

BOOK OF ABSTRACTS

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9th International Conference on Mycorrhiza

30th July – 4th August 2017 | Prague, Czech Republic

BOOK OF ABSTRACTS



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CL = Contributed lecture, **HT** = highlight talk, **IL** = Invited lecture, **KL** = Keynote lecture, **P** = Poster, **ST** = Speed talk, **ST+P** = Speed talk with poster

PLENARY SESSION: Establishing and maintaining mycorrhizas: The molecular interplay

Chair: Paola Bonfante

KL (ID 512)

Reprogramming root cells and altering lipid metabolism to accommodate arbuscular mycorrhizal fungi

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Most vascular flowering plants have the ability to form endosymbioses with arbuscular mycorrhizal (AM) fungi from which they generally gain mineral nutrients while providing carbon to the fungal symbiont. Development of the symbiosis is complex and is initiated by signal exchange that enables growth of the fungus into the root and subsequently into the root cortical cells. Here, reorganization of the cortical cell, coordinated with terminal differentiation of the fungus results in a branched hypha called an arbuscule, enveloped in the plant periarbuscular membrane. This interface is the site of nutrient exchange between the symbionts. We are interested in the molecular basis of development of arbuscular mycorrhizas as well as the mechanisms underlying nutrient exchange between the symbionts. We use Medicago truncatula and Brachypodium distachyon, along with AM fungi, Glomus versiforme and Rhizophagus irregularis, for our studies. Recently, we used a phylogenomic profiling approach to identify plant genes conserved for AM symbiosis that we predict should play significant roles in symbiotic development and/or function¹. This approach revealed at least 138 genes conserved for symbiosis and initial evidence suggests that some of these genes function together in small modules to modify aspects of root cell biology or metabolism to accommodate the fungus within the cell. One such module is comprised of two genes, FatM1 and RAM22, that encode enzymes of lipid biosynthesis. Analysis of M. truncatula fatm and ram2 mutants provided evidence that FatM and RAM2 redirect lipid biosynthesis in the colonized cells to generate C16:0 b-monoacylglycerol, a class of lipid that is typically exported from the cell³. Furthermore, the phenotype and lipid profiles of an ABC transporter mutant, str, suggests that this transporter, which is also conserved for AM symbiosis, may be responsible for export of C16:0 b-monoacylglycerol across the periarbuscular membrane³. Two previous studies have predicted that AM fungi may lack the capacity for de novo fatty acid biosynthesis and therefore may depend on the plant for a supply of fatty acids^{4,5}. Thus, our data support the hypothesis that expression of AM symbiosis-conserved genes enables the root cell to modify lipid biosynthesis to generate a molecule for export to the periarbuscular apoplast, for subsequent access by the fungus.

References:

¹Bravo et al., (2016) Nature Plants. DOI:10.1038/NPLANTS.2015.1208. vol 2.

²Gobbato et al., (2012) Current Biology, 22:2236-2241.

³Bravo et al., (2017) New Phytologist doi:10.1111/nph.14533

⁴Trepanier et al., (2005) Appl. and Environ. Micro., 71:5341-5347

⁵Wewer et al (2014) Plant Journal 79:398-412.

IL (ID 67)

Effector warfare: the role of effector proteins in the establishment and maintenance of mycorrhizal interactions

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In forest ecosystems the roots of most trees and shrubs are colonized by mutualistic ectomycorrhizal (ECM) fungi. Like other mycorrhizal fungi, ECM fungi provide their host with growth limiting nutrients in return for photosynthetically fixed carbon. Thanks to massive community efforts in genomic and transcriptomic sequencing, the last decade has brought us unprecedented understanding of the mechanistic nuts and bolts of how these interactions are established. One of the most striking hallmarks of the 'symbiotic toolbox' encoded by ECM fungi are genes that appear to encode effector-like proteins. Effectors are typically small (<300 amino acids), secreted proteins induced during symbiosis that bear little homology to any other characterized genes. The proteins classified as effectors have been labelled as master regulators of symbiosis due to their ability to effect a change in host signalling or physiology to foster (or force?) symbiosis. I will discuss some of our most recent advances in understanding the biological significance of these enigmatic proteins in establishing and maintaining symbiosis with their host plants. I will finish off with discussing a question that continues to intrigue – if these master regulators are so 'effective', why have ECM fungi not made the evolutionary leap to become pathogenic?

Keywords: protein-protein interactions, hormone signaling, host specificity

IL (ID 115)

Nitrogen metabolism in endomycorrhizal symbioses: is orchid mycorrhiza unique?

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Orchids are peculiar among flowering plants because they form a heterotrophic pre-seed-ling structure, the protocorm, highly dependent on the mycorrhizal fungal partners for supply of organic carbon and other nutrients. Some orchid species remain heterotrophic, and therefore fungus-dependent, also at adulthood. Although orchid tissues are highly N-enriched, we know very little on the N form preferentially transferred to the plant, and on the mechanisms of transfer ^{1,2}.

To study fungal and plant molecular determinants that may be involved in N uptake and transfer in orchid mycorrhiza, we explored genomic and transcriptomic data obtained for the symbiotic association formed between *Tulasnella calospora* (Basidiomycota, Cantharellales) and protocorms of the terrestrial orchid *Serapias vomeracea*³. We also localized fungal and plant transcripts in different cell-type populations collected from mycorrhizal protocorms by laser microdissection (LMD).

Gene expression of a set of fungal and plant genes involved in transport and metabolism of N compounds in asymbiotic and symbiotic conditions suggests that organic N may be the main form transferred to the orchid host. Although we cannot exclude passive N release through digestion of senescent hyphal coils ⁴, plant transcripts coding for amino acid transporters were mainly found in protocorm cells containing viable fungal coils, to indicate active transfer by a living fungus. On the other hand, the intracellular fungus seems to take up ammonium from the apoplastic symbiotic interface, raising intriguing questions on N exchanges in orchid mycorrhiza.

References:

- ¹ Cameron DD, Leake JR, Read DJ (2006) New Phytol. 171:405–416.
- ² Kuga Y, Sakamoto N, Yurimoto H (2013) New Phytol. 202: 594–605.
- ³ Fochi V, Chitarra W, Kohler A, et al. (2017) New Phytol. 213: 365–379
- ⁴ Rasmussen DHN (1995) Terrestrial orchids: From seed to Mycotrophic plant, Cambridge University Press.

Note: RNA sequencing has been carried out at the US Department of Energy (DOE) Joint Genome Institute (contract number DE-AC02-05CH11231) within the framework of the Community Sequencing Project #978 "The Mycorrhizal Genomics Initiative: Exploring the Symbiotic Transcriptomes", coordinated by F. Martin (INRA-Nancy).

Keywords: orchid mycorrhiza, nitrogen, transcriptomics, *Tulasnella*, laser microdissection

IL (ID 310)

Lipid transfer from plants to arbuscular mycorrhiza fungi

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Arbuscular mycorrhiza (AM) symbioses contribute to global carbon cycles as plant hosts divert up to 20% of photosynthate to the obligate biotrophic fungi. Previous experiments suggested that carbon is transferred to AM fungi exclusively in the form of carbohydrates. However, recently sequenced AM fungal genomes lack genes encoding subunits of cytosolic fatty acid (FA) synthase, suggesting that AM fungi may not only depend on sugar but also on FA supply from the host. We identified two Lotus japonicus mutants defective in AM-specifically expressed lipid biosynthesis genes. These mutants perturb arbuscule branching and the formation of vesicles. They also lack emblematic fungal 16:1w5 FAs, indicating that the fungus may lack sufficient amounts of FAs for desaturation. We used ¹³C-glucose labelling followed by 16:0 and 16:1w5 FA isotopologue profiling in roots and in extraradical fungal mycelia to address whether lipids are transferred from host plants to AM fungi. 13C patterns of fungal FAs recapitulated those of two different wild-type plant hosts, indicating cross-kingdom transfer of a 16:0 FA containing lipid from plants to fungi. This transfer of labelled FAs was strongly reduced for the AMspecific lipid-biosynthesis mutants. We conclude that growth and development of beneficial AM fungi is not only fueled by sugars but in addition by energy-rich lipids from plant hosts.

IL (ID 511)

Plant immunity impacting beneficial interactions

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The extent to which immunity functions and defense homeostasis shape and are themselves shaped by microbes remains an open question. New sequencing technologies combined with pathology, cell biology and physiology have enabled a radical re-evaluation of the phylogenetic relationships between saprotrophs, mutualists and pathogens. They also now begin to reveal genomic and transcriptomic signatures of endophytic lifestyles and colonization strategies. As found for well-characterized pathogenic strains, mutualistic fungi have panels of effector-like genes and secreted proteins with the potential to modulate the responsiveness of the host and other microbes. There is also evidence that root-associated microbes enhance plant disease resistance. It is therefore likely that the immune system is a host-specificity determinant of beneficial endophytes. We present here recent advance on molecular communication in root-fungal endophyte interaction.

IL (ID 105)

The arbuscular mycorrhizal transportome, next stop please!

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Understanding how arbuscular mycorrhizal (AM) symbioses establish and function is one of the most important current challenges in microbial ecology. In AM symbiosis, the fungal symbiont and the plant partner benefit from each other in a number of ways. Indeed, availability, uptake and exchange of nutrients in biotrophic interactions will drive plant growth and modulate biomass allocation that are central to plant yield. Understanding mechanisms underlying nitrogen and phosphorus use efficiency by AM plants and carbon allocation in a context of mutualistic biotrophic interactions is critical for plant management. However, the establishment and the functioning of the plant-fungal interface as well as the local adaptation of the fungal mycelium rely on complex and highly coordinated regulations of plant and fungal gene expression that are not well understood. Here, we focus in particular on key players involved in the nutritional exchanges between the mycorrhizal plant and the associated AM fungi, and useful for developing future engineering of new agro-ecological systems.

Keywords: arbuscular mycorrhizal fungi, transportome, nutrition, agroecology

IL (ID 184)

Plant-derived sugar efflux in ectomycorrhizas

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Due to their mycorrhiza-specific induction in whole genome expression analysis of poplar fine roots, members of the SWEET gene family were indicated as candidates for plant sugar efflux carriers in ectomycorrhizal symbiosis. This view was further supported by a plasma membrane localization of selected SWEET proteins after heterologous expression in tobacco leaves. Furthermore, heterologous expression in yeast indicated selected proteins as low affinity glucose facilitators with $K_{\rm M}$ values above 1 mM. Gene expression analysis together with protein function makes it rather likely that glucose is a major fungal carbon source delivered by the plant host upon ectomycorrhizal symbiosis. Surprisingly, mycorrhiza-induced expression of these identified glucose efflux carriers was not restricted to the Hartig net area of ectomycorrhizas but gene expression was also observed in root sections of the developing interface as well as the root tip. An exclusive function of the Hartig net in carbon delivery must thus be questioned.

As ectomycorrhiza-specific SWEET gene expression was obtained with different ectomy-corrhizal fungi, a general mechanism of gene induction can be supposed. We have therefore started the identification of regulatory elements within the promoter region of selected SWEET genes. For fast progress of such promoter analysis we have developed a poplar root transformation protocol that enable the generation of transgenic plants within just one month. Our current data indicate that three different regulatory elements are working together to allow the observed strong and ectomycorrhiza-specific induction of SWEET gene expression.

Keywords: fungal nutrition, sugar, poplar

IL (ID 64)

Sweet talk with endo- and ecto-mycorrhizal symbionts: do they speak the same language?

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Arbuscular mycorrhizal (AM) fungi produce various signaling molecules, including lipochitooligosaccharides (LCOs) and short chitooligosaccharides. These fungal signals are perceived and transduced in host plants via a highly conserved "common symbiosis pathway" (CSP). Genomic data suggested that ectomycorrhizal (ECM) fungi, e.g. Laccaria bicolor, may be capable of producing LCOs and that some ECM host plants like *Populus* spp. contain all the components of the CSP. Based on this observation, we hypothesized that some ECM fungi may produce LCOs and that the CSP could play a role not only in AM but also in ECM associations. We found that hyphal exudates from several ECM fungi but not short chitooligosaccharides can trigger typical root hair branching in Vicia sativa suggesting the presence of non-sulfated LCOs in these ECM exudates. In contrast to sulfated LCOs and negative controls, non-sulfated LCOs triggered an increase in both lateral root formation in hybrid poplar (Populus x canescens) and the total number of root tips colonized by L. bicolor. We utilized RNA interference to silence the expression of core components of the CSP, including CCaMK. Increased lateral root formation induced by nonsulfated LCOs did not occur in the CCaMK-RNAi line. Furthermore, during colonization of poplar by L. bicolor, mantle width, Hartig net depth, and the expression of the phosphate transporter PtPH12 were significantly reduced in the CCaMK-RNAi line compared to a wild-type control. Altogether, our data suggest that LCOs are produced by some ECM fungi and that the CSP has been recruited for the establishment of ECM associations.

Keywords: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, lipo-chitooligosaccharides, common symbiosis pathway

CONCURRENT SESSION: Mycorrhizas in agro- and agroforestry ecosystems

Mycorrhizas are commonly advocated to improve sustainability and productivity of ecosystems from which humans extract resources. These qualities are vital for attenuating the ecological footprint of a growing human population worldwide. In this session we welcome talks on any type of mycorrhiza that give a new insight into ecological functioning of these symbioses in ecosystems under different land-use, with a particular focus on talks that give clear recommendations for the "way forward" in an integrated manner. These can be in the form of a suggested management practice (weighed against alternatives), or through a new understanding of mycorrhizal (community) behavior that informs on their functions in used lands. Whenever possible, talks should extend beyond observations of responses of mycorrhizas to land use (or plants to mycorrhizas), through integrating these into a general conclusion that can be used as a starting point for a discussion to critically evaluate how mycorrhiza can contribute to sustainable land use. Furthermore, we specifically encourage submissions and representation from all continents explicitly including Latin America, Asia and Africa.

Chairs: Amadou Ba, Erik Verbruggen

CL (ID 89)

Arbuscular mycorrhiza induces complementarity in mixtures of maize varieties resulting in overyielding

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Arbuscular mycorrhizal fungi (AMF) have been reported to cause overyielding in plant mixtures, suggesting mycorrhiza-induced complementarity. However, the occurrence of common mycorrhizal networks has also been claimed to increase niche overlap and to reduce complementarity. We grew monocultures and mixtures of maize varieties in a greenhouse and in a field experiment to test these competing hypotheses. In the greenhouse (with one species of AMF) and in the field (with mixed inoculum), variety mixtures showed increased mycorrhizal activity, enhanced P-uptake and overyielding when mycorrhizal but not when non-mycorrhizal. The increase in relative yield total was mainly due to complementarity effects. Mixing also increased size inequalities between varieties. In the field, mixing maize varieties increased species richness of AMF. Our data show that the magnitude of mycorrhiza-induced overyielding in variety mixtures was similar to that reported for two-species mixtures in the absence of temporal niche differentiation.

Keywords: complementarity, overyielding, hyphal length density, mycorrhizal fungal species richness, mycorrhizal networks

CL (ID 164)

Field reality: short-scale soil heterogeneity impacts mycorrhizal contribution to maize P nutrition under contrasted fertilization

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The relationship between root colonization by arbuscular mycorrhiza (AM) fungi and phosphorus (P) fertilization remains controversial and soil characteristics are still rarely considered. To assess the contribution of AM to maize P nutrition in contrasted field conditions, a range of hybrids were grown at two contrasting P levels of a long-term P-fertilizer trial in two adjacent soil types: alkaline and neutral. Root colonization was assessed by microscopic observation and qPCR targeting two common AM species, *Funneliformis mosseae* and *Rhizophagus irregularis*. Functionality of AM colonization was assessed by measuring the expression of two P-transporters by qPCR.

Regardless of the soil type, AM root colonization was highest in the non-fertilized treatment compared to high-P fertilization. Looking closer, this drop was driven by the neutral soil and no variation was observed in the alkaline soil with P fertilization. The non-fertilized treatment was particularly harsh for maize development in the neutral soil, triggering the highest expression of the plant P starvation inducible gene (ZmPT1:3), coupled to the lowest plant growth and P content. Under these conditions, the mycorrhizal P uptake pathway was particularly activated with the highest expression of the AM inducible P transporter (ZmPT1;6). On contrary, its expression remained constant among fertilization levels in the alkaline soil.

The observed resilience to P fertilization in the alkaline soil could partially be due to high abundance of *F. mosseae*, which increased under P fertilization. Our results emphasize the importance of considering soil characteristics which differentially impacted AM symbioses and their implication in plant nutrition.

Keywords: phosphorus fertilization, long term-trial, molecular trait indicators, P transporter, qPCR

CL (ID 242)

Multi-year AMF field application on cereals and pseudocereals: a focus on micronutrients and secondary metabolites

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Production of cereals and pseudocereals often leads to Fe, Zn, and antioxidant deficiency. A better understanding of the potential of field inoculation with AMF to increase micronutrient uptake and production of secondary metabolites is needed.

We investigated by multi-year field experiments (2013–2017) located in central Italy the role of AMF inoculation in Fe-Zn uptake and biosynthesis of antioxidants in cereals (bread wheat, durum wheat and millet) and in pseudocereals (buckwheat).

The effects of AMF inoculation by *Rhizophagus irregularis*, plant genotypes and several agronomic techniques were investigated. AMF inoculum effectiveness was assessed on concentration of Fe-Zn and antioxidant compounds in grain and transformed products. Occurrence of AMF in roots was assessed using morphological and molecular techniques.

In 2014 trial, Fe-Zn grain concentrations were increased by AMF inoculation in durum wheat (53% and 89%) and bread wheat (61% and 90%). Increases in AMF root colonization (durum wheat: 22%; bread wheat: 30%) and modifications in root abundance of AMF sequences, belonging to genera *Rhizophagus* and *Funelliformis*, were reported. In 2015, 2016 and 2017 trials, wheat genotypes differently responded to AMF inoculation for Fe-Zn concentrations (Fe: -26% à 113%; Zn: -22% à 98%), for flavonoids (-29% à 196%), for α -lipoic acid (-28% à 199%), for rutin (3% à 27%) and for quercetin (0.9% à 5.2) in wholemeal flour and transformed products. In 2016 trial, millet and buckwheat genotypes showed a wide range of variation in AMF root colonization.

AMF inoculation represents an agronomic tool to increase food Fe-Zn concentrations and antioxidant compounds.

Keywords: AMF field inoculation, nutraceutical compounds, Fe concentration, Zn concentration, agronomic biofortification

CL (ID 273)

Does inoculation with introduced *Rhizoglomus irregularis* DAOM-197198 change AMF community structure in the field?

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AMF inoculation of agricultural soils has gained popularity in the last decade with the ultimate goal to enhance crop yield and sustainability of agrosystems [1,2]. The effectiveness and establishment of introduced inoculants in the field have shown inconsistency that may be due to many factors influencing crop colonization and efficacy [3-4]. Among these factors, competition with the indigenous AMF and their community structure changes could affect the establishment of these inoculants. In this investigation, we aim to determine the impact of introduced inoculation in the field using Rhizoglomus irregularis on the community structure of indigenous AMF in roots of three major crops: corn, soybean and wheat. Three field trials were conducted with inoculated and non-inoculated plots. Four to ten samples on two growing phases for each crop were collected. Root-colonization and Illumina MiSeq amplicon sequencing using 18S rDNA were performed on 122 samples. After quality trimming and bioinformatic analysis, 4,115,563 reads (35,737 ± 18,296 reads per sample) were kept and analyzed. Taxonomic assignment at 97% of sequence similarity resulted in 103 OTUs that were clustered into 46 virtual VTX. These 46 VTX represented 99.16% of the reads sequenced in each sample. Our results showed R. irregularis was the most abundant VTX in inoculated and non-inoculated plots for all crops. However, we didn't observe any significant difference in AMF relative abundance nor their community structure in the roots in inoculated and noninoculated plots, suggesting that inoculation intervention with R. irregularis didn't changed the native AMF community in the plant roots.

References:

- [1] Hijri 2016. Mycorrhiza 26:209–214.
- [2] Rillig et al. 2016 Frontiers in Plant Science 7: 1625.
- [3] Gosling et al. 2006. Agriculture, Ecosystems & Environment 113:17–35.
- [4] Verbruggen et al. 2013. New Phytologist 197: 1104–1109.
- [5] Schlaeppi et al. 2016. New Phytologist 212: 780–791.

Keywords: AMF inoculant, community structure, agriculture, Illumina MiSeq, amplicon sequencing

ST+P (ID 136)

Locally diverse arbuscular mycorrhizal fungal communities differentially affect plant growth and nutrition

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Functional diversity of arbuscular mycorrhizal fungi (AMF), which plays key roles in plant growth and nutrition, has been rarely investigated by means of whole native AMF communities. A distinctive field site within the UNESCO Biosphere Reserve "Selva Pisana" hosting the AMF highest species richness detected in a single area, with 58 AMF species and 14 genera, was identified as a "hot-spot" of AMF diversity¹.

Here, the soil from such a site, harbouring its native AMF communities, was collected from 6 plots showing uniform chemical-physical characteristics and used to grow three plant species (*Allium cepa*, *Lactuca sativa* and *Capsicum annuum*), which were evaluated for biomass, P and N contents. AMF communities in the roots of plants producing the most contrasting growth and nutritional responses were analyzed by constructing libraries from AML1/AML2 amplicons and sequencing the relevant clones.

AMF species common to agricultural soil, such as *Funneliformis mosseae*, *Claroideoglomus etunicatum* and *Rhizoglomus* spp. and other sensitive to agricultural practices, such as *Racocetra fulgida* were identified. The highest AMF species richness and the most evenly distributed AMF community composition were detected in the roots of plants showing the best growth performances. Moreover, a distinctive host preference was found in some AMF species.

Our work demonstrates that the local diversity of native AMF communities may strongly affect plant responses, a finding to be further explored in the years to come, in order to exploit the potential benefit of mycorrhizal symbionts in sustainable agroecosystems.

References:

¹Njeru E.M. Avio L., Bocci G., Sbrana C., Turrini A., Bàrberi P., Giovannetti M., Oehl F. (2015) Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. Biol Fertil Soils 51:151–166.

Keywords: native AMF community composition, biosphere reserve, local AMF diversity, AMF functional diversity

ST (ID 57)

Bamboo forest expansion increases soil organic carbon through its effect on soil arbuscular mycorrhizal fungal community and abundance

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Aims: Moso bamboo (*Phyllostachys pubescens*) is a fast-growing species that can invade neighboring forests through its vigorous rhizome system, leading to large shifts of plant diversity and soil properties. A primary concern is the response of arbuscular mycorrhizal (AM) fungal communities and related soil C sequestration to bamboo forest expansion.

Materials and Methods: We used a long-term soil chronosequence from primary broadleaved forest to moso bamboo forest to examine the changes in AM fungal abundance and communities, as well as their role in soil C storage. Soil AM fungal abundance was estimated by $16:1\omega 5$ NLFA and community composition was analyzed using high-throughput sequencing technique based on Illumina MiSeq platform.

Results and discussion: AM fungal communities showed strong niche differentiation, and were highly structured by forest type (r^2 =0.647, P=0.001), and marginally correlated to soil pH and organic C. Bamboo forest expansion increased soil AM fungal biomass, easily extracted glomalin-related soil protein (EE-GRSP), and water-stable macroaggregates. We observed that soil AMF biomass contributed substantially to both soil macroaggregates and EE-GRSP, which were tightly correlated with soil organic C. The influence of bamboo forest expansion on soil C sequestration was mainly due to its indirect effect on AM fungal biomass.

Conclusions: Bamboo forest expansion significantly changed soil AM fungal communities and increased AM fungal biomass, which in turn contributed to enhanced soil aggregation and C sequestration.

Keywords: arbuscular mycorrhizal fungal community, moso bamboo forest expansion, soil C sequestration, glomalin-related soil protein, water-stable macroaggregates

ST+P (ID 173)

Exploring functional applications of arbuscular mycorrhizal fungi in sweet cherry and links to fruit quality

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Perennial fruit crops are known to form effective relationships with arbuscular mycorrhizal fungi (AMF) but few studies have been conducted in Australia or with sweet cherry, which is an important fruit crop in Tasmania. The aim of recent research has been to characterize these associations and explore functions related to fruit quality.

Glasshouse trials were established with sweet cherry cuttings inoculated with *Rhizopha-gus irregularis*, and found that survival and growth of cuttings increased significantly. Treatments with reduced and excessive water application sought to determine if presence of the same AMF species could moderate changes in water potential and other physiological indicators of stress, but results were typically not significant.

A field trial was conducted which examined the impact of conventional and alternative fertilizer and weed management on fruit quality in a commercial sweet cherry orchard. A positive and significant relationship was found between root colonization by native AMF and several fruit quality characteristics, including titratable acidity and total soluble solids.

Studies to estimate abundance and diversity of resident AMF species have been conducted in two commercial sweet cherry orchards. Extraction of soil DNA and glomeran-specific quantitative PCR has revealed that strong seasonal differences in abundance of glomeran species can be found, and species diversity via PacBio next generation sequencing will also be presented.

In conclusion, knowledge of the role of AMF in commercial orchards is vital to inform practical management and through a range of ongoing studies this is being advanced.

ST+P (ID 175)

Contribution of arbuscular mycorrhizas to crop nutrition and health in maize agroecosystems

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Abuscular mycorrhizal fungi (AMF) are abundant and diverse in most agroecosystems, but their full potential in crop nutrition and health is yet unexplored. Management of AMF in agroecosystems requires profound knowledge on their ecology especially in terms of interactions with other plant and soil biota and how they respond to agricultural practice.

In a series of greenhouse pot- and field experiments the contribution of native AMF to crop nutrition and health were explored. Also their interactions with other beneficial rhizosphere microorganisms as well as their response to application of mineral and organic fertilizers and chemical pesticides were examined.

In general AMF were compatible with other rhizosphere fungi such as *Candida* spp., *Trichoderma* spp. and *Beauveria bassiana* resulting in improved P nutrition and root health. Strong plant growth promotion and depression was observed without and with mineral P fertilization, respectively. Organic fertilization in terms of green manure, animal manure and compost had no effect on AMF root colonization except green manure with rape, which reduced AMF root colonization. Application of the pesticides Carbofuran, Benomyl and Glyphosate all strongly reduced both AMF root and soil colonization. Field experiments employing Benomyl as non-mycorrhizal control treatments revealed plant growth promotion and depression depending on maize genotype, soil characteristics and fertilization scenario.

In conclusion, native communities of AMF seem to be important in maize agroecosystems in relation to crop nutrition and health, why conservation and management of AMF should be an integrated component when developing crop nutrition and health programs for maize production.

ST+P (ID 318)

Both *in vivo*- and *in vitro*-produced arbuscular mycorrhizal inoculum improve performance of stressed *Malus domestica*

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Application of in vivo- and in vitro-produced mycorrhizal inocula in agricultural systems has become increasingly popular to improve crop performance. The effectiveness of inoculum in agrosystems must be assessed for the continued development of these inoculum production technologies. The goal of this study was to investigate the efficacy of in vivo- versus in vitro-produced MycoApply® inocula in container-grown apple trees. In spring of 2016, commercially available MycoApply® mycorrhizal inoculum containing four arbuscular mycorrhizal species (Rhizophagus intraradices, Funeliformis mosseae, Claroideoglomus etunicatum, Glomus aggregatum) and produced either in vivo (trade name MycoApply Endo) or in vitro (trade name MycoApply EndoMaxx) were applied to bare-root 'Honeycrisp' apple trees at a rate of 6,000 propagules per tree. Single trees were planted into 450 kg tote bags with a calcined granular clay medium and grown under nutrient and water stress. Each treatment contained 6 individual tree replicates. ANOVA analysis was used to determine statistical significance at a P level < 0.05. Both in vivo- and in vitro-produced inocula were effective in increasing mycorrhizal colonization, vegetative growth, relative chlorophyll content, photosynthesis, and chlorophyll fluorescence. Although there were no clear effects on root volume (measured by water displacement), noticeable differences in the nutrient content were observed between the leaves of inoculated trees and the untreated trees. Mineral nutrient content in the leaves increased (N, P, Mn) and decreased (Na, Al, Cu). This study confirmed equal efficacy of in vitro- and in vivo-produced mycorrhizal inocula to improve performance of apple tree as host plant.

Keywords: inoculum production, *Malus domestica, in vivo* vs. *in vitro,* biofertilizer, root colonization

ST+P (ID 98)

The potential of 'biofertilization' and 'bioirrigation' for enhancing legume-cereal intercropping yield

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Legume-cereal intercropping is often used in traditional dry land agriculture, but little is known about the processes by which nutrient and water resources are shared between the crops. Here, we tested whether finger millet, a shallow-rooted cereal, can profit from a deep-rooted neighboring pigeon pea (capable of "bioirrigation", a mechanism by which a deep-rooted plant redistributes water to a neighboring plant) in the presence of "biofertilization" with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), when exposed to drought conditions. We conducted a greenhouse experiment using compartmented microcosms. Pigeon pea was grown in a deep compartment with access to a moist substrate layer at the bottom, whereas finger millet was grown in a neighboring shallow compartment, separated by 25-µm nylon mesh, without direct root-access to the moist substrate layer. In the presence of a common mycorrhizal network (CMN), with or without PGPR, drought conditions had little negative effect on the biomass production of the finger millet plant whereas in absence of biofertilization, finger millet biomass production was less than half compared to well-watered conditions. Isotope tracing (15N and 33P was employed to investigate nitrogen and phosphorus transfer in the context of biofertilization and the different water conditions. Our results show that "biofertilization" with AMF alleviates the negative effects of drought on the finger millet plant and indicate that the pigeon pea plant provides a "bioirrigation" effect in this simulated intercropping system.

CL (ID 28)

Under pressure: overgrazing decreases mycorrhizal colonization of preferred and unpreferred grasses in the Patagonian steppe

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Introduction/Aim: Arbuscular mycorrhizal fungi (AMF) are related to plant community dynamics and ecosystem functioning. Overgrazing can negatively affect plant performance unbalancing the association with AMF. We studied the effect of sheep grazing pressure on AMF colonization for preferred (*Bromus pictus* and *Poa ligularis*) and unpreferred grasses (*Pappostipa humilis* and *Pappostipa speciosa*) in the Patagonian steppe.

Materials and Methods: The degree of AMF colonization (hyphae, arbuscules and vesicles) in ungrazed, moderate and intense grazing sites was quantified. Root samples were taken during two contrasting seasons: summer (active plant growth) and autumn (descending plant growth).

Results: In ungrazed sites, during the summer, *B. pictus* showed the highest degree of AMF colonization (59% *B. pictus* > 29% *P. speciosa* > 24% *P. ligularis* > 16% *P. humilis*). Moreover, in *B. pictus*, the most preferred species, AMF colonization markedly decreased from ungrazed sites to those with moderate and intense grazing. In all species, mycorrhizal colonization was reduced by intense grazing conditions.

Discussion: Decrease of AMF colonization by intense grazing was higher in the most preferred species, but also significant for the unpreferred species. Notably, the reduction of AMF colonization in unpreferred species did not match with their greater abundance under intense grazing conditions, revealing AMF colonization as an indicator of system deterioration.

Conclusion: Our results suggest that benefits of mycorrhizal symbiosis for mineral nutrition and plant survival are being disrupted by overgrazing as an indicator property for the whole system, eventually revealing an increasing degree of ecosystem degradation.

Keywords: arbuscular mycorrhizal fungi, desertification, grazing intensity, herbivore preference, tussock grasses

CL (ID 215)

Improving production of globally important crops by using AMF genetic variability and in vitro production

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Arbuscular mycorrhizal fungi (AMF) have an enormous potential to increase crop yields on a global scale because all globally important plants form this symbiosis. Cassava is the second most important component in the daily diet of about 1 billion people and it is cultivated in acidic soils with low nutrient availability. Enormous challenges lie ahead, in terms of providing food to the growing human population and designing sustainable crop systems. I will show how, by using naturally occurring genetic variation of in vitro-produced AMF, it is possible to use these fungi to greatly increase the production of this globally important crop. Using such variation in the fungi can result in very large reproducible differences in cassava root productivity, in real farming situations, although this is highly dependent on combination of both the AMF genotype and the plant variety. Realistic application of AMF in agriculture should also not have negative impacts on existing AMF communities. I will, therefore, also discuss the impact of genetic variation in AMF on local AMF communities. Our work demonstrates that AMF genetics can be used to greatly increase production of globally important crops in an environmentally sound way. However, our work also shows that crop genetics needs to be considered as well in such applications.

Keywords: cassava, AMF, genetics, crop production, application

CL (ID 117)

Using genetic variability of arbuscular mycorrhizal fungi to improve cassava waterstress tolerance in Africa

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Water scarcity is a problem around the world that negatively impacts global crop yields. Climate change is expected to greatly increase the severity of droughts, thus exacerbating the problem. The use of arbuscular mycorrhizal fungi (AMF) can potentially mitigate the effects of water-deficit stress in plants. Cassava is a crop that feeds almost one billion people daily. In the attempt to produce more food, cassava is increasingly being grown on marginal lands in Africa where the plant suffers water deficit almost annually. Inoculation of cassava with AMF has repeatedly been shown to successfully increase yields of cassava in Colombia. AMF could potentially be applied in many areas of the tropics where cassava is cultivated, including Africa. In a joint Swiss, African and Colombian investigation in Tanzania and Kenya we have been using genetic variation in AMF to improve cassava production and address problems of drought tolerance. Our hypothesis is that progeny from isolates of Rhizophagus irregularis that contain more within-fungus genetic variability will induce a wider range of tolerance responses to drought than progeny with a low within-fungus genetic variability. Here, I will present how genetically variable AMF lines significantly contributed to root production in an improved and a local variety of cassava and how the genetic variability in the fungi affected the drought tolerance of the plants.

Keywords: water-stress, cassava, genetic variability

CL (ID 197)

How reliable are microbial inoculants in agriculture for improving yield and nutrient use efficiency? – a meta-analysis

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The application of microbial inoculants, also called biofertilizers, is a promising technology for future sustainable farming systems in view of rapidly decreasing phosphate (P) stocks and fossil fuel reserves for chemical nitrogen (N) fertilizer production. Rhizosphere microorganisms have evolved together with the plants, and many of them contain genes that are valuable for enhancing plant growth and health. Soils and climates are highly variable, and the success of inoculation is difficult to predict. We have conducted a metaanalysis to quantify benefits of biofertilizers in terms of yield increase, nitrogen and phosphorus use efficiency. A total of 633 peer reviewed publication studies were screened and 169 studies proved to be eligible for meta-analysis enabling us to make 1672 pairwise comparisons, in which we included only studies in which inoculated plots and non-inoculated control plots were compared under identical P and N inputs. Most studies originate from tropical and subtropical countries. Major findings were: 1) the superiority of biofertilizer performance in dry climates over other climatic regions (yield response: dry climate +21.0%, tropical climate +15.9%, oceanic climate +11.3%, continental climate +10.1%); 2) meta-regression analyses revealed that yield response due to biofertilizer application was generally small at low soil P levels; efficacy increased along higher soil P levels in the order AMF, P solubilizers and N fixers. Moreover, success of AMF inoculation was greater at low organic matter content and a neutral pH. We conclude that biofertilisers are already a viable technology to increase yield and thereby P use efficiency.

Keywords: biofertilizer, nitrogen use efficiency, phosphorus use efficiency, agricultural productivity, microbial inoculants

ST+P (ID 299)

Linking fertility amendments, field management, and sorghum genotypes with AM fungi and grain nutritional quality

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Introduction/Aim: Improved partnerships between AM fungi and food crops, such as grain sorghum (*Sorghum bicolor*), could alleviate current agricultural issues such as the staggering rate of farm soil erosion and the unsustainable use of global phosphorus reserves. Our research assesses sorghum genotype and farm management influences on AM fungi, linking these with grain production and nutrition.

Materials and Methods: We determined AM responsiveness in sorghum genotypes under greenhouse conditions. In corresponding field experiments, we assessed the impact of alternative fertility amendments (biochar, worm compost) and farm management practices (intercropping) on AM fungal abundance. We examined how intra-radical (measured microscopically) and extra-radical (measured using phospholipid and neutral lipid fatty acid analyses) AM fungal abundance influenced grain quality (e.g. protein).

Results: In low-fertility soil, landrace (open-pollinated) cultivars were more highly colonized and significantly more responsive to AM fungi, while producing significantly more grain, with greater nutritional quality, compared to commercial (fertilizer responsive) hybrids. Alternative amendments and farm practices maintained or improved yield and grain nutrition, with greater intra- and extra-radical AM fungal abundance, compared to conventional practices.

Discussion/Conclusion: Farm management and genotype selection are key considerations, as some agricultural practices and genotypes can reduce the abundance and benefits of AM fungi. Our results indicate that breeders and farmers may be able to utilize sorghum genetic traits and improved agricultural practices to increase AM biomass and enhance the benefits of AM fungi in agroecosystems, potentially improving grain production and nutrition, decreasing the need for phosphorus amendments, and stabilizing farm soils.

Keywords: intercropping, biochar, worm compost

ST (ID 159)

Conservation Agriculture – Are mycorrhizal fungi also conserved?

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Conservation agriculture (CA) promotes reduction of fertilizers and agrochemicals, reduced tillage (RT) of soil with cover cropping, crop rotation and intercropping management practices. It has been well documented that many conventional agricultural practices negatively affect arbuscular mycorrhizal (AM) establishment and functioning. Could conservation agricultural approaches also conserve or enhance mycorrhizal activity in the soil?

An experimental trial of three replicates plots under CON and RT subdivided into twelve treatments of six crops and two fertilizer inputs was assessed over several growing seasons for various AM fungal parameters such as spore density, percentage root colonisation and easily extractable glomalin related soil protein (GPRS). Cropping combinations were maize monoculture; maize soybean rotation; maize cowpea rotation; maize oats intercropping; maize vetch intercropping.

AM fungal spore densities and GPRS concentrations were very low and colonisation was absent and did not improve over the subsequent growing season. As a result AM fungal inoculation was applied to all plots and in the third growing season resulted in increased mycorrhizal parameters assessed. Overall, fertilizer application and crop type had subsequent implications for mycorrhizal activity.

The absence of mycorrhizal activity in the initial trial stages despite the CA approach may be a result of historical land use practices and current practices and has been noted in similar trials. What has become apparent is that a change in management practices does not translate into an active soil mycorrhizal population and inoculation interventions may be required.

ST+P (ID 206)

Efficiency of six AMF consortia on papaya plants from six different natural ecosystems of Mexico

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Inoculant efficiency has been observed when a mixture of AMF species is used. Therefore, selecting efficient species from native populations for specific target sites is desirable. The goal was to evaluate the efficiency of six consortia from natural ecosystems. Sand dunes, deciduous low forest, tropical forest, xeric shrubland, cloud forest and pineoak woodlands soil was collected. It was then propagated for 10 months. AMF root colonization was determined for each of them. A full randomized experiment was established with 5 replicates and 7 treatments (6 ecosystem inoculant and a control). Carica papaya plants were used because it is highly mycotrophic and their effects were shown 45 days after inoculation. We evaluated, root length AMF colonization, leaf area, total leaf P, and dry weight of the plant. The AMF species that were present in each inoculant were morphologically identified at the beginning and currently a metagenomic analysis is under way by using massive sequencing. A one-way ANOVA was used for each variable. We found significant differences ($P \le 0.05$) among treatments, being the pine-oak woodlands consortium the one that yielded the highest values on dry weight and root AMF colonization. Nevertheless, the total P content in the sampled tissue of the sand dunes consortium showed an 11-folds increase compared with the control, while the low jungle consortium only had 3.5-folds increase respect to control but it showed the highest growth on other variables. These results show that the AMF function varies in a plant species.

ST+P (ID 305)

The coffee agroecosystem highlights possible non-host drivers of diversity in AMF communities

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Which aspects of the environment are key determinants of fungal diversity? For crops such as coffee, grown in tropical ecosystems and largely supplanting them, understanding drivers of diversity is important for maintaining diverse and sustainable agroecosystems. Because coffee fields vary in shade, chemical use, and nutrients, they serve as a natural laboratory for examining effects of non-host differences on fungal communities.

We used Illumina sequencing of the fungal ITS2 and the UNITE database to characterize root communities of 25 coffee fields in Costa Rica, for which we also collected data on environmental characteristics. We used regression, PCA and decision trees to determine which environmental parameters were most strongly associated with diversity of fungi and of AMF in particular.

Both fungal and AMF richness were higher in coffee fields with less available phosphorus, while AMF richness was positively correlated with shade and fungal richness with calcium. AMF richness and abundance were positively correlated, but there was no relationship between fungal richness and abundance. Of 58 AMF species detected, only abundance of *Gigaspora margarita* was negatively correlated with shade.

Greater AMF richness in soils poor in phosphorus may reflect the relative importance of the symbiosis. However, the same pattern for fungi, and for AMF in the shade, suggests that low resource availability may maintain diversity through reducing competitive exclusion. Alternatively, higher AMF richness and abundance in shadier environments may reflect coffee's origin as a forest understory tree. Agroecosystems provide insights into potential drivers of diversity, suggesting factors to test in manipulative experiments.

Keywords: arbuscular mycorrhiza, tropical, coffee agroecosystem, diversity, community

ST+P (ID 163)

Effects of nitrogen and phosphorus addition on ectomycorrhizal community composition and function

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Nitrogen is the main limiting nutrient in boreal ecosystems, but studies in south-western Sweden suggest that certain forests approach phosphorus (P) limitation driven by nitrogen (N) deposition.

To estimate how this will influence the composition of ectomycorrhizal (ECM) communities and N retention, we added N, P or N+P to Norway spruce forest plots to push the system either to N or P limitation. We measured tree growth and needle chemistry, ECM biomass and ECM uptake and leaching of ¹⁵N added to ingrowth mesh-bags. Additionally, we analysed the effect on ECM communities, both in soil and in apatite amended mesh-bags.

Tree growth responded positively to P fertilization and needle P concentrations in the control plots were below deficiency levels suggesting that the system was P limited. ECM biomass was reduced and ¹⁵N ammonium leaching enhanced by N+P fertilization but not by P or N fertilization. The ECM communities in the soil significantly responded to N+P and P fertilization further supporting a more important role of P than of N in regulating growth and activity of trees and ECM fungi in this forest. *Tylospora asterophora* was significantly more abundant in the N+P and P fertilization in soils while *Boletus badius* was significantly more abundant in the apatite-amended mesh-bags, especially in the N treatment where P demand is higher.

The ecological significance of these two species is discussed, and further studies under more controlled conditions will be performed to estimate the roles of these species in tree nutrition under different nutrient regimes.

ST (ID 40)

Does rapid urbanisation pressure in Delhi also threatens biodiversity of Arbuscular Mycorrhizal fungi?

Manju M. Gupta (Sri Aurobindo College, University of Delhi, Malviya Nagar, India)

Urban ecosystems of Delhi forests have been known to harbor rich biodiversity of Arbuscular Mycorrhizal (AM) fungi. In the present study, an effort was made to study the impact of soil and air polluting anthropogenic activities on biodiversity of AM fungi. It was hypothesized that AM fungi would not be able to sustain the urbanization pressure due to upsurge of environmental (air and soil) pollution in Delhi, and the effect would be reflected in different measures of biodiversity and abundance.

AM fungal biodiversity was measured at nine sites located in ancient Aravalli ranges at Delhi, India which were classified as low, moderate and high human intervention sites based on values of measured as air quality index and soil factors. The spores were quantified and characterized from 81 samples collected from these sites.

High alpha, beta and gamma diversity of AM fungi was observed in this area. Significant decrease in AM fungal diversity (measured as Shannon Wiener index and evenness) and abundance (richness and biovolume) was observed in high human intervention sites. Significant differences in AM fungal community structure were depicted with nonmetric multidimensional scaling (nMDS) and PERMANOVA. Members of family Gigasporaceae in subphylum Glomeromycotina were found to be specifically affected by urbanization. Therefore, looking into the overall status of AM fungal biodiversity in these sites of Aravalli forest ranges, long term ecosystem based strategies need to be designed to ensure their protection and conservation.

Keywords: biodiversity, pollution, human intervention, Glomeromycota, arbuscular

POSTERS

P (ID 1)

Elemental Stoichiometry of indicates predominant influence of potassium and phosphorus limitation on arbuscular mycorrhizal symbiosis in acidic soil at high altitude

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The functioning of high-altitude agro-ecosystems is constrained by the harsh environmental conditions, such as low temperatures, acidic soil, and low nutrient supply. It is therefore imperative to investigate the site-specific ecological stoichiometry with respect to AM symbiosis in order to maximize the arbuscular mycorrhizal (AM) benefits for the plants in such ecosystems. Here, we assess the elemental stoichiometry of four Capsicum genotypes grown on acidic soil at high altitude in Arunachal Pradesh, India. Further, we try to identify the predominant resource limitations influencing the symbioses of different Capsicum genotypes with the AM fungi. Foliar and soil elemental stoichiometric relations of Capsicum genotypes were evaluated with arbuscular mycorrhizal (AM) colonization and occurrence under field conditions. AM fungal diversity in rhizosphere, was estimated through PCR-DGGE profiling. Results demonstrated that the symbiotic interaction of various Capsicum genotypes with the AM fungi in acidic soil was not prominent in the study site as evident from the low range of root colonization (21-43.67%). In addition, despite the rich availability of carbon in plant leaves as well as in soil, the carbon-for-phosphorus trade between AMF and plants appeared to be limited. Our results provide strong evidences of predominant influence of the potassium-limitation, in addition to phosphorus-limitation, on AM symbiosis with Capsicum in acidic soil at high altitude. We also conclude that the potassium should be considered in addition to carbon, nitrogen, and phosphorus in further studies investigating the stoichiometric relationships with the AMF symbioses in high altitude agro-ecosystems.

Keywords: stoichiometry, resource limitation, arbuscular mycorrhizal fungi, acidic soil, high altitude

P (ID 3)

The source of Arbuscular Mycorrhizal fungi inoculum improved the growth and nutrient uptake of *Faidherbia albida* (Del.) A. Chev. tree seedlings

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Poor land use management inhibit the growth and establishment of agroforestry seedlings in the dryland areas. Inoculation of plants with AM improves the establishing of agroforestry plants on degraded soils. This paper quantified the AM status of Faidherbia albida trees grown on different land uses and the growth and nutrient uptake of F. albida seedlings inoculated with different AM inoculum sources. Soil and fine root samples were collected from the rhizosphere soils of F. albida trees maintained in different land uses. Completely randomized designs (CRD) with four treatments replicated five times were used to grow F. albida seedlings in a greenhouse. All F. albida trees were colonized by AM fungi. AM root colonization and spore abundance of F. albida trees grown on area exclosure was significantly higher (P<0.0086) followed by grazing land and cultivated land. Glomus was the dominant genus in all land uses AM inoculated F. albida seedlings shown significant (P<0.05) growth enhancements compared to controls. Growth improvement and nutrient uptake was significantly (P<0.05) higher in seedlings inoculated with AM from area exclosure followed by grazing land and cultivated land. This emphasis the importance of native soil AM potentials in area exclosure and grazing land for better combinations of F. albida seedlings with AM fungi to achieve optimum plant growth improvement, environment protection, and rehabilitation of degraded lands.

Keywords: spore abundance, AM colonization, inoculum types, land use types, agroforestry

P (ID 6)

Improving growth performance of *Allanblackia floribunda* cuttings using mycorrhizal association

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The studies aim to assess (1) the mycorrhizal status and community richness in six populations of Allanblackia floribunda (Batibo, Mouanko, Yalpenda, Nkenlikok, Ngoumou and Lomie), two states of the system (disturbed and undisturbed) and two circumference classes (less than 40cm and up to 50cm) of Allanblackia spp. (2) the effect of three inoculums (indigenous soils from root zone of A. floribunda composed of the five fungi taxa mentioned below, one inoculum composed of mixture of Acaulospora tuberculata and Gigaspora margarita and the control) on growth (length of stem) and development (total sugar and proline) of A. floribunda stecklings were carried out. Results showed that A. floribunda is naturally colonized by arbuscular mycorrhizae. Root colonization was significantly (p < 0.01) different in the targeted populations with the highest observed in Yalpenda (57.21 ± 3.175%). State of the system significantly (p = 0.003) affected root colonization. Undisturbed soils displayed the highest root colonization (37.20 ± 1.82%). Circumference classes had significant (p = 0.03) influence on root colonization with the highest observed on circumferences up to 50cm (37.51 ± 1.90%). Five fungi taxa were described at the root zone of A. floribunda: Sclerocystis verrucosa, Gigaspora margarita, Acaulospora tuberculata, Entrophospora infrequens and Glomus manihotis. Twelve months after the inoculations, it was respectively observed that length, total sugar and proline contents were significantly affected by the mycorrhizal inoculation (p < 0.001, p = 0.04 and p < 0.001). From this study, the mycorrhizal inoculation is important for the growth and development of A. floribunda stecklings.

Keywords: arbuscular mycorrhizae, indigenous inoculum, inoculated stecklings, proline, total sugar

P (ID 8)

AM fungi development in not irrigated rice paddy field

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It is known that the population of AM (arbuscular mycorrhizal) fungi weakly developed in rice paddy field. Rice was grown in Ukraine on saline areas of southern chernozem, which also reduce the development of mycorrhizal colonization of plants. Irrigation of rice was organized from the Dnieper River by Northern Crimean Channel. In 2014, the supply of water into the channel was stopped and ceased to rice grow. Other drought-resistant crops are growing now. Therefore, the aim of this work is a survey of AM fungi population in rice paddies in the absence of irrigation.

In 2015, mycorrhiza was determined in 25.8 % root segments of control plants in virgin solonetsous southern chernozem, where the irrigation and the rice grown are never conducted. Intensity of colonization was amounted 2.9 %. Frequency and intensity of colonization of winter wheat and sunflower what grown in rice paddy fields are reached to 55.8-58.3 % and 4.0-11.0 % respectively. Colonization are enhanced because perennial irrigation is contribute to reduction salinity of this soil (Khitrov, 2016). In normal conditions of virgin southern chernozem, frequency and intensity of colonization are achieved to 88.3-94.6 % and 31.0-60.2 % respectively.

Thus, the irrigation had a double effect on mycorrhiza formation: increase of AM fungi development by reason of salt elution, but colonization is not maximum by reason of flooded method of rice cultivation.

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P (ID 19)

Diversity of arbuscular mycorrhizal fungi in Rhizosphere soil of undomesticated sugarcane, Saccharum spontaneum L. in Hainan, China

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All most cultivated sugarcanes around the world were derived from *Saccharum spontaneum* L, a perennial herbage species belonging to the Gramineae family. Because of its strong tillering and adaptation, wide distribution, and tolerance to stresses, this undomesticated species was commonly used in early breeding programs. In this study, we sampled rhizosphere soils from unrelated *S. spontaneum* plants that were collected from three different regions and grown in the same open field at the Hainan Sugarcane Breeding Station. Arbuscular mycorrhizal (AM) fungi were identified by their soil-borne spores morphology and quantified. It was found that rhizosphere soils were rich with AM fungi, with thirteen species including genera of *Scutellospora*, *Archaeospora*, *Glomus* and *Rhizophagus*. Under the same climate and cultivation conditions, rhizosphere soils differed significantly in AM fungi species and spores density among the three unrelated *S. spontaneum* genotypes, however, no significant difference was found within the same genotype. Our data indicate that host genotype can affect colonization of AM fungi. This finding will help guild future breeding activities in selecting proper parent genotypes.

Acknowledgement: This research was funded by NSFC (No.31360356)

Keywords: diversity, arbuscular mycorrhizal fungi, rhizosphere soil, *Saccharum spontaneum* L, undomesticated sugarcane

P (ID 23)

Relationship between arbuscular mycorrhizal fungi and forage grasses under defoliation at different phosphorus supplies

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Introduction/Aim: Defoliation reduces photosynthetic activity resulting in losses of carbon and nutrients. Mycorrhizae can facilitate regrowth by improving plant nutrition. However, post-defoliation recovery of the plants could be influenced by their mycorrhizal responsiveness and soil phosphorus availability. We evaluated mycorrhizae effects on post-defoliation recovery in *Agropyron elongatum* and *Brachiaria brizantha* at different phosphorus supplies.

Materials and Methods: A manipulative experiment with two forage grass species was designed: *A. elongatum* (low responsiveness, temperate) and *B. brizantha* (high responsiveness, tropical). For each species, treatments were: inoculation with arbuscular mycorrhizal fungi (non-inoculated and inoculated), soluble P-supply (low: 0,02mM and high: 1mM) and defoliation (non-defoliated and 60% defoliation). After 22 and 34 days post-defoliation, biomass production, number of tillers per plant and relative growth rate (RGR) were assessed.

Results: In *A. elongatum*, at low P/+Inoc. and high P/-Inoc., no differences were found in total biomass between non-defoliated and defoliated plants. In those treatments, relative growth rate (RGR) of defoliated plants was higher than in non-defoliated ones. Consequently, removed biomass was compensated. At high P, mycorrhizae depressed post-defoliation recovery of plants (32% less tillers per plant). In *B. brizantha*, mycorrhizae had a positive effect in all treatments.

Discussion: In A. elongatum at low P, mycorrhizae favors plants regrowth, while the opposite occurs at high P supply. In B. brizantha, mycorrhizae always promoted plant regrowth.

Conclusion: Our results indicate that the relationship between AMF and defoliated grasses vary with the mycorrhizal affinity of the host species and is modulated by phosphorus supply.

Keywords: Agropyron elongatum, Brachiaria brizantha, arbuscular mycorrhizal fungi (AMF), phosphorus supply, defoliation

P (ID 24)

Effect of commercial mycorrhizal inoculum (Ectovit and Symbivit) on *Quercus suber* grouwth at nursery stage

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The health status of cork oak forest in Algeria is worrying due to the combined action of several natural, historical and anthropogenic factors. These threats make it necessary to produce high-quality seedlings in nurseries for successful reforestation. The present study aims to improve the growth of cork oak (*Quercus suber* L.) by the exploitation of mycorrhizae in the nursery stage.

For this purpose, a controlled mycorrhization test was carried out on cork oak seedlings from aseptically pre-germinated seeds inoculated or not inoculated with two commercial inocula (Ectovit and Symbivit, INOCULUM plus, France), brought in separately or as a mixture. The growing substrate is composed of a mixture of 60% olive pomace and 40% forest humus. It was sterilized in half of the treatments in order to deduce possible synergistic effects of the natural telluric microflora.

After 9 months of growth, the results show that mycorrhizal inoculation (inoculum mixed or not) had significant effects on height growth, fresh aerial and root biomass of the cork oak, on the rate of colonization by ectomycorrhizal and arbuscular symbionts, and the chlorophyll content compared to controls. Sterilization of the substrate revealed a synergistic effect of the natural microflora especially on the root biomass, the aerial biomass / root biomass ratio and on the chlorophyll content. These results show that the use of controlled mycorrhization based on commercial products can be an effective alternative in the case where the local inoculum is not available and saves time.

Keywords: *Quercus suber,* controlled mycorrhization, Ectovit and Symbivit inoculum, growth

P (ID 27)

The role of arbuscular mycorrhizal fungi on soil aggregation and crop nutrition in agroecosystems

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Arbuscular mycorrhizal fungi (AMF) are essential for increasing the sustainability of agroecosystems. However, the importance of AMF in soil aggregation, crop nutrition and productivity under agricultural systems proposed to restore degraded soils is often not quantified. We investigated the relative importance of AMF and roots in influencing soil aggregation, and crop nutrition and production in two field trials differing in management and soil type (Kabete - humic nitisol soil and organic management, Nyabeda - ferrasols soil and conservation agriculture management) in Kenyan agro-ecosystems. We measured aggregate size distribution, crop yield, nitrogen and phosphorus uptake, spore abundance, species richness, hyphal length, glomalin levels, root colonization and inoculum potential (IP). Structural equation modeling (path analysis) indicated that AMF played a role in soil aggregation, N and P uptake, and crop production in the two sites, but their role was dependent on soil conditions, management and agricultural practices. Glomalin was important in explaining soil macro-aggregates in Nyabeda, and both macro-aggregates and micro-aggregates in Kabete. Root length and hyphal length were important in explaining soil aggregates in both sites. AMF activity (colonization, hyphal length & IP) positively correlated with N and P uptake and subsequent crop yield in Kabete, while differences in AMF did not explain crop nutrition and production in Nyabeda. AMF spore abundance and species richness had no effect on soil aggregation, crop nutrition and production. The study highlights the importance of AMF in soil aggregation, crop nutrition and production in agro-ecosystems

Keywords: glomalin, soil structure, arbuscular mycorrhiza fungi, agro-ecosystems, fertilizer

P (ID 29)

Recurrent glyphosate application reduces arbuscular mycorrhizal inoculum potential in a pampean grassland of Argentina

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Glyphosate is a broad-spectrum herbicide used worldwide in crop fields and other agroecosystems. In grasslands of the Pampa region (Argentina), it is usually applied in late summer to increase winter productivity of by reducing competition of forbs and C4 grasses improving the germination of *Lolium multiflorum*.

We conducted a set of experiments to assess soil inoculum potential and arbuscular my-corrhizal colonization of various species after recurrent annual applications of glyphosate at the recommended dose (3 I Glacoxan.ha⁻¹.year⁻¹) in a pampean native grassland.

Under field conditions, we detected a decrease of viable spores of arbuscular mycorrhizal fungi after one application, and 56% reduction of their density after four. Glyphosate application improved establishment and growth of *L. multiflorum* without affecting mycorrhizal root colonization of this cool-season grass. However, soil legacies left by this practice lowered other plant species' ability to form mycorrhizal associations in a microcosm study with soils that received glyphosate. Furthermore, a significant reduction of root colonization was observed in two of five species established in soils that received one application (23% and 6% for the native grass *Melica brassiliana* and the legume *Lotus tenuis*, respectively). But these differences in species response were erased in soils that received glyphosate in four consecutive years (18% average reduction of root colonization).

To conclude, grassland managers should consider that glyphosate application negatively impacts on mycorrhiza. Herbicide effects on non-target organisms and interactions may have long-term consequences for the dynamics and productivity of agroecosystems where nutrient availability is low.

Keywords: Latin-American ecosystems, mycorrhiza spores, herbicide

P (ID 30)

Quantitative assay of ectomycorrhiza of Pinus caribaea in a nursery in Uganda

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Quantitative assay is numerical examination or analysis of a given substance in a particular sample. Keeping in view the undeniable role ectomycorrhiza play in the establishment and growth of Pine seedlings in the field, it is possible to grade the seedlings and establish the most suitable planting-out age of the seedlings through quantitative assay of ectomycorrhiza. This was carried out in order to find out at what age of the seedlings ectomycorrhizal intensity is highest. Seedlings planted out at this age will have the greatest chance of good establishment, growth and survival. The study was carried out in Katugo nursery. The sampled seedlings were of the ages 10, 11, 12, 13, 14, 15, 16, 18, 21, 23 and 25 months. Thirty seedlings of each age were picked randomly from each nursery bed. They were transported to laboratory for ectomycorrhizal root counts. Each seedling was soaked in water so as to remove soil from it. The roots of Pines consist of short and long roots, the former being ectomycorrhizal and the later non-ectomycorrhizal. Ectomycorrhizal root tips are yellowish in colour with swollen end. Non-ectomycorrhizal roots tips are dark brown and not swollen. Both root tips were counted from each seedling and the averages recorded. Average percentage of ectomycorrhizal root tips was calculated. On the basis of percentage of ectomycorrhizal root tips, seedlings should be planted out when they are 13 months old. However, it is recommended that similar study be carried out for seedlings which are 3, 4, 5, 6, 7, 8 and 9 months old.

Keywords: quantitative, assay, ectomycorrhiza, pines, nursery

P (ID 31)

Arbuscular mycorrhizal fungi in stockpile soils of South African coal mines

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Mycorrhizal associations play key roles in the modulation of nutrients in most vascular plants. However, establishment of mycorrhizal associations in plant roots are influenced by prevailing soil condition and management practice. Stockpiling and storage conditions during coal mining operations may impair arbuscular mycorrhizal (AM) fungal spore survival and viability in topsoil, thus undermining post-mining mycorrhizae associations in cultivated rehabilitated soils. In this study, AM fungal spore density and mycorrhization were assessed in stockpile soils obtained from three South African opencast coal mines. Stockpiles were randomly sampled at depths of ≤ 200 mm (topsoil) and >200 mm (subsoil), and used for mycorrhizal trap culturing in maize (Zea mays) plants. AM fungal spore density in pot soils and mycorrhization in roots were assessed using classical staining and targeted amplification and sequencing of the nuclear rRNA gene of the Glomeromycota phylum. AMF spore density was significantly different (P<.05) between soil samples and highest in topsoil from unmined (control) soils. AMF spores were morphologically identified as belonging to genera Acaulospora, Gigaspora and Scutellospora. Nuclear rRNA gene detection in roots revealed that AMF colonisation was more associated with topsoil than with subsoil. Furthermore, species of the genus Paraglomus were the only colonisers of the maize roots in all soils, suggesting a very low diversity of viable AMF spores, poor support for the establishment of root-AMF symbioses or the presence of a single maize (host)-specific AMF symbiont in these soils. Overall, results suggest that stockpile depths may have an effect on spore density and mycorrhization.

Keywords: coal mining, arbuscular mycorrhizal fungi, mycorrhization, stockpiling, soil management

P (ID 37)

Mycorrhizal colonization affects rice gene expression of resistance traits in response to herbivore feeding

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Arbuscular mycorrhizal fungi (AMF) are one example of beneficial soil microbes that play a vital role in mediating plant resistance against insect herbivores. Rice (*Oryza sativa*) also establishes root associations with AMF¹. However, little is known about the underlying molecular mechanisms of these interactions². We studied how AMF colonization leads to changes in rice gene expression, up-regulating/down-regulating resistance-related genes, thereby increasing performance of fall armyworm (*Spodoptera frugiperda*, FAW).

In a greenhouse experiment, we characterized the molecular responses to FAW-damaged and undamaged rice plants colonized or not colonized with mycorrhizae to identify candidate marker genes and gene networks involved in plant defense. Leaves were collected at 24 hours after larvae fed from rice plants subjected to four treatments: mycorrhizae FAW-damaged (MD) and undamaged plants (MUD), and non-mycorrhizae damaged (NMD) and undamaged plants (NMUD). Following the feeding, leaves of the plant were removed and the tissue was prepared for RNA isolation, and synthesis of cDNA.

Transcriptome analysis documented differential gene expression among the four treatments. Validation of altered expression levels of eighteen unigenes that encode plant defense biosynthetic enzymes and transcription factors using quantitative Real-Time PCR (qRT-PCR) will be discussed to reveal novel underlying mechanisms during rice-AMF-FAW interactions. The primary genes of interest were plant defense genes associated with the salicylic acid and jasmonic acid pathways². Our results indicate that AMF colonization causes changes in gene expression related to defense responses, with important consequences for rice-FAW interactions. Ongoing research will provide insights into the underlying mechanisms of plant resistance in rice.

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P (ID 41)

Persistence and effectiveness of *Rhizophagus irregularis* isolate IR27 on the fruit production of jujube trees in a semi-arid field condition

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Arbuscular mycorrhizal fungi (AMF) play a major as biofertilizer for sustainable agriculture. Nevertheless, it is still poorly documented whether inoculated AMF can successfully establish in field soils with exotic AMF and improve plant growth and productivity. Here, we inoculated two provenances (Tasset and Gola) of the fruit tree Ziziphus mauritiana (jujube) with the exotic AM fungus Rhizophagus irregularis isolate IR27. There were four treatments to compare (Tasset non-inoculated, Tasset inoculated, Gola non-inoculated and Gola inoculated). All inoculated seedlings vs non-inoculated seedlings showed a significant increase in growth at the end of the nursery stage and these differences remained after 18 months on growth and fruit production in the field. Molecular analysis of AMF community based on MiSeq Illumina sequencing of 18S rRNA gene, revealed no significant effect of provenance and inoculation factors and their interactions on richness, diversity and abundance of native AM fungal communities whatever the inoculated treatments. The inoculant R. irregularis IR27 was specifically traced and discriminated from native R. irregularis isolates in roots by using qPCR assay targeting a fragment of the RNA polymerase II gene (RPB1) and still accounted for 12% to 15 % of the root colonization. Fruit production was significantly increased in inoculated jujubes compared with non-inoculated controls. Success and sustained positive effects of inoculation with R. irregularis IR27 hold promise for the role that AM fungal inoculum could play within fruit production of jujubes cultivated in semi-arid field conditions.

Keywords: *Ziziphus mauritiana*, fruit farming, Glomeromycota, ecological engineering, Senegal

P (ID 44)

Historical land use determines arbuscular mycorrhizal communities regardless of decades since intensive agricultural conversion

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Arbuscular mycorrhizal fungi (AMF) are key components of soil microbiota. Since intensive soybean (Glycine max Merr.) cultivation was established in Latin America, millions of hectares are deforested and cattle farms converted to monocultures every year. The objective of this study was to investigate the effect of historical land use (HLU) on AMF communities of current soybean fields. Representative soil and root samples were taken from three areas according to the period since agricultural conversion: Forest (<10 years), Livestock (10-50 years), Agricultural (>50 years); from 125 sites of Argentina. AMF spores were morphologically identified and T-RFLP method was used to study AMF patterns in soybean roots. A total of 36 species was found belonging to 7 families: Ambisporaceae, Acaulosporaceae, Claroideoglomeraceae, Diversisporaceae, Entrophosporaceae, Gigasporaceae and Glomeraceae. The most frequently occurring species were Acaulospora scrobiculata, Claroideoglomus etunicatum, Funneliformis mosseae and Glomus fuegianum. Forest sites exhibited significantly higher richness followed by Livestock and Agricultural. The same pattern was found with the number of AMF TRFs in roots. Pairwise comparison of communities based on spore abundances showed that each HLU was different to another. TRFs revealed that soybean grown in Forest sites had a community structure different from Livestock and Agricultural, but the formers did not differed between them. In conclusion, HLU strongly determined AMF community composition and richness in spite of being currently subjected to soybean cultivation practices. Future work should aim to examine how colonizing AMF communities change in order to avoid the loss of specific groups which could affect agriculture sustainability.

Keywords: Glomeromycota, community structure, T-RFLP, 18S RNA, soybean

P (ID 51)

Diversity and richness of AMF in avocado crops

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Composition of AMF (Arbuscular Mycorrhizal Fungi) communities are affected by the agricultural practices such as tillage, fertilization and no crop rotation. The establishment of a monoculture can be related with the decrease of beneficial fungi in high fertility conditions.

The aim of this investigation was to recognize the diversity and richness of AMF in an avocado monoculture.

Diversity and richness of AMF were investigated in avocado crops with high inorganic fertilization. The studio area is located in the municipality of Uruapan, Michoacan, Mexico. At the time of soil collection, the weather conditions were: temperature 26°C, with rain. During the wet season (August 2016), the soil samples were taken from the uppermost until 30 cm under the avocado crop. Spores were isolated from 30g samples and were morphologically identified. Diversity was calculated using the Shannon Wiener index.

Total species richness was fifteen; Entrophospora infrequens, Claroideoglomus clarum, Diversispora spurca, Septoglomus constrictum, Entrophospora nevadensis, Claroideoglomus claroideum, Diversispora aurantia, Glomus macrocarpum, Glomus sp., Funneliformis mosseae, Acaulospora scrobiculata, Glomus citrícola, Funneliformis geosporum, Sclerocystis sinuosa y Sclerocystis rubiformis.

Our results show a low diversity in the study field with a loss of spores in *Septoglomus* and *Acaulospora* an increase in *Glomus* population. We suggest that agricultural practices in this place strongly selects the dominance of few AMF species.

Keywords: monoculture, agricultural, practices, fertilization, rotation

P (ID 69)

Bio-fertilization of pea (*Pisum sativum* L.) with arbuscular mycorrhizal fungi enhanced the seed yield and quality in organic farming

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Arbuscular mycorrhizal (AM) symbiosis could play a key role in the establishment of a sustainable organic farming in the semi-arid region of Mediterranean countries. The aim of the current work is to study the effect of AM fungi bio-fertilization on the productivity of pea (*Pisum sativum* L.) cultivated in organic farming in a semi-arid region of Tunisia.

The experiment was conducted in the field, in rainfall conditions. Six treatments were applied: the control (T): no fertilization; (F): organic amendment (bovine manure); (A): inoculation with a mixture of two indigenous AM fungi species (*Glomus deserticola* and *Glomus* sp.); (S): inoculation with a commercial mycorrhizal inoculum "Symbivit" (mixture of 6 *Glomus* species); (AF): 50% of organic amendment + 50% of the indigenous mycorrhizal inoculum and (SF): 50% of organic amendment+50% of the commercial mycorrhizal inoculum.

Our results demonstrated that AM inoculation increased mycorrhizal root colonization of pea plants. Growth parameters were higher in mycorrhized treatments compared to (T) and (F) treatments. The treatment (AF) produced the highest increase in seed yield: 2 folds higher compared to the control. These results could be explained by the enhancement in nitrogen and phosphorus uptake by103 and 267 %, respectively, in plants inoculated with AM fungi compared to non-inoculated. Also, the crude protein percentage and the amount of total phenolic compounds in pea seeds were ameliorated in mycorrhized plants.

Our study revealed the positive effect of AM bio-fertilization on the seed yield and quality of pea in organic farming, at the field, in rainfall conditions.

Keywords: pea (Pisum sativum L.), AM fungi, organic amendment, bio-inoculation

P (ID 113)

Mycorrhizal inoculation differentially affects Touriga Nacional grapevine performance in Cu-contaminated and non-contaminated soils

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Plant inoculation with arbuscular mycorrhizal fungi (AMF) is increasingly being used to enhance productivity and sustainability in agricultural ecosystems. In the present study, we aimed to evaluate the potential benefits of AMF inoculation in young grapevines replanted in vineyard soils with high Cu concentrations. For this purpose, one year old Touriga Nacional grapevines grafted onto 1103P rootstocks colonized with a consortium of native AMF from a vine nursery were further inoculated with *Rhizoglomus irregulare* (Ri) or *Funneliformis mosseae* (Fm), or were left non-inoculated, and maintained under greenhouse conditions during three months. Plants were then transplanted to containers filled either with an untreated vineyard sandy soil collected in the "Experimental Pole for the Conservation of Autochthonous Grapevine Genetic Variability" (Pegões, Portugal), or with the same sandy soil contaminated artificially with 300 mg Cu/kg.

At the end of the growing season, grapevine's performance was analyzed by measuring root biomass, shoot length, leaf area and leaf nutrients concentration and compared between mycorrhizal treatments (non-inoculated, inoculated with Ri or with Fm) and soil treatments (Cu contamination or non-contamination). Although all plants showed similar levels of root colonization, Ri and Fm inoculated plants showed significantly greater root biomass in the non-contaminated soil. Interestingly, this was not the case in Cu contaminated soil, where non-inoculated plants were most benefited regarding shoot and root development.

Our results indicate that soil Cu levels can modify the outcome of AMF inoculations in young grapevines, disclosing new AMF-plant associations potentially relevant in vine-yards with a tradition of Cu-based fungicide application.

Keywords: symbiosis, arbuscular mycorrhizal fungi, Cu soil contamination

P (ID 156)

Response of rhizosphere microbial diversity in a rotation of cucumber with *Volvariella volvacea*

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Crop rotation can significantly increase yield by improving soil conditions through reducing soil pathogens and increasing the microbial diversity near the root system of the plant. Cucumber is widely consumed across the world, and monoculture of cucumber under greenhouse conditions is often threatened by Fusarium wilt, which is caused by fungi belonging to the genus Fusarium. In Jiangsu province of China, rotating cucumber crops with Volvariella volvacea is used as a way to control Fusarium wilt. Here, we tested how rotating cucumber with V. volvacea influences soil physico-chemical and biological properties. We found that rotating with V. volvacea improved bacterial diversity near the cucumber root system. We also found an increase in nitrogen-fixing bacteria that have been used for biological control of plant pathogens, and a decrease in Fusarium species. In the treated soil, we found increased activity of the soil enzymes catalase, dehydrogenase, polyphenol oxidase and alkaline phosphatase by 2-22%, 14-29%, 16-58% and 9-64%, respectively. Lastly, the total and available nitrogen, phosphorus and potassium content significantly increased one to three weeks after V. volvacea residual substrate was mixed into the soil, up to 39%, 24%, 17%, 28%, 18% and 70, but declined slowly afterwards. These results suggested that rotating cucumber with V. volvacea could aid in resetting the structure of the microbial community of the cucumber root system. Increase in beneficial microbes and improved soil conditions are likely to contribute to improved yield of this commercially important crop.

Keywords: cucumber-*Volvariella volvacea* rotation, rhizosphere microbiota, soil enzyme activity, nutrients

P (ID 170)

Isolates of arbuscular mycorrhizal fungi differentially impact carrot cultivars during water restriction

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Introduction/Aim: Arbuscular mycorrhizal fungi (AMF) impart growth benefits to water-stressed plants. Carrots (*Daucus carota*) readily host AMF and provide an excellent laboratory symbiosis model as root organ cultures; little is known of carrot-AMF symbiosis regarding whole plant response. Water scarcity threatens carrot production due to global climate change, and low-input systems are particularly vulnerable. AMF contribution to plant water uptake likely differs among species, but little is known about whether isolates differ in this respect. The present study screened four carrot cultivars in low-nutrient soil inoculated with AMF isolates from geographically distinct locations.

Materials and Methods: Carrots were greenhouse grown in pasteurized organic field soils amended with perlite and whole inoculants of eight AMF isolates. Mock inoculant served as a negative control. Carrots were well watered during establishment and waterstressed during tuber maturation. Biometric data were recorded for roots and shoots. Fungal structures and hyphal length were quantified in fine roots.

Results: Cultivars differed in water-stress tolerance (heirlooms were more tolerant than hybrids) and in response to inoculation. Some isolates increased tuber growth, and shoot growth decreased for inoculated carrots. Colonization patterns differed between isolates of a single species.

Discussion: Inherent carrot tolerance to water stress likely influences tuber growth response to AMF symbiosis. The proportion of fungal structures in roots may provide insight as to which isolates will likely improve host tuber growth.

Conclusion: Carrot biomass allocation in response to AMF differs from other plant models. Genetic background and inherent tolerance to water stress influenced carrot benefits from AMF.

P (ID 176)

AMF species dependency governs better tree physiology, growth and leaf nutritional quality of mulberry seedlings

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Introduction/Aim: How AMF species within the same or different genera could affect the physiological and growth characteristics as well as leaf nutritional quality of plants including the perennial cash mulberry (*Morus alba* L.) tree remains unclear^{1,2}.

Materials/Methods: Three functionally different AMF species (*Acaulospora scrobiculata*, *Funneliformis mosseae* and *Rhizophagus intraradices*) on a number of physiological and growth characteristics and leaf nutritional quality of 6-month-old mulberry seedlings were addressed in randomly arranged pot studies³.

Results: AMF colonization significantly increased shoot height and taproot length/diameter, leaf and fibrous root numbers, and shoot/root biomass production. Meanwhile, leaf chlorophyll a/b and carotenoid concentrations, net photosynthetic and transpiration rate, and stomatal conductance were significantly greater, while intercellular CO_2 concentration was significantly lower in AMF-colonized than in non-AMF-colonized seedlings. Leaf moisture, total nitrogen, all essential amino acids, histidine, proline, soluble protein, sugar and fat acid were also significantly increased under mycorrhization. Among these three tested AMFs, significantly greater effects on mulberry physiological and growth characteristics ranked as *F. mosseae* > *A. scrobiculata* > *R. intraradices*, whilst on leaf nutrition quality for better silkworm growth as *F. mosseae* \approx *A. scrobiculata* > *R. intraradices*.

Discussion: Significantly greater mulberry biomass production and nutritional quality varied with AMF species and were mainly attributed to AMF-induced positive changes in leaf photosynthetic pigments, net photosynthetic and transpiration rate, and N-containing compounds (methionine, threonine, histidine and proline).

Conclusion: Mulberry is AMF-species dependent and application of *Funneliformis mosseae* or *Acaulospora scrobiculata* in mulberry plantation is promising in promoting silkworm cultivation and textile industry globally.

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Keywords: amino acids, leaf chlorophyll a and b, carotenoid, intercellular CO₂ concentration, net leaf photosynthesis and transpiration rate

P (ID 209)

Mycorrhizal networks between vegetation islands naturally established in metal mine residues

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Previously, our research group reported the persistence of several plants species naturally growing on mine residues; which formed vegetal patches. Gradually, plant colonization has advanced by spreading of these patches and formed several vegetation islands. It is widely recognized that underground fungal networks connecting plants may occur. However, these have not been analyzed in metal polluted soils. This research analyzed the possible underground fungal networks connecting vegetation islands of a non-oxidized mine deposited at Zimapan municipality, a semiarid area with historical mining activity.

Three pair of interconnected islands: two including *Brickellia. veronicifolia-Vigueria dentata* (Bv-Vd1; Bv-Vd2) and one of B. *veronicifolia-B. veronicifolia* (Bv-Bv) were analyzed. Transect between islands core soil samples (25x25 cm) were taken every 25 cm at 0-5 and 5-10 cm deep. No roots were found at 0-5 cm, probably because high soil temperature (55 °C) and mine residue (MR) dryness. Then analyses were performed only with samples from 5-10 cm (T=40 °C, soil moisture from 2%-4.6%). Distance between islands was 125 cm for Bv-Vd1, 100 cm for Bv-Vd2 and 50 cm for Bv-Bv.

Islands are interconnected by fungal mycelium. The highest dry mycelium amount was 9.7 mg/100 g fresh MR in Bv-Vd2. High acidulated water-concentrations of Zn (6.98 mg/kg), Cu (4.71 mg/kg), Cd (1.89 mg/kg), As (128 mg/kg), Pb (12.56 mg/kg) and Sb (16.28 mg/kg) were found on island transects. Mycorrhizal colonization was observed in roots from transects. Significance of results will be discussed according to functional roles of fungal networks and accelerate revegetation on these sites.

Keywords: metal polluted soils, external mycelium, metal sequestration

P (ID 210)

Phytoremediation assisted by mycorrhizal fungi of a Mexican defunct lead acid battery recycling site

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Lead acid battery recycling is a common industry practice to recover Pb from aged batteries of motor cars. Serious pollution of soil may occur when processing and residue disposal are incorrectly carried out. It has been suggested that arbuscular mycorrhizal fungi (AMF) may assist phytoremediation of metal polluted soils; however, scarce information is available for soils polluted by lead acid batteries (LAB). The objective of this research was to evaluate four AMF separately inoculated on *Ricinus communis* on a site polluted by LAB. After assessing pollution levels (Pb, Cu and salts) and, physical and chemical soil characteristics a field experiment was established. *R. communis* seeds were inoculated with *Acaulospora* sp., *Gigaspora gigantea*, *Funneliformis mosseae* or *Glomus* sp.

A non-inoculated treatment (NIT) was also tested. One-month old seedlings were transplanted to the polluted soil mixed with sawsdust and vermicompost (20:20:60). Three shoots and rhizosphere samplings were followed at 5, 10 and 15 months after transplanting. A longitudinal statistical analysis, principal components analysis and Pearson correlations were performed.

Total and DTPA_{Pb} soil concentrations were very high (41, 893 and 6, 246 mg kg⁻¹, respectively). Inoculation with three AMF slightly reduced soil pH (from 8.4 to 7.8). *F. mosseae* stabilized Pb in the rhizosphere (DTPA_{Pb} 2,904 mg kg⁻¹ vs 4,531 mg kg⁻¹ in the NIT). *Acaulospora* sp. decreased shoots Pb concentrations (70 mg kg⁻¹ vs 234 mg kg⁻¹ in the NIT) and improved oil seed quality (palmitic, oleic and linoleic acids). AMF may aid phytoremediation of *R. communis* in soils polluted by LAB.

Keywords: phytostabilization, revegetation, restoration, salinity

P (ID 221)

Comparative study of the efficacy of biofertilizers on performance of TC banana cv. Gros michel

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Inoculation with beneficial microorganisms is one way of protecting tissue cultured (TC) plantlets against environmental stresses hence improving their growth, health and nutrition. Greenhouse and field experiments were conducted to assess the efficacy of commercial microbiological products on growth, nutrition and health of TC banana cv. Gros michel. Plantlets were grown in vertisol, Rhodic ferralsol and Humic nitisols from three banana growing regions in Kenya and inoculated with Rhizatech, EcoT T/T22, PHC Biopak, Subtilex and Mycor products at the acclimatization and nursery phase and subsequently established under field conditions. Growth, nutrient uptake, mycorrhizal colonization and Fusarium suppression were assessed.

PHC Biopak significantly (p≤0.05) increased plant growth in the Vertisol and Rhodic Ferralsol in the nursery phase by 50%. Rhizatech and ECO-T under field conditions significantly (p≤0.05) increased plant growth in the Vertisol by over 100%. Rhizatech had the highest mycorrhizal colonization (76.1%) in the Rhodic Ferralsol while PHC Biopak had the highest in the Vertisol (87.4%). PHC Biopak, ECO-T and Rhizatech reduced Fusarium populations by 47, 68 and 55% respectively in the Humic Nitisol. The findings from this study could benefit post flask management of TC banana plants. However, efficacy of the products is dependent on prevailing soil conditions.

Keywords: tissue cultured banana, commercial microbiological products, mycorrhizal colonization, growth, nutrient uptake, *Fusarium* suppression

P (ID 236)

Freezing effects in Quercus faginea and Quercus ilex seedlings mycorrhizated with different fungi

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Introduction/Aim: In Mediterranean continental climates, frost stress can play an important role in the living status of seedlings. Normally, these areas are favourable lands to plant and to cultivate orchards for truffle production. Truffle production is relationated with the production of carbohidrates in plants.

Materials and Methods: We compared the freezing tolerance in two widespreas coexisting Mediterranean forest trees: *Quercus faginea* and *Q. ilex* subsp *ballota*. Roots of seedlings were associated to three different ectomycorrhizal fungi: *Hebeloma mesophaeum, Tuber aestivum* and *T. melanosporum*. Freezing tolerance was determined in leafs and fine roots (mycorrhizal roots) by relative electrolyte leakage (REL), calculated like percentage damage, from 0°C (control) to -20°C.

Results: The REL test indicated that the lethal temperature for 50% of root samples (LT50) was found at temperatures below -4°C. In leaves, freezing tolerance has high in all temperatures tested. The mycorrhizal treatment with *T. melanosporum* affected positively the freezing tolerance of seedlings.

Conclusion: Despite their ecological differences, both tree species had similar freeze tolerance. Frozen temperatures in soil could produce injury in roots, and subsequently, limit the growth of mycelium and fungi production

P (ID 237)

Mycorrhizal effectiveness from soils with different land-uses from tropical highlands of Colombia

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The impact of land-use over the abundance and mycorrhizal effectiveness (ME) has been studied over the last years^[1,2,3]. In the present study we evaluated the ME from soils with different land uses in Andean montane forest.

To this purpose, soils from five different farmlands located in the department of Antioquia, Colombia, with three land-uses were collected: grassland (with predominance of *Pennisetum clandestinum*), tree tomato (*Solanum betaceum*) and a secondary forest. From each soil sample 25 g (dry basis) were transferred into a plastic pot containing 500 g of a soil substrate (Bw horizon), with a soluble soil P concentration of 0.02 mg/L, optimal for the mycorrhizal association^[4]. For reference, we also included two treatments: an inninoculated control and an inoculated positive control with *Rhizoglomus fasciculatum*^[5]. As indicator plant we used *Leucaena leococephala*, for his very high mycorrhizal dependency^[6]. We monitored leaf phosphorus content every 15 days. After 80 days of growth, dry mass and mycorrhizal colonization was measured.

The results showed that the forest-soil exhibited a significant lower ME (p<0.05) than the other two soils, measured in terms of shoot dry weight, foliar P, and mycorrhizal colonization. Grassland-soil and tree tomato-soil had a high ME comparable with the positive control. The results were also related with the abundance of indigenous mycorrhizal fungi for each soil-use.

We conclude that cultivated soils has a good abundance and effectiveness of arbuscular mycorrhizal, however, these soils have been over-fertilized. It could have negative effects over mycorrhizal functions, soil quality, water resources and production cost^[7].

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Keywords: arbuscular mycorrhiza, mycorrhizal effectiveness, land-use, tropical montane forest

P (ID 245)

Arbuscular mycorrhizae structures in organic coffee leaf litter

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Arbuscular mycorrhizal fungi (AMF) form mutualistic symbiosis with most plants with the major function of nutrient exchange and are important for mineral cycling in tropical ecosystems with high rate of organic matter decomposition and phosphorus-sorbing soils. In Colombia, most crops, including plantain, are stablished in these soils and it is common to pile up the litter below the plant to promote its in situ decomposition and root proliferation. The aim of this study was to describe the presence of AMF in the leaf litter of *M. paradisiaca* found in coffee plots set on organic and organic+chemical inputs management.

Samples were collected from plots set on two different agronomical management systems (organic an organic+chemical inputs) in Cundinamarca-Colombia at 04°58′38″ N, 74° 14′32″ W, and 1,880 m.a.s.l. Leaf litter was collected at three decomposition levels. Conventional techniques adapted to the survey's conditions were used in the sample analysis of AMF.

AMF structures were identified and quantified into and between leaf litter and roots. Vesicles and characteristic hyphaes of AMF were found inside the decomposing leaf tissue.

Statistically significant positive relationship (r=0,90, p=0,03) was found between AMF colonization of decomposing leaf and AMF colonization of root pieces. The high amount of external mycelium, spores and vesicles of AMF in the litter suggest besides an unconventional nutrient uptake and translocation mechanism, a high capacity of dispersion through the soil surface.

Keywords: organic agriculture, litter, Musa paradisiaca, coffee agroecosystems

P (ID 253)

Future regulation of mycorrhiza products in Europe: Biostimulant, microbial, fertilizer, improving nutrition efficiency product?

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Global and European biostimulant market has grown significantly over the past few years and will continue to experience remarkable growth. If microorganisms are formulated in products targeted for use on cultivated plants in the EU, a registration process organises the risk and efficacy assessment of these products. The assessment procedure of Plant Protection Products (PPP) is regulated by the Regulation (EC) No 1107/2009. This regulation covers the modes of action of antagonism, induced resistance and growth regulation, while tolerance induction is not included due to its multi-factorial causes. At present, the legal regulation of biostimulation is not harmonised within the EU but organised on national level, with severe consequences for the producing industry. It is envisaged to consider the aspects of biostimulation in future in the revision process of EU Regulation (EC) 2003/2003 (regulation of fertilizers). Another open question is how to proceed with microorganisms which convey different modes of action to the host plant.

One of the key objectives of this draft fertilizer regulation is to provide access to the Single Market for innovative fertilizing products. The current proposal for a positive list of beneficial microorganisms is unlikely to meet this purpose as it inadvertently provides disincentives to some microbials as innovative products. On that background a clear classification of microbial groups followed by the appropriate registration process is inevitable to place products sustainably on the market.

An updated overview will be given as profound knowledge of future regulation is necessary for both, industry and academia.

Keywords: biostimulants, biologicals, microbials, mycorrhiza fungi, harmonized market

P (ID 254)

Influence of the Arbuscular Mycorrhizal Fungus *Rhizophagus irregularis* on Phosphorus Uptake and Growth of Sorghum and Okra Plants under Water-Deficient Conditions

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Sorghum and okra are important crops in Sudan, especially in the rain fed areas, where drought incidences are frequent. This study aimed to assess the potential of arbuscular mycorrhizal fungi (AMF) to enhance phosphorus (P) uptake in these crops under drought conditions.

Sorghum (Sorghum bicolor L.) and okra (Abelmoschus esculentus L.) were grown under greenhouse conditions in sterilized and fertilized loamy sand, supplied with either 50 mg P (P1) or 100 mg P (P2) per kg dry soil. Plants were either inoculated with Rhizophagus irregularis (+M) or non-inoculated (-M). After four weeks of growth the plants were either kept well-watered at 15% w/w (+W) or the substrate water content was reduced to 7% (-W).

In sorghum plants, a significantly lower percentage of root length colonization (rlc) was observed under (-W) than in (+W) under both P levels. Under high P (P2), plants had lower rlc than under low P (P1). The total content of P and biomass were not significantly affected by AMF inoculation. Okra in contrast showed rlc between 80% and 93%, irrespective of water and P supply levels. Mycorrhizal okra plants had more than double the total content of P than non-mycorrhizal plants in all treatments and significantly higher biomass in treatment (P2/-W) than non-mycorrhizal plants.

While sorghum plants did not show any P uptake or growth improvement by mycorrhiza, okra plants showed a distinct P uptake and growth improvement under the given conditions, indicating that okra was more responsive to AMF inoculation, especially under deficient water supply.

Keywords: mycorrhiza, water-deficient conditions, phosphorus, sorghum, okra

P (ID 255)

Ectomycorrhizal symbionts of silver fir (Abies alba Mill.)

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Introduction: As most European forest tree species, silver fir forms an ectomycorrhizal symbiosis. Currently ectomycorrhizal communities on silver fir remain poorly identified. Most silver fir ectomycorrhiza descriptions were based on morphological and anatomical characteristics and characterized without the exact identification of fungal symbiont. Until now only few studies have been published in which authors had identified ectomycorrhiza on silver fir applying molecular (DNA-based) markers.

Materials and Methods: Samples were (and are still) collected from three different plots in Slovenia on adult's trees of *Abies alba*, once per month. From collected samples ectomycorrhizal fungal symbionts were described based on morphological and anatomical descriptions and characterized as mycorrhizal morphotypes. Further each ectomycorrhizal morphotype were included in molecular markers analysis.

Results: Until now 27,000 ECM root tips of *Abies alba* have been analyzed. With morphological - anatomical method, over 25 different ECM morphotypes of silver fir have been determined. Based on molecular analysis, most abundant silver fir symbionts are (identified on genus level): *Sebacina* sp., *Cenococcum* sp., *Tomentella* sp., *Russula* sp., *Amphinema* sp., *Amanita* sp., *Inocybe* sp., *Boletus* sp., *Lactarius* sp., *Clavulina* sp. etc. As inside the same ECM fungal genus, apparently different species occur, further phylogenetic analysis (in progress) will be used to determine morphotypes by ECM fungal species exact.

Discussion: We have recorded high ectomycorrhizal diversity on *Abies alba*, and a high spatial diversity. An unexpected high temporal diversity in some part of the year, indicates a need for more frequent sampling and diversity analysis.

Keywords: ectomycorrhiza, *Abies alba*, species diversity, molecular analysis

P (ID 265)

Cadmium accumulation and uptake dynamics in AMF inoculated cocoa

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Cocoa is a valuable export product for tropical countries like Colombia. However, in many Latin-American soils where cocoa is cultivated, cadmium (Cd) concentrations are high and this limits its export potential as chocolate is recognised as a source where Cd enters the food chain. It has been demonstrated in other plants that AMF can modify absorption and transport of Cd and, thus, could be used to either prevent Cd accumulation in cocoa or help remove Cd from the soil.

We designed an experiment to determine the temporal dynamics of Cd in the soil and in AMF inoculated versus non-inoculated plants in the nursery. All plants were grown in live non-sterilized soil.

Cocoa seedlings were submitted to different Cd concentrations (according to typical concentrations found in field) and then inoculated with *Rhizophagus irregularis*. Cd-concentration was measured in different soil fractions and in all organs of plants. Morphological and physiological variables in plants were measured as well as AMF-colonization. This was carried out at four successive time points throughout the experiment.

The addition of Cd and AMF inoculum significantly affected plant growth and physiology. Inoculated plants had larger roots and accumulated more Cd than non-inoculated plants. More Cd was removed from the soil or was converted into a fixed form no longer available for uptake by plants in the inoculated treatments.

The information obtained shows an integrated view of the soil and AMF inoculated plants in the presence of Cd and sheds light on future strategies using AMF in cocoa production.

Keywords: arbuscular mycorrhizal fungi (AMF), cocoa, cadmium

P (ID 267)

Ectomycorrhizal hypogeous fungi at the upper timber line – recent findings and new species

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Hypogeous fungi are traditionally known from the Mediterranean. In recent decade, the cultivation efforts and large-scale samplings disclosed that hypogeous fungi are present widely in the northern hemisphere. Commercial truffle hunters never explored the upper timber lane. Out review study is focusing on potential high-altitude areas where hypogeous fungi, in particular true truffles (the genus *Tuber*) are expected to grow.

Applying a purposive sampling approach, we have investigated areas with potential ectomycorrhiza vegetation and preferably on calcium rich rock-bases in SE central Alps, Dinaric Alps and in western Himalayan area. Hypogeous genera were recorded either as ectomycorrhiza or as sporocarps found by specially trained truffle hunting dogs.

The analysed areas were covered with various forest or shrub species. Among commercial truffle species only *Tuber aestivum* was collected in organic soils developed at high altitude (> 1500 m a.s.l.) areas in Macedonia, covered with beech forest or mixed beech and silver fir forests (Grebenc et al., 2011). As similar altitudes, a novel true truffle species *Tuber petrophylum*, a species closely related to *T. melanosporum*, was described (Milenković et al., 2015). Shamekh et al (2013) were first to prove successful cultivated of commercial truffles in boreal zones. Novel ectomycorrhiza species from the genus *Tuber* were retrieved also from *Salix* and *Cedrus* locations in W Himalayas (Ilyas 2013; Jabeen and Khalid, 2014) and with *Salix* in Julian Alps (Slovenia) above 1200 m.asl. The high-altitude areas up to upper timber line are a promising source of yet unrevealed diversity of ectomycorrhizal hypogeous fungi

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Keywords: ectomycorrhiza, hypogeous fungi, distribution, diversity, upper timber line

P (ID 315)

The project fertiledatepalm – bio-inoculation and organic matter management for sustainable date palm propagation and cultivation

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Date palm is an important crop in Morocco, Tunisia and many other drylands with a high agricultural, economic and cultural value. Harsh environmental conditions of those areas, further accelerated by climate change and the spread of root diseases, threaten date palm cultivation. To overcome growth limitations, high inputs of mineral fertilizers, pesticides and irrigation are applied. However, these high external inputs strongly impact the environment and livelihoods.

The project aims at establishing a novel organic bio-fertilizer technology, combining the inoculation of native beneficial soil microorganisms, namely arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) during tissue culture and field propagation of date palms, together with adapted agricultural management practices using organic amendments and intercropping with leguminous nitrogen fixing crops. A culture collection of native AMF and PGPR, isolated from date palm roots and rhizosphere, are currently established, and strains selected for date palm growth promotion, nutrient acquisition and pathogen suppression will subsequently be tested. Customized propagation and application techniques of AMF will be elaborated for tissue culture laboratories and smallholder farmers. The technology, integrating the use of organic amendments and leguminous intercrops, will be developed in a participatory approach with key stakeholders, working at laboratory, on-station and on-farm scale.

In the talk we will present first results of bio-fertilizer isolation, customized propagation approaches and screening assays and experiments using compost combined with bio-fertilizers to ameliorate plant nutrient uptake. Further we will give an overview about the project and its main goals.

Keywords: date palm, drylands, native culture collection, biotechnology

P (ID 325)

Study of an efficient DBP-degrading bacteria and its potential for reducing DBP accumulation in vegetable

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Introduction: Recently, phthalic acid esters (PAEs) pollution in environments has aroused a great of concerns. Dibutyl phthalate (DBP), a typical PAE compound, is one of the most abundant endocrine disruptor chemicals. The widely used plastic film containing DBP in agriculture has caused serious soil pollution and poses risks to human health through food chain.

Materials and Methods: To assess the interactions of DBP-degrading bacteria and plant and their effects on DBP dissipation from soils and DBP accumulation in vegetable, the DBP dissipation, plant accumulation, bacterial survival, and soil enzymes activities were studied.

Results and discussion: A bacterial strain *Microbacterium* sp. J-5, newly isolated from activated sludge, could effectively degrade DBP. Response surface methodology was successfully employed for optimization resulting in 98% degradation of DBP (500 mg L⁻¹) within 10 days. This strain harbored a degradation pathway for complete mineralization of DBP with a maximum specific degradation rate (q_{max}), half-saturation constant (K_s), and inhibition constant (K_s) of 2.87 d⁻¹, 350.8 mg L⁻¹, and 712.4 mg L⁻¹, respectively. Bioaugmentation of DBP-contaminated soils with strain J-5 greatly enhanced DBP dissipation rate. Moreover, this strain could efficiently colonize in the rhizosphere soil of inoculated vegetables and further enhanced DBP degradation (~97%), leading to a significant decrease (>70%) in DBP accumulation in shoots and roots of the inoculated vegetable compared to un-inoculated vegetables.

Conclusion: The results highlighted the roles of DBP-degrading bacteria in simultaneously bioremediating contaminated soils and reducing bioaccumulation of DBP in edible part of the vegetable for food safety.

P (ID 326)

Effects of Arbuscular Mycorrhizal Fungi (AMF) on the Growth, Cd accumulation and Soil Enzyme Activity of Upland rice in Cadmium Contaminated Soil

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The effects of arbuscular mycorrhizal fungi (*Glomus mosseae*, GM) on the growth, Cd accumulation and rhizosphere soil enzyme (urease and invertase) activities of upland rice were investigated using pot experiments under different Cd addition levels (0, 2, 10 mg·kg⁻¹). Results showed that upland rice roots could be infected by GM ranged from 37% to 72% and decreased with increasing Cd concentrations. The mycorrhizal (GM) colonization significantly improved the soil enzyme (urease and sucrase) activities (ranged from 9.6 %~44.5 %) and carbon and nitrogen metabolisms in rhizosphere of upland rice, and thus enhanced upland rice biomasses (ranged from 10.4 %~57.1 %) compared with the nonmycorrhizal upland rice. Furthermore, mycorrhizal (GM) colonization decreased the uptake and transfer of Cd in upland rice, with lowering Cd concentrations in grains of upland rice (ranged from 26.8 %~57.1 %).

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Keywords: soil pollution, cadmium, upland rice, arbuscular mycorrhizal fungi, soil enzyme

P (ID 327)

Diversity index of Arbuscular Mycorrhizal Fungi in Colombian Andean transect

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There are some biotic and abiotic factors that can affect positive or negatively the structure and diversity of Arbuscular Mycorrhizal Fungi communities, such as the interaction with other soil microorganisms, and agricultural practices including cutting down, burning, fertilization and tillage. Also the Arbuscular Mycorrhizal Fungi population could be modulated indirectly by microclimate aspects, topography and plant species. This work was focused in the evaluation of Arbuscular Mycorrhizal Fungi biodiversity in an altitudinal transect (1500 to 3000 masl) cultivated with cape gooseberry (Physalis peruviana) in the Colombian Andean. Samples were collected in two seasonal periods: wet (150 - 300 mm.month⁻¹) and dry (0 - 20 mm.month⁻¹). We evaluated the Arbuscular Mycorrhizal Fungi communities based on species diversity. The estimated diversity indexes corroborate the hypothesis that mycorrhizal communities in these systems are highly heterogeneous with a low/average uniformity levels within and between samples. We found that in the altitudinal gradient, Arbuscular Mycorrhizal Fungi communities showed high diversity, expressed by the number, richness and relative abundance of spores genus and species. This high diversity level founded in cultivate soils at different altitudes with P. peruviana, show that Andean ecosystem has higher diversity compared with other reports for tropical and temperate soils.

Keywords: diversity, functional diversity, arbuscular mycorrhizal fungi

P (ID 330)

Glomaceae number of spores responds to SOC content in karst seasonal tropical forest soils of Yucatán, México

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Agricultural practices, starting with vegetation clearing, cause the depletion of soil organic carbon (SOC) affecting Arbuscular Mycorrhizal fungi (AMF) populations. In seasonal tropical forests Gigasporaceae has been observed to be more abundant in mature vegetation soils while Glomaceae in early successional vegetation soils.

In this study, soil samples were collected in 9 sites of 15- and 30-year-old seasonal tropical forest and agroforestry systems (AS) of Tzucacab, Yucatán, México during the dry and wet seasons. SOC, mycorrhizal infection and number of spores were determined. Data were compared and analyzed using linear regression.

SOC content and number of spores of both Gigasporaceae and Glomaceae were greater in 30-year-old forest. The number of spores of Glomaceae was always higher in all sites but similar between seasons. % infection between SOC and mycorrhizal infection was not statistically different but the regressions were significantly related to *Glomus* spores (p<0.0001, r2=0.82). % infection (hyphae, vesicles) did not change but the total root length increased, thereby increasing total AMF biomass.

We hypothesize that in rotational systems, as the fallow period increases, Glomaceae AMF become more important for nutrient uptake, aggregate formation and stable SOC formation than Gigasporaceae.

We concluded that the number of Glomaceae spores is positively related to the SOC content; thus, the effect of agricultural practices on SOC could be assessed by monitoring the number of *Glomus* spores in soil at any time of the year.

Keywords: arbuscular mycorrhizal fungi, fungi spores, mycorrhizal infection, soil organic matter, soil quality

P (ID 333)

Effect of agronomic practices on arbuscular mycorrhizal root colonisation and inoculum density in different cultivars of spelt wheat (*Triticum spelta*)

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Phosphorus (P) is a major constraint for plant growth and associated with many physiological processes. Since availability of P may be limited in soil; the formation of symbiotic associations with arbuscular mycorrhizal fungi (AMF) can enhance P uptake capability In agroecosystems, the existence and function of AMF is affected by agricultural practices and varies between genotypes.

Two years (2015- 2016) of field plot experiments were conducted to find out the effect of Spelt variety (Oberkulmer Rotkorn, ZOR, Rubiota and Filderstolz) and fertility input type (composted FYM and mineral N) and rate (50 &100 kg N ha⁻¹) on AMF colonisation in spelt roots, inoculum density in the soil, P-uptake and grain yield, under field conditions at Nafferton farm in northeast England. Roots and soil were sampled in June 2015 and July 2016, root staining was conducted, and spores were counted.

The results showed that year (2015 & 2016) had a significant effect on inoculum density and AMF colonisation excluding vesicle colonisation. Vesicle colonisation was higher in the low compost, low mineral, and control treatments compared to fertilisation with high rates of mineral fertiliser. In 2016, the highest inoculum density was recorded at the high rate of compost addition. The fertility x variety interaction had a significant effect on inoculum density in 2015 and 2016 with rankings of varieties at each level of fertility management differing for each year.

Preliminary results indicate that lower levels of fertility input promote vesicle formation, although highest numbers of spores in 2016 were measured at high levels of compost input. Varietal effects on colonisation and spore numbers were variable. Further research is required to determine combinations of varieties and fertility inputs to optimise root colonisation.

Keywords: arbuscular mycorrhizal fungi (AMF)

P (ID 343)

Growth response of four leguminous trees to native arbuscular mycorrhizal fungi from Indonesian forest soils

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Leguminous tree species is expected to be used for reforestation in Indonesia. Inoculation of effective arbuscular mycorrhizal (AM) fungi to leguminous tree species is a strategy to guarantee the success of reforestation in Indonesia. The objective of this study was to isolate and identify effective AM fungi from forest soils in Indonesia and clarify its effect on growth of four leguminous tree species. Soils were collected from five different forests in Indonesia. Paraserianthes falcataria, Calliandra callothyrsus and Cassia siamea were grown on each soils for 90 days. Sixty-one spore morphotypes were collected from the soil and propagated using respective tree species. Propagated spores were identified and inoculated to P. falcataria, C. callothyrsus, Cassia siamea and Sesbania grandiflora. Shoot and root fresh weight, shoot dry weight (DW), shoot P concentration and AM colonization were measured 60 days after inoculation. Mycorrhizal dependency (MD) was calculated based on shoot DW. Five isolates were identified as Diversispora gibbosa (M10-2), Acaulospora sp. (M11-1), Glomus sp. (S6-4), Glomeromycota sp. (M44-3) and Ambispora appendicula (M60-3). Mycorrhizal colonization was ranged from 0% to 99%. Growth of four tree species were the same as control or increased by colonization of AM fungi. Calliandra callothyrsus, P. falcataria and S. grandiflora always showed positive MD upon colonization by AM fungi. Four tree species inoculated with S6-4 showed the highest MD among AM isolates. These findings suggest that growth response of *P. falcataria*, C. callothyrsus, Cassia siamea and S. grandiflora to native AM isolates from Indonesia were different depending on the AM isolates.

P (ID 344)

The effect of AMF inoculum on soil physiochemical properties involved in nutrient leaching from agriculture

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Agriculture faces conflicting priorities of maintaining crop yields while meeting regulations on the use of fertilisers and crop protection products. Predicted changes in rainfall patterns may have a profound effect on crop-water relations and nutrient retention in the soil. Arbuscular mycorrhizal fungi (AMF) are root symbionts that improve water relations and nutrient intake in their plant hosts. They have been shown to alter soil chemical and physical properties, though much of this work has been carried out in controlled pot experiments. The efficacy of AMF amendment at a field scale is less well studied, with many studies focussing on the plant-based benefits. In a field trial set up by the Myco-RhizaSoil consortium at the University of Leeds Farm, Tadcaster, we determined how soil amendment with mycorrhizal inoculum affected the community of fungi colonising the roots of a number of wheat varieties, and whether this had any subsequent effect on both the crop yield and the soil. We tested the hypothesis that AMF inoculum addition would increase yields due to the conferred benefits of mycorrhizal association and confer changes in soil chemical and physical properties related to potential nutrient leaching. These properties include mineral ion content of soluble ammonium, nitrate and phosphate, carbon storage, porosity and water holding capacity of the soil. I will present this data along with DNA sequence data of the mycorrhizal community colonising roots and that of the greater fungal community.

Keywords: AMF, community, leaching, agriculture, field trial

P (ID 349)

Improved Arbuscular Mycorrhizal Root Colonization and Root Health of Wheat by Seed Treatment with Bio-Stimulant

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Arbuscular mycorrhizas are naturally found in cereals, like wheat, barley, rye, triticale and oats. The symbiosis has many benefits for the plant being the most important an improved phosphate (P) uptake. Improved root health was also reported. P-uptake at the start of the growing season can be decisive for cereal yields. To make more efficient and optimum use of such P fertilization one approach can be to stimulate early root growth and arbuscular mycorrhizal development.

We carried out seed treatment field experiments with a bio-stimulant (mixture of seaweed extracts plus *Bacillus* spp., azo-bacteria, *Trichoderma*) with winter and spring wheat in loamy soils in Lithuania. Combined treatments together with fungicides were also tested. Early root and shoot development, chlorophyll content, colonization of roots with pathogens and mycorrhizal fungi were investigated.

The bio-stimulant seed treatment improved early root development and colonization with arbuscular mycorrhiza, chlorophyll and carotinoid content in leaves, and grain yields. Seed treatments with combinations of bio-stimulant with fungicides were less positive for mycorrhizal colonization, while fungicides alone were negative. Bio-stimulant seed treatments increased grain yields.

We concluded that wheat seed treatment with bio-stimulants could be an economic method to increase the root colonization with natively occurring mycorrhizal fungi. This can result in increased root health, better and more efficient nutrient uptake and thus can improve cereals growth in a sustainable way. Use of bio-stimulant for enhancing mycorrhizas in cereals is also more economical than inoculation technologies of cereals with mycorrhizal fungi.

Keywords: azo-bacteria, cereals, Bacillus, natural products, Trichoderma

P (ID 351)

The growth of mycorrhiza research: a quantitative analysis and statistics of global publications

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The mycorrhizal research is making remarkable progress as more and more scientists the world over are turning to it, and are engaged in exploring new and potential species of mycorrhiza that can be used as biofertilizers. In this context, a Mycorrhiza Network was set up at TERI, New Delhi in 1988 with the objective to strengthen research, encourage cooperation, promote exchange of information and germplasm, and facilitate transfer of technology to the field.

The 'Literature Abstracts Database', developed under the Network, has a glorious collection of over 6,000 references serves as a reference tool for scientists engaged in mycorrhizal research. Each literature reference in the database has been classified and assigned with one of the six categories of mycorrhiza, viz., Ectomycorrhiza, Arbuscular mycorrhiza, Orchid mycorrhiza, Ericoid mycorrhiza, Ectendo mycorrhiza, and Mycorrhiza (General). Under each category, the references have been assigned with one or more of the 15 broad subjects, such as • Anatomy • Biochemistry • Biocides • Biological interaction • Ecology • Genetics • Mass production • Methodology • Physiology • Pollution • Soil plant relations • Systematics • Anatomy, histology and ultrastructure, Reviews and General. Majority of these subjects is further divided into relevant sub-subjects to make the data retrieval as specific as possible.

Over 1000 sample literature references published from 2012 to 2016 on different mycorrhizal categories, viz. ectomycorrhizas, arbuscular mycorrhizas, and other mycorrhizas were taken from the database for study; search combinations were performed based on two methods – (i) classification and (ii) database search format. The study, through its quantitative analysis, presents the growth of global mycorrhiza research.

Keywords: mycorrhiza, literature, analysis and statistics, publications

P (ID 353)

Cultivation of Some Ectomycorrhizal Fungi for Mycorrhizations with Seedlings of Some Fagaceae Plant

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The forests of northern Thailand are diverse of ectomycorrhizal (ECM) fungi. Fruiting bodies of ECM fungi are produced from association with the host plants. Some fruiting bodies of ECM fungi are edible mushrooms which are food and income of local villagers during rainy season. Fagaceae is one of the ECM tree families that are potential for forest restoration in northern Thailand. Cultivation of ECM mycelia is difficult compare with saprophytic mycelia. Objective of this research is to cultivate mycelia of some edible ECM fungi for inoculum production for mycorrhizations with seedlings of some Fagaceae plant. The results showed that mycelia of *Amanita princeps* A10 grew well on culture media of Modified Melin Norkans agar mixed with 1.0 g/l yeast extract, and *Gyrodon* sp. B31 grew well on potato dextrose agar. For inoculum production, *A. princeps* A10 and *Gyrodon* sp. B31 could produce in the optimum culture media in two types of liquid media and peat-vermiculite media. The inoculums of the ECM fungi were inoculated to sixmonth-old seedlings of *Castanopsis acuminatissima*. Six months after inoculation, seedlings of each treatment will be evaluated for growth and mycorrhizal formation.

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Keywords: ectomycorrhizal fungi, cultivation, Fagaceae

P (ID 357)

The Use of Mycorrhizal Inoculants in Rice Fields

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Rice is the second most cultivated plants in the world, providing 20 percent of the world's dietary energy supply. The use of mycorrhizal inoculants appears to be a solution to increase yields and improve fertilizers effectiveness.

To demonstrate the benefits of using commercial inoculants for rice production, field trials were done in Ivory Coast during growing seasons 2016 and 2017 in eight irrigated rice fields. Inoculation was made by coating the seeds with AGTIV[™] FIELD CROPS • Powder (Premier Tech Agriculture), giving and average of 2.5 spores per seed. After 3 weeks in nursery, the seedlings were transplanted in fields. Some plots were fertilized according to standard practices and compared to non-fertilized controls.

In nursery, results showed a significant 30 % increase of tillers number per plant. Following transplantation in fields, results showed a significant increase of tillers number and shoot biomass by 11 % and 18 % respectively resulting from mycorrhizal inoculation. Yield increase in paddy rice was 20 % for the inoculated treatment. We observed that mycorrhizal inoculants cannot replace fertilization but increase significantly its effectiveness.

Our results in real rice field conditions show that mycorrhizal inoculants are efficient tools that may help growers to improve the yield and profitability of their crops and can contribute to supply food for the growing population.

Keywords: mycorrhiza, rice, inoculation, Africa

P (ID 358)

Arbuscular Mycorrhizal Fungi Applied Via Seed Coating to Agricultural Crops under Abiotic Stress

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Arbuscular mycorrhizal fungi (AMF) are well known for improving nutrient uptake, plant growth and tolerance to various abiotic stresses. Still, its large scale application has been hampered by the relatively high cost of inoculation per plant. Seed coating allows the use of minor amounts of inocula being a promising tool for a more cost-effective and sustainable AMF inoculation. Different greenhouse experiments were performed with different agricultural crops in order to test seed coating as a feasible AMF inocula delivery system and the inoculation effect in promoting plant growth and yield under different abiotic stresses. The experiments have demonstrated that seed coating system can serve as an efficient vector to apply AMF into the soil with results showing improvements in crop growth, nutrition and resistance to various stresses. The use of this technology is particularly pertinent in low input or organic based agriculture and can be applied under adverse environmental conditions.

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P (ID 361)

Effect of Plant Growth Promoting Endophytic Bacteria and Arbuscular Mycorrhizal Fungi on Rice Growth

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Black rice (Oryza sativa) variety Hom Nil is increasing popularity as healthy food in Thailand. It contains high vitamins and anthocyanin. The consumers concern about health care and demand for high quality of rice products. Many farmers have changed their farming system into organic system. This research is aimed to study the effect of inoculation of endophytic bacteria and arbuscular mycorrhizal (AM) fungi on growth of the black rice variety Hom Nil. The experiment was done in pots with full factorial of inoculation with plant growth promoting endophytic bacteria (PGEB) strain RBR04, AM fungi, and co-inoculation of PGEB strain RBR04 and AM fungi with 20 replications. For the bacterial inoculation, two-week old rice seedlings were submerged in 0.85% NaCl containing with 108 CFU/ml of the PGEB strain RBR04 for 1 hour before transplanting. For AM fungal inoculation, 10³ spore of AM fungi were inoculated in planting hole of the rice seedlings. The results showed that co-inoculation of bacterial strain RBR04 and AM fungi significantly increased shoot dry weight compared to uninoculated control. Moreover, the coinoculation of RBR04 and AM fungi resulted in speed up booting stage compared to the single inoculation and uninoculated control. Inoculation of PGEB strain RBR04 or AM fungi alone were not effects on the growth of black rice variety Hom Nil.

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Keywords: arbuscular mycorrhizal fungi, endophytic bacteria, black rice

P (ID 363)

Abundance and diversity of arbuscular mycorrhizal fungi in cultivated versus wild grapevines

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The aim of this study was to compare AMF abundance and diversity between cultivated grapevine and its wild relative *Vitis vinifera* subsp. *sylvestris*.

AMF abundance ^{1, 2,3} and diversity⁴ were explored in 21 wild grapevine plants in the wide area of Neretva River in southwestern Bosnia and Herzegovina and in eight production vineyards in the neighbouring southern Croatia, whole area with (sub) Mediterranean climate.

AMF colonisation was 90-100% in the wild grapevine versus 40%-50% in conventionally cultivated grapevine or up to 70% in organic vineyards, on *V. berlandieri×V. rupestris* rootstock. Vesicular and arbuscular colonisation of wild grapevine reached 54% and 69% respectively, compared to 21% and 25% in vineyards. AMF spore abundance was similar for both relatives, ~100-1300 per 100 g of soil. In the wild grapevine the majority of the AMF sequences were similar to unidentified *Glomus* species, with only *G. iranicum* identified. In vineyards *G. intraradices/G. fasciculatum*, *G. sinuosum/G. coremioides*, *G. viscosum*, *G. indicum/G. iranicum*, and a group of unidentified sequences were found⁵.

The wild grapevine was determined as highly AMF receptive with colonisation levels well above the levels found for cultivated grapevine in the same region. Data on AMF diversity in wild grapevine remain preliminary at this stage, with only *G. iranicum* identified. Interestingly, some unidentified *Glomus* species both from wild grapevine and from organic vineyards grouped together and further wider sets of sequences will show whether these AMF sequences could be characteristic for grapevine, as to date they have not been recorded for other plant species.

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Keywords: AMF, cultivated grapevine, wild grapevine

P (ID 365)

Arbuscular mycorrhizal fungal abundance and composition shift over life cycle of Sorghum, differ among root, rhizosphere and soil, and were affected by drought

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Introduction: Water availability is the primary limiting factor for biodiversity and ecosystem functioning in semi-arid and arid ecosystems [1, 2], and the drought-adapted *S. bicolor* has a potential to cope with the food and energy crisis under global precipitation change scenarios. Knowing the responses of AM fungi to drought are critical for agriculture of *S. bicolor*. The ITS2 region is an ideal fragment for discrimination of AM fungal species [3, 4], but was rarely used previously [5, 6]. Our analysis showed that the newly published Lee Taylor's ITS2 primers [7] matched well with the sequences used for the Phylogeny and taxonomy of Glomeromycota (http://www.amf-phylogeny.com), suggested that this primers has a potential of unbiased estimation of AM fungal abundance and diversity.

Methods: Using the Illumina Miseq of ITS2, root, rhizosphere and soil AM fungal communities were examined weekly along the life cycles of *S. bicolor* treated with pre-, post-flowering drought, and control.

Results: Arbuscular mycorrhizal fungal reads accounted for ca. 1% of total 30 Million fungal reads, and were clustered into 52 OTUs at 97% similarity cutoff. However, only seven OTUs at 97% similarity cutoff was generated from the flanking 18S region of high similarity reference (>97%) of AM fungal OTUs. Along the life cycle of *S. bicolor*, succession of AM fungal community were demonstrated both at genus-level and at OTU-level within the genus *Rhizophagus*. Both pre- and post- flowering drought decreased the abundance of AM fungi drastically. AM fungal community differed among root, rhizosphere and soil.

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Keywords: drought, SSU, ITS2, Lee Taylor's primer, sorghum

P (ID 371)

Impact of tropical land transformation on root associated fungal communities

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Low-land tropical rain forests are among the most diverse and productive ecosystems of the world. Their ongoing conversion to farmland, especially Oil Palm (*Elais guineensis*) plantations, is one of the major causes of deforestation, leading to severe losses of biodiversity and ecosystem functioning. This is especially the case in Indonesia, one of the biggest palm oil producers today.

While negative impact on above ground biodiversity is well studied, little is known about the effects of such land transformation on the below ground fungal community. We hypothesize that intensive land use leads to severe shifts in both taxonomical and functional diversity of fungal communities and further, that these changes will affect the associated tree community and impair essential ecosystem functions. In an ongoing study located in Jambi (Sumatra, Indonesia) we analyze the impact of land transformation on tree root and soil associated fungal diversity using next generation sequencing methods.

First results reveal functional shifts in the fungal community composition with increasing land use, which was correlated with a decline of tree root health. Currently we are analyzing the temporal variation in the fungal community composition and identifying key environmental variables causing the compositional shifts.

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P (ID 374)

Use of soil microorganisms combined with reduced fertilization to improve bean fruit yield and quality

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Phaseolus vulgaris cv. Billò is an important and valuable crop widely cultivated in western Piedmont. Arbuscular Mycorrhizal Fungi (AMF) are known to improve plant nutrition and hence fruit quality. Rhizobium leguminosarum is a gram-negative bacterium, symbiont of various species of Fabaceae and able to fix atmospheric nitrogen. Aim of this study was to test, in field conditions, the possibility to improve bean fruit yield and quality by means inoculation with AMF and/or rhizobia under conditions of low fertilization.

At harvesting, yield parameters (pod and seed number and weight) and fruit quality (starch, protein, fiber and metal contents) were evaluated. Nodulation and mycorrhizal colonization of roots were assessed.

Yield parameters and fiber content were not influenced by the presence of microorganisms nor by the reduced fertilization, whilst protein concentration was significantly higher in the fruits of rhizobium-inoculated plants in combination or not with AMF. Starch concentration significantly increased in the seeds of plants inoculated with rhizobia alone. Mg, K and Zn concentrations were positively affected by AMF, while Mn concentration was higher in the presence of rhizobia. Ca and Fe levels did not show differences between the treatments.

In conclusion, a low chemical fertilization, an environment-friendly practice, can be associated to inoculation with soil microorganisms to improve fruit quality. Finally, different combinations of microorganisms induced different effects on the fruit characteristics.

P (ID 376)

Effect of indigenous and introduced AM fungi on growth of Allium fistulosum under sterilized field

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Promotion of phosphorus uptake and growth of host plant by AM fungi are well known in pot culture with sterilized soil. However, the effects are not widely observed under field condition, because sterilization of field is difficult. The aim of this study was to investigate effect of indigenous and introduced AM fungi on the phosphorus uptake and growth of Allium fistulosum under sterilized field condition. The field was sterilized with application of fumigant dazomet at the rate of 200 kg ha¹. Superphosphate was applied at the rate of 0, 500, and 100 kg ha⁻¹. A. fistulosum inoculated with Glomus R-10 or uninoculated were transplanted to sterilized field. This field experiment was repeated in 2015 and 2016. AM colonization, shoot P concentration, and shoot growth were measured. AM colonization in unsterilized field ranged from 37% to 76% in uninoculated plant and from 45% to 86% in inoculated plant at 35 days after transplanting, and was not different between inoculated and uninoculated plants until final harvest. AM colonization in sterilized field ranged from 1% to 19% in uninoculated plant and from 45% to 84% in inoculated plant and was mostly higher in inoculated plant than uninoculated plants until final harvest. Shoot growth of inoculated plant was not different from uninoculated plants in unsterilized field, but shoot growth of inoculated plant was higher than uninoculated plants in sterilized field. This result suggested that dazomet treatment suppressed indigenous AM fungi and introduced AM fungi promoted growth of A. fistulosum under sterilized field condition.

P (ID 379)

"Bioirrigation" and biofertilizer based legume-millet intercropping as a tool to mitigate drought-induced crop yield loss in arid and semi-arid tropics

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Hydraulic lift is an important ecological process where roots passively lift up water from deeper soil layers and release it into upper layers. Hydraulically lifted water can be redistributed to a neighbouring plant. In agroecological terms this process is referred to as "biorrigation". The role of beneficial microbial inoculants such as arbuscular mycorrhizal (AM) fungi and plant growth-promoting rhizobacteria (PGPR) as facilitating agents for bioirrigation has often been suggested but is not yet well understood. We conducted both field and pot experiments to reveal the significance of AM fungi and PGPRs for biorrigation using an intercropping system. Specifically, we used the deep-rooting crop species pigeon pea (Cajanus cajan) and the shallow-rooting crop species finger millet (Eleusine coracana) as model plants to understand the role of AM fungi and PGPR as facilitating agents for bioirrigation. Using deuterium labelled water in a pot experiment, and through measuring natural abundance of hydrogen and oxygen isotopes in field experiment, we found evidences for the redistributions of hydraulically lifted water by pigeon pea, in which AM fungi seem to play a key role. In fact, the shallow rooted E. coracana was only able to utilize hydraulically lifted water in the presence of AM fungi and PGPRs. We propose that by designing suitable intercropping system based on bioirrigation and biofertilizers, there is great potential for reducing drought induced crop yield loss in arid and semi-arid tropics.

Keywords: arbuscular mycorrhizal fungi, biofertiliser, bioirrigation, intercropping, sustainable agriculture

P (ID 384)

Cover plants, a tool to manage arbuscular mycorrhizal fungi diversity?

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Due to functional diversity on the benefit conferred to host plants by arbuscular mycorrhizal fungi (AMF) and the difficulties linked to linoculation, strategies are required for managing communities of indigenous AMF associated with different host plants within agricultural cropping systems. The impact of a mycotrophic cover (developer) plant on the mycorrhizal diversity of a successor crop, when the extra-radical mycelium (ERM) was kept intact or not was investigated.

AMF diversity in roots of two species of the Fabaceae (*Ornithopus compressus* and *Trifolium subterraneum*) was compared with those of two species of the Poaceae (*Lolium rigidum* and *Triticum aestivum*). In disturbed soil, the communities of AMF present in the two legumes were clearly different from those of the two members of the Poaceae but were similar within each plant family, consistent with preferential symbioses within an AMF population for a plant group. However, if for example, the extra-radical mycelium (ERM) associated with *L. rigidum* (Poaceae) as the first plants in a succession, was kept intact (undisturbed soil) and functioned as the AMF propagule for colonizing *T. subterraneum* (Fabaceae) as successor crop, the AMF community present in the latter corresponded to that of the first crop, whatever the family group involved. Therefore in a succession of plants (cover crop-main crop or crops in rotation) the choice of the first plant together with use of an appropriate tillage technique, which keeps intact the ERM associated with the first plant, can be used strategically to manage AMF biological diversity within the cropping system.

Keywords: AMF community structure, preferential colonization, abiotic stress

P (ID 388)

Factors affecting effectiveness of arbuscular mycorrhizal fungal inoculation under field conditions

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We conducted field inoculation experiments with a commercial inoculum of arbuscular mycorrhizal fungus R10 (Idemitsu Kosan Co., Japan) for soybean and Welsh onion. The test plants were grown by combining the treatment of inoculation/non-inoculation and 3 levels of phosphorus fertilization. Soybean were directly sown to field with the inoculum and cultivated for 4 months. Welsh onion were sown with the inoculum in nursery bed which did not contain indigenous AM fungi. Six weeks seedlings were transplanted to field and cultivated for 4 months. All experiments were conducted in fields, Kyoto, Japan. Soil was Eutric Fulvisol. For soybean, neither inoculation nor phosphorus fertilization showed significant difference in growth, P uptake and yield. This may indicate rather higher soil P fertility, while AM colonization tended to be increased with inoculation. Pot experiment with sterilized and non-sterilized soil and PCR-DGGE analysis showed that the fungus R10 failed to colonize soybean roots, suggesting that R10 was not able to compete with indigenous fungi. For Welsh onion, on the other hand, the inoculation increased its growth and yield. Inoculation in nursery bed may enable the inoculum, AM fungus R10, to fully colonize the roots and result in competitiveness of the inoculum against indigenous AM fungi in field. These results imply significance of inoculum fungal prevalence in plant roots.

Keywords: field experiment, indigenous fungi, soybean, Welsh onion

P (ID 389)

Commercial mineral-based and chemical fertilisers influence mycorrhizas in pasture plants

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Introduction/Aim: We evaluated the effect of fertilisers of different elemental solubility on mycorrhizal colonisation in relation to root growth for two annual pasture species in a pasture soil.

Materials and Methods: The four treatments were: a commercial mineral-based fertiliser and a commercial chemical fertiliser both applied at 15kgha⁻¹P, a microbial inoculant and a control. Subterranean clover and Wimmera ryegrass were grown separately and roots were examined after 5 and 10 weeks.

Results: By 5 weeks, the proportion of clover roots colonised by AM fungi decreased by 35 and 41% for mineral-based and chemical fertiliser respectively. In contrast, the decrease in proportion of roots of ryegrass roots colonised by AM fungi was only about 20% in each case. The length of colonised clover roots decreased to the same extent with application of both fertilisers, especially after 10 weeks, but the reduction in length of colonised root for ryegrass was much less than for clover. There was little effect of the microbial inoculant on either the proportion or length of roots colonised.

Conclusion: P fertilisers had less effect on root length colonised than on the proportion of roots colonised for two annual pasture plants in this soil. The reduction in the proportion of roots colonised by AM fungi with P fertiliser was not related to differences in their elemental solubility. Differential effects of P fertilisers on naturally occurring AM fungi have potential to influence plant competition and pasture species dominance.

Keywords: mycorrhizal fungi, fertilisers, pasture plants

P (ID 390)

Mycorrhizal colonization and phosphorus absorption in maize fields prepared from grasslands using different tillage systems

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In grasslands dominated by symbiotic plants with arbuscular mycorrhizal (AM) fungi, the inocula of indigenous AM fungi accumulate in the topsoil. We examined the effects of two tillage systems, mold plowing (MP) and rotary tillage (RT), used for converting grasslands to maize fields on P mobilization by AM fungi.

The pot and field experiments comprising two tillage systems and three to four P fertilization levels were conducted to determine the AM colonization, shoot dry weight, and P content of maize in early growth stages.

In the pot experiment using two soils with different P availability, the mean AM colonization for each soil was significantly higher in RT (62, 72%) than in MP (26, 51%) (p<0.01). Additionally, the dry weight and P content of maize were significantly higher in RT than in MP (p<0.05). Although the shoot dry weight of maize decreased with a reduction in P fertilization levels, the rate of decrease was lesser in RT than in MP. In the field experiment in a single field, the dry weight and P content of maize were significantly higher in RT than in MP (p<0.05), but the difference in AM colonization between MP and RT was insignificant (p=0.07).

RT, which retains the indigenous AM inocula in the topsoil, has an advantage over MP, which moves the source of inocula to the lower soil layers.

Thus, RT is more effective than MP because it facilitates AM colonization and P uptake in the early growth stages of maize in fields prepared from grasslands.

Keywords: indiginous arbuscular mycorrhizal fungi, maize (*Zea mays* L.), grassland, phosphorus fertilizer, tillage

P (ID 392)

Effect of preceding crop and weed management history on AM fungal community structure throughout the development of durum wheat

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Although arbuscular mycorrhizal (AM) fungi are recognised as an important component of the plant-soil interphase, the factors that affect their community dynamics in agricultural systems are not well understood.

In this study, we investigated the effects of preceding crop and weed seedbank density on AM fungal community dynamics and root growth in a durum wheat trap crop grown in soils from a long-term rotation study. Soils were removed from treatments where previous crops were flax (*Linum usitatissimum* L.) or canola (*Brassica napus* L.) which have been grown in the same rotation with three different weed seedbank densities.

High weed seedbank densities promoted root length and almost doubled AM fungal root colonisation when integrated over all developmental stages of durum wheat. Conversely, preceding crop had comparatively little impact on these parameters. Over the development of durum wheat, the trajectories of AM fungal communities were influenced by preceding crop and weed seedbank densities and these effects were most prominent at the early vegetative and reproductive stages. Differences in AM fungal communities in response to preceding crop were caused by differences in community richness while differences in response to weed seedbank densities were related to variation in the relative abundance of AM fungal types.

This study shows that some of the outstanding variation in AM fungal community structure in agricultural systems may be explained by weed management history and its interaction with other factors. It is advisable to better document weed populations and seedbank densities when evaluating AM fungal communities in agricultural systems.

Keywords: AM fungi, weed management, durum wheat, agricultural systems

P (ID 394)

Characterisation of a truffle plantation in Burgundy

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Introduction: Truffle production in Burgundy mainly occurs in the wild. Many plantations have been created in the last 10 years. However, production is still limited in these grounds. The aim of this study was to characterise a 10-year-old truffle plantation comprising trees inoculated with *Tuber melanosporum* Vittad. or *T. aestivum var. uncinatum* Chatin, in terms of mycorrhization, brûlé formation and truffle production. In addition, interaction between truffle species and AM fungi was investigated around trees.

Materials and Methods: The presence of brûlés around trees was assessed. Roots of hazel, oak, black pine, hornbeam, birch, cedar and tilia were collected in the plantation for analysis of the level of mycorrhization by ectomycorrhizal fungi. Root colonisation by AM fungi was estimated in *Avena* sp., *Medicago lupulina* L. and *Myosotis* sp. around trees presenting or not brûlés.

Results and discussion: Less than 40% of the trees inoculated with *T. melanosporum* or *T. aestivum var. uncinatum* formed a brûlé and about 5% of the trees produced truffles. The mean level of root colonisation by truffle species was 52% but 46% of the trees were contaminated by other ectomycorrhizal fungi. It is therefore important to adapt cultivation practices in truffle plantations according to the quality of root mycorrhization. No significant difference was identified in root colonisation by AM fungi in *Avena* sp., *M. lupulina* and *Myosotis* sp. around trees whether they presented or not a brûlé. Interaction between truffle mycelium and other soil fungi in soil will be investigated in the future.

Keywords: truffle, AM fungi, agro-ecology

P (ID 405)

Selection of tomato lines tolerant to the infection of *Meloidogyne incognita* using arbuscular mycorrhizae

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Tomato is considered the most important vegetable around the world. One of the critical problem is the production losses resulting from nematodes's attack, which reduce production between 12% and 20% (Hassan *et al.*, 2013)². Arbuscular mycorrhizal fungi (AMF) can reduce damage by enhancing plant growth and/or stimulate the nematode genetic's resistance in infested plants (Azcón-Aguilar y Barea, 1997)³.

The aim of this study was to know the effect of two AMF in the selection of tomatoes' lines with high mycorrhiza dependency and nematodes tolerance.

A bifactorial design was proposed with 50 tomatoes's line and two AMF strains, *Glomus iranicum* var. *tenuihypharum* and *Glomus intraradices*. The mycorrhizal inoculants's application at 3 kg. ha-1 rate was done by irrigation system and several measures of mycorrhizal and plants development were carried out.

All lines of tomatoes showed mycorrhizal colonization with both species, however, from the first sampling the expression of the strain *Glomus iranicum* var. *tenuihypharum* was higher than the strain *Glomus intraradices*, regardless of the line studied. Not every line had the same response to the mycorrhizal process. *Glomus iranicum* var. *tenuihypharum* promoted best behavior than *Glomus intraradices*. Towards the end of the trial, there was a strong correlation between the lines with the highest production of Glomalin and the highest percentages of mycorrhization with *Glomus iranicum* var. *tenuihypharum*. At the end, ten lines were selected with high nematode tolerance and a marked dependency to form mycorrhizae with the specie before mentioned.

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² Hassan, M.A.; Pham, T.H.; Shi, H.; Zheng, J. (2013). Nematodes threats to global food security. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 63(5), 420-425.

³ Barea, J. M., Azcón-Aguilar, C. y Azcón, R. 1997. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems. In: Gange AC, Brown VK (eds) Multitrophic interactions in terrestrial systems. Blackwell Science, Oxford, pp. 65-77.

P (ID 409)

Infection unit density as an index for infection potential of arbuscular mycorrhizal fungi

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For effective use of arbuscular mycorrhizal (AM) functions in agricultural practice, not only AM fungal biological characteristics but also quantitative evaluation of AM fungal infection potential in field soil or in inoculation material would be inevitable. Mean infection percentage (MIP) and most probable number (MPN) methods, both widely used for the quantification of AM fungal infection potential, are indirect assay. It has been previously suggested that number of infection unit (IU) formed on the host plant root after short cultivation period reflect the number of propagules in the soil directly when the pot soil was completely permeated by the host plant root. In the current study, efficiency of IU density, number of IUs per unit root length as an index of AM infection potential was evaluated.

Lotus japonicus, chive or onion were used as test plants. They were grown in sterilized soil-sand mixture inoculated with different amount of AM fungal material. After 12-14 days cultivation, whole root system was stained with DAB and IU numbers were counted under stereomicroscope.

IU density showed linear correlation with the inoculum density. Different from MIP, the value was less affected by host plant species. Processing the stained root for microscopic observation and IU counting took 7 and 5 minutes per sample in average, respectively, which was less time consuming than colonization measurement. Short cultivation period is also advantageous to achieve constant culture condition. Thus, the IU density would be a robust and practically useful index to evaluate the AM fungal infection potential.

Keywords: infection unit, mean infection percentage, most probable number

P (ID 413)

Inoculations of legumes with beneficial soil microorganisms

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The legume production implementing the beneficial soil microbes represents an ecofriendly solution for decreasing the excessive inputs of agrochemicals in a cultivation system as crops can benefit via a better growth, yield and resistance to various environmental stresses thanks to the inoculation of microbes such as arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, Trichoderma sp. etc. These microbes are slowly becoming a key part of a good practice in open field crop productions, although their applications under large scale might be cost and labor sensitive. Series of experiments on legumes in pots (followed a standard inoculation procedure) and in the field (employing a targeted seed coating method) showed positive growth responses. Furthermore, seed coating technique was proved as a functional way of introducing the microbes in the soil, which can be feasible (as needs of inoculum per plant are significantly reduced) both for organic and conventional farming practices.

The authors acknowledge the support of EU-FP7 Project Eurolegume (GA 613781) and Eurostars project E!8275 MicroCoat.

Keywords: arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria, Trichoderma, legumes

P (ID 420)

Interaction effects of Arbuscular Mycorrhizal Fungi, compost and root-knot nematode on tomato landrace's growth from Canary Islands

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Root-knot nematodes are worldwide important pathogens of a wide range of agriculturad crops. In Canary Islands, the effects of root-knot nematodes produce intense damage to tomato crops. Arbuscular mycorrhizal fungi have the potential as biocontrol agent for nematode management when both occur simultaneously in roots and rhizosphere (Jain and Hasan, 1994). Organic fertilizers are known to suppress root-knot nematodes populations and improve crop tolerance (Oka and Yermiyahu 2002).

The aim of this work is to evaluate interactions effect of the arbuscular mycorrhizal fungi and compost application on tomato landrace from Canary Islands "Manzana Negra" affected by root-knot nematodes.

Greenhouse experiment was conducted to assess the influence of local arbuscular mycorrhizal fungus *Glomus mosseae* and compost addition alone and in combination with root-knot nematode *Meloidogyne javanica* and on growth of local tomate landrace.

Negative effects of root-knot nematode on tomato landrace's grows was clearly visible. Tomato plants inoculated with local *Glomus mosseae* showed recovering their normal size, despite being subjected to the negative effects of nematodes. The addition of organic matter produced an increase in plant's vigor, reflected in a significant increase of plant's biomass, number of tomatoes produced and also reduced nematode reproduction rates. Synergistic effects were founded when arbuscular mycorrhizal fungi and compost was applied.

The combined use of arbuscular mycorrhizal fungi and compost produced significative effects on tomato plants such as higher growth, biomass and production and reduced nematode reproduction rates.

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Oka, Y. and Yermiyagu, U. 2002. Suprressive effects of composts against the root-knot nematode Meloidogyne javanica on tomato. Nematology, 4 (8): 891-898.

Keywords: biocontrol, *Glomus mosseae*, compost, *Meloidogyne javanica*, *Solanum lycopersicum* L.

P (ID 421)

Re-evaluating fine root endophyte

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Introduction/Aim: Fine root endophytes (FRE) are morphologically distinct arbuscule-producing endophytic fungi. The presence of arbuscules led to FRE being classified as *Glomus tenue*, an arbuscular mycorrhizal fungus (AMF), phylum Glomeromycota.

Materials and Methods: In this poster we review the state of knowledge on FRE and present key recent findings by our research group.

Results and Discussion: In Australian crops and pastures, colonisation by FRE may often be greater than 20% of root length and may reach up to 80%. In some instances, colonisation by FRE may be higher than that of AMF. This high abundance of FRE is especially interesting as recent sequencing results suggest that FRE belong in the sub-phylum Mucoromycotina, not Glomeromycota. Thus, FRE are the only arbuscule-producing fungus not placed within the Glomeromycota. This difference in phylogeny and the differences in colonisation morphology between FRE and AMF could indicate a different ecological niche. There are some studies that support this contention including some from the Artic region and one with a waterlogging treatment. In these studies, FRE were favoured relative to AMF by the stressful conditions, but only in some host plants.

Conclusion: Further research is required on the functioning of FRE in relation to the environment, host plant and co-occurring AMF and, in particular, to examine whether FRE are important for plant growth in stressful environments. Molecular studies using primers which target AMF (glomeromycotan fungi) may overlook a significant component of the soil mycorrhizal community, therefore targeted primers are urgently needed for further research on FRE.

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P (ID 423)

AMF influence on total, ammonia oxidiser and denitrifiers bacterial communities under different wheat variety

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N₂O is a potent greenhouse gas contributing to both global warming and ozone depletion in the stratosphere. Agricultural land acts as a large source of this gas. Arbuscular Mycorrhizal Fungi (AMF) have been shown to reduce N2O emissions from agriculture through changes in the abundance of key bacterial genes associated with nitrification and denitrification, though the mechanism of this and the effect of AMF on bacterial community structure is still poorly understood. As part of a field trial set up by the MycoRhizaSoil consortium at the University of Leeds Farm, we tested the influence of AMF on soil bacterial communities, looking at the total community and that of ammonia oxidisers and denitrifiers. Soils of six varieties of wheat were amended with a commercial AMF inoculum, and fungal and bacterial community structure was determined through next generation amplicon sequencing. The amendment significantly altered the community structure of all studied groups. The wheat variety was found to be a strong determining factor of the inoculum alterations to fungal community, with a range of AMF species richness changes between varieties. This did not however show the same relation to total bacterial, ammonia oxidizer or denitrifier richness, indicating that this has a different driving mechanism, being the direct influence of AMF presence regardless of variety. The AMF effects on the bacterial functional expression should be investigated in the further studies based on the results.

Keywords: AMF, wheat variety, bacteria, ammonia oxidiser, denitrifiers

P (ID 428)

Dissecting biotic and abiotic soil factors that determine responsiveness of soybean to arbuscular mycorrhizal fungal inoculation in the field with respect to dynamics of inoculum fungus

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The significance of soil biotic and abiotic factors in the effectiveness of arbuscular mycorrhizal (AM) fungal inoculation on yield was evaluated with respect to dynamics of an inoculum fungus in field trials. Soybean was inoculated with a commercial inoculum of Rhizophagus irregularis and grown in three field sites that were differently managed in the previous year: two bare fallowed sites (BF1 and 2) and a palisade grass-grown site (PG). Field soils were collected before fertilizer application, and most probable numbers (MPNs) of AM fungal propagule were estimated. At the time of flowering, the roots were collected, and AM fungal LSU rDNA was amplified from the root DNA extracts and sequenced on the MiSeq platform. Operational taxonomic units (OTUs) were defined in reference to a database composed of 412 AM fungal sequences. Root zone soils were subjected to chemical analysis. Increases in yield and shoot dry weight in response to the inoculation were observed in BF1 and BF2, respectively, but not in PG. Sequence read abundances of the inoculum fungus were correlated negatively with the MPNs and positively with yield responses to inoculation; the less the indigenous fungal population, the more effective the inoculation. Logistic regression analysis indicated that MPN is the most significant determinant of inoculum colonization among the soil factors. Niche breadth analysis of indigenous fungi revealed that those with a broader niche breadth (i.e. general fungi across the three fields) mainly competed against the inoculum fungus and that cultivation of AM fungal host strengthened the competitiveness of them.

P (ID 440)

Investigation of interactions between plant biostimulant and symbiotic microbes and their effects on agricultural crops

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One of the greatest challenges worldwide is the reduction of negative environmental impact of modern agriculture. One often overlooked aspect is the negative impact of intensive chemical inputs on the key ecophysiological features of terrestrial plants, symbioses with mycorrhizal fungi and endophytic microorganisms. The use of environmentally friendly products such as plant biostimulants generated from waste products may be a solution for new sustainable crop production systems.

The poster presents an EU funded project, its background and advances, for development of a new biostimulant based on biorefined waste products. These products, which would otherwise be disposed with the negative impact on the environment, can enhance plant growth and/or stress resistance, and act in synergy with symbiotic microbes and/or promote their growth/functioning due to the content of N-containing compounds influencing plant metabolism. Synergistic effects of tested biostimulant and the symbiotic microbes on the growth promotion of agricultural crops are being studied at the level of plant biometric parameters, biomass yield, nutrients and photosynthetic apparatus. The indirect plant mediated effects of biostimulant foliar treatments on root colonization by symbiotic microbes, their functional enzymatic activities and community structure is being determined.

The project contributes to the increasing awareness of the society on the necessity of development of eco-friendly agricultural products for sustainable crop management, and on the need of biostimulants' regulation by EU laws.

Keywords: plant biostimulant, symbiotic microorganisms, plant growth promotion, sustainable crop management

P (ID 441)

Ericoid mycorrhizal inoculation in organic highbush blueberries: influence on yield and anthocyanins

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Consumer demand and commercial production of cultivated blueberries have strongly increased globally during the last ten years. At the same time, there is a growing demand for organic fruits and vegetables. Between 2011 and 2016, we have conducted a project aiming at optimizing organic production of cultivated blueberries in a Nordic climate.

As a part of the project, we have examined the influence of two ericoid mycorrhizal inocula on growth and anthocyanin content of the highbush blueberry (*Vaccinium corymbosum*) cultivars 'Duke' and 'Reka'. Two-year old plants were inoculated in pots in May 2012 and were cultivated in a greenhouse until transplantation to peat-filled ditches in the field at Rånna Experimental Station, Skövde, in August 2012.

In 2016, 'Reka' was the highest yielding variety. One of the mycorrhizal inocula negatively affected yield for 'Reka', but inoculation did not significantly influence the yield of 'Duke'. While 'Duke' showed the highest berry anthocyanin content, no significant difference was observed between the mycorrhizal treatments. Further analyses are presently conducted on the content of individual anthocyanins and phenolic acids.

In conclusion, the effects of mycorrhizal incoulation on yield of organically grown highbush blueberries were small and depended on inoculum type and blueberry cultivar, while the total amount of anthocyanins was not affected by inoculation.

Keywords: Vaccinium corymbosum, organic cultivation

P (ID 442)

Effect of mycorrhizal seed coating and bacterial inoculation on maize and chickpea early growth

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Seed coating is a technique that facilitates application of microbial inocula to the seeds enabling its feasible use in agricultural practice. It requires a specific coating method for each crop and microorganism used to assure a proper seed germination and inoculum functionality.

Seeds of maize and chickpea have been inoculated with arbuscular mycorrhizal fungi (AMF) and sown into 2L-plastic pots containing sterile growth substrate. The combinations of AMF with two different bacteria have been tested. Coated seeds without AMF and non-coated seeds served as controls. The AMF colonization and the effects of microbes on plant growth promotion have been evaluated after two months of cultivation.

In maize, the germination of seeds was not influenced by coating; in chickpea the germination rate was negatively affected by coating as well as the development of the root colonization by AMF. In AMF treated maize seeds the mean root colonization was the highest (47.5%) in comparison with seeds subjected to the simultaneous AMF and bacterial inoculation, and significantly increased root fresh and dry biomass in comparison with control plants.

These preliminary results indicate that the interaction of various microbial inocula may influence their establishment on plants and affect their efficacy on the crop growth in maize. Applied seed coating resulted as an effective mean to introduce AMF to the maize plants but further studies are necessary to confirm these results under field conditions.

Keywords: seed coating, mycorrhiza, beneficial bacteria

P (ID 443)

Plants talk! - Communication via arbuscular mycorrhizal fungal networks

Carmina Falcato Cabral (Department of Agroecology, Aarhus University, Slagelse, Denmark)

Arbuscular Mycorrhizal fungi (AMF) transport both nutrients and water between plants through the mycelial networks connecting plants underground. Previous studies have shown that plants can use this underground communication system for signaling processes, to warn about imminent pathogen or pest attacks. Nevertheless, both the causes and mechanisms of these transfer processes are scarcely investigated and need further research efforts. Fungal-hyphae mediated allelopathy in response to biotic stress (aphid attacks) via transport of signaling compounds through the AM mycelial networks has been demonstrated. In contrast, the induction of a rapid response against abiotic stress via signaling compounds traveling through these networks has not been investigated. Moreover, the identity and the mechanisms of transport of the putative signaling compounds through or on these AM networks have not yet been described. Applying a tightly connected interdisciplinary approach, ranging from microbiology to plant metabolomics, the present study aims to: i) evaluate the signaling processes between 'donor' and 'receiver' plants, induced by challenging 'donor' plants with different stress types (abiotic/biotic); ii) identify the signaling compounds in the external mycelium connecting the plants and iii) assess if there are changes based on the different stress events/types and/or stress interactions. The identification of these compounds can bring us one step closer to understanding inter-plant communication via arbuscular mycorrhizal networks.

P (ID 454)

The Mycorrhiza Network since 1988: a journey of progress and achievements

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The Mycorrhiza Network at TERI has been actively involved in development and application of mycorrhizal biofertiliser, technology development and its transfer; and information dissemination activities. The Network functions with the objective of helping scientists carry out research in the field of Mycorrhiza and promoting communication among mycorrhiza scientists. The Network also functions as a specialized centre for collection, compilation and dissemination of databases and publications on mycorrhiza, viz., The Network's Literature Abstracts Database has over 7000 classified references; serves scientists who are looking for different aspects of mycorrhizal research. The Centre for Mycorrhizal Culture Collection of the Network provides opportunity to researchers to obtain specific cultures of interest; preserves germplasm available in India and elsewhere; and provides starter cultures. The Centre has a glorious collection of over 600 different isolates of which 257 are ecto mycorrhizal and 350 Arbuscular Mycorrhizal isolates. The Network has developed a Directory of Mycorrhizologists to create a network of scientists associated directly with research on mycorrhiza and to identify institutions and centres where mycorrhiza research is being carried out. The Network's Mycorrhiza Newsletter provides a forum for dissemination, interaction and communication of scientific information on mycorrhizal research. It contains state-of-the-art papers covering the biology, ecology, and other related aspects of mycorrhiza including biodiversity and conservation of mycorrhizae. The Network conducts brainstorming sessions and training programmes for researchers and budding scientists on different aspects of mycorrhizal research.

Keywords: mycorrhiza network, mycorrhiza as biofertilizer, mycorrhiza literature, mycorrhiza culture

P (ID 459)

Indigenous isolate *Glomus mosseae* inoculation enhances fatty acid levels, elemental status of Groundnut (*Arachis hypogaea* L.) oil

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Groundnut is the sixth most important oil seed crop in the world and India is the second largest producing country. Groundnut oil composes a very good lipid profile and being a vegetable oil; it is a good source of plant sterols and possesses high nutritive value.

Ten different indigenously isolated mycorrhizal species viz. Glomus spp, Gigaspora spp, Acaulospora spp were inoculated and its effect on fatty acid profile and elemental status of groundnut seed oil was studied.

Fatty acid composition was evaluated by using GC and GCMS analysis. Elemental analysis was carried out by using ICP-AES. Acid value of oil was calculated according to the AOCS method.

Amongst the ten isolates, *Glomus mosseae was* more efficient in enhancing levels of fatty acids, elemental status and acid value of groundnut seed oil. Oleic (72%) Linoleic acid (39.93 %), Palmitic acid (79.36%) Stearic acid (86.34%), Linolenic acid (95.86%) were more prevalent fatty acids in *Glomus mosseae* inoculated plant seed oil than control. Also, *Glomus mosseae* inoculated plant seed oil had higher levels of Zinc (146.32 ppm), Calcium (4039.58 ppm), Magnesium (489.72 ppm), Manganese (174.38 ppm) as compared to other mycorrhizal isolates. Acid value was also found to be highest in *Glomus mosseae* (79.87 mg KOH/g oil) inoculated plant seed oil than in control.

These results support that, AMF has significant role in enhancement of nutrient profile of groundnut oil.

Keywords: Glomus mosseae, Arachis hypogaea, fatty acid

P (ID 460)

Optimum level of soil available phosphorus for AMF inoculation to Welsh onion in non-allophanic Andosol

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It was demonstrated that inoculation of arbuscular mycorrhizal fungi (AMF) to Welsh onion (Allium fistulosum) was effective to improve its growth and to reduce phosphorus (P) fertilizer (Tawaraya et al., 2012). Andosol is a volcanic soil with high P fixing capacity, so application of AMF is expected for efficient use of P fertilizer. However, it is not yet clarified what level of soil available P is suitable for effective AMF inoculation in Andosol. To clarify the optimum level of soil available P in a non-allophanic Andosol, we conducted field experiments of Welsh onion for 4 years in Field Science Center of Tohoku University, Japan. Welsh Onion was inoculated with a commercial inoculum containing Rhizophagus sp. R10 and grown for about 8 weeks in nursery bed in greenhouse. The seedlings were transplanted to the plots with different levels of soil available P due to previous fertilization. Soil available P of the plots were Very high (VH), High (H), Medium (M), and Low (L). The AMF inoculation significantly increased the yields in the M and H plots, while no effect was found in the VH plot. In the L plot, plant growth was so poor that the inoculation did not clearly improve yields. Additional P fertilizer was applied upon transplanting, but this P fertilization did not much affect the yield of Welsh onion. These results indicate that a medium to high level of soil available P is needed to increase effectiveness of AMF inoculation to Welsh onion in the non-allophanic Andosol.

Keywords: arbuscular mycorrhizal fungi, non-allophanic Andosol, Welsh onion, *Rhizophagus sp.* R10

P (ID 462)

Impact of trees on mycorrhizal abundance and soil fertility in low-input maize cropping systems

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Trees in African low-input maize cropping systems could play a vital role in maintaining arbuscular mycorrhizal fungi (AMF) and as such, improve maize nutrient uptake over the short term and soil fertility in the longer term. The benefits of trees in farmers' fields on soil fertility are established but the role of tree-facilitated AMF in enhancing soil aggregation and soil nutrient content is understudied.

We investigate whether trees maintain AMF during the non-cropping season and subsequently help improve soil fertility. Specifically, we assess the impact of single-standing mango (*Mangifera indica*) and faidherbia (*Faidherbia albida*) trees on AMF hyphal abundance, soil aggregation, soil carbon (C), nitrogen (N), and phosphorus content, maize root colonization by AMF, and maize biomass in Malawian smallholder farmers' maize fields. For this we collected soil samples along a distance-from-tree gradient at the end of the non-cropping season, and maize root and biomass samples.

Aggregation, C content in bulk soil, and C and N content in macroaggregates decrease away from mango but not faidherbia trees. Mycorrhizal abundance in soil and maize roots and maize biomass are hypothesized to decrease with distance from tree. Results are expected by July 2017.

Our preliminary results suggest that mango trees could improve soil fertility in their vicinity while faidherbia trees appear to impact soil properties beyond 15 meter. Correlation between soil fertility and AMF abundance remains to be assessed. In general, this study will provide insight into the potential of tree-facilitated AMF to enhance sustainability of maize cultivation in low-input cropping systems.

P (ID 476)

Enhancing arbuscular mycorrhizal fungi communities to improve drought tolerance in rooftop gardens

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Efforts to enhance global food security promote the inclusion of local urban food systems, which are often more limited by the cost and availability of water than conventional agricultural systems1. Areas of the Wasatch Front (United States) are currently experiencing moderate to severe drought, which can have profound effects on the viability of sustainable urban food systems. Soil microorganism, in particular arbuscular mycorrhizal fungi, have been shown to greatly influence drought tolerance of plants². We investigated how to effectively introduce soil biological complexity found in natural systems into the highly artificial soils of community gardens and green roof gardens, so that crops might be better able to contend with drought-stress. Green peppers (Capsicum annuum L.) were grown in rooftop garden units in a factorial design of three soil - commercial arbuscular mycorrhizal fungi (Glomus intraradices), locally sources arbuscular mycorrhizal fungi from the rhizosphere of Artemisia tridentata, and sterile soils - and two water treatments - continual or pulsed watering. Photosynthetic rates of plants in all treatment combinations were measured throughout the experiment. Photosynthetic rates of peppers grown in continual watering with commercial inoculum were highest at $4.23 + 0.46 \mu mol CO2 m^2 s^1$ and lowest $2.47 + 0.30 \mu mol CO2 m^2 s^1$ in sterile soil under pulsed watering. There was no significant difference in photosynthetic rates between commercial and local AMF communities in continual or pulsed watering. Plants will be grown until fruit production in early July, at which point they will be harvested and measured for aboveground biomass, below-ground biomass, and percent AMF root infection.

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P (ID 482)

The use of AMF to improve commercial horticulture substrate growing systems

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Commercial horticulture is rapidly moving towards protected cropping systems in soil-free substrates to improve production scheduling and reduce the threat of soil-borne pathogens. However, under protection growers are facing increasing restrictions from legislation surrounding water and nutrient input. Key UK horticulture crops such as strawberry and pot-grown herbs are almost entirely grown in substrate under protection. Soil-free substrates, devoid of many microbes, are likely to be an area where the addition of beneficial microbes would lead significant benefits in reducing water and nutrient input. Improving the benefits obtained from soil microbes such as AMF and PGPR may hold an important key to achieving production potentials whilst minimizing input.

Research at NIAB-EMR has shown that inoculation of strawberry and pot grown herbs in substrate with Mycorrhizal fungi increases productivity and confers a greater tolerance of drought. We report the benefit of inoculating strawberry, basil and coriander in substrates under different fertigation regimes. A consistent trend in the increasing fresh weight of strawberry plants and class I fruit yield was demonstrated as well as increased tolerance to drought. Both the number of fruit produced and the average weight of fruit increased with AMF inoculation. Basil and coriander plants showed increased speed of growth, canopy density and fresh weight when inoculated with AMF.

Keywords: horticulture, substrate, strawberry, herbs, water

P (ID 496)

Phosphorus acquisition efficiency by wheat colonized by AMF contrasting in Al tolerance growing in Andisol

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The mechanisms of Al tolerance and P efficiency may be closely related through strategies that decrease the toxicity of the Al3+ and increase P availability in soils. The effects of soils with high Al saturation on P acquisition by wheat have been little studied under field conditions. The aim was to study Al-P interactions on wheat genotypes of contrasting Al tolerance when grown under field conditions in a volcanic soil with high Al saturation (32%) and low pH (5.0). A field-plot experiment was performed with winter wheat genotypes, two Al-tolerant (TCRB14 and TINB14) and one Al-sensitive (STKI14), with application of 0, 44 and 88 kg P ha-1. At the end of tillering and after physiological maturity (90 and 210 days after sowing), plants were harvested and yield and P and Al concentrations in shoots and roots were measured. Soil acid phosphatase, root arbuscular mycorrhizal (AM) colonisation, AM spore number and soil glomalin were determined. Shoot and root production and P uptake were higher in Al-tolerant genotypes than the sensitive genotype. Root AM colonisation and soil acid phosphatase activity were also higher in tolerant genotypes. By contrast, Al concentration in shoots and roots was higher in the sensitive genotype with a concomitant decrease in P concentration. Grain yield of Al-tolerant genotypes was higher than of the Al-sensitive genotype with and without P fertiliser. Overall, the Al-tolerant genotypes were more effective at P acquisition from soil as well as from P fertiliser added, suggesting that plant traits such as Al tolerance, P efficiency, and AM colonisation potential co-operate in overcoming adverse acid soil conditions.

Acknowledgment: FONDECYT 11160385

Keywords: arbuscular mycorrhizal fungi, phosphorus efficiency, aluminium tolerance

P (ID 497)

Biocontrol potential of different Serendipita species against Fusarium wilt in tomato

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The soil-borne pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) causes *Fusarium* wilt in tomato plants (*Solanum lycopersicum* L.) and can lead to severe losses in tomato production worldwide. Endophytic fungi such as *Serendipita indica* can alleviate abiotic and biotic stresses and thus represent interesting candidates for biological control strategies against soil-borne diseases.

In this study, apart from *S. indica* and *S. vermifera*, *S. williamsii* and *S. herbamans* were studied for their potential to reduce *Fol* disease development in *Solanum lycopersicum* (L.) in greenhouse experiments. Furthermore, the selected fungal endophytes were screened for direct inhibitory activity against *Fol* and their impact on root morphological traits in *in-vitro* experiments.

The obtained results showed a reduction of *Fol* disease incidence by 62.78, 54.51, 44.03 and 35.76 % in tomato plants inoculated with *S. vermifera*, *S. indica*, *S. herbamans* and *S. williamsii*, respectively. However, direct antagonistic effects of the applied *Serendipita* spp. on *Fol* were not evident in *in-vitro* experiments. The analysis of the scanned root systems with the software WinRhizoTM revealed an increase in root length in the *S. indica*, *S. herbamans* and *S. vermifera* treatments compared to the control plants. Furthermore, root surface area was increased by all tested endophytic fungi.

This data set suggests great potential of other *Serendipita* species than *S. indica* in *Fol* disease control and tomato plant growth promotion and should be investigated in greater detail.

Keywords: Serendipita spp., biological control, Solanum lycopersicum (L.), fungal endophytes

P (ID 498)

Mapping the mycorrhiza community distribution along natural habitat patches in an agricultural landscape

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More investigations on a landscape scale are required to better understand the role of environmental and management factors shaping the mycorrhiza community and their role in ecosystem services in intensive used agricultural landscapes.

The aim of the European research project BASIL (Balancing Agroecosystem Services In Landscapes) is to balance agroecosystem services for maximal environmental and socioeconomic sustainability in agricultural landscapes. Intensive agricultural management decreases the arbuscular mycorrhizal diversity (AMF) and related services, e.g. regulation of water and nutrients. We tested the influence of natural elements such as in-field ponds and hedgerows within intensive agricultural used fields on the diversity of the AMF. Our hypothesis is that landscape heterogeneity increases AMF diversity and that AMF diversity and abundance decreases from natural elements into the agricultural fields

AMF in roots of winter wheat plants, AMF diversity in soil and spore abundance and diversity were investigated along transects from hedgerows and in-field ponds into agricultural fields.

First results show a significantly linear decrease in the percentage of roots colonized with AMF along transects from the edge of the fields bordering natural elements to 50 m into the wheat fields. The Spore abundance pattern along the transect were different dependent on the type of the natural element.

Further analyses include high-throughput sequencing, community coalescence and so-cio-economic aspects.

The deeper understanding of the relative influence of land use and habitat heterogeneity on mycorrhiza diversity at the Landscape, as the scale for human activities and decisions, will be helpful to formulate management strategies for a sustainable land use.

Keywords: AMF diversity, landscape scale, ecosystem service, in-field ponds, hedgerows

P (ID 501)

Arbuscular mycorrhizal fungi induced acquired systemic resistance against *Botrytis cinerea* in *Cucumis sativa* via modulation of reactive oxygen species

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Gray mold disease (GMD) caused by Botrytis cinerea, is one of the most destructive disease of cucumber (Cucumis sativa), worldwide. The GMD is controlled with the application of fungicides with deteriorating effects on health and environment. Arbuscular mycorrhizal fungi (AMF) are considered as an alternative biologically strategy for GMD suppression and plant growth promotion in cucumber. In this study, defense related mechanisms induced by AMF against GMD were studied. The gray mold caused destabilization of biomolecules via an increase of proline, glycine, betaine, total protein, total soluble sugar (TSS), hydrogen peroxide (H₂O₂), lipid peroxidation (MDA) and NADPH oxidase over the control. However, application of AMF was found to modulate such attributes in cucumber seedlings. The activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) was increased over the control in plants under disease stress. The antioxidant enzyme activity was further increased by the AMF inoculation. A significant negative correlation between AMF colonization and incidence of GMD was observed. On the other hand, a significantly positive correlation was documented between AMF and antioxidant enzymes activities. The synergistic interaction between biomolecules and antioxidant enzyme activities reported in this study, probably adds to the biocontrol potential of AMF via induction of acquired systemic resistance in cucumber against GMD.

Keywords: gray mold, plant disease, *Botrytis cinerea*, *Cucumis sativa*, arbuscular mycorrhizal

P (ID 502)

Combined application of biofertilizers and inorganic nutrients improves sweet potato yields

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Sweet potato (Ipomoea batatas (L) Lam) yields currently stand at 4.5 t ha⁻¹ on smallholder farms in Uganda, despite the attainable yield (45-48 t ha-1) of NASPOT 11 cultivar comparable to the potential yield (45 t ha⁻¹) in sub-Saharan Africa (SSA). On-farm field experiments were conducted for two seasons in the Mt Elgon High Farmlands and Lake Victoria Crescent agro-ecological zones in Uganda to determine the potential of biofertilizers, specifically arbuscular mycorrhizal fungi (AMF), to increase sweet potato yields (NASPOT 11 cultivar). Two kinds of biofertilizers were compared to different rates of phosphorus (P) fertilizer when applied with or without nitrogen (N) and potassium (K). The sweet potato response to treatments was variable across sites (soil types) and seasons, and significant tuber yield increase (p<0.05) was promoted by biofertilizer and NPK treatments during the short-rain season in the Ferralsol. Tuber yields ranged from 12.8 to 20.1 t ha⁻¹ in the Rhodic Nitisol (sandy-clay) compared to 7.6 to 14.9 t ha⁻¹ in the Ferralsol (sandy-loam) during the same season. Root colonization was greater in the short-rain season compared to the long-rain season. Biofertilizers combined with N and K realized higher biomass and tuber yield than biofertilizers alone during the short-rain season indicating the need for starter nutrients for hyphal growth and root colonization of AMF. In this study, N0.25PK (34.6 t ha⁻¹) and N0.5PK (32.9 t ha⁻¹) resulted in the highest yield during the long and the short-rain season, respectively, but there was still a yield gap of 11.9 and 13.6 t ha⁻¹ for the cultivar. Therefore, a combination of 90 kg N ha⁻¹ and 100 kg K ha⁻¹ with either 15 or 30 kg P ha⁻¹ can increase sweet potato yield from 4.5 t ha⁻¹ to >30 t ha⁻¹. The results also show that to realize significance of AMF in nutrient depleted soils, starter nutrients should be included.

Keywords: arbuscular mycorrhizal fungi, drought stress, root colonization, sweet potato, yield gap

P (ID 504)

Investigations of arbuscular mycorrhiza in context with apple replant disease

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The phenomenon called replant disease is typical for members of the family Rosaceae. It is defined that after a repeated replanting of a certain crop the soil loses the ability to serve as a growing medium for this species¹. A frequent replanting occurs in large scales in apple nurseries. Typical symptoms are a reduced vegetative growth, dark and decayed root systems and reduced yields which leads to economic losses². After soil disinfection the plant growth can be restored. But former used chemical soil disinfectants are now banned due to their environment toxicity. The development of alternative approaches is absolutely essential to maintain a sustainable soil productivity. Although apple replant disease is known for the centuries the actual cause is still unknown³. Therefor apple replant disease is studied in the BONARES joint Project ORDIAmur.

Arbuscular mycorrhiza have the potential to counteract apple replant disease by promoting the plant growth. Therefor the relationship between apple replant disease and mycorrhiza colonization is studied in this project.

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Keywords: arbuscular mycorrhiza, apple replant disease, plant health

P (ID 523)

Investigating the effect of Arbuscular Mycorrhizal Fungi (*Glomus etunicatum*) and air pollutants on growth parameters of Maize (*Zea mays* L.)

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In order to investigate the effect of *Glomus etunicatum* and air pollutants and acidic rain on growth parameters of maize plants (*Zea mays* L.), a completely randomized experiment with ten replications was conducted from February 2016 to April 2017. Experimental treatments included four categories containing the plants treated by Mycorrhiza Fungus irrigation by acidic rains, plants treated by Mycorrhiza Fungus irrigation by control water with (PH=7), witnessing plants irrigation by acidic rain and witnessing plants irrigation by control water.

Results from analysis of variance revealed that the effect of mycorrhizal inoculation on chlorophyll, protein and carotenoid content of leaves, plant height, leaf dry wt, leaf fresh wt, root fresh wt, fruit number and leaf number and surface, were significant (p<0.05). Mycorrhizal Inoculation enhanced all parameters significantly in comparison to the witnessing plants and the highest value for these traits obtained by the plants inoculated with Mycorrhiza irrigation by control water and the least obtained in witnessing plants irrigation by acidic rain water. Furthermore, the research revealed that the amount of these parameters in plants inoculated with Mycorrhiza irrigation by acidic rain, is significantly more than witnessing plants irrigation by control water. In general inoculation by Mycorrhizal Fungi in addition to enhancing growth parameters can enhance the photosynthesis and production of oxygen in maize even under acidic rain circumstances and air polluted environments, compared to non-mycorrhizal plants in regular circumstances.

Keywords: mycorrhiza, maize, air pollutants, acidic rain, chlorophyll, protein, carotenoid, plants growth

CONCURRENT SESSION: Soil and climate feedbacks in mycorrhizal biogeography and ecology

Mycorrhizal fungi (MF) are ubiquitous soil fungi that can form symbiotic associations with the majority of terrestrial plants. MF can facilitate plant uptake of mineral nutrients and water, organic matter mineralization and stabilization, buildup and stabilization of soil aggregates, and improve plant resistance to various environmental stresses such as drought, pathogens and soil contamination. As the physiological and ecological functions of MF could be predetermined by their ecological adaptability, it is important to reveal the MF biodiversity and their response to environmental changes and anthropogenic pressures. With the development and application of molecular-based methodologies, remarkable progress has been achieved in the fields of mycorrhizal ecology and biogeography in recent years. This session will thus focus on:

- 1. mycorrhizal biogeography at different spatial scales, and influences of biotic and abiotic factors on the distribution of MF
- 2. responses of MF community structure and function to climate change and anthropogenic activities
- 3. interplay between plant and fungal communities along environmental gradients (e.g., soil and climate)
- ecological significance of MF in mineral weathering, soil aggregation, biogeochemistry of carbon, nitrogen and phosphorus, and water conservation and utilization, in different environmental settings.

Chairs: Baodong Chen, José Ignacio Querejeta

CL (ID 201)

Harnessing the power of mycorrhiza-enhanced mineral weathering for mitigating soil degradation and climate change

<u>Jonathan R. Leake</u> (Department of Animal & Plant Sciences, The University of Sheffield, Sheffield, UK), **Joe Quirk** (The University of Sheffield, Sheffield, UK), **Rachel M. S. Thorley** (The University of Sheffield, Sheffield, UK), **Lyla L. Taylor** (The University of Sheffield, Sheffield, UK), **Dimitar Epihov** (The University of Sheffield, Sheffield, UK), **David J. Beerling** (The University of Sheffield, Sheffield, UK)

The evolutionary rise of land plants in symbiosis with mycorrhizal fungal partners, over 400 million years ago, transformed global biogeochemical cycles through increasing the weathering of silicate minerals. This released phosphorus, increasing plant productivity, and accelerated the export of Ca and Mg into the oceans, causing draw-down of atmospheric CO₂ into carbonate sediments and global cooling. It has recently been proposed that artificially enhancing mineral weathering through the applications of calcium-rich silicate rock grains such as basalt over large areas of agricultural land and forestry could be used as tool to remove CO₂ from the atmosphere and reduce ocean acidification^{1,2}. This could also address threats to agricultural sustainability arising from the nexus of soil erosion and degradation, nutrient depletion, rising fertilizer costs, increasing severity of pests and diseases, and climate change, while global food demand is predicted to double this century. In this presentation we address the critical importance of mycorrhizal fungi in partnership with plants in enhancing mineral weathering processes³, and consider their potential contribution to more sustainable agriculture and forestry through facilitating "remineralizing" soils with basaltic rock grains, to improve crop nutrition, increase uptake of silica to give resistance to pests and diseases, and provide more sustainable sources of phosphorus fertilizer. Increased understanding of the role of mycorrhizas in earth system science is providing vital insights into how we may be able to harness their power to transform minerals and facilitate biogeochemical processes that deliver benefits to people and the planet, restore degraded soils and sequester atmospheric CO2.

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¹Taylor LL *et al.*, (2015). Enhanced weathering strategies for stabilizing climate and averting ocean acidification. Nature Climate Change 6: 402-406, DOI:10.1038/NCLI-MATE2882

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http://dx.doi.org/10.1098/rsbl.2017.0149

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Keywords: global biogeochemical cycles, geochemical carbon cycle, pedogenesis, mycorrhiza-driven weathering, sustainable agroecosystems

CL (ID 303)

Seasonal dynamics of arbuscular mycorrhizal communities in tropical dry forests

Benjamin S. T. Morgan (Program in Plant Biology and Conservation, Northwestern University, Evanston, USA), <u>Louise M. Egerton-Warburton</u> (Plant Sciences, Chicago Botanic Garden, Glencoe, USA)

Introduction: Elucidating AMF responses to changing precipitation is critical to improving our understanding of ecosystems under future climate change scenarios. Climate models predict substantial changes in rainfall (decreased) and drought intensity (increased) in seasonally dry tropical forests (SDTF). SDTFs are vulnerable to drought but how AMF communities respond to these conditions is poorly understood. We asked: are AMF sensitive to changes in rainfall, or potentially resilient and adapted to soil moisture deficits?

Materials and Methods: We examined seasonal variations (wet versus dry) in AMF communities over two years using adapted AMF barcoding primers NS31/AML2 for custom dual-indexed Illumina sequencing. Reads were processed using a QIIME bioinformatics pipeline, and analyzed in R.

Results: We recovered >200 distinct AMF OTUs covering eight AMF families. *Glomus* species dominated AMF communities, and most taxa were detected in both wet and dry seasons. Apart from *Archaeospora*, AMF taxa were more abundant, and root and soil AMF communities more diverse in dry relative to wet seasons in both years. Shifts in community composition were correlated with soil moisture, NO₃ levels, and spatial position.

Discussion and Conclusions: Higher AMF abundance and diversity in the dry vs. wet season supports the possibility that AMF communities are adapted to soil moisture deficits, and comprise taxa with life history traits or physiological strategies to cope with drought. Nevertheless, increased tree mortality with extended drought or changes in timing or length of seasons could alter AMF resilience in ways that are distinct from AMF responses to predictable (seasonal) water deficits.

Keywords: next generation sequencing, diversity, precipitation

CL (ID 269)

Environmental drivers of ectomycorrhizas at large scales

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There is considerable uncertainty about what are the most significant drivers of mycorrhizas at the large scales where global changes are happening. Over the last decade, the ICP Forests intensive long-term monitoring network has provided us a unique opportunity to study ectomycorrhizal diversity and distribution at a continental scale and to determine the major drivers of ectomycorrhizal diversity, which dominant fungi respond to key environmental variables, what are the environmental thresholds for large-scale ectomycorrhizal changes, and whether ectomycorrhizal traits show plasticity in response to environmental changes.

From 137 plots dominated by Scots pine, Norway spruce, European beech or temperate oaks, and covering all European climatic regions, we individually examined and analyzed over 30,000 ectomycorrhizas. About half of these are formed by fungi that form no, inconspicuous, or belowground reproductive structures. From 170 foliar, soil, atmospheric, meteorological and spatial variables measured *in situ*, we tested the relative importance of key determinants of mycorrhizal diversity and identified their major indicator species.

Detailed ectomycorrhizal data and intensive forest monitoring data can be combined across large scales with different predictions of environmental change to understand the processes that control below-ground symbioses and their likely fate in our changing forests.

CL (ID 151)

Experimental drought and soil depth interactively influence fungal community composition in piñon-juniper woodland

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Changes in the structure of mycorrhizal communities due to disturbances such as fire or drought likely influence post-disturbance vegetation dynamics. However, such microbial legacies are understudied empirically and have yet to be incorporated into climate-vegetation models. Climate change models predict increasing intensity and duration of drought events in aridlands globally, which could enhance mycorrhizal legacy effects. At the same time, disturbance intensity is mediated by soil depth.

We address the potential for mycorrhizal legacies following drought by sampling soils from a long-term moisture manipulation experiment in piñon-juniper woodlands within the Sevilleta LTER site of central New Mexico. Fungal community composition at two soil depths was compared in irrigated, control and drought plots using Illumina ITS amplicon sequencing.

We found that surface soils had higher fungal diversity but lower relative abundances of ectomycorrhizal taxa than did deeper soils. The drought treatment caused declines in fungal species richness in both surface and deep soils, while the irrigation treatment had little impact. Moreover, the drought treatment caused a statistically significant shift in community composition and a decline in relative abundance of ectomycorrhizal taxa relative to paired control plots. SIMPER analyses revealed that the most responsive species were ectomycorrhizal taxa, with an array of basidiomycete taxa essentially disappearing under drought.

We speculate that death of host piñon pines had a larger impact on the fungal community than did soil moisture deficit. Our results suggest that piñon regeneration following drought may be delayed due to loss of ectomycorrhizal fungi in a depth and habitat-dependent fashion.

Keywords: drought, ectomycorrhiza, pine, juniper, experiment

CL (ID 109)

Geothermic soil warming has little effect on ectomycorrhizal growth and community composition in a Sitka spruce plantation in Iceland

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A planted *Picea sithchensis* forest in a geothermally warmed area in Iceland was used to study effects of elevated temperatures (0-6 °C above ambient) on ectomycorrhizal (EMF) growth and community composition, as well as EMF induced carbon sequestration using ingrowth mesh bags with ¹³C enriched organic material (maize compost).

In contrast to what we expected, warming up to 6°C above ambient temperature did not influence the production of EMF in the mesh bags. An average accumulation of 650±60 Kg C of EMF origin was estimated at the first harvest (5 months), and 1030±170 Kg C at the second harvest in the top 10 cm of the soil (12 months). The EMF community composition was not significantly influenced by soil warming in our study, though we did observe increased abundance of ascomycetous EMF and total Ascomycete abundance, as well as a shift in the saprotrophic fungal community composition in the mesh bags. The community was extremely species poor in comparison with studies done in the boreal region. The total number of OTUs was 20, dominated by *Amphinema byssoides* which on average contributed with 50% of the sequences in the mesh bags.

In conclusion, elevated temperature may influence growth and composition of the EMF community through indirect effects on soil moisture, nitrogen availability and plant growth. However, when these indirect effects are kept at a minimum, as in the present experiment, elevated temperatures up to 6°C above ambient do not seem to influence EMF growth or community composition.

Keywords: soil warming, ectomycorrhizal, *Amphinema*, Sitka spruce

CL (ID 97)

Ectomycorrhizal fungal community assembly along a latitude gradient in Chinese forest ecosystems

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Mycorrhizal fungi form symbiotic associations with most terrestrial plant species and play important role in species coexistence and community assembly. However, ectomycorrhizal (EM) fungal community assembly in different forest ecosystems has been less documented. In this study, EM fungal community was examined in 12 permanent plots along a latitude gradient in Chinese forest ecosystems using Illumina Miseq pyrosequencing of ITS1 sequences. A total of 2709 EM fungal operational taxonomic units (OTUs) were obtained at 97% sequence similarity, dominated by Russulales, Thelephorales, Agaricales and Sebacinales. With the increase of latitude, EM fungal OTU richness significantly increased and peaked at 40°N. The linear mixed effect model analysis showed that EM fungal OTU richness was significantly related to EM plant species richness in temperate and subtropical-tropical forests and to EM plant species richness and the interaction between EM plant species richness and mean annual temperature (MAT) in whole forest. PERMANOVA revealed significant difference in EM fungal composition between the temperate and tropic-subtropical forests. The final multiple regression of distance matrices models showed that EM fungal composition was explained by EM plant community (13.32%), MAT (7.21%) and altitude (3.76%) in the temperate forest; by soil (8.82%), MAT (4.52%), geographic distance (3.61%) and EM plant community (1.61%) in the tropic-subtropical forest; and by EM plant community (4.10%), geographic distance (4.65%), MAT (4.65%), mean annual precipitation (5.42%) and soil (1.20%) in the whole forest. This study suggests that the EM fungal community assembly varies in different forest ecosystems.

Keywords: ectomycorrhizal fungi, diversity, community, pyrosequencing of ITS1, forest ecosystem

CL (ID 99)

A trait-based understanding of mycorrhizal fungal dynamics in Australian environments

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Introduction: We lack an understanding of what drives the distributions of mycorrhizal fungi beyond broad generalisations regarding their roles in particular biomes. High-throughput sequencing has attempted to shed light on this, but in a way that is arguably independent of existing autecological understanding.

Methods: Using information obtained from the Biomes of Australian Soil Environments (BASE) database (https://downloads.bioplatforms.com/base/), we mapped the distributions of arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi in Australia. Then, to determine whether environmental filtering might be acting on specific traits associated with these fungi, we integrated information regarding spore traits (namely colour and size, from http://invam.wvu.edu/ and from species descriptions in the literature) with species of AM fungi identified and observed in BASE and calculated community weighted means for each sample.

Results: We found that the relative abundances of ITS sequence reads and the richness of operational taxonomic units (OTUs) belonging to AM and EM fungi were responsive to environmental conditions (e.g., vegetation type and soil fertility) in ways that were expected based on current knowledge. However, for both AM and EM fungi, turnover in OTU composition among samples was extremely high suggesting a largely stochastic community assembly process. Despite this, we found evidence for climate, land use, and edaphic properties influencing AM fungal community composition via selection on spore traits.

Discussion and conclusions: This study emphasises how integrating data generated from DNA-based environmental surveys with trait data obtained at the species level is a promising approach to generate mechanistic knowledge of fungal adaptations to different environments

CL (ID 59)

Multi-scale patterns and drivers of AM fungal communities

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Arbuscular mycorrhizal fungi (AMF) form diverse communities and are known to influence aboveground community dynamics and biodiversity. However, the multi-scale patterns of AMF composition and diversity, and the abiotic and biotic drivers, are still poorly understood. In this study we aimed to assess the spatial scales at which communities vary and how this is influenced by several abiotic and biotic factors.

Root and soil samples of *Plantago lanceolata* plants were sampled at several spatial scales in the naturally fragmented populations of *P. lanceolata* in the Åland Islands. We compared samples among neighbouring plants, within populations, among nearby populations and regions. Within each population we also measured soil nutrients, temperature and moisture and the neighbouring plant communities.

While the diversity and composition of AMF communities varied significantly at all spatial scales, most of the variation in AMF community composition was found among neighbouring plants, with decreasing variation explained with increasing spatial scale. We also found that abiotic drivers, such as nutrients and temperature influenced AMF community composition. Furthermore, we found a strong link between above plant and belowground AMF community composition.

We show that even neighbouring plants are exposed to a heterogeneous landscape of AMF, which indicates that at small spatial scales AMF could be dispersal limited. In addition we find that certain communities of AMF correlate with certain communities of plants, showing ecological specificity between these two organism groups.

We show that both the spatial scale, the environment and aboveground plant communities are driving AMF community composition.

Keywords: aboveground-belowground, arbuscular mycorrhizal fungi, community composition, distribution, spatial structure

CL (ID 191)

Nitrogen fertilization decouples roots and rhizosphere microbes more in ectomycorrhizal than arbuscular mycorrhizal forests

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Introduction/Aim: While nitrogen (N) deposition has enhanced the growth of most temperate forest trees, its impacts on belowground C cycling vary considerably across forests. Differences between ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) trees in the strength of root-microbial interactions may explain this variation. Given that ECM trees send more C to rhizosphere microbes to stimulate N mobilization than AM trees, we hypothesized that N fertilization would weaken root-microbial interactions and decomposition more in ECM than AM stands.

Materials and Methods: We measured root-microbial interactions in ECM and AM dominated plots across two long-term N fertilization studies, the Fernow Experimental Forest, WV and Bear Brook Watershed, ME. We measured the activity of hydrolytic and oxidative enzymes, and examined the bacterial and fungal metagenome and metatranscriptome in rhizosphere, bulk, and organic horizons. We then linked these to assays of plant-C investment belowground including fine roots, mycorrhizal colonization, and root exudation.

Results/Conclusions: N fertilization decreased fine root biomass, root exudation, and mycorrhizal colonization to a greater degree in ECM than AM stands. In ECM stands, these declines in belowground C allocation were mirrored by shifts in fungal community composition and gene expression that were tightly coupled to reductions in lignolytic and hydrolytic enzyme activity. By contrast, the soil meta-genome and -transcriptome were more resilient to decreases in belowground C allocation in AM stands. Collectively, our results indicate that N fertilization may reduce soil decomposition and enhance soil C storage to a greater extent in forests dominated by ECM than AM trees.

Keywords: temperate forests, rhizosphere, ectomycorrhizae, arbuscular mycorrhizae, metagenomics

CL (ID 153)

Root traits and altered precipitation regimes effects on grassland arbuscular mycorrhizal fungal communities

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Introduction: Future climatic scenarios predict changes in rainfall variability and amount. These changes are expected to affect plants via effects on the expression of root traits associated with water and nutrient uptake. Associated mycorrhizal fungi may also respond to these new precipitation regimes, either directly in response to changes in the soil environment or indirectly in response to altered root traits.

Methods: We characterised arbuscular mycorrhizal (AM) fungal communities in an Australian grassland exposed to experimentally altered rainfall regimes predicted for the region (increased dry periods and decreased rainfall). We used DNA-based tools to assess the responses of AM fungal communities associated with four plant species both within individual root systems (using Terminal Restriction Fragment Length Polymorphism) and among plants (using Illumina sequencing) sampled from the different treatments.

Results: AM fungal communities were highly variable even within individual root systems. The extent of this variation depended on the host plant species, but not the rainfall manipulation. Community variation was greatest for *Senecio madagascariensis* and least for *Paspalum dilatatum*. Specific root length (SRL) was observed to influence AM fungal richness, while the root chemistry was observed to influence AM fungal composition. The rainfall manipulation had significant but small effects on AM fungal communities.

Discussion and conclusions: These results suggest that climate change and its effects on rainfall may influence the structure of AM fungal communities, with potential functional consequences, but that inherent spatial variation in AM fungal communities may limit the magnitude of these changes.

CL (ID 92)

Three years of experimental summer drought: quantitative losses vs. qualitative stability of ectomycorrhizal community responses

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Introduction/Aim: A rainfall exclusion experiment was established in a mature mixed stand of *Fagus sylvatica* and *Picea abies* to study effects of repeated severe summer drought¹ on ectomycorrhizal (ECM) community composition and functions of these two physiologically contrasting tree species and if growing in mixture influences drought effects².

Materials and Methods: Six out of twelve trenched plots were equipped with roofs closing automatically upon rainfall. ECM samples were collected at the end of the vegetation periods 2013-2016 from pure beech, pure spruce, and the mixture zone between both in each plot. We determined community diversity, abundance (morphotyping and illumina sequencing) and as a functional trait extracellular enzyme activities (EA) involved in the release of nutrients through breakdown of organic macromolecules ³.

Results: Drought reduced diversity and altered ECM community composition. Diversity changed earlier in beech but then declined more strongly in spruce. The decline was slightly attenuated in mixture. We observed a strong negative effect of drought on laccase activity due to a loss of ECM taxa with this activity. The observed severe and progressing decline of all enzyme activities per soil volume over the three drought years was caused by a sharp decrease of vital fine root tips.

Discussion: Most effects became only significant after the second or third consecutive drought period demonstrating the need for long-term measurements. EA results confirmed high functional redundancy in ECM communities (except laccase activity) but also limited plasticity for compensation of quantitative losses.

Conclusion: Positive mixture effects need further mechanistic explanations.

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Keywords: repeated drought, tree mixture, ECM community composition, ECM extracellular enzyme activities

CL (ID 112)

Functional and phylogenetic structure responses of fungal communities to the fire regime in Mediterranean forests

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The evolutionary history of Mediterranean ecosystems is tightly linked to fire. Recurrent fire affects above and belowground diversity, as well as mechanisms crucial for the fitness and survival of trees (e.g. mutualistic interactions) with costs that remain largely unknown. Wildfire frequency increase predicted with global change may alter soil fungal communities and trees with important consequences at the ecosystem level.

We evaluated whether the fire regime (low/high fire recurrence) affected the structure and functioning of soil fungal communities in forests of a representative Mediterranean pine species, *Pinus halepensis* Mill., and if tree population genetics influenced the assemblage of fungi. High-throughput sequencing, phylogenetic methods and soil enzymatic tests were used.

Recurrent fire induced a clustering of the soil fungal community (i.e. significantly less phylodiverse), and in parallel, soil activities related with carbon, and nutrient cycling increased. In fact, the phylogenetic structure of the soil fungal community explained activities implicated in the mobilization of nitrogen and phosphorous. Besides, the genetic structure of trees correlated with the assemblage of specific fungal guilds (i.e. Basidiomycetes and ectomycorrhizal).

Our results indicate that fire can filter certain fungal groups and affect relevant ecosystem functions, pointing out to fire-induced changes in tree population genetics as a major driver structuring fungal communities in Mediterranean pine forests, which can be of main importance for the ecology and resilience of these vulnerable ecosystems.

Keywords: fire regime, fungal phylodiversity, soil enzymes, *Pinus halepensis*

CL (ID 130)

Biogeography of plant root associated fungal communities in the North-Atlantic region mirrors climatic variability

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Polar and alpine areas are particularly subject to increasing temperatures and altered precipitation patterns linked to climate change. Current distributions of fungal communities over large biogeographic scales along climatic gradients can provide valuable insight into the potential effects of climate change on these communities. In this study, we investigate root associated fungi (RAF) in the North Atlantic region and focus on how climatic variation influences composition and how species-environment relationships correlate with selected fungal traits. Amplicon pyrosequencing of the ITS1 region was used to characterize the RAF of 297 Bistora vivipara individuals from Iceland, Jan-Mayen, Scotland, Svalbard, and high alpine localities in Norway and Austria. The RAF communities exhibited strong biogeographic structuring, and both compositional variation and fungal species richness correlated with annual temperature and precipitation, as well as temperature during the wettest quarter of the year. In accordance with general island biogeography theory, the least speciose RAF communities were found on Jan Mayen, a remote and small island. Spore length, width, volume and shape, as well as mycelial exploration type were summarized at a community level across the region. However, only average spore shape and mycelial exploration type of those ectomycorrhizal taxa present correlated with climatic variability. The average spore shape (length:breadth ratio) became more elongated with increasing latitude. Hydrophilic exploration types were associated with warmer temperature during the wettest quarter of the year.

CL (ID 222)

Impacts of Long-Term Elevated Atmospheric CO₂ on AM Fungal Communities in a Temperate Grassland

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The ecological implications of long-term elevated atmospheric CO₂ (eCO₂) levels on communities of arbuscular mycorrhizal fungi (AMF) remain poorly understood. Obligatory biotrophic AMF form symbiosis with the majority of terrestrial plants. Thus any changes in photosynthate availability due to eCO2 could alter both AMF communities and their influence on plant community dynamics. We used high-resolution amplicon sequencing (Illumina, HiSeq) to quantify the AMF communities from plant roots from the longest running Free Air Carbon dioxide Enrichment (FACE) experiment in the northern hemisphere in Giessen (GiFACE), providing samples from an old-growth (>100 years) grassland subjected to >15 years of eCO₂. eCO₂ significantly increased AMF diversity, yet had a minimal impact on overall AMF community composition. However, while broad changes in the composition of AMF communities were not observed, subtle responses of specific AMF taxa, with their populations both increasing and decreasing in abundance in response to eCO₂ were found. Furthermore, taxa from three families (Archaeosporaceae, Claroideoglomeraceae, and Glomeraceae) were significantly more abundant under eCO₂. This demonstrates the need to consider individual populations, alongside broad shifts in community composition when assessing how soil microbes respond to environmental change. AMF communities are likely to increase in diversity in the eCO2 world, but other factors involved in global change (e.g. water availability and changes in plant host fitness) may impact their communities significantly more than eCO₂ per se.

Keywords: arbuscular mycorrhizal fungi, community composition, elevated CO₂, diversity, next generation sequencin (NGS)

CL (ID 218)

Root vs. mycorrhizal allocation relates with nitrogen and phosphorus nutrition in grassland species

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In oligotroph ecosystems, such as dry sandy grasslands, selective pressure for life under nutrient deficiency has promoted the dominance of plant species with efficient nutrient uptake mechanisms, like extensive and widely branched root systems. However, some prominent species only develop coarse and narrow root systems, with their successful establishment likely being related to resource allocation into arbuscular mycorrhizal fungi (AMF). A trade-off between belowground investment into either roots or AMF is likely in oligotroph habitats and perhaps a driver of species-specific responsiveness to AMF. Investments into AMF do not only comprise carbon allocation to the fungus, but also to root-structures, such as an extensive cortex, to allow for residence of fungal structures. Further, the allocation trade-off between absorptive root-surface vs. AMF-surface might bring about differential advantages related to efficient nitrogen (N) vs. phosphorus (P) nutrition. We investigated mycorrhizal responsiveness together with root morphological characteristics and plant P- and N-relations in 13 grassland species. We found significant relationships of fine-root proportions and root cortex thickness with mycorrhizal responsiveness. Additionally, we observed a negative correlation of plant N content and a positive correlation of plant P content with mycorrhizal responsiveness over the 13 species. The results confirmed our hypotheses on the relevance of the allocation tradeoff between root and mycorrhizal surface for efficient N- and P-nutrition in grassland species, with this trade-off potentially having direct consequences for competitive interactions and the occurrence of root vs. mycorrhizal strategists over the course of succession in grassland habitats.

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Keywords: arbuscular mycorrhizal fungi, root morphology, mycorrhizal responsiveness, nitrogen, phosphorus

ST+P (ID 71)

Plant identity exerts stronger effect than fertilization on soil-dwelling arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi form symbiotic associations with the majority of land plants and play crucial roles in grassland ecosystem. Understanding the responses of belowground AM fungi in alpine meadow facing anthropogenic manipulation may aid sustainable grassland management. Here we studied AM fungal responses to fertilization (urea and manure) and plant identity (grasses Avena sativa and Elymus nutans and legume Vicia sativa) in a sown pasture on the Qinghai-Tibet Plateau. AM fungal extraradical hyphal density, compared with unfertilized control, was significantly increased by urea and manure under A. sativa and by manure only under E. nutans plantation. AM fungal spore density was not significantly affected by plant identity or fertilization. Forty-eight AM fungal operational taxonomic units (OTUs) were obtained through high-throughput sequencing of 18S rRNA gene. The AM fungal diversity including OTU richness and Shannon index were significantly higher under E. nutans than V. sativa cultivation condition, but not significantly affected by fertilizer urea or manure regardless of plant species. Non-metric multidimensional scaling along with structural equation model results revealed that AM fungal community composition was significantly separated by three plant species and structured directly by plant identity only and indirectly by both urea addition and plant identity through soil total nitrogen content. Taken together, our findings highlight that plant identity exerts stronger effect than fertilization on soil-dwelling AM fungi in the converted pastureland from the similar alpine meadow.

Keywords: AM fungi, community, fertilization, plant identity, sown pasture

ST+P (ID 181)

Arbuscular mycorrhizal fungal diversity along a precipitation gradient in coast redwood forests using next-generation sequencing

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Introduction: Early work showed that coast redwood (*Sequoia sempervirens*) associate with AMF¹, yet we know little about the identity of these symbionts. It is clear, however, that this iconic keystone tree species largely determines a unique understory habitat, home to a number of threatened and endemic fungal species^{2,3}. Interestingly, it has been asserted that grasslands harbor greater AMF diversity than forests⁴; however, only one study has investigated AMF diversity in forests dominated by an AMF-associated gymnosperm⁵. Our study sought to understand 1) patterns of diversity in such forests and 2) how these patterns might be influenced by environmental drivers.

Methods: Utilizing a strong precipitation gradient, we investigated the role of environmental factors and host identity on AMF community composition. Eight sites were sampled across the gradient and the ITS-2 region⁶ was amplified with Illumina-adapted primers. Select samples were also sequenced with SSU for comparison.

Results: Global models of AMF diversity would predict a sample richness of 12–14 for an entire plot (based on SSU)⁴. However, even with the more conservative SSU as a marker, we find 14 OTUs associated with a single root and 18 OTUs in the corresponding soil sample. Consistent with other findings, preliminary data show precipitation an important factor in community composition of soils⁷. Interestingly, root samples between sites display a high degree of convergence and markedly less richness than soils, highlighting for potential selection of AMF by roots in this ancient system. These data highlight the need for further exploration of AMF in forest systems.

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Keywords: arbuscular-mycorrhizal fungi (AMF), coast redwood, Illumina next-generation sequencing, diversity

ST (ID 80)

AMF biogeography at regional scale in northern China

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts of terrestrial plants and play key roles in regulating various ecosystem processes, but limited information is available as for AMF biogeography at regional scale. This study aims at exploring the key predictors of AMF communities across a 5000-km transect in northern China. We determined the soil AMF species richness and community composition at 47 sites representative of four vegetation types (meadow steppe, typical steppe, desert steppe and desert) and related them to plant community characteristics, abiotic factors and geographic distance. The results showed that soil pH was the strongest predictor of AMF richness and phylogenetic diversity. However, abiotic factors only have a low predictive effect on AMF community composition or phylogenetic patterns. By contrast, we found a significant relationship between community composition of AMF and plants, which was a surprising result given the extent of heterogeneity in the plant community across this transect. Moreover, the geographic distance predominantly explained the AMF phylogenetic structure, implying that history evolutionary may play a role in shaping AMF biogeographic patterns. This study highlighted the different roles of main factors in predicting AMF biogeography, and bridged landscape-scale studies to more recent global-scale efforts. Compared to AMF distribution patterns, much less is known about the functionality of AMF communities. Further studies are now needed to elucidate linkages between AMF function and biogeography.

Keywords: arbuscular mycorrhizal fungi, biogeography, Glomeromycota, community ecology

ST+P (ID 90)

Vertical and seasonal patterns of AM fungal wind dispersal

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Introduction/Aim. Biogeographical patterns of arbuscular mycorrhizal fungal (AMF) species are shaped by biotic and abiotic environmental conditions, as well as the intrinsic capability of species to disperse. Recent work documents large-scale spatial distributions of AMF taxa, yet dispersal mechanisms remain poorly studied. As a result, inferences regarding endemism, invasions, or the need for conservation and restoration efforts remain speculative. This study investigates wind dispersal of AMF taxa in varied locations, seasons, and vertical heights.

Materials and Methods. We assessed air-borne AMF spores in urban and wildland settings using BSNE passive dust samplers. In the urban experiment, we sampled air monthly from rooftops in Chicago, USA, 20m above ground level. In the wildland experiment, we sampled air seasonally from semi-arid shrublands in Utah, USA, at ground level. Spores were measured, enumerated, and identified according to morphological traits. The viability of wind-borne urban spores was examined using in vitro germination trials.

Results. Diverse communities of AMF spore morphospecies were observed in air samples regardless of height, season, or measurement duration. Patterns in spore abundance, size, diversity, and seasonality, as well as spore viability, are explored.

Discussion. The presence of AMF spores at greater heights suggests potential long-range wind dispersal and a lack of endemism for certain AMF taxa. Seasonal patterns and spore traits may elucidate climate or taxon-specific wind dispersal.

Conclusion. Wind may be an important dispersal mechanism for certain AMF taxa, contributing to their large-scale biogeographical patterns. Future research will integrate traits, viability, and DNA-based identification to explore AMF wind dispersal.

Keywords: biogeography, dispersal, endemism, diversity, scale

ST+P (ID 132)

Mycorrhizal diversity in the tropical rainforests: feedbacks between root-fungal symbioses and soil phosphorus partitioning

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High diversity is a striking feature of South-East Asian Tropical Rainforests. A wide variety of mechanisms have been proposed to explain the origin and maintenance of tropical forest diversity. One hypothesis is that resource partitioning for soil phosphorus (P) contributes to tropical plant diversity and coexistence. Southeast Asian tropical forests are distinct as their biomass is dominated by a single family of ectomycorrhizal trees (Dipterocarpaceae), while most of the diversity of the tree community is represented by species of arbuscular mycorrhizal trees in >100 other families. There is potential for specialization between and within mycorrhizal associations, this may be one mechanism that facilitates partitioning for soil P. Our work has linked soil edaphic factors to environmental sampling of AM and ECM communities across multiple scales from the cm to the km within tropical forests using high-throughput molecular techniques, linking fungal taxa to plant taxa using barcoding of the roots and utilising well characterised data on above ground tree diversity. This work combined with nursery microcosms has increased our understanding of rainforest mycorrhizal diversity and function within rainforest ecosystems. Showing that soil type and chemistry drives both tree and mycorrhizal diversity in the rainforest and that AM and ECM trees respond in significantly different ways to different phosphorus forms. Despite the dipterocarps dominating we see a high diversity of ecotomycorrhizal taxa but a comparatively low diversity of AM fungal taxa when compared to the sheer diversity of AM fungal trees, this work will have important implications for forest restoration and conservation.

Keywords: rainforest, arbuscular mycorrhiza, ectomycorrhiza, phosphorus, nutrient partitioning

ST+P (ID 499)

The structure of arbuscular mycorrhizal fungal community in the environment contaminated with toxic organic pollutants

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Aim: The aim of research was the analysis of structure and biodiversity of AMF community in the environment contaminated with phenol and PAHs, in comparison to the non-polluted site.

Materials and Methods: Root and rhizosphere soil samples were obtained from two plant species: *Poa trivilais* and *Phragmites austalis*, collected from the contaminated area around Kalina Pond (Świętochłowice) and from the control site (Kokotek Lake, Lubliniec). Samples where subjected to the assessment of root mycorrhizal colonization, extrametrical hyphal length, number of spores, $16:1\omega$ 5c PLFAs/NLFAs and glomalin concentration. AMF biodiversity was analyzed with nested-PCR-DGGE method, using a fragment of 18S rDNA gene and by sequencing of the 28S rDNA fragment on MiSeq platform (Illumina).

Results: Mycorrhizal colonization and the quantity of AMF in soil was much lower in the contaminated site, when compared to non-polluted one. The number of AMF sequences obtained from sequencing of the contaminated soil was 67498, whereas considering uncontaminated soil, 547966. Totally, 241 OTUs where obtained, with 141 OTUs in the contaminated soil and 262 in the non-polluted on. The rhizosphere of plants grown around Kalina Pound was highly dominated by: *Paraglomus sp., Funneliformis sp., Claroideoglomus sp.* and *Rhizophagus sp.* In the control soil, the most abundant species where: *Archaeospora sp., Glomus sp. and Paraglomus sp.*

Conclusions: The presence of PAHs and phenol had undoubtedly negative influence on biodiversity and symbiotic activity of AMF. It also favored a selection of some PAH/phenol-tolerant strains which will be further isolated and tested in an experiment on rhizoremediation.

Keywords: arbuscular mycorrhizal fungi, PAHs, phenol, next generation sequencing

ST+P (ID 53)

Anthropogenic disturbance leads to the homogenization of arbuscular mycorrhizal fungal communities

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Arbuscular mycorrhizal (AM) symbiosis is among key plant-soil microbe interactions, and should, therefore, be a target of soil biota conservation strategies. AM fungal richness is highly influenced by disturbance, but the response patterns have shown to be contrasting, depending upon the character of the particular taxa, disturbance, and ecosystem under investigation.

In this global study, we aimed to (1) compare AM fungal richness in unwooded and wooded ecosystems, (2) assess the effect of disturbance on AM fungal richness and composition, and (3) determine whether disturbance enhances ruderal AM fungi.

We addressed AM fungal communities on paired intact and disturbed ecosystems on six unwooded and ten wooded sites distributed across five continents. AM fungi were identified in soil samples by 454-sequencing the NS31-AML2 amplicon of the SSU rRNA gene.

AM fungal richness was higher in unwooded than wooded sites. In the presence of disturbance, AM fungal richness reduced in naturally rich sites and increased in naturally poor sites. In addition, disturbance changed AM fungal community composition, increasing the share of ruderal fungi.

We confirmed the previous finding of high AM fungal richness in unwooded sites. Disturbance excluded some taxa from naturally rich AM fungal community and created new niches for pioneer taxa in naturally poor habitats. In addition, mechanical disturbance may have broken AM hyphal networks, which fostered ruderals.

We conclude that the protection of AM fungal richness is not enough for their conservation. Instead, the focus should be put on preserving the functional structure of intact AM fungal communities.

Keywords: mycorrhizal biogeography, functional traits, unwooded and wooded ecosystems

ST+P (ID 250)

Influence of soil type on oak mycorrhiza in a temperate forest: a pot experiment

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Ectomycorrhizal fungi are major ecological players in European temperate forests. It is known that more than 80% of terrestrial plant species interact with mycorrhizal symbionts that are crucial for the survival of the plant and oaks are no exception. The main objective of this study was to compare mycorrhizal fungal communities in oak roots grown in different soils and the effect of oaks on fungi in the rhizosphere and bulk soil.

Soil was collected from stands of oak, beech and spruce in a temperate forest in the Czech Republic. Oak seeds were introduced to each soil-type in a pot experiment and oaks were allowed to grow for 4 and 16 months. Roots, rhizosphere soil and bulk soil were collected, DNA was extracted and the ITS2 region of fungal rRNA gene was amplified and sequenced on Illumina MiSeq platform. In addition to pots with oaks, pots without trees were used as controls.

Results indicate that the presence of young oak trees affects significantly the composition of fungal community in soil, the effect being considerably stronger in the second year of sampling. The differences between soil treatments also increased with time and the effects of trees were most pronounced in roots, followed by the rhizosphere and bulk soil. Our work shows a complex feedback between soil and plant where the soil represents a latent pool of microorganisms and the plant contributes to the selection of those that establish as dominants. Symbiotic ectomycorrhizal fungi are apparently an important component of this feedback.

References:

Suiz L. et al., Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests., Mol Ecol. 2014 Nov;23(22):5628-44. doi: 10.1111/mec.12947. Epub 2014 Nov 7.

Keywords: oak, mycorrhiza, temperate forest, rhizosphere

ST+P (ID 316)

A comparative analysis of ectomycorrhizal fungal communities in Scotland and Scandinavia

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Most forest trees in northern Europe form essential symbiotic associations with ectomy-corrhizal (ECM) fungi. A number of factors are known to influence the local or regional distribution of these ECM fungi but broad scale distribution patterns across Europe are still poorly understood. The main objective of this study was a comparison between ECM fungal communities in Scotland with those in Scandinavian countries. The comparison utilised data from 1123 species from 31 genera. Scotland had the lowest number of species (614) compared to Denmark (682), Finland (741), Norway (866) and Sweden (981). The Scottish community was most similar to that found in Norway, sharing 61 % of species. Host diversity did not appear to be a major factor in determining ECM species richness. However, a combination of host and soil conditions was a significant factor: in particular conifers growing on calcareous soils in Scandinavia support 131 species that were missing from the Scottish community. Other influencing factors include post-glacial history, forest area and climate.

Keywords: ectomycorrhizal fungi, fungal community composition, Scotland, oak, birch

POSTERS

P (ID 16)

Why are mycorrhizal communities of wide floodplain forest ecosystems in middle Balkans (Serbia) so specific?

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The specific climate, geographical position and geological origin of the flood plains in middle Balkan Peninsula have created peculiar ecosystems with very specific soil processes. The evolutionary phases of soils formed by the river deposits and succession of ground water seasonal variations caused the formation of forests that, on the first glance, look very similar to other gallery forests in the volleys of European rivers. Beside the highest plant diversity rates among the forests in the region and high biomass production, they are characterized by the presence of some plant species that are indicators of Mediterranean climate, which implies specific soil processes and unusual mycorrhizal communities.

In this contribution, we present investigation held in the selected forests aiming to explain vegetation and soil parameters that have influenced appearance of specific mycorrhizal fungi. We have followed seasonal changes of soil microenvironments by measuring soil water content and temperature, as well as concentrations of some macronutrients assumed to be important factors for formation of mycorrhiza (available nitrogen forms, phosphorus, and potassium). In addition, we have done very detailed analyses of forms of phosphorous and some biologically important ions in the soil complexes, as well as soil structure and some enzymes that indicate soil processes. We will discuss the factors that may have caused the differences between sampling forest locations, as well as between forest and adjoined agricultural soils in order to detect the changes that have happened during the exploitation. The detailed analyses of mycorrhizal communities in investigated forests are ongoing.

Keywords: Floodplain ecosystems, soil seasonality, soil complexes, soil microclimate, mycorrhizal diversity

P (ID 32)

Diversity and global biogeography of mycorrhizal fungi in Mexico revealed by Next-Generation Sequencing

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Mexico is a ~2 million Km² megadiversity country and a large repository of mycorrhizal fungal diversity with potential genetics resources on Earth. Mexican Neotropical forests contain important "hotspots" and unique unexplored habitats for fungi and plants, establishing complex networks with over and understory plants. Because the scant reliable information available on Mexican mycorrhizal fungal communities, we present for the first time a comprehensive survey based on a consistent global sampling protocol¹ by using Next-Generation Sequencing [NGS].

We collected 160 soil samples from 4 Neotropical forest soils: 2 tropical coniferous montane forests/East-Central and /Southwestern; 1 dry tropical forest/Southeastern; 1 chaparral/North-Central Mexico. We randomly select 20 trees located 8m apart in each 2,500 m² circular sampling plots and 40 soil cores were collected. Soil samples were processed and analysed with 454 Life Sciences pyrosequencing, bioinformatics, quantitative and biogeographic methods.

Metabarcoding analysis of Mexican Neotropical forest soils revealed more than 3,000 species-level MOTUs mycorrhizal fungi of which the predominant were ectomycorrhizal. Changes in the relative proportion among mycorrhizal communities was observed in the forest sites. The richest forests were *Quercus potosina – Arctostaphylos pungens –* chaparral/North-Central followed by the dry tropical forest, and the poorest the tropical coniferous (*Pseudotzuga-Abies-Pinus*) montane forests in the Southwestern and the East-Central region.

Mexican Neotropical mycorrhizal biota was related with North America, Europe and East Asia biogeographic regions but idiosyncratic towards the tropics.

This is the first report of native mycorrhizal fungal communities in contrasting Neotropical Mexican forest soils using NGS and revealing that the chaparral was the richest studied biome.

References:

¹Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Quang Thu P, Suija A, *et al.* (2014). Global diversity and geography of soil fungi. *Science*, 346: 1256688.

Keywords: Mexican neotropical forests, Mexican mycorrhizal diversity, next-generation sequencing, mycorrhizal ecology, mycorrhizal biogeography

P (ID 125)

Different responses of arbuscluar mycorrhizal fungi to short-term and long-term soil warming in Iceland grassland

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Aim: Plants allocate plenty of photosynthate to arbuscular mycorrhizal fungi (AMF) in exchange for nutrients, and this is expected to increase under warming. Thus, the aim of this study was to evaluate the effect of short-term (7 years) and long-term (>50 years) geothermal warming on growth and C sequestration by arbuscular mycorrhizal fungi in grasslands in Iceland.

Materials and Methods: Mesh bags (mesh size: $50\mu m$, only the hyphae enter) which were filled with sand and 13 C enriched maize compost (C4-C, 1%) and incubated in the soil for 2 years. The biomarker $16:1\omega 5$ of phospholipid fatty acids and neutral lipid fatty acid was used to measure AMF biomass. The C isotopic composition of the mesh bag content (C4-C from maize and C3-C from AMF) was used to estimate C import from AMF to the mesh bags.

Results: The results showed that the mean concentration of AMF was 0.6 ± 0.3 nmol g⁻¹ (PLFA) and 3.9 ± 1.4 nmol g⁻¹ (NLFA), however, there was no significant difference of warming (P>0.05) in neither the short-term nor the long-term warming sites. The C imported from AMF was 0.80 ± 0.33 mg g⁻¹ (equal to 1280 ± 94 kg ha⁻¹), which accounted for ~1.1% of the total C in soil.

Conclusion: Our results illustrated that AMF appears to be more tolerant to elevated temperatures than roots since root biomass declined significantly in the experiment. (Leblans 2016, PhD thesis, University of Antwerp). The amount of extraradical mycelium produced per root biomass has thus increased with elevated temperatures.

Keywords: arbuscular mycorrhizal fungi, warming, soil carbon sequestration

P (ID 139)

Nitrogen deposition changes ectomycorrhizal communities in Swiss beech forests

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Atmospheric pollution has implications for the health and diversity of temperate forests that cover large parts of central Europe. Long-term elevated anthropogenic deposition of nitrogen (N) is driving forest ecosystems from N limitation to phosphorus (P) limitation and is found to affect tree health and ectomycorrhizal fungi (EMF), which most trees depend on for nutrient uptake. However, the consequence of changes in EMF communities for tree growth and nutrient uptake remains unclear. Therefore, we investigated changes in EMF communities on root tips and in soil of beech forests along a N deposition gradient ranging between 16 and 33 kg N ha⁻¹ a⁻¹, where high levels of N deposition were found to negatively affect tree growth and nutrient levels. The most important factors significantly explaining variation in EMF community composition were increased N deposition and base saturation. With increasing N deposition, root colonization, EMF diversity, and production of extramatrical mycelium in soil decreased significantly. Meanwhile, foliar K and P were positively associated with increasing EMF diversity and we found EMF community composition to be associated with foliar N and P and other nutrients. Moreover, the abundance of the important species Cenococcum geophilum and of species with high biomass decreased significantly with increasing N availability, indicating repercussions for carbon sequestration. Our study supports the hypothesis that the decrease in nutrient uptake in beech forests across Europe is related to changes in EMF communities and suggests that continued high N deposition may change soil carbon and nutrient cycles, thereby affecting forest ecosystem health.

Keywords: Fagus sylvatica, soil acidification, carbon sequestration, phosphorus, mesh bags

P (ID 198)

Nutrients affect fungal growth and specialization in orchid mycorrhizal associations

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One of the questions in the study of mycorrhizas is about the evolution of specialization. It has been observed that environmental conditions can affect the diversity and composition of mycorrhizal fungi associated with plants. One of these conditions are soil nutrients, especially nitrogen and phosphorus. In a past study we observed that soil N and P were negatively correlated with diversity of mycorrhizal fungi associated with the orchid Bipinnula fimbriata, and also that soil P affected fungal composition. Nevertheless, it was not clear if this was due to different fungal choices from plants, or different fungal performances under different soil nutrients content. To evaluate this, we studied the effect of nutrients on fungal growth and on the establishment of the mycorrhizal association. We selected four OTUs of Ceratobasidiaceae and four OTUs of Tulasnellaceae isolated from B. fimbriata, and measured their growth under four nutrient treatments. Then we placed B. fimbriata seeds with the eight fungal OTUs under the same treatments and recorded the seed development. We observed that nutrients affected growth of most fungal OTUs, independently of the family, and that this effect was related with seed germination. In higher nutrients media only two OTUs could germinate seeds, the other 6 promoted seed germination only under low nutrient media. Our results suggest that nutrients affect fungi which in turn affect the establishment of the mycorrhizal association; a less number of the fungal OTUs germinate seeds under high nutrients, which could explain the mycorrhizal specialization under high nutrient availability.

Keywords: mycorrhizal specialization, soil nutrients, orchid mycorrhiza, symbiotic germination, Chilean orchid

P (ID 202)

Spatial patterns of ectomycorrhizal fungal diversity in a Mexican subtropical pine-oak forest

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Introduction. This study aimed to describe local-scale spatial diversity patterns and controlling factors of ectomycorrhizal fungal (ECMF) community composition in a subtropical pine-oak forest in Mexico. We hypothesized that, due to the high plant-fungus specificity, host is more important than edaphic factors in shaping ECM fungal communities.

Methods. We established a nested square sampling design with three plots of 8x8 subplots (10x10 m), and we sampled 32 subplots in each. This allowed us to scale up with the same sampling representativeness. Vegetation, fungi and soil parameters were measured in the 96 squares. Fungi were identified in soil samples by Illumina MiSeq sequencing of ITS2 region. ECM fungal OTUs were determined using FUNGuild software.

Results and discussion. We found 674 ECMF OTUs in total and 65 per sample on average. ECMF species richness increased exponentially to 80 m in linear distance and to 400 m2 in area and continued, less pronounced, to the largest linear distance (350 m) and area (12800 m2) without reaching an asymptote. Seventy percent of OTUs were detected in only 1-4 samples. We found less than 25% similarity between plots, separated linearly by

Conclusion. We found highly diverse and structured ECMF communities showing a weaker association to host species and to soil properties than expected.

Keywords: ectomycorrhizal, diversity, biogeography, soil, fungi

P (ID 285)

Ecological meaning of spore size variation on the arbuscular mycorrhizal symbiosis

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Recognizing patterns of resource allocation to offspring represents a way to understand the ecology of establishment and survival of an organism at earlier juvenile stages. In arbuscular mycorrhizal fungi (AMF), adult mycelia can confer resources to their offspring during spore formation, analogous to resource allocation from adult plants to offspring via seed endosperm. Despite its importance, little to no research in this area has been done for AMF. To address this gap, we gathered several lines of evidence to determine the ecological significance of spore size in AMF. We did this by: (1) quantifying spore size variation across all described AMF species, synthesizing data from taxonomic literature and culture databases; (2) determining whether disturbance—a known driver of propagule size variation in plants—explains AMF spore size variation, by combining the AMF spore data to published AMF community surveys along disturbance gradients; and (3) reviewing ecological literature to identify similarities between ecological consequences of propagule size variation between plants and AMF. We found that, similar to plants, AMF propagule size varies five orders of magnitude across species, but failed to detect any effect of disturbance to explain AMF spore variation. These patterns and our literature review, help us to identify major knowledge gaps that need to be addressed to advance the study of the ecology of reproductive investment in AMF. Based on this work, we argue that this unexplored research area would not be only beneficial for understanding AMF ecology, but also will benefit the theory of reproductive ecology in general.

Keywords: reproductive ecology, spore size, propagule size

P (ID 306)

Response of Ectomycorrhizae to Soil Nitrogen Amendments and SNC Disease Three Years After Treatment

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We measured Douglas-fir (*Pseudotsuga menziesii*) ectomycorrhizal root density and species richness across a gradient of climate and Swiss Needle Cast disease. Some normally common ectomycorrhiza types were reduced in frequency on sites highly impacted by Swiss Needle Cast disease. The variable responses of ectomycorrhizal fungi to carbon availability and soil nutrient status provides a context for understanding the strong relationship between ectomycorrhizal species richness and needle retention. We confirmed our previous hypothesis that knowledge of ectomycorrhizal fungal community structure would reveal responses associated with particular fertilizer treatments.

Three years after treatment, the nitrogen (urea) treatment decreased feeder-root density by 62% (p = 0.008), and decreased ectomycorrhiza type richness by 33% (p = 0.047). However, the richness effects varied by block. For control plots, ectomycorrhizal fungus species richness was significantly correlated with root density; R^2 = 0.82, p = 0.0003. For urea plots, ectomycorrhizal species richness was not correlated with root density; R^2 = 0.09, p = 0.39. For control plots, ectomycorrhizal species richness was significantly correlated with Douglas-fir needle retention; R^2 = 0.76, p = 0.001. For urea plots, ectomycorrhizal species richness was not correlated with Douglas-fir needle retention; R^2 = 0.09, p = 0.43. For control plots, ectomycorrhizal root density was significantly correlated with Douglas-fir needle retention; R^2 = 0.59, p = 0.01. For urea plots, ectomycorrhizal root density was not correlated with Douglas-fir needle retention; R^2 = 0.006, p = 0.84. We speculate there is a threshold of root density where species richness stabilizes at about two ectomycorrhizal types per 350 cc soil core volume.

References:

Luoma, D.L., Eberhart, J.L. 2014. Relationships between Swiss needle cast and ectomy-corrhizal fungus diversity. *Mycologia*. 106: 666-675. doi: 10.3852/12-180.

Keywords: fertilization, climate change

P (ID 320)

Environmental characteristics shape the ectomycorrhizal fungal community of two varieties of *Pinus clausa*

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The soil biota is diverse, yet the ecological and evolutionary processes that regulate species diversity and abundance of soil microorganisms across space and time remains elusive. In forest ecosystems, periodic disturbances which operate at different time scales represent one possible avenue by which diversity in the soil biota may be maintained due to their effect on genetic and phenotypic characteristics of the host and associated environment. To investigate the degree to which host variety and environmental characteristics shape the belowground ectomycorrhizal (ECM) fungal community, we sampled ten populations of a single pine species that has diverged into two genetically distinct varieties as a result of disturbance history (*Pinus clausa* var. *immuginata* and var. *clausa*) and used multivariate analysis to relate the community matrices of ECM fungi to host and environmental characteristics. Host variety was an important determinant of ECM fungal community structure and diversity at both the species level and when fungi were grouped into categories based on their substrate exploration and nutrient acquisition strategies independent of environmental characteristics. Overall, our results suggest that periodic disturbances which shape the host populations are also important for structuring the ECM fungal community independent of environmental effects.

Keywords: fungal diversity, exploration type, foraging trait, conifer

P (ID 339)

CulturTruf: optimizing truffle orchards cultivation by recording soil mycelium dynamic, hydric regime and soil temperature

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Truffles belonging to *Tuber* genus are Ascomycetes fungi forming ectomycorrhizal symbioses with trees and shrubs. Since 1970s, it is possible to inoculate seedlings with truffles in well-controlled conditions. High added value agroforestry ecosystems with inoculated seedling have been implanted worldwide. Truffle orchards represent an agroecological culture promoting biodiversity. However, for its development, truffle industry is requesting innovation for defining clear guidelines for truffle orchards management.

Truffle growers adopt various management practices (e.g. watering, soil tilling, tree pruning) often empirical to overcome climatic constraints such as summer climatic deficit. In this context, French agriculture ministry (FranceAgriMer) financed a national experimental project, CulturTruf, to determine the optimal soil hydric potential and truffle mycelium dynamic for truffles production. In the frame of this project we are analysing the seasonal dynamic of *Tuber melanosporum* and *T. aestivum* mycelium in the soils under 45 truffle productive trees of 13 experimental sites in France. Soil hydric potential and temperature are recorded under each tree and four soil cores are harvested each month. Truffles are heterothallic species and we quantified the mycelium of both mating type strains in the soils. The quantity of mycelium varied during the year and both mating type mycelium were frequent in spring or summer suggesting sexual reproduction initiation occurs in this period. This analysis highlights also the effect of soil tilling on soil mycelium. The correlation of management practices, soil temperature, hydric regime and soil mycelium dynamic will allow us to optimize the truffle production.

Keywords: climate, truffle, soil mycelium dynamic, soil temperature, soil hydric potential

P (ID 342)

Diversity and Abundance of Arbuscular Mycorrhizal Fungi (AMF) in Native and Managed Landscapes in Tasmania, Australia

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Despite the significant role of arbuscular mycorrhizal fungi (AMF, Glomeromycota) in supporting plant functioning and soil health, the identity of individual species and their relative abundance is largely unknown in most regions of Australia. To contribute to knowledge in this area, we are studying the identification and relative abundance of AMF taxa by two main methods; glomeran-specific quantitative PCR (qPCR) and Pac-Bio sequencing of 18S rRNA gene (rDNA) amplicons. We are examining the AMF communities in different native and managed landscapes in Tasmania to illustrate how key factors (i.e., land use, season, soil type and agricultural management practices) may influence AMF abundance and diversity. We have sampled 20 sites including cherry orchards, vineyards, pastures and native vegetation for comparison, in the South and North of Tasmania. In the first study, DNA amplification by gPCR revealed that the abundance of AMF in the soil varied with land use and season. In relation to the influence of land use on AMF abundance, Glomeromycota abundance was highest in improved pasture, followed by the cherry orchard and then native bush. The seasonal impact on AMF abundance showed a similar trend, regardless of land use. A rapid increase in spring compared to winter, was followed by a gradual reduction in summer. In autumn, abundance in pasture declined more rapidly than in the cherry orchard. We conclude from the results that AMF communities are affected most significantly by different land use management and seasonal changes, while evident, were not as great.

P (ID 345)

Soil inhabiting fungal communities in French Guiana and their relationship with phosphate

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Tropical rainforests on the Guiana shield in the neotropics function despite extremely low phosphate (P) availability. The scarcity of phosphate could potentially be a strong driver of soil fungal community composition, through plant reliance on mycorrhizal fungi and phosphate acquiring strategies of decomposers.

In order to better understand how low P conditions affect fungi we sampled soils at two different depths at 12 plots along a gradient in P availability varying from approx. 0.1 to 4 ppm available P. From these soils we isolated DNA and subjected it to PCR with fungal specific primers and high-throughput sequencing.

We find that the community composition of fungi is significantly affected by local fertility, with the strongest effects of nitrogen (N) and P. Relative abundance of arbuscular mycorrhizal (AM) fungi was found to decrease with increasing P and to increase in deeper soils. Relative abundance of ectomycorrhizal fungi was not related to soil fertility and is potentially more strongly determined by host occurrence.

We conclude that even at extremely low P levels, tropical trees appear to rely on AM fungi more with decreasing P. Furthermore, P and N are important drivers of the soil fungal community composition at large.

Keywords: tropical rainforests, mycorrhiza, phosphate, mutualism

P (ID 348)

Decomposed leaf litter suppresses arbuscular mycorrhizal symbiosis in Salix caprea understory

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Little is known on the mechanisms driving dynamics of mycorrhizal fungi at sites undergoing primary succession, despite a significant effect of these fungi on the course of plant succession. We extensively sampled the Podkrušnohorská spoil bank (Czech Republic) encompassing differently aged patches of vegetation up to 50 years old and found a dramatic drop in arbuscular mycorrhizal (AM) fungal abundance at sites ca 30 years old, dominated by ectomycorrhizal (EcM) *Salix caprea* trees that produce much of leaf litter. We hypothesized that AM suppression is caused either by competition with ectomycorrhizal (EcM) fungi, or by allelopathic effects of decomposing litter.

In a rhizobox system, EcM-host *Betula pendula* grew in the middle, AM-hosts (*Tripleu-rospermum inodorum* and *Calamagrostis epigejos*) in the lateral compartments. Plants either interacted via extraradical mycelia or not. The soil was amended with ground raw leaves (RL), fermentation horizon (FH) of decomposing litter or left unamended.

Presence of EcM mycelia radiating from *B. pendula* affected neither colonization nor richness of AM fungi in the AM-hosts' roots. FH decreased AM colonization, but did not eliminate any AM fungal species from the host plants' roots. Plants produced more biomass in this treatment. As FH represents generous source of nutrients, increased nutrient availability probably devalued AM fungi as a resource provider and supported direct plant nutrient uptake. Contrary, RL stimulated AM colonization including arbuscule abundance, but had no effect on plant growth.

Our results indicate that leaf litter suppresses AM colonization rather through changes in nutrient relations than by releasing allelopathic compounds.

Keywords: succession, spoil bank, leaf litter, arbuscular mycorrhiza, ectomycorrhiza

P (ID 352)

Which ectomycorrhizal fungal traits follow elevation gradient at the treeline?

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The treeline is an important ecotone of the elevation gradient. Ectomycorrhizal (ECM) fungi are supposed to be key players there, because they influence tree fitness under suboptimal conditions by providing nutrients in exchange for carbon.

Three elevation transects of 5 plots (500 m²) across an ecotone formed by *Picea abies* and *Pinus mugo* were established in Krkonoše Mts (Czech Republic) to study ECM community. We used three approaches: monitoring of fruit bodies, analysis of ectomycorrhizae (Sanger sequencing) and mycelia in soil (Illumina sequencing). Altogether we got data on 152 ECM taxonomical units (105 of them were identified into species level), 22 species were present in all three datasets. To find a trait based pattern following the elevation gradient, data on fruit body type, exploration type of ectomycorrhizae, spore size, spore colour and spore surface were assigned to each ECM species, according to literature and our observation.

The most abundant species at the tree line were of contact or short distance exploration types and formed crustothecia with round spores and pronounce ornamentation. We found, that type of fruit body (ECM fungi with holothecia and hypogeous fruit bodies occur more in low elevated plots) and to some extent also exploration type are associated with elevation, but spore character seems to be independent.

Keywords: ectomycorrhiza, fungal trait, elevation gradient, treeline

P (ID 354)

Temperature-mediated local adaptation alters the symbiotic function in arbuscular mycorrhiza

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Introduction: Variation in the symbiotic function of arbuscular mycorrhizal fungi (AM fungi) has been demonstrated among distinct biotic and abiotic interactions. However, there is little knowledge on how local temperature conditions influence the functional divergence of AM symbionts in alpine ecosystems.

Materials and Methods: We conduct a reciprocal inoculation experiment to explore the three-way interactions among plants, AM fungal inoculum and temperature at sites of contrasting elevation.

Results: Evidence of local adaptation of plant growth was found only under low temperature conditions. The origin of either the plant or the inoculum relative to the temperature was important in explaining symbiotic function. Specifically, when inoculum and temperature were sympatric but allopatric to the plant, poor adaptation by the plant to the novel environment was clearly found under both temperature conditions.

Discussion: Sympatric combinations of plant, inoculum and temperature conditions yielded the highest host biomass under the lower temperature conditions. This indicates that temperature may be an important driver mediating plant response to inoculation under local conditions. Positive feedback will help the AM associations to alleviate the stress when the relative fitness of the plant and that of the fungus are positively correlated under the lower temperature conditions (Bever, 1999; Johnson et al, 2013).

Conclusion: These results suggest that local adaptation represents a powerful factor in the establishment of novel combinations of plant, inoculum and temperature, and confirms the importance of taking into account both biotic and abiotic interactions in the prediction of the response of symbionts to global environmental change.

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Keywords: AM fungi, temperature, symbiotic function, geographic origin, local adaptation

P (ID 362)

AM fungi increase soil carbon and reduce N₂O and CH₄ emissions under warming condition

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Introduction: Arbuscular mycorrhizal (AM) fungi play key roles in the cyclings of nitrogen (N) and carbon (C). However, the influence of AM fungi on the emissions of nitrous oxide (N_2O) and methane (CH₄) are still not well understood. To test the influence of AM fungi on N_2O and CH₄ emissions under warming condition, an *in situ* experiment was conducted in a temperate meadow.

Materials and methods: The warmed plots were heated continuously using infrared radiators. In the The indigenous AM fungi were suppressed using benomyl. The N_2O and CH_4 were collected using static chambers measured using LGR (913-1054).

Results: AM fungi significantly decreased N_2O emissions under warming. AM fungi did not affect CH_4 emissions under warming. Under warming condition AM fungi significantly reduced soil total N. AM fungi increased soil total C under warming.

Discussion: The reduction of N_2O could partly be explained by accelerated the mineralization of N and increased N uptake under warming. The decline of CH_4 might be related to the decrease of C:N. The increase of soil C might be related to the acceleration of litter decomposition caused by AM fungi.

Conclusion: AM fungi can reduce the emissions of N₂O and CH₄ and increase soil C, and then accelerate the C and N transform from aboveground to belowground under warming condition.

Keywords: arbuscular mycorrhizal fungi, carbon storage, global change, greenhouse gas, N mineralization

P (ID 364)

Molecular diversity of arbuscular mycorrhizal fungi associated with two plant species in the Tibetan Plateau

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Aim: AMF form mutualistic associations with most land plants. AMF can increase plant tolerance to environmental stresses and maintain the diversity of plants, especially in harsh fragile alpine ecosystem. In order to explore the molecular diversity of AMF in alpine grasslands, an investigation was conducted in Qinghai Province.

Materials and Methods: The root and rhizosphere soil of two dominant plant species, *Kobresia* sp. and *Leontopodium pusillum* were collected at ten sampling sites. The dominant plant species are the target to be researched in each site. AMF diversity in root and soil communities was determined by phylogenetic analysis of 18S rRNA sequences.

Results: A total of 30 operational taxonomic units (OTUs) were identified. *Glomeraceae* was found dominant in roots, while both *Glomeraceae* and *Diversisporaceae* were frequently present in soil. Distinct AMF community was found in roots and soil. Specifically, five OTUs were only found in *L. pusillum* roots and ten OTUs were only found in *Kobresia* sp.

Discussion: The total of 30 OTUs in seven families detected supports the assertion that AMF have a broad ecological range and are an important component in grasslands^[1]. The three dominant families (*Glomeraceae*, *Diversisporaceae* and *Acaulosporaceae*) and some dominant OTUs had a noticeably biased occurrence in roots or in soil, which is similar to the finding at the HBAMERS^[2].

Conclusions: Theses results showed a relatively high diversity of AMF existed in the eastern region of the Tibetan Plateau, and distinct AMF community existed in roots and soil.

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Keywords: arbuscular mycorrhizal fungi, diversity, community composition, Tibetan plateau

P (ID 366)

Ectomycorrhizal fungal communities associated with alpine relict populations of *Pinus pumila* over Japan

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Alpine ecosystems are recognized to be vulnerable to global warming, but we know little about ECM fungal communities in alpine forests. *Pinus pumila* is distributed in fragmented alpine regions of Japan, and often forms mono-dominant forest stands. Here, we examined ECM fungal communities of *P. pumila* forests throughout Japan to reveal factors affecting the community structures in alpine regions.

Soil cores containing ECM roots were collected from *P. pumila* mature forests located in central to north-eastern Japan. The soils were separately excavated from litter and mineral soil layers. ECM root tips were morphologically classified and subjected to molecular analyses. Fungal species were identified based on the similarity of rDNA ITS sequences.

In total, 147 ECM fungal species were detected from 4134 sequenced ECM root tips. Estimated species richness was positively correlated with soil pH and total soil nitrogen in litter and mineral soil layers, respectively. Redundancy analysis revealed a larger contribution of climatic conditions to ECM fungal communities than those of soil environments and spatial distance.

Our results indicate that ECM fungal diversity is negatively affected by high-acidic and poor-nutrient soils, which are typical under alpine climates. Moreover, the significant effect of climates on the composition of ECM fungi associated with the same alpine tree species implies that ECM fungi could respond to the climate change prior to the aboveground community changes. The preceding changes in belowground ECM fungi may eventually affect vulnerable above-ground alpine plant communities under the ongoing global warming.

Keywords: alpine forest, ectomycorrhizal fungi, *Pinus pumila*

P (ID 378)

Ectomycorrhizal fungal communities of Scots pine along a 1,300 km latitudinal gradient

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Both host tree and environmental conditions shape the ectomycorrhizal fungal (EMF) communities. Still, it is poorly understood how one tree species affects own EMF community along a latitudinal MAT (mean annual temperature) gradient. Low temperature may hinder litter decomposition and associated nutrient soil availability. Hence, we hypothesize that a tree species is able to cope with soil infertility in a boreal forest through specific EMF assemblages.

To answer the question, we analyzed ectomycorrhizae of *Pinus sylvestris* along the 1,300 km latitudinal gradient in Sweden.

EMF communities were identified using ITS1 rDNA sequencing. The indexed libraries representing each population were sequenced with Ion Torrent PGM System. Sequence data were analyzed using Geneious R10.0.9 and operational taxonomic units were compared to UNITE, GenBank and MycoBank databases. In total 6,308,178 reads were analyzed, ranging from ca. 3,200 to 601,000 for each population.

In total we analyzed 60,779 of ectomycorrhizal root tips which resulted in 53 of EMF species. Number of EMF species in each population varied among sites from 2 to 17, although EMF species richness was unrelated to the mean annual temperature of the site.

Our data suggest that the EMF communities of *P. silvestris* are unaffected by the temperature along the latitudinal gradient in Sweden. Even though dominating EMF species were found across the pine forests, no clear pattern emerged that could explain pine adaptations to MAT and thereby soil infertility in colder areas of Sweden.

P (ID 383)

Spatial distribution of arbuscular mycorrhizal fungal spores in a hot spot field site

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms establishing mutualistic symbioses with most land plants and playing key roles in plant nutrition and health. Their spatial distribution patterns have been investigated at global and regional scales, while little is known of their community composition at smaller scales.

In this work, a small field previously identified as a large repository of AMF diversity, was investigated in order to determine spatial variation in AMF spore communities. Spore number and identity were assessed in soil collected from 12 sampling points across the experimental field (9x134 m). Spores were identified to species level and their distribution was correlated with soil structure and characteristics.

A total of 47 species belonging to 14 genera were detected. Mantel test showed that AMF community composition was significantly correlated with soil characteristics. AMF and soil variables were autocorrelated within a distance of 10 m, suggesting the occurrence of a small scale spatial heterogeneity. Most taxa displayed aggregated distribution, as revealed by the variance to mean ratio. Soil characteristics differentially affected distribution patterns of individual taxa within the field, both at the family and species level.

Differential responses of individual AMF taxa to edaphic factors play a role in shaping the distribution and heterogeneity of AMF communities, consistently with the small scale spatial patterns of soil physical and chemical properties.

Studies on the spatial patterns of whole AMF communities and individual taxa are fundamental for improving our knowledge on the ecological drivers of native AMF distribution at the local scale.

Keywords: arbuscular mycorrhiza, spore distribution patterns, spatial heterogeneity, soil physico-chemical properties

P (ID 396)

Do plants actively shape arbuscular mycorrhizal fungal communities according to the environmental conditions to optimize their symbiotic benefits?

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Generally, it is well established that abiotic conditions influence both the composition of arbuscular mycorrhizal fungal (AMF) communities and the host plant's benefits from mycorrhiza. However, the relationship between AMF community composition and mycorrhizal benefits in different abiotic conditions remains little explored. Particularly, it is unknown to which extent abiotic conditions influence the quantitative ratios of AMF species within communities and whether such changes could be regarded as a means of optimization of plant fitness under varying environmental conditions.

To address the concept of adaptive mycorrhizal association, we established a two-stage greenhouse experiment with *Medicago truncatula* as host plant, which was inoculated with a synthetic community of five AMF species. In the first stage, the system was exposed to contrasting abiotic conditions including water shortage, light shortage and high phosphorus availability assuming that the treatments would generate AMF communities of different composition. In the second stage, these different AMF communities were inoculated to *M. truncatula* plants and subjected to the same stresses in a cross-factorial design to test whether they differed in their symbiotic efficiency.

We found that all AMF communities were dominated by the same species, while abiotic conditions influenced the abundance of the subdominant species. Mycorrhizal responses of *M. truncatula* were affected by the environmental conditions, but not by AMF community history in the second stage. Thus, species ratios within the AMF community were relatively robust against changes induced by abiotic conditions, and AMF community composition did not contribute to conditions-specific mycorrhizal responses.

P (ID 407)

Functioning of arbuscular mycorrhizal symbiosis under soil water deficiency

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Drought spells are becoming more frequent and more severe in some parts of the world due to rearrangements of precipitation patterns in consequence to the global climatic changes. Therefore, it is very timely to disclose the natural mechanisms of nutrient and water cycling between soil and plants, including root physiology and rhizosphere-associated microbes, to provide solutions for the pressing environmental and agricultural challenges in the years to come. Particularly, we should improve our understanding of the functioning of arbuscular mycorrhizal (AM) symbiosis related to water. Its role in plant water uptake remains still rather controversial and functional aspects of AM symbiosis under soil water deficiency are only little understood.

When soil water becomes depleted, the water remains only in pores of smaller sizes, not directly accessible to roots or root hairs. Since mycorrhizal hyphae often have only about 2 μ m in diameter, they can penetrate even into some of the micropores, exploiting the water (and soluble nutrients) available there. The hyphae could contribute not only to direct uptake of water from soil to plant, but also play a part in hydraulic lift, i.e. the redistribution of water in soil profile through the activity of plant roots. These issues are just starting to be addressed in a new project, benefiting from availability of mycorrhizal mutant plants, molecular quantification of mycorrhizal fungal development and a range of stable isotopes and radioisotopes.

Keywords: arbuscular mycorrhiza, drought, water uptake, stable and radioisotopes, hydraulic lift

P (ID 415)

Effects of 15 Years of Nitrogen Fertilization on Ericoid Mycorrhizal Colonization at Whim Bog

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Introduction/Aim: At the long-term fertilization experiment at Whim bog, Scotland, the effects of nitrogen deposition on peatlands are studied. The predominant groundcover plants are ericoid species, which rely on ericoid mycorrhizal fungi (ERM) for access to organic sources of nutrients such as Nitrogen (N) and Phosphorous (P). As ERM symbiosis is directly related to nutrient availability these species and their fungal symbionts are indicators of these effects. By measuring vegetation diversity, abundance and mycorrhizal colonization rates, we aim to examine how atmospheric N deposition changes peatland plant-mycorrhizal relationships.

Materials and Methods: The sampled plots comprised triplicate controls and treatments of either NH₄Cl or NaNO₃ at 6.4 g N m⁻² y⁻¹ with and without additional K₂HPO₄. Aboveground vegetation diversity and abundance was estimated by point-intercept method. Roots from ericoid plants, *Calluna vulgaris* and *Erica tetralix*, were sampled and microscopically quantified for mycorrhizal colonization, as described by Kiheri et al.¹.

Results/Conclusion: All nutrient addition treatments displayed a shift in abundance from ericoids to the sedge *Eriophorum vaginatum*. Unexpectedly, ericoid mycorrhizal colonization rates increased under treatment compared to controls, while the presence of Dark Septate Endophytes (DSE) decreased. Increased ERM colonization indicates that the role of mycorrhizal fungi is changing under high N availability. Further analyses are necessary to identify key causes and effects in this ecosystem.

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¹Kiheri H, Heinonsalo J, Timonen S. Staining and Microscopy of Mycorrhizal Fungal Colonization in Preserved Ericoid Plant Roots. Journal of Berry Research. In press August 2017.

Keywords: ericoid mycorrhiza, microscopy, peatland, nitrogen deposition

P (ID 416)

Investigating changes to mycorrhiza-crop relationships in wheat cultivars under elevated levels of atmospheric CO₂

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Arbuscular mycorrhizal fungal (AMF) associations are formed in many globally important crops and as such, may have potential in reducing usage of chemical fertilisers in sustainable agriculture. Moreover, climate change is now negatively impacting agricultural productivity and while there is evidence to suggest that AMF can help mitigate these effects (e.g. drought alleviation), little is known about how increasing atmospheric CO_2 will change the functionality of mycorrhiza-crop relationships. This is an important knowledge gap as there is now substantial evidence to suggest that atmospheric CO_2 is a powerful regulator of mycorrhizal function.

I assessed the physiological responses of three wheat cultivars, Skyfall, Cadenza and Avalon, following inoculation with a commercial *Rhizophagus irregularis* inoculum. The wheat cultivars were grown in controlled environment chambers at atmospheric CO₂ concentrations of 440 ppm or 800 ppm, in line with IPCC projections for projected future CO₂ levels. The crops were grown in the presence of the inoculum or a non-mycorrhizal control inoculum and harvested after 8 weeks to assess growth and level of colonisation.

Results confirm that growth benefits from mycorrhizal inocula varies between wheat cultivars. In addition, atmospheric CO_2 concentration can affect the plant growth benefits gained through commercial AMF inocula, but this is also cultivar dependant.

In conclusion, my research shows the variability of responses to AMF inoculation between wheat cultivars and across CO₂ concentrations, which is an important first step in understanding how the relationship between AMF and wheat will change in a future climate with rising CO₂ levels.

P (ID 417)

Community structure of arbuscular and ectomycorrhizal fungi in plants grown in frost-treated soils

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Previously we reported after-effects of a soil frost exposure on subsequent formation of arbuscular and ectomycorrhizas. Even extremely low temperatures did not affect boreal ectomycorrhiza (EM) formation, but arbuscular mycorrhiza (AM) formation was reduced. Here the aim is to show whether there was a change in the community structure within either fungal type.

We collected and homogenized surface soil from sites with diverse EM and AM vegetation in Eastern Finland in late autumn and exposed soil batches to a range of temperatures in controlled freezing tests: +5 °C (control), -12 °C, -25 °C, -48 °C and -130 °C. Seeds of silver birch (*Betula pendula*), grey alder (*Alnus incana*) or white clover (*Trifolium repens*) were sown in the treated soils. Plants were grown in similar, favourable conditions in a growth room and harvested after 15 weeks. DNA was extracted from harvested roots, and the ITS2 fungal barcode was amplified and sequenced using Illumina MiSeq amplicon sequencing. Data were analysed using the QIIME pipeline and the UNITE database for fungal OTU delimitation, and the R package vegan for community analysis.

Data will be presented showing whether i) there is a change in species composition due to low temperature exposure, and ii) whether there is a decline in diversity with temperature. Understanding the changing interaction between AM and EM at different temperatures will inform predictions of ecosystem functions in cool climatic regions.

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Kilpeläinen J., Vestberg M., Repo, T. & Lehto, T. 2016. Arbuscular and ectomycorrhizal root colonisation and plant nutrition in soils exposed to freezing temperatures. Soil Biology & Biochemistry 99, 85–93.

Keywords: arbuscular mycorrhizal fungi, cool climate, ectomycorrhizal fungi, frost, fungal species

P (ID 419)

The role of different mycorrhizas in the drought resistance of grey alder (*Alnus incana*) seedlings

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Arbuscular mycorrhizas (AM) are more common in soils with higher available-N:P ratio and higher pH than ectomycorrhizas (EM). However, the climatic conditions, which drive soil formation, may affect the mycorrhiza types also directly. We suggest that AM fungi (AMF) in general are more adapted to warm and dry climates and EM fungi (EMF) to cold and humid climates, and accordingly, benefit their host plants especially in those conditions. Here, we tested the drought resistance of AM, EM and nonmycorrhizal (NM) grey alder (Alnus incana) seedlings.

Alder seedlings were grown from seeds in pots in a controlled-environment chamber. They were inoculated with two species of either AMF or EMF. The AMF were *Rhizophagus intraradices* and *Glomus hoi*, and the EMF were *Paxillus involutus* and an ascomycete isolated from mycorrhizas of grey alder. After three months, half of the seedlings were left without water and the other half were watered. Net photosynthesis (A), stomatal conductance (g_s) and shoot water potential (Ψ) were determined.

In the watered treatment, the AM plants had higher g_s and A, but when the soil dried, the AM plants closed their stomata as soon as the EM and NM plants. Conservative water use was probably the reason for the higher (less negative) Ψ in the droughted AM plants than EM and NM. When compared at the same soil moisture level, there was no difference between the mycorrhiza treatments. In conclusion, the AM plants had more control over their water use than the EM and NM.

P (ID 429)

Effect of low-intensity fires on ectomycorrhizal fungal communities in a dry dipterocarp forest in Thailand

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Fires are a major form of disturbance of dry dipterocarp forests (DDF) in Thailand and elsewhere in Southeast Asia. Most annual fires are of a low intensity and may have shaped the structure and dynamics of this forest ecosystem type^{[1][2]}. However, the impact on the ectomycorrhizal (ECM) fungal community, an integral symbiosis with dipterocarp host trees, has not yet been explored^[3]. Thus this study investigated the effect of fires on the ECM fungal community in three DDF sites in Saraburi Province, Thailand.

Soil samples were collected every three months from December 2013-September 2014. Illumina MiSeq data covering the ITS1 region^[4] were used to examine the ECM fungal communities in the study sites, two of which were burned in January 2014.

A total of 66 operational taxonomic units (OTUs) were detected, belonging to 11 ECM fungal lineages. Most common ECM lineages were /tomentella-thelephora (19 OTUs) and /russula-lactarius (15 OTUs). The patterns of temporal change in ECM fungal communities in the burned and unburned areas were different, especially the emergence of new ECM fungal OTUs. In the burned sites, most of the new OTUs were found within 2-5 months after the fires. In the unburned site, however, most of the new OTUs were detected after 8 months and some of them were the same newly-emerged OTUs in the burned sites.

Therefore, forest fires could provide newly available microhabitats for ECM fungi while the absence of fire apparently extended the emergence time of some ECM fungi.

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Keywords: temporal change, next generation sequencing

P (ID 430)

Community Structures of Ectomycorrhizal Fungi in *Abies koreana* Along Environmental Gradients

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Climate changes have important consequences for plant communities and their fungal root symbionts. Climate change is known to alter the distribution of ectomycorrhizal fungi (ECM) in temperate, boreal, and tropical biomes Abies koreana is climate-sensitive indicator species found in the Korean peninsula; it is native to sub-alpine mountains and is designated as an endangered species by the IUCN. Symbiosis with ECM is vitally important for the survival of A. koreana, as the host plant provides the fungal partners with carbon assimilated via photosynthesis in exchange for a range of benefit, including enhanced water and nutrient uptake, protection from soil pathogens, and heavy metal tolerance. The purpose of this study was to determine the distribution of ECM fungi colonizing roots of A. koreana along environmental gradients. Root sampling of A. koreana was done at altitudes in 100 m increments in Mt. Halla in Jeju-Island and various environmental factors were simultaneously investigated. We found that soil environmental factors affecting the distribution of ECM fungi were nitrogen, organic matter, and phosphorous concentration gradient. The nitrogen concentration gradient was found to remarkably affect ECM colonization rates. We suggested that increased nitrogen deposition in forests might cause great changes in the ECM community structure and functioning, which, in turn, might lead to reduced nitrogen uptake by roots and fungi. These results could help in understanding the distribution and conserving A. koreana, which is declining because of climate change

Keywords: Abies koreana, environmental gradient, ectomycorrhiza, nitrogen deposition

P (ID 437)

Where do we find the mushrooms? Spatio-temporal drivers of West African woodland ectomycorrhizal fruitbody production

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Fruiting bodies of ectomycorrhizal fungi are a seasonally important food source for rural inhabitants of woodlands in West Africa. In this study, we investigate the drivers of spatial and temporal variation in ECM community composition and fruitbody production in woodlands in central Benin, including soils, vegetation, and microclimate.

Our study area consists of nine 50x50 m plots sorted into three different vegetation types, each dominated by one ECM tree species. Within each plot, we harvest and weigh all ECM mushrooms twice weekly during the mushroom season from June – October, 2015 – 2018. Voucher specimens are preserved and later DNA barcoded to verify morphological identification of species. We have also collected spatial and temporal environmental data from the sites including soil physical and chemical properties, forest structure, and soil and air meteorology.

Sampling is complete for the years 2015 and 2016, and 2017 sampling is underway. 179 morphologically identified species were collected in 2015 and 2016, although this number is likely to change after the barcoding of specimens is completed. Total yield ranged from 1 kg/ha/yr to 30 kg/ha/yr (dry weight) in different plots and years.

This poster presents our preliminary results regarding the phenology of these communities and discusses the prospect for identifying seasonal and interannual climate effects as additional years of data become available. We also summarize preliminary results regarding the drivers of spatial variation in fruitbody community composition, species richness, diversity, and total annual fruitbody yield.

Keywords: West Africa, ectomycorrhiza, phenology, wild edible fungi, climate

P (ID 439)

Mycorrhizal and saprotrophic sporocarp communities have contrasting responses to moth outbreak in a subarctic mountain birch forest

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Outbreaks of folivorous insects cause large-scale defoliation in forest ecosystems. In the subarctic mountain birch forests, outbreaks of geometrid moths cause occasionally landscapescale forest damage and even death of individual trees and forest stands. Here, we studied how aboveground macrofungal sporocarp communities respond to moth herbivory and relate to previously described understory vegetation, soil nutrients and mountain birch ectomycorrhizal communities.

We compared intact mountain birch forests with once and repeatedly defoliated forests just after a massive outbreak of geometrid moths. Aboveground macrofungal sporocarps were surveyed in about 2 week intervals during autumnal fruiting seasons for 3 years.

The sporocarp communities were heavily affected by moth outbreak. Repeated moth herbivory decreased ectomycorrhizal sporocarp production by 90% and increased saprotrophic sporocarp production by 200% compared to the intact sites. Also, the number of ectomycorrhizal species decreased dramatically, whereas the number of saprotrophic species increased.

Defoliation of mountain birches decreases the amount of photosynthesized carbon available for ectomycorrhizal fungi, which has a negative effect on ectomycorrhizal sporocarp production. Increased quantity as well as altered quality of litter benefit the sporocarp production of saprotrophic fungi. These results add to the evidence that insect outbreaks shift the main carbon route from trees to soil: from a mycorrhizal channel, to a saprotrophic channel.

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Keywords: herbivory, host carbon limitation, sexual reproduction

P (ID 477)

How climate and edaphic controllers shape the distribution of arbuscular mycorrhizal fungi in California

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms with critical roles in ecosystems, notably as plant root symbionts. The development of molecular methods has made it possible to identify AMF taxa directly from plant roots and soil, instead of relying on morphological characteristics of spores, and offers new opportunities to investigate the molecular diversity and spacial distribution of AMF. The goal of this study is to determine which controller is more important in shaping the distribution of AMF: soil characteristics, climate, or host plant.

We selected the plant genus Avena, a naturalized annual grass in California highly colonized by AMF, that is present at multiple sites. We collected rhizosphere and bulk soils from three mediterranean grasslands that varied substantially in terms of soil taxonomies and texture, climate, and dominant Avena species, either barbara or sativa. We extracted DNA from rhizosphere and bulk soil, and sequenced it with Illumina sequencing. We used of a combination of primer pairs, ITS70/ITS4, which spans a more variable region, and WANDA/AML2, which spans a more conserved region of AMF rDNA. This use of primer pairs with complementary strengths leads to better resolution and inclusion during molecular analyses, and more robust information for understanding AMF biogeography.

If AMF in bulk soils are more similar to each other than their corresponding rhizosphere samples, it will indicate that roots are stronger drivers of AMF community composition than the conditions of the background soil. Conversely, if AMF in rhizosphere soils are more similar to each other than their corresponding bulk samples, it will suggest that background soil conditions are more important controllers.

P (ID 484)

Arbuscular mycorrhizal fungi in the arid Himalayas

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Little is known about arbuscular mycorrhizal fungal (AMF) diversity and distributional patterns in high altitudes and in very cold or dry habitats characterizing high elevations. Our aim was to describe diversity patterns of AMF along an elevational gradient and to determine the abiotic and biotic factors shaping the richness and community composition of AMF.

Our study area is situated in Ladakh, on the dry flanks of NW Himalayas. In total, we sampled fine roots of 344 plant individuals (ca 200 species) in 85 sites across a range of 1940-6100 m asl. The sites were located in six major habitat types (from lowest to highest): forests, cold deserts and steppes, alpine grasslands, screes, lower and upper subnival zones. DNA-based virtual taxa (VT) of AMF were identified using 454-sequencing of the SSU rRNA gene. We included a number of biotic and abiotic factors into the analyses.

In total we detected 101 AMF VT (mean 5.6; range 0-27 VT per sample) spanning across nine Glomeromycota genera, most common of them being Glomus (66 VT), Acaulospora (10 VT) and Diversispora (7 VT). There was a significant negative relationship between AMF VT richness and altitude (P<0.001; Spearman rho=-0.27), with highest richness around 2200 m and 4300 m asl corresponding to forest and alpine grassland habitat types, respectively.

The results of this study will significantly advance our understanding of microbial, and specifically AMF diversity patterns and their underlying mechanisms at multiple spatial scales across a range of habitat types.

Keywords: arbuscular mycorrhizal fungi, high-elevation, dry habitats, diversity, 454 sequencing

P (ID 486)

Characterization of mycorrhizal potential of the soils envahed by *Nicotiana glauca* Graham in Marrakesh Safi region (Morocco)

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Particular attention has been paid to the negative effects of invasive species on the environment, but studies that quantify their beneficial effects are scarce.

The objective of this study is to evaluate the consequences of invasions by *Nicotiana glauca* Graham on soil fertility in the Marrakesh Safi region by examining (i) its contribution to soil mycorrhizal potential and (ii) its impact on the richness and diversity of the arbuscular mycorrhizal community. This study was carried out on 5 stations dispersed in this region, which are known as sites heavily invaded by *Nicotiana glauca* Graham, Including two stations upstream and downstream of the banks of Oued Tensift and a station on the banks of Oued Lahjar.

The results reveal a particularly important mycorrhizal potential in the rhizospheric soils of *Nicotiana glauca* Graham, representing mycorrhizal propagules fungi density present in the soil such as spores, mycelium or vesicles able to form mycorrhizae.

The most probable number (MPN) of mycorrhizal propagules per 100 g of dry soil ranged from 12.21 (bare soil) to 1213.73 (*Nicotiana glauca* Graham).

From the point of view of sporadic diversity, three genera of Arbuscular mycorrhiza could be isolated: Glomus, Gigaspora and Sclerocystis with a high abundance of spores of the genus Glomus than those of other genera.

The ecosystems invaded by *Nicotiana glauca* Graham could be of great interest to restore degraded soils of ruderal areas.

Keywords: *Nicotiana glauca* Graham, invasive species, mycorrhizal potential, arbuscular mycorrhiza, Marrakech Safi, Morocco

P (ID 487)

Root-associated fungal communities at a nutrient enriched arctic bird-nesting cliff

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Arctic terrestrial ecosystems are frequently nutrient poor with low primary productivity. The vegetation rich areas beneath bird-nesting cliffs are an exception to this. Nesting birds transport nitrogen from sea to land through guano deposition, creating both a nutrient and pH gradient surrounding their nesting sites.

The fungal communities associated with the roots of *Salix polaris* growing in ornithogenic soils along a pH and nutrient gradient beneath a bird-nesting cliff on Svalbard (77.6N, 14.7E) were investigated using illumina amplicon sequencing of the internal transcribed spacer 2 (ITS2) region of ribosomal DNA.

Although species richness remained constant across the gradient, fungal community composition varied with the main abiotic and biotic gradients. The total root fungal community was structured by soil pH, carbon, and nitrogen content, as well as the surrounding plant community. Guild-specific responses were observed, with the ectomycorrhizal community primarily responding to host nitrogen and carbon content, soil pH, and the surrounding plant community. By contrast, the *Salix* root-associated saprophytic community was more strongly influenced by edaphic properties.

Despite the absence here of the strong nutrient limitations typically found in arctic terrestrial ecosystems, the root associated fungal community was still primarily structured by variation in abiotic edaphic properties. The relative importance of biotic factors, including host C:N content, in structuring ectomycorrhizal root associated communities is unsurprising given their near obligate association with the host plant. The stronger role of edaphic factors in structuring the root-associated saprophyte community suggests the possibility of priority effects during their assembly.

P (ID 500)

A recycling method of afforesting infertile soil using a soil amendment and incorporation of *Pisolithus tinctorius*

<u>Hye Young Yun</u> (Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea), **Kyung Joon Lee** (Braintree Biotechnology Institute, Seoul, Korea)

The present research is focused on developing a recycling method for afforesting marginal, infertile soil using the mycorrhizal fungus *Pisolithus tinctorius* as a fertilizer and adding a soil- improving compound. This afforestation method helped seedling growth, thereby potentially making forest or landscape trees grow faster and healthier. In these experiments, various soil amendments were incorporated to supply nutrients for cultivation of seedlings and growth of *Pinus densiflora*. It was determined that, when *Pisolithus tinctorius* was cultured and introduced into the seedbed soil used for cultivating seedlings, significantly improved seedlings were produced compared to controls. In addition, when seedlings were cultivated by introducing the soil-improving agent alone, without *Pisolithus tinctorius*, the growth rate of seedlings was not greatly enhanced. Further, it was found that growth rates of *Pinus densiflora* varied depending on the amount of soil-improving agent used. When the ratio of soil-improving agent to culture medium was 1:5 (v/v), excellent height and growth was exhibited. Both rate of mycorrhiza formation and growth rate of seedlings were enhanced by using *Pisolithus tinctorius* mycorrhizae together with a soil-improving agent.

CONCURRENT SESSION: Acquisition, assimilation and transport of nutrients and carbon in mycorrhizal symbioses

Well-functioning mycorrhizae are established and maintained on condition of mutual nutrient exchange. Understanding the nutrients for photosynthesis-derived sugar trade has been a challenge and source of debate for ages. This session will focus on the physiological and molecular-genetic mechanisms by which mycorrhizal fungi regulate homeostasis of macro-, micronutrients and sugars, determine the nutrient offer at the plant-fungal interface and impact on nutrient balances of the host plant. Nutrient foraging, assimilation, storage, translocation, transfer, metabolism and their regulation in distinct external conditions as well as interspecific differences, specialization and adaptation towards particular environments are included.

Chairs: Joske Ruytinx, Katsuharu Saito

CL (ID 75)

Zn homeostasis and adaptive Zn tolerance in the ectomycorrhizal fungus Suillus luteus

<u>Joske Ruytinx</u> (Hasselt University, Diepenbeek, Belgium), **Laura Coninx** (Hasselt University, Diepenbeek, Belgium), **Hoai Nguyen** (Hasselt University, Diepenbeek, Belgium), **Jan Colpaert** (Hasselt University, Diepenbeek, Belgium)

Suillus luteus is a cosmopolitan fungal species, symbiotically associated with pine trees. In primary successions of pines this species is abundant and involved in seedling establishment. On severely metal-contaminated soils, Zn-tolerant S. luteus populations evolved by natural selection. Tolerant individuals effectively protect their host tree from Zn toxicity on these soils. However, the molecular-genetic mechanism underlying adaptive Zn tolerance in S. luteus is unknown. We hypothesize that the tolerance phenotype is due to an adaptation in the common Zn homeostasis network. By comparative and functional genetics we identified several S. luteus genes encoding transporters involved in Zn homeostasis. One of these transporters, SIZnT2, a CDF family transporter exhibits a differential gene expression among Zn-tolerant and Zn-sensitive phenotypes. Zn-tolerant S. luteus isolates show a high expression whereas Zn-sensitive isolates show a near to background expression of the gene. The difference in expression level seems to be partly caused by an extensive gene multiplication, partly by differences is cis-regulation. SIZnT2 copy number in Zn-tolerant isolates ranges between two and seven. Zinc-sensitive isolates have one copy. Three different promoter genotypes were identified; one of these was exclusively found in Zn-tolerant isolates. SIZnT2 is predicted to be localized on the tonoplast and to move Zn from the cytoplasm into the vacuole. However, we could not confirm this function. In conclusion, Zn-tolerant S. luteus isolates are selected for high expression of SIZnT2, a CDF family transporter of unknown function. Further functional analyses are required to define the role of high SIZnT2 expression in Zn tolerance.

IL (ID 213)

Influence of ectomycorrhiza on decomposition across biomes

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Some ectomycorrhizal fungi have been proposed to be directly involved in decomposition, as a way to mobilise organically bound nutrients. On the other hand, most ectomycorrhizal fungi lack efficient enzymes to decompose complex organic matter and have rather been proposed to affect decomposition negatively, by competing with free-living saprotrophs (the "Gadgil effect"). Field studies from different boreal settings indicate that the influence of ectomycorrhiza on decomposition is context dependent. Towards the heathland – boreal transition, ectomycorrhizal fungi tend to stimulate decomposition relative to the otherwise dominant stress-tolerant ascomycetes. Towards the nemoral boreal transition, ectomycorrhizal fungi tend to restrict decomposition by constraining the distribution and activities of more efficient saprotrophic decomposers. Increased understanding of the mechanisms by which ectomycorrhizal fungi mediate feedbacks between plants and soil organic matter is urgently needed, to enable predictions of how different ecosystems respond to climate changes and intense exploitation. Competitive interactions between fungal guilds mediate positive feedbacks between decomposition and ecosystem fertility, which may contribute to variation in the forest landscape and amplify responses to disturbance, but also drive long-term retrogression of undisturbed forest ecosystems.

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Keywords: forest ecology, decomposition, Gadgil effect, nitrogen cycle

CL (ID 296)

Trading carbon for nitrogen: reciprocal resource exchange in beech ectomycorrhiza at subcellular scales

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The controls of resource exchange between plant roots and their mycorrhizal partners are still under debate. While a reciprocal exchange of C and nutrients has been suggested for the arbuscular mycorrhizal symbiosis, much less is known about 'terms of trade' in the ectomycorrhizal symbiosis.

We exposed young beech trees ($Fagus\,sylvatica$) associated with natural mycorrhizal fungal communities to a $^{13}CO_2$ atmosphere and added ^{15}N -labelled amino acids to a compartment exclusively accessible to mycorrhizal hyphae. Within two days after ^{15}N and $^{13}CO_2$ application, fine roots and mycorrhizal root-tips were harvested and analyzed by isotope-ratio mass spectrometry. Associated fungal communities were identified by ITS sequencing. Selected mycorrhizal root tips were analysed by nano-scale secondary ion mass spectrometry (NanoSIMS) to visualize the spatial distribution of ^{13}C and ^{15}N in root cross-sections at the subcellular scale.

¹³C and ¹⁵N were weakly correlated across collected mycorrhizal root-tips. The relatively high variance within this correlation was seemingly driven by fungal colonization pattern. At the subcellular scale, however, NanoSIMS imaging revealed a highly significant spatial correlation between ¹³C and ¹⁵N across both plant and fungal cells. Individual fungal hyphae "hotspots" that were highly enriched in ¹⁵N were at the same time extraordinarily enriched in ¹³C.

Our results provide first evidence for a reciprocal exchange of C and N between roots and ectomycorrhizal fungi at the subcellular scale. They indicate that a mechanism may exist at this scale that directs plant C flow specifically into N-delivering mycorrhizal hyphae.

IL (ID 200)

Phosphate unloading in ectomycorrhizae: why is the *Hc*PT2 transporter of *Hebeloma* cylindrosporum a good candidate?

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It is well known that ectomycorrhizal (ECM) fungi improve the growth of their hosts by providing growth-limiting nutrients and particularly inorganic phosphate (Pi). However, the molecular players driving the Pi allocation from the fungus to the host are largely unknown thus far. In the ECM association between Pinus pinaster and Hebeloma cylindrosporum, we hypothesized that one of the three Pi transporters identified in the fungal genome (named HcPT1.1, HcPT1.2 and HcPT2) could be more specifically involved in Pi unloading in the Hartig net. To address this hypothesis, we combined microscopy, physiology and fungal genetic approaches to unravel the role of each Pi transporter. Immunolocalization of Pi transporter proteins showed that only HcPT1.1 and HcPT2 are localized in the Hartig net, indicating that these two proteins could play a role in Pi efflux in the ECM. We produced transgenic lines, either down- (RNAi) or over- (OE) expressing one of the two proteins. We used them to measure in vitro Pi fluxes as well as P accumulation in young P. pinaster seedlings. Our experiments showed that only OE-PT2 lines were able to increase in vitro Pi effluxes and shoot P accumulation of pine seedlings whereas the OE-PT1.1 lines increased only root P accumulation. Taken as a whole, these results support that the HcPT2 Pi transporter is a key component of the fungal Pi unloading machinery in the Hartig net of ECM.

Keywords: ectomycorrhizal symbiosis, phosphate transporter, immuno-localization, phosphate efflux, agrobacterium-transformed fungal strains

ST+P (ID 522)

On genes and mechanisms underlying metal (hyper)accumulation phenotypes in ectomycorrhizal *Amanita*, *Russula* and *Hebeloma* species

<u>Pavel Kotrba</u> (Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Prague, Czech Republic), **Milan Gryndler** (Institute of Microbiology, v.v.i., Czech Academy of Sciences, Prague, Czech Republic), **Jan Borovička** (Institute of Geology, v.v.i. & Nuclear Physics Institute, v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic)

The high concentrations of heavy metals accumulated in their sporocarps indicate that fungi substantially contribute to the cycling and environmental sequestration of these elements. Mycorrhizal fungi further play an important dual role in plant metal homeostasis: scavenging of metal micronutrients and their supply to the host; detoxification of both the excess essential and physiologically irrelevant metals. The delineation of molecular basis of metal uptake and tolerance in metal-accumulating species may allow rating their metal cycling and host-protection/stimulation capacity, with certain significance for bioremediation purposes. In the sporocarps of ectomycorrhizal fungi grown in pristine environments is the Ag hyperaccumulation threshold of 100 mg kg⁻¹ only rarely exceeded and Zn levels typically range from 50 to 150 mg kg⁻¹. This report focuses on the handling of metals in species that surpass these limits: Ag-hyperaccumulating Amanita strobiliformis, in which copper transporters (CTRs) recognize also Ag for transport, and all the accumulated Ag occurs complexed by metallothioneins (MTs); Zn accumulators of Russula spp., particularly R. atropurpurea, which has a high affinity ZIP transporter for the acquisition of Zn, and CDF transporters for compartmentalization and unusual MT-like peptides for complexation [of a substantial portion] of overaccumulated Zn; and Hebeloma mesophaeum that preferentially funnels excess Zn (and Cd) into subcellular compartments, although it has the capacity to produce MTs. A brief overview of the inventory of metal-related functions identified in course of transcriptome sequencing projects will be also presented.

Work currently supported by the Czech Science Foundation (16-15065S).

Keywords: ectomycorrhizal fungi, heavy metal, metal uptake, metal binding, metal transporters

ST+P (ID 233)

Arbuscular mycorrhizal fungi affect legume tree uptake of trace metal from substrate with coal-mine tailings

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Arbuscular mycorrhizal fungi (AMF) help plant growth in the restoration of areas degraded by mining. In Brazil, where coal mine tailings lower soil pH and increase trace metal availability, AMF are being tested for land restoration with native trees. *Enterolobium contorstisiliquum* and *Parapiptadenia rigida* were grown in a substrate containing coal mine tailings, which increased Cu, Zn, and Mn availability (11±0.4, 42±2.5, and 64±2.2 mg kg⁻¹, respectively). Plants were inoculated with *Rhizophagus clarus* (isolate SCT720A = Rc) or *Acaulospora colombiana* (isolate SCT115A = Ac). Seedling Dickson quality index, remediation efficiency (ratio between total amounts of each element in plant and soil) and bioaccumulation factor (ratio between concentrations of each element in plant and soil) were evaluated.

Rc promoted higher colonization, but Rc-inoculated plants did not differ from control while Ac increased seedling quality of both plant species. Rc promoted higher content, remediation efficiency and bioaccumulation of Cu in both plants species, while Ac-inoculated plants decreased those indexes, mainly in E. contorstisiliquum. Zn extraction depended on plant-fungus combinations; Rc increased remediation efficiency and bioaccumulation of Zn in E. contorstisiliquum, while Ac decreased these values. Ac increased both indexes for Mn in P. rigida, which has a 10-fold higher Mn concentration than E. contorstisiliquum.

Selection of AMF is necessary, due to differences in isolates ability to increase trace metal uptake by woody plants.

Improvement of plant growth and extraction of trace elements from the soil are independent abilities of mycorrhizal fungal isolates and also depend on symbiont combinations

Keywords: pacara earpod tree, cola waste, zinc, copper, manganese

ST+P (ID 144)

Exo-chitinolytic activities: phylogenetic conservatism of a functional trait in the ecto-mycorrhizal fungi

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Chitin is one of most abundant organic and renewable biopolymers in soils and ecologists have suggested that it is also a recalcitrant compound in fungal necromass that compose soil organic matter (SOM). In fungi, chitinases play important biological and physiological roles, including autophagy, nutrition, morphogenesis or biotic interaction functions.

While ectomycorrhizal (ECM) fungi have been described as a source of dead fungal biomass in forest soils, they are also able to product high exo-chitinolytic activities and they could decompose the necromass of other fungal ecological guilds. In this study, we aimed to measure the exo-chitinolytic activities of a large panel of ECM fungal strains and to evaluate the potential phylogenetic conservatism of this functional trait within more than twenty different genera.

A functional screening was applied on 40 ECM fungal species mainly belonging to Basidiomycota. The analysis of the regulation of exo-chitinolytic activities clearly separated two major Orders of ECM fungi: Boletales and Agaricales. Particularly, Agaricales strains (n=16) studied in this functional screening revealed an overexpression of the N-acetylglucosaminidase activity in presence of chitin. Conversely, Boletales strains (n=14) shown a negative feedback of this activity in the presence of the substrate. The potential involvement of this chitinolytic activity for fungal and host-plant nutrition has been studied with experiments monitoring the regulation of these activities during C / N starvation.

Finally, the phylogenetic conservatism of this functional trait will be discuss in relationship with different biological and physiological roles of ECM fungi.

Keywords: functional trait, chitinase, regulation, phylogenetic conservatism

ST+P (ID 101)

Switching from ammonium to proteins as main nitrogen source induces the Fenton reaction in ectomycorrhizal fungi

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Hydroxyl radicals produced by the Fenton reaction are known to participate in the extracellular decomposition of lignocellulose by brown rot wood decomposing fungi. In these fungi, nitrogen starvation triggers ligninolytic activity. Recently, the Fenton reaction has been shown to be involved in the decomposition of plant litter by ectomycorrhizal fungi as well. However, it is still unclear under which environmental conditions the Fenton reaction is induced in ectomycorrhizal fungi.

We investigated whether the production of hydroxyl radicals by *Paxillus involutus* can be induced under different nitrogen nutritional conditions. *P. involutus* cultures were grown on nitrogen-free or nitrogen containing organic model compounds that resemble major components of plant litter in the presence or absence of ammonium.

Results show that the induction of the Fenton reaction in *P. involutus* coincides with a switch from ammonium to proteins as the main nitrogen source. This specific induction of the Fenton reaction by nitrogen nutritional conditions suggests that the Fenton reaction has evolved in ectomycorrhizal fungi to improve nitrogen acquisition under ammonium-limiting conditions. Potential mechanisms by which the Fenton reaction may improve nitrogen acquisition by ectomycorrhizal fungi are discussed.

Keywords: ectomycorrhiza, ammonium, proteins, fenton reaction

ST+P (ID 241)

Mechanisms of generalist host range in the ectomycorrhizal symbiosis

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Introduction/Aim: Most ectomycorrhizal fungi can associate with many species of host plant, often simultaneously, but it remains unclear how these fungi maintain relationships with such diverse partners. We have presented a generalist ectomycorrhizal fungus, *Thelephora terrestris*, with six distantly related host plants. We are interested in two main questions: How does the fungal chemical strategy, particularly its use of effectors, change with each new host plant? And does its quality as a mutualist vary across hosts?

Materials and methods: We are using RNA sequencing and stable isotope labeling to answer these questions. We are currently enriching plants and fungi with carbon-13 and nitrogen-15 to track resource exchange, and harvesting plant, fungal, and symbiotic tissue for RNA sequencing. Transcriptomic analysis of infected roots will reveal fungal genes that are differentially expressed relative to soil hyphae and across different hosts.

Results: We hypothesize that fungal gene expression and symbiotic performance will be most similar on closely related hosts, and will become less similar with increasing phylogenetic distance among the plants. We anticipate that a core symbiotic gene set will be expressed across host environments, while putative effectors and signaling-associated genes will differ by host.

Discussion: Measuring gene expression in symbiotic tissues while tracking resource allocation will reveal genes associated with generalist host preference and improved symbiotic performance, providing a powerful link between gene expression, physiological function, and the ecology of these important mutualists.

Conclusion: This project will provide insight into how generalist host range works in ectomycorrhizal symbiosis.

Keywords: ectomycorrhiza, mutualism maintenance, stable isotope enrichment, transcriptomics, host generalism

ST+P (ID 15)

Preference for mycorrhizal or direct P uptake pathways of maize plant can be adjusted by soil P levels

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Arbuscular mycorrhizal(AM) plants have two pathways to acquire phosphorus(P) from soil, i.e., the direct pathway (DP). The two pathways interact each other when they supply P to and gain photosynthate from shoots, which forms an integrated P acquisition efficiency (PAE) of a mycorrhizal plant. Understanding the interplay mechanisms between DP and MP is crucial issues. We tested the following hypothesis that PAE of MP and PAE of DP might be different in response to soil available P levels, which may adjust plant preference for the two pathways in acquiring P from soil. We selected two maize cultivars, an old landrace (HMY, released before 1950s) and a hybrid (XY335, released in 2000s) and used 32P labeling combined with qPCR method to quantify PAE basing on per unit length of root (PAE-DP) or hyphae (PAE-MP) and the expression of the plant PHT genes involved in the DP (ZmPHT1;1, ZmPHT1;3 and ZmPHT1;4) and MP (ZmPHT1;6) at 4.5 (low), 8 (medium and optimized P level for maize) and 50 (high) mg kg-1 Olsen-P levels. Our results showed that PAE-DP was the highest at high Olsen-P level, while the PAE-MP was the highest at medium olsen-P level. The preference index which was defined as the ratio of P uptak of MP against P uptake of DP was higher than 1 at the medium Olsen-P level. We concluded that Olsen-P level influence plant trade-off between two pathways in term of P acquisition, plant prefers MP at a medium P level.

Keywords: mycorrhizal P uptake pathway, direct P uptake pathway, preference, P acquisition efficiency, soil Olsen-P

ST+P (ID 231)

Novel functions of fungal TOK channels: putative roles in symbiotic potassium transfer

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Nutrient exchanges are an essential characteristic of mycorrhizal symbioses (Garcia et al., 2016). One of the elements involved in this dialog between the plant and the fungus is potassium (K⁺), a major nutrient which is not only responsible for many cell processes but also plays a role in the tolerance to environmental stress. Previous research has stated that the ectomycorrhizal (ECM) fungus Hebeloma cylindrosporum greatly enhances K⁺ absorption of its host *Pinus pinaster* when it is scarce in the culture medium (Garcia et al., 2013). Our aim is to characterize the fungal proteins that transport K⁺ in the ECM symbiosis, focusing on the fungus-plant interface. TOK genes coding for twopore K⁺ channels with outwardly rectifying activity in yeast (Ketchum et al., 1995) have been identified as good candidates for the transfer of K⁺ towards the plant. We have studied the three H. cylindrosporum TOK channels (HcTOK1, HcTOK2.1 and HcTOK2.2) with multiple approaches. Their function has been analysed by two-electrode voltageclamp and yeast complementation, while their subcellular and tissue localization have been unraveled by expression in yeast, in situ hybridization and confocal microscopy. We have also analysed their physiological role in the ECM using pine seedlings inoculated with HcTOK overexpressing lines. Furthermore, we count on transcriptomic data of their expression in pure culture and ECM (Doré et al., 2015, 2017). Our results suggest that mainly HcTOK2.2 is likely to be involved in symbiotic K⁺ transfer to the plant, while the others could play different roles in the fungus.

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Keywords: Ectomycorrhizal symbiosis, *Hebeloma cylindrosporum*, plant potassium nutrition, two-pore potassium channels (TOK)

ST+P (ID 143)

Fungal necromass in soil forest: are ectomycorrhizal fungi scavengers?

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In temperate forests, dead wood is a relevant source of nutrients for lignolytic fungi, but it hosts also an important abundance of ectomycorrhizal (ECM) species during the late decay stages.

Consecutively, it has been suggested that ECM fungi could be involved in the decomposition of the lignolytic fungal necromass (Buée et al. 2007). Because fungal necromass is significantly composed of chitin, we tested the N-acetyl-glucosaminidase activity of ECM fungi in presence of pure chitin and fungal necromass (*Postia placenta*). Then, in sterile pure culture, we monitored the capacities of the ECM fungus *Paxillus involutus* to mobilize C and N from the labelled necromass of *P placenta*. Finally, in an axenic microcosm experiment, the transfers of ¹⁵N and ¹³C from labelled fungal necromass (*P. placenta*) were measured within the different compartments of ectomycorrhized tree (*P. involutus* X *Pinus sylvestris*.).

The significant ¹⁵N and ¹³C labelling of *P. involutus* mycelium showed that this ECM fungus was able to mobilize C and N from the necromass of *P. placenta*. Nevertheless, the high ¹⁵N enrichment in the different parts of the host-plant revealed that carbon remains mainly in the ECM fungal compartments (extrametrical mycelium and ECM root tips) and proved that the ECM fungus transferred only N from the necromass to the tree.

These results demonstrate that dead mycelium can be a nutrient source for the ECM fungus and the associated host tree.

Keywords: fungal necromass, chitinolytic activity, 15 N / 13 C labelling, C / N transfert, decomposition

IL (ID 20)

The Ties That Bind: The Arbuscular Mycorrhizal Contribution to Plant Potassium Nutrition

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Potassium (K⁺) supply for crops is becoming an emerging issue in agriculture due to the constant increase of K⁺-deprived soils across the globe. K⁺ participates in various crucial processes such as plasma membrane polarization, growth, stomatal aperture or adaptation to environmental changes.^{2,3} Plant K⁺ use efficiency can be improved by understanding the strategies developed by plants to cope with K⁺ deprivation, including their interactions with soil microbes. 4,5 We used the model association between Medicago truncatula and Rhizophagus irregularis to unravel the contribution of the arbuscular mycorrhizal (AM) symbiosis to the physiological and transcriptional responses of roots to K⁺ deprivation.⁶ We showed that AM plants display a higher K⁺ content than non-mycorrhizal ones under K⁺-limiting conditions. Non-mycorrhizal plants displayed a reduction of root biomass, and an increase in sodium concentration compared to AM plants. Using RNA-Seq, we identified candidate genes specifically regulated in AM roots, including one putative K+/H+ transporter. The AM status of M. truncatula prevented part of the responses normally triggered during K⁺ deprivation. These results indicated that the AM symbiosis helps the plant to cope with this abiotic stress. Finally, we found that two M. truncatula accessions with very different genetic backgrounds are not similarly affected by K⁺ deprivation, revealing different adaptation strategies. Investigating the impact of AM symbiosis on physiological and transcriptional responses to K⁺ deprivation in M. truncatula accessions will allow understanding how the genetics of the host can affect the mycorrhiza-dependent K⁺ acquisition.

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IL (ID 95)

Molecular mechanism of long-distance phosphorus transport in arbuscular mycorrhizas as revealed by virus-induced gene silencing

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Arbuscular mycorrhizal (AM) fungi translocate polyphosphate over a long distance to deliver to the host, which is a major rate-limiting step of phosphate delivery through the mycorrhizal pathway. So far, only a simple diffusion model has been applied to solute transport in filamentous fungi. More than three decades ago, however, deceleration of phosphate delivery to the host due to suppression of host transpiration was observed, leading to the hypothesis that transpiration provides a primary driving force for the delivery processes via creating water flow through hyphae in which fungal aquaporin(s) is involved.

Lotus japonicus was inoculated with Rhizophagus clarus and grown in the mesh-bag two compartment culture system, and the virus-induced gene silencing technique was applied for knockdown of a fungal aquaporin gene. R. clarus aquaporin 3 (RcAQP3) was most highly expressed in intraradical mycelia, and heterologous expression in yeast showed that RcAQP3 is responsible for water transport activity across the plasma membrane. Knockdown of RcAQP3 with the cucumber mosaic virus vector, as well as the suppression of host transpiration, decelerated polyphosphate translocation in proportion to the levels of knockdown and suppression, respectively, supporting the hypothesis.

In this study, the feasibility of virus-induced gene silencing in AM fungi by a plant viral vector, in which transformation of plant/fungus is unnecessary, has been demonstrated. Further, given that AM fungi colonize multiple plants under natural conditions, our findings provide a new interpretation for the regulatory mechanism of nutrient allocation from the fungi to the plants, highlighting the importance of plant transpiration.

Keywords: arbuscular mycorrhizal fungi, aquaporin, polyphosphate, translocation, virus-induced gene silencing

CL (ID 5)

Beyond nutrients: A meta-analysis of the effects of arbuscular mycorrhizal fungi on plants and soils

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Arbuscular mycorrhizal fungi (AMF) can increase plant fitness under certain environmental conditions. Among the mechanisms that may drive this mutualism, the most studied is provisioning of nutrients by AMF in exchange for carbon from plant hosts. However, AMF may also provide a suite of non-nutritional benefits to plants including improved water uptake, disease resistance, plant chemical defense, soil aggregation, and allelochemical transport and protection. Here, we use a meta-analysis of 93 studies to assess the relative effect of AMF on nutritional and non-nutritional factors that may influence plant fitness. We find that the positive effects of AMF on soil aggregation, water flow and disease resistance are equal to the effect of AMF on plant nitrogen and phosphorus uptake. However, AMF had no effect on the uptake of other nutrients, plant water content, allelopathic transport or production of chemical defense compounds. We suggest future research directions, including experimentally assessing the relative contribution on plant fitness of AMF interactions by untangling the independence of alternative benefits of AMF from an increase in nutrient uptake. This will lead to a more holistic view of the mycorrhizal-plant association and a more accurate picture of the net impact on the plant or plant community in question.

Keywords: meta-analysis, disease resistance, soil aggregation, nutrient uptake, water flow

CL (ID 76)

Root transportome in mycorrhizal Medicago truncatula - the sweet part

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Carbon (C) fluxes in mycorrhizal symbiosis remain elusive in spite of rapid progress in molecular biology and long research focus on this subject and the fact that large amounts of C are possibly transferred from plant to the fungus across the symbiotic interface. Here we aimed at identification of genes involved in C transfer within M. truncatula - Rhizophagus irregularis symbiosis by means of gene expression profiling of mycorrhizal and nonmycorrhizal plants, both colonized by compatible rhizobia. To this end we identified a couple of gene candidates using Affymetrix microarrays and Genevestigator search tools. Further, we designed qPCR markers for these genes and conducted a well-replicated time-series analysis as well as a short-term shading experiment to specifically address regulation of expression of the focal genes. Whereas expression of some of the recently described SWEET transporters were not significantly upregulated by mycorrhizal symbiosis, the expression of others indicated their possible role in mycorrhiza carbon exchanges – and appeared to follow a similar trend as expression of the mycorrhiza-specific phosphate transporter MtPT4. Other (plastid-bound) transporters appear to be differentially upregulated in shoots and roots of the plants by mycorrhizal symbiosis. Now, it appears important to ascertain the indispensability/importance of the individual genes in functioning of mycorrhizal symbiosis by targeted mutagenesis/gene silencing. Further, response of the gene transcription shall be described to shifting environmental conditions (e.g. fertilization, shading, interplant competition) in order to match their transcription profile to previous ecophysiological observations of the dynamics of mycorrhizal C fluxes.

Keywords: gene activity, transcriptome analysis, *Medicago truncatula*, shading, carbon flux

CL (ID 45)

A novel plant-fungus symbiosis benefiting the host without forming mycorrhizal structures

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Most terrestrial plants form mutually beneficial symbioses with specific soil-borne fungi known as mycorrhiza. In a typical mycorrhizal association, fungal hyphae (i) colonise plant roots, (ii) explore the soil beyond the rhizosphere and (iii) provide host plants with nutrients that might be chemically or physically inaccessible to root systems. In contrast to these common features, we report a plant growth promoting symbiosis between the basidiomycete fungus *Austroboletus occidentalis* and jarrah (*Eucalyptus marginata*) which has quite different characteristics. By combining nutritional, radioisotopic (³³P) and genetic approaches, we elucidate the nature of this novel symbiosis. We show (i) the fungal partner does not colonise plant roots, (ii) hyphae are localised to the rhizosphere soil (and vicinity) and consequently (iii) do not transfer nutrients located much beyond the rhizosphere. This novel symbiosis challenges our current view of mycorrhizal associations and rhizosphere ecology and may lead to a reassessment of ectomycorrhizal relationships.

CL (ID 86)

A multi-element stable isotope natural abundance approach indicates partial mycoheterotrophy for Central European arbuscular mycorrhizal *Equisetum* species

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Introduction/Aim: Mycoheterotrophy occurs in orchid and ericoid but also in arbuscular mycorrhizal (AM) plants. In most cases, these plants acquire carbon and nutrients from a fungus associated with another mutualistic mycorrhizal partner. While achlorophyllous gametophytes of basal AM-plants were unmasked as fully mycoheterotrophic evidences for partial mycoheterotrophy in AM-plants are currently scarce, even though in orchids partial mycoheterotrophy is ubiquitous. *Equisetum* species are considered as at least habitat-dependent AM-plants. The aim of this study was to evaluate the mycoheterotrophic status of the last recent representatives of the carboniferous Equisetaceae.

Materials and Methods: Microphylls and spread leaves of six *Equisetum* species and fully autotrophic co-occurring plants (FAP) were collected in NE-Bavaria, Germany. Carbon and nitrogen stable isotope natural abundances were analysed. Additionally, for *E. palustre* and *E. sylvaticum* and their FAPs hydrogen and oxygen stable isotopes were measured.

Results: Microphylls of *Equisetum* were enriched in ¹³C and ²H compared to FAPs. They also were more enriched in ¹⁵N (except for *E. arvense*) and mostly had higher nitrogen concentrations than FAPs.

Discussion: The isotope abundance and nitrogen concentration patterns found for *Equisetum* are known from green orchids and pyroloids to indicate partially mycoheterotrophic carbon and nutrient gain. Thus, *Equisetum* has to be considered as a new clade of partially mycoheterotrophic plants.

Conclusion: During the era of the carboniferous coal forests 30-m-tall scale bark trees overtopped 10-m-tall *Equisetum* ancestors. The potential of mycoheterotrophy could explain their carbon gain under these light-limited conditions.

Keywords: arbuscular mycorrhiza, mycoheterotrophy, Equisetaceae, stable isotope (C, N, O, H)

POSTERS

P (ID 81)

Arbuscular mycorrhizal fungi improve the growth, nodulation and phosphorus uptake of beans plants fertilized with compost of rock phosphate fed dung

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The combined use of compost and beneficial soil microbes like arbuscular mycorrhizal fungi (AMF) are the possible substitutes to commercial fertilizers for increasing plant growth and phosphorus (P) solubility in P deficient soils. In view of this, completely randomized design experiment with six replicates per treatment was conducted in pots at greenhouse conditions. Inoculation effect of AMF on P solubility from composted rock phosphate (RP) fed dung and their successive uptake by mung bean plants was assessed in alkaline silty clay loam soil. The native powdered RP fed dung compost was used alone or/and in combination with inoculum having six different species of AMF, although the idea of composted fed dung with AMF has never been confirmed before. The results showed that the association of AMF with RP composted fed dung had a positive effect on mung bean plants growth parameters, chlorophyll (a, b), carotenoid contents and nutrients uptake (N and P) unlike to the plants in control. Similarly, the percent roots colonization and nodulation of mung bean plant roots and their post-harvest soil properties were also improved by the inoculation of AMF alone and in combination with composted RP fed dung. It is concluded from this study that the combined application of AMF with composted RP fed dung has almost the same effect as commercial fertilizers for improving mung bean plants growth and their nutrients uptake. Moreover, AMF inoculants have a great prospect to be used as a suitable biofertilizers for improving P solubility from compost in alkaline soils.

Keywords: mycorrhizal fungi, fed dung compost, phosphorus uptake, Mung bean, colonization and nodulation

P (ID 133)

Mobilization of goethite-bound orthophosphate and phytate by *Lycopersicum esculentum* mycorrhized with *Rhizophagus irregularis*

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Phosphorus (P) is an essential element for food production but its availability is often restricted. P can be fixed in large quantities in soil by complexation with minerals, which imposes problems in agricultural production. We explored the mobilization of free and goethite-bound orthophosphate (OP) and phytate (PA) by the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* in order to evaluate the possible role of AM in improving the P availability for the host plant by a 91 day mesocosm experiment. Our hypothesis was that (i) AM can provide substantial amounts of P from these complexes to the host plant, and that (ii) the plants investments of photosynthates in turn are higher the less accessible the P source is.

All AM plants incorporated P from the four sources, in decreasing order of their assumed bioavailability (OP 30.4 %, PA 10.4 %, goethite-OP 6 %, and goethite-PA 2.1 % of the total P offered, respectively). All plants that had access to a P source showed significantly higher photosynthetic rates. $16:1\omega$ 5c PLFA and NLFA AM biomarker showed a significantly higher accumulation when P was mobilized from the goethite complexes as well as a positive correlation between the P delivered to the plant and the uptake rates over the four P sources.

This is the first report where an organic P source bonded to a secondary mineral has been mobilized via the AM uptake pathway and made available for the plant, suggesting mycorrhizal management of crops as a promising option in P efficient sustainable agriculture

Keywords: goethite, phytate, secondary mineral, phosphorus, arbuscular mycorrhiza

P (ID 172)

Nurturing or abusing the children? Mycorrhizal network effects on pine seedling performance and root endophytes

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Research on common mycorrhizal networks indicate a potential for facilitation, resource sharing, and even communication between trees mediated by connections to the soil ectomycorrhizal fungal mycelium. We set out to examine the effect of connection to the mycorrhizal network on fitness and resource exchange between pine seedlings and their mycorrhizal fungi. We performed two experiments in Northern Sweden using 1-year-old scots pine seedlings outplanted into the field,

In experiment 1 we utilized forest clearings to manipulate light availability and connection strength to the common mycorrhizal network. Trenching increased the dominance of ectomycorrhizal fungi, greatly increased the recovery of ¹⁵N tracer (added to the soil) in pine needles, and moderately increased foliar N contents. Shading was associated with significantly higher N transfer to the host. Trenching and light availability had relatively minor effects on the ectomycorrhizal community, but greater distance from mature trees significantly altered the root mycorrhical community.

In experiment 2 we utilized root and mycorrrhizal exclusion manipulations across nitrogen fertilization plots. Root exclusion had no effect while root + mycorrhizal exclusion had a significant effect on ectomycorrhizal community composition. High N additions significantly impacted root fungal communities and increased seedling mortality, while moderate N additions had no effect on root fungal communities. Root exclusion was associated with increased seedling nitrogen content and biomass, while root + mycorrhizal exclusion was not.

Our results indicate benefits of connections to common mycorrhizal networks, costs of competition with mature trees, and mycorrhizal control over nitrogen for carbon exchange with plants.

Keywords: common mycorrhizal network, Pinus sylvestris, boreal forest, nitrogen

P (ID 177)

Growth, nodulation and nutrient concentrations of lucerne in response to inoculation with abscular mycorrhizal fungus and rhizobium

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Acid soils are P deficient and widespread in South China. Inoculation with acid-tolerant rhizobia and abscular mycorrhizal fungi could be considered as one of important strategies to improve the growth and nutrition (especially nitrogen and phosphorus) of lucerne. Therefore, a greenhouse pot experiment examined the effects of inoculation with Glomus intraradices Smith & Schenck (Beg number 141) and acid-tolerant Sinorhizobium meliloti (strain 1.163) on growth, nodule formation and nutrient concentrations in the shoots of lucerne (cultivar Victoria) grown in an acid soil (pH 5.5) with a factorial design with 6 replications. Shoot biomass of lucerne changed in the sequence: mixture inoculation of S. meliloti and G. intraradices > S. meliloti inoculation > Glomus intraradices inoculation > control. Few nodules were formed on the roots of control and G. intraradices inoculated seedlings, while the seedlings nodulated well when inoculated with S. meliloti, particularly with S. meliloti and G. intraradices together. Compared with sole S. meliloti inoculation, G. intraradices increased the number of root nodules/plant but reduced both mean nodule weight and total weight of nodules/plant in the presence of S. meliloti. Higher P concentration were detected in shoots of lucerne with G. intraradices inoculation than control. S. meliloti inoculation significantly increased both N concentrations and accumulation in shoots of lucerne, particularly inoculated with S. meliloti and G. intraradices together. These results indicated that inoculation with G. intraradices and acid-tolerant S. meliloti together boosted production of both biomass and protein formation of lucerne when grown in acid soils.

Keywords: rhizobia, abscular mycorrhizal fungus, lucerne, nodule

P (ID 178)

Huge differences in abilities of thirteen *Suillus* species to mobilize potassium and promote the growth of *Pinus massoniana* seedlings

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Growth, oxalate efflux, potassium (K) absorption and soil K depletion by thirteen of Suillus species originally isolated from Pinus massoniana forests in Wuling Mountain, Chongqing, China, were compared in pure liquid culture using soil as sole K source. Then eight of ectomycorrhizal fungi with high and low K accumulation rates from the soil substrate in the *in vitro* experiment were then selected to inoculate *Pinus massoniana* seedlings grown in the same soil to study the growth and K utilization by mycorrhizal trees. Ectomycorrhizal fungi varied in growth rate, K accumulation and oxalate efflux. The fungal growth reflected K uptake and thus there was a positive correlation between fungal biomass and K absorption (r = 0.934, P < 0.01). The highest concentration of oxalate in fungal culture solution was about six times that of the lowest. Oxalate effused from the fungi may have led to biological weathering of the minerals in soil, especially under conditions of limited exchangeable K supply. Mycorrhizal plants had higher biomass and extracted more K from the soil than non-mycorrhizal control. Fungal K uptake in vitro showed a significant correlation with plant K uptake (r = 0.847, P < 0.05). Therefore, the patterns and mechanisms of K extraction from soil pools by the fungal isolates might be similar to those exhibited by the mycorrhizal plants. Mycorrhizas decreased significantly K in mineral structures unavailable to non-mycorrhizal plants, indicating the importance of the fungi in K mobilization and utilization by mycorrhizal trees.

Keywords: ectomycorrhizal fungi, potassium, mobilization, oxalate

P (ID 225)

Do Horticultural Plant Species are Depending on Mycorrhizal Inoculation?

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Since 1995 several field experiments were conducted to search effect of selected and indigenous mycorrhizae spores for horticultural plant species production and nutrient uptake in the Mediterranean region. The effect of several mycorrhizal species inoculation on seedling survives and plant growth also has been studied. Mycorrhizal dependency and growth response of several plants were calculated under several P level applications with several selected exotic mycorrhizae species.

Control, *G. Mosseae*, *G. Etunicatum*, *G. Clarium*, *G. Caledonium*, Indigenous Mycorrhizae, and Cocktail mycorrhizae species used for, Solanaceae, Cucurbitaceae and Alliaceae family. In another experiment with and without P application were used with and without mycorrhizal inoculation. After several years under field experiment, mycorrhizal inoculation effectively infected plant root and increased plant yield. Since in the area soils have phosphorus and zinc deficiency usually plant tissue P and Zn concentration is under critical levels. And with mycorrhizae inoculation P and Zn concentration erased up to critical levels.

Under filed conditions for several years with several mycorrhizae specials plant response to mycorrhizae depend on soil ecological conditions, mycorrhizae and plant species. After several years' field experiments, it has been concluded that for horticultural fruits plants are significantly response to mycorrhizal species. In general, horticultural fruits plants such as melon, watermelon, cucumber, pumpkin, green pepper, eggplant, onion, garlic, leek are mycorrhizal dependent plants. Dependency is pronounced more for P nutrition rather than Zn nutrition.

This work was supported by TÜBİTAK-TOVAG-1120785 projects

Keywords: mycorrhiza dependency, horticultural plants, mycorrhizae species

P (ID 308)

Rhizophagus irregularis improves Pi acquisition by Medicago truncatula plantlets in presence of benzo[a]pyrene under semi-hydroponic cultivation system

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The release in soils of benzo[a]pyrene (B[a]P) causes detrimental effects on flora and fauna, and could affect human health. Nowadays, remediation of polluted soils with plants (i.e. phytoremediation) represent a cost-effective and environmental-friendly strategy. However, its success depends on the plant resistance/tolerance against pollutant. One option to improve this plant resistance/tolerance against B[a]P is the association with arbuscular mycorrhizal fungi (AMF). These fungi are well-known to improve plant mineral nutrition under non-stressed conditions and increase plant resistance/tolerance to B[a]P. However, it remains mostly unknown whether and how AMF could help plants to acquire Pi in presence of pollutant.

A semi-hydroponic circulating system was developed to study (1) impact of B[a]P on the Pi uptake by non-mycorrhized (NM) or pre-mycorrhized (PM) *Medicago truncatula* plantlets with the AMF *Rhizophagus irregularis* and (2) if the presence of AMF could help plant to acquire Pi in presence of B[a]P. Pi depletion in a nutritive solution circulating through plant containers during 48h as well as P concentration in plantlets were measured using ICP-AES.

In presence of B[a]P, plant growth as well as short-term Pi acquisition was decreased in plantlets. Interestingly, root colonization with the AMF was not affected by B[a]P and resulted in a better Pi uptake by PM plantlets in comparison with NM plantlets. As a consequence, B[a]P-stressed PM plantlets accumulated more P than NM plantlets.

The innovative semi-hydroponic cultivation system developed was adequate for shortand long-term studies of Pi acquisition by PM plants, allowing the possibility to measure other nutrients under diverse environmental conditions.

Keywords: phosphorus, polyaromatic hydrocarbons, semi-hydroponic circulation system, arbuscular mycorrhizal fungi, Medicago truncatula

P (ID 337)

Allocation of nitrogen and carbon is regulated by nodulation and mycorrhizal networks in soybean/maize intercropping system

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Soybean/maize intercropping has remarkable advantages in increasing crop yield and nitrogen (N) efficiency. However, little is known about the contributions of rhizobia or arbuscular mycorrhizal fungi (AMF) to yield increases and N acquisition in the intercropping system. Plus, the mechanisms controlling carbon (C) and N allocation in intercropping systems remain unsettled. In the present study, a greenhouse experiment combined with 15N and 13C labeling was conducted using various inoculation and nutrient treatments. The results showed that co-inoculation with AMF and rhizobia dramatically increased biomass and N content of soybean and maize, and moderate application of N and phosphorus largely amplified the effect of co-inoculation. Maize had a competitive advantage over soybean only under co-inoculation and moderate nutrient availability conditions, indicating that the effects of AMF and rhizobia in intercropping systems are closely related to nutrient status. Results from 15N labeling showed that the amount of N transferred from soybean to maize in co-inoculations was 54% higher than that with AMF inoculation alone, with this increased N transfer partly resulting from symbiotic N fixation. The results from 13C labeling showed that 13C content increased in maize shoots and decreased in soybean roots with AMF inoculation compared to uninoculated controls. Yet, with co-inoculation, 13C content increased in soybean. These results indicate that photosynthate assimilation is stimulated by AM symbiosis in maize and rhizobial symbiosis in soybean, but AMF inoculation leads to soybean investing more carbon than maize into common mycorrhizal networks (CMNs). Overall, the results herein demonstrate that the growth advantage of maize when intercropped with soybean is due to acquisition of N by maize via CMNs while this crop contributes less C into CMNs than soybean under co-inoculation conditions.

P (ID 346)

Role of plant-fungal nutrient trading and host control in determining the competitive success of ectomycorrhizal fungi

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Multiple ectomycorrhizal fungi (EMF) compete to colonise the roots of a host plant but it is not known whether their success is under plant or fungal control, or a combination of both. We assessed whether plants control EMF colonization by preferentially allocating more carbon (C) to more beneficial partners in terms of nitrogen (N) supply or if other factors drive competitive success.

Using a microcosm-based system, we combined stable isotope labelling and RNA sequencing approaches to characterize nutrient exchange dynamics between the plant host *Eucalyptus grandis* and three *Pisolithus* isolates when growing alone and when competing either indirectly (with a physical barrier) or directly.

Overall, we found that N provision to the plant does not explain the amount of C that an isolate receives nor the number of roots that it colonises. Differences in nutrient exchange among isolates were related to differences in expression of key fungal and plant N and C transporter genes. When given a choice of partners, the plant was able to limit colonization by the least cooperative isolate and therefore influence the outcome of EMF competition. This reduction in colonization was not explained by a reduction in allocated C. Instead, our results suggest that partner choice in EMF could operate through the upregulation of defence-related genes against those fungi providing fewer nutrients, a mechanism that deserves further investigation.

Keywords: preferential allocation, isotope labelling, RNA sequencing, *Pisolithus, Eucalyptus*

P (ID 356)

Physiological and molecular analyses of extraradical mycorrhizal mycelium produced in an in vivo whole-plant system

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Arbuscular mycorrhizal (AM) fungal extraradical mycelium (ERM) represents the fundamental structure involved in mineral nutrient absorption and translocation from the soil to host plants. With the aim of obtaining *in vivo* produced ERM, a whole-plant experimental system was devised, characterized by the physical separation of the roots from the substrate and of ERM from the roots.

Plant roots were wrapped in a nylon net to obtain a flat mesh pocket, allowing the spreading of ERM from the nylon net onto membranes for its easy collection. Micronutrients content and expression of genes encoding for ammonium transporters (AMT) were analysed in ERM produced by three different AM fungi, *Funneliformis mosseae*, *Rhizoglomus irregulare* and *Funneliformis coronatus*, growing in symbiosis with *Cichorium intybus* var. *foliosum*.

ERM fresh biomass ranged from 1 to 7 mg per plant, depending on the identity of the symbiont. RT-qPCR analyses of AMT genes showed different expression levels in the ERM of the three fungal isolates. ERM analysis by atomic absorption spectrophotometry and laser induced breakdown spectroscopy (LIBS) showed detectable amounts of micronutrients, such as copper, iron, manganese and zinc.

The whole-plant system producing viable and intact ERM represents a useful research tool for morphological, physiological and molecular analyses.

Our experimental tool, successfully mimicking mycorrhizal symbioses occurring in natural conditions, is suitable for a virtually limitless range of AM fungi and host plant species.

Keywords: arbuscular mycorrhizal fungi, extraradical mycelium, transfer of mineral nutrients

P (ID 359)

Arbuscular mycorrhizal colonization enhances organic phosphorus hydrolysis ability of watermelon root

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Organic manure is beneficial to crop quality. Organic phosphorus in manure need hydrolyse to inorganic phosphorus by phosphatases, which secretes from plant root and microbe in rhizosphere. AtPAP10, AtPAP12, AtPAP15, AtPAP26 are coded as secretory phosphatases in arabidopsis root. Arbuscular mycorrhizal colonization enhances phosphorus acquisition in watermelon. It is unclear whether phosphorus acquisition by hydrolysis of organic phosphorus. Pot experiment was carried out to investigate effects of AM colonization on phosphatase activity and coded gene expression in watermelon root. Gene expression of ClaPAP10, ClaPAP12, ClaPAP15 and ClaPAP26 is detected by qPCR method. Acid phosphatase activity on root is determined by 5-bromo-4-chloro-3-indolylphosphate (BICP) staining method. The results showed that arbuscular mycorrhizal colonization up-regulates secretory PAP genes expression in watermelon root. The up-regulated expression of PAPs induces the improvement of the coded acid phosphatase activity. It suggests that AM colonized plant secreted more acid phosphatase to hydrolyse more organic phosphorus in rhizosphere. Therefore AM colonization enhances plant ability of hydrolysis and utilization of organic phosphorus to improve P absorption. It is demonstrated that overexpression of PAP genes increases extracellular organic phosphorus utilization in rice. And the Improvement of phosphatase activity increases phosphorus acquisition in legume. In conclusion, Arbuscular mycorrhizal colonization up-regulates ClaPAP genes expression, enhances hydrolysis ability of organic phosphorus and improves P acquisition in watermelon.

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Keywords: arbuscular mycorrhiza, acid phosphatase, phosphorus, organic manure, watermelon

P (ID 373)

Low-phosphorus conditions increase release of acid phosphatase from extraradical hyphae of *Rhizophagus clarus*

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Arbuscular mycorrhizal (AM) fungi increase plant phosphate (P) uptake. Organic P accounts for 30-80 % of total soil P. Some plants hydrolyze organic P by releasing acid phosphatase (ACP) from roots, especially under low-P conditions. The previous study demonstrated that Rhizophagus clarus releases ACP from extraradical hyphae. However, the effect of soil P level on the ACP release has been unknown. Allium fistulosum were inoculated or uninoculated with R. clarus and grown on a two-compartment pot filled with sterilized andosol to which 0 (P0) or 0.5 g P₂O₅ kg⁻¹ (P5) was applied. Soil solution was collected from the hyphal compartment 40, 45, 50, and 55 days after sowing using mullite ceramic tubes. The solutions were subjected to quantitative assessment of ACP activity by using disodium p-nitrophenylphosphate and to qualitative analysis with ACP activity staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. AM colonization, shoot dry weight, and shoot P concentration were also measured. AM colonization in mycorrhizal treatment was 70-80%. ACP activity in the inoculated treatments was higher than that in the uninoculated treatments and further, the activity in the P0-inoculated treatment was higher than that in the P5-inoculated treatments throughout the experiment. ACP at 187 kDa was absent in the uninoculated treatments, but was the main activity in the inoculated treatments, in which staining intensity was likely to be greater at P0 than at P5. These results demonstrate that R. clarus release the ACP from the extraradical hyphae in response to soil P status.

P (ID 381)

Lycopodiella inundata: insights into plant-fungal associations in early vascular plants

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Recent studies have revealed that extant basal vascular plants associate with a wide range of Mucoromycotina and/or Glomeromycota fungi, paralleling the same in non-vascular liverworts and hornworts. This dispels the long-held paradigm that these early diverging lineages harbour Glomeromycota exclusively. Endophytes belonging to both fungal lineages have also been reported, for the first time, in a Devonian plant (*Horneophyton ligneri*). Together these discoveries point to much more diverse plant-fungus interactions in early vascular plants than previously assumed, however our understanding of these remains limited.

In order to gain further insights into these key partnerships, especially those involving the early diverging Mucoromycotina, we are developing the lycophyte *Lycopodiella inundata* as an experimental system. *L. inundata* sporophytes have been shown to harbour solely Mucoromycotina fungi but equally fundamental, the identity of its gametophyte endophyte remains unknown.

Using molecular and cytological approaches, we confirm that young *L. inundata* sporophytes are colonized exclusively by Mucoromycotina and show that the cytology of colonisation – consisting of both inter- and intracellular phases – closely resembles that in Haplomitriopsida liverwort-Mucoromycotina partnerships and the corm of *H. ligneri*. Our current isolation, resynthesis and molecular studies will provide further insights into both host and fungi specificity.

P (ID 382)

P nutrition and AMF inoculation modulate growth and photosynthetic rate of tomato plants

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AMF colonize the roots of most plant species, improving their nutrition (especially the phosphatic one) and promoting photosynthesis. Phosphorus has a key role in plant physiology, also affecting the photosynthetic process, as it is involved in sugar/carbon metabolism. Since AMF can act as a sink of carbohydrates, they can also influence carbon metabolism.

The aim of this work was to evaluate the growth and photosynthetic parameters of *Solanum lycopersicon* plants inoculated or not with *Funnelliformis mosseae* and grown in controlled conditions at different P levels (32, 96 and 288µM) for 40 days.

Fresh and dry weight of different plant organs, mycorrhizal colonization and the concentration of photosynthetic pigments were assessed. Chlorophyll(a) fluorescence, biochemical analysis of thylakoid proteins and fractionation of thylakoid membranes were evaluated in order to assess the photosystem I and II activities. Results were statistically analyzed by one- and two-way ANOVA followed by Fisher's post hoc test.

Root and shoot biomass were positively correlated with P concentration in the growth media. Inoculation did not significantly affect growth parameters, also in the plants grown at $32\mu M$ of P, despite their higher mycorrhizal colonization. AM symbiosis was strongly inhibited at the highest P level. No significant differences were observed between the various treatments for photosynthetic pigment content, while some differences were observed in terms of quantum yield, NPQ (Non-Photochemical-Quencing) and LHCII (Light-Harvesting-Complex) fractionation.

Data relative to chlorophyll fluorescence and western blot analyses suggested that P and AMF affected NPQ and electron transport efficiency, probably modulating the xanthophyll cycle.

P (ID 520)

Biomass allocation patterns in arbuscular mycorrhizal and non-mycorrhizal plants as affected by P supply

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Arbuscular mycorrhizal (AM) fungal root colonisation has frequently been observed to modify plant biomass allocation. Biomass allocation is mainly shifted towards the shoot, but so far no readily interpretable pattern has emerged.

We used data from two separate plant growth response experiments. Subterranean clover was grown in two different soils, supplied with multiple P levels and inoculated or not with the AM fungus *Rhizophagus invermaius*. Regression curves fitted to the responses of shoot P content as well as of root/shoot biomass ratios to increasing levels of added P were plotted against each other to obtain plant P status dependent biomass allocation patterns.

Plants grown under no P limitation had the lowest root/shoot biomass ratios which were similar in mycorrhizal and non-mycorrhizal plants. All plants increased relative allocation to root biomass at growth-limiting P levels. Under these conditions, mycorrhizal plants allocated less biomass towards roots than non-mycorrhizal plants, even when of similar P content.

Based on these results, we hypothesize that only root/shoot biomass ratios greater than that of non-P-limited plants indicate a P-limited plant investment into roots for P acquisition. We further suggest that when comparing this investment between non-mycorrhizal and *R. invermaius*-colonised plants of equal P content, a constant factor is obtained by which severely to slightly P-limited non-mycorrhizal plants invest relatively more into root biomass.

P (ID 406)

Molecular basis for mycorrhizal functioning in plant survival and establishment in acidic soil

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Increased aluminum availability in acidic soil inhibits root elongation and thus water and nutrient uptake. Mycorrhizal formation drastically improves plant survival and establishment in acidic soil, but the molecular mechanism underlying is largely unknown. Here, we employed the dual RNA-Seq approach for comprehensive understanding of the enhanced acid tolerance of mycorrhizal plants. Lotus japonicus MG-20 was inoculated with an acid-tolerant fungus Rhizophagus clarus RF1 and grown at pH 3.5, 3.8, 4.2, and 4.9. in the mesh bag culture system for 7 weeks. RNA was extracted both from extraradical mycelia and mycorrhizal roots, and 150 bp paired-end reads were generated on the illumina NextSeq platform. Root growth was strongly inhibited with decreasing pH, probably reflecting aluminum availability in the soil, while shoot growth and P content were maximum at pH 4.2. Gene expression involved in P uptake and polyphosphate biosynthesis/translocation in extraradical mycelia was significantly correlated with shoot growth, indicating that plant growth in acidic soil highly depends on functioning of extraradical hyphae. Whereas expression of the plant sucrose synthase gene responsible for carbon supply to the fungus was down-regulated at pH < 4.2, during which gene expression of the fungal hexose transporter for hexose uptake in intraradical mycelia was maintained. These results suggest that, although mycorrhizal functioning i.e P uptake and subsequent polyphosphate biosynthesis/translocation in extraradical mycelia largely regulate plant P acquisition in acidic soil, these processes are energy-dependent and thus regulated also by host carbon investment determined by a cost-benefit trade-off of the association.

P (ID 408)

Different responses of various crop species to arbuscular mycorrhizal inoculation and P fertilization

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The responsiveness of plants to arbuscular mycorrhizal (AM) colonization has been suggested to vary with plant species, as well as with environmental conditions such as soil P fertility. The effect of AM inoculation on 9 different crop species was examined in different soil P levels.

Soybean, maize, cucumber, sunflower, welsh onion, komatsuna, carrot, tomato and oat crops (all crops other than komatsuna being AM hosts) were grown in the sterilized soil applied 0.1, 1.0 or 5.0 g P_2O_5 /pot of superphosphate (0.1P, 1.0P, 5.0P). Pots (900 g soil) for AM treatments received 20 g/pot of AM inoculum (Glomus sp. R10, Idemitsu Kosan Co., Japan), whereas control pots received autoclaved AM inoculum (20 g/pot). Ammonium sulfate, potassium sulfate and microelements were applied to all the pots consistently.

Growth of each crops except for komatsuna responded to P application significantly. AM inoculum significantly increased the growth of welsh onion at 1.0P, cucumber at 5.0P, komatsuna at 1.0P and 5.0P, tomato at 0.1P, carrot at 0.1P and 1.0P, and significantly decreased the growth of maize at 5.0P. Responsiveness of soybean, maize, welsh onion, sunflower, tomato and carrot to AM inoculum was higher at lower P levels.

Soil inorganic N was significantly affected by AM treatment, 30-50 % of inorganic N was NO₃-N at welsh onion harvest (8 weeks after sowing) in AM treatment, but almost all the inorganic N was NH₄-N in nonmycorrhizal control. This might be causes of positive responses of nonmycorrhizal komatsuna and negative responses of maize to AM in this study.

Keywords: arbuscular mycorrhiza, responsiveness, P fertilization, inorganic N

P (ID 414)

Contrasting accumulation of Ag and Cu by *Amanita strobiliformis* from two distinct sites

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The ectomycorrhizal mushroom *Amanita strobiliformis* is known to hyperaccumulate Ag in sporocarps, with concentrations up to 1,200 mg/kg in dry weight. However, contrasting accumulation of Ag and Cu was observed at two sites in Prague: Klíčov and Jinonice. At Klíčov, average concentrations of Ag and Cu in fruit-bodies reach 292 mg/kg and 79 mg/kg, respectively. In contrast, average concentrations of both metals at Jinonice are much lower: 72 mg Ag/kg and 30 mg Cu/kg. At their disposal and irrespective of the locality, the fungi have the transporters of the copper transporter (CTR) family to import both Cu and Ag into the cells and metallothioneins to bind and detoxify the accumulated metals in the cytoplasm.

Distribution of mycelium under individual sporocarps at both Klíčov and Jinonice sites was investigated by RT-PCR with specific primers to the soil depth of 30 cm. At both sites, the highest density of mycelium (reaching 247 μ g/g dry soil) was found within the soil depth of circa 0-10 cm.

At both sites, the 206 Pb/ 207 Pb isotopic ratio measured in the sporocarps varied between 1.169 and 1.172 which corresponds to the "labile" Pb fraction in the horizons Ahk at Klíčov and Oak-Ahk at Jinonice where the highest density of mycelium was observed. This would indicate that *A. strobiliformis* preferably accumulates Pb from those soil horizons.

The investigation of metal distribution and mobility in the top-soils at both sites (currently being conducted) will possibly enable us to explain the striking accumulation of metals in *A. strobiliformis* at both sites.

Keywords: Amanita, silver, copper, RT-PCR, accumulation

P (ID 426)

Mycorrhiza alters the expression of phosphate-transporters without changing growth and phosphorus contents in P-starved-soybean plants

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Soybean is a good model to study tripartite symbiosis between plants, arbuscular mycorrhizal (AM) fungi, and rhizobia. Scarce phosphorus availability may constrain nodulation and plant performance. Depending on P deficiency severity AM may overcome plant P demand and positively influence P uptake, nodulation and N2 fixation or result in not net benefits. To evaluate AM influence on the expression of root and nodule phosphate transporters, nodulated soybean plants were exposed to P deficiency (50 µM) and inoculated or not with the AM fungus Glomus macrocarpum. Non-AM plants were grown with sufficient P (500 μM) were also grown; with twelve replicates per treatment. Plants were grown till the flowering stage. Under P-starvation, mycorrhizal plants showed low intraradical colonization and did not differ significantly in terms of growth, nodulation and plant P contents with respect to that non-mycorrhizal, indicating severe P limitation. However, the expression profile of PHT1 transporters was altered by the AM symbiosis, in both roots and nodules. Phosphorus starvation highly induced the expression of several GmPHT in roots and nodules. AM symbiosis significantly repressed GmPHT1;6, 7 and 10 in roots and GmPHT1;3, 5, 7, 8, and 10 in nodules. Interestingly, AM changed acid phosphatase activities in roots and leaves. It is concluded that even under a low degree of colonization the AM symbiosis significantly modulated overall plant responses to P deficiency. In addition, PHT1 expression profile suggested different pathways of Pi acquisition in mycorrhizal and non-mycorrhizal plants during P starvation, even in the absence of plant growth promotion.

Keywords: PHT1 transporters, inorganic phosphate, nodulation, acid phosphatases

P (ID 432)

PHO1-type transporter of arbuscular mycorrhizal fungi mediates phosphate export in the plant-fungal interface

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Arbuscular mycorrhizal (AM) fungi play a predominant role in phosphate nutrition of most land plants. Information about the molecular mechanism of phosphate transfer from the fungi to the host, however, is limited. Here, we demonstrate that a PHO1-type transporter of an AM fungus is responsible for the process by the virus-induced gene silencing technique¹, as well as by a complementation experiment with a model filamentous fungus. Rhizophagus clarus HR1 (MAFF520076) was grown in the mesh-bag separated two-compartment culture system in association with Nicotiana benthamiana. R. clarus PHO1-1 (RcPHO1-1) that is a homologue of the endomembrane-type phosphate exporter gene AtPHO1 was exclusively expressed in intraradical mycelia, and the expression was significantly correlated with that of a mycorrhiza-specific phosphate transporter gene in N. benthamiana. Knockdown of RcPHO1-1 by the plant viral vector decreased plant growth and increased polyphosphate accumulation in intraradical mycelia in proportion to RcPHO1-1 expression levels. Interestingly, the knockdown of RcPHO1-1 also decreased arbuscule development in the roots. An in vitro assay with the rice blast fungus Pyricularia oryzae revealed that phosphate efflux from the fungal cell to the culture medium was decreased in delta-PHO1 mutants of P. oryzae, but was recovered to the level of the wild type in RcPHO1-1-complemented delta-PHO1 mutants. These results strongly suggest that a PHO1-1-type transporter in AM fungi is responsible for phosphate transfer to the host plant in AM symbiosis.

References:

¹Kikuchi et al. (2016) New Phytol 211: 1202-1208

P (ID 433)

Estimation of phosphate acquisition through the mycorrhizal pathway via transcriptome responses

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Arbuscular mycorrhizal fungi deliver P to the host through the mycorrhizal pathway. The contribution of the fungi to P uptake of the plants could easily be assessed in laboratories/greenhouses, but not in the field. Our aim is to establish a plant molecular marker to estimate P uptake through the mycorrhizal pathway. The model plant Nicotiana benthamiana and Welsh onion (Allium fistulosum), a highly mycorrhiza-responsive Allium crop, were inoculated with Rhizophagus clarus HR1 at 0 - 500 spores and grown in the two-compartment culture system. Fifty to one thousand µM P was applied to the root compartment of non-mycorrhizal plants (direct pathway) or to the hyphal compartment of mycorrhizal plants (mycorrhizal pathway) every other day. Total RNA was extracted from the shoots and roots after 6 weeks, and single-end 50-bp sequencing for read mapping (N. benthamiana and A. fistulosum) and paired-end 100-bp sequencing for de novo assembling (A. fistulosum) were performed with Illumina HiSeq. In N. benthamiana a molecular marker consisted of 8 root-expressed genes that are up- or down-regulated specifically in response to P uptake through the mycorrhizal pathway, but not to that through the direct pathway, was constructed by LASSO modeling. A marker with shootexpressed genes could also be constructed, but its accuracy was lower than that of the root marker. In A. fistulosum a comprehensive database that covers expressed genes both in the shoots and roots was constructed for marker development. Our study demonstrates the technical feasibility for the assessment of mycorrhizal functioning in the field.

P (ID 447)

Catalytic properties of vacuolar transporter chaperon 4 (VTC4) protein in arbuscular mycorrhizal fungus *Rhizosphaqus irreqularis*

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Polyphosphate (polyP) is the major storage and translocation form of P in arbuscular mycorrhizal fungi. In the budding yeast *Saccharomyces cerevisiae*, polyP is synthesized by the vacuolar transporter chaperone (VTC) complex localized in vacuolar membrane¹. The complex consists of accessory subunits (VTC1, VTC2 and VTC3) and a catalytic subunit (VTC4). We investigated catalytic properties of VTC4 in an arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *R. irregularis* has *RiVTC1*, *RiVTC2* and *RiVTC4* in the genome², which expressed in both extraradical and intraradical hyphae. We purified the recombinant RiVTC4 using the bacterial protein expression system. RiVTC4 catalyzed polyP biosynthesis using the gamma-phosphate of ATP and GTP as the phosphoryl donor, which exhibited a dual pH optimum of 7.5 and 5.0. The catalytic activity of RiVTC4 required divalent cations including Mn²⁺, Mg²⁺ and Zn²⁺. These results confirm that VTC4 is responsible for polyP biosynthesis in arbuscular mycorrhizal fungi.

References:

¹Hothorn, M., et al. (2009) Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. Science 324:513-516.

²Tisserant, E., et al. (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. PNAS 110: 20117-20122.

P (ID 449)

Does the composition and symbiotic efficiency of AMF communities depend on species ratios in the inoculum pool?

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Establishment of communities of symbiotic arbuscular mycorrhizal fungi (AMF) involves competition among the root- and soil- colonizing AMF species, but we are far from understanding the factors that affect the outcome in terms of AMF community composition and symbiotic efficiency. The composition of the soil inoculum pool may be one important factor as more abundant species gain initial advantage in the root colonization process.

Thus, we asked whether AMF species ratios in the inoculum pool affect the composition and symbiotic efficiency of the developing AMF communities using a model system of *Medicago truncatula* and five AMF species. We created inoculum pools with different species ratios and compared the dynamics of the developing AMF communities and the host plant's mycorrhizal benefits with the natural state, represented by an inoculum pool obtained by previous co-cultivation of all five AMF species.

The naturally established inoculum pool and the subsequent root-colonizing AMF community was characterized by pronounced overdominance of one AMF species, the ruderal *Rhizophagus irregularis*. The other inoculum pools dominated by the other species transiently promoted abundance of the particular species, but did not reverse the dominance of *R. irregularis*. Mycorrhizal growth response was positively related to root colonization levels, with highest values recorded for the *R. irregularis*-dominated communities. We conclude that AMF species traits appear more important determinants of AMF community composition than the propagule ratios in the soil inoculum pool. The latter factor, however, affects root colonization levels and the host plant's mycorrhizal benefits.

Keywords: interspecific competition, species ratios, quantitative PCR, mycorrhizal growth response

P (ID 466)

Nitrogen fertilisation effects on the form and function of arbuscular mycorrhizal fungi in barley

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Although most staple crop species form symbioses with arbuscular mycorrhizal fungi (AMF), fungal contribution to plant nutrient uptake in the field is poorly understood. Most of our current understanding of AM-route nutrient uptake comes from greenhouse experiments using individual plants, colonised by single AMF species. Field studies quantifying plant nutrient uptake via indigenous AMF are comparatively lacking.

AMF contribution to nitrogen (N) uptake in barley (*Hordeum vulgare*) was tested as part of a wider field study into N fertiliser application rates. Two cultivars, Maris Otter and Meridian were investigated, receiving two N fertilisation rates (60 or 280 kg Ha⁻¹ yr⁻¹) in a factorial design. Isotopic ¹⁵N was added to zones of soil which excluded either roots or roots and AMF. Comparative ¹⁵N enrichment of barley shoots indicated AM-route uptake. N source preference was tested by adding ¹⁵N as nitrate (¹⁵NO₃⁻) or ammonium (¹⁵NH₄⁺). Cultivar identity and N application rate effects on AMF community composition (within *H. vulgare* roots) were investigated using terminal restriction fragment length polymorphism (TRFLP).

AMF access to the ¹⁵N-labelled exclusion zones increased ¹⁵N enrichment of Meridian grown in high-N plots when added as nitrate but not ammonium. No ¹⁵N enrichment above controls was seen in low-N Meridian. No treatment saw AMF contributions of ¹⁵N to Maris Otter greater than in controls. TRFLP data suggest N application rate and cultivar identity influence AMF community assemblages in *H. vulgare* roots, while isotope data suggest these factors also influence the extent to which AMF contribute to plant N uptake.

P (ID 470)

Climate change impacts on carbon-for-nutrient exchange between winter wheat (*Triticum aestivum*) and arbuscular mycorrhizal fungi

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Dramatic increases in agricultural productivity during the 20th Century were driven primarily by the production and application of non-renewable synthetic fertilizers and pesticides. However, yields of key crops have now plateaued, with intensification practices compromising soil fertility and health. Encouraging the formation of arbuscular mycorrhizal (AM) associations in cultivated crops has been proposed as a means of reducing chemical inputs within sustainable agricultural systems. AM fungi can enhance plant-nutrient uptake in exchange for photosynthetic carbon, and may 'prime' host-plant defence pathways against insect herbivores.

Nonetheless, how the environment influences the functioning of crop-mycorrhizal interactions in terms of carbon-for-nutrient exchange and how it may impact upon mycorrhiza-crop-herbivore interactions has never before been quantified. Previous studies have recorded reduced phosphorous uptake efficiency in mycorrhizal vascular plants following atmospheric CO2 ($[CO_2]_a$) enrichment; observations of consequence given severe IPCC projections estimate $[CO_2]_a$ to exceed 800 ppm by 2100.

By supplying an isotopically-labelled nutrient solution (33 P and 15 N) to fungal symbionts within mesh-walled cores and 14 CO₂ to host plants, we show the first quantification of carbon-for-nutrient exchange between winter wheat (*Triticum aestivum*) and the AM fungus *Rhizophagus irregularis* under both present-day [CO₂]_a (440 ppm) and projected future [CO₂]_a (800 ppm).

Further experiments will evaluate how biotic interactions - specifically insect herbivory - impact mycorrhizal functioning in terms of nutrient trade under both CO₂ atmospheres, and lead to an improved understanding of how AM fungi might be used most effectively within future sustainable agro-ecosystems.

P (ID 471)

The SIZRT1 Gene Encodes a Plasma Membrane-Located ZIP Transporter in the Ectomy-corrhizal Fungus *Suillus luteus*

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Suillus luteus is an ectomycorrhizal fungus of which isolates with a variable tolerance towards environmental Zn exist. Zn tolerance in these isolates is believed to be due to an adaptation of the Zn homeostatis network. Zn uptake in eukaryotic cells is known to be predominantly governed by members of the ZIP (ZRT/IRT-like protein) family of Zn transporters. The aim of this study was to identify and characterize membrane transporters involved in Zn uptake in S. luteus. SIZRT1, a S. luteus ZIP transporter, was identified and investigated in silico. A phylogenetic analysis was performed to predict the function of the protein. Bio-informatics data illustrate that SIZRT1 has a ZIP signature sequence and that it most likely functions as a plasma membrane localized Zn importer. Heterologous expression in yeast was performed to confirm the predicted function and localization of the protein. The wild-type phenotype of the zinc-uptake-deficient yeast strain Δzrt1Δzrt2 was restored by heterologous expression of SIZRT1. A GFP fusion protein revealed that SIZRT1 is targeted to the plasma membrane. Finally, a gene expression analysis via RTqPCR was performed to establish whether SIZRT1 expression in S. luteus is affected by external Zn concentrations. Exposure to elevated concentrations of Zn resulted in a significant downregulation of SIZRT1 gene expression. All together, these data support a role as cellular Zn importer for SIZnT1 and indicate a key role in cellular Zn uptake of S. luteus. Further research is needed to understand the eventual contribution of SIZRT1 to Zn tolerance.

P (ID 474)

Biological markets control resource exchange in tripartite interactions of legumes

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Legumes form tripartite interactions, and are simultaneously colonized with nitrogenfixing bacteria and arbuscular mycorrhizal (AM) fungi. These tripartite interactions can substantially contribute to the nutrient efficiency of legumes, and increase the fitness of both the host and the different root symbionts. A synergy of benefits can occur, and the host plant can gain more from tripartite interactions, than from single inoculations with either symbiont. However, antagonistic effects can also occur, and it has been suggested that an auto-regulatory mechanism controls the extent of root colonization by both symbionts possibly to limit the high carbon costs of these interactions. We used split root systems and stable isotope labeling to track resource exchange dynamics in tripartite interactions to examine how the host plant controls its carbon investment into each root symbiosis under different nutrient supply conditions for the host and for the fungus. We found that Medicago plants in tripartite interactions allocated assimilated carbon depending on their nutrient demand, and that under nitrogen stress the plants invested more carbon into their root nodules, while under P stress available carbon resources were equally shared among both symbiotic partners. The colonization of the root system with nitrogen-fixing bacteria changed the nutrient allocation pattern of the fungus, and affected the ability of the fungus to compete for carbon with a supply of nitrogen. The results are discussed in the context of biological markets in which nutrient supply and demand affect resource exchange dynamics among partners in tripartite interactions.

Keywords: biological markets, tripartite interactions, legumes, root symbiosis

P (ID 475)

Effects of Mycorrhizal Inoculation and Phosphorus fertilization on Tomato, Eggplant, Green and Bell Pepper plants yield and nutrient uptake under field conditions

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This study was undertaken, under field conditions to evaluate the impact of mycorrhizal fungi inoculation and different amount of P fertilizer on the fruit yield and P, Zn, Fe, Mn and Cu uptake of tomato, green pepper, bell pepper and eggplant. The study was carried out at the Research Farm of the Cukurova University in the eastern part of the Mediterranean region of Adana-Turkey. Three levels of phosphorus control, 50 kg and 100 kg P2O5 ha-1 were applied with and without mycorrhizae inoculation. After harvesting the nutrient concentration of the leaves, mycorrhizal inoculation percentage (MI), inoculation effectiveness (MIE) and fruit yield were determined. The results of the experiment revealed that all mycorrhizal inoculated with P fertilizer application showed better results than non-inoculated plants and non P fertilizer in terms of plant yield, P, Zn, Fe, Mn and Cu concentration in the leaves. Mycorrhizal inoculation with P fertilizer application especially moderate amount of P (50 kg ha -1 P2O5) fertilizer has been able to increase the green pepper, bell pepper and tomato fruit yield compared with non-inoculated plants and non P fertilizer application treatments. The root colonization was in the highest range (≥ 53 %) in the all four plant species with mycorrhizal inoculated treatments. In addition, in the same plant species highest yield was observed, especially in the plants which were inoculated with mycorrhizal fungi. Increasing P level application reduced MIE. At 100 kg P2O5 ha-1application MIE was negative except eggplant and green pepper.

Keywords: mycorrhizal inoculation, P fertilizer, nutrient uptake, pepper, tomato

P (ID 489)

Trehalase activity in orchid mycorrhiza tightly colocalizes with living pelotons

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Orchids rely on mycorrhiza, especially during the stage of heterotrophic protocorms. In this special endomycorrhiza type, fungi form dense intracellular hyphal coils called pelotons. It is unknown which compounds ensure the energy and carbon transfers and whether the plant acquires carbon from living pelotons or takes it up after peloton lysis.

To address these questions, we selected *Dactylorhiza majalis* and *Ceratobasidium* sp. as the main model species. We focused on disaccharide trehalose which was hypothesized previously to play a role in carbon and energy transfers. Utilization of this saccharide depends on trehalase action and we therefore sought to localize trehalase in mycorrhizal tissues. We developed a histochemical trehalase localization method and used it for the first time.

Our results show, that trehalase activity tightly colocalizes with mycorrhizal structures. Intensive activity was detected in orchid tissues containing living pelotons, but not in non-infected tissues. Trehalase activity was missing in senescent pelotons. The same pattern was observed in completely heterotrophic protocorms as well as in roots of adult plants.

The results suggest that fungal trehalose can be hydrolysed by trehalase directly in mycorrhizal tissue. Trehalose degradation seems to be involved in biotrophic carbon transfer from living pelotons.

The work was supported by Ministry of Education, Youth and Sports of the Czech Republic (project NPU1: LO1417).

P (ID 490)

Visualising Carbon and Nitrogen exchange at the cellular scale in the ectomycorrhizal symbiosis using NanoSIMS

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A large proportion of plant-assimilated carbon (C) is allocated belowground and passed on to mycorrhizal fungi shortly after photosynthesis. Mycorrhizal fungi deliver nutrients in return, with the ectomycorrhizal symbiosis being especially important for nitrogen (N) nutrition of plants. However, little is known about how carbon and nutrients are transferred across the mycorrhizal interface (including fungal mantle, Hartig net and plant cells) and whether their exchange is regulated at the cellular scale.

The aim of this study was to visualize the *in-situ* flows of recently assimilated plant photosynthates and nitrogen delivered by the associated fungus through the ectomycorrhizal interface. After pulse-labelling ectomycorrhizal beech plants with $^{13}CO_2$ and ^{15}N (NH₄+, NO₃-, AA mix), each accessible only by one symbiotic partner, we applied nano-scale secondary ion mass spectrometry (NanoSIMS) on a cross section of a mycorrhizal root tip, allowing us to measure isotope ratios in different types of ectomycorrhizal tissue at a high spatial resolution (30nm).

We found significant correlations between ¹³C and ¹⁵N in all tissues, which indicate a coupled transfer of C and N. Correlations were mostly reaching a plateau for ¹⁵N at a certain threshold of ¹³C. However, different ectomycorrhizal tissues (fungal mantle, hartig-net, external hyphae, plant cells) exhibited different trends (regression functions and breakpoints), that could be associated with different transport mechanisms in the corresponding tissues.

Our results highlight the potential of NanoSIMS for investigating element flow at the cellular scale in the mycorrhizal symbiosis to explore spatial pattern of resource exchange.

Keywords: ectomycorrhiza, carbon, nitrogen, stable isotope probing, NanoSIMS

P (ID 505)

Mycorrhizal networks affect seedling emergence in mycorrhizal and non-mycorrhizal plants

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Introduction and aims: An ecologically important aspect of arbuscular mycorrhizal symbiosis is the proliferation of mycelial networks that colonise the roots of more than one plant, thereby forming a belowground network linking numerous plant roots with common fungal hyphae. In this study we tested whether the presence of an established network of common mycorrhizal hyphae would affect the emergence of seedlings of key species from the ecologically unique Jarrah forests of Western Australia.

Methods: Leek plants were inoculated with either one of two arbuscular mycorrhizal fungi (*Glomus* sp. or *Scutellospora* sp.). These were used to establish mycorrhizal networks and control treatments were also included (non-mycorrhizal). Once networks were established, seeds of ten Jarrah forest species were sown into the soil. The rate of seedling emergence and early seedling vigour were measured.

Results: Some plants such as *Bossiaea eriocarpa* responded positively (in terms of emergence rate and biomass production) to mycorrhizal networks whereas other species such as *Acacia celastrifolia* responded negatively. Mycorrhizal and non-mycorrhizal plants were affected by the presence of the soil-borne fungal networks.

Conclusions: Mycorrhizal networks affected the emergence and early seedling vigour of Jarrah forest species, whether mycorrhizal or not. The effect can be positive or negative depending on the plant species concerned.

Keywords: common mycorrhizal networks, seedling emergence

P (ID 510)

Photosynthesis and mycorrhizal functioning in epiphytic tropical orchids

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Introduction: Orchids are associated with mycorrhizal fungi (OMF) during the initial stages of germination, where fungi used provide the carbon. In the adult stage, fungus provides mineral nutrients from the environment. It is still debated whether they always, in exchange, receives photoassimilates from the host. This is especially unknown in epiphytic orchids that abound in tropical ecosystems. Concentrated on epiphytic orchids, we evaluated (i) the distribution of CAM, drought-adapted orchids, *versus* C3 orchids along a height gradient on a phorophyte, and (ii) based on ¹³C enrichment of the fungal pelotons recovered from roots, whether the fungus received plants photosynthates.

Material and methods: 22 samples of orchid, roots and leaves, and of other plant species, growing on the same phorophyte between 2 to 18 m, were collected in an Atlantic Forest State Park (Serra do Brigadeiro). Pelotons were picked up from the orchid roots. All samples were dried, ground and analyzed in GC-IRMS for estimating ¹³C abundance.

Results: No influence of height was observed in the distribution of CAM *versus* C3 orchids (nor for other plant species). As expected, due to higher drought and light availability, C3 plants (orchids and other species) had increasing ¹³C enrichments with increasing elevation, but CAM plants poorly responded to elevation. In all, 77% of the orchids were CAM and 23% were C3, but the prediction that higher position correlated with higher CAM frequency was not validated. All orchids were mycorrhizal. We observed that fungi had similar ¹³C enrichments to the plant harboring them, a correlation supporting C-transfer from orchids to fungus.

Discussion and conclusion: Epiphytic orchids are associated with OMF throughout life, especially in epiphytic species growing where there is little availability of nutrients. There seems to be a carbon flux from orchid to OMF, and the relative height from the soil, which is related to the light availability, did not influence this result, nor the distribution of like-CAM *versus* C3 plants.

Acknowledgements: CAPES, CNPg, Fapemig, MNHN

PLENARY SESSION: Diversity and biogeography of mycorrhizal symbioses

Chair: Dirk Redecker

KL (ID 189)

Biogeography and evolution of ectomycorrhizal plant lineages

Leho Tedersoo (Natural History Museum, University of Tartu, Tartu, Estonia)

Based on literature, 331 ectomycorrhizal (EcM) plant genera (ca. 8500 species) were assigned into 30 monophyletic lineages [1,2]. Considering low representation of EcM habit in several Australian lineages, we estimate that 6000-7000 plant species from 250-300 genera are able to establish EcM symbiosis. EcM plants thus constitute 1.7-2.4% of all accepted higher plant (Embryophyta) species. In addition to 30 gains of symbiosis, several potential losses of EcM habit may have occurred in Fagales, two groups of Fabales, two groups of Asterales and Myrtoideae that could be attributed to shifts to association with nitrogen fixing bacteria, shrubby or herbaceous life form or wetland habitat. There is still much confusion about the mycorrhizal status in multiple families where conflicting reports exist and incorrect assignments have rooted themselves deeply in the literature.

EcM habit evolved in Pinaceae in the Early Jurassic. Most other shifts to EcM habit occurred from the Late Cretaceous to Mid-Miocene [1,3]. I demonstrate maps about natural distribution of each EcM plant lineage and show that the present and historical distribution patterns may have little overlap in certain groups. The distribution data from EcM plant lineages identifies SW and SE Australia and mountain ranges in Mexico and Central Europe as phylogenetic diversity hotspots for EcM plants [3]. Fossil records and phylogeographic studies of fungi indicate that EcM plants have experienced three major events of symbiont interchange: 1) North Africa in the Eocene, 2) the Isthmus of Panama in the Late Pliocene and Pleistocene, and 3) Malesia in the Pleistocene.

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Keywords: ectomycorrhiza, evolution, biogeography

IL (ID 232)

Functional diversity and evolutionary stability of mycorrhizal and mycorrhiza-like symbioses

Katie J. Field (Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, UK)

Given the ancient origins of mycorrhizal symbioses, it has been hypothesized that for these mutualistic interactions between plants and fungi to persist, they require stabilization in the form of "reciprocal rewards". According to biological market theory, symbionts engaged in mutualisms exchange goods in the same way as commodities are traded in human economic markets. Under this framework, mycorrhizal fungal partner selection and evolutionary stability are underpinned by the proportional bidirectional exchange of photosynthates for nutrients, where the most "cooperative" partners are "rewarded" with resources, while so-called "uncooperative partners" face nutritional "sanctions". For such a rewards/sanctions-based mechanism to exist, each symbiont must detect variation in benefit received, and be in a position to withhold nutrients until maximum reward for their investment is achieved. However, this model does not account for the persistence of a diverse array of mycorrhizal (mycorrhiza-like in rootless plants) symbioses across plant life histories, in particular in those exhibiting heterotrophy. Lab and field experiments demonstrate significant diversity in function, with bidirectional exchange of carbon-for-nutrients between plants and fungi not always being proportional, with environment and ecology also playing critical roles. Here, I will discuss the hypothesis that stabilizing selection in mycorrhizas does not occur as a function of instantaneous bidirectional fluxes of resources between symbionts, but is a function of the net effect of these processes on the fitness of each partner. It is crucial we take a holistic view of the lifetime fitness benefit accrued by each partner rather than rely upon immediate measurements of resource exchange.

Keywords: functional diversity, mutualism, carbon-for-nutrient exchange, evolution

IL (ID 120)

Inbreeding, low dispersal and habitat-driven orchid mycorrhizal associations in Tulasnella

<u>Celeste Linde</u> (Ecology and Evolution, Australian National University, Canberra, Australia), **Monica Ruibal** (Australian National University, Canberra, Australia), **Yann Triponez** (Australian National University, Canberra, Australia), **Rod Peakall** (Australian National University, Canberra, Australia)

Fundamental life history processes of mycorrhizal fungi with inconspicuous fruiting bodies can be difficult to elucidate. In this study we investigated the species identities and life history of the orchid mycorrhizal Tulasnella fungi, which associate with the south eastern Australia orchid genus Chiloglottis. Tulasnella prima was the primary partner and was found to be associated with all 17 Chiloglottis species across a range of >1000 km, and to occur in the two edaphic conditions investigated (soil and sphagnum hammocks). Another Tulasnella species (T. sphagneti) appears to be restricted to moist conditions of alpine sphagnum hammocks. The population genetic structure of the widespread species T. prima, was investigated at 10 simple sequence repeat (SSR) markers and at four crossamplified SSR loci for T. sphagneti. For both taxa, no sharing of multilocus genotypes was found between sites, but clones were found within sites. Evidence for inbreeding within T. prima was found at 3 of 5 sites. Significant genetic differentiation was found within and between taxa. Significant local positive spatial genetic autocorrelation was detected among non-clonal isolates at the scale of two metres. Overall, the population genetic patterns indicated that in Tulasnella mating occurs by inbreeding and dispersal is typically restricted to short-distances.

Keywords: orchid mycorrhizae, life history, inbreeding, habitat-driven diversity

IL (ID 252)

Symbiont switching and alternative resource acquisition strategies but not shifts to parasitism drive breakdown of the plant – arbuscular mycorrhizal mutualism

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The plant-arbuscular mycorrhizal symbiosis is the world's most important terrestrial mutualism, and is thought to have facilitated the invasion of the land by plants. Yet, while many plant species could not survive without interacting with arbuscular mycorrhizal (AM) fungi, about 15% of all land plants have lost the ability to form the symbiosis. However, we lack empirical data on the drivers of this loss of plant-fungal cooperation and consequentially do not understand why the mutualism breaks down. Compiling a global database of plant-mycorrhizal status of almost 4,000 plant species, we used ancestral state reconstructions to study the persistence and loss of mutualism and identified ~25 independent mutualism losses across the seed plants. We quantified the relative importance of potential routes driving these breakdowns and found the ancestral mutualism is three times as likely to be lost when: (1) host plants switch to other root symbionts and (2) they evolve alternative resource acquisition strategies. In contrast, (3) we found no evidence for shifts towards parasitism driving mutualism loss. This suggests that parasitism is not a main factor undermining stable cooperation among plants and AM fungi in nature. Studying the potential for soil and climate variables as drivers of AM mutualism loss, we found higher soil nutrients and lower temperatures to be significantly associated with collapses of the symbiosis. Our analyses reveal that even ancient and very fundamental plant symbioses can be abandoned if there is an alternative for their function.

Keywords: mutualism, symbiosis loss, cheating, cooperation, macro-evolution

IL (ID 220)

Biogeography of mycorrhizal symbiosis: using plant mycorrhizal traits in space and time

Mari Moora (Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia)

Functional type- and trait based approaches to understanding community dynamics and ecosystem functioning are gradually gaining popularity. However, plant mycorrhizal traits are rarely considered in plant trait databases and are almost neglected in trait based plant community studies, despite that more than 80% of the land flora is mycorrhizal. In addition, knowledge about plant mycorrhizal traits in combination with plant distribution data gives an opportunity to study the importance and dynamics of mycorrhizal symbiosis across environmental gradients in space and time.

In this talk I shall discuss and define the mycorrhizal traits of plant species, notably mycorrhizal type, mycorrhizal status and mycorrhizal flexibility. I demonstrate ways of using these plant traits for understanding the dynamics and distribution of mycorrhizal symbiosis (1) across space at continental scale in Europe; (2) across time during the last 50,000 years in Arctic; and last but not least (3) for understanding the effect of mycorrhizal symbiosis on plant realized niche.

IL (ID 294)

Nitrogen pollution shifts forest mycorrhizal biogeography at continental scale

<u>Colin Averill</u> (Boston University, Boston, USA), <u>Michael C. Dietze</u> (Boston University, Boston, USA), <u>Jennifer M. Talbot</u> (Boston University, Boston, USA)

Most trees on Earth form a symbiosis with either ectomycorrhizal or arbuscular mycorrhizal fungi. The type of association has demonstrated importance for understanding ecosystem carbon (C) and nitrogen (N) cycling. Given this, it becomes important to understand where different mycorrhizal associations are, what controls their distribution, and where they will be in the future. Here we analyze ~3,000 forest inventory plots from the United States, and determined the relative abundance of arbuscular and ectomycorrhizal associated trees. We model this abundance as a function of climate, soil chemical properties, and atmospheric N deposition. We hypothesized that N pollution in the United States has affected the relative abundance of different forest mycorrhizal associations.

Models showed that climate, soil chemistry, and N deposition were important for predicting the relative abundance of ecto- and arbuscular associated trees. Ectomycorrhizal trees were more abundant in cold and wet climates compared to hot and dry. Low soil pH and high soil C:N ratios were also associated with an increase in the relative abundance of ectomycorrhizal trees. Most interesting, N deposition reduced the abundance of ectomycorrhizal compared to arbuscular mycorrhizal associated trees independent of climate and soil chemistry. Given the known associations between ectomycorrhizal dominance and soil C stabilization, we argue that N pollution in the United States has shifted the forest microbiome in a way that may have large implications for ecosystem C balance. Future changes in atmospheric N deposition will likely alter forest community composition and C balance via interactions with the forest microbiome.

Keywords: nitrogen deposition, soil carbon

IL (ID 453)

Mycorrhizas in a changing world: globalization effects on composition, networks, and function

Ian A. Dickie (School of Biological Sciences, Bio-Protection Research Centre, University of Canterbury, Christchurch, New Zealand)

The worldwide transport of plants and mycorrhizal fungi outside their native ranges profoundly alters plant and fungal communities. This is particularly true in the context of invasions of ectomycorrhizal plants and fungi. Using both molecular methods and sporocarp surveys we are beginning to understand how these linked plant-fungal invasions proceed, including similarities and differences across different plant species. Using data from New Zealand and the United Kingdom, we find the introduced plant-fungal networks show less modularity than native networks and, in NZ, lower functional diversity. Recent data using environmental barcoding at multiple scales suggests plant introductions also change the predicatbility and spatial structure of fungal communities at local, regional, and national scales. As a whole, the results suggest alien plants are largely, but not entirely, invading using non-native fungal associations. These alien-plant associated fungal communities are more variable in function across species, more inter-connected, and more predictable at large spatial scales than native plant associations. Overall, the results show that understanding the effects of globalisation on plant-fungal interactions requires considering a broader interaction-network context at multiple spatial scales.

IL (ID 249)

The global diversity and importance of mycorrhizal and nonmycorrhizal plants

Mark Brundrett (Biological sciences, University of Western Australia, Crawley, Australia)

On a global scale the majority of plants are mycorrhizal, 72% with AM (7% inconsistent AM), >2% are ectomycorrhizal (EM), <2% have ericoid mycorrhizas and 10% have orchid mycorrhizas. Nonmycorrhizal (NM) and NM-AM plants typically occur where plant productivity is limited. Miss-assignment of mycorrhizal types is common in databases and can have serious consequences on the validity of results of meta-analyses.

Australia is a global diversity hotspot for root evolution, with about 1/3 of EM plants, many NM plants and 1/4 of all carnivorous plants. There is strong phylogenetic consistency for within plant families, but also exceptions including Australian members of the Fabaceae and the Myrtaceae, which have many switches from AM to EM, EM-AM or NM roots.

Globally, the two main evolutionary trends are from AM to NM (~45,000 spp.), or from AM to EM (>6000 spp.). Three waves of mycorrhizal evolution are evident, starting when AM plants colonised land. The second wave was in the Cretaceous for the Orchidaceae, Ericaceae, as well as EM, NM, parasitic, carnivorous and nitrogen fixing plants. The recent origin of New Complex Root (NCR) clades in Australia (and elsewhere) is a third wave of mycorrhizal evolution starting in the Pleistocene. NCR lineages have diverse roots types within a genus and show explosive diversification in the past 30 Ma coinciding with continental aridification and highly unfertile soils in southwestern Australia. These conditions resulted in an advantage for plants with new or variable root types and provide a preview of future conditions elsewhere on earth.

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Keywords: global diversity, nonmycorrhizal plants, evolution

CONCURRENT SESSION: Mycorrhizas in plant and fungal invasions

Invasions of alien species are a major driver of global change, of concern in both natural and managed ecosystems. Mycorrhizal fungi can determine the success or failure of plant invasions and shape the impacts of invasive plants on ecosystem function, as are invasive species in their own right. Research on linked plant-fungal invasions provides unique insights into mycorrhizal ecology, including the ecology of non-mycorrhizal and facultatively mycorrhizal plants, specificity between plants and fungi, interactions between mycorrhizal fungi and other biota, and effects of mycorrhizas on ecosystem functions. Notwithstanding this potential, there remain major gaps in our knowledge. Correct classification of the mycorrhizal status of plants, clear species concepts for fungi, and better knowledge of fungal species origins and distributions are essential to advancing the field. This session will cover multiple mycorrhizal types and their role in invasions, and highlight emerging mycorrhizal paradigms and critical research gaps in plant and fungal invasions.

Chair: Ian Dickie

CL (ID 161)

The pantropically introduced Polygonaceae *Coccoloba uvifera* was followed by specific, pseudovertically transmitted *Scleroderma spp.*

<u>Marc-Andre Selosse</u> (Institut de Systématique, Evolution, Biodiversité (UMR 7205), Muséum national d'Histoire naturelle, Paris, France), **Seynabou Sene** (Laboratoire Commun de Microbiologie, IRD/UCAD/ISRA, Dakar, Senegal), **Mathieu Forget** (Institut de Systématique, Evolution, Biodiversité (UMR 7205), Muséum national d'Histoire naturelle, Paris, France), **Amadou Ba** (Laboratoire de Biologie et Physiologie Végétales, Faculté des Sciences Exactes et Naturelles, Université des Antilles, Pointe-à-Pitre, France)

Coccoloba uvifera (Polygonaceae) is an ectomycorrhizal tree endemic to Caribbean beaches. It has been introduced pantropically for ornament, fruits production and stabilization of sandy soils. Investigating three Caribbean regions of origin (ROs) of C. uvifera (Guadeloupe, Martinique and Porto Rico) and six regions of introduction (RIs) of the tree (Brazil, Senegal, Guyana, La Réunion (Pacific Ocean), Okinawa and Malaysia), ITS barcoding revealed that C. uvifera associates with diverse fungi mostly in ROs but with single species of semi-hypogeous Scleroderma in RIs. These species, also present in ROs, are an unknown Scleroderma in Brazil, Japan and Malaysia, and Scleroderma bermudense in Guyana, Senegal and La Réunion. To further assess whether S. bermudense was introduced or native in RIs, we analyzed populations using six microsatellite markers. No founder effect was observed in RIs, but the pattern of isolation by distance was congruent with independent introductions to Senegal and La Réunion. These two populations were less divergent from each other than expected, and very close to some ROs' populations. Moreover, in Senegal, C. uvifera cannot associate with local ectomycorrhizal fungi, but forms spontaneously ectomycorrhizas with S. bermudense, even in sterile soil. Scanning electron microscopy revealed that some dried fruit aggregates sand and spores, likely during the drying process on soil, likely transmitting the fungus with limited founder effect. Specific ectomycorrhizal Scleroderma species from the RIs were thus introduced with C. uvifera seeds, in an accidental microbial co-introduction with plants, thanks to a hitherto undescribed pseudovertical transmission of mycorrhizal symbionts.

Keywords: introduction, population genetics, scleroderma, seagrappe, specificity

CL (ID 169)

Plant provenance vs. functional group identity: The arbuscular mycorrhizal fungal perspective on plant invasions

Ylva Lekberg (MPG Ranch and University of Montana, Missoula, USA)

Arbuscular mycorrhizal fungi (AMF) may facilitate plant invasion by increasing the competitive ability of exotic plants or by reducing the biotic resistance of native plants. Wellstudied systems involving spotted knapweed (Centaurea stoebe) and garlic mustard (Allaria petiolata) illustrate both strategies, but whether one is more prevalent among invasive plants and differ fundamentally from mycorrhizal associations of native plants are unknown. I will present data from field surveys in intermountain grasslands in Montana that shows disparate responses by AMF to different invaders. Specifically, the annual cheatgrass (Bromus tectorum) reduced AMF abundance whereas the perennial forbs spotted knapweed and leafy spurge (Euphorbia esula) increased AMF abundance, richness and diversity relative to grass-dominated native communities Thus, AMF responses to – and potential role in – plant invasions may be highly dependent on plant functional group identity of the invader relative to the community being invaded. A meta-analysis of peer-reviewed articles provided further support that plant-AMF interactions are best predicted by plant functional group, not provenance. Ongoing field experiments that evaluate the functional consequences of invader-mediated shifts in AMF communities will be discussed, as will comparisons of AMF communities across native and invasive ranges.

CL (ID 219)

Reduced mycorrhizal responsiveness leads to increased competitive tolerance in an invasive exotic plant

<u>Lauren Waller</u> (Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand), Ragan Callaway (University of Montana, Missoula, USA), John Klironomos (University of British Columbia, Okanagan, Canada), Yvette Ortega (USDA Forest Service, Rocky Mountain Research Station, Missoula, USA), John Maron (University of Montana, Missoula, USA)

Many exotic invasive species associate weakly with AM fungi, which may be beneficial in disturbed habitats where competition for resources is low. However, less is known about how weak responsiveness to AM fungi may benefit invaders in a competitive environment. Furthermore, since some exotic plants interact differently with AM fungi in their new ranges, range-based shifts in AM responsiveness could influence competitive interactions between exotic and resident plants, although this remains poorly studied. We explored whether genotypes of the annual exotic Centaurea solstitialis (yellow starthistle), collected from populations across the native and non-native ranges, differed in responsiveness to AM fungi in the introduced range and whether range-based differences in mycorrhizal responsiveness affected how strongly C. solstitialis tolerated competition with the North American native bunchgrass, Stipa pulchra. Grown alone, C. solstitialis from both ranges derived only weak benefits from AM fungi. However, association with AM fungi was costly to plants when grown in competition with S. pulchra. The magnitude of the suppressive effect of AM fungi was greater for genotypes from native versus introduced populations. This suggests that AM fungi may contribute to invasion resistance in established native communities, but range-based shifts in the way exotic genotypes respond to AM fungal partners may counter such biotic resistance.

CL (ID 289)

Effect of plant invasion on assembly rules in arbuscular mycorrhizal fungal communities

<u>Petr Kohout</u> (Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic), **Kateřina Štajerová** (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), **Martin Hejda** (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), **Radka Sudová** (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), **Zuzana Kolaříková** (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), **Maarja Öpik** (Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia), **Petr Pyšek** (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic)

Many plant species have been introduced to new habitats throughout the world, and some also spread and grow in abundance and extend beyond original points of introduction. Plant species that spread extensively in their novel habitats are termed invasive. Many invasive plants affect populations of native species, resident communities and influence ecosystem properties. Despite the salient role of arbuscular mycorrhizal fungi (AMF) in plant life, studies exploring the changes in host plant-AMF relationship during the invasion processes are scarce. To better understand the effect of plant invasions on assembly rules in arbuscular mycorrhizal fungal communities, we conducted a cross continental study focused on AMF communities associated with three host plant species (Cirsium arvense, Tanacetum vulgare and Leucanthemum vulgare), which are native in Europe and invasive in North America. Besides the above mentioned plant, we also sampled representative amount of all surrounding plant species on each study site, to determine the local AMF species pool. The study was conducted on nine sites in North America (USA: Wyoming, Montana, Minnesota; Canada: Ontario) and nine sites in Europe (Czech Republic, Poland and Switzerland). Illumina sequencing of SSU region of rDNA was used for characterisation of the AMF community composition in all 550 samples. We found significantly smaller host plant effect on AMF community composition in introduced, compare to native host plant range. Besides that, invasive plants differed in their AMF partner choice from the local species pools between introduced and native range.

ST+P (ID 62)

Fungal bio-fertilizers may suppress local plant communities

<u>Vasileios Kokkoris</u> (Biology, University of British Columbia Okanagan, Kelowna, Canada), <u>Miranda Hart</u> (Biology, University of British Columbia Okanagan, Kelowna, Canada)

Bio-fertilizers are microbes used to improve crop performance, but we know little of how they affect wild plant communities. Arbuscular mycorrhizal (AM) fungi form important root symbioses with most plants and are increasingly applied as bio-fertilizers to many crops around the world. Due to the intimate connection between plants and their AM fungi, we asked if commercial inoculants interact differently with native plants versus crop plants. We hypothesized that commercial inoculants would stimulate crop plants but suppress wild plants, which should perform better with locally adapted fungi.

We grew a commercial AM fungal isolate and a closely related wild relative with 10 different plants (five cultivars and five wild plants), and measured plant growth (biomass, seeds, leaf phosphorus content) and fungal performance (spore number, mycelial size and fungal structures).

Contrary to our hypothesis – plants responded similarly, but negatively, to the commercial isolate compared to the wild isolate. Plants inoculated with the commercial isolate allocated more biomass above ground while plants inoculated with the wild isolate allocated more biomass to roots. Leaf phosphorus was significantly lower for plants inoculated with the commercial isolate, suggesting the commercial isolate was less mutualistic. The commercial isolate produced significantly higher number of spores compared to the wild one independently of the host.

Such differences have the capacity to lead to long term changes in plant communities, thus the use of commercial inoculum must be considered from an ecological perspective.

ST+P (ID 174)

Arbuscular and Ectomycorrhizal Fungi in a Warmer, Fertilized Forest Colonized by an Invasive Plant

<u>Mark A. Anthony</u> (Natural Resources, University of New Hampshire, Durham, USA), **Serita D. Frey** (Natural Resources, University of New Hampshire, Durham, USA), **Kristina A. Stinson** (Environmental Conservation, University of Massachusetts, Amherst, USA)

The mycorrhizal component of temperate forest soils influences carbon (C) and nitrogen (N) cycling and may be sensitive to simultaneous global change stressors. To assess this, we introduced invasive Alliaria petiolata into forest plots that had been experimentally warmed (+5°C), fertilized (+50 kg N ha-1 yr-1), or both for ten years. Arbuscular mycorrhizal (AMF) and ectomycorrhizal (EMF) fungal communities were assessed in soil samples collected in 2016 alongside soil C and N stocks and fluxes. Our results show that all stressors reduced AMF and EMF diversity compared to ambient plots. EMF also had lower relative abundance across all stressors compared to ambient plots, with the three most abundant EMF taxa being Russula, Amanita, and Cortinarius. These genera were sensitive to invasion in control, warmed, and fertilized plots but this effect was ameliorated in warmed × fertilized plots. At the same time, AMF diversity was lowest in plots with all three stressors compared to all other plots and had enhanced dominance by Glomus at the expense of less common taxa, like Paraglomus, ultimately reducing community heterogeneity. Plots with all three stressors also had lower N-mineralization compared to ambient plots, and N-mineralization was positively correlated with C-mineralization and AMF richness. Our results suggest that the global change factors studied reduced mycorrhizal diversity and C and N-mineralization rates in forests invaded by A. petiolata. A. petiolata invasion into a warmer, fertilized forest may lead to local extinction of mycorrhizae by enhancing dominance of abundant taxa and homogenizing community composition.

Keywords: global change, climate change, nitrogen deposition, invasion, biogeochemistry

ST+P (ID 309)

Suillus lakei and Aureoboletus projectellus in native and invasion range

<u>Marcin Pietras</u> (Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Gdansk, Poland), **Marta Kolanowska** (Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Gdańsk, Poland)

Recently the species distribution niche modeling was successfully used to estimate the current potential and feature range of numerous animal and plant species. So far this method was not applied to estimate distribution of any non-pathogenic fungi. Therefore, here, the maximum entropy method was used to create models of suitable niche distribution and future range of two ectomycorrhizal fungi: *Suillus lakei* and *Aureoboletus projectellus*. This research indicated that the area of potential habitats of *S. lakei* in native and invasive habitat acreage will decrease by 2080. The limiting factor for further expansion of *S. lakei* in invasion range will be disjunctive occurrence of its ectomycorrhizal partner – Douglas fir. In each scenario of climate change available niches of *A. projectellus* will decrease in native range (from 214 000 km² nowadays to 31 932 km² in 2080), but surprisingly, increase in invasion range (from 95 000 km² to 289 000 km² in the same period). Most decisive factors for *A. projectellus* occurrence are precipitation of driest month and annual mean temperature.

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Keywords: fungal invasion, Niche modeling, Aureoboletus projectellus, Suillus lakei

ST+P (ID 239)

Soil fungal community in grassland with and without Fritillaria meleagris

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The Uppsala Kungsäng Nature preserve is a species-rich grassland founded in 1943 to protect Scandinavias largest population of *Fritillaria meleagris*. The reserve stretches from a wet unmanaged area along the Fyris river with low Fritillaria density into a drier area managed by annual mowing where Fritillaria is abundant. The botanical compositions change dramatically from the wet western part to the drier eastern part of the filed. In this study, we explored the soil fungal communities associated with this shift.

Soil samples were taken at five locations along two parallel 30 m transects (west and east). At each location two soil samples were collected, one by a Fritilaria plant and another at least 50 cm from a Fritillaria plant. After DNA extraction, ribosomal genes were amplified using ITS1-F/LR5-R primer-set. The resulting 1500 bp amplicons were sequenced using PacBio. This produced a dataset with 53 057 reads after quality control and de-multiplexing. The entire reads were clustered using Usearch and taxonomically identified by blasting of LSU region to Silva database, identifying 378 operational taxonomic units (OTUs). On average, 73 OTUs across 699 fungal reads were recovered from each soil sample.

Principle component analysis demonstrated a clear separation of fungal communities from the two sides of the field, with species from Pleosporales and Mortierellales dominating the east side and species from Capnodiales and Hymenochaetales dominating the west side. Further, we observed a separation of fungal communities from Fritillaria and non-Fritillaria soil samples in the east part of the field.

Keywords: soil, grassland, Fritillaria meleagris

ST+P (ID 301)

What do we know about mycorrhizae role in exotic conifers' invasion of Nothofagaceae forests in Patagonia, Argentina?

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Mycorrhizae associations are vital for Nothofagaceae forests and P. menziesii plantations' establishment and development. In Patagonia, P.menziesii is being planted close or within native forests, showing an aggressive invasion behaviour. We studied the mycorrhizal status of seedlings along Nothofagaceae+P.menziesii invasion matrices to investigate their role in this process. Our results present evidence that P. menziesii invasion of Nothofagaceae forests occurs with the co-invasion of their mycorrhizal partners. Also, soil environments located beyond invading conifers seedlings have shown to hold ectomycorrhizal inoculum capable to associate with incoming new plants. We have evidence that P. menziesii invasion could produce maladaptation of native ectomycorrhizal communities. Hebeloma mesophaeum, a Wilcoxina sp. (early-stage and common P. menziesii's ectomycorrhiza) and a Pyronemataceae sp. (widely associated with Nothofagaceae spp.) were found shared by P. menziesii and Nothofagaceae spp. Interestingly, Hebeloma hiemale and Wilcoxina sp., common partners for P. menziesii in Patagonia although not registered from Nothofagaceae forest, were found associated with N. antarctica, this is the first report for both fungal species. Pseudotsuga menziesii seedling seems to have the ability to form different AM colonization types (Paris-Arum-Both-Intermediate-type) depending on sites conditions. Significant high presence of Intermediate-type was found in seedling grown in invaded soils, where the colonization was less abundance. The presence of different mutual association gives P. menziesii a strong ability for seedlings establishment. The nursery mycorrhizal effect has been observed consistently in different environments, therefore, this fact should be considered in the design, site selection and invasion management of fast growing exotic plantations in Patagonia.

Keywords: Ectomycorriza, arbuscular mycorrhizas, Patagonia, Nothofagus forest

ST+P (ID 461)

Is *Pennisetum setaceum* altering the arbuscular mycorrhizal fungi communities during the invasion process?

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The involvement of mutualistic plant-fungal interactions in invasion processes, especially in some climatic regions including arid and semiarid areas, has not been sufficiently investigated. To provide an insight into the possible role of arbuscular mycorrhizal fungi (AMF) during invasion, we compared the AMF communities harbored by the invasive plant *Pennisetum setaceum* with those from the co-occurring native *Hyparrhenia hirta* at five different Mediterranean semiarid locations with different edaphic characteristics. Illumina high throughput sequencing technology was used to investigate AMF communities. The subsequent multivariate analysis showed that native and non-native host plants shared a similar AMF community, whereas the invaded locations differed significantly in such fungal communities. Simultaneously, and according to the canonical correspondence analysis results, the variability in the AMF communities between sampling sites was related to changes in soil total carbon, electrical conductivity, respiration, and protease and urease activities. These findings reveal the unspecificity and adaptability of *P. setaceum* in relation to its association with the AMF community encountered in the invaded locations, which could have facilitated its successful establishment and spread.

Keywords: invasive plants, mycorrhizal symbiosis, microbial functioning, semiarid environment, Illumina sequencing

POSTERS

P (ID 367)

Invasion shifts soil fungal community composition in California chaparral

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Invasion persistence is likely driven by multiple interacting mechanisms, such as the a priori presence of both mutualistic and parasitic soil organisms or alteration of the belowground community by the invasive species. We hypothesized that (1) invasive annuals will associate with an 'early successional' AMF community; (2) native perennials will likely associate with 'late successional' AMF species; and (3) invasive grasses will have a relatively lower pathogen abundance. We collected roots and soils in March 2016 from two sites underneath individuals of a dominant native shrub (Adenostoma fasciculatum) and invasive grasses (Bromus diandrus and Avena fatua). We extracted DNA from root and soil sub-samples, amplified ITS2 and SSU rDNA using using primers specific to all fungi (ITS2) or to AMF (SSU). We sequenced amplicons on an Illumina MiSeq, and processed reads using QIIME. ITS sequences were aggregated to functional guilds using FUNguild. Host-plant invasive status drove differences in fungal community composition. NMDS shows clear grouping of both ITS and AMF communities by host-plant identity. Invasion increased abundance of Acaulosporaceae, in support of the first hypothesis. In contrast to the third hypothesis, invasives had a greater relative abundance of pathotrophic OTUs, whereas natives had a greater relative abundance of symbiotrophic OTUs. Invasion resulted in an increase of 'early successional' AMF that primarily have intra-radicle colonization, which may decrease the presence of extra-radicle foraging hyphae in the soil. These natives depend on associations with foraging hyphae to increase access to resources; a loss of this inoculum may reduce success of re-establishment

P (ID 328)

Effect of Arbuscular Mycorrhizal Fungi on vascular wilt control caused by Fusarium oxysporum in goldenberry plantlets

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Colombia is one of the largest cape gooseberry producers in the world. The pathogenic fungi Fusarium oxysporum affects seriously the production and quality of the fruits, causing losses that reach 80 to 100% of the culture. In addition, there are not effective ways to control the vascular wilt caused by this pathogen, because the commercial fungicides has low effectiveness, and high economical and environmental cost, plus the pathogen has high persistence in soils and physiological versatility. The Arbuscular Mycorrhizal Fungi, can exert a positive control in the effect of vascular wilt. This study was aimed to evaluate this effect in greenhouse conditions in a cape gooseberry plantlets. A complete random blocks design with three treatments, one inoculated with a mixture of Arbuscular Mycorrhizal Fungi (Glomus sp. + Acaulospora mellea) and two controls with a complete and half conventional chemical fertilization was used in this experiment. All plantlets were inoculated with Fusarium oxysporum in a 104 microconidia⁻¹ per liter concentration. We analyzed plant-root development as well as incidence and severity of vascular wilt, finding that plants that were biofertilized with a mixture of Arbuscular mycorrhizal Fungi had a low incidence and severity disease levels compared with controls and show a regular plantlets development. Results allow us to consider that the use of Arbuscular Mycorrhizal Fungi could be implemented as part of an integrated strategy model to control the effect of vascular wilt caused by Fusarium oxysporum

Keywords: arbuscular mycorrhyzal fungi, *Fusarium oxysporum*, *Physalis peruviana*, vascular wilt

P (ID 483)

Impact of invasive plants to fungal community structure and soil characteristics

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The NAKI II project is a cooperation of several research groups to study biotic risks which may occur in parks and their surroundings. Our group's main focus lies on designing sustainable management procedures for cultivated alien plant species in chateau parks and landscape gardens, by studying newly introduced plants and the subsequent danger of potential pathogens to fungal communities. Invasive plant species are known to influence diversity, e.g. by reshaping soil properties (Gibbson et al. 2017) and can alter fungal communities (e.g. EcMF, AMF and pathogens) between native and non-native sites (Kivlin et al. 2011, Yannarell et al. 2011). This may suggest that they even influence soil ecology on larger scales than their own rhizosphere. By sampling of soil in different parks distributed all over the Czech Republic, we want to find out the difference between human shaped chateau gardens and nearby natural areas (forest). Thus will enable us to identify potential treatments to prevent potential risks of invasive species, such as application of fungicides, etc. The park sampling in the Czech Republic will be done in ca. 50 gardens, soil cores are taken of different tree individuals with according metadata (GPS, tree spp., tree cover, etc.). Soil characteristics will be analysed of the plant rhizosphere and fungal communities will be screened by Illumina sequencing. Preliminary results of the first 10 parks sampled are shown.

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Keywords: invasive plants, fungal community

P (ID 187)

Stress acclimatization and melanin production of Dark Septate Endophytes

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Endophytes are considered as microorganisms living inside the tissues of their host plants without causing any disease symptoms. One large group of root endophytic fungi is the so called Dark Septate Endophytes (DSEs), which are frequently isolated from environments with strong abiotic stress. They are characterized by the formation of specific microsclerotia within plant cells.

In the current study, we first monitored the responses of two model DSEs, *Periconia macrospinosa* and *Cadophora* sp. to different abiotic stresses. DSE growth and morphology on different culture media subjected to high temperature, salt and heavy metals suggested that both endophytes have a high competence to tolerate abiotic stress.

In a second step, DSEs were cultivated at moderately increased concentrations of NaCl. Such acclimatized strains were more tolerant to increasing salt concentrations and interestingly, showed also a differential response to other abiotic stresses.

DSEs are usually characterized by high levels of melanin pigmentation. Our analyses indicated that among three pathways, previously identified in ascomycetes, the 1,8-dihydroxynaphthalene (DHN) pathway was used for melanin biosynthesis in the current model DSEs. Melanin content increased in response to the presence of salt stress.

We conclude that our two DSE models can tolerate severe stress conditions and that this tolerance can be even increased during adaptive cultivation. This might lead to their application as biological agents in plant production systems conferring abiotic stress tolerance to crops. However, it should be tested whether melanin pigmentation is a good proxy for screening this potential.

P (ID 216)

Differential interaction of dark septate endophytes and fungal pathogens in vitro and in planta

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Dark Septate Endophytes (DSEs) is a group of widespread root-colonizing fungi. The role of these endophytes in ecosystems and their interactions with plants and phytopathogens are not well understood. In the current study, we assessed the biocontrol potential of the two model DSEs *Periconia macrospinosa* and *Cadophora* sp. against the tomato pathogens *Rhizoctonia solani*, *Pythium aphanidermatum* and *Verticillium dahliae*. To investigate the interaction between DSEs and fungal pathogens we performed *in vitro* assays followed by a greenhouse experiment in which tomato plants inoculated with one of the DSEs or control, were challenged by one of the three pathogens. The pathogens and the DSE fungi were quantified in the roots using qRT-PCR assays.

Both DSE species inhibited the growth of the three fungal pathogens *in vitro* most probably by compounds secreted by the DSEs into the medium. On the other hand, a negative effect of the pathogens on the DSEs was also detected. In roots, these negative effects could not be observed, e.g. root colonization by *V. dahliae* and *P. aphanidermatum* was increased in the presence of *Cadophora* sp.- and *Cadophora sp.* can form more biomass in roots colonized by pathogens.

Keywords: dark septate endophytes, Ascomycota, phytopathogens, biocontrol

P (ID 13)

Efficacy of arbuscular mycorrhizal fungi in different integrated disease management approaches on carnation wilt

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Wilt (Fusarium oxysporum f. sp. dianthi) of carnation is a serious disease in India. Effect of conjoint application of Arbuscular mycorrhizal (AM) fungi, Azotobacter chroococcum and Trichoderma viride was evaluated in soil solarised with transparent polyethylene mulch (25 µm thick) and amended with neem (Azadirachta indica) cake (10 tones/ha) on the incidence of Fusarium wilt, growth of the plants and important quality characteristics of the flowers in carnation. Conjoint application of native isolates of AM fungi (5g of inoculums/ plant, raised on Vigna radiata), A. chroococcum (5g slurry/ plant as root inoculation of cuttings) and T. viride (0.5g talc based formulation mixed in 25g of farmyard manure/ 5kg of pot soil) as soil application before transplantation of the cuttings was found most effective with 97.2 per cent reduction in disease incidence in comparison to unsolarized control. This treatment combination also resulted in 242.1, 45.3, 43.0 and 150.0 per cent increase in important plant quality parameters like number of flowers per plant, flower size, length of flowering stem and stem strength with 'A' grade flowers, respectively in comparison to unsolarized control. Conjoint application of AM fungi, A. chroococcum and T. viride was also found effective in unsolarized soil with 80.0 per cent reduction in the plant mortality with appreciable increase in plant growth and flower quality characteristics in comparison to control. This treatment resulted in 23.8 times higher spore count of AM fungi in solarized soil in comparison to unsolarized control and also resulted in 21.6 per cent root colonization.

Keywords: arbuscular mycorrhizal fungi, *Azotobacter chroococcum*, *Trichoderma viride*, soil solarization, *Fusarium oxysporum f. sp. dianthi*

P (ID 171)

Remediation of degraded post-opencast mine land with tree species inoculated with arbuscular mycorrhizal fungi in Indonesia. Remediation of degraded post-opencast mine land with tree species inoculated with arbuscular mycorrhizal fungi in Indonesia

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Deforestation is occurring owing to over logging, forest fire, forest conversion into agricultural land and opencast mining in Indonesia. Opencast mining removes vegetation and soil of natural forests and results in increasing atmospheric CO2. Immediate remediation of degraded post-opencast mine land is required. Arbuscular mycorrhizal (AM) fungi can increase growth of tree species in disturbed fields. The objective of this study was to investigate the effect of inoculating native AM fungi on the growth of Albizia saman and Paraserianthes falcataria in a nursery and a post-opencast coal mine field. Seeds of A. saman and P. falcataria were inoculated with three native AM fungi, Rhizophagus clarus, Gigaspora decipiens, and Scutellospora sp., and sown in sterilized compost under nursery conditions for six months. Non-inoculated seeds were used as control. Inoculated seedlings were transplanted in a post-opencast coal mine field and grown for seven months. AM colonization, shoot nitrogen (N) and phosphorus (P) concentration, stem diameter, and shoot dry weight were measured both in the nursery and in the field. AM colonization was 3-82% under nursery conditions and increased shoot P content and dry weight. Stem diameter, shoot N content, shoot P content, shoot dry weight, and survival rate under field conditions were higher in inoculated seedlings than in control seven months after transplanting. The results suggest that inoculating tree species with AM fungi promotes remediation of post-opencast coal mine field.

Keywords: remediation, opencast mining, Indonesia

P (ID 157)

Two exotic plant species affect negatively the dynamic of mycorrhizal fungi in Madagascar natural forests

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Mycorrhizae are among the most sensitive microbial community to vegetation change in forestry ecosystems. The goal of the study was to describe the evolution of mycorhizal fungi community dynamics within two natural forest ecosystems respectively invaded by two exotic plant species, *Pinus patula* and *Grevillea banksii*, in Madagascar.

Soil samples were collected under each exotic plant species. We considered as controls, the soil samples from the non-invaded zones of each natural forest. The dynamic of MF propagules were assessed by counting the number of endomycorrhizal spores or infected root fragments in soil, by describing the mycorrhizal diversity and by measuring the soil mycorrhizal infectivity (SMI). In the same time, the soil phosphatase activity and the development of two native plant species, *Uapaca bojeri* and *Dalbergia trichocarpa*, in the invaded soils were evaluated.

We recorded that for the two sites, the invaded soils were significantly rich in mycorrhizal propagules with 11043 AMF spores for *Pinus patula* soil against only 8716 AMF spores for the non-invaded soil and 590 AMF spores for *Grevillea banksii* soil against 477 AMF spore for the non-invaded soil. However, the SMI and the diversity did not follow this tendency because they were significantly lower in the invaded soils when compared to the non-invaded soils. We observed also that soil phosphatase activity, plant development and the root infection for the two native plant species cultivated on the invaded soils were significantly low compared to those planted on non-invaded soils.

This suggests that exotic species select mycorrhizal associate which are only beneficial for them but not adapted for the native plant species.

Keywords: mycorrhizae, exotic plant species, *Uapaca bojeri*, *Dalbergia trichocarpa*, soil mycorrhizal infectivity

CONCURRENT SESSION: Population genetics/genomics/ DNA polymorphism

Molecular genetic approaches have opened new possibilities to look at phylogenetic relatedness of microbes, microbial community composition and ecology. Yet, it also uncovered a number of dissimilarities to other (previously sequenced) organisms, and it still remains a challenge to identify mating systems, modes of reproduction, the level of genetic variation within individuum, species and higher taxonomic units, for different loci and different fungal groups, particularly with respect to the Glomeromycotina. Recent advances in population genetics from different groups of mycorrhizal fungi will be discussed and compared in this session, addressing whether Mendelian genetics apply (and at which level) to studies of mycorrhizal fungi, what clonality means for (mycorrhizal) fungal genetics and ecology and which challenges need to be conquered if we are to produce stable mutants/transgenic mycorrhizal fungi in the different types of mycorrhizal symbioses.

Chairs: Nicolas Corradi, Martina Peter

IL (ID 214)

Demonstrating the link between genomic variation in *Rhizophagus irregularis* and significant differences in plant growth

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Plant breeders have used naturally occurring genetic variation in plants to develop improved varieties, leading to the massive increases in food production seen in the 60's and 70's. Several published studies show considerable genetic variation among isolates of the fungus Rhizophagus irregularis, as well as their effects on plant growth and P uptake capacity. We have shown that manipulating this fungus to generate genetically novel lines induces very large significant differences in the growth of the globally important plant rice (and in cassava in the field – see presentation at ICOM by Alia Rodriguez). However, in such experiments, there is no established causal link between genomic variation among the fungi and the variation in plant growth response. We have already proposed that R. irregularis is an ideal species for an AMF genetic improvement program because of high within-species genetic variation and the ability to mass produce clean inoculum of this fungus in an easy-to-deliver carrier. However, the use of AMF genetic variation depends on a clear established link that genetic variation in the fungus causes differences in quantitative growth traits of both the fungus and the plant. In this presentation, using a combination of NGS techniques and experiments on plant growth, we demonstrate for the first time a clear link for a genetic basis in the fungus causing significant variation in plant growth. Our results have strong implications for both the development of AMF inocula for improving crop growth and for the ecological role of genetic variation in natural plants.

Keywords: genome variation, AMF, NGS, plant growth response, phylogenetics

CL (ID 186)

Five years investigation of female and male genotypes in Périgord black truffle (*Tuber melanosporum* Vittad.) revealed contrasted reproduction strategies

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Tuber melanosporum, is a heterothallic ectomycorrhizal ascomycete having two mating-type idiomorphs. The small-scale genetic structure of female genotypes has already been analyzed and it has been found they are linked to the tree via the ectomycorrhizae. On the contrary, this was not yet analyzed for male genotypes and the origin of the male structures is still unknown.

The aim was to characterize the small-scale genetic structure of male and female genotypes in order to unravel the sexual reproduction strategy of *T. melanosporum* and, the origin and behavior of male genotypes. For that, 241 ascocarps, 475 ectomycorrhizae and 20 soil cores were harvested along five seasons, in a 25-year-old truffle orchard in France. The samples were mapped and genotyped using microsatellites and mating-type genes. We obtained the genotypes of female and male ascocarp partners, of mycorrhizas and the mating-type present in soil samples.

Isolation by distance analysis revealed pronounced small-scale genetic structure for female and male genotypes. The genotypic diversity was higher for male genotypes with numerous small-size genotypes, suggesting an important turnover due to ascospore recruitment. Larger and perennial female and male genotypes were also detected. Three genotypes were found as both female and male genotypes (hermaphrodites) while most were detected only as female or male (dioecy).

Our results suggest that germinating ascospores act as male genotypes, but also soil mycelium could be a reservoir of male genotypes. These findings open new perspective in the cultivation of truffle, to improve production by the implementation of methodologies promoting truffle mating.

Keywords: Tuber melanosporum, mycorrhiza, sexual reproduction, mating-type, genetics

CL (ID 70)

Genetic diversity and population analysis of Cenococcum geophilum

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Cenococcum geophilum (Dothideomycetes, Ascomycota) is one of the most common ectomycorrhizal fungi in boreal, temperate and some subtropical regions. Cenococcum geophilum was originally considered as a single anamorphic species but accumulating evidence suggests that C. geophilum is a diverse species complex that also likely undergoes cryptic recombination. Here we provide an overview of what is known on the genetic diversity and population structure of C. geophilum and we suggest important future directions for studies of Cenococcum. Recent molecular data indicate that the genetic diversity within Cenococcum can be incredibly high, even at the scale of a single soil core. Given this high diversity, it is important to phylogenetically characterize Cenococcum samples prior to population studies so that cryptic taxa are not admixed together in the analyses. Also, low correspondence between genotypes from sclerotia and ectomycorrhizas was found in an isolation-based study, indicating appropriate sampling design and sampling effort are also critical for elucidating the population and phylogenetic diversity of Cenococcum. A recent population study targeted one Cenococcum lineage found no spatial autocorrelation at the forest stand level but found evidence for a pattern of isolation by distance at larger spatial scales. These observations are consistent with the possibility of cryptic recombination. Another recent phylogenetic study found that several Cenococcum lineages are widely distributed across multiple regions and continents. This indicates that some lineages within Cenococcum may be ancient or that cryptic long-distance dispersal is ongoing.

Keywords: cryptic species, ectomycorrhizal fungi, recombination, species complex

CL (ID 118)

Exploration of mating strategies in arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil fungi that colonize the roots of vascular plants, increasing the plant's uptake of essential nutrients. These fungi are classified as ancient asexuals, however, recent discoveries from our lab indicate the presence of cryptic mating processes in these organisms. Specifically, a conserved region identical to those controlling mating in other fungi (MAT-loci) was found in the genome of 27 strains of the model AMF Rhizophagus irregularis. The region is composed of two homeodomains and up to six putative MAT-loci have been defined. Of these 27 strains, 3 harbor two distinct mating types in their mycelium; a dikaryon-like structure providing compelling evidence of genetic exchange. 1,2 My project aims to identify the distribution and diversity of mating types present in both in vitro cultures and the environment, in addition to understanding spore compatibility and what drives the shift to sexual reproduction in AMF. Here, I show that AMF strains always exhibit one or two mating types, with no evidence of greater karyosis (e.g. tri-tetra or more). I also found that spores of dikarvotic strains can contain one of each compatible MAT-locus or a combination of both. Furthermore, by analysing the MAT-locus in various soil samples, I provide preliminary data showing that more than 6 main MAT-locus lineages exist in natural communities of Rhizophagus spp. Overall, this research provides further stepping stones for understanding the origin of genetic diversity in AMF. Such knowledge is crucial to direct the creation of new AMF strains optimized for expanded roles as bio-fertilizers

References:

- Ropars, J. et al. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. Nat. Microbiol. 16033 (2016). doi:10.1038/nmicrobiol.2016.33
- Corradi, N. & Brachmann, A. Fungal Mating in the Most Widespread Plant Symbionts? Trends Plant Sci. 22, 175–183 (2017).

Keywords: arbuscular mycorrhizal fungi, mating, karyosis, compatibility

CL (ID 275)

Intraspecific genomic variation in the ectomycorrhizal symbiont *Laccaria bicolor* links to variation in ecosystem functioning

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Ectomycorrhizal fungi (EMF) are important for forest ecosystem functioning. Recent evidence from a plant-soil microcosm experiment with manipulated EMF diversity highlighted the importance of inter- but remarkably also intra-specific diversity in influencing ecosystem functions¹. To gain a better understanding of the importance of EMF intraspecific diversity, comparative genomic analyses of ectomycorrhizal basidiomycete Laccaria bicolor strains were conducted to identify genomic features that could be responsible for the observed functional variation between the strains. Eight L. bicolor strains were sequenced and assembled to reference genome L. bicolor S238N-H82 v2.0 (JGI MycoCosm database). Phylogenetic and single nucleotide polymorphism (SNP) analyses using homologous sequences were conducted, including transporter and KEGG genes that have been shown to be upregulated during mycorrhizal symbiosis. Two distinct clusters of L. bicolor strains were identified. Cluster 1 contained greater genomic variability and also greater strain diversity effects on functioning and physiology metrics in comparison to the less genetically diverse cluster 2. Of 60 transporter and 216 KEGG genes used in SNP analyses, only five and twenty genes, respectively, were highly conserved across all strains. Substantial variability in SNP rate was found across 33 gene families investigated with the proportion of genes containing non-synonymous SNPs in cluster 1 greater (average 59%) compared to cluster 2 (17%). Intraspecific genetic variability of L. bicolor is considerable. Functional variation between the strains could not be related to a specific gene or gene family indicating genetic variation reflected overall genetic distance between strains rather particular genetic elements being responsible for specific functional attributes.

References:

¹Hazard C, Kruitbos L, Davidson H, Taylor AFS, Johnson D (2017) Contrasting effects of intra- and interspecific identity and richness of ectomycorrhizal fungi on host plants, nutrient retention and multifunctionality. New Phytologist 213, 852-863.

Keywords: ectomycorrhizal fungi, *Laccaria bicolor*, genomic comparison, intraspecific, ecosystem function

CL (ID 192)

Insights from the genome of symbiotic fungus *Rhizophagus proliferum*, the second species of AMF to be sequenced to date

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Introduction: Arbuscular mycorrhiza fungi (AMF, phylum Glomeromycota) are ecologically important component of soils and form symbiotic association with almost 70% of the terrestrial plant species in all major ecosystems [1-2]. Species of AMF influence plant growth response, plant diversity and plant community structure [3]. Genetic architecture of AMF has largely remained undetermined, despite recognition of its potential application in sustainable agriculture. AMF produce aseptate hyphae and form multinucleate spores, in which the extent of nuclear heterogeneity within an isolate has remained contentious. Whole genome sequencing studies attempted to date on the only AMF species, *Rhizophagus irregularis*, have generated contrasting results for within isolate nuclear genetic composition [4-5]. To attain further insights into AMF genetic structure and function, we carried out whole genome sequencing of the second AMF species, *Rhizophagus proliferum*.

Materials and Methods: *De novo* whole genome sequencing was done using Illumina HiSeq2500 platform. The Genome was assembled by the MSR-CA assembler and investigated for completeness, evolutionary relationship, within isolate nuclear homogeneity, and protein families important in establishing AMF-host interaction especially small secreted proteins (SSP).

Results and Discussion: Study of the genome of *R. proliferum* provides further understanding of genomic architecture and evolution of AMF species. Low nuclear heterogeneity observed in this study corroborates the observation made by Tisserant et al [4] for *R. irregularis* genome. Putative genes of the fungal-partner that could be involved in establishing symbiosis with plants are identified.

Conclusion: The present study is of fundamental importance to the field of AMF functional-genomics and population-genetics.

References:

- 1. Davison et al., 2015; 2. van der Heijden et al., 2015; 3. van der Heijden et al., 1998;
- 4. Tisserant et al., 2013; 5. Boon et al., 2015

Keywords: AMF genome, evolution, nuclear homogeneity, proteome, secretome

POSTERS

P (ID 226)

Investigating the intraspecific polymorphism of the nuclear ribosomal operon of arbuscular mycorrhizal strains from GINCO-CAN

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Most of the species of arbuscular mycorrhiza (AM) described during the last 15 years have been sequenced using nuclear ribosomal markers. These markers are easy to amplify thanks to the succession of conserved and variable regions along the ribosomal RNA operon and due to their organisation in tandem repeat arrays. Nevertheless, an important disadvantage of targeting the RNA operon is that the multiple copies of the rRNA genes may vary within each nucleus leading to high levels of intraspecific variation. The goal of this study is to analyse the level of intraspecific polymorphism in rRNA genes for 16 AM cultivated under in vitro conditions, including 12 strains from the Glomeromycota In vitro Collection (GINCO-CAN) hosted in Ottawa Research and Development Centre. A 3 kb fragment representing the 18S, ITS1, 5.8S, ITS2, and partial 28S regions was amplified for each strain. PCR free libraries were constructed and indexed libraries were size selected to 500bp and sequenced on MiSeq platform (Illumina). The record of all the putative variants of rRNA genes for well-characterized cultures is expected to facilitate AM identification both at the species and community level.

Keywords: genetic variation, nuclear ribosomal operon, culture collections, intraspecific polymorphism

P (ID 411)

Sequence-based phylogeny of Glomeromycota: from virtual taxa to phylospecies and global ecological and biogeographical patterns

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Arbuscular mycorrhizal fungi (AMF) are ecologically and agronomically important, but global data on their species abundance and diversity are scarce. From ~290 morphologically described AMF species only a part is sequenced or available in culture collections, due to their obligate symbiotic lifestyle. The amount of 'taxa' based on environmental DNA sequences largely exceeds the number of described species. Thus, research is needed to further define the AMF species on the molecular level and to understand their genetic variability.

We aim to sequence the almost complete rDNA spanning the SSU, complete ITS region and a large part of the LSU (~2.5 kb) from morphologically described taxa in cultures representing main AMF lineages and selected AMF species-rich field samples from across the globe. This will enable us to determine bioinformatic thresholds for species, genus, family and order-level resolution for the different marker regions used (SSU, ITS and LSU) for all main glomeromycotan lineages, to conduct a robust backbone phylogeny, to compare results of AMF community studies targeting different rDNA regions and search for general patterns in AMF biogeographic and ecological distribution at global scale.

An optimized nested PCR approach for amplification of the proposed fragment is used followed by the SMRT (Single Molecule Real Time) sequencing, which in combination with the circular consensus reads (5 repeats) enables sequencing of long reads with high accuracy. The results will improve the environmental sequence assignment to the species-level and thus serve for a better interpretation of AMF community data from next generation sequencing studies.

P (ID 131)

Reproductive biology of the black truffle *Tuber melanosporum*: contrasting ecologies of the two parents

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The black truffle *Tuber melanosporum* is an ectomycorrhizal ascomycete of commercial value. Few is known about the reproductive biology of this famous edible mushroom. It is potentially hermaphroditic but reproduction - i.e. formation of the fruitbody (ascocarp) and production of meiotic spores (ascospores) within the later - requires mating between two individuals with opposite mating types 1,2 . At fruiting, a maternal parent forms the ascocarp, whereas the paternal one only leaves genes in the ascospores. In addition, beyond ascocarps, maternal genotypes also form surrounding ectomycorrhizas, suggesting that they are established as tree symbiont³.

We summarize here novelties issuing from five years of sampling over France. In order to characterize the genetic structure of populations for both parents, we conducted genotyping analyses using 12 microsatellites to characterize maternal and paternal genotypes in ascocarps from seven truffle grounds⁴. This revealed high inbreeding and a strong isolation by distance for maternal and paternal genotypes, ruling out the possibility of gametic gene flow. Maternal individuals were bigger and more perennial than paternal ones, more numerous and transient. Paternal genotypes were never found on surrounding ectomycorrhizas. This suggests that the two partners have different developments and niches.

We also investigated herbaceous plant roots as a potential niche for the two paternal partners by barcoding and, when possible, by genotyping *T. melanosporum* from these roots. Although the black truffle infects the herbaceous plants of truffle grounds, only maternal genotypes were again found here. We thus suggest that germinating ascospores could act as paternal parent⁵.

References:

- ¹ Riccioni et al. (2008), *Tuber melanosporum* outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. New Phytologist 180, 466–478.
- ² Martin et al. (2010), Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464, 1033–1038.

Keywords: black truffle, fungal dioecism, ectomycorrhizas, life-cycle, population genetics

³ Murat et al. (2013), Fine-scale spatial genetic structure of the black truffle (*Tuber melanosporum*) investigated with neutral microsatellites and functional mating type genes. New Phytologist 199, 176–187.

⁴ Taschen et al. (2016), How the truffle got its mate: insights from genetic structure in spontaneous and planted Mediterranean populations of *Tuber melanosporum*. Molecular Ecology 25, 5611–5627.

⁵ Selosse et al. (2013), Do black truffles avoid sexual harassment by linking mating type and vegetative incompatibility? New Phytologist 199: 10–13.

P (ID 479)

Comparative genomics reveals functional diversity in two dark septate endophytic (DSE) fungi of grasslands

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Dark septate endophytes (DSE) compose a form group of mainly anamorphic ascomyceteous root endophytes with melanized septated intraradical hyphae. DSE fungi are found worldwide and they seem to be relatively frequent in harsh and nutrient-poor environments such as arid and semiarid areas. Nevertheless, our knowledge of their role in ecosystem functioning is limited. *Cadophora* sp. and *Periconia macrospinosa* are common DSE fungi found in semiarid grasslands, representing distinct phylogenetic lineages and different host preferences. The genomes of these two species were sequenced and analyzed to gain insight into the enigmatic lifestyle of DSE fungi.

The genomes of *Cadophora* sp. and *P. macrospinosa* were assembled into 1,193 (1,092 > 2 kb) and 1,566 (1,217 > 2 kb) scaffolds, with expanded genome size of 70.46 Mb and 54.99 Mb including 22,766 and 18,750 gene models. Families of carbohydrate active enzymes (CAZymes), secreted proteases and lipases, and genes linked to secondary metabolism, meiosis and melanin synthesis pathways were analyzed across the two DSE genomes and other 32 ascomycetes with different lifestyles. The results showed significant expansion of certain gene families, e.g. both root endophytes possess increased numbers of plant cell wall-degrading enzymes. Genome-wide reconstruction of gene duplication and loss histories revealed high numbers of species-specific gene duplications in *Cadophora* sp. and *P. macrospinosa* and low levels of convergence in gene family evolution, which suggests functional diversity in species classified as dark septate endophytes.

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P (ID 18)

Molecular diversity of arbuscular mycorrhizal fungi in rhizosphere soils of sugarcane in southern China

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Guangxi province has the largest sugarcane growing area in China (about 93 million hectares). Due to continuous cropping over more than twenty years, the soils are seriously deteriorated. In our study, 104 samples of sugarcane roots and rhizosphere soils, representing seven different types of soils from 26 counties/districts in Guangxi were collected. Arbuscular mycorrhizal (AM) fungi were identified/quantified by molecular approach and their diversity was investigated. It was found that 61.5% of the 52 collected soil samples had a pH value that was 6.0 or lower. In contrast, 25% of the samples had a pH value ranging from 6.1 to 7 and 13.5% had a pH value higher than 7. The organic matter in the soil was very low. AM fungi colonized sugarcane root epidermis extensively. Following nested-PCR amplification of AM fungal 18S rDNA from sugarcane roots and rhizosphere soils, all sequences of AM fungi were grouped into 32 operational taxonomic units (OTUs). Clustering analysis indicated that they belong to 7 families, 13 genera, and 32 species of AM fungi, including Glomeraceae (5 genera, 17 species), Diversisporaceae (2 genera, 3 species), Acaulosporaceae (1 genera, 1 species), Gigasporaceae (1 genera, 1species), Claroideoglomeraceae (1 genera and 5 species), Archaeosporaceae (1 genera and 2 species) and Paraglomaceae (1 genera and 2 species). Among the 32 molecular species, 7 had the same morphology. Glomeraceae was the most dominant family in all soil types in the study, followed by Claroideoglomeraceae and Paraglomaceae. Rhizophagus was the most dominant genera.

Acknowledgement: This research was funded by NSFC (No.31360356)

Keywords: molecular diversity, arbuscular mycorrhizal fungi, rhizosphere soils, sugarcane, southern China

P (ID 494)

Genome analysis of Endogone pisiformis and Sphaerocreas pubescens in Mucoromycotina

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Endogonalean fungi are members of the subphylum Mucoromycotina, an early divergent fungal lineage. This fungal group includes several endomycorrhizal and endophytic fungi of land plants. Endogonales is a key fungal taxon for understanding the evolution of mycorrhizal symbioses. In this study, we sequenced genomes of Endogone pisiformis and Sphaerocreas pubescens, closely related to uncultured Mucoromycotina endophytes. Genome size of E. pisiformis and S. pubescens was estimated 71.1 and 59.2 megabases based on k-mer analysis, respectively. We comprehensively predicted 19,725 proteincoding genes of E. pisiformis and 16,798 genes of S. pubescens. Genome-wide phylogenetic analysis showed that these two fungi are phylogenetically closely related. In the genome of S. pubescens, tyrosine kinase-like domains are highly diversified, which has been also observed in other endomycorrhizal fungi, Rhizophagus irregularis and Tulasnella calospora. Comparative genome analysis of diverse fungal species revealed that genes encoding resistance-like proteins and transient receptor potential Ca²⁺ channels are highly expanded in both E. pisiformis and S. pubescens genomes. The genome information of E. pisiformis and S. pubescens can serve as a useful tool for the study of symbiotic Mucoromycotina fungi.

P (ID 39)

Single nuclei sequencing on Arbuscular Mycorrhizal Fungi

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The genetic structure and organization of the multinucleate arbuscular mycorrhizal fungi (AMF) spores has been an unresolved topic of debate over the last decades. Unlike other eukaryotes, AMF have no single nucleate state in their life cycle and are assumed to be asexual. Furthermore, hyphal phusion and nuclear mixing occurs, that may result in heterokaryosis (Croll *et al.*, 2009). Multiple fingerprint methods have supported the hypothesis that AMF have a dynamic heterokaryotic system for heritability and adaptation. However, recent studies revealed that AMF do have meiotic genes and, perhaps, a cryptic sexual cycle with recombination as the origin of heterokaryosis (Ropars *et al.*, 2016).

Now, a new technique opens the way to finally assess how the genetic variation is organized in AMF; by using a Fluorescence-activated cell-sorting (FACS) instrument we can isolate multiple individual nuclei from crushed spores. Subsequent whole genome amplification of the nuclei allows us to sequence genomes of different nuclei from the same organism, and even from the same spore. Results from a pilot study on *Clareidoglomus candidum* will be presented.

References:

Croll D, et al. (2009) Non-self vegetative fusion and genetic exchange in the arbuscular mycorrhzial fungus *Glomus intraradices*. New Phytol.

Ropars J, et al. (2016) Evidence for the sexual origin of heterokaryosis in Arbuscular mycorrhizal fungi. Nature Microbiology.

Keywords: genomics, single nuclei sequencing, whole genome amplification, *Clareidoglomus candidum*, fluorescence-activated cell-sorting

P (ID 287)

Detecting the phylogenetic position of tropical edible Boletaceae from Thailand by an ITS-LSU combined analysis

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Introduction/Aim: The Boletaceae is a large family with worldwide distribution. Most species are important ectomycorrhizal fungi in forest ecosystems, and many of them are edible. In Thailand, there are many diverse genera and species but only a few studies based on nucleotide sequences are available. In this study, two loci were investigated from edible boletoid mushrooms found in Thailand and used to construct the phylogenetic tree (ITS and LSU nrDNA).

Materials and Methods: In this study, DNA sequences of two loci (ITS and LSU nrDNA) were newly obtained from representative specimens of Boletaceae collected in Thailand.

Results & Discussion: Most of Thai boletes belong to 2 subfamilies of Boletoideae (*Boletus* and *Tylopilus*) and Xerocomoideae (*Aureoboletus*, *Boletellus*, *Heimioporus*, *Sinoboletus* and *Xerocomus*). Interestingly, many nucleotide sequences especially ITS exhibited low % similarity varying from 82 to 96 % against sequences from GenBank and Unite databases. The morphological characters of these mushrooms are unique and specimens correspond to unidentified species.

Conclusion: The nucleotide sequences obtained from this study are not only increasing the number of boletes sequences from the tropics in databases, but new records of boletes awaiting for describing.

Keywords: Boletales, ectomycorrhiza, wild edible mushrooms, Thailand

P (ID 369)

Phylogenetic position of ectomycorrhizal species in genus *Ramaria* from tropical Thailand

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Ramaria is a large clavarioid genus in the Gomphaceae with a worldwide distribution and includes several important ectomycorrhizal species both in temperate and tropical geographical areas. This work concentrates on a small group found in northeast Thailand which have been analysed morphologically and anatomically, and by molecular techniques using nuclear large subunit and internal transcribed spacer sequences. The representatives of Ramaria samples were investigated and constructed a phylogenetic tree with closely related species obtained from GenBank and Unite databases. Interestingly, the results showed clear separation into six new subclades of unidentified Ramaria species from Thailand with high bootstrap support. Blast results also revealed low percentage of similarity, less than 97%, comparing to all identified species. There are at least 10 species found in our study and some are new records from Thailand. This study was the first report exhibited the phylogenetic position of Ramaria species from Thailand based on nucleotide sequences analysis.

Keywords: Ramaria, coral fungi, phylogenetics relationship, DNA sequences, Thailand

P (ID 347)

Sieving Soil, Destroying Diversity? The Impact of Processing on Fungal Communities in CPP Degradation Studies

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Efficacy of Crop Protection Products (CPP) is determined by their availability in the soil, which is mediated in part by microbial degradation. Fungi are hypothesised to be important in the breakdown of CPPs yet their role is poorly understood. Regulations laid down by the OECD (and practiced by most countries) require soil used for tests to be sieved and moisture adjusted before use. In addition soil samples are regularly stored for periods of up to 12 weeks before use in trials due to sampling regimes. Recent studies at Syngenta have found a significant link between these preparatory techniques and the rate of degradation of CPPs.

To investigate the impact of soil processing on the fungal communities cores were taken during the spring, summer and autumn seasons from agricultural and pasture sites. To test the effect of soil processing on fungal diversity, DNA was extracted from the cores before they were processed in accordance with OECD guidelines and put into a CPP degradation test environment for a month before the second DNA soil extraction. Fungal community structure and abundance were determined by ITS region fungal barcode via PCR using fungal ITS1F and ITS4 primers. We will present data comparing unprocessed and processed communities, elucidating the significant impact these regimes have upon the native fungal community. Understanding the effect soil processing has on fungal diversity is the first step in a larger study that seeks to determine the role of soil fungi in driving CPP biodegradation and efficacy.

Keywords: sieving, degradation

P (ID 446)

Estimating Fungal Barcode Variation Using an in silico Genomics Approach

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The Internal Transcribed Spacer (ITS) region exists as a cassette of tandem repeats and has long served as the primary barcoding locus for fungi. While the repetitive nature of the ITS region makes it highly amenable to amplification, variation in the number of ITS repeats among different fungal species may cause misrepresentations of abundance in amplicon-based 'metabarcoding' studies. Data derived from whole genome sequencing efforts have the potential to identify variation in ITS copy number, however, most genomes to date have been sequenced on short-read platforms, which either fail to align repetitive regions or collapse them into a single representation during assembly. To overcome this shortcoming, we have adopted a sequence read depth-based approach to compare the number of ITS sequence reads to those of many single copy genes. Here, we present a pipeline for estimating fungal copy number from raw short-read genome sequence data and validate the method using an in silico mock genome. We then analyze the genomes of a diverse suite of 11 ectomycorrhizal fungi and show that ITS copy number varies widely among fungal taxa, ranging from 9 to 166. Finally, we discuss the results of a metabarcoding analysis of an ectomycorrhizal mock community for which DNA template concentrations have been adjusted based on estimated copy number variation.

Keywords: ITS, Copy number, genomics, barcode, metabarcoding

CONCURRENT SESSION: Modelling of mycorrhizas

Most plants transfer carbon to mycorrhiza to increase their access to limiting nutrients. As such, mycorrhizas are increasingly being integrated into our understanding of how coupled carbon and nutrient cycles respond to global change. Recent data syntheses have showed that mycorrhizas drive ecosystem and global scale patterns of soil carbon and nutrient cycling as well as the ability of ecosystems to sustain productivity gains under elevated CO2. Despite their relevance, integrating mycorrhizal dynamics into predictive ecosystem models has remained a challenge. Empirically, this challenge reflects the difficulties in producing data that can inform and validate models. Models of mycorrhizas have been developed from the spatial scale of a single hypha up to global scale models that incorporate mycorrhizal effects. At different levels of complexity, they describe hyphal proliferation, colony development, and the exchange of nutrients and carbon between the partners. However, there remains a need for modelers to mine the complexity of fine scale models to generate the simplifications necessary to model mycorrhiza at larger scales. We invite contributions from both empiricists and modelers focused on efforts to produce datasets to inform models, generate conceptual frameworks that prioritize mycorrhizal function for model integration, and model mycorrhizal dynamics across multiple scales.

Chairs: Andrea Schnepf, Edward R. Brzostek

IL (ID 74)

Including ectomycorrhizal fungi in a forest ecosystem model: importance for the P cycle

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Recently reported growth decline and decreased foliar nutritional status suggest that water and nutrient shortage, especially P, could be major constraints to forest productivity in the future. Whereas significant progress has been made in developing mechanistic models simulating the water, C and N cycles in forest ecosystems, the cycling of P has received little attention. This greatly handicaps models' potential to accurately predict forest growth under changing environments. Forest ecosystem models generally include a simple representation of soil functioning. To get realistic results such models need to simulate an unrealistically large availability of P to the plants. By including the interaction with ectomycorrhizal fungi a more realistic representation of tree P uptake is possible. This can lead to new insights concerning the thresholds for P-limitation of forests. We show how including explicit representation of EM fungi improves the results of simulating a Scot pine forest with the forest model ANAFORE. The sensitivity of the model to EM-parameters is further analysed.

IL (ID 211)

Colonization-competition tradeoffs structure ectomycorrhizal diversity

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Introduction: Fungi vary in key traits, and interest is growing in trait-based approaches to fungal community ecology that differ from frameworks based mainly on neutral processes¹. In ectomycorrhizal fungi, research supports tradeoffs between production of propagules and ability to compete for root tips², which could affect succession and help to create diversity, but the influence that these phenomena have on ectomycorrhizal community structure *in situ* remains unclear. Synthesizing nearly a decade of research at Point Reyes National Seashore in California, we combined empirical data from field experiments with mathematical modeling to explore the role played by colonization-competition tradeoffs in ectomycorrhizal communities.

Methods: Using well-established soil bioassay techniques, we examined an inoculum gradient at a forest edge in order to determine whether ectomycorrhizal species distributions showed evidence of tradeoffs between colonization and competition. Then, we used a model incorporating colonization-competition tradeoffs to determine whether they alone could give rise to the patterns of diversity seen in ectomycorrhizal communities.

Results/Discussion/Conclusion: We found significant evidence supporting the importance of tradeoffs between colonization and competition as a structuring force in ectomycorrhizal communities, demonstrated both by patterns of species abundance along the inoculum gradient, and also by the ability of our model to reproduce empirical data. We thus show that these tradeoffs strongly affect not only spatial and temporal variation in ectomycorrhizal community composition, but also the creation and maintenance of ectomycorrhizal diversity in general.

References:

- 1 Crowther TW, Maynard DS, Crowther TR, Peccia J, Smith JR, Bradford MA. 2014. Untangling the fungal niche: The trait-based approach. Frontiers in Microbiology 5: 1–12.
- 2 Kennedy PG, Higgins LM, Rogers RH, Weber MG. 2011. Colonization-competition tradeoffs as a mechanism driving successional dynamics in ectomycorrhizal fungal communities. PLoS ONE 6.

Keywords: ectomycorrhiza, diversity, trait-based ecology, modeling, colonization-competition tradeoffs

CL (ID 108)

Mycorrhiza enhances substrate water holding capacity and conductivity by filling up voids and altering pore size distribution

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Arbuscular mycorrhizae (AM) proliferate in pore spaces and increase plant drought tolerance. Their influence on physical properties of substrates remains less clear. We hypothesized that (i) AM change pore size distribution via colonization of particular pore spaces and, (ii) increase the substrate hydraulic conductivity.

We carried out experiments with tomato that created variation in colonization by harvest date or by mixed pots with WT plants and mycorrhiza resistant (RMC) mutants. Sampling cores were introduced and used to assess substrate moisture retention dynamics and state-of-the-art modeling of substrate water retention and hydraulic conductivity.

Under exclusion of roots, AM reduced total pore space, especially those spaces from where water would be lost by drainage. In exchange, AM increased the amount of pores in the bulk substrate with smaller diameters that contribute to water holding capacity. This is strongly correlated to the biomass of extraradical mycelia. The mean pore diameter of capillary pores increased in AM substrates and, hence, allows a higher hydraulic conductivity when water is sucked from those pores. When root proliferation is allowed, AM effects decrease gradually with rooting densities, because roots will occupy most of the pore space.

The pore loss in AM substrates is discussed as the cause for increased water repellency and the gain of capillary pores as a result of AM ingrowth into coarse pores.

We conclude that irrigation is differently effective in AM and NM substrates and moisture stress of a host is decreased in colonized substrates at the same level of substrate water content.

Keywords: arbuscular mycorrhiza, drought, soil moisture retention, hydraulic conductivity, pore size distribution

POSTERS

P (ID 155)

Mycorrhiza and Obesity, are they related

Meenakshi Sharma (Delhi University, New Delhi, India & London, Canada)

Mycorrhiza, the benefits are unquestionable, but has something being neglected while studying Mycorrhiza and Rhizobium for utilizing as a fertilizer? If yes, then what is it, and why is it essential to discuss the problem now.

Cancer and obesity are two very ubiquitously found problems now-a-days, the reason for which has not been found. These problems existed previously also but the population was not so heavily impacted by these two medical issues. While undertaking some theoretical research after my MPhil. work and analyzing the data in front of me with a different manner of understanding, it was found that Rhizobium and Mycorrhiza could be and most probably are infection of the plant, not necessarily providing the required nutrients to humans. The reason for my analysis was based on the following questions, is bigger always better, while increasing nutrient uptake, is mycorrhiza increasing nutrients within economically important parts of the plant, is it increase in size only due to water retention or excessive water uptake? Is the fungal body increasing within the plant or is it only plant tissue adding to the plant size? Is it better to increase number of fruits instead of the size of it, same genome behaving differently? My research answered these questions, to my dismay, it did not provide me with an answer that was beneficial to utilize Mycorrhiza as a bio fertilizer. During my presentation I will discuss a theoretical model that I have written to explain a possible correlation between Obesity and Mycorrhiza.

Keywords: Mycorrhiza, Obesity

P (ID 180)

Desert truffle cultivation: A bioclimatic model for suitable management and production

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Desert truffles are edible hypogeous fungi from the *Pezizaceae* family. Ascocarps of *Terfezia* and *Tirmania* have been an important food and medicinal resource for thousands of years in arid and semiarid regions such as Mediterranean Basin, Middle East, countries across the Sahara and Kalahari Deserts and the Australian Outback. Nowadays *Terfezia* cultivation with *Helianthemum* spp. host plants is a reality, being *Terfezia claveryi* one of the few species of mycorrhizal fungi that is being cultivated ¹. However, there is still a lack of information regarding the orchard management in order to ensure a constant truffle production. Our hypothesis was that there are certain meteorological parameters which influence dramatically the orchard performance.

The present work builds up a fourteen years study of the first productive desert truffle plantation initiated in 1999. We sought to model the relationship among the fourteen years meteorological data and the *T. claveryi* truffle production. Pearson correlation analysis and multiple comparisons were made on all bioclimatic parameters *versus* annual desert truffle yields.

Positive and negative correlations were found with almost all the climatic parameters, but only autumn precipitation and spring temperatures were able to explain more than 85% of variability. A mathematical model has been made that combines several climatic parameters and explains more than 95% of variability of desert truffle production. This model allows to propose different managements to increase and buffer truffle production along the years and adapted to the global climate change, as well as to estimate the potential truffle production in future plantations.

References:

¹ MORTE A., ANDRINO A., HONRUBIA M., NAVARRO-RÓDENAS A., 2012. *Terfezia* cultivation in arid and semiarid soils. In: Edible Ectomycorrhizal Mushrooms. Soil Biology, (Zambonelli A., Bonito G.M., eds) vol 34. Springer-Verlag Berlin. Heidelberg, pp. 241-263.

Acknowledgements: This work was supported by projects 19484/PI/14 (FEDER and Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia, Spain) and CGL2016-78946-R (AEI/FEDER, UE). ANR thanks the University of Murcia for a postdoctoral contract. JEMG thanks MINECO for a PhD grant (DI-14-06904).

Keywords: desert truffle, Terfezia claveryi, cultivation, bioclimatic model, turmiculture

P (ID 493)

Hundred years of mycorrhizal research

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In 1895 Frank introduced the term mycorrhiza and research in the field has grown tremendously since. Network analysis of plant fungal associations has taught us much about host specificity and species distribution in (arbuscular mycorrhizal fungi) AMF and (ecto-mycorrhizal fungi) EMF communities. Similarly, we can learn much about ourselves as a scientific community by studying the networks that we form. We analyzed mycorrhizal publication from 1917–2017 (Title: *mycorrhiz* in Web of Science on May 9th 2017) and used network analysis to explore citing patterns. Specifically we wanted to know if geographical citation patterns could be observed indicating distinct scientific communities of American mycorrhizal scientists (AMS) vs. European mycorrhizal scientist (EMS).

Many mycorrhizal publications originate from Europe (41%), followed by North America (25%), Asia (18%), Oceania (9%), South America (5%) and Africa (3%). 14 % of the publications are intercontinental. Interestingly Europe dominates publications on AMF with few intercontinental publications while EMF publications are dominated by North America with the majority of publications being intercontinental. Up until the first ICOM in 1993 strong geographic citations patters were observed for AMS and EMS. These temporarily dissolved following the first mycorrhizal meeting. Continental citation patterns were largely dissolved by the start of the 21-century. Asia is now emerging as the strongest publication force in the community contribution to increased intercontinental citation.

Keywords: publication, network analysis, citation

P (ID 49)

Modelling the Root Age Structure of Perennial Woody Plants

<u>Tyler Poppenwimer</u> (Ecology and Evolutionary Biology, The University of Tennessee, Knoxville, USA), **Louis Gross** (Ecology and Evolutionary Biology, The University of Tennessee, Knoxville, USA), **Joseph Bailey** (Ecology and Evolutionary Biology, The University of Tennessee, Knoxville, USA), **Megan Rúa** (Biological Sciences, Wright State University, Dayton, USA)

Root age and nutrient uptake efficiency are typically inverse. It has been suggested overall growth is enhanced by maintaining a large population of younger roots while minimizing biomass allocation to older roots which have low efficiency. However, there are trade-offs in senescing scheduling for new roots and a root age distribution could be skewed towards older roots. Root turnover and root production have been proposed as two methods by which plants control the age distribution of roots. Unfortunately, direct examination of these hypotheses is not experimentally tractable as measuring root age can be difficult. However, an age based theoretical model with varying cost-benefit ratios for roots of different ages could be used to examine the impact on overall uptake of alternative root age distributions. The ability of this model to analyze how root age distribution affects growth may enhance our capability to project whole-plant growth. To our knowledge, no such model has been constructed.

We apply an integral equation approach using varying death, survivorship, and cost-benefit curves to analyze the age distribution of the roots. To simulate growth and aging, we include feedbacks between roots and shoots to incorporate the additional influx of nutrients from roots and carbon from shoots as the plant ages. An objective is to observe the impacts on plant growth of changing root age structure and mycorrhizal associations throughout the life cycle, and determine conditions for which age distribution dynamics tends towards a steady state, a cycle, or if the age distribution fluctuates.

CONCURRENT SESSION: Advances in biological conservation through a better understanding of mycorrhizal ecology

The loss of biodiversity and functional diversity due to land conversion, invasive species and local or global pollution has increased our awareness about the importance of biological conservation. Yet, despite the codependency of plants and mycorrhizal fungi, their interactions have largely been overlooked in the context of habitat restoration and conservation. In recent years, many studies have increased our knowledge on the diversity and biogeography of mycorrhizal fungi; they also have enhanced our knowledge on the interactions and dependency between plants and mycorrhizal fungi at the ecosystem level. In this session, the coevolution and specificity of the mycorrhizal symbiosis will be discussed with respect to the conservation of both plants and mycorrhizal fungi. Questions such as: can mycorrhizal symbioses explain rarity (e.g. in orchids) and threatened status? What role do mycorrhizal interactions play in ecosystem disturbances, such as plant invasions? Can mycorrhizal fungi increase the success of restoration and conservation, *in* or *ex-situ*? Will be addressed by a group of expert speakers.

Chair: Marc-André Selosse

IL (ID 26)

They matter! Mycorrhizal fungi in ecosystem restoration: A local and global perspective

<u>Lena Neuenkamp</u> (University of Tartu, Tartu, Estonia), **Mari Moora** (University of Tartu, Tartu, Estonia), **Maarja Öpik** (University of Tartu, Tartu, Estonia), **John Davison** (University of Tartu, Tartu, Estonia), **Rachel Standish** (Murdoch University, Perth, Australia), **Suzanne Prober** (CSIRO Land and Water Flagship, Perth, Australia), **Jodi Price** (Charles Sturt University, Albury, Australia), **Maret Gerz** (University of Tartu, Tartu, Estonia), **Minna Männistö** (Finnish Forest Research Institute, Rovaniemi, Finland), **Martin Zobel** (University of Tartu, Tartu, Estonia)

Mycorrhizal fungi have a key position among soil microorganisms at the plant-soil interface and are thus important to consider in ecosystem restoration. While several studies have investigated the impact of mycorrhizal fungi on plant community composition and plant performance, studies exploring their role in restoration projects are less common but increasing. We present evidence for the importance of mycorrhizal fungi in ecosystem restoration at two scales. First, we describe a meta-analysis summarizing the effect of mycorrhizal inoculation on the outcomes of ecosystem restoration, considering plant growth and plant richness, as well as the context-dependency of these effects. Findings from our meta-analysis show that mycorrhizal inoculation promotes plant growth and plant richness, but effects are strongly context-dependent on host plant characteristics, soil fertility and inoculum complexity.

Second, to illustrate the conclusions from our meta-analysis, we describe the results of a field study investigating covariation of plant and arbuscular mycorrhizal fungal (AMF) communities during secondary succession of semi-natural grasslands. Findings from covariation analysis imply that plant and AMF communities are clearly correlated in grasslands but diversity as well as strength of correlation between plant and AMF communities decline with shrub and tree encroachment. This indicates that AMF might be an important factor in structuring grassland plant communities with AMF diversity promoting plant diversity. Thus, restoration of grassland plant communities might be retarded by a lack of proper symbiotic AMF, either due to a lack of local fungal propagules, or due to the presence of profoundly altered local AMF communities.

Keywords: plant-fungal interaction, ecosystem conservation, mycorrhizal inoculation, grassland restoration, arbuscular mycorrhizal fungi

IL (ID 281)

The importance of the soil context for understanding arbuscular mycorrhizae in conservation scenarios

<u>Catherine Zabinski</u> (Land Resources and Environmental Sciences, Montana State University, Bozeman, USA), **Brian Ohsowski** (Institute of Environmental Sustainability, Loyola University, Chicago, USA), **Mitchell Greer** (Biological Sciences, Fort Hays State University, Hays, USA), **Bala Chaudhary** (Environmental Science and Studies, DePaul University, Chicago, USA), **Chris Allen** (Civil Engineering, Montana State University, Bozeman, USA)

Our understanding of mycorrhizal ecology has been incorporated into conservation efforts, from rare species management, to habitat conservation, and—to move outside of the traditional consideration of conservation—into the realm of sustainable agriculture. The justification for inoculation is often focused on the beneficial effects for host plants: to enhance seedling success, to contribute to plant community diversity, or to bolster crop production. Most experimental approaches test the effects of AM inoculation on host plant growth. The influence of soil-environment feedbacks on the plant-mycorrhizal symbiosis is often assumed but not tested. In this talk, we review findings that underscore the importance of soil characteristics, such as nutrient levels and soil organic matter, on AM function in conservation scenarios. In addition, a meta-analysis of 55 restoration studies yielding 169 experimental units showed that the effects of AM inoculation on host plants were the most pronounced in severely disturbed soils. In this analysis, AM plants were more than twice the size of non-mycorrhizal plants when growing in contaminated soils, and about 1.4 times as large when growing in soils with lesser disturbances. Those effects were mediated by the addition of other soil amendments, including fertilizer and soil organic matter, with an increase in response to mycorrhizae with fertilizer addition. Framing the question of mycorrhizal function within the broader context of the soil ecosystem could support both our basic understanding of this symbiosis and our capacity to manage mycorrhizae in ecosystems varying in disturbance type and soil condition.

Keywords: conservation, restoration, soil ecology

IL (ID 129)

Fire effect on ectomycorrhizal symbiosis: Lessons for biological conservation

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Among the millions of hectares of ectomycorrhizal (ECM) forests, those that are fire-prone concentrate an astonishing diversity of mutualistic soil fungi that are pivotal for seedling establishment and forest functioning. In these ecosystems, the anthropic foot-print drastically alter fire regime through direct management practices (*e.g.*, prescribed burning) and ecosystem modification (*e.g.*, land use change). Therefore, fire-prone ECM forests are a crossroad of conversation issues for plants, fungi and ecosystems. Here, we explore the links between fire regime, including prescribed fires, and ECM fungal diversity using a two-step approach.

We first review the published literature that directly address fire effect on ECM symbiosis. A remarkably low number of field studies (73) documented this topic in a restricted geographic area that partially represents the geography of both ECM biodiversity stakes and fire risk. The analyzed literature consensually reports long-term shifts in the composition of ECM fungal communities after fire. Contrastingly, the effects of fire on fungal diversity and richness at the local scale lacks of consensus among researchers.

Second, we document fire effects on ECM diversity in the island of Corsica. Using NGS of soil samples collected 15 years after large wildfires, we detected the signature of wildfires on soil ECM fungal communities. Fire negatively impacts numerous ECM OTUs but promotes a few of them in both mineral and organic layers. However, destructive fires do not affect ECM fungal diversity while surface fire increase this diversity. We discuss operational and practical considerations for stakeholders and managers.

Keywords: wildfire, prescribed burning, conservation, ectomycorrhizal symbiosis

IL (ID 302)

Restoration methods alter AM fungal abundance and fungal community composition

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Introduction/Aim, Materials and Methods: Arbuscular mycorrhizal fungi can improve restoration outcomes by facilitating establishment of native plants. Yet, we know little about the effects of restoration methods on AM fungal communities. We examined how invasive plant management methods via adding topsoil from a reference ecosystem (i.e., salvage topsoil) might alter AM abundance and fungal communities in a coastal sage scrubland. Removing and burying seeds of invasive plants via the addition of salvage topsoil, which includes both AM propagules and native AM host-plant seeds, could potentially improve AM growth and diversity. However, some restoration practices, such as stockpiling salvage topsoil or removing thatch might harm AM fungi. Indeed, removing thatch may expose soil surfaces to increased solar radiation and stockpiling salvage topsoil may hinder hyphal growth, damage AM spores, or alter AM fungal community composition. In a restored coastal sage scrubland, we measured plant and fungal community composition at plots with stockpiled or freshly-deployed salvage topsoil.

Results, Discussion, Conclusion: Although restoration treatments reduced invasive plant cover, only freshly-deployed salvage topsoil addition improved native plant richness and cover. Stockpiling salvage topsoil limited fungal diversity, while adding fresh topsoil not only promoted AM diversity and abundance but also increased AM host-plant diversity. Overall, depending on methods used by practitioners, salvage topsoil additions may deter non-native plant invasion and help re-establish the AM community in restored ecosystems.

Keywords: reference ecosystem, invasive plants, restoration, fungal communities, salvage topsoil

CL (ID 298)

Grassland restoration: Does plant functional group influence AMF?

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Introduction: While the overall goal of restoration ecology focuses on assisting the recovery of ecosystems that have been degraded, damaged, or destroyed, the most successful path to recovery is uncertain. Conversion of tallgrass prairie to row-crop agriculture can result in state changes and biotic or abiotic ecological legacies that inhibit return to grassland state and require human intervention (restoration).

Methods: Our study assessed ecosystem processes, such as soil aggregate stability, soil organic carbon, relative abundance of soil microbial functional groups such as arbuscular mycorrhizal fungi (AMF), and nematode community structure following seeding of an agricultural field with diverse mixtures of either native cool-season (CSG) or warm-season grasses (WSG). These replicate restoration plots were compared with adjacent undisturbed grassland.

Results: Eight years following seeding, AMF biomass associated with WSG was greater than CSG, yet remained significantly lower than undisturbed grassland soils. Soil aggregate stability and SOC were also greater in WSG soils compared to CSG, but likewise continued to reflect degradation relative to undisturbed grassland soil. Both AMF and nematode communities under CSG or WSG restorations remained fundamentally different from that associated with undisturbed grassland. For example, AMF communities of the restored prairie exhibited greater relative abundances of Gigasporaceae and relatively lower abundances of Glomeraceae, compared to undisturbed grassland.

Conclusion: Our research indicates ecosystem processes are highly complex and may require extensive time for recovery. Nonetheless, restoration practices that include native warm-season grasses differentially promote AMF biomass, which has well-documented downstream influences on soil structure, SOC, and soil food webs.

CL (ID 148)

Isolation and characterization of arbuscular mycorrhizal and dark-septate endophytic fungi: towards their use in the phytomanagement of metal-contamined sites?

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Introduction/Aims: High tolerance of AM and endophytic fungi to metal pollution and their relatively high abundance in contaminated habitats suggest that they might have an important function for host survival in these extreme conditions¹. Both fungi could affect trace element (TE) uptake of their host plants and increase plant metal tolerance. Therefore, in the context of phytomananagement assisted by beneficial microorganisms, we studied these fungi into more details.

Materials and Methods: Four AMF belonging to *Funneliformis mosseae* and eleven endophytes belonging to Helotiales and Sebacinales were isolated from poplar roots and propagated *in vitro*^{2,3}. The ability of strains to promote plant growth in TE-contaminated soil was evaluated (i) either with herbaceous or woody plants, (ii) on different soils, (iii) in single or dual-inoculation (AMF/endophyte)⁴. Plant biomass and elemental contents were evaluated. Moreover, root colonization by fungi was recorded by using both microscopic and molecular methods. Finally, the activity of alkaline phosphatase (AP) was used to study the impact of inoculation on P cycle.

Results: AMF and endophyte inoculations resulted in the increase of plant biomass and in the decrease of TE contents in shoots. AMF promoted AP activity, resulting in the increase of P mobility in soils. Moreover, when the AMF was co-inoculated with an endophytic strain, both fungi could normally colonize plant roots and plant growth was not affected.

Discussion and Conclusion: The usefulness of symbiotic inocula for biomass production on TE-polluted sites, as well as their potential for improving the efficiency of phytoremediation will be discussed.

References:

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²Lacercat-Didier L, Berthelot C, Foulon J, Errard A, Martino E, Chalot M, Blaudez D (2016) New mutualistic endophytic fungi isolated from poplar roots display high metal tolerance. Mycorrhiza 26: 657–671.

³Berthelot C, Leyval C, Foulon J, Chalot M, Blaudez D (2016) Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal polluted poplar phytomanagement sites. FEMS Microbiology Ecology 92(10): fiw144.

⁴Berthelot C, Blaudez D, Leyval C (2017) Differential growth promotion of poplar and birch inoculated by three dark septate endophytes in two trace element-contaminated soils. International Journal of Phytoremediation (*in press*).

Keywords: arbuscular mycorrhizal fungi, endophytes fungi, phytomanagement, trace element

CL (ID 126)

Pre-inoculation with AMF as a management tool for reintroduction of rare species

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The number of abandoned agricultural fields strongly increased in the last 25 years, but plant communities established on them have lower plant diversity than surrounding grasslands. Many projects thus modify the course of secondary succession by transplanting target species to the abandoned fields, but some species are not able to survive there. One possible explanation could be the shift in the AMF community after long term previous agriculture.

We tested if preinoculation of species by grassland AMF community could enhance their performance on abandoned field.

We selected 10 species pairs according to their grassland specificity: one species occurring only on grassland (specialist) and one occurring on both sites (generalist). Plants were preinoculated by grassland or field AMF community. Two months old seedlings were transplanted to the abandoned field and their survival and growth were monitored for 2 years. At harvest, root and shoot biomass and percentage of root colonization were measured.

Specialist had higher aboveground biomass than generalists independent of AMF, while generalist were larger when inoculated by AMF from abandoned field. Higher survival rate was observed for specialists and plants inoculated by AMF from abandoned field. Percentage of root colonization was species specific.

Better plant performance of species inoculated by field AMF could be explained by higher mycorrhizal inoculation potential of field AMF leading to quick establishment of root colonization. It suggests that inoculation by AMF with high infectivity could enhance the success of plant reintroduction.

Keywords: arbuscular fungi, rare species, dry grasslands, abandoned field, plant conservation

POSTERS

P (ID 338)

Diversity of arbuscular mycorrhizal fungi (AMF) in an impacted mining tailings areas in Mariana-MG, Brazil

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Arbuscular mycorrhizal fungi (AMF) are very important in recovery processes following environmental disasters, such as the rupture of the tailings dam occurred in Mariana, Brazil, on November 2015. The objective of this work was to evaluate the community profile of AMFs in soil from the Rio Doce river hydrographic basin.

Four areas were selected: one undisturbed forest (UND) and three areas under different stages of recovery (REC 1, 2 and 3). Soil samples were collected in triplicate, at different period of times (Feb/2016; Sep/2016 and Feb/2017). DNA was extracted and PCR-DGGE performed using AML1- AML2 and Glo1- NS31 (Nested) primers. The bands of DGGE gel were analyzed and normalized in Bionumerics and after statistical analyses the Shannon diversity index by PAST. Each band was considered as an OTU (operational taxonomic unit), dendrograms were generated using DICE-WARD index.

In the three period of samples, UND were agrouped with similarity above 65%. The FMA community was influenced by the re-vegetation process and a greater diversity was observed in UND and in the advanced re-vegetation areas (REC2 and REC3). The REC1 in the first sampling it was totally covered by tailings and without any plant, and DNA did not amplify. In the subsequent samplings, there were some plants, and an increase in OTUs were observed.

AMFs are dispersed in all disturbed areas, and although no difference (p<0.05) was observed, there was an increase in the AMF diversity in these areas throughout the re-vegetation progress, showing the importance of this association for recovery process.

Keywords: undisturbed (UND), recovery (REC), mining tailings, PCR-DGGE, iron mine residue

P (ID 469)

Mycorrhizal Colonization in Forested Wetlands

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Forested wetlands are wooded habitats with soils that are at least seasonally water saturated. The mycorrhizal ecology of these environments is not well understood, and it was presumed that the low oxygen content of the soil would impede fungal growth. However, recent evidence suggests that mycorrhizae are more common in wetlands than previously thought. Nevertheless, colonization by arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi appears to decrease as soil moisture increases. Even less is known about the ericoid mycorrhizae (ERM) of wetlands, but we suspect that they may be less affected by high soil moisture than other mycorrhizal groups. We are examining patterns of mycorrhizal colonization in plants that form AM, ECM and ERM as well as the relationships between mycorrhizal colonization levels, soil moisture, pH, and soil and foliar nutrient contents. Root samples from two species of AM, ECM and ERM plants were collected from upland, intermediate, and wetland forest plots in Nova Scotia. Mycorrhizae are quantified using clearing and staining and soil and foliar nutrient contents are being related to colonization levels to give an indication of the efficiency of wetland mycorrhizae in nutrient uptake. Our goals are to understand how mycorrhizal relationships are adapted to forested wetland habitats, and how this might be reflected in plant nutrient uptake. Mycorrhizae are an important component of forested wetlands, and the adaptations of ERM may be particularly important in water saturated habitats where ericaceous plants thrive.

Keywords: Ericoid, mycorrhizal quantification, soil moisture, low oxygen conditions

P (ID 17)

Plant-arbuscular mycorrhizal fungi mutualism is reinforced in soil from abandoned fields

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Introduction/Aim: After abandonment of agricultural fields, some grassland plant species colonize these sites (generalists) while other are missing (specialists). We aimed to understand this absence by focusing on changes in AMF communities and soil abiotic properties caused by previous cultivation.

Materials and Methods: We performed a greenhouse experiment with six species pairs, each composed of one specialist and one generalist species. The plants were grown in dry grassland or abandoned field soil inoculated with AMF from one or the other site. Plant growth, abundance of mycorrhizal structures and plant response to AMF were evaluated.

Results: In the abandoned field soil, the increase in plant biomass was lower and the extraradical fungal mycelium (ERM) length shorter while the degree of mutualism was higher than in grassland soil. The AMF from dry grassland produced more arbuscules and vesicles. However, the AMF from abandoned field ensured higher phosphorus nutrition. Specialists had more arbuscules than generalists and, in abandoned field soil inoculated with AMF from dry grassland, they had more ERM.

Discussion: Even if the N:P ratio was lower in abandoned field soil, AMF from this soil were more efficient. This is likely due to faster infectivity of AMF from abandoned field. The lower response of specialists to AMF inoculation could be due to their costly tightly link with AMF.

Conclusion: Because mutualism is being reinforced in the soil and by AMF from abandoned field, inoculation of this soil with AMF from grassland is not a solution to allow settlement of specialists.

Keywords: native AMF, internal and external fungal structures, mycorrhizal growth response, nitrogen limitation, phosphorus content

P (ID 103)

What does limit seedling establishment of four mycoheterotrophic species?

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The mycoheterotrophic plants produce huge number of tiny seeds and are considered as dispersal unlimited. In contrast to this general assumption, many theoretically suitable sites stay unoccupied. In addition, several studies showed they are able to germinate on this unoccupied sites.

We focused on three mycoheterotrophic species: *Neottia nidus-avis* (fully mycoheterotrophic), *Cephalanthera rubra* and *Epipactis atrorubens* (both partly mycoheterotrophic). In study area (SW Bohemia), all study species occur only in beech and pine forests on limestone. As a control, we included *E. helleborine* (partly mycoheterotrophic) occurring in a wide range of forest and shrub habitats. To study their germination success, we used seed batting technique. The seeds packets were buried in 36 sites combining occupied (beech and pine forest on limestone), unoccupied but putatively suitable (beech and pine forest on limestone) and unoccupied but putatively unsuitable (spruce forest on limestone and beech, pine, spruce and scree forest on acidic substrate) habitats. The packets were collected after two years and seedling success was determined to evaluate the availability of suitable mycorrhizal fungi, DNA was isolated from orchid seedlings and adult's roots, soil and ECM root tips followed by NGS.

We found out differences among species in the impact of habitat type, presence of adult plants and distribution of mycorrhizal fungi on germination success. Nevertheless, all species showed very low germination rate on most unoccupied sites (both putatively suitable and unsuitable).

Our results suggest, that the germination success is influenced mainly by habitat type, what corresponds more with habitat than dispersal limitation.

P (ID 321)

Symbiotic orchid seed germination is regulated by nitrates at extremely low concentrations

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Currently, many orchid species are seriously endangered. The reasons for disappearing of many of them from natural habitats remain often unclear. Very little is known about orchid physiological specifics including the regulation of seed germination. Moreover, mature seeds of several terrestrial species do not germinate in vitro, making their cultivation for scientific and rescue purposes impossible. As we have shown previously, nitrates are able to strongly inhibit asymbiotic orchid seed germination. In this work, we tested whether orchid seed germination is sensitive to nitrates also in the presence of mycorhizzal fungi. We performed symbiotic in vitro germination of two species, Dactylorhiza majalis and Gymnadenia conopsea, on cultivation medium containing different concentrations of nitrates. Two strains of Ceratobasidium, one of Tulasnella and Sebacina fungi were tested. The response differed markedly between orchid taxa. Germination of G. conopsea on higher nitrate concentrations was induced by symbiotic fungi while D. majalis germination was negatively influenced by nitrate presence similarly as on asymbiotic culture media. These results suggest that mycorrhizal fungi exhibit various effects on different orchid species. We also show that nitrates can strongly inhibit even the symbiotic germination and therefore might be responsible for recent decrease in number of European orchid sites.

The study was supported by the Charles University, project GA UK No. 365115.

Keywords: orchidaceae, nitrate, seed, germination, in vitro

P (ID 375)

Is the mycorrhizal fungi distribution limiting the orchid establishment in restored meadows?

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In the Czech Republic, vast areas of former species-rich grasslands converted into arable land are undergoing restoration using seeding of regional plant species and regular mowing. Recovery of species with complicated life cycles, such as orchids, is particularly slow though the seed source populations grow in close vicinity. Orchids are obligately mycorrhizal plants which need mycorrhizal fungi (mainly saprotrophic fungi from Tulasnellaceae, Ceratobasidiaceae or Serendipitaceae) for germination. We focused on seven both rare and common orchid species and investigated (i) in situ germination and (ii) in vitro germination and specificity. Only two rather common orchid species (Neottia ovata and Gymnadenia conopsea) germinated in the restored grasslands while others germinated only in undisturbed natural grasslands with established orchid populations. Gymnadenia conopsea showed also lowest specificity to mycorrhizal fungi during in vitro germination. Thus, diversity of orchid mycorrhizal fungi in restored habitats may be limiting to establishment of some orchid species. The slow restoration of orchid species due to absence of appropriate fungi could be potentially overwhelmed by targeted introduction of fungi and substrate amelioration of restored sites.

Keywords: orchid, germination, restored grassland

P (ID 427)

Does forest age matter? Ectomycorrhizal communities in Mediterranean oak forests of contrasted history

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Introduction/Aim: During past millennia, Mediterranean forests dramatically reduced due to high anthropogenic pressures. Only few ones, called ancient forests, continuously conserved their tree cover during the 19th century forest minimum. These ecosystems are scarce and their biodiversity is undocumented. We assessed the species richness, spatial structuration and community composition of ectomycorrhizal (ECM) fungi in forests that differ in regard to their ancientness (ancient vs. post-agricultural secondary forests) and soil conditions.

Materials and Methods: ECM communities were sampled in six downy oak forests, including two ancient humid, two ancient dry and two recent humid forests.

Results: By sequencing fungal ITS, we identified 50 species in 1083 sampled ECM root tips, with higher species richness in ancient forests as compared to recent ones. In ancient forests, only slight differences in species richness were found between humid and dry soil conditions, but the composition markedly differed at various taxonomic levels. In all forests, the community was dominated by Thelephoraceae which colonized 42% of the root tips and represented the most species rich family. The relative abundance of Inocybaceae was higher in recent compared to ancient forests, while Sebacinaceae and Tuberaceae were more abundant in dry compared to humid soil conditions.

Discussion/Conclusion: Our results show that both forest history and environmental conditions shape the composition of ECM fungal communities in Mediterranean oak forests. In this region, integrative conservation strategies of the fungal diversity need to take into account the ecological range of focal tree species and the history of forests.

Keywords: ectomycorrhizal communities, ancient forests, *Quercus pubescens*, Mediterranean, abiotic soil conditions

P (ID 506)

The effect of simulated eutrofication on the growth and mycorrhizal symbiosis of Mediterranean tuberous orchids

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It is widely acknowledged that eutrofication causes decline of orchid populations, namely in temperate or Mediterranean climatic zone. Surprisingly, very little information is available about its mechanisms. We aimed to test the effect of simulated eutrofication on the survival, growth, and mycorrhiza formation of several orchid taxa.

In a series of long term (3 yrs) greenhouse experiments we cultivated *Serapias lingua*, *Ophrys speculum*, *Ophrys lutea*, *Ophrys bombyliflora*, and ×*Serapicamptis capitata*, without competition with any co-cultivated plant species. The strongest eutrofication was simulated by applying the solution containing 17.1 mM N-NO₃, 5.4 mM N-NH₄, 4.5 mM P, 18 mM K, 5.8 mM Ca and 4.6 mM Mg, which for the other treatments was gradually diluted; the least concentrated solution applied contained only 1.2% of the above nutrients. To compare the effect of eutrofication on orchids with the effect on the growth and arbuscular mycorrhiza development, *Plantago lanceolata* associated with *Rhizophagus intraradices* were cultivated under the same conditions and subjected to the same treatments.

In contrast with our initial expectations, the higher supply of mineral nutrients favored the biomass formation, flowering, and vegetative propagation of all taxa studied, except the higher mortality observed in plants treated with the most concentrated solution. The orchid mycorrhiza formation, both root colonization and extraradical mycelium formation, was unaffected by nutrient supply, in contrast to AMF associated with plantains.

These results strongly contradict the opinion that eutrofication affects orchids or their mycorrhizal symbiosis directly. The possible competition mediated effect of eutrofication on orchids should be thus tested.

Keywords: orchid mycorrhiza, eutrofication, *Ophrys, Serapias*

P (ID 33)

Manipulating below ground diversity for above ground diversity: the application of arbuscular mycorrhizal fungi in vegetation restoration

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The loss and fragmentation of habitats is one of the main drivers of deteriorating ecosystem functioning and services. This has induced a growing need for conservation and increasingly more — restoration. A central part of land-ecosystems is soil biota with arbuscular mycorrhizal (AM) fungi being a key constituent. In soils where disturbance has lead to the loss of soil cover or a drastic change in plant community composition, the absence of suitable AM fungal symbionts can lead to slow post-restoration plant community recovery, with negative implications on ecosystem functioning for decades. Because of this, the manipulation of soil AM fungal composition for restoring plant communities is a highly promising perspective.

To test the applicability of this idea, we have set up field inoculation experiments in three ecosystem restoration scenarios in Estonia: restoration of alvar grassland vegetation following clearing; restoration of wooded meadow vegetation following clearing; restoration of vegetation cover in depleted oil shale quarries. Native soils and plant seeds were collected from target ecosystems in a good state and trap cultures were set up to obtain bulk inoculums. These inoculums were used with native plant seeds in 18 restoration sites across Estonia. The experiment will be monitored for the desired effect and temporal changes in above and belowground diversity, giving valuable insights into the practical implication of AM fungi for ecosystem restoration. An overview of the hypotheses, methodology and practical boundries of this experiment will be given, highlighting knowledge gaps and their possible solutions.

P (ID 391)

Interaction between dark-septate-endophytes and ectomycorrhizal fungi in the context of trace-element contaminated sites

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Dark septate endophytes (DSE) are ascomycetous fungi that colonize living plant without causing apparent negative effects. They are characterized by melanized hyphae and microsclerotia within plant roots. DSE are widely distributed throughout the world and are often reported to co-habit with other microorganisms such as ectomycorrhizal fungi (ECM). High tolerance of DSE to trace element- (TE-) pollution and their relatively high abundance in contaminated habitats suggest that DSE might have an important function for host survival in these conditions. Therefore, in the context of phytoremediation assisted by symbiotic fungi, we studied these groups of fungi into more details. We first isolated seven DSE strains from poplar roots from TE-polluted sites¹. Fungal isolates were identified as members of the Phialophora, Phialocephala, Cadophora and Leptodontidium genera. A screening of the strains was performed to select the best promising symbionts. They were characterized for their plant-growth-promoting abilities through different tests such as production of indol-3-acetic acid, release of volatile organic compounds and TE-tolerance. Further, we also investigated the interaction between DSE and ECM (Hebeloma cylindrosporum and Paxillus involutus) and the interaction between these fungi and pathogens (Heterobasidion annosum, Phytophotora citricola, and Pythium ultimum) through an in vitro pairwise assay. For the most interesting strains, an inoculation experiment was performed to monitor the effect of the fungi on the growth of poplar and birch on metal-contaminated soils². Finally, the use of DSE and ECM in the phytomanangement of metal-contaminated soils will be presented.

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¹Berthelot C, Leyval C, Foulon J, Chalot M, Blaudez D (2016) Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal polluted poplar phytomanagement sites. FEMS Microbiology Ecology 92(10): fiw144.

²Berthelot C, Blaudez D, Leyval C (2017) Differential growth promotion of poplar and birch inoculated by three dark septate endophytes in two trace element-contaminated soils. International Journal of Phytoremediation (*in press*).

Keywords: dark septate endophyte, ectomycorrhizal fungi, pathogen, plant growth promoting, phytomanagement

P (ID 135)

Soil fungal mycelium species composition and biomass: ectomycorrhizal and saprotrophic guilds under the influence of forest type and environmental factors

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Ectomycorrhizal and saprotrophic fungi dominate temperate forest soils driving essential ecosystem processes (nutrient cycling, C storage). The two guilds coexist into a competitive relationship (Bödeker et al. 2016), likely sensitive to changes in the environmental factors, or forest tree composition.

We investigated species composition and biomass of ectomycorrhizal and saprotrophic guilds in three regions of Germany across a gradient of 48 independent environmental factors, including vegetation, soil, root, forest and climatic properties. The study considered forest stands of pure beech, beech mixed with other deciduous species, beech mixed with coniferous, and pure coniferous.

The results indicated that in the ectomycorrhizal traditional dominated soil depth, at the interface between mineral horizon and humus (Lindahl et al. 2007), the mycelial biomass was dominated by saprotrophs. Environmental predictors either for community composition or biomass were different between the two fungal guilds. Forest type had a significant influence, particularly on saprotroph's biomass, which were higher in coniferous that in deciduous forests. The region effect was also significant for fungal community and biomass composition.

By alteration of the relationship between ectomycorrhizal and saprotrophic fungi, dramatic shifts in ecosystem processes may ensue. Therefore, identification of the effects of forest type (species selection) and other environmental factors on this relationship is a major factor in the development of sustainable forest management strategies.

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Keywords: saprotrophs, mineral horizon, biomass, competitive interactions, temperate forest

P (ID 467)

Role of arbuscular mycorrhizal fungi in the sustainability of a green fertilization practice

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The practice of sown biodiverse permanent pastures rich in legumes (SBPPRL) is a strategy to increase grassland productivity and carbon sequestration. However, the sustainability of SBPPRL is unclear, as legumes are susceptible to drought, overgrazing and nutrient limitation (particularly phosphorus (P)). The implementation of arbuscular mycorrhizal fungi (AMF) may mitigate these factors and increase success and sustainability of SBPPRL.

We tested the effects of AMF (*Rhizophagus irregularis*) on the performance of five legume species (*Trifolium subterraneum*, *T. resupinatum*, *T. incarnatum*, *T. vesiculosum* and *Ornithopus sativus*) commonly used in SBPPRL in three pot experiments with manipulation of (i) phosphorus availability, (ii) grazing pressure, and (iii) water availability.

All species showed strong positive mycorrhizal growth dependencies (MGDs). (i) Under low P-conditions AMF allowed for biomass increases of ~100%. (ii) Frequent clipping of 50% of leaf area led to similar regrowth in AM- and NM-individuals, indicating that compensatory regrowth capacity was unrelated to mycorrhization. However, MGD was increased in all species when clipped. (iii) Drought effects on productivity were not generally mitigated through mycorrhiza.

Our results indicate that (i) P-fertilization in SBPPRL may be reduced markedly by implementing AMF, and that (ii) herbivory through grazing and (iii) drought may be the major problems for SBPPRL legumes, explaining lacking sustainability of the practice, with AMF not adding much help in tackling these problems. Nevertheless, AMF was shown to increase biomass even under severe drought and herbivory conditions, pointing to a larger positive effect through P-delivery than through carbon drain.

Keywords: arbuscular mycorrhizal fungi, herbivory, biomass production, drought effects, phosphorus acquisition

P (ID 492)

Molecular diversity of arbuscular mycorrhizal fungi in the South American mediterranean ecoregion

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The mediterranean ecoregion correspond to a small area in the world that supports 20% of the plant species on Earth. At least half of them are endemic and are threatened by agriculture and urbanization. Most of conservation guidelines consider only the aboveground compartment for their recommendations and the belowground compartment is thought as a black box. One of the key organisms in this compartment are mycorrhizal fungi, that forms a symbiosis with more than 90% of terrestrial plants and in recent years they began to be recognized by playing a key role in shaping diversity, distribution and abundance of plant species. There are five ecosystems in the world composing the mediterranean ecoregion; for four of them mycorrhizal fungi research has arisen, however almost nothing is known for the South American mediterranean ecosystem. In this research, is aimed to describe, for the very first time, patterns of molecular diversity and community structure of AMF present in soil and plant roots of one of the most threatened habitat of South American mediterranean ecosystem: sclerophyllous forest. Also, is aimed to understand how those ecological patterns are affected by host plant species, soil physicochemical factors and seasons of the year. So far, the system has shown to have a molecular species richness of 118 virtual taxa. In terms of richness and abundance, this is significantly affected by soil physicochemical factors and seasons of the year, but not by plant species. The results obtained from this research will be useful for conservation purposes of the mediterranean ecoregion.

Keywords: arbuscular mycorrhizal fungi, mediterranean ecoregion, South America, molecular ecology, mycorrhizal ecology

P (ID 223)

AMF species diversity in the Three Gorges Reservoir's drawdown zone under different fertilization histories

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Introduction/Aim: Annual winter and summer water-flow regulation of the world largest Three Gorges Reservoir (TGR, 1084km²) since 2003 has shaped a 350km² huge hydrofluctuation or drawdown zone, where riparian vegetation and associated microbes have been stressed by drying-wetting alternations¹.². However, how arbuscular mycorrhizal fungus (AMF) species diversity could respond to such hydrological stresses remain unclear.

Materials/Methods: Responses of AMF community structure and diversity were monitored by morphological/taxonomical characteristics at three different fertilization history areas (Area-A, Area-B and Area-C for 5-, 3- and zero-year fertilization) in the TGR's drawdown zone³.

Results: From 10 dominant plant species in all three areas, 50 AMF species were totally identified within 13 genera (*Glomus*, 20; *Acaulospora*, 8; *Funneliformis*, 4; 3 for *Ambispora*, *Claroideoglomus* and *Septoglomus*; 2 for *Scutellospora* and *Diversispora*; each for *Gigaspora*, *Entrophospora*, *Paraglomus*, *Rhizophagus*, *Sclerocystis*)³. More abundant AMF species were within *Acaulospora*, *Ambispora*, *Entrophospora* and *Paraglomus* in Area-A and Area-B, while within *Claroideoglomus*, *Diversispora*, *Sclerocystis* and *Septoglomus* in Area-C. Shannon-Wiener and evenness indexes ranked significantly greater at Area-A>Area-C>Area-B, positively correlated with each other (*P*=0.05) and were highest volumes under *Phytolacca americana* rhizosphere soils.

Discussion: Plant host, fertilization history or edaphic properties could affect the distribution of AMFs. As main drivers affecting AMFs, soil C/N ratio, available phosphorus and potassium significantly correlated (*P*<0.001) with AMF community composition in these three fertilization history areas³.

Conclusion: Positive relationships between AM fungal community and edaphic properties demonstrate important functions of AMFs for ecological restoration of riparian vegetation, particularly *Phytolacca americana*, in the TGR's harsh drying-wetting drawdown zones.

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Keywords: AMF morphology, drying-wetting alternation, hydro-fluctuation zone, soil C/N ratio, soil available phosphorus and potassium

P (ID 387)

The effect of nutritional limitation by fungicide treatment on growth of mixotrophic Pyrola japonica

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Pyrola japonica is understory plants living at temperate forests in East Asia. Since carbon stable isotopic ratio (δ^{13} C) of the plant was intermediate between autotrophic and mycoheterotrophic plants, nutritional mode is mixotrophic, i.e. can acquire carbon from both photosynthesis and plants' mycorrhizal fungi. However, little direct evidence is available about the extent of mycorrhizal dependency of *P. japonica*. The aim of the study was to clarify the functional significance of mycorrhizal fungi on growth of *P. japonica* by experimentally reducing the mycorrhizal colonization.

In an evergreen broad-leaved forest, P. japonica plants were subjected to in situ fungicide treatments. After 4 months, the plants were collected and their biomass as well as δ^{13} C of leaves and fruits were measured. In addition, root samples were assessed for mycorrhizal colonization and for taxonomic affiliation of associated fungi by cloning sequences of the ITS region.

All plants from fungicide and control treatments plants formed arbutoid mycorrhizas without fungal mantles. The rate of fungal colonization in the fungicide treatment (75.3%) was lower than that in the control (85.0%). Associating fungi detected were ectomycorrhizal fungi (e.g. Russulaceae and Thelephoraceae). Leaf dry weights of plants in the treatment were lower and their fruits appeared to be more withering than those in the control. δ^{13} C values of leaves and fruits in the treatment were lower than those in control.

These results suggested that *P. japonica* depends on its fungi for shoot and fruit formation but partially improves photosynthesis when the access to mycorrhizal fungi is limited.

Keywords: arbutoid mycorrhiza, Ericaceae, stable carbon isotopes, ITS barcoding

P (ID 434)

Distribution of Arbuscular Mycorrhizal Fungi According to Salinity in Reclaimed land in Korea

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Dry climate is known to increase salinity of soil worldwide. In particular, reclaimed land has higher salt concentration than grasslands or forests, and is unfavorable for plants. Arbuscular mycorrhizal fungi (AMF) are known to enhance plant adaptability in harsh environments. The purpose of this study was to investigate the symbiotic relationship between AMF and plants in saline soils. We collected host plants and rhizosphere soils at different distances from the sea in a reclaimed land of Korea. To investigate the diversity of AMF species, DNA was extracted from the plant roots and cloned, and restriction fragment length polymorphism (RFLP) was performed. Molecular and morphological analyses on AMF spores extracted from the field soils were performed. We found that *Paraglomus occultum* was the dominant AMF species in the plant roots. In field soils, we identified *Ambispora leptoticha, Acaulospora longula, Glomus eburneum* and Diversispora sp.[Eom1]. These results indicated a difference in species diversity between AMF in the field soils and in plant roots. Further studies using AMF to improve the adaptability of plants in highly saline soils will be necessary.

Keywords: Paraglomus occultum, reclaimed land, arbuscular mycorrhizal fungi, salinity

P (ID 436)

Changes in Ectomycorrhizal Community Structure after Forest Tending

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Ectomycorrhizal fungi (EMF) are important microorganisms that contribute to nutrient cycling in forest ecosystems. EMF extends mycelia into the intercellular space of plant roots and forms a symbiotic relationship with the host plant. Forest tending is one of the ways to increase the forest production and to maintain and manage forest ecosystems. Forest tending could cause changes in the plant community structure, which in turn results in changes in the community structure of EMF in symbiotic relationships with the host plants. This study was conducted to investigate the species diversity and community structure of EMF by studying soil samples from natural forests and tending area of the study site in Korea. We identified EMF based on morphological characteristics and internal transcribed spacer (ITS) DNA sequences analysis. We found that Russula was the dominant genus in both natural and tending areas. However, Suillus became the dominant genus over time followed by Tomentella. We also confirmed that forest tending reduced species diversity. Therefore, we suggested that forest tending could affect the community structure of EMF.

Keywords: ectomycorrhizal fungi, forest tending, *Russula*, species diversity

P (ID 263)

Characterization of Mycorrhiza Fungi associated with the Genera of Borneo *Phalaenopsis* Orchid

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Phalaenopsis is a popular orchid in the trade, through the development of many artificial hybrids. It is native to southern China, the Indian Subcontinent, Southeast Asia, New Guinea, the Bismarck Archipelago, and Queensland based on Kew Garden databases. This genus is no longer common in nature because it is highly prized by poachers or has vanished as the habitat is destroyed, thus more easily found outside their habitats. Phalaenopsis has become an important cut-flower crop, with large, heavy-substanced flowers possessing unusual long-lasting qualities. The species that can be found in Borneo is known as Phalaenopsis Borneo. Being an epiphyte, information on the genera Phalaenopsis and symbioses with mycorrhiza fungi is lacking. The objectives were firstly to isolate culturable endofungi from protocorm and characterize using morphological description and DNA sequencing. The second objective was to verify of existence of symbioses between Phalaenopsis orchid and fungi. Thirty samples of wild Phalaenopsis species coded based on the location from various parts of Sarawak conserved in the ethnobotanical garden were screened. Thirty-four culturable fungi were isolated. There was no trend of specificity among the samples and no significant differences among apex, middle and mature zones of the protocorm in colonization. Two fungi colonized more than six samples of orchids while seven fungi colonized in only two samples while the rest were only in one type of orchid. Two orchids did not have colonization. The characterization of the mycorrhizal fungi indicated the diversity and functional preferences of symbioses due to specificity of host-fungi relationship.

Keywords: Phalaenopsis orchid, mycorrhiza, symbioses, culturable fungi, protocorm

P (ID 36)

Evidence from soil fungal community ecology suggest that *Abies religiosa* could be nursed by *Pinus montezumae* in central Mexico

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Abies religiosa forests in central Mexico are the overwinter refuge of Monarch butterfly and they also provide important ecosystem services. These forests have lost 54.6% of extension in the last 40 years. Assisted migration has been proposed a strategy to avoid its extinction due to climate change. For these reason, it is necessary to consider its fungal symbiosis to facilitate its establishment in new areas. Our objective was to analyze the fungal diversity in soils of forests with close relative hosts in order to identify which are the best potential habitats according to their mycobiome.

We sampled soil from five temperate conifer forests (*A. religiosa*, *Pinus montezumae*, *P. hartwegii*, and conifer mixed forest) and sequenced the ITS2 region with IlluminaMiSeq. Sequences were grouped at 97% and assigned to MOTUs and functional guilds.

We obtained 6286 MOTUs, in which Ascomycota was the most common Phylum and Agaricales the most common Order. The species turnover between forest types fluctuated 0.543 – 0.681. The MOTUs with highest ecological importance value were *Russula aff. olivobrunnea*, *Mortierella* sp. and *Piloderma* sp. The core-diversity had 314 MOTUs dominated by Russulaceae and Clavulinaceae. The fungal communities composition were significantly influenced by site altitude.

As *Pinus montezumae* forests hosted the highest number of shared ectomycorrhizal fungi and have a distribution adjacent to *A. religiosa*, we propose further research to validate the assisted migration of *Abies religiosa* into *P. montezumae* forests using this pine as nurse plant.

Keywords: fungal community ecology, plant community ecology, forest management, environmental decision making

P (ID 63)

Vulnerability of soil microbial community to land use changes in a Mexican tropical rain forest

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Land use change has important effects on the soil microbial community (MC), mainly affecting their diversity and vulnerability to environmental changes. Los Tuxtlas tropical rain forest in Mexico offers a suitable framework to test the effect of land use change on the soil microbial communities including factors as the seasonal variation.

We analyzed the whole cell fatty acid of the MC in soils from four land uses: cattle ranching, crops, primary and secondary tropical rain forest, and during the dry and rainy seasons. Soil physicochemical properties were additionally analyzed at each land use.

We identified twenty eight individual fatty acids, representing the whole functional groups of soil MC: Gram+ bacteria, Gram- bacteria, actinobacteria, fungi, arbuscular mycorrhizal fungi (AMF), oomycets.

The abundance of the functional groups of microorganisms were affected by land use but not by seasonality; specially AMF had highest values in soils from cattle ranching in comparison with those of crops and rain forest. Also, the land use influenced the assemblage of the soil MC, especially during the rainy season. Nitrate, soil organic matter and phosphorus explained a great percentage of the axis after a NMDS analysis.

The results show that actual agriculture practices are noxious to the soil MC, because of the excessive use of fertilizers and herbicides. The soil MC belonging to the secondary forest had the same pattern in terms of assemblage and vulnerability as the mature forest, indicating a high potential of recovery of the soil functions associated with the microbial community.

Keywords: soil microbial community, arbuscular mycorrhizal fungi, whole cell fatty acids, tropical rain forest, community

P (ID 336)

Colonization and species richness of arbuscular mycorrhizal fungi in roots of *Cedrela* odorata and *Swietenia macrophylla*

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Introduction: Cedrela odorata L. and Swietenia macrophylla King (Meliaceae) are globally important timber species. Unfortunately, illegal logging, shoot borer (Hypsipyla grandella Zeller), anthropogenic and natural disturbances have affected both forestry species. Arbuscular mycorrhizal fungi (AMF) have been proposed as a biotechnological alternative in ecological restoration and in agroforestry systems. However, ecology and inoculation of plants with native species of AMF in Meliaceae species are still poorly understood.

Aim: The aim of this study was to evaluate the effect of inoculation of native AMF, the level of colonization, richness AMF in mahogany and cedar Spanish trees in Yucatan Peninsula, Mexico.

Materials and Methods: *Soil and roots* samples were collected from ten individuals of cedar and mohogany per site, with at least 100m away from each other; a total of 200 cm of roots were morphological analyzed. Partial SSU rDNA fragment were amplified using AM1 and NS31 primers.

Results: The morphological AM colonization pattern was considered a Paris-type. Dark septate fungi, with septate hyphae and microsclerotia, were present within the roots of mahogany. The percentages of AMF colonization in roots varied between species, and range from 25 to 35 % in cedar and 68 to 90 % in mahogany. In both species, two Glomeraceae phylotypes were identified by sequencing, and two Diversisporaceae phylotypes were only present in the roots of mahogany.

Conclusion: The knowledge of the diversity of arbuscular mycorrhiza fungi in natural populations of these two economically important species would help to the selection of the potential species in sustainable agriculture and conservation.

Keywords: arbuscular mycorrhizas, rain forest, phylotypes

P (ID 401)

Ectomycorrhizal fungal communities in protected and managed mixed coniferous forests in Poland

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The main objective of the studies was to compare above- and below-ground community structure of ectomycorrhizal (ECM) fungi in protected and managed continental mixed coniferous forests (Querco roboris-Pinetum) in Poland. The studies were performed at three pairs of 80 years old forest stands, located within the forest reserves and mature managed forest. To determine ECM community structure, traditional fruiting bodies inventories were used along with molecular identification (Sanger sequencing) of ECM morphotypes. Aboveground fruiting bodies surveys and molecular identification of mycorrhizas revealed in total up to 140 ECM fungal taxa. Molecular analysis of ECM morphotypes revealed higher fungal species richness than fruiting bodies inventories. After four seasons of research (spring and autumn 2015 and 2016) we found that ECM fungal species richness did not differ significantly between managed and not managed forests. However a tendency to higher fungal diversity in managed stands was observed. Individual pairs of stands were differentiated in terms of species composition and abundance. This appears to be due to the greater volume of dead wood in protected forest in comparison to the managed forests. Dead wood provides specific ecological niches allowing for the development of the fruiting bodies of some specialized ECM fungi (e.g. Tomentella, Pseudotomentella). Investigations planned for the approaching seasons (spring and autumn 2017) using NGS should complement our present studies to assess the full diversity of fungal community, not only ECM fungi.

This research is financially supported by a grant from the National Science Center, Poland (grant 2014/13/B/NZ9/01992).

Keywords: community structure, forest management, forest reserves

P (ID 167)

Ectomycorrhizal functional traits mediate plant-soil feedback processes in trace elements contaminated soils

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The role of ectomycorrhizal (ECM) fungi in the interface between plant and soil environment is key in explaining ecosystem processes and functions, in particular in the recovery of ecosystem services in degraded environments. Using a trait-based approach, we aimed to study how the effects of soil contamination by trace elements on individual trees were mediated by changes in the community composition and functional traits of ECM symbionts.

Soil abiotic properties and ECM patterns of colonization were characterised on holm oak trees (*Quercus ilex* subsp. *ballota*) in areas with different levels of contamination in the Guadiamar Green Corridor, in SW Spain. A variety of morphological traits were characterised for both the trees and their associated ECM fungal species, as well as the molecular characterisation of ECM fungal communities.

ECM communities' composition was found to be dependent on soil pH, nutrients and contamination levels. The two most abundant ECM fungal taxa, *Hebeloma cavipes* and *Thelephora terrestris*, had contrasting distributions: *H. cavipes* was found in non-contaminated soils while *T. terrestris* was associated with acidic soils. Among the studied fungal traits, frequency of emanating hyphae was found to be clustered among species in the communities in contaminated soils. The reduction of emanating hyphae in contaminated soils had an effect on the associated trees by reducing their phosphorous uptake.

In conclusion, soil contamination acted as an environmental filter to ECM fungal communities which decreased their functional diversity posing a negative effect on holm oaks performance.

Keywords: ecosystem processes, morphological traits, emanating hyphae, functional diversity, heavy metals

P (ID 457)

Global Fungal Red-listing and the contribution we need from you!

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A critical way to help politicians and citizens be more aware of the importance of fungi and the need to conserve them is to have fungal species included in the IUCN (International Union for Conservation of Nature) Global Red List. This list is a compilation of rigorous assessments of the extinction risk for individual species made using strict universal criteria and categories (www.iucnredlist.org). Fungi face the same threats as animals and plants – habitat loss, pollution, and climate change.

Until recently, some thought that it was not possible to rigorously assess the conservation status of fungal species using IUCN criteria because of the unique biology of fungi and insufficient information on their taxonomy, distribution and ecology. However, much progress has been made to address these challenges.

The Global Fungal Red List Initiative aims to facilitate and coordinate efforts by the global mycological community to get species of threatened fungi assessed and included in the global IUCN Red List. The goal of the initiative is to raise awareness of fungal conservation among mycologists, the conservation community, policy makers and the general public. So far 33 fungi, plus 20 to be in 2017 (21 are ectomycorrhizal) have been added and presented in the poster.

The global red-list assessment critically depends on many to contribute with knowledge of distributions, ecologies and population trends of individual species as well as checking facts and suggested assessments.

Check the web-page of the Global Fungal Red List initiative. Your contribution is needed.

References:

Dahlberg A & Mueller G. 2011. Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. Fungal Ecology 4: 1–16

P (ID 11)

Simplified ex vitro symbiotic seed germination method as a tool for orchid conservation

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Two biggest threats to wild orchids are the loss of habitat and illegal collection. For some threaten and endangered species where *in situ* conservation is no longer possible, *ex situ* conservation is the only option. *Ex situ* orchid conservation, however, has many constraints including the lack of easy, efficient and economical propagation methods that can engage members of the local communities that are otherwise potential smugglers in the conservation program. *Ex vitro* symbiotic seed germination is an attractive orchid propagation method that does not require axenic condition during seed germination step. Aewsakul et al. (2013) reported a simple *ex vitro* orchid germination method that used common orchid cultivation substrates as the germination medium. We have modified the method to reduce the problem of fungal and mite contamination and to make it more feasible to amateur orchid growers by optimizing the method for preparing the germination substrate and using plastic food containers with lids vented with air-permeable materials. The method has been used successfully to germinate *Spathoglottis affinis* de Viese, *Dendrobium friedericksianum* Rchb. f., and *Eria javanica* (Sw.) Blume.

Keywords: *ex vitro*, symbiotic seed germination

P (ID 304)

Burn, baby, burn: Soil microbial community responses to fire in alternate states of tallgrass prairie

<u>Laura A. Mino</u> (Division of Biology, Kansas State University, Manhattan, USA), **Ari Jump-ponen** (Division of Biology, Kansas State University, Manhattan, USA)

Current estimates state that as little as 1% of the historic range of the North American tallgrass prairie remains intact. One of the greatest threats to this prairie, and grasslands worldwide, is the encroachment of woody species. Suppression of fire disturbance facilitates a transition from grassland to shrub- and/or woodland, resulting in two alternate ecosystem states. The woody encroached state reduces ecosystem productivity and biodiversity, renders pastureland unsuitable for grazing, and alters key ecosystem functions. Prescribed fire acts as an attractor for the non-encroached grassland state, but restoration of woody encroached states using fire have been unsuccessful. Soil microbes are known to play critical roles in determining plant community structure; however, little research exists on potential differences between soil microbial communities associated with these two alternate ecosystem states.

We examine soil fungal and bacterial community composition pre- and post-fire in both encroached and non-encroached states of tallgrass prairie. While data are preliminary, we utilize a novel sampling approach to assess soil microbial responses on a high temporal resolution scale. We characterize fungal and bacterial biomass and community composition using qPCR and Illumina MiSeq (16S and ITS), with emphasis on mycorrhizal taxa and other microbial symbionts. Ancillary data such as soil respiration and nutrient dynamics are also measured.

Mycorrhizae and other symbiotic microbes have the potential to hinder or facilitate the rapid transition of grasslands to shrubby ecosystem states; understanding these communities' functional responses to fire may offer insights into conservation of remaining grasslands and restoration of encroached woodlands.

Keywords: prescribed fire, prescribed burn, fire, woody encroachment, grasslands

P (ID 123)

Mycorrhizal specificity in rare and common Caladenia orchid species

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Although Orchidaceae is noted as one of the most species-rich plant families, many species in this family are listed as endangered. One of the factors that are suggested to influence orchid rarity is mycorrhizal specificity. We examined mycorrhizal specificity in two closely related Caladenia species, the rare Caladenia procera and the common Caladenia pectinata by integrating phylogenetic analysis and symbiotic seed germination. All fungal isolates and sequenced fungal clones belong to Serendipita vermifera, with only two Operational Taxonomic Units (OTUs) identified from each of the rare C. procera or the common C. pectinata. The two species has one out in common. To compare germination ability of the two orchids, we used 20 fungal isolates representing seven OTUs obtained from various Caladenia species. From the symbiotic germination trials seeds of the rare C. procera were germinated by 14 (6 OTUs) of the 20 fungal isolates, while the common C. pectinata seed were germinated by 10 (4 OTUs) of the 20 fungal isolates. Seeds from both species utilized more OTUs than were found in adult plants, suggesting fungal specificity play a lessor role during germination. Given the fact that the rare C. procera could utilize an identical OTU to C. pectinata, it suggests that its distribution may be limited by factors other than mycorrhizal specificity and that mycorrhizal availability should not limit the distribution of C. procera. For conservation efforts of C. procera, the data presented here give a useful insight into the importance fungal specificity for future ex situ orchid propagation.

Keywords: Serendipita, specificity, conservation, rarity

CONCURRENT SESSION: Emerging technologies to make new discoveries in mycorrhizal physiology and ecology

Scientific advances are often dependent on available technologies. Whereas microscopy and molecular genetic approaches have revolutionized entire biology at earlier and more recent times, respectively, and mycorrhizal physiology and ecology make hardly any exception in this respect, there is still a number of issues to be solved. What is often missing in mycorrhiza research is high spatial resolution and temporal dynamics of the processes related to recognition of symbiotic partners, transport of metabolites and nutrients across symbiotic interface, redistribution of elements and enzymes in the mycorrhizosphere, and direct insights into cascading interactions within complex microbial communities and ecosystem processes at different time scales (from diurnal variation to geological time scales). Whole array of approaches promising new insights into mycorrhizal physiology and ecology shall be discussed in this session such as microdissection — transcriptomics, NanoSIMs or designing chronosequence studies to uncover long-term ecosystem consequences of mycorrhizal symbiosis.

Chairs: Barbara Drigo, Ian Anderson, Petr Kohout

CL (ID 165)

¹³C-tracers added to hyphae resulted in different destinations of host organelles in orchid symbiotic protocorms

<u>Yukari Kuga</u> (Hiroshima University, Higashihiroshima, Japan), **Naoya Sakamoto** (Hokkaido University, Sapporo, Japan), **Kousuke Nagata** (Hokkaido University, Sapporo, Japan), **Hisayoshi Yurimoto** (Hokkaido University, Sapporo, Japan), **Chie Katsuyama** (Integrated Arts and Sciences, Hiroshima University, Higashihiroshima, Japan)

Cellular localization of stable isotopes (SI) in tracer experiments has led to new insights into the translocation of elements, a central function of mycorrhizal symbioses. In the previous study of orchid symbiotic protocorms (Spiranthes sinensis – Ceratobasidium sp.), simultaneous labeling of ¹³C-glucose and ¹⁵N-NH₄NO₃ demonstrated transfers of C and N in intact and senescing stages of fungal pelotons¹⁾. In this study, ¹³C-acetate and ¹³C¹⁵Narginine, and a mixture of ¹³C-glucose and ¹⁵N-NH₄NO₃ were used as tracers in the same symbiotic orchid protocorm system. After four days labeling, protocorms were chemically fixed, ethanol dehydrated and embedded in a resin. Localization of SIs in resin sections was subjected to secondary ion mass spectrometry (IMS1270 with SCAPS), and isotope ratios were analyzed on recognizable structures using a set of the SI images. In all three treatments, 13C/12C and 15N/14N were elevated in uncolonized cells of the host tissue; however, ¹³C/¹²C elevation patterns of organelles showed contrasts between them. In the acetate treatment, ¹³C was transferred to amyloplasts but not to nuclei. In the arginine treatment, however, ¹³C/¹²C was increased in both organelles but was low in the amyloplasts. The glucose treatment showed high ¹³C/¹²C in both organelles. This study showed that tracer compounds added only to the symbiotic hyphae resulted in the different destinations of the host organelles, which suggested that several compounds were involved in the membrane transfer at the interface.

References:

1) Kuga, Y., Sakamoto, N., Yurimoto, H. New Phytologist 202: 594-605 (2014).

Keywords: secondary ion mass spectrometry, stable isotope imaging, ¹³C¹⁵N-arginine, ¹³C-acetate

CL (ID 141)

Imaging symbiotic networks across scales

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Arbuscular Mycorrhizal Fungal (AMF) hyphae form extensive underground webs, foraging for nutrients and connecting roots of different plant species. These adaptive networks are critical for the movement of nutrients around ecosystems. However, to image and characterize quantitatively fungal networks is a challenge. The topological and transport dynamics occur many orders of magnitude apart in space and time. While transport takes place on the millisecond timescale inside the 10-micrometre wide hyphae, network topology changes in hours or days and spans distances of meters. By combining biophysics and ecology, our group is developing modern imaging tools to visualize how symbiotic fungi build their networks with high spatial resolution. By coupling with multi-colored phosphate-coated quantum dots, we are able to visualize how fungi transport and trade nutrients with plants with unprecedented time resolution. Our images link quantitatively transport and topology by directly visualizing how biotic and abiotic factors impact mycorrhizal physiology. Ultimately, our aim is to understand how the network structure reflects conflict and cooperation in AMF-plant symbiosis.

Keywords: arbuscular mycorrhizal fungi, fluorescence microscopy, network science, transport, symbiosis

CL (ID 307)

Challenging soil fungi foraging in micro-engineered Soil Chips

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From the point of view of microorganisms, the soil environment is an enormously complex labyrinth with paths and dead-end streets, where resources and shelters are unevenly distributed.

Inspired from findings of arbuscular mycorrhizal fungi growing through the pores of the inert material biochar, we manipulated growth habitat microstructure with lab-on-a-chip techniques. We designed complex environments with channels and obstacle at dimensions of the size of hyphae, and constructed them in the transparent, gas-permeable polymer PDMS. We filled those with air, different nutrient solutions or combined them with mineral nutrient gradients, and inoculated them with soil organisms. We studied foraging strategies of soil fungi, and the possibility of physio-spatial stabilization of organic matter by "hiding" in occluded soil spaces.

We compared different soil litter decomposers and *Rhizophagus irregularis* for their ability to forage through complex air-gap structures and attempt to classify them into functional traits concerning their mycelium directionality, space-exploring approach and ability to grow through acute angles and narrow constrictions. We identified structures which are very difficult to penetrate for most species, and compounds located behind such features may thus be spatially unavailable for decomposers.

We discuss our approach in comparison to soil pore space tomographic analyses and our findings in the pore space of colonized wood biochar.

Keywords: foraging strategies, soil structure, organic matter stabilization, microfluidics, mycelium

ST+P (ID 207)

Nitrogen uptake capacity by ectomycorrhizal fungi as an exogeneous trait: insights from MIFE microelectrode ion flux measurements

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Nitrogen (N) uptake capacity of conifer roots is a critical component of plant fitness. Ectomycorrhizal fungi (ECM) are known to enhance N uptake, but differences in this functional trait among ECM species has rarely been quantified.

Using MIFE technology (microelectrode ion flux measurement), we compared uptake of ammonium and nitrate among the suites of ECM found on Douglas-fir roots in temperate rainforests, from a variety of sites of contrasting fertility and N form availability.

Rates of ammonium uptake by ECM were consistently higher than by non-mycorrhizal white root tips, and rates of uptake varied widely among ECM species. ECM taxa associated with N rich sites, for example, had up to 3 times higher ammonium uptake than taxa from low N sites. All native fungi associated with Douglas-fir roots had limited capacity for nitrate uptake, however, even on soils with high rates of nitrification. Our results emphasize that inorganic N uptake capacity, particularly ammonium uptake, is not a property of host root physiology but an exogenous trait dictated by ECM species.

The MIFE system is a powerful tool for assessing simultaneous net fluxes of different ions over the mantle of fungal root tips, and allows elucidation of key traits that govern ECM species' function and distribution. Ongoing work will examine the competitiveness of ECM trees vs. arbuscular mycorrhizal trees in relation to soil N availability, and the degree to which host tree species may control N uptake capacity by the symbiont.

Keywords: nitrogen uptake, Ectomycorrhizae, microelectrode ion flux measurement

ST+P (ID 424)

GFP and mCherry expression in *Laccaria bicolor* – a plasmid toolkit for flexible use of fluorescent protein markers in ECM basidiomycetes

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An efficient expression of fluorescent proteins have resulted challenging in basidiomycetes. This is partly due to frequent requirement of introns by these fungi, and also for the lack of "easy-to use" cloning plasmids for constructing translational fusions adapted specifically for basidiomycetes

Laccaria bicolor histone (H2B) genomic coding region together with 1000 bp upstream sequence was fused to N-terminal of GFP and mCherry. These translational fusion constructs were cloned into pHg¹ and used for ATMT of the dikaryotic strain S238N of Laccaria under hygromycin B selection.

The vast majority of the transformed fungal strains tested showed clear GFP and mCherry signals in their nuclei. Especially intense signal was detected in cells going through cell division and clamp connection formation. This is the first time that fluorescent protein expression has been established in the model ECM basidiomycete *L. bicolor*.

This successful GFP and mCherry expression in *Laccaria* has led to construction of a set of marker protein fusion cloning vectors (pN-GFP, pN-mCherry, pCEBN-GFP and pCEBN-mCherry) with maximum technical flexibility and compatibility with ATMT. We hope that our plasmid toolkit will expand the use of GFP and mCherry as *in vivo* fluorescent markers in ECM research, and in basidiomycete studies in general.

¹ Kemppainen & Pardo 2010. Microbial Biotechnology 3(2):178–200.

ST+P (ID 124)

Dissecting differential mycorrhizal growth responses with high throughput phenomics

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Introduction/Aim: Land plants have evolved diverse resource acquisition strategies that may alter the degree of resource limitation they experience, and plant resource limitation has been hypothesised to be a primary driver of mycorrhizal growth responses. We ask the question whether different resource acquisition phenotypes of Brachypodium dystachion, a model cereal, are associated with alterations in the mycorrhizal growth response under particular nitrogen (N) and phosphorus (P) resource supply conditions.

Materials and Methods: We applied high throughput plant phenotyping to monitor daily plant phenotypic changes in two plant genotypes subjected to three N:P supply ratios in the presence and absence of AM fungi.

Results: We identified that the two genotypes had different N and P acquisition phenotypes such that one genotype was more N responsive and the other was more P responsive. In the presence of AM fungi, the two genotypes experienced differential time-dependent mycorrhizal effects on leaf yellowing and growth. Moreover, these differential growth responses were related to a coordinated response of plant traits including specific leaf area, mass fraction, and tissue N:P ratio, which suggests a link between mycorrhizal growth response and plant resource utilisation strategy.

Discussion: Based on these data, plants that require more N for growth and are more susceptible to N limitation may experience greater mycorrhizal growth depressions under low N, but may also be more likely to experience positive mycorrhizal growth responses under low P.

Conclusion: High-throughput plant phenotyping is a useful tool to uncover plant genotype-dependant traits relevant to mycorrhizal growth responses.

Keywords: phenomics, plant traits, mycorrhizal growth response

ST+P (ID 68)

Ericoid plant species shape fungal communities in boreal forest soil and plant roots

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Boreal forest soils are limited by the available nutrients, and to improve the nutrient uptake, most plants form symbioses with mycorrhizal fungi ¹. In boreal forest, trees form mycorrhizal associations typically with ectomycorrhizal (ECM) fungi, and ericoid shrubs form symbioses with ericoid mycorrhizal fungi (ERM) ^{1,2}. However, the results of host specificity of ERM fungi are differing. Some studies indicate that regionality shapes the fungal community of ericoid roots with no host specificity ^{3,4} while others suggest that ERM fungi show host preference ^{5,6}.

We aimed to study the differences and similarities of the fungal communities of typical boreal forest plants *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Calluna vulgaris* and *Pinus sylvestris* growing on originally similar boreal forest soils in individual microcosms for 18-months. Further, with $^{13}\text{CO}_2$ -labeling and DNA stable isotope probing ($^{13}\text{C-DNA-SIP}$), we identified those root associated fungi, which interacted with the studied plants and obtained photosynthetic carbon.

Our results indicate that some root associated were host specific while others were inhabiting the roots of all the studied plants. Based on ¹³C-DNA-SIP, the majority of the detected fungi in the washed plant roots obtained photosynthates from their hosts. Additionally, some common root endophytes without confirmed mycorrhizal status obtained photosynthetic ¹³C.

Several fungal species co-occurred in the roots of all the studied plants. Thus, our results highlight that more knowledge of the co-occurrence and co-functioning of ERM, ECM and endophytic fungi, and the functions of potential mycorrhizal network between plants are still needed.

References:

¹ Smith & Read. 2008. Mycorrhizal symbiosis. Cambridge, UK: Academic Press

² Read. 1996. The structure and function of the ericoid mycorrhizal root. Annals of Botany 77: 365–374.

Keywords: boreal forest soil, ¹³C-DNA-stable isotope probing, ectomycorrhiza, ericoid mycorrhiza, fungal community

³ Kjoller *et al.* 2010. Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. Fungal Ecology 3: 205–214.

⁴ Walker *et al.* 2011. Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New Phytologist 191: 515–527.

⁵ Bougoure *et al.* 2007. Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. Molecular ecology 16: 4624–36.

⁶ Ishida & Nordin. 2010. No evidence that nitrogen enrichment affect fungal communities of *Vaccinium* roots in two contrasting boreal forest types. Soil Biology and Biochemistry 42: 234–243.

ST+P (ID 140)

Transcriptional profiles of sunflower roots during mycorrhizal colonization as revealed by RNA-SEQ

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Sunflower (*Helianthus annuus* L.) is an important crop cultivated worldwide and represents a non-model plant species, whose mycorrhizal status was recently assessed in wild accessions, cultivars and inbred lines¹. Previous sunflower transcriptome analyses revealed gene differential expression in response to biotic/abiotic stresses², while no data are available on changes induced by arbuscular mycorrhizal (AM) fungi. Here, for the first time, an RNAseq approach was used to investigate transcriptional changes in mycorrhizal versus nonmycorrhizal sunflower roots and gene differential expression at early and late stages of mycorrhizal establishment.

Sunflower plantlets were inoculated with the AM symbiont *Rhizoglomus irregulare* and total RNA was isolated from roots 4 and 16 days after inoculation. Twelve cDNA libraries were built and sequenced by Illumina technology. A *de novo* transcriptome was established by assembling Illumina reads and annotating contigs. After quantitative analyses by mapping reads against the *de novo* assembled transcriptome 1,037 differentially expressed putative transcripts (DEs) between mycorrhizal and control plants were identified. The number of overexpressed DEs was much higher than under-expressed ones, increasing over time. Gene Ontology analyses showed that several DEs were specifically involved in known mycorrhizal processes, such as membrane transport (ABC transporters, ammonium transporters), cell wall shaping (subtilisins, ascorbate oxidases), gene networking (DELLA, ERF) and defense response (germin-like protein, pathogen-related proteins). We also identified new mycorrhizal-induced putative transcripts, such as BAHD-acyltransferase and Nph3. This work provides novel data on gene expression during the establishment of AM symbiosis in a non-model plant species, useful for future comparative functional genomics analyses.

References:

- ¹ Turrini A., Giordani T., Avio L., Natali L., Giovannetti M., Cavallini A. (2016). Large variation in mycorrhizal colonization among wild accessions, cultivars, and inbreds of sunflower (*Helianthus annuus* L.). EUPHYTICA 207: 331-342
- ² Ramu V., Anjugam P., Venkategowda R., Basavaiah M., Makarla U., Muthappa S. (2016). Transcriptome analysis of sunflower genotypes with contrasting oxidative stress tolerance reveals Individual- and combined-biotic and abiotic stress tolerance mechanisms. PLOSone 11(6): e0157522. doi:10.1371/journal.pone.0157522

Keywords: RNA-seq, arbuscular mycorrhiza, *Helianthus annuus*, mycorrhizal-induced gene expression, non-model plant

ST+P (ID 212)

New method for arbuscular mycorrhizal fungi identification based on spore-proteomic biotyping by MALDI-TOF-MS

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Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are mutualistic symbionts associated with the great majority of land plants. These fungi are essential in plant production, but their taxonomic identification remains a crucial challenge for culture academic research, collections and inoculum producers. A reliable and simple technique for identification for these organisms could help removing a road block on the way to large scale AMF usage.

An alternative approach to DNA-based isolate characterization, which to our knowledge has never been applied to AMF, is proteomic-based chemotaxonomic biotyping using Matrix-Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS). This technique is already used in the fields of medical diagnosis principally for bacteria identification. In this study MALDI-TOF-MS proteomic biotyping approach was used for AMF identification. Spores of nineteen AMF isolates belonging fourteen species were studied. A comparison between molecular identification method and proteomic biotyping by MALDI-TOF-MS was carried out.

The studied isolates were globally differentiated by MALDI biotyping at the interspecific and intraspecific level. We could show that the proteome profiles we obtained are highly reproducible. MALDI spectra did not vary significantly with culture age, growth stage or growth substrate. AMF identification by MALDI biotyping could then be useful, for research, agricultural and environmental applications.

MALDI biotyping approach for AMF characterization is a fast, accurate and inexpensive technic and could be an alternative to conventional morphological and molecular methods for AMF identification.

Keywords: AMF identification, MALDI-TOF-MS, biotyping, interspecific level, intraspecific level

ST+P (ID 509)

High-resolution community profiling of arbuscular mycorrhizal fungi

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Community analyses of arbuscular mycorrhizal fungi (AMF) using ribosomal small subunit (SSU) or internal transcribed spacer (ITS) DNA sequences often suffer from low resolution or coverage. We developed a novel sequencing based approach for a highly resolving and specific profiling of AMF communities. We took advantage of previously established AMF-specific PCR primers that amplify a c. 1.5-kb long fragment covering parts of SSU, ITS and parts of the large ribosomal subunit (LSU), and we sequenced the resulting amplicons with single molecule real-time (SMRT) sequencing. The method was applicable to soil and root samples, detected all major AMF families and successfully discriminated closely related AMF species, which would not be discernible using SSU sequences. In inoculation tests we could trace the introduced AMF inoculum at the molecular level. One of the introduced strains almost replaced the local strain(s), revealing that AMF inoculation can have a profound impact on the native community. The methodology presented offers researchers a powerful new tool for AMF community analysis because it unifies improved specificity and enhanced resolution, whereas the drawback of medium sequencing throughput appears of lesser importance for low-diversity groups such as AMF.

Keywords: AMF community analyses, AMF inoculation, arbuscular mycorrhizal fungi (AMF), Glomeromycota diversity, microbiome, single molecule real-time

ST+P (ID 190)

PacBio metabarcoding of fungi: biases, perspectives and analysis tool PipeCraft

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High-throughput sequencing methods have enormously advanced our understanding about biodiversity and ecology of microorganisms. Second-generation sequencing platforms 454, Ion-Torrent and Illumina enabled to increase the number of samples analyzed and taxa recovered by 1-2 orders of magnitude. Yet, these methods enable analysis of mini-barcodes (ITS1 and ITS2 in case of fungi) that are suboptimal for accurate taxonomic identification.

We developed a protocol of Pacific Biosciences (PacBio) third-generation sequencing for fungi and other eukaryotes using 15 primer pairs (250-2500 bp amplicons) from soil and mock communities. The sequence quality was strongly related to the number of circular passes and stabilized at 8-12 passes. Random substitution errors by the polymerase outnumbered the sequencing errors of insertions and deletions accumulating in homopolymers. The data of the novel Sequel model were more error-infested compared with the RSII model. We also recovered multiple biases during PCR (amplicon length), priming (mismatching taxa), library loading (amplicon length), sequencing (error profile), ITS extraction (divergent taxa, 5.8S 3' HMMs) and reference-based identification (UNITE, SILVA). Sequencing of full ITS and SSU+ITS (not LSU!) proved highly useful for more accurate high-level taxonomic assignment. Longer barcodes have greater capacity for accumulating random errors without generating spurious richness and enable use of evolutionary tools [1].

We also generated a software compilation PipeCraft for analysis of custom high-throughput sequencing data (including PacBio). In a user-friendly graphical interface, users can select multiple options regarding sequencing platform, data input format, demultiplexing, filtering, chimera-removal, clustering and taxonomic annotations and run these in an automated pipeline [2].

References:

- [1] Tedersoo L, Tooming-Klunderud A, Anslan S. 2017. PacBio metabarcoding of fungi and other eukaryotes: biases and perspectives. Submitted.
- [2] PipeCraft: Flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. Molecular Ecology Resources, in press.

Keywords: Pacific Biosciences SMRT sequencing, Soil eukaryotes, primer bias, bioinformatics analysis

POSTERS

P (ID 393)

Laser microdissection protocol for DNA sequence-based confirmation of trophic status of ectomycorrhizal fungi

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Ectomycorrhizal trophic status is ascribed to fungi with corresponding combination of morpho-anatomical characters and molecular phylogenetic evidence. Molecular phylogenetic evidence is sometimes unreliable as certain fungal taxa can function both as ectomycorrhizal partners and saprotrophs. In such cases ectomycorrhizal status can be confirmed only by time-consuming reestablishment of ectomycorrhiza in axenic conditions. To avoid this step, laser microdissection approach was used to confirm that the identity of the fungal part that is growing as a mantle or mantle like-structure on the root surface is the same as a fungal part, growing inside the root as Hartig net.

Ectomycorrhizal roots were fixed in ethanol – acetic acid mixture. Longitudinal sections of paraffin embedded samples were transferred onto membrane slides and deparaffinized by immersing slides in xylene and ethanol series. PALM Zeiss laser microdissection system was used to dissect mantle and Hartig net portion of ectomycorrhizal root separately. For extraction of DNA, Arcturus PicoPure DNA Extraction Kit was applied. ITS1f/ITS2, ITS3/ITS4 and ITS1f/ITS4 primer pairs were tested in combination with KAPA Biosystems HiFi hotstart DNA polymerase for PCR amplification of the ITS1, ITS2 and ITS1-5.8SrDNA-ITS2 region of fungal ribosomal operon to determine the optimal combination of amplification efficiency and sequence length.

Freshly fixed samples yielded enough PCR products for direct sequencing with all three primer pairs, while samples of lower quality (lower quality of starting material, longer storage in fixative) amplified best with primers ITS3/ITS4.

The presented protocol is highly advantageous as it practically eliminates uncertainty regarding the source of isolated DNA.

P (ID 386)

Development of a random mutagenesis method for *Rhizophagus irregularis* for research and sustainable agriculture

<u>Kenza Samlali</u> (Bioscience Engineering, KU Leuven, Leuven, Belgium), **Soon-Jae Lee** (Biology, Université de Montréal, Montreal, Canada)

The lack of a molecular biotechnological toolset that can efficiently transform arbuscular mycorrhiza (AM), limits basic research on the genetics and physiology of AM. Here, a first method for random mutagenesis on AMF *R. irregularis* was developed to resolve this. The effect of the mutagen ethyl methanesulfonate (EMS) on *R. irregularis* spores is being evaluated.

The goal to determine an optimal treatment condition for treatment of *R. irregularis* DAOM-197198 spores with EMS, can be obtained by treating the spores under different concentrations and incubation periods. The treatment will result in viable strains, proven by an adequate germination and mycorrhization capacity. The resulting protocol for the treatment of AM spores should be reproducible and prove the genetic mutational strength of EMS.

Spores are treated under time and concentration series with EMS. Two viability assays, a germination assay and a tetrazolium salt assay (MTT), allowed for verification of the cytotoxicity of EMS, followed by germination and mycorrhization testing with *D. carotus* Ti-DNA transformed monoxenic cultures.

Optimal spore treatment was obtained. The treated spores showed germination, and mycorrhization of in vitro *Daucis carota L.* Ri T-DNA transformed root organ culture (ROC) (4.12% in original, 100% in F1).

Novel studies on AM basic biology require molecular biotechnological methods. This study marks a first successful step in the development of random mutagenesis method for AMF strains. Treatment has an adequate success rate for larger scale and can be used for several applications, like the development of novel strains or for revealing genes otherwise not found with comparative studies.

Keywords: EMS, random mutagenesis, Rhizophagus irregularis, arbuscular mycorrhiza

P (ID 324)

Plant colonization by indigenous Arbuscular Mycorrhizal Fungi in multi-contaminated sites in the Czech Republic

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Colonization by Arbuscular Mycorrhizal Fungi (AMF) was quantified in the roots of 16 plants (8 species from 4 families) growing in multi-contaminated soils, in order to observe AMF colonization on plant species tolerant to contamination. The soils are highly polluted with metals by former mining and smelting activities. The roots were digested in 10% KOH and 5% HCl, and stained by 0.05% trypan blue to observe colonization. The concentrations of the target risk elements (As, Zn and Pb) in the rhizosphere and in plant tissues were measured after acid digestion and determined by ICP-OES. The results showed that all the species evaluated are colonized by highly frequent mycorrhizae in the root system (86 to 100 %) but with a minor abundance of arbuscules (7 to 34 %). The rhizosphere samples yielded high amounts of Pb, up to 2,102 ppm, As up to 152 ppm, and Zn up to 2,392 ppm. Moreover, all the plant species showed high levels of Pb and Zn in root tissues, up to 742 and up to 2,633 ppm, respectively. However, the concentrations of Pb and Zn in shoot tissues, and the presence of As in both roots and shoots are more heterogeneous. The results concur with other evaluations that have proven colonization of AMF in tolerant plants, especially grasses. From these results, we are currently evaluating the behaviour and colonization of indigenous AMF over the 3 dominant grass species, and in the future we expect to analyse its response to engineered amendments for assisted phytostabilization.

Keywords: contamination, phytostabilization, tolerant

P (ID 83)

Diversity of dark septate endophytes in Yunnan metal mine areas, southwest China and their functional roles in the enhanced metal tolerance of host plants

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Dark septate endophytes (DSEs) ubiquitously colonize the roots of plants spontaneously growing in the metal mine areas. While the diversity of DSEs and their functional roles in the metal tolerance of host plants remain obscure. In the present study, about 600 fungal strains from 32 genera, including the known DSE fungi, such as Cladosporium, Cladophialophora, Exophiala, Leptodontidium, Phialocephala and Phialophora were isolated from the plant roots in Yunnan metal mine areas. These fungal isolates exhibited a relatively high resistance to Pb, Zn and Cd in vitro, especially it's true for the members of Exophiala, e.g. E. pisciphila H93 with 15% Pb, 16% Zn and 4.9% Cd accumulation in fungal mycelia (dry weight), under the EC50 concentrations (800 Pb, 1010 Zn, 111 mg/L Cd, respectively). Comparative transcriptomics revealed that diverse strategies, involved in the 10 well-known metal-tolerant pathways of 575 differentially expressed genes, conferred the extreme Cd tolerance of H93. In vivo, we found that H93 inoculation significantly enhanced the tolerance of maize to Pb, Zn and Cd by the restricting metal translocation from roots to shoots. Besides, H93 inoculation also triggered the activities of antioxidant enzymes, enhanced the contents of antioxidants, increased Cd accumulation in cell wall and altered Cd into inactive forms in maize shoots and roots via the specific regulation of genes related to metal uptake, translocation and chelation.

Keywords: dark septate endophyte (DSE), metal mine areas, diversity, metal tolerance

P (ID 360)

Arbuscular mycorrhizal fungal DNA that are in physiologically active colonization in roots

<u>Yoshihiro Kobae</u> (National Agriculture and Food Research Organization, Sapporo, Japan), **Ryo Ohtomo** (National Agriculture and Food Research Organization, Sapporo, Japan), **Norikuni Oka** (National Agriculture and Food Research Organization, Sapporo, Japan), **Sho Morimoto** (National Agriculture and Food Research Organization, Sapporo, Japan)

Even a few centimeters of roots in field soils can be colonized by genetically diverse arbuscular mycorrhizal (AM) fungi. The DNA sequences of AM fungi in roots suggest the fungal identities; however, it is almost impossible to know which DNA sequences detected are related to the physiological activity. In this study, we took advantage of the characteristics of rice (Oryza sativa L.) mycorrhizal roots, in which active colonization in roots is easily detected via histochemical staining of fungal succinate dehydrogenase activity (vital staining) and individual active colonization regions (infection units) in roots rarely coalesce. Root segments (< 3 mm) containing an active infection unit were dissected and squashed, large subunit (LSU) ribosomal RNA genes (rDNAs) were amplified using fungal universal primers and the sequences were directly determined by Sanger sequencing. All obtained sequences of colonization regions were of glomeromycotan origin. Phylogenetic analysis revealed that the levels of LSU-rDNA heterogeneity within an active colonization region were different among different clades. The methodology presented in this study offers researchers a novel tool for investigating the DNA information of physiologically active AM fungi in roots, whereas the factors that affect genetic diversity among active colonization remain to be clarified.

References:

Kobae Y, Ohtomo R, Oka N, Morimoto S. (2017) A simple model system for identifying arbuscular mycorrhizal fungal taxa that actively colonize rice roots grown in field soil. *Soil Science and Plant Nutrition*, 63: 29-36

http://www.tandfonline.com/doi/full/10.1080/00380768.2016.1277156

Keywords: arbuscular mycorrhizas, infection unit, physiologically active colonization, vital staining

CONCURRENT SESSION: Mycorrhizal carbon fluxes, carbon sequestration

Carbon is the universal currency supporting mycorrhizal symbiosis and mycorrhizal networks. This session will cover carbon fluxes between atmosphere, plants, mycorrhizal fungi, soil microbiota and soil organic matter and shall increase our understanding of the kinetic parameters and regulation of carbon fluxes between the symbiotic partners, the mycorrhiza-aided buildup and turn-over of soil organic matter, and the roles of different types of mycorrhizal symbiosis in global carbon cycling. The session should also include contributions on ecological consequences of reverse carbon flow in mycorrhizas formed by achlorophyllous plants and the importance of common mycorrhizal networks in crossfeeding different plants within a plant community.

Chairs: Iver Jakobsen, Martin Lukac

CL (ID 266)

Mucoromycotina fungi are mutualistic with vascular plants

<u>Grace A. Hoysted</u> (Centre for Plant Sciences, University of Leeds, Leeds, UK), Alison Jacob (Department of Life Sciences, Imperial College London, London, UK), Jill Kowal (Department of Life Sciences, National History Museum, London, UK), William Rimington (Department of Life Sciences, Imperial College London, London, UK), Martin Bidartondo (Department of Life Sciences, Imperial College London, London, UK), Jeff Duckett (Department of Life Sciences, National History Museum, London, UK), Silvia Pressel (Department of Life Sciences, National History Museum, London, UK), Sebastian Schnornack (Sainsbury Laboratory, University of Cambridge, Cambridge, UK), Katie Field (Centre for Plant Sciences, University of Leeds, Leeds, UK)

It has long been postulated that fungi played a critical role in facilitating the transition of plants from an aquatic to terrestrial lifestyle >475 million years ago, and we know that associations with fungi from two early-branching lineages — the Glomeromycotina and the Mucoromycotina - are widespread amongst extant early-diverging land plants. However, the physiological and molecular basis of the functioning of fungal symbiosis in early vascular plants are unknown.

We measured bi-directional transfer of carbon-for-nutrients between the lycophyte, *Lycopodiella inundata* (representative of an early evolutionary transition from non-vascular to vascular plants) and Mucoromycotina fungi using radio- and stable-isotope tracers under both modern and Paleozoic-like atmospheres of 440 ppm and 800 ppm [CO₂], respectively. Quantification of ³³P was achieved through acid digestion and scintillation counting, ¹⁵N transfer determined using compound specific-isotope ratio mass spectrometry (CS-IRMS), and movement of plant-fixed carbon to fungi by sample oxidation and liquid scintillation. We show for the first time, transfer of nutrients from Mucoromycotina fungi to a lycophyte in exchange for plant-derived carbon. There were no significant differences in ³³P transferred from fungus to plant under the two different CO₂ concentrations.

By demonstrating carbon-for-nutrient exchange between an ancient lineage of vascular plants and Mucoromycotina fungi, this investigation changes our understanding of the role of diverse fungal symbionts in the initial colonisation of the terrestrial environment, and subsequent plant diversification through the Paleozoic. Furthermore, our research can play a crucial role in predicting the responses of these partnerships to ongoing changes in atmospheric CO₂.

CL (ID 224)

Mycorrhizal Net Primary Production and Respiration in a Tropical Rainforest: Impact of Leaf Cutter Ants

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Introduction: Tropical Rainforests are the lungs of the earth. We studied soil net primary production (NPP) and soil respiration (Rs).

Methods: A Soil Ecosystem Observatory utilized sensors for CO2, O2, H2O, and temperature. Fluxes and production were modeled using Fick's Law and daily images using automated minirhizotrons of direct observation.

Results: Mean root standing crop =120g/m2 with lifespan =60 days, equaling a fine root NPP 720g/m2/y. Mean fungal hyphal standing crop =3.5g/m2, lifespan =24.9 days equaling a fungal NPP of 52.9g/m2/y. AMF NPP =24.3g/m2/y, or 1.6% of the total NPP. Rs =1720g/m2/y; AMF hyphal respiration =140kg/m2/y, or 8%. During La Niña (wet) phase of ENSO standing water reduced Rs to ~0; soils emitted up to 0.3mg/m2/day of methane-C. During El Niño (dry) phase, soils consumed 1.3mg/m2/day of methane-C and respired 31g/m2/s of CO2-C. Both roots and hyphae form air channels connecting to nests of Leaf Cutter Ants (LCA) where O2 and CO2 are exchanged. Hyphal standing crop was unchanged but hyphal lifespans declined to 7.1 days, for an AMF hyphal NPP of 188g/m2/y.

Conclusions: Dynamic belowground NPP nearly equals that aboveground. AMF directly process nearly 10% of soil C and affect soil chemistry. CO2 diffuses through root/hyphal channels from soil-to-nest. LCA nests thus form a chimney for CO2 from soil through the forest canopy and are an important contributor to mycorrhizal and rainforest C dynamics. Under climate change scenarios, precipitation may increase or decrease altering ant activity, mycorrhizae and greenhouse gas cycling.

Keywords: mycorrhiza net primary production, tropical rainforest, soil respiration, leaf cutter ants

CL (ID 230)

Fungal regulation of organic matter accumulation along a coniferous soil fertility gradient

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Plant-soil interactions provide nutritional feed-backs on ecosystem fertility, and soil microorganisms play a central role as mediators of these interactions, but mechanistic understanding is still largely lacking. Here we show that interplay between fungal functional guilds regulated organic matter accumulation across a fertility gradient in a Swedish conferous forest.

With higher soil fertility and relaxed environmental stress, the relative abundance of fungal species shifted within all functional groups. The representation of saprotrophic litter fungi among the Agaricomycetes increased in a depth-dependent manner. In particular, the genus *Mycena* increased in relative abundance and moved deeper into the profile with increasing fertility. Towards the less fertile end of the gradient, spatial separation between litter and root associated fungi was more pronounced and ectomycorrizal fungi dominated strongly deeper in the profile.

Organic matter accumulation correlated negatively with Mn-peroxidase activity, which in turn correlated positively with litter basidiomycetes (primarily *Mycena* spp.), highlighting this functional group as pivotal in driving organic matter turnover across the gradient. A negative connection to ectomycorrhizal fungi supported that these indirectly suppressed enzymatic oxidation by competing with litter saprotrophs.

In conclusion, our results supported that fungal-driven enzymatic oxidation constrains belowground C accumulation and drives ecosystem fertility, by preventing N retention in organic pools. Further, we found that these processes were regulated by a fertility-related interplay between fungal guilds with contrasting decomposing capacity. The mutual interdependency of organic matter accumulation and fungal decomposition closes a positive feed-back loop, which may drive ecosystem development in different directions under changing environmental conditions.

CL (ID 137)

The impact of plant-derived C flow and mycorrhizosphere microbiology on boreal forest soil organic matter chemistry

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Boreal forest soils are globally a large carbon reservoir and therefore the response of boreal soil organic matter (SOM) to climate change is of great interest¹. There is increasing evidence that residues of soil microbes and mycorrhizal fungi form large part of SOM² but also the importance of mycorrhizal fungi in SOM decomposition is highlighted³.

To study plant and mycorrhizal influence on SOM decomposition and formation, we established a three-year long dual experiment in a boreal Scots pine forest where the C flow to natural SOM was controlled and SOM chemistry (e.g. mass loss, N pools, condensed tannins) and microbiology (PLFA, MiSeq for fungi and bacteria, GeoChip®) were investigated. Following treatments were established: the mesh size of 1 μ m excluded both tree roots and fungal hyphae, the mesh of 50 μ m allowed fungal hyphae to penetrate and the 1 mm mesh (or no mesh) allowed unlimited C flow to enter. Larger scale study consisted of 1 m² trenching plots, smaller scale of 10x10 cm SOM-filled mesh bags.

Our results suggest that plant-mycorrhizal interactions increased the recalcitrant pool of organic N in SOM due to root-derived tannins. Although mycorrhizal plant-soil interaction seems to strongly affect the formation of recalcitrant SOM, the net decomposition is not hindered by these chemical changes. The detailed microbial community data will be discussed in relation to SOM formation and decomposition. Our study underlines that plant-soil feedbacks and especially soil chemistry behind the interactions are decisive factors for estimating changes in SOM decomposition rates.

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Keywords: boreal forest soil, SOM formation, SOM decomposition, recalcitrant, mycorrhizal fungi

ST+P (ID 319)

Ectomycorrhizal community composition is a driver of mycelial biomass turnover across a *Pinus sylvestris* chronosequence

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In boreal forest soils biomass turnover of ectomycorrhizal fungal mycelium is a fundamental process regulating standing mycelial biomass and necromass flux into soil organic matter. Input via roots and associated fungi are a major contributor to the build-up of organic matter stocks, but the role of mycelial turnover in regulating boreal carbon sinks is currently not well understood, neither how turnover may be driven by successions in ectomycorrhizal fungal communities.

We quantified mycelial turnover and assessed fungal community composition in a chron-osequence of eight, 12- to 158-years-old, managed *Pinus sylvestris* forests. Turnover was estimated by determining mycelial biomass, using ergosterol as a proxy, in sequentially harvested in-growth mesh bags and applying mathematical models. Fungal communities were identified using Pacific Biosciences sequencing of fungal ITS2 amplicons.

Turnover decreased with forest age, from 7.0 yr⁻¹ in the youngest stand to 0.3 yr⁻¹ in the oldest stand. Fast turnover rates in the younger stands were associated with *Tomentella radiosa* and *Wilcoxina* species. Intermediate turnover rates were primarily associated with *Piloderma*, *Suillus* and *Cortinarius* species, whereas slow rates of turnover in the older stands were weakly associated with *Lactarius* and *Russula* species.

There was a strong forest age effect on both ectomycorrhizal community composition and turnover across the chronosequence. Mycorrhizal community composition is therefore proposed to drive the observed changes in mycelial turnover, with potential importance for carbon sequestration.

Keywords: mycelial turnover, ITS2 amplicons, community composition, carbon sequestration, boreal forest

ST+P (ID 293)

Transitions in fungal communities during the decomposition of ectomycorrhizal fine roots

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Fine roots are relatively short lived and their turnover represents a large input of resources to the soil community. Although they harbour various groups of fungi including ECM, endophytic and saprobic species, relatively little is known about changes in fungal assemblages as fine roots decompose.

Plots were established within mature *Abies balsamea* and *Picea rubens* forests in Nova Scotia. In the spring of the first year, understory seedlings were either severed at the root collar, or left as controls. Seedlings were then collected over 16 months and assessed for loss of fine roots and the integrity of ECM mantles on remaining roots. Degrading mantles were grouped into broad categories based on texture, pigment and exploration type. We also monitored the total fungal community over time by ITS cloning-sequencing.

Prosenchymous, diffusely pigmented mantles of short to medium distance exploration type ECM (e.g. *Cortinarius*) were lost during the first year. However, synenchymous, melanised mantles of short distance types (e.g. *Cenococcum*) maintained integrity throughout the experiment. Synenchymous mantles with diffuse pigmentation of contact type ECM (e.g. Russulaceae) and prosenchymous mantles with intense pigmentation of medium distance types (e.g. *Piloderma fallax*) degraded at intermediate rates. Cloning-sequencing did not detect any clear changes in ECM communities over time, even though mantles were degraded. Very few obviously lignocellulolytic taxa were detected, implying that the plant component was not yet under attack. However, fungal root endophytes belonging to the Helotiaceae increased in abundance during the first year and decreased thereafter, suggesting a possible role in mantle decomposition.

Keywords: Fine root turnover, fungal root endophytes, ECM mantle structure

ST+P (ID 258)

Ectomycorrhizal fungi may contribute to the formation and sorptive stabilization of soil organic matter

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Recent studies have shown that ectomycorrhizal (ECM) fungi have some capacity to decompose soil organic matter (SOM) using oxidative mechanisms. Most likely the major benefit of this activity is to release nutrients including nitrogen that are embedded in the SOM. Here we investigated whether the processing of boreal forest SOM by an ECM fungus and a closely related saprotrophic fungus increase the reactivity of the organic matter towards mineral surfaces and thereby may contribute to the stabilization of SOM. The experiment was performed by growing the ECM fungus Paxillus involutus and its closely related saprotrophic fungus (Hydnomerulius pinastri) on a boreal forest SOM extract supplemented with ¹³C-glucose. Both the undecomposed and decomposed SOM were characterized in detail using spectroscopic and isotopic measurements and their sorption onto goethite was then examined. In the presence of glucose and when acquiring nitrogen, both fungi slightly oxidized and decreased the molecular size of the SOM extract. During the decomposition, both fungi secreted a substantial amount (>10% new biomass C) of aromatic metabolites. The retention of the SOM extract onto goethite enhanced after decomposition due to oxidation and production of surface-reactive metabolites. The results suggest that the production of metabolites along with the oxidation of organic carbon during SOM decomposition by ECM fungi may contribute to the stabilization of organic matter in boreal forest soils.

ST+P (ID 107)

Stable isotopes elucidate more and more facets of mycoheterotrophic carbon gain among plants

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Introduction/Aim: In the mycorrhizal symbiosis plants usually exchange photosynthates for mineral nutrients acquired by fungi. This mutualistic arrangement has been subverted by plants producing dust seeds and relying on fungal hosts for carbon supply in the seedling development stage (initial mycoheterotrophs, IMH). A few hundreds of IMH plants remain achlorophyllous as adults and continue relying on fungal carbon (full mycoheterotrophs, FMH). During the last decade additionally an increasing number of green plants has been identified as gaining carbon simultaneously from two sources, own photosynthesis and mycorrhizal fungi (partial mycoheterotrophs, PMH). Here we aim separating mycoheterotrophic plants not only according to their respective functional groups (IMH, FMH, PMH), but also according to their taxonomic relationships and to the functional groups of their respective fungal hosts.

Materials and Methods: We used a stable isotope abundance database of mycoheterotrophic and accompanying autotrophic plants and molecular data on the identity of the mycorrhizal fungi to test the different functional groups for significance of differences in their isotope abundance patterns.

Results: We succeeded in separating IMH, FMH and PMH from autotrophic plants^{1,2}. We furthermore separated (i) FMH from PMH^{1,3}, (ii) FMH mycorrhizal on ECM, SAP litter- and wood-decomposing and AM fungi^{3,4}, (iii) PMH on ECM basidiomycetes, ECM ascomycetes and rhizoctonias^{5,6} and (iv) FMH and PMH orchids and ericoids⁷.

Discussion: Isotope natural abundance is an ideally suited tool to elucidate fungus-to-plant carbon fluxes and to identify functional diversity among mycoheterotrophic plants.

Conclusion: Mycoheterotrophy in mycorrhizal symbioses is much more widespread and diverse than previously assumed.

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Keywords: carbon, mycoheterotrophy, mycorrhiza, stable isotopes

ST+P (ID 88)

Many ways to exploit mycorrhizas: the mycoheterotrophy continuum in orchids

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Introduction: Mycorrhizal carbon flow is not only reversed in achlorophyllous, fully mycoheterotrophic (FMH) plants but also in putatively autotrophic plants participating in mycorrhizal symbioses. Partially mycoheterotrophic (PMH) plants utilise carbon both from photosynthesis and a fungal source. All 28,000 species in the largest angiosperm family, the Orchidaceae, produce dust seeds and are initially mycoheterotrophic; they need a mycobiont for nutrient provision in early life stages. Thus, orchids may be predisposed to a PMH nutrition due to their initial FMH protocorm stage.

Materials and Methods: We investigated the ecophysiology of the heterotrophic nutrition of 30 orchid species native to Central Europe using stable C, N and H isotope analysis and molecular identification of orchid mycorrhizal fungi, and a further 40 PMH and FMH Ericaceae and Orchidaceae species from a database.

Results: We found (i) a varying degree of PMH in rhizoctonia-associated meadow orchids¹; (ii) a correlation between local light climate and ¹³C enrichment in rhizoctonia-associated orchid species; (iii) the identity of the fungal partner (rhizoctonia, ECM basidiomycete, ECM ascomycete) associated with *Epipactis* taxa to drive plant ¹⁵N enrichment²; (iv) that FMH seedlings of mature green-leaved orchids associated with rhizoctonia fungi are useful to assess the organic matter gain derived from fungi; and (v) taxonomic patterns of mycoheterotrophy³.

Discussion: Our findings demonstrate that PMH in orchids is a trophic continuum between the extreme endpoints of autotrophy and FMH, ranging from marginal to pronounced PMH.

Conclusion: In rhizoctonia-associated orchids, PMH plays a far greater role than previously assumed, even in full light conditions.

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Keywords: orchid mycorrhiza, partial mycoheterotrophy, rhizoctonia, stable isotopes, ITS sequencing

ST+P (ID 128)

The evolution of mixotrophy in Ericaceae

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Whereas most mycorrhizal plants trade photosynthates for mineral nutrients with their fungal symbionts, mycoheterotrophic species have evolved the ability to divert carbon compounds from their mycorrhizas¹. Mycoheterotrophy is permanent in some species that have lost photosynthetic capacities, but adds up to photosynthesis in a few green plants, resulting in a partially mycoheterotrophic nutrition called mixotrophy². Mixotrophy, which is considered to be an adaptation to shaded forest habitats, is described for Orchidaceae and Ericaceae species. Mixotrophic orchids have been well-studied and shown to plastically increase their use of fungal carbon in young shoots before leaf expansion and in shaded conditions^{3,4}. Moreover, in orchids, mixotrophy is considered as a likely transition step towards full mycoheterotrophy.

Although orchids include most known mixotrophic species, Ericaceae stand as a good counterpoint. Early-diverging lineages within this family encompass species showing the whole nutrition continuum, from autotrophic in arbutoids, to mycoheterotrophic in monotropoids, and mixotrophic in pyroloids¹. Based on combined plastidial and nuclear markers, we present an up-to-date phylogenetical frame, which brings the three above-mentioned clades in a monophyletic group and argues for two independent emergences of mycoheterotrophy⁵. What has predisposed to mycoheterotrophic nutrition during Ericaceae's evolution remain an intriguing issue to investigate. We complete this work with field assessment of variations in heterotrophy level in five pyroloids from Estonian boreal forests in response to shoot age and light availability. The absence of noticeable changes in mycoheterotrophic levels, based on stable isotopes abundances, contrasts with co-occurring orchids and suggests unexpected diversity of mixotrophic nutritional strategies.

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Keywords: mycoheterotrophy, mixotrophy, Ericaceae, phylogeny, isotopes

POSTERS

P (ID 468)

Ectomycorrhizae as a food source for collembola

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Although there is an abundance of information on the initiation and development of ectomycorrhizae (ECM), there is a lack of knowledge on their breakdown, which releases carbon and nitrogen back into the soil environment. Decomposition rates of ECM vary across fungal species, with some, such as the highly melanized Cenococcum geophilum, remaining intact for up to four years after root tip mortality, and others, such as Cortinarius spp., degrading much more rapidly. One potential mechanism in the breakdown of ECM is the grazing of the ECM mantle by microarthropods, specifically collembola. To determine the importance of ECM fungal species on collembola grazing, a series of feeding trials were performed in which fresh pine ECM were collected, identified by ITS sequencing, and used as food sources. Folsomia candida (a model collembola species) were presented with pairs of different ECM root tips including Cenococcum geophilum, Piloderma fallax, Lactarius sp., and Suillus sp., to determine the hierarchy of ECM preference. After a one week incubation period, the feces of the collembola in close proximity to each ECM type were counted, and the amount of ECM mantle grazed was measured digitally. Although Folsomia appear to feed on most ECM types to varying degrees, they will not feed on the darkly melanized ECM formed by C. geophilum, even when it is the only food source available. This is congruent with the observed longevity of C. geophilum ECM in the soil, but seems to contradict the commonly held idea that microarthropods prefer melanized fungi.

Keywords: ectomycorrhizal decomposition, microarthropods, selective grazing, springtails

P (ID 150)

The contribution of genetic variability among *Rhizophagus irregularis* isolates on soil respiration in tropical soils under cassava cultivation

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Soil degradation is increasing worldwide and will become a major constraint to sustainable agriculture for future generations. Soil is the largest terrestrial source of organic carbon (2,344 Gt) and tropical soils account for approximately 30% of the global carbon pool. Soil respiration results in the release of CO2 into the atmosphere and is the most meaningful indicator of soil microbial activity. Arbuscular mycorrhizal fungi (AMF) act as stabilizers of soil structure through physical and chemical soil aggregation (e.g. hyphae or exudates) by encapsulating organic carbon within aggregates and protecting it from microbial activity. In previous studies we have observed very large differences in cassava root productivity when inoculated with genetically diverse isolates of Rhizophagus irregularis, suggesting that carbon dynamics belowground could be greatly influenced by the genetics of the fungus. We studied the effects of genetically different isolates of Rhizophagus irregularis on carbon dynamics, accumulation and emissions to the atmosphere in soils under cassava cultivation; separately measuring heterotrophic, autotrophic and mycorrhizal fungal contributions to total soil respiration. This research forms part of an extensive collaborative project aiming to study the effects of natural genetic variation of AMF on cassava cropping and its environmental effects.

Keywords: soil respiration, AMF, cassava, soil aggregation, carbon storage

P (ID 455)

Reduction of C-flow to AMF by P-fertilization or shading – is it caused by plant?

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Both root colonization by arbuscular mycorrhizal fungi (AMF) and carbon flow to AMF can be reduced under environmental conditions where AMF might become less beneficial for hosts, e.g. P-fertilization or shading. We investigated conceivable causes of this phenomenon including reduced C flow to roots or reduced C flow specifically to AMF.

Leek (*Allium porrum*), medic (*Medicago truncatula*) and ryegrass (*Lolium perenne*) populations, inoculated with field soil, were subjected to two soil P- levels or to a partial short-time shading in two glasshouse experiments. The C flows were traced by 13 C isotope labelling from shoots down to the AMF-signature fatty acid $16:1\omega5$ in roots.

Relative plant C allocation to AMF was decreased by P-fertilization in leek and ryegrass, but not in medic. The decrease was caused by lower C allocation to AMF compared to roots (explained by both decreased abundance and $^{13}\text{C-enrichment}$ of $16:1\omega5)$ and not by lower C allocation to roots in general. In contrast, sudden shading decreased C allocation to AMF in medic, but not in leek (not studied in ryegrass). The decrease was caused by reduced C allocation to roots in general, while C allocation to AMF compared to roots did not change.

Our results suggest that some plants might be able to suppress C flow specifically to AMF due to P-fertilization. This notion is supported by the reduced C flow to AMF compared to roots, linked with both reduced AMF mass and reduced C income per unit of AMF mass.

Keywords: arbuscular mycorrhiza, carbon allocation, phosphorus fertilization, 13 C isotope labelling, $16:1\omega 5$ fatty acid

P (ID 503)

Mycorrhizal symbiosis induces plant carbon re-allocation differently in C₃ and C₄ Panicum grasses

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Arbuscular mycorrhiza (AM) symbiosis involves a broad range of plant taxa with both C₃ and C₄ photosynthesis types. The C₄ plants have been established to be generally more responsive to establishment of AM symbiosis than the C₃ plants in terms of their nutrition and growth promotion. However, it is not known whether the type of photosynthesis affects the symbiotic carbon (C) fluxes. To achieve phylogenetically unbiased comparison, closely related C₃-Panicum and C₄-Panicum have been grown with a synthetic community of AM fungi at two levels of available phosphorus (P), and the whole plant C budget was assembled based on ¹³CO₂ pulse chase labeling and sequential harvesting. On average, mycorrhizal plants allocated 3.9% more recently fixed C belowground than their non-mycorrhizal (NM) counterparts. Whereas mycorrhizal C₃-Panicum allocated relatively less recently fixed C to aboveground respiration as compared to their NM controls, mycorrhizal C4-Panicum allocated more of the recent C into the substrate and aboveground respiration as compared to their NM controls, and relatively less to the belowground respiration as compared to the mycorrhizal C₃-Panicum. Mycorrhizal C allocations measured here are at the low end of previously published records and were not significantly different between the two plant species. Yet, the *Panicum* grasses with C₃ and C₄ types of photosynthesis showed different strategies to reallocate recently fixed C due to their mycorrhizal status, and this is probably only partly explainable by improved mineral nutrition or increased belowground C sink strength.

Keywords: carbon (C) budget, C3 and C4 photosynthesis, ¹³C labeling, *Panicum sp.*, phosphorus

P (ID 243)

Tillage intensification affects AMF diversity, SOC and enzymatic activities within soil aggregates at various scales

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Long-term agricultural management may change soil C sequestration and alter soil organic matter content, structure and biological activity. The objective of this study was to investigate the impact of tillage and N fertilization within a field experiment with a soybean/wheat rotation, originally established in 1982 in Central Italy.

Treatments were ploughing at 30-cm depth (P30) and minimum tillage (MT) in combination with two N fertilizer rates to wheat, 0 (N0) and 200 kg N ha-1 (N200). In spring 2016, soil samples were collected from the 0–15 and 15–30 cm soil layers. Bulk density (BD), NH4-N and NO3-N concentration were assessed. After wet-sieving fractionation, SOC, total N and available P, enzymatic activities and AMF diversity were assessed in both bulk soil and microaggregates within macroaggregates (mM). AMF diversity was characterized by SSU-ITS-LSU fragment.

At both soil layers, BD did not vary between tillage intensities, but was 7% higher in N200 than N0. At 15-30 cm depth, the proportion of mM was 21% higher in MT than P30, while no differences were detected in the surface layer. Tillage did not change soil total N and available P, whereas N fertilization affected NH4-N (15%) and NO3-N (28%) concentration at both soil layers. SOC, enzymatic activities and AMF diversity in bulk soil and in mM changed in P30 respect to MT.

Our results showed co-occurrence patterns in SOC, enzymatic activities and AMF diversity of bulk soil and mM fraction, suggesting that the contribution of soil biota to C sequestration within aggregates varied with tillage.

Keywords: tillage intensification, soil fertility, soil aggregates, AMF community diversity, soil enzymatic activity

P (ID 458)

A Serengeti Without Arbuscular Mycorrhizas: Quantifying Interactions Between Migratory Mammals and Fungal Symbionts

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Both mycorrhizas and grazing mammals influence phosphorus (P) availability and primary productivity of grassland ecosystems. Grasses are estimated to obtain as much as 90% of their P from arbuscular mycorrhizas, and these symbioses are particularly important in acquiring P from the highly weathered soils which typically occur in tropical grasslands, such as the Serengeti. Large, migratory ungulates have been shown to translocate nutrients and increase the availability of P for grassland vegetation. The P content of vegetation and the abundance of arbuscular mycorrhizal (AM) fungi are highest in regions with the highest soil P. The concentration of P in plant tissues may influence the migratory patterns of ungulates in the Serengeti; during the gestation period, when nutritional requirements are increased, grazing mammals seek areas containing plants with higher P concentrations. The purpose of this study was to analyze the interactive roles of AM fungi and mammalian grazers on the P cycle and primary production in the Serengeti. Bayesian linear regressions were used to infer the primary factors that influence AM fungal abundance in the experimental plots. The best model indicated that total soil P was the strongest predictor of AM fungal biomass, it was significantly and positively correlated with the AM fungal biomass in grazed plots, but not in ungrazed plots. Biomass measurements of AM fungi were combined with published data on the abundance and physiology of large ungulates in a customized Madingley model to simulate P dynamics and net primary production of the Serengeti ecosystem.

Keywords: mycorrhizas, Serengeti, macroorganisms, biomass, model

WORKSHOP: *In-situ* mycorrhizal function – how do we get relevant data from a messy world?

This workshop will bring together conference participants with an interest in working in field settings. Field systems are "messy" and can yield equally messy data. The aim is to share approaches to designing and implementing field studies that identify and quantify mycorrhizal **function**, beyond proxies such as plant biomass accumulation or community change. There will be "flash talks" of 5 minutes to give examples of various approaches, or that highlight particular issues. Structured group discussion will follow, allowing participants to draw on the expertise in the room. The discussion will focus on the following areas:

- 1. Definitions: what key variables define mycorrhizal function?
- 2. Measurement: What measurements do we need? Are there general measures that can be applied across all mycorrhizal associations? How do we move beyond proxies to direct measurement of function?
- 3. Horizon Scanning: What are the barriers to determining function? Can we learn from other disciplines? What technologies are there that we could seek to adopt?

The close of the workshop will be a synthesis of the event. We aim to prepare an open access perspectives paper to encourage debate on this topic within the mycorrhizal research community beyond the conference close.

Chairs: Thorunn Helgason, Ylva Lekberg

WORKSHOP: Species concept of Glomeromycota

Species are ultimately a human construct, and for many uses our working definitions are sufficient and provide a common metric for comparison. However, unlike other taxonomic categories species ideally define the level at which genetic exchange is common. When species concepts approach this ideal they become appropriate categories for better understanding basic biology and population genetics, and they improve the validity of comparisons across groups.

Species in the Glomeromycotina have traditionally been assumed to be clonal and have been defined by spore morphology and rDNA sequences. Recent genomic data, however, has shown that Glomeromycotina have intact mating loci, tracts of recombination, and high variability. These results challenge the idea they are asexual, and open up new avenues for species recognition.

The goals of this workshop are:

- 1. to bring together a broad audience interested in understanding species within arbuscular mycorrhizal fungi (AMF)
- 2. to clarify what the current species concept in AMF represents and to identify its appropriate uses and limitations
- to discuss ways that we can develop a species concept that recognized units of genetic exchange

Chairs: Tom Bruns, Maarja Öpik, John Taylor

IL (ID 48)

Rhizophagus irregularis: what's in a name?

Nicolas Corradi (University of Ottawa, Ottawa, Canada)

Rhizophagus irregularis (also known as Rhizoglomus irregulare, and previously referred to as Glomus intraradices) represents the most widely used arbuscular mycorrhizal fungus (AMF) for scientific research. Genome analyses of many strains of R. irregularis have shown that the mycelium of this species is either homokaryotic (contains highly similar nuclei with one MAT-locus), or dikaryotic (harbors two populations of nuclei, each with a different MAT-locus). Past analyses have also shown that different R. irregularis strains (also known as isolates) are both phenotypically and genetically different, and can affect the host plant in different ways. However, the reasons for this variation is unknown, and so is but the amount and nature of divergence that exists between the genomes of these isolates. Here, we compare the structure and gene content of six R. irregularis genomes and identify large inter-isolate genome diversity at all levels; both coding and non-coding. The extent of inter-isolate functional and structural genomic divergence raises intriguing questions regarding the conspecificity of the isolates we analysed, and warrants caution in the interpretation of past population-based analyses of these symbionts based on one genome reference.

IL (ID 280)

Discontinuities in morphospace vs. reproductive isolation: species descriptions in the Glomeromycota

Dirk Redecker (UMR Agroécologie (INRA, Université de Bourgogne Franche Comté, Agro-Sup Dijon, CNRS), Dijon, France)

About 250 species have been described in Arbuscular Mycorrhizal Fungi (AMF, Glomeromycota), many of them exclusively based on morphological characters of the spores. Considering the limited set of morphological features of AMF spores this continues to represent a challenge, in particular as many descriptions are based on field-collected material.

Recently, it has become common practice to use nuclear ribosomal DNA sequences as additional evidence for species descriptions. However, there are no commonly-accepted strict criteria to define species based on sequences, which may not even be possible using a single gene. Intra-isolate variation of the nuclear ribosomal genes seems to additionally complicate interpretation of the sequence data.

An overview will be given on the current state of the art of the practice of species description in the Glomeromycota, highlighting problems and possible ways forward.

IL (ID 521)

Molecular taxon concepts of AM fungi used in community ecology

Maarja Öpik (University of Tartu, Tartu, Estonia)

Molecular (DNA-sequencing based) identification has revolutionized understanding about arbuscular mycorrhizal (AM) fungal biodiversity, and its patterns in ecosystems. Sequencing targeted amplicons of AM fungi from different kinds of samples—soil or roots, spores or mycelia—provides DNA sequences that can be classified into molecular taxa or species proxies. In this introductory presentation to the workshop, to provide seed for discussion among all attendants, I will summarise the most common molecular taxon concepts currently used in community ecology of AM fungi, indicating the assumptions behind them as well as knowledge gaps.

Keywords: arbuscular mycorrhizal fungi, taxon recognition, taxon identification, community ecology, taxon concepts

WORKSHOP: Mycorrhiza for human welfare – Past, present and future

There is a growing awareness of the beneficial roles played by mycorrhizal symbioses in human nutrition and welfare and of the economic importance of ecosystem services they provide in agriculture, forestry and society. Mycorrhizal symbioses are strategically important as they enhance primary productivity and provide fundamental services by regulating ecosystem processes, such as soil formation, quality and health, bioremediation, nutrient cycling, soil carbon sequestration, pest control, plant nutrient acquisition and health. In addition, several mycorrhizal food, medicinal and aromatic plant species show increased content in phytochemicals, such as polyphenols, anthocyanins and carotenoids, that play a fundamental role in promoting human health. Yet mycorrhizal symbioses are often overlooked when it comes to decision-making on agriculture and forestry management practices, which should aim at maintaining and enhancing the mycorrhizal potential of soils. In the Workshop, fundamental studies on the genetic, ecological and functional traits of host plants and fungal symbionts will be discussed in order to answer the question as to whether mycorrhizal symbioses may represent suitable tools for improving crop productivity and environmental quality, reducing the use of pesticides and synthetic fertilizers, and producing safe and healthy food to feed the megacities in the years to come.

Chairs: Manuela Giovannetti, Joyce Jefwa

IL (ID 517)

Is African Agriculture Ready?

Joanna F. Dames (Department of Microbiology and Biochemistry, Mycorrhizal Research Laboratory, Rhodes University, Grahamstown, South Africa)

In developed countries a revolution embracing the use of microbial products in agriculture is growing with large corporations investing in these technologies. Microbial solutions to problems of nutrient-poor, low-yielding degraded agricultural lands are a best-kept secret that has not become rooted in African agriculture. African agriculture contributes 32 % to the GDP involving 65-85 % of the workforce which is largely based on small holder and subsistence farmers. Nutrient deficient, acidic and degraded soils reduce crop production and food security contributing to malnutrition. It is well established that arbuscular mycorrhizal (AM) fungi play a crucial role in sustainable agriculture, reducing agrochemical reliance, improving soil health and crop production and quality. Why has mycorrhizal technology not been embraced and adopted as a standard agricultural practice in Africa?

The reasons are varied and may be due to challenges in production of AM inoculants. Market knowledge, farmer perception, technology costs and inability of resource poor farmers, in particular, to successfully exploit these technologies contribute to this problem in Africa. An African strategy should promote the translation of research conducted in greenhouse trials to more extensive subsistence field trials in order to economically assess the true mycorrhizal benefits to local communities, while also promoting an understanding of soil microbiology for improved soil health.

This workshop presentation will examine these challenges and suggest alternative approaches such as "on farm" production of quality inoculants and highlight areas of research requiring attention in the African context.

IL (ID 208)

Prospects of mycorrhizas and their role in agroforestry systems: An analytical framework

Degi Harja Asmara (1Centre for forest Research and Institute for Integrative Systems Biology, Université Laval, Quebec, Canada), **Suzanne Allaire** (Soils and Agro-Environmental Engineering, and Centre for Horticultural Research, Université Laval, Quebec, Canada), **Damase P. Khasa** (Centre for forest Research, Institute for Integrative Systems Biology, and Canadian Research Chair in Forest and Environmental Genomics, Université Laval, Quebec, Canada)

Mycorrhizas play an important role in soil nutrient cycling, and plant-soil interactions, but their full contribution is complex and requires the identification of the best conditions to get the most benefit of these associations. Few researches explore mycorrhizal interactions with multi-plant species. The outcomes are often ambiguous, with confounding factors because the interactions may occur at multiple scales. Mycorrhizal fungal networks rather than single interactions linking numerous plant roots in an ecosystem could be more functional. The networks add complexity to the system and open a new paradigm on plant interactions, which mostly focuses on direct below and aboveground resources sharing. The mycorrhizal networks are known to be either accelerating the competition or balancing the resources sharing within the connected plants. The potential impact may range along a broad continuum from mutualism to parasitism. A better understanding of the mechanisms and interactions between crop genotypes and species, the abiotic stressors, the integrated pest management, and the links between above- and below-ground systems, is crucial. A modeling framework may be required to fully understand interactions within the plant-plant, and plant-microbe-soil interactions. The framework can be useful for exploring best scenarios and management on tree-soil-crop interactions and mycorrhizal fungal networks and how to optimize these interactions. The application on intercropping agroforestry system can be used for sustainable low- or high input agricultural systems, or restoration of drastically degraded ecosystems. A decision support software WaNuLCAS is presented as a model of water, nutrient and light capture in various agroforestry systems.

Keywords: agroforestry systems, ecological modelling, mycorrhizal networks, symbiosis, WaNuLCAS software

IL (ID 314)

Can designer mycorrhizal communities enhance soil carbon sequestration in forestry applications?

Colin Averill (Boston University, Boston, MA, USA)

Microbial community composition can transform ecosystem function. This has been demonstrated time and time again in fields as diverse as soil ecology, human medicine and pollinator conservation. There is mounting evidence that ectomycorrhizal fungi can slow soil C cycling and enhance C sequestration in soil. While our community is still in the early stages of *describing* how mycorrhizal composition can transform function, we are only beginning to understand how to *apply* this information.

In this presentation I describe how ectomycorrhizal fungi can slow soil C cycling and enhance ecosystem C sequestration. I demonstrate how experimentally adding single mycorrhizal community members to soil can turn on and off this critical ecosystem function. Given there is likely variation in the effect of particular mycorrhizal species, and among mycorrhizal consortia, I suggest there is enormous potential to bio-prospect for and design mycorrhizal communities to enhance ecosystem C sequestration in forestry applications. Globally, forestry represents ~500 billion USD in annual revenue, and most forestry species associate with ectomycorrhizal symbionts. Given that managed forestry covers ~1.2 million km², the C-sequestration potential is large. Furthermore, depending on future C-offset valuation, this type of microbial management may be economically viable.

CL (ID 55)

Arbuscular mycorrhizal fungi and medicinal plants: prospects for the enhanced production of phytochemicals promoting human health and for sustainable agriculture

Szymon Zubek (Institute of Botany, Jagiellonian University, Krakow, Poland)

The impact of AMF on selected species of medicinal plants and the influence of medicinal plants under cultivation on these microorganisms were studied ¹⁻⁸.

The laboratory experiments showed that some of AMF species applied increased the concentrations of thymol and anthraquinone derivatives in *Inula ensifolia* and *Hypericum perforatum*, respectively, as well as phenolic acids and flavonoids in *Viola tricolor* ^{5,6,8}. The enhanced production of secondary metabolites in the plants might be due to improved mineral nutrition by AMF and/or a result of general plant defense reaction to fungal colonization. Regardless of the mechanisms, these AMF species may be used to improve the quality of herbal materials.

The field experiments revealed that the long-term monocultures of mycorrhizal plants (*H. perforatum, Levisticum officinale, Mentha ×citrata* subsp. *citrata, Thymus vulgaris*) had no effect on the level of AMF propagules, however, affected AMF species composition. The cultivation of nonmycorrhizal *Chelidonium majus* reduced the number of AMF propagules, which can be detrimental to medicinal plants dependent on symbiosis and grown subsequently in this area ⁴. The aim of the second stage of field experiments was to investigate the combined effect of mineral or organic fertilization and three mycorrhizal plants under cultivation (*Lavandula angustifolia, Melissa officinalis, Salvia officinalis*) on the diversity and abundance of AMF. The effect of the type of fertilization depended on the plant species. Moreover, mineral fertilizers used in moderate amounts did not have a negative impact on the number of AMF propagules, while having a positive effect on species diversity ⁷.

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Keywords: AMF species richness and composition, arable soils, cultivation, medicinal plants, plant secondary metabolites

IL (ID 152)

Arbuscular mycorrhizal fungi and the production of health-promoting foods: perspectives for the future

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Arbuscular mycorrhizal fungi can positively affect plant health and growth. This is possible because AMF help their host plants to take up minerals and water from the soil and promote defense responses against pathogens. As a consequence, mycorrhizal plants require lower chemical inputs in the form of fertilizers and pesticides, and the use of inoculated plants (or the management of the existing AMF populations in the soil) can significantly contribute to the development of more sustainable agricultural practices. Also, the yield of crop plants colonized by AMF can be higher than that of plants without AMF.

In recent years, increasing attention has been devoted not only to the amount of food production, but also to the quality of food. Indeed, the awareness of the importance of food quality has increased more and more and it has been associated to the development of the idea of functional foods. These are foods containing health-promoting compounds, decreasing the risk of chronic or acute diseases.

AMF have been shown to affect the quality and quantity of secondary metabolites in plants; such molecules can act as health-promoting compounds in humans. Increasing evidence indicates that AMF, alone or in combination with plant growth-promoting bacteria, can improve the quality of crop products. In this presentation, relevant examples from the literature and some original results will be shown, together with the discussion of future perspective and possible applications.

Keywords: arbuscular mycorrhiza, crop quality, functional foods, plant growth-promoting bacteria

IL (ID 91)

Perspectives of plant-fungal symbioses for bioremediation of metal polluted sites

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Metal pollution has drastically increased during the last centuries and it is expected to rise in the future. Fungi, from many distantly related groups, have evolved the ability to associate with plants even in habitats extremely unfavorable due to extreme temperature, low nutrient availability and high abundance of toxic metals. The best described symbiosis between plants and fungi is the association of plant roots with mycorrhizal fungi. This relationship gives the mycorrhizal plants a competitive advantage over others, that are devoid of properly selected microbiota. Recently, the complexity of plant microbial associations has been recognized. These symbiotic associations may be useful during introduction of plants to unfavorable environments. The establishment of an effective plant microbiota in such conditions is usually a very long process, thus introducing plants, associated with carefully selected consortia of microorganisms can significantly accelerate this process. Several field and laboratory experiments have shown that for successful phytoremediation consortia of mycorrhizal, endophytic fungi and endophytic as well rhizospheric bacteria have to be applied. These complex inocula have better chance to stimulate plant growth, photosynthesis, pathogen resistance and abiotic stress attenuation.

Our results clearly indicate that inoculation with selected microbial consortia has a beneficial effect on various species of plants, mycorrhizal and nonmycorrhizal and thus, has the potential to improve growth of whole plant communities. The inocula can be used in phytostabilisation and phytoextraction of such elements as Ni, where the proper selection of microbes can result in improving the efficiency of phytomining.

IL (ID 313)

Impact of land-use intensification on arbuscular mycorrhizal fungal species abundance decay, beta-diversity and multi-trophic interaction in grasslands

Tesfaye Wubet (UFZ-Helmholtz-Centre for Environmental Research, Department of Soil Ecology, Halle/Saale, Germany)

Arbuscular mycorrhizal (AM) fungi are ubiquitous and important soil organisms with multiple ecosystem functions. Their diversity and community structure is mainly influenced by both soil and plant related parameters and involve in direct or indirect interactions with a range of grassland communities. Here results from multi-species studies including AM fungi to assess the influence of land-use intensity (LUI) on the species abundance decay (Simon et al., 2017) and on the beta-diversity and multi-trophic interactions (Gossner et at., 2016) in grassland ecosystems will be presented.

The studies were carried out in three geographically separated regions within the frame of the German Biodiversity Exploratories project (www.biodiversity-exploratories.de).

Of the three species abundance decay (SAD) descriptors tested, abundance decay rate became steeper with increasing land-use intensity across groups. Overall, belowground groups had more even abundance distributions than aboveground groups. The observed strong differences between the two groups indicated that no single taxonomic group could serve as an indicator for effects in other groups (Simons et al. 2017). High land-use intensity reduced α -diversity for most aboveground groups, but had neutral or positive effects on belowground organisms. Analysis of the effects of land-use on β -diversity revealed a strong evidence for biotic homogenization. Belowground groups, including AM fungi were homogenized at high LUI even though their α -diversity increased (Gossner et al., 2016).

The implication of these results will be discussed in line with the impact of land-use intensity on multi-trophic homogenization and the role of AM fungi for human welfare in a changing world.

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Keywords: arbuscular mycorrhizal fungi, species abundance decay, land-use intensity, grassland ecosystem

IL (ID 312)

Sustainability of habitats and AMF biogeography: a case study in Kenya

Joyce Mnyazi Jefwa (The National Museums of Kenya, Department of Botany (Herbarium), Nairobi, Kenya)

The biogeography of AMF in Africa is yet to be unravelled, data is still scarce and the tools for conducting the studies are still limited. The increase in population and changing land use types is a major threat to the diverse natural biogeochemical landscapes whose sustainability is not clearly understood. Africa is characterised by highly diverse climatic and geological landscapes, defining unique biodiversity habitats. Kenya has distinct biogeographical regions ranging from sea-level coastal region, high altitude snow-capped mountainous ecosystems, drylands savannas and desserts to highly wet tropical equatorial ecosystems. The mycorrhizal symbiosis is key to sustenance of plant communities. with ectomycorrhizal symbiosis highly confined to specific biogeographical region sustaining a major biome rich in biodiversity. The Arbuscular mycorrhizal symbiosis is the most widespread in all regions in Kenya and is limited information on the nature of distribution of AMF across the biogeographical regions. A synthesis of voucher specimen from the National Museums of Kenya of AMF isolated from several biogeographical locations in Kenya is presented. The data has been compiled from a range of studies conducted by the National museums of Kenya. The regions represented are the tropical equatorial of western and rift valley, coastal lowlands and highlands, high altitude mount Kenya and the semi-arid drylands of Kenya. The presentation distinguishes the different morphotypes of AMF from the different biogeographical regions, which indicates distinct AMF communities. The presentation will also highlight opportunities and challenges of data collection, maintenance AMF collections and tools for biogeographical studies.

WORKSHOP: Common mycorrhizal networks – how common and how important they are?

In the top 10 cm of soil globally, external mycorrhizal mycelium extends over distances that total about half the width of our galaxy in a complex web that interlinks plant roots and soil particles. In this web, common mycelial networks in which hyphae connect plant individuals/species are likely to be important in evolution, fitness of individuals, assembly of communities, and biogeochemical cycles - and there remains much to be discussed and to be discovered. Common mycelial networks can transport carbon-energy, nutrients, and water, and provide a putative pathway for inter-plant communication. Recent studies of common mycorrhizal networks in inter-plant defence signaling, in mycorrhizal communities formed in crops grown with companion or cover crops, and progress in network analyses methods and theoretical networks as applied to mycorrhiza, are making important advances in our understanding of this field. This session invites contributions that address these themes, in talks that showcase important experimental and theoretical developments and insights, followed by a 30-minute roundtable structured discussion of the wider implications and applications of this knowledge to evolutionary biology, ecosystems and agriculture, and the priority research questions that still need to be addressed.

Chair: Jonathan Leake

IL (ID 518)

Inter-plant signalling through common mycorrhizal networks

David Johnson (School of Earth and Environmental Sciences, The University of Manchester, Michael Smith Building, Manchester, UK)

Common mycorrhizal networks (CMNs) inter-connect plants, and thus have potential to act as conduits for movement of resources and signal molecules. One key process that has been little studied is the role of CMNs in transferring signals generated by plants in response to herbivore attack. Here, I discuss evidence that mycorrhizal mycelia act as conduits for signalling between plants, acting as an early warning system for herbivore attack. Insect herbivory causes systemic changes in the production of plant volatiles, particularly methyl salicylate, making bean plants, Vicia faba, repellent to aphids but attractive to aphid enemies such as parasitoids. These effects can also occur in aphid-free plants but only when they are connected to aphid-infested plants via a CMN. This underground messaging system allows neighbouring plants to invoke herbivore defences before attack. A likely mechanism of transfer is direct movement of signalling molecules within hyphae. However, electrical signals, which can be induced by wounding, may also enable signalling over relatively long distances because the biophysical constraints imposed by liquid transport in hyphae and interaction with soil are relieved. We do not yet understand the ecological, evolutionary and agronomic implications of inter-plant signalling via CMNs. Identifying the mechanism of inter-plant signalling will help address these gaps.

IL (ID 290)

Kin recognition through mycorrhizal networks in Douglas-fir

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Mycorrhizal networks create pathways for movement of resources and information molecules belowground. Mycorrhizal networks can link members of the same or different species, and are formed by both arbuscular and ectomycorrhizas. Work in the Simard lab at University of British Columbia in Canada has mapped the extent of the mycorrhizal network in a Douglas-fir (Pseudotsuga menziesii) forest. Experiments have demonstrated movement of resources in response to source-sink dynamics, seasonality, and differences in age of linked plants. Furthermore, the network appears to act a conduit for chemical information, for example the transfer of defense chemicals in response to herbivory or pathogen attack. Because of recent evidence demonstrating that carbon transfer between seedlings, and colonization of Douglas-fir by ectomycorrhizas, varies based on tree relatedness, we aimed to determine whether Douglas-fir would preferentially transfer nutrients to kin over 'strangers' when under herbivore attack. Using seedlings with or without access to a mycorrhizal network, we used stable isotope pulse labeling to track carbon in the system. One seedling of a pair was designated as the 'donor' and a defoliation treatment was applied immediately prior to photosynthesizing with 99%-13C-CO₂. Transfer was determined by measuring δ^{13} C in tissues (needle, stem, root) of kin and stranger seedlings. Data was analyzed using linear mixed models. Significantly greater ¹³C enrichment was detected in kin with access to mycorrhizal networks than in non-defoliated controls. We conclude that preferential carbon transfer through mycorrhizal networks occurs between kin in Douglas-fir and is amplified by herbivory stress.

Keywords: mycorrhizal networks, Douglas-fir, kin recognition, carbon transfer, ectomy-corrhizas

IL (ID 66)

Network structure and its drivers of arbuscular mycorrhizal fungi in Chinese forest ecosystems

Liang-Dong Guo (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China)

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with most terrestrial plant species, and play important roles in species coexistence and community assembly. However, the network structure and its drivers of AM fungi remain unclear. In this study, AM fungal community was examined from plant root and soil samples in Chinese forest ecosystems using pyrosequencing of 18S rRNA gene. The bipartite network analysis showed the woody plant-AM fungal networks to be highly interconnected and nested, but in anti-modular and anti-specialized manners in a Chinese subtropical forest. Furthermore, the non-random pattern in the woody plant-AM fungal network was explained by plant and AM fungal phylogenies, with a tendency for a stronger phylogenetic signal by plant than AM fungal phylogeny. Besides, the co-occurrence network inferred from soil AM fungi in 12 forest ecosystems along latitudinal gradient showed small-world and scale-free characters. The Pearson's correlation results showed that the node numbers, edge numbers, connectance, average degree, average betweenness, average path length, clustering coefficient, betweenness centralization, and degree centralization were significantly negatively correlated with latitude, but modularity was significantly positively correlated with latitude. This result indicated that AM fungi had closer relationship and higher interaction at low latitude than at high latitude. Moreover, the topological features of network, except for modularity, significantly correlated with climate factors, soil PSD, and AM plant diversity positively, but with geographical factors, soil nutrients, and non-AM plant diversity negatively. This study suggests that AM symbionts could contribute to community assembly in forest ecosystems.

Keywords: arbuscular mycorrhizal fungi, network structure, co-occurrence pattern, network driver, forest ecosystem

IL (ID 516)

The role of intact extraradical mycelium in managing indigenous arbuscular mycorrhiza

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Arbuscular colonisation (AC) of the second plant in a succession starts earlier and develops faster if the extraradical mycelium of the arbuscular mychorriza fungi (AMF) associated with the first plant is kept intact and is the preferential propagule type. When abiotic or biotic stressors are present in the soil, the benefits conferred by the AMF are greatly enhanced. Thus, under manganese (Mn) toxicity, compared with any other type of AMF propagule, the presence of an intact ERM enhanced AC by 1.82 and 3.3 times, in wheat and subterranean clover, respectively, 21 days after planting (DAP). In consequence, the growth of both crops was more than doubled and was significantly correlated with a reduction in the Mn concentration of shoots of wheat and in the roots of subterranean clover. However, when the first plant in the succession was a member of the Fabeacea (Ornithopus compressus L.) shoot growth of both crops increased by 1.6 times relative to that determined if the first plant was from the Poacea (Lolium rigidum L.), even though no differences were observed in AC or Mn concentration. These results seem to be associated with the AMF assemblage in the roots of the first plant that was passed to the second plant when the ERM was kept intact; whatever was the combination of plant families. The presence in the soil of an intact ERM also induced better growth of tomato plants (3.2 times at 21 DAP) after infection with Fusarium oxysporum (109 conidia/plant).

CL (ID 138)

AMF-network effects on seedling growth as influenced by plant type, plant community richness and succession stage

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Overyielding in diverse plant communities can be due to reduced growth suppression by soil-borne pathogens or increased benefit of beneficial soil biota such as AMF. Grassland plant communities establish AMF hyphal-networks that could promote the growth of new seedlings in plant species mixtures. This effect may depend on the plant species richness and/or successional stage of the plant community. We tested the hypotheses that (1) access to an established AMF-network promotes the productivity of new seedlings, (2) AMF-networks of high-diversity plant communities and of later successional stages promote seedling biomass more than in low plant species richness and in early successional stages.

In order to test the contribution of AMF networks to production of seedling biomass we grew 8 plant species (4 grasses, 4 forbs) in 30um mesh tubes that were inserted into the soil at two different locations: the long term biodiversity experiment in Jena (Germany) across a plant species richness gradient and a time series of abandoned arable lands at the Veluwe (The Netherlands) in 3 early and 3 late-successional stage plots. Half the cores were rotated every 3rd day in order to disrupt AMF hyphae growing through the mesh.

Preliminary results show that absence of core rotation did not promote seedling biomass of grasses and forbs, irrespective of plant community richness or successional stage. Instead, growth was suppressed when cores were not rotated. These results suggest that, in the field, seedling growth is suppressed through connectivity with the mycorrhizal network of the surrounding plant community.

Keywords: common mycorrhizal networks, plant functional groups, plant-soil feedback

CL (ID 122)

The interchange of arbuscular mycorrhizal fungal hyphae helped chilli pepper and cheated sweet corn in an intercropping system

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The roles of arbuscular mycorrhizal (AM) fungal hyphae in coordinating the nutrient competition between intercrops are unclear. A 4-month greenhouse pot experiment was established to compare the nutrient acquisition by chilli pepper and sweet corn in an intercropping system. The two intercrops cultivated in an unsterilized soil were absolutely separated by PVC layer, or semi-separated by nylon cloth with the pore size of 30 μm. The mycorrhizal colonization rates of corn roots were much higher than those of pepper roots, regardless of the separation of the intercrops. However, the semi-separated treatment had significantly higher root colonization rates with both pepper and corn than the separated one. However, the interchange of mycorrhizal hyphae increased the N, P, and K acquisition amounts by 42, 46, and 47% with pepper, and 7, -34, and -21% with corn, respectively. As a result, the interchange greatly increased the N, P, and K acquisition ratios of pepper from 5.9, 21, and 44% to 7.5, 38, and 59%, respectively. Furthermore, the interchange also significantly increased the average biomass of pepper fruit by 176%, and thereby decreased the average biomass of corn ears by 30%. In conclusion, in terms of nutrients alone, the interchange of AM fungal hyphae helped pepper and cheated corn in the intercropping system.

Keywords: nutrient competition, semi-separation, mycorrhizal colonization, acquisition amount, acquisition ratio

WORKSHOP: Specificity in mycorrhizal symbioses: an evolvable trait?

Specificity is a fundamental variable in the web of life, so it has been the focus of extensive observational, experimental and theoretical research. This workshop will present and discuss the latest advances in the study of specialization in both endo- and ectomy-corrhizal symbioses, across diverse plant and fungal groups, from ecological, molecular and evolutionary viewpoints. In particular, the field has been recently revolutionized by network analysis, next-generation sequencing and links to the rhizobial symbiosis.

Chairs: Martin I. Bidartondo, Marcel Bucher

IL (ID 519)

Partitioning host specificity of arbuscular mycorrhizal fungi reveals the independent contributions of biogeography and evolutionary history

<u>Stavros Veresoglou</u> (Freie Universität Berlin, Berlin, Germany), **Annette Manntschke** (Freie Universität Berlin, Berlin, Germany), **Jeff R. Powell** (University of Western Sydney, Sydney, Australia), **Stefan Hempel** (Freie Universität Berlin, Berlin, Germany), **Matthias C. Rillig** (Freie Universität Berlin, Berlin, Germany)

There are numerous reasons why a better understanding of the association patterns of Glomeromycotan fungi can be enlightening for plant community ecologists. In here we aimed at accessing the extent to which Glomeromycotan fungi exhibit different forms of host specificity and how these correlate to each other. Contrary to most existing literature on mycorrhizal interactions we adopted a mycocentric approach and used metadata that had been standardized to carry out the analysis. We detected significant basic host specificity (BHS) and geographic host specificity where we were able to demonstrate that Glomeromycotan families differed with regards to BHS. We could detect no tradeoffs between the different forms of host specificity. This represents the very first attempt to adapt a well-established framework from parasitology to a mutualistic interaction.

CL (ID 473)

Inter- and Intraspecific Diversity in the Arbuscular Mycorrhizal Symbiosis and the Consequences for the Composition of Arbuscular Mycorrhizal Communities

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Arbuscular mycorrhizal (AM) fungi are obligate symbionts, and the fact that a relatively low diversity of AM fungi (< 200 fungal species) is able to colonize the majority of land plants has led to the overall assumption that plant-AM fungal associations must have a low specificity. However, host plant benefits (in terms of for example phosphate or nitrogen nutrition) can be highly fungal specific. Plants are colonized by communities of AM fungi, but the mechanisms that control the composition of these communities, and the colonization of roots by competing fungal symbionts are currently unknown. We studied the inter- and intraspecific diversity in AM fungi and whether host plant demand and the access of AM fungal symbionts to specific nutrients plays a role for the AM community composition. We found that the intraspecific diversity can be as high as the interspecific diversity in fungal benefits, and that the colonization with high-quality fungi led to higher mycorrhizal growth responses and an improved phosphate and nitrogen nutrition. The nutrient demand of the host plant and the fungal access to specific nutrients have an impact on the AM community composition and a low-quality fungus can dominate the AM community composition, when the fungus has access to a nutrient that the host plant is in demand of. We discuss the mechanisms that contribute to this high fungal diversity, and will correlate the competitiveness of low-quality fungi in AM communities to biological market dynamics in the AM symbiosis.

Keywords: common mycorrhizal networks, phosphate, nitrogen, fungal competition, arbuscular mycorrhizal community composition

CL (ID 47)

Host- and stage-dependent transcriptome of Rhizophagus irregularis

Tian Zeng (Molecular Biology, Wageningen University & Research, Wageningen, the Netherlands), **Rens Holmer** (Molecular Biology, Wageningen University & Research, Wageningen, the Netherlands), **Elio Schijlen** (Bioscience, Wageningen University & Research, Wageningen, the Netherlands), **Ton Bisseling** (Molecular Biology, Wageningen University & Research, Wageningen, the Netherlands), **Erik Limpens** (Molecular Biology, Wageningen University & Research, Wageningen, the Netherlands)

In general terms there is very little host-specificity in arbuscular mycorrhizal symbiosis, indicating an extremely broad compatibility. However, host preferences as well as varying symbiotic efficiencies have been observed, the molecular basis of which is still largely unknown.

To study how AM fungi interact with different hosts, we analysed the transcriptome of *Rhizophagus irregularis* DAOM197198 in three evolutionary distantly related plant species; *Medicago truncatula*, *Nicotiana benthamiana* and the monocot *Allium schoenoprasum*. In addition we used laser microdissection in combination with RNAseq to study gene expression at different stages of the interaction. We especially focussed on secreted proteins (SPs) that may act as fungal effectors to control symbiotic efficiency in a host-dependent manner.

Our data indicate that the vast majority of SPs show equal expression levels in all three host plants. In addition, a subset of the genes are differentially regulated depending on the host plant. This host-dependent expression appears to be controlled locally in the hyphal network in response to host metabolic cues.

Overall, we will present a comprehensive analysis of the *R. irregularis* transcriptome to get insight in how AM fungi manage to interact with such a wide variety of plant species.

Keywords: transcriptome, laser microdissection, effector, *Rhizophagus irregularis*, specificity

CL (ID 121)

The phylogenetics of mycorrhizal specificity: understanding the impacts of scale, sampling, and the ecological niche

Richard P. Shefferson (Organization for Programs on Environmental Sciences, University of Tokyo, Meguro-ku, Tokyo, Japan)

Introduction: Much of the literature on mycorrhizal specificity has explored patterns in specialization, identifying specialist taxa, and whether these interactions correlate to any ecological drivers of evolution or conservation potential. However, I argue that such studies typically make assumptions that lead to strongly contingent results, most notably that phylogenetic scale is not relevant, that phylogenetic sampling is sufficient, and that the ecological niches of hosts are equivalent.

Materials and Methods: I phylogenetically analyze a 65 species database of mycorrhizal interactions in the orchid subfamily Cypripedioideae to show the impacts of these assumptions on the inferences made.

Results: I show: 1) that evidence of specialization depends on the phylogenetic scale of analysis, 2) that imperfect phylogenetic sampling often leads to artefactual evidence for phylogenetic signal, and 3) that breaking the assumption of niche equivalence can show evidence of specialization even in generalism.

Discussion: Investigations into interaction specificity need a high level of phylogenetic sampling, and great care in considering phylogenetic scale and the niche of hosts. In the Cypripedioideae case, certain key fungi and their closest relatives from the core mycorrhizal hosts, suggesting that they form what may be considered a functional group. Further, we found evidence of phylogenetic signal adjusted for niche. We artefactually found phylogenetic signal when we undersampled the Cypripedioideae phylogeny.

Conclusion: A greater accounting for the assumptions underlying studies of interaction specificity can lead to greatly increased power to unravel the ecological and evolutionary dimensions of ecological interactions such as the orchid mycorrhiza.

Keywords: phylogeny, macroevolution, scale, niche

CL (ID 288)

Is specificity the rule or the exception in northern European ectomycorrhizal associations?

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The degree of specialisation of ectomycorhizal (ECM) fungi on particular hosts reported in the literature is often contradictory with suggestions ranging from slight to well-developed. There have in fact been surprisingly few empirical studies examining this phenomenon across a broad taxonomic range of ECM fungi. Here, we used data from northern Europe for agaricoid, boletoid, clavarioid, and gastroid ECM genera within the Basidiomycota to examine the question of host specialisation. The data are based upon observations of fruit body occurrence and cover a total of 1168 taxa from 30 fungal genera. There were 31 host categories with most representing individual species or genera, with the broadest categories being conifers and broadleaf hosts or generalist with no evidence of specialisation. Over 60% of fungal taxa associated with only one or two host genera. No evidence was found of fungal species to host species specialisation, although some fungal taxa occur only with single species within host polytypic genera where that species grows in a specialised habitat e.g. alpine/arctic ecosystems. The greatest degree of specialisation was observed within some boletoid genera where individual fungal taxa associated with subgeneric sections within a host genus. Specialisation within fungal genera was highly variable, even with closely related genera e.g. Russula and Lactarius. The data support the view that specialisation is highly developed within ECM and that it is a major evolutionary driver which has been a significant factor in the diversification of ECM fungal taxa.b

CL (ID 238)

Modification of host specificity 'rules' in light of forest history: the curious case of host generalism for *Suillus subaureus*

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Fungi in the genus *Suillus* are thought to associate exclusively with plants in the family Pinaceae and are routinely employed in ECM host specificity studies. Using a combination of molecular based field sampling and pair-wise inoculation assays, we characterized host specificity patterns among three *Suillus*-host species pairs. Specifically, we sought to characterize the influence of 1) spore vs. mycelial colonization, 2) neighborhood effects, and 3) host removal on ECM formation. We found a novel pattern of host specificity for the species *S. subaureus*, which successfully formed ectomycorrhizas with angiosperm hosts at both our field sites and under greenhouse conditions. The colonization of an angiosperm host by *S. subaureus* in the inoculation assays, however, required coplanting with a Pinaceae host. In addition, *S. subaureus* formed ectomycorrhizas with two phylogenetically distant Pinaceae hosts, suggesting it is likely a host generalist. Since our field sites occur in areas where Pinaceae hosts were once present but are currently absent, our results suggest the patterns of host specificity in ECM fungal communities should be viewed not only in light of current host community composition, but as a function of community change over time.

Keywords: Suillus, specificity, bioassay, host

IL (ID 127)

Host shift dependent diversification in ectomycorrhizal fungal genera *Strobilomyces* and *Afroboletus*

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The evolution of mutualistic interactions can drive biogeographic dynamics, in which a species expands its distribution range and population size, promoting allopatric speciation and/or reducing extinction probability. Previous biogeographic studies indicated geographical distributions of ectomycorrhizal fungi are strongly controlled by those of host plants, presumably due to strong host preference of these fungi. Therefore, my hypothesis is that host shifts may have allowed ectomycorrhizal fungi to undergo substantial expansion of their distribution ranges, potentially promoting allopatric speciation. The present study aimed to test for whether acquisition of new host lineages could increase diversification rates of the monophyletic ectomycorrhizal fungal genera, Strobilomyces and Afroboletus. Using a Bayesian tree inferred from 23,027-base nucleotide sequences of 80 single-copy genes, the ancestral host sates were inferred and then the host-shift dependent diversification of these fungi was tested. The results indicated that these fungi were initially associated with Caesalpinioideae/Monotoideae in Africa, acquired associations with Dipterocarpoideae in tropical Asia, and then switched to Fagaceae/Pinaceae and Nothofagaceae/Eucalyptus. Fungal lineages associated with Fagaceae/Pinaceae were inferred to have much greater diversification rates than those associated with other host lineages. This study suggests that host-shift events, particularly those occurring with respect to Fagaceae/Pinaceae, can provide ecological opportunities for the rapid diversification of Strobilomyces-Afroboletus.

HT (ID 181)

Arbuscular mycorrhizal fungal diversity along a precipitation gradient in coast redwood forests using next-generation sequencing

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Introduction: Early work showed that coast redwood (*Sequoia sempervirens*) associate with AMF¹, yet we know little about the identity of these symbionts. It is clear, however, that this iconic keystone tree species largely determines a unique understory habitat, home to a number of threatened and endemic fungal species^{2,3}. Interestingly, it has been asserted that grasslands harbor greater AMF diversity than forests⁴; however, only one study has investigated AMF diversity in forests dominated by an AMF-associated gymnosperm⁵. Our study sought to understand 1) patterns of diversity in such forests and 2) how these patterns might be influenced by environmental drivers.

Methods: Utilizing a strong precipitation gradient, we investigated the role of environmental factors and host identity on AMF community composition. Eight sites were sampled across the gradient and the ITS-2 region⁶ was amplified with Illumina-adapted primers. Select samples were also sequenced with SSU for comparison.

Results: Global models of AMF diversity would predict a sample richness of 12-14 for an entire plot (based on SSU)⁴. However, even with the more conservative SSU as a marker, we find 14 OTUs associated with a single root and 18 OTUs in the corresponding soil sample. Consistent with other findings, preliminary data show precipitation an important factor in community composition of soils⁷. Interestingly, root samples between sites display a high degree of convergence and markedly less richness than soils, highlighting for potential selection of AMF by roots in this ancient system. These data highlight the need for further exploration of AMF in forest systems.

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Keywords: arbuscular-mycorrhizal fungi (AMF), coast redwood, Illumina next-generation sequencing, diversity

HT (ID 71)

Plant identity exerts stronger effect than fertilization on soil-dwelling arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi form symbiotic associations with the majority of land plants and play crucial roles in grassland ecosystem. Understanding the responses of belowground AM fungi in alpine meadow facing anthropogenic manipulation may aid sustainable grassland management. Here we studied AM fungal responses to fertilization (urea and manure) and plant identity (grasses Avena sativa and Elymus nutans and legume Vicia sativa) in a sown pasture on the Qinghai-Tibet Plateau. AM fungal extraradical hyphal density, compared with unfertilized control, was significantly increased by urea and manure under A. sativa and by manure only under E. nutans plantation. AM fungal spore density was not significantly affected by plant identity or fertilization. Forty-eight AM fungal operational taxonomic units (OTUs) were obtained through high-throughput sequencing of 18S rRNA gene. The AM fungal diversity including OTU richness and Shannon index were significantly higher under E. nutans than V. sativa cultivation condition, but not significantly affected by fertilizer urea or manure regardless of plant species. Non-metric multidimensional scaling along with structural equation model results revealed that AM fungal community composition was significantly separated by three plant species and structured directly by plant identity only and indirectly by both urea addition and plant identity through soil total nitrogen content. Taken together, our findings highlight that plant identity exerts stronger effect than fertilization on soil-dwelling AM fungi in the converted pastureland from the similar alpine meadow.

Keywords: AM fungi, community, fertilization, plant identity, sown pasture

HT (ID 229)

Arabidopsis-mycorrhiza, an ambiguous relationship

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Around 18% of all terrestrial plant species are unable to establish arbuscular mycorrhizal (AM) symbioses. The mechanisms that underlie this incompatibility remain large unknown. Here we study the interaction between the non-host plant *Arabidopsis thaliana* and the AM fungus *Rhizophagus irregularis*.

In vitro-grown cultures of Rhizophagus, the fungal pathogen Fusarium oxysporum, and the fungal endophyte Trichoderma harzianum were used to study the specificity of early plant-microbe-interactions in Arabidopsis roots. A bi-compartmental microcosm system with a Rhizophagus fungal network provided by the host Medicago truncatula was used to study late stages of the Arabidopsis-Rhizophagus interaction. The transcriptomic profile of Medicago and Arabidopsis roots colonized by Rhizophagus was analyzed by high-throughput RNA-sequencing (RNA-seq) and compared with non-colonized control plants.

In early stage of the *Rhizophagus-Arabidopsis* interaction, *Rhizophagus* activated the *Arabidopsis* strigolactone biosynthesis genes *CCD7* and *CCD8*, whereas *Fusarium* and *Trichoderma* did not. Conversely, *Fusarium* and *Trichoderma* activated the early plant defense marker genes *MYB51* and *CYP71A12*, while *Rhizophagus* did not. At late stages of the interaction *Arabidopsis* roots colonization by *Rhizophagus* was detected with a shoot biomass reduction associated. AM fungal genes characteristic for nutrient exchange were not expressed in *Arabidopsis* roots and arbuscules were absent, indicating that no functional symbiosis was established. Furthermore, *Arabidopsis* roots colonized by *Rhizophagus* showed a plant defense response activation, which was not observed in colonized *Medicago* roots.

These results indicate that early signaling events between *Rhizophagus* and *Arabidopsis* are not totally impaired and that the incompatibility is conferred at later stages, coupled to plant defense responses.

Keywords: arbuscular mycorrhiza, non-host plants, *Arabidopsis thaliana*, *Rhizophagus ir-regularis*, RNA-sequencing

HT (ID 123)

Mycorrhizal specificity in rare and common Caladenia orchid species

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Although Orchidaceae is noted as one of the most species-rich plant families, many species in this family are listed as endangered. One of the factors that are suggested to influence orchid rarity is mycorrhizal specificity. We examined mycorrhizal specificity in two closely related Caladenia species, the rare Caladenia procera and the common Caladenia pectinata by integrating phylogenetic analysis and symbiotic seed germination. All fungal isolates and sequenced fungal clones belong to Serendipita vermifera, with only two Operational Taxonomic Units (OTUs) identified from each of the rare C. procera or the common C. pectinata. The two species has one out in common. To compare germination ability of the two orchids, we used 20 fungal isolates representing seven OTUs obtained from various Caladenia species. From the symbiotic germination trials seeds of the rare C. procera were germinated by 14 (6 OTUs) of the 20 fungal isolates, while the common C. pectinata seed were germinated by 10 (4 OTUs) of the 20 fungal isolates. Seeds from both species utilized more OTUs than were found in adult plants, suggesting fungal specificity play a lessor role during germination. Given the fact that the rare C. procera could utilize an identical OTU to C. pectinata, it suggests that its distribution may be limited by factors other than mycorrhizal specificity and that mycorrhizal availability should not limit the distribution of C. procera. For conservation efforts of C. procera, the data presented here give a useful insight into the importance fungal specificity for future ex situ orchid propagation.

Keywords: Serendipita, specificity, conservation, rarity

HT (ID 198)

Nutrients affect fungal growth and specialization in orchid mycorrhizal associations

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One of the questions in the study of mycorrhizas is about the evolution of specialization. It has been observed that environmental conditions can affect the diversity and composition of mycorrhizal fungi associated with plants. One of these conditions are soil nutrients, especially nitrogen and phosphorus. In a past study we observed that soil N and P were negatively correlated with diversity of mycorrhizal fungi associated with the orchid Bipinnula fimbriata, and also that soil P affected fungal composition. Nevertheless, it was not clear if this was due to different fungal choices from plants, or different fungal performances under different soil nutrients content. To evaluate this, we studied the effect of nutrients on fungal growth and on the establishment of the mycorrhizal association. We selected four OTUs of Ceratobasidiaceae and four OTUs of Tulasnellaceae isolated from B. fimbriata, and measured their growth under four nutrient treatments. Then we placed B. fimbriata seeds with the eight fungal OTUs under the same treatments and recorded the seed development. We observed that nutrients affected growth of most fungal OTUs, independently of the family, and that this effect was related with seed germination. In higher nutrients media only two OTUs could germinate seeds, the other 6 promoted seed germination only under low nutrient media. Our results suggest that nutrients affect fungi which in turn affect the establishment of the mycorrhizal association; a less number of the fungal OTUs germinate seeds under high nutrients, which could explain the mycorrhizal specialization under high nutrient availability.

Keywords: mycorrhizal specialization, soil nutrients, orchid mycorrhiza, symbiotic germination, Chilean orchid

HT (ID 68)

Ericoid plant species shape fungal communities in boreal forest soil and plant roots

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Boreal forest soils are limited by the available nutrients, and to improve the nutrient uptake, most plants form symbioses with mycorrhizal fungi ¹. In boreal forest, trees form mycorrhizal associations typically with ectomycorrhizal (ECM) fungi, and ericoid shrubs form symbioses with ericoid mycorrhizal fungi (ERM) ^{1,2}. However, the results of host specificity of ERM fungi are differing. Some studies indicate that regionality shapes the fungal community of ericoid roots with no host specificity ^{3,4} while others suggest that ERM fungi show host preference ^{5,6}.

We aimed to study the differences and similarities of the fungal communities of typical boreal forest plants *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Calluna vulgaris* and *Pinus sylvestris* growing on originally similar boreal forest soils in individual microcosms for 18-months. Further, with $^{13}\text{CO}_2$ -labeling and DNA stable isotope probing ($^{13}\text{C-DNA-SIP}$), we identified those root associated fungi, which interacted with the studied plants and obtained photosynthetic carbon.

Our results indicate that some root associated were host specific while others were inhabiting the roots of all the studied plants. Based on ¹³C-DNA-SIP, the majority of the detected fungi in the washed plant roots obtained photosynthates from their hosts. Additionally, some common root endophytes without confirmed mycorrhizal status obtained photosynthetic ¹³C.

Several fungal species co-occurred in the roots of all the studied plants. Thus, our results highlight that more knowledge of the co-occurrence and co-functioning of ERM, ECM and endophytic fungi, and the functions of potential mycorrhizal network between plants are still needed.

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Keywords: boreal forest soil, ¹³C-DNA-Stable isotope probing, Ectomycorrhiza, ericoid mycorrhiza, fungal community

³ Kjoller *et al.* 2010. Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. Fungal Ecology 3: 205–214.

⁴ Walker *et al.* 2011. Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New Phytologist 191: 515–527.

⁵ Bougoure *et al.* 2007. Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. Molecular ecology 16: 4624–36.

⁶ Ishida & Nordin. 2010. No evidence that nitrogen enrichment affect fungal communities of *Vaccinium* roots in two contrasting boreal forest types. Soil Biology and Biochemistry 42: 234–243.

HT (ID 241)

Mechanisms of generalist host range in the ectomycorrhizal symbiosis

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Introduction/Aim: Most ectomycorrhizal fungi can associate with many species of host plant, often simultaneously, but it remains unclear how these fungi maintain relationships with such diverse partners. We have presented a generalist ectomycorrhizal fungus, *Thelephora terrestris*, with six distantly related host plants. We are interested in two main questions: How does the fungal chemical strategy, particularly its use of effectors, change with each new host plant? And does its quality as a mutualist vary across hosts?

Materials and methods: We are using RNA sequencing and stable isotope labeling to answer these questions. We are currently enriching plants and fungi with carbon-13 and nitrogen-15 to track resource exchange, and harvesting plant, fungal, and symbiotic tissue for RNA sequencing. Transcriptomic analysis of infected roots will reveal fungal genes that are differentially expressed relative to soil hyphae and across different hosts.

Results: We hypothesize that fungal gene expression and symbiotic performance will be most similar on closely related hosts, and will become less similar with increasing phylogenetic distance among the plants. We anticipate that a core symbiotic gene set will be expressed across host environments, while putative effectors and signaling-associated genes will differ by host.

Discussion: Measuring gene expression in symbiotic tissues while tracking resource allocation will reveal genes associated with generalist host preference and improved symbiotic performance, providing a powerful link between gene expression, physiological function, and the ecology of these important mutualists.

Conclusion: This project will provide insight into how generalist host range works in ectomycorrhizal symbiosis.

Keywords: ectomycorrhiza, mutualism maintenance, stable isotope enrichment, transcriptomics, host generalism

HT (ID 301)

What do we know about mycorrhizae role in exotic conifers' invasion of Nothofagaceae forests in Patagonia, Argentina?

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Mycorrhizae associations are vital for Nothofagaceae forests and P. menziesii plantations' establishment and development. In Patagonia, P. menziesii is being planted close or within native forests, showing an aggressive invasion behaviour. We studied the mycorrhizal status of seedlings along Nothofagaceae + P. menziesii invasion matrices to investigate their role in this process. Our results present evidence that *P. menziesii* invasion of Nothofagaceae forests occurs with the co-invasion of their mycorrhizal partners. Also, soil environments located beyond invading conifers seedlings have shown to hold ectomycorrhizal inoculum capable to associate with incoming new plants. We have evidence that P. menziesii invasion could produce maladaptation of native ectomycorrhizal communities. Hebeloma mesophaeum, a Wilcoxina sp. (early-stage and common P. menziesii's ectomycorrhiza) and a Pyronemataceae sp. (widely associated with Nothofagaceae spp.) were found shared by P. menziesii and Nothofagaceae spp. Interestingly, Hebeloma hiemale and Wilcoxina sp., common partners for P. menziesii in Patagonia although not registered from Nothofagaceae forest, were found associated with N. antarctica, this is the first report for both fungal species. Pseudotsuga menziesii seedling seems to have the ability to form different AM colonization types (Paris-Arum-Both-Intermediate-type) depending on sites conditions. Significant high presence of Intermediate-type was found in seedling grown in invaded soils, where the colonization was less abundance. The presence of different mutual association gives P. menziesii a strong ability for seedlings establishment. The nursery mycorrhizal effect has been observed consistently in different environments, therefore, this fact should be considered in the design, site selection and invasion management of fast growing exotic plantations in Patagonia.

Keywords: ectomycorriza, arbuscular mycorrhizas, Patagonia, Nothofagus forest

HT (ID 61)

Unlocking the Door for Mycorrhizal Symbioses: Do Endo- and Ecto-mycorrhizal Fungi Use the Same Key?

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Arbuscular mycorrhizal (AM) fungi produce various signaling molecules, including lipochitooligosaccharides (LCOs) and short chitooligosaccharides. These fungal signals are perceived and transduced in host plants via a highly conserved "common symbiosis pathway" (CSP). Genomic data suggested that ectomycorrhizal (ECM) fungi, e.g. Laccaria bicolor, may be capable of producing LCOs and that some ECM host plants like Populus spp. contain all the components of the CSP. Based on this observation, we hypothesized that some ECM fungi may produce LCOs and that the CSP could play a role not only in AM but also in ECM associations. We found that hyphal exudates from several ECM fungi but not short chitooligosaccharides can trigger typical root hair branching in Vicia sativa suggesting the presence of non-sulfated LCOs in these ECM exudates. In contrast to sulfated LCOs and negative controls, non-sulfated LCOs triggered an increase in both lateral root formation in hybrid poplar (Populus x canescens) and the total number of root tips colonized by L. bicolor. We utilized RNA interference to silence the expression of core components of the CSP, including CCaMK. Increased lateral root formation induced by nonsulfated LCOs did not occur in the CCaMK-RNAi line. Furthermore, during colonization of poplar by L. bicolor, mantle width, Hartig net depth, and the expression of the phosphate transporter PtPH12 were significantly reduced in the CCaMK-RNAi line compared to a wild-type control. Altogether, our data suggest that LCOs are produced by some ECM fungi and that the CSP has been recruited for the establishment of ECM associations.

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Keywords: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, poplar, lipo-chito-oligo-saccharides, common symbiosis pathway

PLENARY SESSION: Belowground diversity and ecosystem functioning

Chair: Nancy C. Johnson

KL (ID 513)

Soil Biodiversity, Underground Networks and Ecosystem Functioning

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Soil biota represent the unseen majority of life on Earth. A handful of soil contains billions of bacterial cells, kilometers of fungal hyphae and a significant fraction of life's genetic diversity. Mycorrhizal fungi are an important component of the soil ecosystem, in terms of abundance, and due to their impact on plant growth, nutrient and carbon cycling. Here I will summarize recent findings related to the significance of mycorrhizal fungi and soil biodiversity for ecosystems. Results will be presented that show that soil life and soil biodiversity regulate ecosystem functioning, plant community dynamics and the stability of ecosystem services. I present examples of how underground microbial and mycorrhizal networks influence grassland and forest systems and present some first thoughts on the role that mycorrhizal fungi play in structuring and regulating the soil and plant root microbiome. Finally, I will highlight avenues for further research and identify some of the major challenges that I consider to be of significance for soil ecology and mycorrhizal ecology, including the use of soil biota to enhance ecosystem sustainability and sustainable food production.

IL (ID 277)

Contrasting ecology of ectomycorrhizal and ericoid mycorrhizal symbioses as drivers of the northern carbon sink

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In a global perspective, northern arctic and boreal biomes act as major belowground carbon sinks. However large variation in carbon stocks exists among northern ecosystems, and we hypothesize that this is largely related to the relative dominance of the three major fungal guilds – the saprotrophic, ectomycorrhizal and ericoid mycorrhizal fungi.

Communities of litter saprotrophic and mycorrhizal fungi are vertically stratified within boreal forest soils. In a recent study we transplanted organic substrates vertically in soil profiles in order to test the decomposition capacities of saprotrophic versus mycorrhizal fungal guilds. We observed that although both guilds colonized both litter and humus substrates, litter decomposed more rapidly when colonized by saprotrophic fungi, suggesting that ectomycorrhizal fungi can delay early stage litter decomposition.

In another study we transplanted organic substrates horizontally between a subarctic mountain birch forest and a nearby ericoid-dominated heath tundra to investigate the decomposition capacities of microbial communities at the two sites. Heath humus decomposed faster than forest humus substrates, irrespective of where they were incubated, suggesting that the large carbon sink in the heath was not driven by low quality of the organic matter. Furthermore, when tree roots and ectomycorrhizal fungi – but not ericoid roots and associated fungi – were excluded from the decomposing matter, incubated sample mass increased, suggesting sustained belowground input, but decreased decomposition rate. Our results support the idea that the presence and relative decomposition capacity of ectomycorrhizal fungi, rather than different input quantities or qualities, determine long-term carbon sink strength across northern ecosystems.

IL (ID 42)

Mycorrhizal fungi affect orchid distribution and population dynamics

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Introduction: The absence of appropriate mycorrhizal fungi can limit where plants can grow, but little is known about how the abundance and diversity of appropriate mycorrhizal fungi can affect plant growth and population dynamics. We hypothesized that the distribution, abundance, and emergence of orchids, which are particularly dependent on their fungi, would be driven at least partly by the abundance and diversity of their mycorrhizal fungi.

Methods: We used specific PCR primers, quantitative real-time PCR, and spatially nested soil samples in three independent studies to measure the distribution and abundance of mycorrhizal fungi that associated with five temperate, terrestrial, forest-dwelling orchid species.

Results: We found that the distribution and population dynamics of each orchid were affected by the distribution, abundance, and in some cases, genetic identity of their mycorrhizal fungi in the soil.

Discussion: Molecular techniques are allowing increased insight into below-ground diversity and its effects on above-ground diversity. Despite our findings with these forest orchids, other studies have found less relationship between orchid and fungus distribution, suggesting that this pattern may not be universal.

Conclusion: We conclude that attempts to understand the drivers of plant population dynamics need to also consider the diversity, distribution, and dynamics of the mycorrhizal fungi they require.

Keywords: orchid, *Tulasnella*, *Ceratobasidium*

IL (ID 268)

Does genotypic and species diversity of mycorrhizal plants and fungi matter for ecosystem functioning?

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Biodiversity encompasses many different levels including functional groups, species and genotypes that often have complex spatial distributions. Yet genotypic diversity and how it interacts with other levels of diversity is rarely considered from a functional perspective, especially in mycorrhizal systems. This lack of knowledge is surprising given the vast quantity of genomic data that continues to be generated from mycorrhizal systems, which has generated many possibilities to better understand how components of genetic diversity of mycorrhizal plants and fungi impact key ecosystem processes. Here, we outline some of the interacting processes that can affect ecosystem processes both directly and indirectly, and go on to show that genotypic diversity of mycorrhizal fungi affects several key ecophysiological processes and functions1. We highlight the need to link information on genomic variation of isolates to key functions of fungi when in symbiosis with host plants. Finally, we highlight key gaps in understanding of biodiversity-ecosystem function interactions from hyper-diverse plant communities such as tropical forests, where consideration of the spatial distribution of species is critical. These systems present additional experimental challenges but targeted sampling and advanced statistical analytical approaches offer promise to generate new insights into the functional diversity of mycorrhizal fungi in these systems.

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¹Hazard C, Kruitbos L, Davidson H, Taylor AFS, Johnson D (2016) Contrasting effects of intra- and interspecific identity and richness of ectomycorrhizal fungi on host plants, nutrient retention and multifunctionality. New Phytologist 213, 852-863. DOI 10.1111/nph.14184

Keywords: biodiversity, ectomycorrhiza, nutrient cycling, ecology, tropical forests

IL (ID 93)

Diverse *Sorghum bicolor* lines differ in their responsiveness to colonisation by different species of mycorrhizal fungi

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Sorghum bicolor (sorghum) is an agronomically important crop that forms associations with arbuscular mycorrhizal fungi (AMF) and can obtain phosphate (Pi) from the soil via the mycorrhizal pathway of uptake. We hypothesised that inoculation with AMF would improve growth and tissue mineral nutrition of sorghum grown in Pi-limited soil, and responsiveness in terms of growth and tissue nutrition would vary with sorghum genetic variation and AMF species diversity.

Eighteen diverse sorghum lines, the parent lines of a NAM (nested association mapping) population, were grown in low nutrient soil and inoculated with five different AMF treatments which included four different AMF species (*R. irregularis, G. versiforme, Gi. gigantea, G. candidum*), a mixed inoculation, and a mock-inoculation treatment. The experimental design was completely factorial. Measurements of plant growth, shoot nutrition and root phosphate transporter gene expression were taken.

The growth, nutrition, and gene expression of the sorghum plants were affected by the sorghum line x AMF species interaction. The diverse sorghum lines ranged widely in their capacity to benefit from the mycorrhizal symbiosis, both in terms of growth and tissue nutrition. Expression of AM-induced and constitutive phosphate transporter genes in sorghum roots was variable depending on the line and colonising AMF species.

There is potential for AMF to improve the growth and uptake of numerous mineral elements in sorghum, but the specific combination of sorghum line with AMF species was very important in determining the level of plant responsiveness, as growth and tissue nutrient content, to AMF colonisation.

Keywords: arbuscular mycorrhiza, phosphate, sorghum

IL (ID 78)

Suppression of the root external arbuscular mycorrhizal fungi (AMF) mycelium by the soil microbiota

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The activity of AMF may be suppressed in non-sterile soils¹ resembling the phenomenon of 'disease suppressive soils' ². Microbial groups and microbial metabolites responsible for suppression of other fungi have been identified ^{3,4}, while organisms and metabolites suppressive towards AMF are unknown. This study aims to assess whether suppression of the AMF by unsterile soil is common and if it can be ascribed to specific taxonomic groups of soil microorganisms.

Suppression of AMF was investigated in a *Medicago truncatula* model system with ³³P-labelled patches of soil where the patches could be accessed by hyphae but not roots. ³³P uptake into shoots was used as a proxy for AMF activity. A range of soils were compared. For selected soils, effects of liming and pasteurization were evaluated. Data of ³³P uptake and hyphal growth were compared to microbiome profiles assessed by PLFA markers and 16S-rRNA gene targeted sequencing.

We found a large variation in AMF suppression across soils and suppression was mitigated by soil pasteurization. Besides, suppression was greater at low than at high pH. PCA analysis of PLFA and of 16S RNA amplicon sequencing data revealed a clear separation between AMF suppressive and conducive soils. Acidobacteria subgroup 1 was more abundant in suppressive than in conducive soils and may constitute a potential AMF suppressor.

Ongoing work aims to identify and isolate AMF suppressive microorganisms and to identify the metabolites responsible for suppression. The perspective is to develop new field management strategies for improving the role of AMF in plant nutrition.

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Keywords: suppressive soils, arbuscular mycorrhizal fungi, microbial metabolites

IL (ID 52)

Mycorrhizas and plant-soil feedbacks in temperate forests

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Introduction: Mycorrhizas can have strong effects on plant populations and communities. One mechanism for these effects is plant-soil feedback (PSF): the net effect of accumulated soil organisms on growth and recruitment. Mycorrhizas promote positive PSF by increasing nutrient access and pathogen protection for the plant; however, ectomycorrhizas (EM) provide greater protection from pathogens than arbuscular mycorrhizas (AM). PSF may also be regulated by plant growth strategies and the environment; however, if EM plants are protected from pathogens, then the same relationships may not hold.

Methods: We collected seed and soil from 550 populations belonging to 55 North American temperate forest tree species (30 EM and 25 AM) to test for PSF. I then tested whether mycorrhizal type altered the relationship between PSF and plant strategies (using plant traits) or the local environment.

Results: AM trees experienced negative PSF, while EM trees experienced positive PSF, likely due to differences in pathogen protection. Plant strategies also helped explain PSF; however, the relationships differed between AM and EM trees. Fast growing AM species experiencing more negative PSF, especially in wetter environments; whereas EM species exhibited opposite or neutral relationships.

Conclusions: In temperate forests, mycorrhizal type is the primary determinant of the direction of PSF and influences how PSF relates to plant strategies and the local environment. Consequently, mycorrhizal types need to be considered to in any study of PSF. A more complete understanding of how traits and the environment shape mycorrhizal benefits may also be required to understand forest dynamics.

IL (ID 323)

Could Mycorrhiza research provide new insights for sustainability between economic and ecological systems?

Nikhil Joshi (Lifel, Mumbai, India)

Introduction/Aim: This study explores a broad, multilevel view of the organization of flow networks in living systems spanning ecological and socioeconomic systems¹. It compares organization of Mycorrhiza in soil-plant ecological flow networks²⁻⁶, gut-bacteria in plant-heterotroph ecological flow networks⁷⁻¹⁰, and financial investment networks in human resource flow networks in socioeconomic communities.¹¹⁻¹⁴

Materials and Methods: New definitions of classes of coupled-composite systems, decoupled-composite systems, alphabetic catalysts, and the concept of modulator system have been used to discern common patterns in the organization of flow networks across multiple levels¹.

Results & Discussion: Initial findings revealed interesting similarities in the organization of flow networks across three levels. This led to a hypothesis that organization of subsoil Mycorrhizal networks and gut bacterial networks in ecosystems, and financial investment networks in socioeconomic communities could form a common multilevel organizational pattern (CMOP) spanning across levels. Can these similarities be leveraged to explore new avenues for sustainability between economic and ecological systems? This is discussed in recent publications. ^{1,15}

Conclusion: This study is a part of a collaborative, interdisciplinary research effort to develop multilevel models of organization in living systems, with a broader aim to develop new scalable solutions to enable multisystem sustainability between economic and ecological systems. It is hoped that initial ideas presented here motivate researchers to look beyond their domains and collaboratively explore a "big-picture" view of organization in living systems. Such an endeavor will not only be a significant contribution to science, but also allow researchers to contribute towards addressing pressing problems in sustainability.

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Keywords: mycorrhiza, financial markets, sustainability

CONCURRENT SESSION: Integrating mycorrhizas into plant community ecology

Mycorrhizal fungi are ubiquitous in terrestrial ecosystems and have demonstrable effects on the outcomes of plant-plant interactions, on the effects of trophic interactions involving plants, and on the diversity and productivity of plant communities. These observations justify the inclusion of mycorrhizal fungi into theoretical frameworks of plant community ecology, but there has been limited progress on this front. One reason for this is that phenomenological observations of mycorrhizal effects are difficult to integrate into these frameworks without a mechanistic understanding of the processes underlying these observations. This session will focus on recent research into the roles that mycorrhizal fungi play in dynamical aspects of plant communities. An aim, in particular, in this session is to disseminate research and to facilitate a dialogue that can enhance the uptake of mycorrhizal research into the advancement of plant ecology in general.

Chairs: Jeff Powell, Jim Bever

CL (ID 85)

Arbuscular mycorrhizal fungi can influence plant community ecology by altering herbivore parasitism via multiple mechanisms

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Introduction/Aim: Arbuscular mycorrhizal (AM) fungi alter plant interactions and community ecology. AM fungi alter host plant quality for insect herbivores by altering plant nutrition and/or priming plants for enhanced inducible and constitutive plant defences. This produces two pathways by which AM fungi could influence herbivore-parasitoid interactions: First, plant quality effects on herbivores could cascade to higher trophic levels influencing natural enemy control of insect pests. Second, via priming, AM fungi could alter indirect defences by altering volatiles attracting parasitoids.

Materials and Methods: We addressed the relative importance of these two pathways by manipulating the presence of AM fungi, Solanum species and genotype, potato aphid genotype, and parasitism of potato aphids. We conducted two types of parasitism assays to assess the influence of both pathways.

Results: We found that AM fungi altered parasitism via both pathways: As suggested by the first pathway, AM fungi increased parasitism success of aphids when they were parasitized in a neutral environment, and the parasitoid received no cues from the host plant. However, as we would expect from the second pathway, parasitoid attraction to plants hosting AM fungi was much greater than attraction to plants that did not host AM fungi. However, in both cases, the influence of these pathways was dependent upon Solanum species and genotype.

Discussion/Conclusion: Thus AM fungi influenced parasitism via multiple mechanisms, but this influence depended upon host plant. Therefore AM fungi influence plant community ecology by altering plant trophic interactions regardless of individual host plant responses to AM fungi.

Keywords: plant defense, plant nutrition, indirect defense, parasitism, trophic interactions

CL (ID 84)

Small-scale spatial variability in the distribution of ectomycorrhizal fungi affects plant performance and fungal diversity

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The effects of spatial heterogeneity in negative biological interactions on individual performance and species diversity have been studied extensively. Notably, little is known about the respective effects involving positive biological interactions, including the symbiosis between plants and ectomycorrhizal (EM) fungi. Using a greenhouse bioassay, we explored how spatial heterogeneity of natural soil inoculum influences the performance of pine seedlings and composition of the EM fungi colonizing their roots. We illustrated that when the distribution of soil inoculum was clumped it allowed competitively inferior EM fungal taxa, which in this case were also inferior mutualist, to persist. This increased EM fungal community diversity, but resulted in reduced growth of individual plants. Our results highlight the significance of small scale spatial variation in the distribution of EM fungal inoculum for the establishment and growth of ectomycorrhizal host plants; they also suggest that the trade-off between competitive ability and mutualistic capacity of mutualists does not necessarily exist.

Keywords: ectomycorrhizal fungi, priority effects, spatial heterogeneity, Pinus halepensis

CL (ID 56)

Unearthing mechanisms behind growth rate in Norway spruce – role of diversity of ectomycorrhizal fungi

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Our studies focus on the relationship of ectomycorrhizal (ECM) fungi and genetically determined growth traits of Norway spruce (Picea abies). A long-term field experiment revealed that high ECM species richness was associated with fast-growing spruce families1. ECM fungal richness increased functional complementary of the nutrient acquisition potential of the host by diversifying the production of fungal derived exoenzymes². Carbon allocation to above- and below-ground parts varied also between fast and slow families, especially in response to increasing fungal richness³. In contrast to our hypothesis on the underlying mechanisms, genetic differences in susceptibility to ECM symbiosis formation in the host did not contribute to the variation in ECM fungal diversity. Instead, we found systematic, genetically controlled variation in root characteristics between the spruce families⁴. Still even-sized but in future fast-growing seedlings showed a higher number of root tips, more frequent branching of root orders as well as allocation of root tips further away from the base of the seedling compared to slow-growing seedlings⁵. This provides an advantage in nutrient limited, heterogeneous forest soil by enhancing exploration and contact with the soil microbiota. We conclude that over time, small differences in these specific allocation patterns for root growth create positive self-reinforcing mechanisms that may underlie the higher ECM fungal diversity and long-term growth performance of some spruce origins.

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⁵Hamberg et al. submitted.

CL (ID 79)

Arbuscular common mycorrhizal networks mediate plant intra- and interspecific interactions

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Introduction/Aim: Over the last century of ecological research, it has remained elusive when plant interactions will be competitive, neutral, or facilitative. This enigma may persist because most investigations have failed to consider the role of ubiquitous common mycorrhizal networks (CMNs). We examined how CMNs affect intra- and interspecific interactions within and between populations of *Andropogon gerardii*, a highly mycorrhiza-dependent, C4, dominant prairie grass and *Elymus canadensis*, a weakly dependent, C3, subordinate prairie species.

Materials and Methods: In microcosms, we grew *A. gerardii* and *E. canadensis* alone and together, with individual root systems isolated but either interconnected by CMNs or with CMNs severed weekly.

Results: CMNs improved survival of both *A. gerardii* and *E. canadensis*, but intensified intraspecific competition among *A. gerardii*. In mixture with *E. canadensis*, *A. gerardii* over-yielded in the presence of intact CMNs but not when CMNs were severed. CMNs improved manganese nutrition of both host species, with the largest plants receiving the most manganese. Enhanced growth in consequence of improved mineral nutrition led to large *E. canadensis* in intact CMNs being water-stressed as revealed by ¹³C isotope abundance.

Discussion/Conclusion: Our findings suggest that in prairie plant communities, CMNs may influence mineral nutrient distributions, water relations, within-species size hierarchies, and between-species interactions. If our results extend to crop plants, then CMNs may provide a novel explanation for over-yielding in polycultures.

Keywords: common mycorrhizal networks, arbuscular mycorrhizal fungi, competition, manganese, ¹³C

CL (ID 77)

Effects of mycorrhizae abundance, richness and phylogenetic diversity on plant facilitation

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Introduction/Aim: Plant facilitative interactions enhance co-occurrence between distant relatives, due to a limited overlap in resource requirements, or non-exclusively, to the enhancement of facilitation through their functional complementarity. We test whether mycorrhizae abundance, richness and phylogenetic diversity explain plant facilitation between distantly related species, and its effects on the structure of local communities within fragmented landscapes.

Materials and Methods: In field experiments, we tested whether nurse and facilitated plants exchange nutrients through fungal networks using stable isotopes. Then, we used molecular tools and network analyses to assess the effect of richness and phylogenetic patterns in plant-fungi interactions on the persistence of facilitative interactions and the species co-existence in local communities.

Results: Soil fungi enhance nitrogen transfer among plants, which increases between distantly related plants. Additionally, the persistence of facilitative interactions is enhanced when plant species harbor contrasting mycorrhizae that increase their phylogenetic diversity. Finally, the phylogenetic community structure of plants and mycorrhizae are inter-related across vegetation patches and their interaction networks lose robustness as communities' decrease in size.

Discussion: Nutrient transfer towards distant relatives results from evolutionary conservatism of nutrient content, establishing steeper source-sink gradients across distant relatives. Within patches, shifts in the phylogenetic composition of plants and AMF suggest an inter-related succession dynamic, and across communities, mycorrhizae symbionts increase their vulnerability to plant species loss in small-size communities.

Conclusion: Mycorrhizae symbiosis can drive plant facilitation, not only by enhancing plants nutrient-status but also plant communities' phylogenetic diversity. Not only mycorrhizae abundance but its richness and phylogenetic diversity can influence plant-plant interactions.

Keywords: plant facilitative interactions, plant-mycorrhizal networks, nutrient transfer, community ecology, phylogenetic community ecology

CL (ID 65)

Mycorrhizal allocation determines their function across varying environmental contexts

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Arbuscular mycorrhizal (AM) fungi are obligate symbionts to plants. Resource limitation may favor stronger mutualisms, where resource abundance may result in diminished mutualism or even parasitism. Co-adapted plants and mycorrhizal fungi have been documented to have greater mutualistic function. The mechanisms for these differences are not well understood, though it has been proposed that fungal allocation to different structures may have a role. Within plant roots, these fungi establish sites for nutrient exchange (arbuscules) and storage (vesicles). It is likely that greater investment in storage reduces their ability to gather and/or exchange nutrients and thus makes them less mutualistic and vice versa. We conducted a greenhouse study with *Boteloua gracilis* to test mycorrhizal allocation and how it varies based on environmental context. We manipulated both plant and AM fungi origin as well as created two levels of simulated drought. We also conducted a field transplant experiment to test our findings in more dynamic environments. In the greenhouse, we found that plants consistently grew larger and survived drought longer when they were paired with sympatric AM fungi. Additionally, after 2 years in the field, sympatric plant-AM fungi pairings yield the greatest plant growth regardless of environment. In sympatric pairings, there was also more external AM fungal hyphae and a greater percentage of plant roots were colonized by hyphae and arbuscules. In allopatric pairings, plant roots were more heavily colonized by vesicles. These findings demonstrate potential mechanisms for how AM fungi can behave as either mutualists or parasites depending on environmental context.

Keywords: mycorrhizal allocation, drought, mycorrhizal function, plant ecology, co-adaptation

CL (ID 274)

Linking AM fungi and pollinator interactions in agro-ecosystems: An ecological network approach

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Ecological networks are being increasingly used to study mycorrhizal fungi, as in other systems they have previously described interactions between species, the underlying structure of communities, and the function and stability of ecosystems. However, we do not know whether AMF-plant networks influence aboveground networks (such as plant-pollinator networks), or if factors such as interaction duration and type have functional consequences for yields.

Over two years, we explored the influence of AMF and plant cultivar on pollinator visitation rates, foraging activities, network structure, and yield using three cultivars of strawberry (*Fragaria* × *ananassa*), and four AMF communities. We measured pollinator visitation / community composition, and collected strawberries to determine yield quantity and quality.

Altering the AMF community influenced the number of flowers produced, the frequency and duration of visits by *Bombus terrestris* Audax, as well as the frequency and duration of pollen and nectar foraging visits. Furthermore, the wider plant-pollinator network was influenced by AMF community, with plant genotype mediating these influences. AMF influenced yield quantity, but not sugar content, or human perceived taste quality.

These results indicate that AMF can not only shape the overall plant-pollinator network, but also influence the nature of the interactions that occur, which can determine crop yields. With declines in pollinator communities and increasing pressures on crop production, research into this field may form part of an important toolset for sustainable increases in food security, as well as helping us to gain a deeper understanding of the fundamental biology that influences ecological networks.

Keywords: pollinators, ecological networks, above-belowground interactions, food security, strawberry

CL (ID 43)

A legume erases mycorrhizal negative effects on an endophytic grass increasing N transference

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Plant coexistence can be affected by the interaction of multiple plant symbionts. The outcome of co-infection with arbuscular mycorrhizal fungi (AMF) and foliar endophytic fungi (FEF) in grasses is variable and may range from antagonism to synergism. Here we tested whether a nitrogen-fixing legume affects grass response to the presence of both types of symbionts by enhancing N nutrition.

The grass (Lolium multiflorum), either associated or not with FEF (Epichloë occultans), grew in monocultures or in mixtures with legumes (Trifolium repens inoculated with rhizobia) in pots with sterilized soil. In half of the pots, we added AMF inoculum (Simiglomus hoi, Funneliformis mosseae, Rhizophagus irregularis). We measured plant biomass and the transference of atmospheric-N fixed from the legume to the grass.

Legume biomass and the amount of N fixed were affected neither by AMF inoculation nor by FEF presence in the neighboring grass. In mixtures, AMF inoculation increased more than three times the amount of N-fixed transferred to both the FEF-associated and the FEF-free grass, but increased 26% the biomass of only the FEF-free grass. In monocultures, it reduced 18% grass biomass irrespective of FEF presence.

The presence of nitrogen-fixing plant erases the parasitic effect of AMF on a grass by enhancing the transference of fixed N, but this emergent property was not enough to be positive for FEF-associated grass plants. In conclusion, biotic context can influence the outcome of the co-infection with two types of symbionts and thus the level of antagonism between AMF and FEF within the grass.

Keywords: grass-legumes systems, nitrogen dynamics, mycorrhizal networks, multisymbiosis

ST+P (ID 114)

Mycorrhizal fungi exacerbate plant responses to experimental climate change in a semiarid shrubland

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Warmer and drier conditions associated with ongoing climate change will increase abiotic stress for plants and mycorrhizal fungi in Mediterranean drylands, thus reducing vegetation cover and productivity and increasing the risk of land degradation and desertification.

We conducted a four-year manipulative study in a semiarid shrubland to assess the effects of warming (~2.5°C; W), rainfall reduction (~30%; RR) and their combination (W+RR) on the performance of native shrubs (*Helianthemum squamatum*) and their associated mycorrhizal fungi.

Warming, RR and W+RR drastically reduced the relative abundance of ectomycorrhizal fungi (EMF), a response associated to decreases in leaf N and P contents under these treatments. Warming decreased photosynthetic rates by one third despite concurrent increases in stomatal conductance, leading to sharp decreases (~50%) in water use efficiency in W and W+RR plants. Warming (W and W+RR) reduced shoot biomass production by ~36% and decreased survival in a dry year. Plants under RR showed moderate decreases in photosynthesis, stomatal conductance and shoot growth.

The interdependent and mutually exacerbating responses of plants and EMF to warming and rainfall reduction may lead to a detrimental feedback loop on vegetation productivity, which could amplify the adverse impacts of climate change on ecosystem functioning in EMF-dominated drylands.

ST+P (ID 82)

Dispersal and primary succession of plant and arbuscular mycorrhizal communities

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Studies of natural succession of communities have largely focused on plants, while the dynamics of their symbiotic partner, arbuscular mycorrhizal fungi (AMF), is largely unknown. One of the impediments is the lack of primary successional scenarios, i.e. newly formed terrestrial lands without AMF propagules, where the sequential change of species over time can be traced. Here, we surveyed the succession of plant and associated AMF communities on the artificial island Peberholm, constructed in 1998 from sea floor sediments.

During last six years, roots of plant communities on the island of Peberholm were sampled (2010–2016). To allow a comparison with an older scenario, the neighboring island Saltholm has also been sampled using the same design. Plant and AMF communities were characterized using illumina MiSeq sequencing technology. Data on soil properties and spatial positions of samples were also collected. A parallel survey of AMF spore communities was carried out to determine morphological and habitat preference traits of the most abundant AMF species.

First, we traced the change of composition and rank abundance distribution of species along time. Second, we analyzed the contribution of biotic (plant community), abiotic (soil properties) and spatial factors to AMF distribution to detect the spatial signature produced by dispersal process. Third, we applied a trait-based approach to identify traits responsible of causing dispersal limitation on AMF communities.

Our findings will improve the understanding of how plant and AMF communities assemble during primary succession and the possible effect that dispersal limitation of one group can have on the other.

Keywords: community assembly, spore traits, species abundance distribution, dispersal limitation

ST+P (ID 166)

Linking Ericaceae with conifers: DNA-based prospects and in vitro hopes vs. the in natura reality

Martin Vohník (Institute of Botany CAS/Charles University, Průhonice/Prague, Czech Republic)

Ericaceae shrubs often form understory in boreal and temperate coniferous forests and many authors hypothesized that both plant guilds could be linked through common mycorrhizal networks (CMNs). This idea has become very popular over past ca. 20 years and anticipates the existence of dual root mycobionts which are able to simultaneously form ericoid mycorrhizae and ectomycorrhizae. To meet the criteria of the Koch's postulates, such CMNs have to be firstly observed under natural conditions, then the respective mycobionts have to be isolated into pure culture from both host types and subsequently, these have to form CMNs between both host types under controlled conditions. In reality, however, these criteria have never been met and our current knowledge is limited to the observations that 1) DNA of identical fungi is present in the roots of neighboring ericads and conifers and 2) some mycobionts isolated from conifer ectomycorrhizae may form simultaneously ericoid mycorrhiza and ectomycorrhiza under in vitro conditions. In contrast, a growing number of reports suggest that under natural conditions, the potential to form CMNs between Ericaceae and ectomycorrhizal conifers is low and may be even inhibited when members of both plant guilds co-occur together. I will review the existing literature on this topic, summarize the arguments in favor of and against the existence of such mycorrhizal links and present results of a microcosm experiment which challenge our current views on the identity and functioning of the potentially shared mycobionts.

Keywords: ericoid mycorrhiza, ectomycorrhiza, Ericaceae, *Rhizoscyphus ericaceae* aggregate, common mycorrhizal networks

ST+P (ID 35)

Changes on soil and eucalypt ectomycorrhizal communities across a climatic gradient in south-east Australia

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Mycorrhizal communities and their responses to climatic variability are critical unknowns for the majority of natural ecosystems in Australia. How they respond to climate may have significant implications on their plant hosts under climate change. Aim of this study is to explore the changes in soil and eucalypt ectomycorrhizal richness across an elevation and climate gradient in south-east Australia.

Ectomycorrhizal (ECM) tips from fine roots were collected from each eucalypt species present at twelve plots located along an elevation gradient ranging from 200 to 1600 m.a.s.l. Soil samples beneath each sampled tree were collected and pooled for each plot. ECM colonization and species identity were determined by morphological and molecular characterization of mycorrhizal tips. Soil ECM species were identified using Next Generation Sequencing. Soil chemical and physical properties were also determined. Multivariate ordination techniques were used to explore changes in ECM communities in relation to climate, host species and soil properties.

ECM richness on soil and eucalypts changed along the climatic gradient with lower fungal species richness at higher elevations. ECM richness in many eucalypt species increased along the elevation gradient. Similarity of ECM communities is higher at lower elevations compared to mid and higher elevations. Host and mycobiont specificity of the associations and changes in composition and structure of ECM communities will be assessed across the studied soil and climatic gradient.

Changes in ECM richness along the elevation gradient suggest that climate change may have direct impacts on ECM communities which may have implications on their host plants.

Keywords: ectomycorrhizal communities, soil, eucalypts, climatic gradient, south-east Australia

ST+P (ID 278)

Relationships between fungal soil diversity, ecosystem services and forest management in boreal forests

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The roles of fungal diversity in the stability, resilience and function of forest ecosystems are poorly understood. Fungi have pivotal ecological roles at the same time as forest management affects the occurrence and abundance of species. This may subsequently impact fungal ecosystem processes with consequences for tree biomass growth and other ecosystem services.

The aim is to establish relationships between forest management practices and fungal diversity in Swedish boreal forests and to estimate the role of fungal diversity in the provision of ecosystem services.

To do so, we coordinate fungal soil sampling with the survey of permanent forest plots by the Swedish Forest Soil Inventory (SFSI) and the Swedish National Forest Survey (SNFS). Thus, we have the unique opportunity of combining data of soil and forest properties with the fungal community on a national scale and to establish relationships between fungal diversity and environment, stand and soil variables. We used PacBio sequencing and analyzed the identified fungal community together with the data collected by SFSI and SNFS. We will present Preliminary results will be based on 463 stands collected 2014-15; collecting continues with 250 stands annually.

The results picture common soil fungi in Sweden with *Piloderma sphaerosporum* as the most frequent ectomycorrhizal species, occurring in 56% of the stands. We will present preliminary result where we test the hypothesis that forest increment partly can be explained by the composition of ectomycorrhizal fungi accounting for e.g. stand location, forest age, soil fertility and tree species mixture.

ST+P (ID 270)

Ancient land plants and their open relationships with fungi

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Views on the origins of mycorrhizas are changing. The consensus that arbuscular mycorrhizal fungi (Glomeromycotina) were solely responsible for forming the first fungal symbiosis with plants no longer exists. Glomeromycotina symbiosis may have been preceded by symbiosis with Mucoromycotina fungi and early land plants likely utilised a variety of symbiotic options to colonise land. The paucity of early land plant fossils requires the use of alternative investigative approaches to studying the evolution of fungal symbioses in plants and how these facilitated plant terrestrialisation. Non-vascular plants are the closest living relatives of the first land plants and offer us a unique window into early land plant evolution. Focusing on liverworts (Marchantiophyta), we investigated the diversity of fungal symbionts in over 700 globally-collected members of this widespread and ancient plant lineage. We have previously shown that some non-vascular plants can enter into mutualisms with both Glomeromycotina and Mucoromycotina, sometimes simultaneously. We now present evidence that colonisation by both Glomeromycotina and/or Mucoromycotina, of much greater diversity than previously known, is the norm in earlydiverging liverworts. Arbuscular mycorrhizal fungi from all four Glomeromycotina orders were found to colonise liverworts. Network analyses and species delimitation methods have been employed and new Glomeromycotina and Mucoromycotina virtual taxa and interactions with plants have been discovered. This fungal diversity combined with the common occurrence of dual colonisations in the earliest diverging liverworts support the notion that the symbiotic options available to the first land plants were more varied than previously assumed.

Keywords: Mucoromycotina, Glomeromycotina, liverworts, plant terrestrialisation

ST+P (ID 183)

Soil fungal assemblages in Chilean temperate rainforests: geological history, forest mycorrhizal dominance, and altitude effects on taxonomical, functional, and phylogenetic diversity

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Chilean temperate rainforests, located in two mountain systems (Andes and Coast) with contrasting geological histories, feature three vegetation types: Nothofagus spp. forests (dominated by ectomycorrhizal (EM) trees), Valdivian and coniferous forests (dominated by arbuscular mycorrhizal (AM) trees). By soil resource partitioning/competition, the dominant trees mycorrhizal type, or 'mycorrhizal dominance', likely affects other nonmycorrhizal fungal guilds. This study aimed to test the effects of mountain system, mycorrhizal dominance, edaphic conditions, and altitude on soil fungal taxonomic, functional, and phylogenetic diversity. Here we describe soil fungal communities of temperate rainforests using ITS2 Illumina sequencing. There was a significant effect of mountain system on the community composition of all, saprotrophic, EM, and AM fungi. In addition to affecting the community composition of all, saprotrophic, and EM fungi, mycorrhizal dominance affected EM and AM phylogenetic diversity. Redox potential, C, N, plant available P, Ca, K and Mg were the edaphic variables that significantly affected all fungi community composition. The composition of saprotrophs and AM fungi were affected by similar edaphic variables while EM composition was affected by pH and K. Only AM richness was lower at higher altitudes. Saprotroph and EM abundances were negatively related. Overall, mycorrhizal dominance significantly affected non-mycorrhizal soil fungal guilds.

Keywords: altitude, functional diversity, geological history, mycorrhizal dominance, phylogenetic diversity

ST+P (ID 251)

Is plant community mycorrhization decreasing along a gradient of anthropogenic pressure?

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Anthropogenic influence has long been recognized as the main cause of biodiversity loss, thus contributing to ecosystem degradation. At the same time, mycorrhizal symbiosis plays a crucial role in multiple ecosystem services and often favours plant diversity. Although it is known that some human impact can decrease mycorrhization at plant individual level, it has not yet been tested how increasing anthropogenic influence translates into changes in plant community mycorrhization at larger scales. In this study, we ask how the anthropogenic impact changes plant community mycorrhization at a regional scale. We expect community mycorrhization to decrease with increasing human influence, potentially leading to the sensitivity of ecosystems to plant invasions or changes in nutrient dynamics.

Accumulating plant mycorrhizal trait data (mycorrhizal status and type) makes it possible to start estimating the contribution of the symbiosis at larger scales. For this, we quantified the importance of mycorrhizal symbiosis in 170 (semi)terrestrial habitat types in the Netherlands, using the Dutch National Vegetation database¹ - the largest regional vegetation database available. The prevalence of mycorrhizal symbiosis in these habitats was quantified by plant community mycorrhization index² using plant mycorrhizal status data. In this talk, the changes in plant community mycorrhization with respect to anthropogenic influence, and potential processes behind these patterns are discussed.

References:

¹Schaminée, J.H.J., Hennekens, S.M. & Ozinga, W.A. (2012) The Dutch National Vegetation Database. Biodiversity & Ecology, 4, 201–209

² Moora, M. (2014) Mycorrhizal traits and plant communities: perspectives for integration Journal of Vegetation Science, 25, 1126–1132

Keywords: anthropogenic impact, plant community mycorrhization, plant mycorrhizal traits, plant functional traits, mycorrhizal status

ST+P (ID 244)

Ectomycorrhizal Fungal Communities in a Changing Boreal Forest

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Introduction: There is a well-documented phenomenon of alternative successional pathways in the boreal forest of Interior Alaska following climate change induced increases in fire severity^[1,3]. However, the impacts of a changing fire regime and concomitant shifts in vegetation in the boreal forest of Interior Alaska on ectomycorrhizal fungal (EMF) composition and potential below-aboveground feedbacks remains unknown. In this study we investigate how fire severity and host tree species influence EMF composition, and how this may affect further ecosystem change through influences on hosts.

Materials and Methods: 2004 was the largest fire year on record in Alaska, with over 5 million hectares burned. In 2005, replicates of four species of host trees were planted into these burns. In 2011 and 2013, seedlings and roots were harvested, aboveground biomass was measured and roots were sequenced to determine EMF composition.

Results: There was a significant interactive effect of burn severity and host tree identity on the composition of EMF. Amongst the tree species planted - aspen, black spruce, white spruce, and lodgepole pine, aspen EMF communities were the most unique.

Discussion: As EMF community composition shifts with changes in the fire regime and increases in cover by aspen, there is potential to greatly alter forest function through species specific differences in fungal contributions to processes such as nutrient cycling, carbon storage, and benefits conferred to host trees^[2].

Conclusion: Concurrent changes in EMF composition with shifts in aboveground tree dominance have the potential to affect boreal forest function and responses to climate change.

References:

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- 2. Koide, R. (2014). Determining place and process: Functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, 201(2), 433-439. doi:10.1111/nph.12538
- 3. Mann, D. (2012). Is Alaska's boreal forest now crossing a major ecological threshold? *Arctic, Antarctic, and Alpine Research*, 44(3), 319-331. doi:10.1657/1938-4246-44.3.319

Keywords: fire, ectomycorrhiza, boreal, forest, Alaska

ST+P (ID 279)

Determining plant mycorrhizal types for large datasets: Comparing the phylogenetic vs the empirical approach

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Our knowledge about plants and their symbioses with fungal partners forming mycorrhiza is quickly increasing, leading to new opportunities to answer general and global questions about the ecology of mycorrhiza. However, the determination of plant mycorrhizal traits (i.e. mycorrhizal type) is time demanding, being a limiting factor for studying the mycorrhizal symbiosis for complete floras and plant communities at larger scales. In these cases, an alternative determination of plant mycorrhizal types has been commonly used based on phylogenetic expert-based assumptions¹. This approach firstly extrapolates species mycorrhizal information to higher taxonomical levels, such as plant genus, family, order, etc., and then interpolates that information to other species within the same taxonomic level previously used. Despite of its need and use, we lack any comparative study to assess the strength and weakness of this common approach in relation to the use of the most updated collation of empirical research data from each species (species based information approach²). In this study, we compare the plant species empirical approach2 with the phylogenetic approach1 to determine plant mycorrhizal types for the plant species of the European Flora. We compared the trait data obtained with the two approaches and assessed their differences in the distribution patterns of mycorrhizal types in Europe. In this talk, I will present the comparison of both approaches and discuss the best strategies to overcome the lack of information depending on the scale and the question of the study.

References:

Keywords: plant mycorrhizal traits, arbuscular mycorrhiza (AM), ectomycorrhizal (ECM), ericoid mycorrhiza (ERM), non-mycorrhizal plant species (NM)

¹ Brundrett, M.C. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil (2009) 320: 37. doi:10.1007/s11104-008-9877-9

² Bueno CG, Moora M, Gerz M, et al. Plant mycorrhizal status, but not type, shifts with latitude and elevation in Europe. Global Ecol Biogeogr. (2017) In press. https://doi.org/10.1111/geb.12582

ST+P (ID 257)

Tree genotype modulates nutrient cycling through its root-associated microbiota in Mediterranean pine forests

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Plant genetic variation determines the diversity and composition of belowground microbial communities. Thus, the genotype of the host plant may leave a phylogenetic fingerprint in its root-associated microbiota that could finally determine the rates of microbial-driven ecosystem functions.

To test this hypothesis, we sequenced molecular markers and reconstructed the phylogeny of symbiotic ectomycorrhizal (ECM) fungi and rhizospheric bacteria associated with different genotypes of *Pinus pinaster* Ait. trees planted in three 45-year-old common garden experiments. Different enzymatic activities related to carbon turnover and the mobilization of nutrients were used to evaluate the ecosystem functioning.

The tree genotype determined the plant productivity and shaped the phylogenetic structure of its root-associated microbial communities regardless of the planting site. The phylogenetic community structure of ECM fungi and bacteria further predicted the rates of nutrient cycling. Fungal phylogenetic assemblages dominated by Basidiomycetes that co-existed with evolutionarily distant fungi, showed increased cellulolytic activity, while those with an overrepresentation of Ascomycetes co-occurring with closer relatives, increased peptidase activity. Bacterial phylogenetic assemblages dominated by Proteobacteria that showed low phylogenetic distances to their neighbours, showed an increased enzymatic hemicellulose-degrading activity and recalcitrant nitrogen compounds. The fact that fungi and bacteria explained the activity of different enzymes suggests functional complementarity.

Different plant genotypes shape the phylogenetic structure of their belowground ECM fungi and bacteria with crucial ecological consequences at the community and ecosystem levels.

POSTERS

P (ID 7)

AMF association contributes to Cr accumulation and tolerance in plants growing on Cr polluted soils

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Arbuscular mycorrhizal fungi (AMF) are found in mutualistic association with plants in heavy metal (HM) polluted soils.¹ This study has been undertaken to elucidate the AMF status and its contribution to the host plants growing in chromium (Cr) sludge deposit.

Various mycorrhizal parameters and Cr accumulation in the roots has been assessed and correlated through principle component analysis. The structural adaptions found in AMF were also studied.

Cr sludge deposit was dominated by *Cynodon dactylon*, *Parthenium hysterophorus*, *Croton bonplandianum* and *Prosopis juliflora*. High mycorrhizal colonization and low spore population was recorded. A total of seven types of AMF were isolated. *Funneliformis mosseae*, *Rhizophagus intraradices*, *Funneliformis geosporus* and *Glomus sinuosum* were recovered from two hosts, while, *Rhizophagus fasciculatus*, *Glomus aggregatum* and *Acaulospora scrobiculata* from single host. Diversity of AMF in HM polluted soils is low due the selection pressure of HM². Degree of mycorrhizal colonization and the abundance of arbuscules and hyphal coils were in strong influence of Cr accumulation in the roots. AMF of the study site were consisting of adaptive features like compact sporocarp, very thick and dark colored spore wall, sloughing outermost layer and formation of water stable aggregates. Native AMF provide protection to the host plants in HM polluted soil by various means¹.

Results of correlation analysis between various parameters and the presence of structural adaptive features in AMF clearly indicated, that these AMF have contributed to the Cr accumulation and tolerance to the host plants.

References:

- 1. Khade SW, Adholeya A (2009) Arbuscular mycorrhizal association in plants growing on metal-contaminated and non-contaminated soils adjoining Kanpur tanneries, Uttar Pradesh, India. Water Air Soil Pollut 202:45-56
- 2. Yang Y, Song Y, Henrik V et al. (2015) Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil Biol Biochem 86:146-158

Keywords: arbuscular mycorrhizal fungi, chromium accumulation, heavy metal tolerance, structural adaptation, Technosols

P (ID 34)

Ectomycorrhizal fungal community hosted by Caryophyllales in a hyperdiverse Neotropical dry forest

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The ectomycorrhizal (ECM) fungal studies commonly carried out in temperate forests have currently been extended to tropical habitats; with the result of an ample discovery of new symbioses and fungal species. We have been exploring a tropical dry forest (TDF) which is an endangered terrestrial ecosystem. Since Fabaceae is the main plant family in this environment, we tested whether leguminous hosts were the main ECM hosts.

We sampled 98 soil cores in Chamela, Mexico. To recognize the ECM fungal-plant symbionts we sequenced the fungal ITS rDNA region and the plant *rbcL* and *trnL* cpDNA regions.

This study identified 21 plant species belonging to 10 families that hosted 20 previously unknown ECM fungal species from 6 lineages. The majority of ECM fungi were associated with Caryophyllales, nor with Fabaceae. *Achatocarpus gracilis* and *Guapira petenensis* were the main ECM hosts. *Achatocarpus, Coccoloba, Guapira, Pisonia* and *Ruprechtia,* demonstrate that the ECM symbiosis evolved at least in 4 independent clades within Caryophyllales.

All the ECM fungi found are new species. We had already described *Tomentella brunne-oincrustata*, *Thelephora versatilis*, *T. pseudoversatilis*, and new *Clavulina* species are still in revision. The unique and small ECM fungal diversity in this TDF could be explained because the scattered distribution of a high diversity of non-abundant ECM hosts. Our results emphasize the importance of exploring the ecology and evolution of plants and fungi in tropical habitats.

Keywords: tropical dry forest, Caryophyllales, ectomycorrhiza, *Achatocarpus*, new fungal species

P (ID 38)

Is Above- or Below- Ground Interaction is important: A case study of an Orchid *Malaxis* acuminata D. Don

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Malaxis acuminata D. Don (syn. Crepidium acuminatum (D.Don) Szlach.) is a threatened and medicinally important species belonging to family Orchidaceae. Majority of orchids rely on symbiotic relationships with pollinators for sexual reproduction (above ground interaction) and mycorrhizal fungi for seed germination (below ground interaction) to complete their life cycle. To study the above ground interactions: floral morphology, phenology, biochemistry and pollination aspects were carried out. For below ground interaction: roots were analysed to assess the extent of mycorrhizal invasion using ultrastructural studies. Molecular characterization was also done to identify associated fungi using ITS1F/ITS4 and ITS1/ITS4tul primers from root samples. ITS sequences were analyzed with BLAST against the NCBI sequence database to know the closest sequence matches in the GenBank database. Samples were identified to be belonging to three main genera i.e., Aspergillus, Penicillium and Tulasnella. The results obtained make it a comprehensive study that above ground interaction doesn't lead to a reproductive success but below ground interaction helps seeds to germinate and to get nutrition. Since, Orchids possess non-endospermous embryo which lacks the food reservoir. Hence, orchid roots harbour a large diversity of fungal species to obtain carbon and other mineral nutrients to complete their life cycle. Thus, the identification of associated mycorrhizal fungi will help us to conserve this plant as well as to maintain the ecological balance.

Keywords: Malaxis acuminata, orchidaceae, mycorrhiza, diversity, conservation

P (ID 58)

Sequestration and detoxification of metal ions by metallothioneins in ectomycorrhizal fungus *Suillus himalayensis*

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Metallothioneins (MTs) are small, cysteine-rich peptides involved in intracellular sequestration of heavy metals. We examined the role of two MTs (*ShMT1* and *ShMT2*) in metal homeostasis and detoxification from the ectomycorrhizal fungus *Suillus himalayensis*.

Two MT genes *ShMT1* and *ShMT2* were cloned and characterized from *S. himalayensis*. The response of these MTs to elevated copper and cadmium stress was studied by qPCR analysis. Heterologous complementation of *ShMTs* were performed in metal-sensitive yeast mutants to confer the metal tolerance.

Sequence analysis revealed that *ShMT1* cDNA contains 105 bp ORF coding for 34 amino acids with a predicted molecular mass of 3.4 kDa and isoelectric point at 5.88 with 7 cysteine residues forming 3 C-x-C motifs. *ShMT2* cDNA contains 108 bp ORF coding for 35 amino acids with molecular mass of 3.5 kDa and isoelectric point at 4.14 with 6 cysteine residues forming two C-x-C motifs. The sequence features of *ShMTs* showed homologous to region-specific traits of known short MTs from basidiomycetes. qPCR analysis showed that the expression levels of both *ShMT1* and *ShMT2* increased as a function of increased external Cu and Cd concentrations. *Suillus himalayensis* also showed high tolerance to Cu and Cd and able to accumulate when grown in presence of these metals. Heterologous complementation assays in metal-sensitive yeast mutants indicated that both *ShMT1* and *ShMT2* encode peptides capable of conferring higher tolerance to both Cu and Cd. Present study results indicated that *S. himalayansis* encodes two MTs involved in the detoxification of Cu and Cd.

P (ID 168)

Assessment of Indigenous Arbuscular Mycorrhizal Fungal Colonization Status in Selected Plants in Kuwait Desert

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An investigation of arbuscular mycorrhizal fungal community associated with the roots of selected plants from different vegetation types in Kuwait desert areas has been conducted in this pioneer study. In desert environments, mycorrhizal associations are obvious and a critical factor for the survival of plants and play a fundamental role in ecosystem dynamics and productivity. Nevertheless, a little effort has been offered in Kuwait to investigate AM fungal community structure with native plant species and its identification. The main aim of the study was to investigate the mycorrhizal community associated with representative plant root samples that were collected from different species from Kuwait desert and to obtain a baseline data on AM fungal community structure.

Root samples were analyzed for a suite of characteristics including 16S rDNA and ITS sequence identifications, and PCR based cloning and sequencing technique for the identification of AM fungal community. The presence of and the rate of AM fungal colonization data revealed that samples collected during winter had relatively higher colonization rates when compared to summer samples. A total of 172 clones were sequenced. However, only 148 clones could be identified in the Glomeromycota group. Other clones mostly belong to Ascomycota with fewer belonging to Basidiomycota and Viridiplantae. Phylogenetic relationships were developed and showed the evolutionary relationship of arbuscular fungal taxa. Most of the identified AM belongs to *Glomus, Septoglomus, Rhizophagus, Funneliformis, Claroideoglomus* and a large number of uncultured genus. Our research is currently focused on AM community study using more advanced next generation sequencing tools to identify up to the species level.

Keywords: native vegetation, arbuscular mycorrhizal fungi, molecular identification, root samples

P (ID 193)

Contribution of mycorrhizas to plant community ecology - a commercial perspective

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Introduction: Commercial success of any product is the ultimate yardstick of its usefulness. Mycorrhizas are multi utility, multidimensional, versatile and nearest to nature. How do these score on commercial scale for the manufacturer, trader, farmer, plant community and ecosystem management?

Methodology: This paper is entrepreneur's perspective and not that of a scientist. It is summing up of observations and analysis of 7 years data of actual experience by manufacturer on challenges in production of Mycorrhizas, its storage, sale, farmers' views, effects on environment and ecology.

Results: It is possible and feasible to overcome the challenges of obtaining pure culture, do contamination free bulk production, make different Concentrations and Formulations to suit the needs of terrain, crops and farmers. It is financially viable for manufacturer, profitable for traders, economical to farmer and immensely beneficial to plant ecosystem due to communication through Mycelium for nutrition and plant immunity.

Discussion: Mycorrhiza (Frank 1885) has taken more than 100 years to become commercial. Culture based Technology followed by Sai Bio Organics has preserved purity of VAM and also grown it in aexinic culture for contamination free spores suspension in bulk inoculants. Our in house developed special Recipe of additives to culture has improved the end results in actual field conditions. This has made our product surpass all other technologies to contribute immensely to plant community ecology through "WOOD WIDE WEB" as per journal Nature.

Conclusion: Mycorrhizas produced with quality consciousness are a commercial and ecological success story.

Keywords: mycorrhizas, plant community, ecology, commercial, aspect

P (ID 205)

¹³⁷Cs transition between fungi and plants in a pine forest within a year

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The Earth's biosphere has been significantly contaminated with 137 Cs as a result of nuclear weapons testing and accidents at nuclear power plants. The accident at the Chernobyl NPP stimulated studies on accumulation of 137 Cs by mycorrhizal fungi and their role in transferring 137 Cs to symbiotic plants.

The aim of this investigation was to study the impact of seasonal fluctuations in the content of ¹³⁷Cs in fungi on its accumulation by symbiotic plants.

The research was carried out on the territory of the exclusion zone of the ChNPP (Ukraine). Objects of research were fruiting bodies of *Cantharellus cibarius*, as well as 1-year-old needles and branches of *Pinus silvestris*. Content of ¹³⁷Cs was measured by gamma spectrometry.

Maximum levels of ¹³⁷Cs specific activity in fungi were fixed in October. The peak of ¹³⁷Cs content in needles and branches occurs at the time of their active growth in May-July.

Mycorrhiza is a barrier to the penetration of 137 Cs into symbiotic plants (Dighton, 2003). As a result of the research, it was found that the lowest levels of 137 Cs in needles and branches are observed during the period of autumn intensive growth of the mycelium. The 137 Cs content in needles and branches is comparable to its content in fungi with intensive growth of plant.

Conclusions: The unrelated fluctuations in ¹³⁷Cs content in fungi and plants indicate that ¹³⁷Cs circulates between the fungi and the plant throughout the year and passes from one symbiont to another during the intensive growth of each of them.

References:

Dighton J., 2003. Fungi in Ecosystem Processes. New York.

Keywords: 137Cs, fungi, plants, circulation

P (ID 227)

From grassland to forest: Interaction between arbuscular mycorrhizal and ectomycorrhizal plants

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In the course of primary succession in temperate and boreal zones, herbaceous vegetation and grasses of early succession stages forming arbuscular mycorrhizal (AM) symbiosis are later replaced with woody plants predominantly colonized by ectomycorrhizal (EcM) fungi. Such shift from a grassland dominated by Calamagrostis epigejos (AM host) to young forest of Salix caprea (predominantly EcM host) has been explored at post-mining sites. We tested the hypothesis that EcM fungi can negatively affect AM fungi and, consequently, also their host plants, contributing to vegetation change. We sampled soil from spots of S. caprea or C. epigejos monocenosis and their contact zone, as well as roots of these plants and studied communities of root associated fungi (RAF), mycorrhizal colonization and EcM and AM inoculation potentials of the soils. The coexistence of the two plant species significantly affected their RAF communities, but more in C. epigejos roots. Presence of S. caprea also significantly decreased AM fungal abundance in the soil and colonization and species richness of AM fungi in C. epigejos roots. Further, to study the effect of interaction of AM and EcM mycelia on seed germination, seedling recruitment and establishment of AM and EcM plant species, we conducted a rhizobox experiment, where the middle compartment was affected by living mycelium of either AM fungi, EcM fungi or both fungal groups. Preliminary result show differences in seedling establishment and growth depending on presence of specific AM and EcM fungal mycelia.

Keywords: root associated fungal communities, primary succession, arbuscular mycorrhiza, ectomycorrhiza

P (ID 228)

Ectomycorrhizal diversity and community structure in stands of *Quercus baloot* in the dry temperate forests of Kalam, Pakistan

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Ectomycorrhizal fungi are essential for plant growth and ecosystem structure. During this investigation, ectomycorrhizal diversity and community structure of Oaks in dry temperate forest, Kalam, KP, Pakistan was analyzed. Ectomycorrhizal root tips were sampled four times during 2014-2015. Sequencing of the nuclear ribosomal ITS region from ectomycorrhizal root tips and sporocarps identified 43 taxa in Basidiomycota and 8 taxa in Ascomycota. A total of 27 species in the form of macrofungi and 39 species in association with roots have been identified, some being shared above ground and below ground. Ectomycorrhizal communities on root tips were dominated by *Inocybe* sp.1 followed by *Cortinarius rubricosus*. This is the first study of the ectomycorrhizal community composition of Oaks from Pakistan. Our results provide baseline data for species richness and ecology of ectomycorrhizal fungi associated with *Quercus baloot*. This study will help in the establishment and reclamation of forests of *Quercus* trees in Pakistan.

Keywords: Hindu Kush range, fungal ecology, mycorrhizal fungi

P (ID 311)

Isolation and morpho-molecular identification of root plant symbionts of Quebec's arctic and alpine tundra

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The development of energy and natural resources-rich Northern Quebec in the coming decades may have a negative impact on the ecological integrity of terrestrial and aquatic ecosystems. For example, mine waste dumps established in the Schefferville area in the 1960-1970s, due to iron ore exploitation, are still bare of vegetation. Acquiring new knowledge on the ability of northern ecosystems to absorb and remove contaminants and to regenerate is crucial for sustainable development of the Quebec mining industry. Our research hypothesis is that the use of root plant symbionts can enhance the uptake and removal of contaminants and the establishment of plant communities on drastically disturbed land. The objective of our work was to isolate root symbionts from healthy arctic and alpine tundra host plants from Schefferville area, using selective media. Morphotypes were characterized using molecular techniques. DNeasy Plant kit (Qiagen) was used to isolate DNA from pure cultures. 16S rDNA and Internal Transcribed Spacer (ITS) bi-directional Sanger sequencings were used to identify bacterial and fungal symbionts, respectively. The sequences were blasted using the blastn algorithm in the NCBI database. Phylogenetic analysis was performed by Maximum Likelihood (ML) implemented in Mega 7.0.21 and Bayesian inference (BI) in MrBayes 3.2.6 softwares to generate genus-species level classification. Preliminary results using morphological and molecular typings will be presented. This project will allow us to create a microbial bank that can be used as microbial inoculants for reclamation of iron ore waste dumps in Schefferville.

Keywords: northern ecosystems, plant symbionts, microbial inoculants, mycorrhiza

P (ID 317)

Scleroderma areolatum ectomycorrhiza on Fagus sylvatica L.

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Introduction: Despite its broad host range and distribution and its potential applications in commercial plantation forests, comprehensive descriptions of *Scleroderma* ectomycorrhizae are available only for *S. citrinum*, *S. bovista* and *S. sinnamariense*.

Materials and Methods: This study provides a morphological and anatomical description of naturally occurring ectomycorrhizae of *S. areolatum* on *Fagus sylvatica*, obtained from a tree nursery in Slovenia and grown for several years in a climatized room. Additionally a phylogenetic position of the newly described ectomycorrhizal was performed.

Results: Ectomycorrhizae of *S. areolatum* were silvery-white with abundant rhizomorphs; all mantle layers were plectenchymatous, rhizomorphs of type E, with prominent emanating hyphae with thick cell wall. The distal ends of emanating hyphae of rhizomorphs were inflated and often merged with other emanating hyphae. All parts of the mycorrhiza were clampless. In hyphae of the outer mantle layer, rhizomorphs and emanating hyphae, oily droplets were observed that did not stain in sulfo-vanillin and disappeared in lactic acid after a few hours. The phylogenetic position of the newly described ectomycorrhiza placed the fungal partner in a single polyphyletic species hypothesis taxon SH005470.07FU in clade with *S. verrucosum* and *S. cepa*, while the ectomycorrhiza can still be separated from these two morph-species based on rhizomorph type.

Discussion: To adequately describe and identify *Scleroderma areolatum* ectomycorrhizae, there is a need to use a combination of all proposed approaches and a worldwide consideration of the available reference material.

Keywords: pioneer ectomycorrhizal fungi, morphological and anatomical description, long-distance exploration type, rhizomorphs, European beech

P (ID 341)

Relationship between diversity of plants and arbuscular mycorrhizal fungi in response to land-use change

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In Mexico, tropical montane cloud forests account for 1% of the land area, but support the highest diversity of plants and animals of any Mexican ecosystem¹. However, the coverage of cloud forests has decreased mainly due to land-use change for coffee crops². Because arbuscular mycorrhizal (AM) fungi can contribute to the restoration of degraded ecosystems, understanding the effects of land-use change on AM diversity and their potential host plants is a challenge.

Here, we investigated land-use change effects on the diversity of plants and AM spores in the cloud forest and their associated coffee crops in Nayarit, México. We had ten plots of 5x5 m along a transect located by a vegetal community. In the dry season, we registered the diversity of plants and collected soil from five points within each plot, the soil was then homogenized.

Our preliminary results showed that the abundance of plants was greater in the coffee crops, but the richness was higher in cloud forest and this one presented more species evenness. For AM morphospecies the abundance and richness were higher in the coffee crops than cloud forest.

These results are in agreement with a previous study³. It is probable that the diversity of AM fungi and plants may not correlate but, the ecotone could explain the greater diversity of AM fungi in the coffee crops. Further research is needed to determine the role of land-use change on the diversity of AM fungi and plants in the cloud forests.

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²Gual-Díaz M, Rendón-Correa A (comps.). 2014. Bosques mesófilos de montaña de México: diversidad, ecología y manejo. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. 352 pp.

³Arias RM, Heredia-Abarca G, Sosa VJ, Fuentes-Ramírez LE. 2011. Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee production systems and in a tropical montane cloud forest patch in Veracruz, México. Agroforestry Systems 85:179–193.

P (ID 350)

Distribution characteristics of AMF and DSE in mangrove along a tide gradient

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Previous studies have proved the common presence of arbuscular mycorrhizal fungus (AMF) and dark septate endophytes (DSE) in mangrove roots. But their distribution characteristics in mangrove forest is not yet clear. Given the various environment in intertidal zone, we predict different distribution of AMF and DSE along tide level.

Sampling sites were divided into three tide levels: (1) mangrove edge (ME) which were not swamped; (2) high tide (HT) and (3)mid tide (MT) regions with daily flooding hours of around 1-3 h and 3-5 h, respectively. Both soil and root samples were collected for determination of fungal composition, colonization rates, and AMF spore density.

We found similar fungal composition in soil but different in roots. Homologous fungal communities were found among HT root samples. In addition, higher mycorrhizal colonization was observed in HT. However, AMF spore density was similar among different groups.

Our work demonstrates higher mycorrhizal diversity and quantity in the high tide zone, which seems to be contradictory to the fact that these fungi are aerobic. The fungal distribution might affect the mangrove community patches.

In conclusion, mycorrhizal distribution is different along tide gradient, and their eco-function in mangrove should be explored further.

Keywords: arbuscular mycorrhizal fungus, dark septate endophytes, mangrove, mycorrhizal distribution, tide gradient

P (ID 368)

Characterization of arbuscular mycorrhizal fungi along volcanic slopes with respect to disturbance tolerance

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Arbuscular mycorrhizal (AM) fungi play a significant role in the establishment of vegetation during early primary succession. Slopes of active volcanoes are a unique ecosystem in which plant and soil are frequently disturbed by ash falling and erosion.

In this study, AM fungal community in the slopes of Mt. Tarumae volcano, one of the active volcanoes in Japan, is characterized with respect to disturbance tolerance.

Ten individuals of the grass pioneer species *Miscanthus sinensis* were selected from each of the three habitat types, slope, gully, and grassland, along the slopes towards the crater (30 plants in total), and two soil core samples were collected from each individual. One of the two core samples were sieved (disturbed treatment), and the other was not (intact treatment). *M. sinensis* seedlings were grown in these soils for two months, and the roots were subjected to molecular analysis for identification of AM fungi. Sixty PCR products were sequenced by the illumina MiSeq platform, and 113 AM fungal operational taxonomic units (OTUs) across 7 families were identified.

Mantel test revealed that elevation is a significant environmental factor that drives the communities. Dissimilarity analysis showed that the disturbance treatment had a significant impact on the communities, but the impact was smaller at higher elevations and smallest in the slope habitat. The fourth-corner analysis revealed that significant positive correlation of disturbance-tolerant OTUs with elevation.

These results indicated that the communities are structured along the elevation gradient and disturbance-tolerant AM fungi are more abundant at higher elevations.

P (ID 385)

Extreme responses to ectomycorrhizas limit geographic range size in trees

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Introduction/Aims: Many mechanisms have been proposed to explain geographic range sizes. Though the importance of biotic interactions has a long history in biogeography, soil microbes are typically ignored from this perspective. We tested whether response to ectomycorrhizal (EM) inoculation explains range size of trees. To examine mechanisms underlying the relationship between range size and response to EM inoculation, we also examined how the extent of sympatry between host and fungi influenced this relationship.

Methods: Using a dataset of 1295 existing inoculation studies archived in MycoDB, we calculated the mean total biomass response (effect size) of 62 tree species to EM inoculation. We extracted host range area from digitized maps of native distributions, and we determined whether hosts were naturally sympatric with fungal species used as inoculum by searching herbaria databases with geospatially referenced data.

Results: Tree species with effect sizes falling above or below the average response tended to have significantly smaller ranges compared to species with 'average' responses. Host species inoculated with fungi that were allopatric in geography had a higher positive response than those inoculated by sympatric fungi.

Discussion/Conclusion: Here we show that mycorrhizas may underlie host biogeographical distribution patterns at the continental scale. Our study is novel in the scope of species and scale tested, and points to a mechanism underlying this pattern related to the process of mutualism breakdown accruing over time at local geographical scales. For EM tree species, performance may in fact increase when exposed to fungal partners without a recent shared evolutionary history.

P (ID 397)

Spatial-temporal variations of sporulation of arbuscular mycorrhizal fungi (AMF) in Mediterranean sand dunes

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Arbuscular mycorrhizae (AMF) are very important in ecosystems with low nutrient availability, such as the sand dunes on the Mediterranean Coast. The main goal of our study was to determine optimal conditions for AMF sporulation in this ecosystem. To this end, samples from the rhizosphere of four structuring plants were collected in two sand dune habitats (2110 and 2120, according the directive 92/43/CEE) and their ecotones in six geographical locations along the coast, in each season over two years (2014-2015; 2015-2016). A preliminary analysis of the results using a Generalized Linear Model showed that the probability of finding AMF spores differed according to locality, habitat type, and sampling season (higher sporulation in spring). However, no significant effects of plant species were found on the probability of finding AMF spores. Focusing on AMF species composition, Redundancy Analysis showed a significant ordination model, after forward selection of particular localities, plant species, habitats and sampling seasons, where locality and habitat had the strongest effect on which AMF taxa were found. Further analysis of soil environmental features will help understanding whether the effects of the studied categorical variables can be related to particular differences in soil environmental traits such as humidity or nutrient availability.

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P (ID 399)

AM fungi enhance grassland sustainability by improving plant productivity and reducing N₂O emission under nitrogen deposition

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Introduction: Nitrogen (N) deposition and arbuscular mycorrhizal (AM) fungi have a big impact on plant community structure and ecosystem functioning. The interactive effects of AM fungi and N on grassland communities are still unclear. To test the influence of AM fungi and N addition on grassland stability and functioning, a 4-year *in situ* experiment was conducted in a temperate meadow in China.

Materials and methods: This study included two factors, with and without N deposition and with and without AM fungal suppression. In the N deposition treatment, plots received ammonium nitrate (10 g m⁻² yr⁻¹). The abundance and activity of AM fungi was suppressed using benomyl. Plant species composition, productivity and N_2O were measured.

Results: N deposition strongly reduced plant species diversity and evenness, but increased aboveground biomass and N₂O emission. Under N deposition, AM did not affect plant species diversity and richness, but significantly altered the functional groups coverage and increased aboveground biomass. Moreover, AM fungi significantly decreased N₂O emission and increased soil N content.

Discussion: AM fungi did not affect plant diversity and richness which might be related to the community composition. In the studied ecosystem, the dominant species has lower mycorrhizal dependence. The reduction of N_2O could partly be explained by accelerated the mineralization of N and increased plant N uptake by AM fungi.

Conclusion: AM fungi play key roles in maintaining ecosystem stability via increasing plant productivity, reducing N₂O emission under N deposition in temperate meadow ecosystem.

Keywords: AM fungi, diversity, greenhouse gas, N cycling

P (ID 402)

Distribution patterns of AMF in the roots of co-occurring arbuscular mycorrhizal and non-host wetland plants

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Introduction/Aim: Although AMF colonization of non-mycorrhizal (NM) plants has been known for decades, our knowledge of AMF community structure in NM plants is only fragmentarily known.

Materials and Methods: We compared AMF species composition and community structure in the roots of co-occurring NM and AM plants in two wetlands. A fragment covering partial SSU, ITS and partial LSU rDNA region was amplified, cloned and sequenced, and used to characterize the AMF community structure in the roots of 16 putative NM and 18 AM plant species.

Results: AM hyphal and vesicle structures were observed in both NM and AM plants, and molecular data confirmed their presence. Totally 971 AMF sequences were obtained, and these were finally assigned to 28 OTUs based on phylogenetic analysis. The AMF diversity in non-host plants was not lower than in AM hosts, and ten OTUs were only detected in the NM species. The root-colonizing AMF community was significantly different between the AM and NM plants.

Conclusion: NM plants represent a potential source of "hidden" diversity, and the AMF global diversity may possibly been under-estimated. The host quality may be an important driver of AMF community assembling; and neighboring AM roots may not be the primary carbon source for AMF in non-host plants.

Keywords: arbuscular mycorrhizal fungi (AMF), distribution pattern, hidden diversity, non-host (non-mycorrhizal) plant, wetland

P (ID 403)

A giant mycoheterotrophic orchid, *Erythrorchis altissima*, is associated with a wide range of wood-decaying fungi

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The orchid *Erythrorchis altissima* is known as one of the largest mycoheterotrophs. It is a climbing, perennial hemi-epiphytic species without foliage with a subterranean root system. To study its mycorrhizal fungal diversity and specificity, we firstly investigated mycorrhizal fungi of 26 individuals by molecular identification and found 36 fungal groups belonging to eight orders. Most of the fungal groups are wood-decaying fungi, but underground roots have ectomycorrhizal *Russula*. Secondly, we isolated the mycorrhizal fungi from root tissues and used them for symbiotic germination assays to confirm their mycorrhizal ability. Two fungal isolates induced seed germination and following seedling development. Thirdly, natural stable isotope abundances of carbon and nitrogen were analyzed to reveal the pathways of nutrient acquisition in *E. altissima*. The measurement of C and N stable isotope abundances revealed that *E. altissima* is a full mycoheterotroh whose carbon mainly originates from wood-decaying fungi. This study is the first to demonstrate that a giant mycoheterotrophic orchid, *E. altissima*, is associated with a divergent set of wood-decaying fungi.

Keywords: mycoheterotroph, orchid, wood-decaying fungi, stable isotope

P (ID 418)

Root-trait responses to arbuscular mycorrhizal associations in *Brachypodium distach-*

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Arbuscular mycorrhizal (AM) responses are highly conditional, with plant-fungal interactions being determined in part by plant root architecture [1][2]. Root architecture is an important determinant of plant susceptibility to nutrient deficiency and pathogen infection, and there is evidence to suggest AM associations can influence these root traits. However, most studies have focused on comparisons among plant species with large architectural differences. To improve our understanding of how mycorrhizal-plant ecology might be dependent on intraspecific variation in root architecture, plant traits were characterised and compared between two accessions of the model cereal *Brachypodium distachyon*, BD-21 and Kah-1, to explore correlations between root traits and AM inoculation.

Plants were inoculated (or not) with a four-species mixture of AM fungi. Plant responses included root trait (WinRhizo) and biomass measurements. Multivariate analyses were performed to confirm differences in root traits between accessions (P = 0.04), primarily driven by differences in leaf nodal roots. The AM treatment had little effect on root trait development, the most notable exception being the reduced emergence of crown nodal roots in AM plants (P = 0.007). These responses were consistent in the two accessions.

These results suggest that, while a general AM effect was observed, most notably on the emergence of crown nodal roots, *B. distachyon* responses to AM inoculation did not vary between the two tested genotypes. This study also suggests that intraspecific variability in AM root architectural trait responses may be low but that this should be confirmed with a broader survey of genotypes and their responses.

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Keywords: arbuscular mycorrhiza, root traits, root architecture, *Brachypodium distachyon*, context dependency

P (ID 422)

Profiling ectomycorrhizal fungal community recovery in seedlings of south China native masson pine and introduced slash pine

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Soil fungal spore banks are reservoirs of dormant ectomycorrhizal (ECM) fungal propagules. As pine trees are reliant on ECM fungal symbionts for growth and survival, the availability of existing ECM fungi spore banks is likely to be important for establishment of alien pine species in a new habitat.

We took advantage of a large forest site in Guizhou, China that includes both conserved native pine (*Pinus massoniana*) forests and introduced pine (*Pinus elliottii*) plantations. Pine seedling bioassays were employed to examine ECM fungal root tip communities using high-throughput sequencing of pooled roots. Field-collected soil cores were treated one of three ways: kept intact (undisturbed), homogenized, or autoclaved. We collected ECM root tips for two times and found that the pattern of recovery of ECM fungal communities differ from the original soil spore bank: a) pioneer colonizers such as *Rhizopogon* and *Tomentella* dominated the bioassay communities in all treatments; b) intact soil treatment increased the survivorship for late successional ECM fungal species such as *Russula*; c) ECM fungal community of the introduced pine was comprised of a different ECM fungal community to the native pine along a chronosequence developed from soil spore bank.

Thus, our results show that soil disturbance levels could provide various subset of the mature forest soil fungal community for host seedlings to establish. Yet, they may be extremely important in fungal colonization after large-scale disturbances of reforest area and conventional experimental bioassay system.

Keywords: microbial seed bank, resistant propagule community, ectomycorrhizae, host preference, *Pinus*

P (ID 431)

Community Structure of Arburscular Mycorrhizal Fungi in Post-mining Areas in Korea

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Arbuscular mycorrhizal fungi (AMF) are the most ubiquitous plant-fungal symbiont on global ecosystem. Plant-AMF symbiosis is important in ecosystems with unfavorable soil because AMF improve the phosphorus absorption ability, tolerance to stress, and resistance to pathogens of host plants. Because post-mining areas are not properly treated or subjected to environmental restoration after abandoning the mine, soil contamination with the remaining heavy metal components is a serious issue. The purpose of this study was to investigate the differences in the AMF communities in soil samples from natural forests and abandoned metal mines in Korea. We collected 200 rhizosphere soil samples from abondoned metal mines and natural forest areas in Korea. AMF species were identified using morphological and molecular characteristics of the spores extracted from the soil samples. Twenty-one species of AMF from 11 genera were identified. Four AMF species were found only in abandoned mines and there were significant differences in relative abundance of AMF between abandoned mines and natural forests. Acaulospora mellea was dominant in abondoned mines and Ambispora leptoticha was dominant in natural forests. When different types of mining were compared, a difference in species diversities based on the type of mining was also confirmed.

Keywords: arbuscluar mycorrhizal fungi, mycorrhiza, post-mining area, heavy metal tolerance, diversity and community

P (ID 435)

Symbiotic cornucopia: Diversity of Mucoromycotina from vascular and non-vascular plants

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The discovery that diverse non-vascular plants are symbiotic with Mucoromycotina (e.g. Endogonales) rather than Glomeromycotina has sparked debate about which partnership represents the ancestral state of mycorrhizal symbiosis. Recent investigations have shown mutualistic Mucoromycotina-liverwort associations and Mucoromycotina occurring throughout hornworts, liverworts and lycopods.

Recent DNA sequences, from plant endosymbionts, has hugely expanded knowledge of *Endogone* diversity a genus within the Endogonales, previously thought to comprise only saprotrophic and ectomycorrhizal pea-truffles. One clade is dominated by fruitbody-derived sequences, another by endosymbiotic "*Endogone*-like" sequences. Our aims are to discover the real diversity and host ranges of the Endogonales, to improve phylogenetic resolution and to select model taxa for further physiological and cytological studies.

We screened liverworts, hornworts, lycopods and monocots for Mucoromycotina. Phylogenetic reconstructions were performed using maximum likelihood and Bayesian inference approaches and species delimitation was performed using the generalized mixed Yule coalescent model and Poisson tree processes.

We found that most sequences cluster with known groups within the larger *Endogone*-like clade. However, we also detected a new group of sequences derived from a lycopod and identified *Endogone*-like sequences in monocots.

Phylogenetic reconstruction supports division of the Endogonales into two putative families indicating that the order needs revision. Furthermore, monocots warrant further study to determine the nature and extent of their interactions with Endogonales.

P (ID 444)

Do biotically- and abiotically-dispersed plants rely equally on mycorrhizal associations? A case study in Gorongosa-Mozambique

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Mutualistic interactions like those established between plants and mycorrhizal fungi and seed dispersers are fundamental to determine plant population dynamics and ecosystem composition, structure, and function. Despite their importance for plant colonization and establishment, both mutualisms have rarely been explored together. To integrate the study of seed dispersal and mycorrhizal interactions, a tripartite network quantifying the interactions between arbuscular mycorrhizal fungi, plants, and dispersers was constructed for Gorongosa National Park, GNP. Data for seed dispersers and plant dispersed were obtained from a larger study on seed dispersal conducted during one year (2014-2015) in GNP. Arbuscular mycorrhizal communities (AMF) were assessed in the roots of the 15 plant species most commonly dispersed and in 15 other common plant species in GNP that were not animal-dispersed. Mixed root samples for each host species were subjected to DNA extraction amplification of the SSU rDNA and pyrosequencing. This is the first time that a tripartite network of this type is assembled. Comparing both mutualisms for dispersed plants, we found a higher specialization for dispersers than for AMF. 71 AMF virtual taxa (VT) were detected mostly from Glomeraceae family, 30 VT exclusive of dispersed plants and 14 of plants not found to be dispersed by animals while 27 VT were shared by both groups. Our results show that plants dispersed by animals tend to have a lower diversity of AMF virtual taxa in their roots than those dispersed by abiotic vectors.

Keywords: arbuscular mycorrhizal fungi, ecological networks, seed dispersal, Africa

P (ID 445)

Community structure of arbuscular mycorrhizal fungi associated to *Argania spinosa* trees from Algeria

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The study aims to evaluate the diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of argan trees (*Argania spinosa* L. Skeels) growing in the region of Chlef and that of Mostaganem (Algeria). The identification of these fungi has been performed by microscopic analysis of stained roots by trypan blue. The isolation of spores was done by wet sieving. Observation of root system of the argan trees under the microscope revealed the absence absorbent pans; this character shows the dependence of the argan to mycorrhizal fungi for the absorption of water and minerals. The observation of root fragments taken from the two regions has helped to highlight the presence of arbuscular mycorrhiza. The study also reveal the identification of four mycorrhizal species, three species belong to the genus *Glomus* (*Glomus aggregatum*, *Glomus constrictum* and *Glomus sp.*) and one specie belongs to the genus *Gigaspora* (*Gigaspora maragrita*). The results of isolation and identification of spores are similar in both regions in terms of species and different types of side number of spores; Whereas the ecological factors such as climate, rainfall, temperature, and biotic factors: soil type, the phosphorus content, the floristic surrounding the tree trunk.

Keywords: mycorrhiza, Argania, Glomus, Algeria

P (ID 448)

Ectomycorrhizal diversity in stands of Quercus dilata from Upper Dir, KPK, Pakistan

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Most conservation efforts in dry tropical forests have overlooked less obvious targets for conservation, such as mycorrhizal fungi, that are critical to plant growth and ecosystem structure. We documented the diversity of ectomycorrhizal (EMF) fungi of *Quercus dilata* (Fagaceae), in Lamati, Upper Dir, KPK, Pakistan. Sequencing of the nuclear ribosomal ITS region in EMF root tips identified 15 genera with 28 species. The result showed that *Inocybe* was highly diverse with five different species. *Lacatrius mediterranensis* was found dominant. This is the first study of the ectomycorrhizal composition of oaks from Upper Dir. Information collected from this study can be used as baseline information for future studies to investigate the impacts of different ectomycorrhizal fungi on endemic oak.

P (ID 456)

Primary succession and functioning of arbuscular mycorrhiza in a Danish coastal dune system

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The succession of plants is well studied while there is still much uncertainty on how mycorrhizal functions develop in ecosystems over time. In this study, we focus on the succession of arbuscular mycorrhizal (AM) fungal traits over a well-defined successional gradient. By examining orthophotos from 1995-2016 we were able to trace the establishment of the vegetation in a new coastal system in southern Zealand, Denmark. A pilot study revealed a clear increase of AM extraradical hyphae and organic matter content with time, and succession of the plant community. We plan to study this further and include other AM traits, such as root colonization of arbuscules, vesicles, hyphae and the number of spores in the rhizosphere. Taking advantage of trait-based approaches and the different functional significance of the measured traits, we will trace the functional change along ecosystem formation at this particular site. Knowledge generated in this study will be helpful in determining which primary factors drive the successional development of natural vegetation.

Keywords: arbuscular mycorrhiza, traits, succession, community assembly

CONCURRENT SESSION: Mycorrhizal microbiomes

Complex microbial communities including both prokaryotes and eukaryotes colonize and interact with mycorrhizal fungal hyphae, sporocarps/fruitbodies, or the mycorrhizosphere. These microbial communities could well be responsible for positive, as well as negative interactions with the establishment, development and function of mycorrhizal symbioses. Research on mycorrhizal microbiomes has been particularly facilitated by recent explosion of cultivation-independent and high-throughput molecular genetic approaches to identify total and active microbial communities at the scales from single cells to ecosystems, and by using isotope labeling and advanced microscopy techniques. This session shall cover a range of subjects, focusing on the strength of association of mycorrhizal fungi and other microbes (from loose to tight), including mycorrhizal-helper bacteria, facilitation and competition for resources with saprophytic soil microbes, cross-feeding, trophic cascades as well as endocellular symbionts (bacteria-like organelles) within the hyphae of mycorrhizal fungi, stressing the perspective on function and spatial arrangements.

Chairs: Christina Kaiser, Mika Tarkka

IL (ID 199)

The intracellular microbiota of arbuscular mycorrhizal fungi

Paola Bonfante (Life Science and Systems Biology, University of Torino, Torino, Italy)

As elements of the same plant microbiota, fungi and bacteria largely interact in the rhizosphere. These inter-domain associations cover different levels of complexity, but the most intimate interaction takes place when bacteria live inside the fungal cells as endobacteria.

The presentation will summarize our current knowledge on the endobacteria living inside the arbuscular mycorrhizal fungi (AMF, Glomeromycotina), using them as a paradigm to better understand the diversity and origin of fungal endobacteria as well as the interkingdom interaction they originate.

To detect the reasons why many fungal strains have maintained their bacterial guests for hundreds of million years, we have developed a stable endosymbiont-free strain of *Gigaspora margarita* (B –), which has been cured of its *Candidatus* Glomeribacter gigasporarum. We have applied multiple morphological and 'omics' approaches to directly compare the B– line with the wild-type strain hosting the endobacterium (B+ line). The coupling of transcriptomics and proteomics with physiological and cell biological approaches demonstrated that the Gigaspora endobacterium raises the bioenergetic capacity of the fungus, increasing its ATP production and respiration, and eliciting mechanisms to detoxify reactive oxygen species. We hypothesize that such changes may provide a better ecological fitness to the mycorrhizal fungus.

CL (ID 259)

The response of plants and soil microbial communities to drought

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Soil fungal, bacterial and protist communities interact heavily with each other in soil food webs. Therefore, changes in one group may trigger drastic changes in others. As our climate is rapidly changing, it is critical to understand how soil food webs respond to repeated disturbances. We theorize that the presence or absence of arbuscular mycorrhizal fungi (AMF) will have an impact on the soil microbial food web and the response of plants when subjected to drought events. To test our hypothesis, we have set up a mesocosm experiment with three different mycorrhizal treatments (0, 1 or 3 taxa) and two different drought intensities. Through analyzing several plant parameters as well as the soil fungal, bacterial and protist community through high-throughput sequencing, we hope to gain an understanding of the robustness of soil microbial communities and their interactions with plants when faced with repeated major environmental disturbances. Our results indicate that strong observed advantages of AMF disappear or are reversed in droughts, with both mycorrhizal treatments experiencing a higher mortality compared to non-mycorrhizal treatments. However, all plants seem to benefit greatly from the presence of mycorrhiza for their recovery after the extreme drought event. Our genetic analysis shows that soil food webs exhibit a shift from the control treatment compared to drought treatments, but not with AMF treatments. Therefore we conclude that, although in our experiment AMF did not induce a significant shift in the soil food web, they still play a significant role for plants during and after extreme events.

Keywords: soil microbial food webs, arbuscular mycorrhizal fungi, drought

CL (ID 246)

Shaking up the microbiome? How field inoculations with mycorrhizal fungi affect rhizosphere communities

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Producing yields to feed a growing global population without deteriorating the environment is a major challenge for mankind. Inoculations of arable fields with beneficial arbuscular mycorrhizal (AM) fungi could be used to enhance crop nutrition in a sustainable way. However, it is unclear how the introduction of non-native fungi affects native AM fungal and microbial communities.

We tested, whether *Rhizoglomus irregulare* inoculated into Swiss maize fields can establish in maize roots. Inoculation success of the fungus was determined by specially-designed, strain-specific qPCR. We found an overall increase of the inoculant in maize roots across all field sites. However, the increase in *R. irregulare* copy numbers was not necessarily reflected in enhanced root colonization, suggesting that the inoculant successfully competed with native AM fungal communities for niche space. Establishment success was negatively correlated to native AM fungal abundance. In a greenhouse essay, we tested effects of AM fungal inoculation on native AM fungal community composition by single molecule real-time (SMRT) sequencing targeting a 1.3 kb long fragment of the ribosomal gene. We confirmed that *R. irregulare* could outcompete native AM fungi and dominate the AM fungal community. However, inoculation with an isolate of *Funneliformis mosseae* did not significantly affect native communities.

Our results indicate that inoculated fungi can be competitors with native communities, raising concerns about potential invasiveness. Consequences for the biosafety of AM fungal inoculations are discussed. Interactions of AM fungi with the wider root microbiome are currently being investigated and results will be presented.

Keywords: field inoculation, agriculture, sequencing, biosafety, microbiome

ST+P (ID 188)

The virome of the arbuscular mycorrhizal fungus Gigaspora margarita

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As very ancient and widespread in nature, arbuscular mycorrhizal fungi (AMF) are key components of the plant microbiota. Recent publication of genomic and transcriptomic data shed light on the biology and evolution of AMF. The genetic complexity of AMF is increased by the presence of endobacteria which live inside many AMF¹. A further component of such genetic complexity is the virome associated to AMF, whose knowledge is still very limited^{2,3}. Here, by exploiting recently published transcriptomic data⁴ we describe the virome associated to Gigaspora margarita. Viral RNA-dependent RNA polymerases (RdRp) are markers of mycoviruses with an RNA genome. A Blast search for RdRp sequences allowed us to identify four mitoviruses (so called because they replicate in mitochondria), one narnavirus (Ourmia-like), one Giardia-like virus, and two sequences related to Fusarium mycoviruses. Northern blot and RT-PCR assays on RNA from germinating spores confirmed the authenticity of all the sequences with the exception of the Fusarium-related ones. All the mitoviruses are replicative and functional since both positive strand and negative strand RNA are present. Interestingly, in all the four mitoviral sequences 100% of tryptophan residues are encoded by the TGG codon: apparently a functional RdRp can be translated both in cytosol and mitochondria. In addition we report, for the first time, DNA fragments corresponding to mitovirus sequences associated to the presence of viral RNA: mitoviral DNA sequences are not integrated in the genome, but likely exist as extrachromosomal genetic units. Phylogenetic analyses show evidence of co-evolution of mitoviruses with their AMF hosts.

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³Kitahara R, Ikeda Y, Shimura H, Masuta C, Ezawa T (2014) A unique mitovirus from Glomeromycota, the phylum of arbuscular mycorrhizal fungi. Arch Virol (2014) 159:2157–2160.

⁴Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P (2016) Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. ISME J. 10(1): 130–44.

ST+P (ID 116)

The non-mycorrhizal control in cultivation experiments with AM symbiosis: microbiome perspective

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Experimentation with AM fungi (AMF) involves application of inocula of various complexity that mostly contain complex microflora potentially affecting the results of experiments. Inocula containing pure AMF are rarely used. The aim of our work was to evaluate the effects of two types of inocula (pot cultured and monoxenic) and of different control treatments on host growth and communities of fungi and prokaryotes in the substrate.

To this end, we grew *Andropogon gerardii* in pots with sterilized substrate. The treatments comprised uninoculated control, inoculation with complex blank inoculum lacking AMF, filtrate of blank inoculum, complex mycorrhizal inoculum, filtrate of this mycorrhizal inoculum, axenic mycorrhizal inoculum and axenic inoculum combined with filtrate of blank inoculum.

Plant growth was stimulated by AMF irrespectively of the inoculum type. Any type of non-mycorrhizal inoculation resulted in plant biomass being higher than that of uninoculated control. Molecular analysis of microbial communities in the substrate revealed some microorganisms abundant in treatment inoculated with complex mycorrhizal inoculum but lacking in other treatments.

This indicates that, in our experiment, physiological effects of mycorrhizal inoculation were consistently expressed. However, no control with microflora similar to that in the treatment with complex mycorrhizal inoculation was obtained. Thus, treatments receiving nonmycorrhizal inoculation (blank inoculum, filtrates) can only be taken as appropriate control from the plant physiological perspective.

The type of non-mycorrhizal control should be chosen according to the experiment purpose and axenic inocula combined with the inoculation by saprotrophs should preferentially be used in molecular studies of substrate microbial communities.

Keywords: inoculum, control, arbuscular mycorrhiza, microbial community, Andropogon

ST+P (ID 261)

SiolBioHedge: Using arable leys as highways for soil quality reconstruction by microbial soil engineer species

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Since the mid 90s, wheat yields in the UK and across the global have plateaued, after half a century of dramatic increases(1). Possible explanations include changes in agronomic practices, decreased plant nutrition, reduced soil organic carbon, increased soil compaction and destruction of soil food webs through conventional tillage practices(2). To overcome this plateau while meeting increased pressure to reduce inputs may require rebuilding soil quality that contribute holistically to plant health. Hedgerows are well known to be reservoirs of above-ground biodiversity in agricultural landscapes (3). Could connecting these reservoirs to arable field centres using grass & clover ley strips act as "highways" to rapidly rebuild soil quality parameters, such as soil engineer species and arbuscular mycorrhizal fungi? Here we present data from a field scale experiment in the UK that monitored the performance of grass/clover leys in arable fields and suggests that short term leys, whether connected to the field margin or not, increase soil fertility and the abundance of earthworms. Hedgerow and field margin below ground soil microbiomes appear to reflect the above-ground biodiversity, in that there is a greater species diversity in hedge and margin fungal and bacterial communities than in-field communities. While there is a decrease in species abundance between the field margin and the field-proper, there does not appear to be an effect of distance on the arbuscular mycorrhizal fungal communities along the length of the ley.

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Keywords: mycorrhiza, hedgerow, agriculture, ley, eathworm

ST+P (ID 398)

Effect of AMF inoculation and fertilization on the bacteria and AMF communities inhabiting pulse roots

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Fertilization practices and arbuscular mycorrhizal fungi (AMF) inoculants could change root-associated microbial assemblages in agricultural soils. The aims of this study were to 1) assess the effect of fertilization regimes and AMF inoculation of lentil and pea on the diversity and community structure of bacteria and AMF inhabiting the roots of the crops, and 2) explore the relationships between microbial assemblages and crop yield. Two field studies were conducted in Swift Current, SK, and Beaverlodge, AB. In a conventional system, plants received three levels of mineral fertilization (0%, 50%, and 100% of the recommended N and P rates), while in the organic system, the plants were fertilized with three levels (0%, 75% and 150%) of certified organic P-fertilizers (rock-phosphate or composted manure). Total DNA was extracted from plant roots and amplicons of the bacterial 16S and AMF 18S rRNA genes were sequenced using the Illumina MiSeq platform. The composition of bacterial and AMF assemblages varied more with location and crop species than with fertilizer regime or AMF addition. Strong positive correlations were found between the abundance of the AMF taxa Funneliformis geosporum and the yield of non-inoculated pea receiving 75% of the recommended rate of composted manure, and between the abundance of Glomus sp. Glo4, and the yield of pea receiving 100% of the recommended rates of mineral N and P. We conclude that a positive contribution of AMF taxa to crop yield depends on fertilizer level or form, AMF inoculation, crop species and environmental conditions.

Keywords: AMF, bacteria, fertilizers regime, Illumina MiSeq sequencing, crop yield

CL (ID 235)

Mycorrhizal Competition Release? Fungal and bacterial communities in native and nonnative productive truffle orchard soils

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Given their irresistible aroma and unpredictable supply, truffles in the genus Tuber are one of the most expensive foods. These ectomycorrhizal fungi are also cultivated successfully worldwide. Recently, Australia has become the top producer of Tuber melanosporum (black truffle), a species native to Europe. When a species is introduced into a non-native habitat it may thrive due to the release from competition and natural enemies. With this study we aimed to test this competition release hypothesis by characterizing fungal and bacterial communities in truffle plantations in native (European) and non-native (Australian) habitats. In total, we sampled 650 soils from 27 orchards (n=20-25/orchard). DNA was extracted from soils and the 16S and ITS ribosomal DNA Illumina was sequenced with Illumina MiSeq resulting in 23,860,471 ITS and 30,941,017 16S demultiplexed reads. Sequences were quality filtered and clustered into 13,168 fungal and 16,559 bacterial Operational Taxonomic Units (OTUs) with the UPARSE pipeline. NMDS and PERMANOVA showed that fungal and bacterial communities are structured by country of origin. Tuber melanosporum had a higher relative abundance in Australian soils (10.4%) compared to European soils (7.0%), but was the most dominant species under productive trees in both continents. Truffle microbiomes at productive sites in both continents also included the non-mycorrhizal fungi Fusarium, Cryptococcus, Mortierella, and Ilyonectria spp. and bacterial (Chloroflexi) and archeal (Nitrososphaeraceae) taxa. Overall, ectomycorrhizal fungi had three times fewer OTUs and a lower relative abundance in Australian soils, consistent with the competition release hypothesis.

Keywords: microbiome, biogeography, ectomycorrhizal, tuber, Nitrososphaeraceae

CL (ID 46)

Arbuscular mycorrhiza alters the community structure of ammonia oxidizers at high fertility via competition for soil NH₄+

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Nitrification represents a central process in the cycling of nitrogen (N) which in high-fertility habitats can occasionally be undesirable. Here we explore how arbuscular mycorrhiza (AM) impacts nitrification when N-availability is not limiting to plant growth. We wanted to test which of the mechanisms that have been proposed in the literature best describes how AM influences nitrification. We manipulated the growth settings of *Plantago lanceolata* so that our soil no longer contained ammonia-oxidizing (AO) archaea and we could control the mycorrhizal state of our plants. AM induced no changes in the rates of nitrification potential or the estimates of AO bacteria. However, we could observe a shift in the community of ammonia-oxidizers which matched the shift we saw when comparing hyphosphere to rhizosphere soil samples and mirrored well changes in the availability of ammonium in soil. We interpreted our results as support that it is competition for N that drives the interaction between AM and AO. Our experiment sheds light on an understudied interaction which is pertinent to usual management practices in agricultural.

Keywords: 454 sequencing, ammonia monooxygenase gene community structure, arbuscular mycorrhiza, nitrification, RT-PCR

CL (ID 149)

Nitrogen and root mycobiome in temperate forests along a biogeographic gradient

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Root fungal communities, especially mycorrhizas, are of importance for plant nitrogen nutrition which is often limited in temperate forest ecosystems. However, knowledge about the impact of root mycobiome on tree nitrogen at forest ecosystem level is scarce.

The objective of this study was to assess the diversity and trophic modes of root fungal communities in relation to root and soil nitrogen at a large scale. We hypothesized that concentrations of root and soil nitrogen differ among biogeographical areas and forest types, and that concentrations of root nitrogen are higher and those of soil nitrogen are lower with increased diversity of mycorrhizal fungi.

To test these hypotheses, we sampled fine roots from 150 forest plots (100mx100m) differing in tree composition in three areas across Germany: Schorfheide-Chorin (SCH), Hainich-Dün (HAI) and Schwäbische Alb (ALB) in the frame of the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/). Root and soil concentrations of NH₄⁺ and NO₃⁻ were measured. After root DNA extraction, ITS2 region of the fungal rDNA was amplified using primers ITS3KYO2/ITS4. High throughput sequencing (Illumina MiSeq) of the PCR amplicons was conducted to detect root-associated fungi.

Our preliminary results showed that root N concentration was significantly higher in ALB than in SCH and HAI. Sequence analysis revealed 4525 fungal OTUs. Fungal phyla contained Basidiomycota (72.6%), Ascomycota (22.6%), Glomeromycota (2.1%), Zygomycota (0.2%) and unidentified fungi (2.5%). Data on the relationship between concentrations of root and soil nitrogen and diversity and trophic modes of root fungal communities will be presented and discussed in the context of forest composition.

Keywords: root fungal communities, mycorrhizal diversity, root nitrogen, soil nitrogen, Illumina MiSeq

CL (ID 162)

Fructose in hyphal exudate as a signal molecule to trigger the AMF-bacteria cooperation

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Introduction: Cooperation is a prevalent phenomenon and how it originates and maintains is a fundamental question in ecology^{1,2}. The widely spread association between arbuscular mycorrhizal fungi and bacteria represents a suitable model for studying the cooperation between two species³. Here we investigated if the exchange of carbon and phosphorus is pivotal to the cooperation between an arbuscular mycorrhizal fungus *Rhizophagus irregularis* and a phosphate solubilizing bacterium (PSB) *Rahnella aquatilis*.

Materials and Methods: Four different experiments, using hair root and medicago plant *in vitro* culture systems, were conducted to study how AMF influence the processes related to metabolism and phosphatase production (the expression of sugar transporter, excretive phosphatases genes, key genes in cell division and involved in secretory systems) in PSB and whether PSB could influence the phosphate transporter and polyP synthesis genes expression in the extraradical hyphae of the AMF.

Results: Fructose exuded by the AMF stimulated the phosphatase genes expression in the bacterium as well as the efficiency of phosphatase secretion into the environment by regulating its protein secretory system. The phosphatase activity was subsequently increased, promoting the hydrolyzation of organic phosphorus into inorganic phosphorus, stimulating simultaneously the processes involved in phosphorus uptake by the AMF.

Conclusion: Our results demonstrated for the first time that fructose not only represent a carbon source, but also play a role as a signal molecule to trigger bacteria-mediated organic phosphorus mineralization processes. It highlighted the molecular mechanisms by which the hyphal exudates play a role in maintaining the cooperation between AMF and bacteria.

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Keywords: carbon catabolite repression, cooperation, fructose, phosphatase genes expression, protein secretory systems

ST+P (ID 73)

Mycorrhiza in association with mycorrhiza helper bacteria suppressess basal stem rot in oil palm

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Ganoderma boninense is an important fungal pathogen of the oil palm (Elaeis guineensis Jacq.) that causes serious economic losses in Southeast Asia region. The fungus causes the disease known as basal stem rot.

This study attempts to explore the efficacy of two endophytic microbial applications to control the disease both in the lab, nursery and field. Two endophytic microorganisms; Arbuscular Mycorrhizal Fungi (AMF) and endophytic bacteria were tested. *Glomus intraradices* and *G. clarum* represents AMF while *Pseudomonas* strain UPMP3 and *Burkholderia* strain UPMB3 represents the endophytic bacteria. The endophytic bacteria are classified as Mycorrhiza Helper Bacteria (MHB) based on the findings through the *in vitro* assessment. The study further investigates the application of these endophytic microorganisms through nursery and field conditions. Gene expression and biochemical work was also investigated for induced disease resistance within oil palm.

Vegetative assessments in nursery found to be promising for the oil palm seedlings treated with a single application of *G. intraradices* (T2), followed by treatments with both AMF species with UPMP3 (T4). In field condition however, found combinations of AMF species and UPMP3 treated seedlings gave the highest vegetative readings. These results also indicate that the response of rhizosphere populations to AMF cannot be generalized through nursery trials and possibly to the microbial diversity within the rhizosphere suggesting different AMF may effect differently on the oil palm growth. Nevertheless the study provides an innovative approach for a prophylactic treatment in reducing the onset of BSR disease by exploring the potentials of these endophytic microoganisms.

Keywords: arbuscular mycorrhiza, oil palm, endophytic bacteria, *Ganoderma boninense*, biocontrol agent

ST+P (ID 146)

Large-scale demonstration of increased production of poplar biomass by mycorrhizal inoculation at metal-contaminated phytomanagement sites, and investigation of associated fungal communities

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Introduction: Afforestation of trace element (TE)-contaminated soil, notably with fast growing trees producing large biomass has been demonstrated to be an attractive option for their bioremediation. Mycorrhizal fungi can form symbiotic associations with trees, contributing to TE tolerance and participating actively in bioremediation processes.

Materials and Methods: We studied the effects of mycorrhizal inoculation on the growth of two poplar clones to large-scale. Two TE-contaminated sites of ca. 1 ha were planted with 2200 trees, and were either inoculated with a mixed commercial inoculum or not-inoculated and allometric parameters were determined after 2 and 4 years of growth¹. Moreover, the soil fungal communities were also analysed from different management regimes [short rotation coppice (SRC) *versus* very short rotation coppice (vSRC)] and different poplar genotypes using Illumina-based sequencing^{2,3}.

Results and Discussion: Planting poplars increased the proportion of symbiotic/endophytic fungi in the rhizosphere, whereas saprotrophic fungi dominated in all samples. Only minor alterations in the relative abundance of taxa and OTUs were noted between SRC and vSRC management regimes, although mycorrhizal colonization of poplars varies greatly between species and genotypes.

The inoculation with mycorrhizal fungi significantly impacted the biomass production of the Skado clone at both sites, despite striking differences in soil structure and contamination. Interestingly, the filtering TE capacity of inoculated poplar roots was significant for several metals in Skado leaves, only at the most contaminated areas. In overall, this bioaugmentation option with mycorrhizal fungi influenced more poplar growth than soil contamination and highly improved its biomass production.

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Keywords: phytomanagement, poplar, metal polluted sites, large scale inoculation, mycorrhizal communities

CL (ID 145)

Are Streptomycetes members of the mycorrhiza microbiome steering committee?

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Streptomyces Actinobacteria are prolific producers secondary metabolites and commonly antagonistic against other microbes. They have also been described to be beneficial and growth promoting for eukaryotic hosts. We'll present evidence that the streptomycetes can protect mushrooms, mycorrhizas, and plants. Mushrooms of ECM fungi in the order Boletales are commonly associated with bacteria and infected by molds of the mycoparasitic genus Sepedonium. From a diverse bacterial community, the fruiting body associated streptomycetes were identified as the most potent inhibitors of the mycoparasite, and the search of responsible secondary metabolites and the respective gene clusters in a Streptomyces strain have started. In ectomycorrhizas, the associated streptomycetes commonly inhibit the growth of extrinsic fungi and bacteria, but do not affect the growth of the host mycorrhizal fungus. Interaction of pedunculate oak clone DF159 with mycorrhization helper bacterium Streptomyces strain AcH 505 leads to extensive changes in the structure of mycorrhizosphere microbial community, and reduces host plant damage by a parasitic nematode Pratylenchus penetrans. Strain AcH 505 protects pedunculate oak from pathogenesis by oak powdery mildew Erysiphe alphitoides, and the oak defense priming by Streptomyces involves the induction of a large array of defense-related genes. In contrast, the gene expression response to Streptomyces is attenuated when a beneficial mycorrhizal fungus is co-inoculated [1]. We conclude that the streptomycetes contribute to disease protection, bringing about mutual benefit to the host and symbiont.

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Herrmann et al. Perspectives in Plant Ecology, Evolution and Systematics. 2016, 19:40–48

Keywords: helper bacteria, biocontrol, induced resistance, mushroom, ectomycorrhiza

POSTERS

P (ID 355)

Phosphorus forms mediate characteristic of hyphosphere microbiome driven organic phosphorus turnover

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The objective of this study was to explore functional groups (alkaline phosphomonoesterase ALP and β-propeller phytase BPP) of microbes driving soil organic P turnover. Bacterial ALP- and BPP-encoding genes in the hyphosphere of Allium porrum were studied with the combination of compartmented microcosms and high-throughput sequencing technologies. Leek was grown with or without Funneliformis mosseae in root compartment, and the extraradical mycelium of F. mosseae was allowed to grow into a separate hyphal compartment containing no P, KH₂PO₄ or phytin. Illumina MiSeq sequencing revealed community compositions of ALP- and BPP-harboring bacteria using the specific primer pairs. Different P forms significantly changed the community structures of ALPharboring bacteria, but not for BPP-harboring bacteria. The more abundant community compositions of ALP-harboring bacteria were Sinorhizobium, Bradyrhizobium, Pseudomonas and Ralstonia, and specifically, organic P treatments significantly increased relative abundance of Pseudomonas compared with control and inorganic P treatments. Likewise, the predominant community composition of BPP-harboring bacteria was Pseudomonas, moreover, the highest abundance was presented in organic P treatments. These results show that community compositions on bacterial ALP and BPP genes are meditated by P forms, which might drive the turnover of soil P in bacterial communities and consequently manipulate the biogeochemical cycle of soil P.

Keywords: alkaline phosphatase, β -propeller phytase, community composition, hyphosphere, P forms

P (ID 22)

Use of cactus microbiome for improving grapevine rootstock tolerance to abiotic stresses

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The effects of AMF and PGPRs are being studied for the first time to improve the tolerance of the grapevine rootstock 140Ru (Vitis rupestris × Vitis berlandieri). Indeed, 140Ru is among the rootstocks commonly used in Tunisia and North Africa and considered as tolerant to salinity and drought. However, due to the climate change effects and deterioration of the soil quality during last decades, the efficacy of this rootstock is being compromised. Farmers are so far looking for new alternative rootstocks. Thus, the inoculation of 140Ru with mycorrhizae and PGPR would boost its performances and promote its sustainable use. Two months old plants (issued from cutting) were inoculated with a rhizosphere collected under cactus (Ficus indica) from a Tunisian arid area. Inoculated plants were cultivated under greenhouse (70% humidity, 25-28°C, 14-16h photoperiod) and subjected to salt stress, phosphorus limitation and water shortage. Growth, water and osmotic potentials, metabolites and differential gene expression were monitored under the different stresses. Preliminary results show better growth of inoculated plants than controls and were much less affected by the applied stresses. The water and osmotic potentials were substantially less affected in the presence of AMF and PGPRs. Results from metabolomics and gene expression will further clarify these data and would support our hypothesis that the cactus rhizosphere would contain advantageous microorganisms such as AMF and PGPRS able to promote growth and enhance sustainable tolerance of this rootstock to abiotic stresses and extend its use.

Keywords: grapevine, AMF, PGPR, cactus, abiotic stresses

P (ID 106)

Formation of Mycorrhiza in Urban Trees after Transplanting to Roadsides

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Mycorrhizal associations are essential for trees. In natural habitats colonization is guaranteed. Urban trees derive from nurseries and are transplanted baled into artificial substrates with low inoculum potential, and mostly without contact to other trees. Restricted rooting zones, a disturbed water and air balance of the substrate and multiple pollutants may be adverse to mycorrhization.

To select future urban trees, a long-term project was started at our institute in 2010. 6-8 individuals of the test species, trunk perimeter 16-18 cm, were planted in three differing climates. Representing different mycorrhizal preferences, some of the test species were chosen for monitoring their mycorrhization. In root balls and from 2011 to 2014 selected specimen were scrutinized every year in spring and autumn. Besides microanalysis, fungal species identification was performed by conventional sequencing and in addition deep amplicon-sequencing for exclusive root samples taken in 2012 and 2014.

In all trees tested both ecto- and endo- mycorrhizal fungi could be detected, except for *Magnolia*. Frequency of mycorrhization was higher than 50% showing seasonal changes. Intensity of endomycorrhizal colonization averaged 15%. Sequence analyses revealed a complex, tree species specific fungal community. Conventional sequencing was able to identify the main ectomycorrhizal partners but rarely identified endomycorrhizal fungi. A few fungal species were specific for one cultivation site.

Our studies demonstrate that trees in an urban environment display a differentiated mycorrhization representing the tree species specific communities. Based on these results the mycorrhizal inocula for urban trees require a tree species and site specific design.

Keywords: Quercus, Carpinus, Fraxinus, Tilia, Ostrya

P (ID 182)

Accelerated reclamation of drastically disturbed Canadian mine and oil sands areas using mycorrhizal and actinorhizal plant symbioses

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The coevolution of plant-microbe symbionts has played a fundamental role in the colonization of vascular plants on terrestrial ecosystems. Arbuscular mycorrhizae evolved more than 400 million years ago while ectomycorrhizae evolved about 225 million years ago and ericoid mycorrhizas about 100 million years ago. The mycorrhizal fungi are known to enhance mineral and water absorption by plants and protect them against biotic and abiotic stress. The nitrogen-fixing root symbioses evolved about 100 million years ago) and absorb atmospheric dinitrogen (79% of air on earth) by formation of nodular symbioses of dicots from Eurosid I clade with rhizobia (Alpha-and Beta-proteobacteria) and with Actinobacteria (Frankia spp.). Over the last 15 years of land reclamation research and development, we have made significant progress in the use of bioinoculants to enhance reclamation and the establishment of plant communities on drastically disturbed oil sands and gold mine sites. In all cases inoculated plants with selected biofertilizers outperformed uninoculated plants as measured by the seedling volume and plot volume indices in the nursery and the field, respectively. In addition, physico-chemical soil properties, microbial diversity and catabolic activity or phytobial stabilization were improved as revealed by conventional methods (physico-chemical soil analyses, DGGE, acetate mineralization, most probable number) and next generation pyrosequencing of the bacterial 16S rRNA gene and the fungal ribosomal internal transcribed spacer (ITS) region. The use of bioionoculants combined with green fertilizers and nanofertilizers is an economically, socially and environmentally acceptable promising biotechnology towards building Canada's green sustainable reclamation industry for the 21st century.

Keywords: actinorhizal plants, biofertilizers, mine and oil sands industry, mycorrhizal plants, reclamation

P (ID 185)

Analysis of mycelial growth and fruit body characteristics of Sparassis latifolia strains

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Sparassis latifolia is called "Cauliflower Mushroom" and is an edible mushroom that have high content of ß-glucan. Recently, S. latifolia is began to artificial cultivation by bottle, plastic bag and wood cultivation in Korea. However it is not spreaded because there are low incubation ratio and yield. For the high efficiency of production, we aim to find the superior strains and media for better mycelial and fruit body growth.

First, we analyzed the genetic relationship among 31 strains and divided five groups with three kinds of URP primers. And then ten strains were selected from five groups for the experiment of mycelial growth. The suitable media for mycelial growth is different according to media type. The suitable solid and liquid media for mycelial growth of *S. latifolia* isolates were selected PDA and M2, respectively. In addition, the larger C/N ratio increased the mycelial growth until C/N 160.

Second, we investigated that fruit body of strians with plastic bag cultivation. The substrate is mixed larch sawdust, corn flour, and wheat flour (8:1:1, v/v). And moisture content of substrate was controlled about 60% with 10% molasses solution. The 19 isolates were formed primordia of total 31strains. The eight strains were showed higher fresh weight of fruit body above 140g/1kg substrate.

In the future, we have the research project of *S. latifolia* breeding and culture system for stable production of a new item.

Keywords: Sparassis latifolia, mycelial growth, cauliflower mushroom, fruit body

P (ID 234)

AMF spore microbiomes differ from those of host plant roots and rhizospheres in petroleum-contaminated soil

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Improving the efficiency of phytoremediation requires a thorough knowledge of the microbial diversity associated with plant roots. This study aims to assess fungal ITS and bacterial 16S rDNA diversity using amplicon sequencing in rhizospheres and roots of three species Solidago canadensis, Populus balsamifera and Lycopus europaeus, growing spontaneously in three petroleum hydrocarbon polluted sedimentation basins. Microbial community structures of rhizospheric soils and roots were compared with those of microbes associated with arbuscular mycorrhizal fungal (AMF) spores harvested from the same roots to determine the links between the root and rhizosphere communities and those associated with AMF. Our results showed a difference in OTU richness and community structure composition between soils and roots for both bacteria and fungi. We found that petroleum hydrocarbon concentrations have a significant effect on fungal and bacterial community structures in both soils and roots, whereas plant identity showed a significant effect only on the root communities for bacteria and fungi. Our results also showed that the community composition of bacteria and fungi in soil and roots differed from those associated with AMF spores harvested from the same plants. This suggests that in petroleum hydrocarbon contaminated soils, AMF may release chemical compounds by which they recruit beneficial microbes to tolerate or degrade the petroleum hydrocarbon pollutants present in the soil.

Keywords: microbiome, petroleum hydrocarbon contamination, amplicon sequencing, biodiversity, arbuscular mycorrhizal fungi

P (ID 240)

Resolving fungal taxa using long environmental amplicons – a case study using Archaeorhizomycetes

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The class Archaeorhizomycetes (Taphrinomycotina, Ascomycota) represent soil fungi with a wide geographical distribution, occurring in terrestrial ecosystems on several continents. Based on similarity to available environmental DNA sequences, the class is likely to comprise hundreds of cryptic species. Since taxonomic classification requires biological type materials only two species, *Archaeorhizomyces finlayi* and *Archaeorhizomyces borealis*, have been formally described. Using PacBio sequencing of long environmental amplicons of ribosomal genes (SSU-ITS1-5.8S-ITS2-LSU), this study aimed at identifying phylogenetic distinct taxa of Archaeorhizomycetes from a well studied field site in mid Sweden.

After chimera checking unique sequences were aligned and a tree was generated. Clustering were collapsed based on 98% homology across the ITS region to resolve taxa. Habitat specific distribution across different soil layers, e.g Organic, Illuvial (E) and Alluvial (B) mineral soils, was analyzed to determine if taxa are ecologically distinct. We verified the method by also analyzing well known groups of fungi, ie. Russula in the dataset.

We resolve nine well supported taxa of Archaeorhizomycetes, eight of which are also distinct at 98% homology across the ITS region. We find a strong signal of soil horizon specific distribution among the nine taxa suggesting that they represent ecologically distinct taxa. Sequences representing *A. finlayi* were significantly more abundant in the B horizon, which is also the soil layer it was isolated from.

Based on these results, we propose that using a combination of phylogenetic and ecological species concepts is a feasible method to approach the description of fungal taxa using environmental sequences.

P (ID 297)

Rhizophagus clarus and Lysinibacillus fusiformis interaction in biochar amended soil for Zea mays nutrients uptake

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Mycorrhizal fungi and phosphorus solubilizing bacteria are important organisms in the rhizosphere to collaborate together for nutrient availability and uptake, particularly phosphorus. Their combination in presence of biochar facilitates plant root growth which absorbs nutrients from the soil more easily and shows optimum nutrient concentrations in the above-ground part of the plant. Moreover, strong linkage of mycorrhizal fungi to the plant roots ensures minimum nutrient loss and plant quality improvement.

In this study, two biochars from Phragmites and sawdust were prepared at 350° C and used in Lithic xerorthent soil. Two rates of P_2O_5 (0 and 0.433g kg⁻¹ soil) application were used along with different combinations of arbuscular mycorrhizae fungi (AMF) and phosphorus solubilizing bacteria PSB. Nutrient concentration in the roots and shoots were evaluated by dry ash method using ICP-OES along with root infection percentage.

Maximum 80% of root infection was observed in the AMF and PSB inoculated treatment with sawdust biochar. In AMF and PSB inoculated maize plants, more nutrient uptake was observed in both biochar amended soils.

Previous studies reported that AMF application increase plant nutrient uptake and also biochar assist the plants in root growth development which increase plant growth and nutrient uptake (Ortas 2012).

The combination of AMF, PSB, and biochar has not been evaluated previously and their combination can prove a major milestone for organic farming and food security.

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Keywords: mycorrhizae, phosphorus, bacteria

P (ID 340)

Diversity and specificity of AM fungi in the rhizospheres of six common plants in Songnen grassland, China

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Introduction/Aim: Arbuscular mycorrhizal fungi (AMF) have mutualistic relationships with the majority of plant species. However, the diversity of AMF is not very high and the host-specificity is not the strong interaction^[1]. Moreover, the parallel studies on diversity and specificity of AMF are seldom conducted in grasslands. Thus, we investigated the AMF community composition in the rhizospheres of six common plant species, in Songnen grasslands of North China to compare the diversity of AMF colonizing the different roots, and to determine the host-specificity.

Materials and Methods: Six common plant species in *Leymus chinensis* meadow steppe (*L. chinensis, Calamagrostis epigejos, Lespedeza hedysaroides, Vicia amoena, Artemisia lavandulaefolia,* and *Carex duriuscula*) are selected as our target plants. Their roots and rhizosphere soils were collected. The indentification and classification of AMF spores mainly based on their morphological characteristics. The spore density and infection rate were further measured.

Results: 24 AM fungal species were identified and classified into 6 genera. *Acaulospora* and *Glomus* were the dominant genus. Species richness of AMF in the rhizospheres of six plants was not significantly different. *Glomus melanosporum* and *Glomus claroideum* were the mutual dominant species of the rhizospheres of six plants. However, the subdominant species and companion species are different for AMF community in the rhizospheres of six plants. The AMF spore density, spore density and infection rate in the rhizospheres of six plants were different of significance.

Discussion: The difference in chemical composition of the litter and root exudates is likely to be one of the most important reason, leading to the difference in AMF community characteristic from plant rhizospheres^[3].

Conclusion: AMF species richness is not obviously different. However, the change in subdominant species, spore density and infection rate still could exhibit a trend towards the high host-specificity.

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Keywords: diversity of AM fungi, specific selection, host plant species, songnen grassland, *Leymus chinensis* meadow steppe

P (ID 372)

Defense-related phytohormones alter the structuring of the root microbiome in Grey poplar

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Tree roots are colonized by ectomycorrhizal (EcM) fungi and rhizospheric/endophytic bacteria and fungi - the microbiota. EcM fungi play a key role in the host nutrition, whereas endophytic associates may modulate the plant resilience to biotic and abiotic stresses. The molecular mechanisms shaping the tree microbiota are not known. Here, we aim to determine how the plant defense signaling pathways regulate the root microbiome in Grey poplar (Populus tremula x P. alba). We surveyed the root microbiota from poplar lines constitutively expressing the fungal protein MiSSP7 (Mycorrhizal-induced Small Secreted Protein of 7 kDa). MiSSP7 is released by the EcM Laccaria bicolor, enters root nuclei and interacts with the transcriptional regulator JAZ6, the jasmonate co-receptor. By preventing JAZ6 degradation, MiSSP7 represses the triggering of the jasmonate-related defense signaling pathways. Wild type (WT) and MiSSP7-overexpressing poplar lines were grown in natural soil. Rhizospheric and bulk soils, and roots were sampled after 10 days and 6.5 weeks. Metabolite profiles of WT and MiSSP7 lines were measured by gas chromatography-mass spectrometry, whereas the root fungal and bacterial communities were surveyed by rDNA metabarcoding. We show that the fungal colonisation of MiSSP7-overexpressing roots was 30-fold higher than WT lines, suggesting that the alteration of JA signalling by MiSSP7 facilitated fungal colonization. Metabolites accumulated in roots colonized by EcM fungi were also more abundant in MiSSP7-overexpressing lines. Our results suggest that MiSSP7 may not only play a role in the formation of mycorrhizal symbiosis, but it could also influence the structuring of root-associated microbial communities.

Keywords: microbiome, DNA metabarcoding, jasmonic acid, poplar, MiSSP7

P (ID 377)

AM fungal community associated to *Vitis vinifera* cv. Pinot Nero treated with integrated pest managements

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Vitis vinifera (L.) is an economically important crop whose value largely depends on fruit quality that can be influenced by soil migroorganisms, among which arbuscular mycorrhizal fungi (AMF). AMF, able to establish symbiotic associations with vine roots, have beneficial effects on grapevine performance, including water use efficiency and replant success. Most grapevine varieties are susceptible to diseases, whose control can be performed by different approaches, including integrate pest practice (IPM). Previous reports suggested a host specificity among grapevine and AMF and the importance of soil characteristics on this association. In the present study, we examined the AMF communities in the rhizospheric and bulk soil of V. vinifera cv Pinot Nero, subjected to IPM, by using 454 Roche sequencing technology. The bulk and the rhizospheric soil of the grapevines were sampled before and after grape production. Genomic DNA was amplified, after extraction, according to the methods for pyrosequencing, by nested PCR using AMF specific primers of the large ribosomal subunit (LSU rDNA). Sequences were compared with both NCBI and an AMF LSU rDNA reference databases. Our data showed different AMF communities in the rhizospheric and bulk soil of V. vinifera and the importance of the sampling time in regulating AMF biodiversity.

P (ID 380)

Community characteristics of Arbuscular mycorrhizal fungi associated with Chinese fir in different age stages

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Arbuscular mycorrhizal fungi (AMF) play an important role in plant-fungi communities. However, studies about AMF associated with Chinese fir (a majority timber plants in China) are scarce. To establish an understanding of AMF community dynamics associated with Chinese fir, 30 root samples were collected from Chinese fir stands with five different age stages in Jiangle National Forestry Farm, Fujian province. A total of 150 AMF operational taxonomic units (OTUs) were identified. AMF diversity changed as the host plant grows, and a significantly higher Observed otus and Shannon index were present in the age of nine than others. Non-metric multi-dimensional scaling (NMDS) analysis revealed that significant cluster were observed among young, adolescent and three mature stages, while no significance among near mature, mature and over mature. 48 OTUs of AMF were found to be indicator species, belonging to Glomus, Archaeospora and Acaulospora mellea. According to partial least squares path modeling (PLS-PM), AMF community was mainly affected by the age of host plant, total phosphorus may exert a strong positive effect on AMF community diversity, and available potassium may negatively affect AMF composition. Exploring the changes of AMF communities along host plant age stages could help us better elaborate managing forests.

Keywords: Illumina high-throughput sequencing, arbuscular mycorrhizal fungi (AMF) community composition, Cunninghamia lanceolata, indicator species, soil factors

P (ID 404)

Disentangling microbial interactions upon utilization of soil organic N by arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi (AMF) can significantly contribute to plant nitrogen (N) uptake from complex organic sources in soil, most likely in concert with activity of soil saprotrophes releasing the N bound in the organic compounds. Yet, since most of the model experiments have so far been carried out only with isotopically labeled plant litter, utilization of organic N from other sources by the AMF hyphal networks remains little understood. Here we tested whether AMF (Rhizophagus irregularis) hyphal networks show any preferences towards different forms of organic N (chitin of fungal or crustacean origin, DNA, clover biomass, or albumin) administered in spatially discrete patches in a single pot. Further, by direct ¹⁵N labeling, we quantified the flux of N from the organic soil amendments through the AMF hyphae to the plants (Andropogon gerardii). Mycorrhizal plants grew 6.5 fold larger and accumulated, on average, 19 fold more ¹⁵N originating from the organic sources (fungal chitin or clover biomass) than their non-mycorrhizal counterparts. The AMF hyphae (as per quantitative PCR analyses) colonized patches supplemented with any organic N significantly more than patches with no (organic or mineral) N amendment. Molecular profiling of microbial communities in the different patches indicated consistently higher abundance of ammonia oxidizers in the organic N patches, whereas Acanthamoeba protists (key functional group in soil microbial loop) were detected almost exclusively in clover or albumin- amended patches. Other protists (e.g., Vermamoeba sp.) might have replaced Acanthamoeba in chitin-amended patches, as suggested by preliminary metatranscriptome analyses.

P (ID 410)

The impact of Aridity on Microbial Diversity of Opuntia ficus-indica

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Many native microbes such as AMF and PGPR are known to enhance stress tolerance in a range of crop species and to help plants to prosper in degraded arid and semi-arid areas. However, the associations Opuntia cacti establish with microorganisms and the rules governing microbial community assembly remain poorly understood.

In order to characterize the cactus stress-specific microbe association mechanisms under adverse climatic conditions and its microbial symbiont effects, microbial diversity of Tunisian rhizospheric cacti were investigated using cultivation-independent (DGGE) and high next-generation sequencing (Miseq-Illumina) approaches. Soil and roots samples we collected from cacti following a bioclimatic gradient from low to high arid conditions.

High-throughput sequencing (Mi-seq) demonstrated that the abundant OTUs from soil and root samples were related to *Proteobacteria* and *Actinobacteria* phyla. In addition, *Firmicutes* and *Chloroflexi* phyla, *Bacillus* and *Rubrobacter* genera were also abundant in soil samples. However, *Cyanobacteria* phylum and bacterial species belonging to *Burkholderia*, *Pseudomonas* and *Erwinia* were rather abundant in roots. The identities of the fragments of the fungal rRNA-ITS region showed that the dominant fungal genera detected in soil and roots samples were *Fusarium* and *Penicillium*. *Alternaria*, *Aspergillus* and *Neosartorya* were abundant and detected in cacti roots.

The composition and assembly of microbial communities associated with Cacti were highly influenced by the bioclimatic conditions and soil physicochemical proprieties. The microbial diversity was specifically higher in upper arid areas as compared to humid, semi-arid and lower arid areas. Furthermore, soil exhibited the highest levels of microbial diversity whereas the root part showed the lowest.

P (ID 425)

Root mycobionts of the seagrass *Posidonia oceanica* in the central Adriatic – microscopy, culturing and 454-pyrosequencing

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A significant part of the hitherto unexplored fungal diversity is hidden in the marine environment. At the same time, plant tissues host endophytic communities often dominated by yet undescribed fungal lineages. Here we focused on the Mediterranean endemic seagrass Posidonia oceanica and screened its root mycobionts at eight localities in the Croatian central Adriatic Sea using (stereo-)microscopy, culturing from surface sterilized root segments and 454-pyrosequencing. Our microscopic observations revealed that roots from all investigated localities possessed the typical dark septate endophytic association recently reported in this seagrass in the northwestern Mediterranean Sea. Both culturing and pyrosequencing detected very narrow fungal communities lacking typical terrestrial root endophytes. Similarly to the NW Mediterranean, these were dominated by the hitherto undescribed slow-growing mycobiont from the Pleosporales (one OTU $^{\sim}$ ca. 92% from the total 430 isolates, and two MOTUs $^{\sim}$ ca. 92% from the total 393 fungal sequences) and also comprised mycobionts from the Lulworthiales (two OTUs ~ ca. 8% of isolates, and one MOTU ~ 1.3% of sequences). Such a narrow, single-species dominated root mycobiont spectrum is unusual for photoautotrophic vascular plants and points at a close symbiotic relationship between the dominating pleosporalean mycobiont and the dominant Mediterranean seagrass. Additionally, the apparent lack of typical terrestrial root endophytic fungi as well as their probable substitution by the pleosporalean mycobiont so far not known from other hosts or ecosystems implies relatively long specific coevolution of both marine organisms.

Keywords: seagrasses, marine fungi, dark septate endophytes, Pleosporales, Lulworthiales

P (ID 438)

Resilience of root fungal communities under experimental warming and northern light climate

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Global warming is driving plant range shifts to higher latitudes. However, plants encounter different light environment in the north. Elongation of many tree species dependent on photoperiod, which varies substantially across latitudes. Moreover, spectral composition of light is different between high and low latitudes. We conducted a reciprocal transplantation study, where coniferous seedlings from southern and northern tree populations were grown under similar experimental temperature conditions in southern (60°N) and northern (69°N) Finland. Growth chambers in northern site were heated according to real-time temperature signal from south. Norway spruce and Scots pine seedlings were grown for 3 months in experimental chambers, after which plant biomass was recorded and root-associated fungal communities determined by sequencing fungal rDNA on Ion Torrent PGM platform. In both species shoot elongation was higher in southern populations, while northern populations allocated more biomass below ground. Light conditions of the experimental sites had less impact on plant growth. Norway spruce and Scots pine supported largely different root fungal communities, although Thelephora/Tomentella clade was dominant in both. Composition of root-associated fungi in Norway spruce differed between northern and southern populations, likely due to different nurseries they originated. In roots of Scots pine, fungal community composition and ectomycorrhizal fungal (EMF) reads showed site x population interaction. Abundance of EMF was highest in southern pine populations in south and in northern populations in north. Our results indicate that plant growth and composition of root-associated fungal communities vary between populations but may be more resilient to changes in light conditions.

Keywords: global warming, range shift, tree population, root-associated fungi, ectomy-corrhizal

P (ID 450)

Ectomycorrhizal carbon allocation to soil bacteria in the mycorrhizosphere of Fagus sylvatica

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Current hypotheses suggest transfer of recently photoassimilated carbon (C) via mycorrhizal hyphae to adjacent soil bacteria. Hyphal C exudation may be benefitial for both mycorrhizal fungi and plants, as labile C facilitates decomposition of complex organic compounds by soil saprotrophs, which increases availability of limiting nutrients like nitrogen (N) or phosphorus. However, only limited experimental evidence is available, especially for ectomycorrhizal systems. Here, we tested plant-derived C allocation in the mycorrhizosphere of *Fagus sylvatica*, and applied spatially restricted N fertilisation to investigate microbial community responses.

Stable isotope probing with $^{13}\text{CO}_2$ was applied to trace the fate of recent photosynthates. Plant roots were divided into two soil compartments, each connected to a hyphae-exclusive litter compartment separated by a mesh (35 μ m). N fertilisation was applied to only one of two litter compartments.

After a 17 h labelling period, we found significant ¹³C enrichment in PLFAs specific for fungi and bacteria in the hyphae-exclusive litter compartment. Nanoscale secondary ion mass spectrometry (NanoSIMS) imaging enabled visualisation of plant-derived C both within and as accumulated exudation products in bacterial cells outside extraradical hyphae.

Our results show rapid transport of recent photosynthates via ectomycorrhizal hyphae to associated soil bacteria. A decline in biomass of Actinobacteria and other Gram positive bacteria with N fertilization suggests to be a reaction of ectomycorrhizal fungi acting on associated soil bacteria, as the effect is also observed in soil compartments where fertilized N would only be available for bacteria through hyphal transport.

Keywords: ectomycorrhizal (EM) fungi, hyphal carbon transfer, mycorrhizosphere priming, PLFAs, NanoSIMS

P (ID 480)

Geosiphon pyriformis a unique arbuscular mycorrhizal fungus

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Geosiphon pyriformis is an exceptional example of an arbuscular mycorrhizal (AM) fungus forming a unique endosymbiosis with the cyanobacterium *Nostoc punctiforme*, in which the membrane, enclosing the cyanobacteria cells, is homologous to the fungal plasma membrane and the occurring nutrient exchange corresponds with the plant-fungal symbiotic interface in AM.

Up to now it is still not known, whether *G. pyriformis* forms an AM beside the symbiosis with cyanobacteria. We aim to show if *G. pyriformis* forms intraradical structures, to verify the position of *G. pyriformis* within the AM fungi, and its potential as a model system for Glomeromycota, due to easy mRNA separation between the fungal and the cyanobacterial partner (polyA+ selection).

So far, we established monoxenic cultures of the Geosiphon-Nostoc symbiosis from symbiotic bladders obtained from the stable habitat in Bieber (Spessart, Germany), and will use the spores of *G. pyriformis* in a greenhouse experiments with different plant hosts, to detect potential intraradical structures.

Furthermore, we will study the genome of *G. pyriformis*, which will give us a deeper insight into the genes involved in the obligate biotrophic lifestyle and the AM symbiosis itself. This knowledge will be enlarged with RNA-Seq data, to determine the differentially expressed genes in the different symbiotic states of *G. pyriformis*, as the bladders (fungus-cyanobacteria), the fungal-plant part and the fungal spores.

The Geosiphon-Nostoc symbiosis is of interest not only for functional characterization of the AM, but also as a valuable model to uncover evolutionary aspects of AM fungi.

Keywords: Geosiphon pyriformis, arbuscular mycorrhiza

P (ID 481)

Root-associated fungi respond more strongly than rhizosphere-soil fungi to N fertilisation in a boreal forest

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Pinus sylvestris forms ectomycorrhizal associations with many fungi, but there are still few studies of the fungal microbiomes associated with its roots and rhizosphere soil, or of how these vary between different soil horizons. Ectomycorrhizal fungi mobilise N and P from organic polymers and P and base cations from mineral substrates that are weathered at different rates. However, the roles of individual taxa and their location within the soil profile are still poorly understood. We used high throughput sequencing to investigate fungal community structure in roots and rhizosphere soil from the O, E and B horizons of an 85-year-old boreal pine forest with an unfertilised control and a fertilised treatment (150 kg N ha⁻¹ 16 months prior to sampling). Statistical analyses revealed distinct fungal communities associated with roots and rhizosphere soil in all horizons, irrespective of N-fertilisation. There was a significant effect of soil horizon on both root and rhizosphere soil fungal communities. Fungal species richness decreased with soil depth in both rhizosphere soil and root samples. In soil, N-fertilisation increased fungal diversity in the O horizon but had no effect in the mineral horizons. In roots, N-fertilisation decreased fungal diversity significantly in all horizons. Significant differences between soil and root-associated fungal community structure in all horizons, and the stronger effect of N on root-associated fungal diversity, implying that root-colonising fungi may be more sensitive to N fertilisation, suggest that both soil and root-associated fungal communities should be analysed for accurate monitoring of environmental perturbations.

Keywords: N-fertilisation, *Pinus sylvestris*, podzol horizon, fungal communities, rhizosphere soil and root

P (ID 485)

Mycorrhizal phosphate uptake pathway affects maize root-associated microbiome

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Phosphorus (P) is an essential nutrient for plants and often limits growth performance. Arbuscular mycorrhizal (AM) fungi as part of the root-associated microbiota are beneficial for maize growth on soils with low nutrient availability, especially P. Plants utilize mycorrhiza-specific orthophosphate (Pi) transporters to take up fungus-derived Pi. In maize the *Pht1;6* gene encodes a mycorrhiza-specific Pi transporter. Loss of function of *pht1;6* mutant plants are impaired in AM symbiosis and thus display reduced growth under Pi-deficient conditions.

In the present study, the impact of *Pht1;6* knock-out on fungal microbial community structure and composition in maize roots were investigated. To this end, root and the corresponding rhizosphere materials from *pht1;6* and wild type (WT) plants grown in soil pots in a greenhouse trial were sampled and then analysed by ITS2 amplicon sequencing. We observed substantial differences in fungal community structure and relative abundance of fungal orders between *pht1;6* and WT roots. These thus suggest and important role of mycorrhizal phosphate uptake in fungal microbiome development.

Future research will include a large-scale study at the transcriptome, metabolome, ionome, and microbiome levels to decipher the complex relationships between mycorrhizal phosphate uptake and soil microbial communities and their function in maize development and growth.

P (ID 488)

Fungi diversity associated to root of Zygopetallum maxillare (Orchidaceae)

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Orchid mycorrhizal fungi are mainly represented by fungi from Sebacinaceae, Tulasnel-laceae and Ceratobasidiaceae families. *Zygopetallum maxillare* is an epiphytic orchid species, which grows exclusively on two phorophyte fern species: *Cyathea delgadii* and *Dicksonia sellowiana*. Here, we investigated the fungal community in root samples of *Z. maxillare*.

The orchid roots were collected in plants growing on both phorophytes, growing in three sites of Atlantic Forest, in Minas Gerais and Rio de Janeiro States. The whole fungal community was sequenced by Illumina MiSeq using the primers ITS1F e ITS4.

The Ascomycota fungi were the dominating phylum and *Cladophialophora* were the more frequent genus, while *Capronia*, *Rhinocladiella*, *Phialocephala* and *Taphrina* (Ascomycota) and one unidentified Mucoraceae (Zygomycota) were the genera shared in all samples. Two sites of Rio de Janeiro exhibited the same OTUs richness, but this was higher in Minas Gerais and in orchids on *C. delgadii*, even after rarefaction. Among Basidiomycota, *Trechispora*, *Auriculariales*, *Sebacina* and *Ceratobasidium* genera displayed frequency greater than 2%. The latter two are well-known orchid mycorrhizal fungi although showed low or no shared OTUs between sites and phorophytes.

The high number of ascomycetes has been reported in orchid roots, as endophytes, with unknown function. The little sharing of mycorrhizal fungi, between the sampling sites and phorophytes, highlighted the influence of these factors shaping the fungi occurrence pattern. These findings show that *Z. maxillare* associates with *Sebacina* and *Ceratobasidium* mycorrhizal genera and that most OTUs are restrict to one site.

P (ID 491)

The fine scale distribution of orchid mycorrhizal fungi of the leafless epiphytic orchid, Dendrophylax lindenii

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All orchids depend on mycorrhizal fungi for seed germination, and based on stable isotope data, at least some also depend on fungi as a carbon source as adults. The role that these fungi play in driving fine scale distribution patterns of orchids remains unclear. To assess the role of mycorrhizal fungi in phorophyte (host tree) specificity, we investigated the root microbiome of a leafless epiphytic orchid Dendrophylax lindenii, the ghost orchid. Florida individuals of D. lindenii are restricted to two phorophyte species. We tested the hypothesis that the source of D. lindenii root microbiota was from its phorophytes using amplicon sequencing of ITS on an Illumina miSeq (2 x 250). We collected root samples and subtending bark from individuals of D. lindenii and other epiphytic orchids growing on their preferred phorophytes (Fraxinus caroliniana and Annona glabra) from the Florida Panther National Wildlife Refuge in south Florida, USA. Preliminary results of roots identified 708 fungal OTUs (i.e. 97% sequence similarity). Epiphytic orchids associated with a mix of eight putative orchid mycorrhizal fungi including Ceratobasidium, Sebacina, Tomentella and Tuber plus a mix of saprobic species. Subtending bark of phorophytes hosted mycorrhizal fungi and potentially serve as the source of the root mycorrhizal fungi.

CONCURRENT SESSION: Molecular programming of mycorrhizal symbioses

Currently available technologies and massive flow of sequencing data from ongoing projects is allowing unprecedented insights into general patterns and specificities of mycorrhizal symbiosis across the different partner combinations and mycorrhizal types. This session shall offer a platform to discuss parallels and/or uniqueness of processes related to partner recognition (early communication and signaling events), molecular dialogue between symbiotic partners, mechanisms of exchange of nutrients, carbon and signal molecules between the partners, as well as possible evolution of mycorrhizal functioning from or to facultative parasitic interactions. This session will also offer overview of associated technologies and methods including gene silencing, symbiotic mutants and simplified ex-vivo models.

Chairs: Annegret Kohler, Pierre-Emmanuel Courty

CL (ID 60)

Reprogramming root cells for arbuscular mycorrhiza

<u>Didier Reinhardt</u> (Biology, University of Fribourg, Fribourg, Switzerland), **Mélanie K. Rich** (Biology, University of Fribourg, Fribourg, Switzerland), **Pierre-Emmanuel Courty** (Biology, University of Fribourg, Fribourg, Switzerland)

Background & Aims: Development of arbuscular mycorrhiza (AM) requires a fundamental reprogramming of root cells for symbiosis. A recently identified GRAS-type transcription factor in *Petunia hybrida*, ATA/RAM1, is required for gene induction in the host, and for morphogenesis of the fungal endosymbiont. To better understand the role of RAM1 in symbiosis, we set out to identify all genes that depend on activation by RAM1 in mycorrhizal roots.

Methods & Results: We have carried out a transcript profiling experiment by RNAseq of mycorrhizal plants vs. non-mycorrhizal controls in wild type and *ram1* mutants. The results show that the expression of early genes required for AM, such as the strigolactone biosynthetic genes and the common symbiosis signalling genes, is independent of RAM1. In contrast, genes that are involved at later stages of symbiosis, for example for nutrient exchange in cortex cells, require RAM1 for induction. RAM1 itself is highly induced in mycorrhizal roots together with many other transcription factors, in particular GRAS proteins.

Discussion & Conclusion: We conclude that RAM1 acts as an early transcriptional switch that induces most AM-related genes, among them genes that are essential for the development of arbuscules, such as STR, STR2, RAM2, and PT4. Taken together, these results indicate that the defect in the morphogenesis of the fungal arbuscules in *ram1* mutants may be an indirect consequence of functional defects in the host, which interfere with nutrient exchange and possibly other functions on which the fungus depends.

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Keywords: RAM1, transcriptome

CL (ID 322)

Evolutionary conservation of mycorrhiza-specific phosphate transporter gene regulation in *Lotus japonicus* by CTTC MOTIF-BINDING TRANSCRIPTION FACTOR1 (CBX1)

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The periarbuscular membrane (PAM) is plant derived and harbors a specific set of membrane proteins. By surrounding growing and mature arbuscules it serves signaling and the reciprocal exchange of nutrients and metabolites in the arbuscular mycorrhizal symbiosis. We are interested in the protein composition underlying PAM functionality and therefore performed RNA-sequencing with mycorrhizal Lotus roots. A set of 43 mycorrhiza-regulated transcription factors were screened for binding the cis-regulatory CTTC element which is required for mycorrhiza-specific regulation of phosphate transporter genes. We identified AP2 family transcription factor CTTC MOTIF-BINDING TRANSCRIP-TION FACTOR1 (CBX1) which activates Lotus japonicus phosphate transporter 4 (LjPT4) and the mycorrhiza-inducible gene encoding H⁺-ATPase (LjHA1), implicated in energizing nutrient uptake across the peri-arbuscular membrane. The CBX1-CTTC regulatory mechanism is conserved in different mycorrhizal host plant species. Genome-wide binding profiles of CBX1 reveal a subset of a regulatory network in the AM symbiosis. Our finding defines the role of CBX1 in the activation of a gene module essential for the mutualistic AM symbiosis and supports an evolutionary conservation model on the control of mycorrhizal phosphate uptake in the eudicots.

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CL (ID 104)

Metal transport in arbuscular mycorrhizas

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As a result of the essential but toxic nature of the transition metals Cu, Fe and Zn, all organisms have evolved specialized mechanisms to control precisely the levels of these metals within cells. Arbuscular mycorrhizal fungi play an important role in modulating plant metal acquisition in a wide range of soil metal concentrations. Moreover, given the essential roles played by metals in all living organisms, a lack or excess of essential metals should have a profound effect not only on the plant but also on the fungus and, by extension, in their interaction. However, we are far from understanding the key molecular determinants of metal homeostasis in arbuscular mycorrhizas.

To get some insights into these mechanisms and since transporters represent the first line of defence to perturbations of cellular and subcellular metal homeostasis, we are currently studying the plant and fungal metallotransportomes.

We are characterizing metal transporters in *Rhizophagus irregularis* extraradical mycelia and in maize mycorrhizal roots.

On the fungal side, we have observed an expansion of some families of metal transporters and up-regulation in the *R. irregularis* intraradical mycelium of a certain number of genes encoding transporters mediating metal uptake and the mobilization of the vacuolar stores. On the plant side, we have also observed a specific activation of some metal transporters in mycorrhizal roots. The most recent progresses in the identification and characterization of these transporters will be presented and discussed.

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Keywords: metal transport, Rhizophagus irregularis, copper, iron

ST+P (ID 61)

Unlocking the Door for Mycorrhizal Symbioses: Do Endo- and Ecto-mycorrhizal Fungi Use the Same Key?

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Arbuscular mycorrhizal (AM) fungi produce various signaling molecules, including lipochitooligosaccharides (LCOs) and short chitooligosaccharides. These fungal signals are perceived and transduced in host plants via a highly conserved "common symbiosis pathway" (CSP). Genomic data suggested that ectomycorrhizal (ECM) fungi, e.g. Laccaria bicolor, may be capable of producing LCOs and that some ECM host plants like Populus spp. contain all the components of the CSP. Based on this observation, we hypothesized that some ECM fungi may produce LCOs and that the CSP could play a role not only in AM but also in ECM associations. We found that hyphal exudates from several ECM fungi but not short chitooligosaccharides can trigger typical root hair branching in Vicia sativa suggesting the presence of non-sulfated LCOs in these ECM exudates. In contrast to sulfated LCOs and negative controls, non-sulfated LCOs triggered an increase in both lateral root formation in hybrid poplar (Populus x canescens) and the total number of root tips colonized by L. bicolor. We utilized RNA interference to silence the expression of core components of the CSP, including CCaMK. Increased lateral root formation induced by nonsulfated LCOs did not occur in the CCaMK-RNAi line. Furthermore, during colonization of poplar by L. bicolor, mantle width, Hartig net depth, and the expression of the phosphate transporter PtPH12 were significantly reduced in the CCaMK-RNAi line compared to a wild-type control. Altogether, our data suggest that LCOs are produced by some ECM fungi and that the CSP has been recruited for the establishment of ECM associations.

References:

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Keywords: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, poplar, lipo-chito-oligo-saccharides, common symbiosis pathway

ST+P (ID 134)

A functional approach towards understanding the mitochondrial respiratory chain role in an endomycorrhizal symbiosis

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Organism fitness and adaptation to the changing environment can be driven by the modulation of mitochondrial respiratory chain. Nevertheless, the role of the respiratory function in endomycorrhizal relationships remains largely unexplored. We hypothesized that the alternative oxidase (AOX) and the cytochrome oxidase (COX) pathways are involved in specific mycorrhizal behaviors.

At the pre-symbiotic phase (axenic conditions), we studied phenotypic responses of *Rhizoglomus irregulare* spores applying AOX and COX inhibitors (SHAM and KCN, respectively) and two growth regulators (ABA and Ga₃). At the symbiotic phase, we analyzed phenotypic and transcriptomic (genes codifying for proteins involved in respiration, transport, and fermentation) responses in *Solanum tuberosum/Rhizoglomus irregulare* biosystem (glasshouse conditions): we monitored the effects driven by ABA, and explored modulations induced by SHAM and KCN under five phosphorus concentrations.

KCN and SHAM inhibited *in vitro* spore germination while ABA and Ga₃ induced differential spore germination and hyphal patterns. ABA promoted mycorrhizal colonization, strong arbuscule intensity and positive mycorrhizal growth dependency (MGD). In ABA-treated plants, *R. irregulare* induced down-regulation of *StAOX* gene isoforms and upregulation of genes involved in plant COX pathway. Respiratory inhibitors induced opposite mycorrhizal patterns *in planta* in all phosphorus concentrations: KCN induced higher *Arum*-type arbuscule density, positive MGD but lower root colonization compared to SHAM, which favored *Paris*-type formation and negative MGD.

Following our results and current state-of-the-art knowledge, we discuss metabolic functions linked to respiration that may occur within mycorrhizal behavior. We highlight potential connections between AOX pathways and fermentation, and we propose new research and mycorrhizal application perspectives.

Keywords: mitochondrial respiratory chain, phosphorus, mycorrhizal growth dependency, *Rhizoglomus irregulare*, *Solanum tuberosum*

ST+P (ID 260)

How hormone interplay, carbon partitioning and plant priming affect the endomycorrhizal symbiosis: a theory

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AMF, as obligate biotrophs, depend on plant-to-fungus energy trade for their growth within roots. The regulation of AMF root colonisation is driven by a myriad of various signalling events, where particular hormones, phosphorus and fungal effector molecules play key roles *in planta* by modifying plant energetic metabolism including mitochondrial respiration.

The physiological state of the plant is one of the main drivers of AMF development and function. Here, we propose a model which describes the favourable (low P) and unfavourable (high P) metabolic context for AMF behaviour by linking phytohormone interplay with carbon partitioning (including glycolysis, TCA cycle, mitochondrial respiration, fermentation).

This model reveals that two antagonistic groups of hormones *in planta* drive the differential response under low and high P. One group of hormones, promoting AMF development at low P, seems connected with a reduced glycolytic flow and a more engaged fermentation and alternative oxidase pathways. Another group of hormones, inhibiting mycorrhizal development at high P, seems to be involved in an active glycolytic flow providing electrons to the TCA cycle and the cytochrome oxidase pathway.

Moreover, an IRS- or SAR-like system may be implemented in promotion or inhibition of AMF development by these two antagonistic situations. Understanding this system could lead to innovative biotechnologies based on plant priming that induces a plant physiological context able to promote mycorrhizal development and function.

Keywords: hormone interplay, phosphorus, carbon partitioning, priming, ISR/SAR

ST+P (ID 2)

Epigenetical inheritance in Plant-mycorrhizal interaction: Re-establishing the co-evolution

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Phenotypic plasticity or ecological adaptation has indeed rendered the arbuscular mycorrhizae (AM) symbiosis more robust and sustainable since the evolution. However, the credit for this triumph cannot solely be given to the genetics as because, this interaction involves phenotypic arrangement which reportedly doesn't allow much adaptation opportunities to the symbiotic partners, in the ecological context. So, in finding the rationale, here we propose an idea and a whole new perspective of possible involvement of epigenetics in the establishment and maintenance of mutual interaction for greater ecological adaptations. Epigenetics reportedly provides greater flexibility to the adapting organism under different stressful conditions which may be the case for plant-AM fungal interaction that compelled both the partners to co-evolve and established mutualism for several trans-generations. However, not much attention has been paid to the epigenetics for plant-AM fungal symbiotic co-evolution yet. In this article, emphasis has been made to explore the possibilities for the involvement of epigenetics in the plant-AM fungal symbiosis by discussing DNA methylation, chromatin modification, and gene expression level along with measurement of somatic homologous recombination as potential epigenetic traits. These hypotheses provide a novel approach and a different perspective to understand the ecological sustainability of plant-AM fungal interaction that warrants empirical investigation, and therefore, may re-establish the co-evolutionary aspect of one of the most successful ecological interactions. Further, it will be helpful in exploiting the interrelations between epigenetic modifications, phenotypic plasticity and ecological adaptations among model ecological interactions such as plant-AM fungal symbiosis to significantly improve their application for agro-ecosystem services.

Keywords: epigenetics, arbuscular mycorrhizae, ecological adaptations, agro-ecosystem, trans-generation

CL (ID 142)

Cell wall remodeling during ectomycorrhiza development

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The cell wall machinery is highly evolved to perform molecular talk between the plant and their interacting microbial partners. All types of mycorrhizal developments require an extensive level of cell wall modification of the interacting partners at the contact surface area to achieve a full spectrum of symbiotic functionality. In ectomycorrhizal symbiosis, the Hartig net, formed through fungal invasion of the apoplastic space between root cells, enables nutrient exchanges between the partner. Hartig Net formation and fungal invasion of the root requires modification of plant and fungal cell walls. Our study aims at understanding the nature of cell wall remodeling required for successful colonization and Hartig Net formation in the Populus/Laccaria bicolor ectomycorrhizal symbiosis and to identify the underlying molecular actors from plant and fungus. Existing transcriptome data show that a set of pectin modifying plant and fungal CAZy genes are significantly induced during this symbiosis. We hypothesize that these candidate CAZymes are potentially associated with cell wall loosening and, later during colonization, stiffening, and act as auxin-mediated down-stream players controlling fungal root colonization. At present we are examining the cell wall remodeling process using biochemical and various microscopic techniques including Raman micro-spectroscopy (1) and immuno-localization with antibodies against cell-wall polymer epitopes. Our immuno-localization data indicates that the cell wall remodeling through pectin demethylesterification is indeed involved in ectomycorrhiza development. The importance of pectin modification for Hartig Net development is revealed by studying *Populus* roots colonized by either *L. bicolor* wildtype or mutants with restricted Hartig Net formation capacity.

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Keywords: ectomycorrhiza, cell wall, pectin, immuno-localization, Raman micro-spectroscopy

CL (ID 160)

The role of protein methylation on the development of ectomycorrhizal symbiosis between Pisolithus and Eucalyptus

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Introduction: Post-translational modifications have profound effects on the structure, localization and function of proteins. Methylation at arginine residues catalyzed by protein arginine methyl transferases (PRMTs) is well studied in mammalian systems, but research into its role in plant systems is more recent and primarily restricted to annual model plants. Even less is know about the effects of protein methylation in plants on negotiating interactions with microbes.

Materials/Methods: Using a combination of chemical inhibition techniques, genetic modification, proteomics and transcriptomics, we consider the effect of protein methylation on the development of the symbiosis between *Eucalyptus grandis* roots and the ectomycorrhizal fungus *Pisolithus albus*.

Results: Intriguingly, we find that two plant PRMTs, PRMT10 and PRMT1, exhibit contrasting roles in the establishment of mycorrhizal root tips: overexpression of PRMT1 fosters symbiosis while PRMT10 overexpression reduces root colonization. Our results suggest that PRMT1 is involved in cell wall and cytoskeleton arrangement and PRMT10 in affecting the transcription of genes involved in hormone signalling and defence pathways.

Discussion/Conclusion: This work is the first highlighting the important role of PRMTs in the development of ectomycorrhizal symbiosis. We show that PRMTs act as major regulators of transcription and protein function, affecting numerous signalling and developmental pathways. These results demonstrate the importance of a proper balance in PRMT activity for the establishment of mycorrhizal symbiosis and the need for future research in the area of post-translational modifications.

Keywords: post-translational modifications, ectomycorrhizal symbiosis, asymmetric arginine methylation

CL (ID 271)

Mycorrhizal reprogramming affects pest resistance of poplar

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The symbiosis between plants and mycorrhizal fungi results in reprogramming of plant metabolism and architecture, affecting plant fitness. Ectomycorrhizal colonization strengthens the plants' defence against aboveground herbivores. The underlying regulatory networks and biochemical mechanisms how mycorrhizal symbiosis at the root tips influences aboveground plant-herbivore interactions are unknown. Here, we will present data on the tripartite interaction between Populus x canescens, the ectomycorrhizal fungus (EMF) Laccaria bicolor and the specialized leaf herbivore Chrysomela populi. We found that feeding damage and the oviposition of C. populi were less pronounced in mycorrhizal compared with non-mycorrhizal plants. We integrated data on transcriptomic, metabolomic and volatiles of mycorrhizal, non-mycorrhizal and infested plants into a network, in which metabolic fluxes were approximated by advanced bioinformatics and metabolomic tools. Our results suggest that the improved protection from leaf beetles results in metabolic changes from phenolics to aldoxime biosynthesis. We also conducted studies with a generalistic herbivore supporting that mycorrhizal inoculation enhances poplar fitness. Our results demonstrate that subtle reprogramming of plant metabolism by belowground plant-microbe associations mitigates aboveground biotic stress.

CL (ID 110)

Petunia as model for breeding mycorrhiza-responsive crop plants

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The advantage of arbuscular mycorrhizas for plants is based on nutrient exchange and increased resistance or tolerance against pathogens and abiotic stress. Whether the interaction results in increased performances of the plant does not only depend on environmental factors, but also on the genotype of both partners. This leads to considerable differences between cultivars of crop plants in the response to colonization by a mycorrhizal fungus. If mycorrhiza should be applied in future production systems, mycorrhiza responsiveness of plants has to be a trait to breed for.

Petunia hybrida is used since ten years as model in mycorrhiza research. Gene maps and markers exist for a long time, but sequencing of the genomes of the progenitor species open up new ways in breeding research.

In a first step, the response of wild species to inoculation with a mycorrhizal fungus was compared. Differences were revealed concerning root colonization, biomass changes and in mycorrhiza-induced resistance (MIR) against a root-pathogenic fungus. First genes which are hypothesized to be involved in MIR have been identified. In ongoing experiments, the phenotypes of crossing populations are analyzed in order to map QTLs for the different responses. Using the genome sequences, further genes can be identified which are involved in the responses of petunia to mycorrhization. Due to the availability of a natural transposon insertion population it is possible to confirm the role of the candidate genes. These genes can be used in future breeding programs as functional markers for achieving new mycorrhiza-responsive cultivars.

Keywords: mycorrhiza responsiveness, mycorrhiza-induced resistance, breeding

CL (ID 282)

Identification of *Medicago truncatula* genes involved in the arbuscular mycorrhizal symbiosis with whole genome sequencing

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Improving the efficiency of the arbuscular mycorrhizal (AM) symbiosis in crop plants offers an excellent opportunity to improve crop nutrition in developing countries and reduce the demand on dwindling phosphate reserves. To engineer an improvement to the AM symbiosis, we need to understand its genetic basis in plants. The common symbiosis pathway (CSP), shared with the root nodule symbiosis in legumes, is well understood, but there is much less information known about the genetic basis required to form the structures like arbuscules unique to the AM symbiosis.

To remedy this, we have been using the Noble Foundation's *Medicago truncatula* collection, mutagenised with the tnt1 transposon. We have identified a number of lines that had a reduced AM colonisation phenotype, but retained normal nodulation, therefore having a non-CSP mutation. These lines have a large (>20) number of transposon insertions, making previous PCR based methods of transposon discovery very time consuming. The rapid decrease in whole genome sequencing (WGS) cost offers a great opportunity, as simple computational methods can be used to locate all transposon insertions in a plant line. We demonstrate this by describing several *M. truncatula* lines with 40-60 copies of the tnt1 transposon and reduced AM colonisation. We used WGS-driven genotyping to show the causal mutations in these lines, including a novel locus of previously undescribed genes at the telomeric end of [MS1] [TI2] Chr7q are causing this phenotype.

CL (ID 229)

Arabidopsis-mycorrhiza, an ambiguous relationship

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Around 18% of all terrestrial plant species are unable to establish arbuscular mycorrhizal (AM) symbioses. The mechanisms that underlie this incompatibility remain large unknown. Here we study the interaction between the non-host plant *Arabidopsis thaliana* and the AM fungus *Rhizophagus irregularis*.

In vitro-grown cultures of Rhizophagus, the fungal pathogen Fusarium oxysporum, and the fungal endophyte Trichoderma harzianum were used to study the specificity of early plant-microbe-interactions in Arabidopsis roots. A bi-compartmental microcosm system with a Rhizophagus fungal network provided by the host Medicago truncatula was used to study late stages of the Arabidopsis-Rhizophagus interaction. The transcriptomic profile of Medicago and Arabidopsis roots colonized by Rhizophagus was analyzed by high-throughput RNA-sequencing (RNA-seq) and compared with non-colonized control plants.

In early stage of the *Rhizophagus-Arabidopsis* interaction, *Rhizophagus* activated the *Arabidopsis* strigolactone biosynthesis genes *CCD7* and *CCD8*, whereas *Fusarium* and *Trichoderma* did not. Conversely, *Fusarium* and *Trichoderma* activated the early plant defense marker genes *MYB51* and *CYP71A12*, while *Rhizophagus* did not. At late stages of the interaction *Arabidopsis* roots colonization by *Rhizophagus* was detected with a shoot biomass reduction associated. AM fungal genes characteristic for nutrient exchange were not expressed in *Arabidopsis* roots and arbuscules were absent, indicating that no functional symbiosis was established. Furthermore, *Arabidopsis* roots colonized by *Rhizophagus* showed a plant defense response activation, which was not observed in colonized *Medicago* roots.

These results indicate that early signaling events between *Rhizophagus* and *Arabidopsis* are not totally impaired and that the incompatibility is conferred at later stages, coupled to plant defense responses.

Keywords: arbuscular mycorrhiza, non-host plants, *Arabidopsis thaliana*, *Rhizophagus irregularis*, RNA-sequencing

CL (ID 262)

Oligosaccharides as signals: a new opportunity for promoting mycorrhizal development and effectiveness in revegetation programs?

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AMF are key soil components providing several ecosystem services. However, mycorrhizal inoculum application faces challenges, such as variability in product quality (in terms of richness and effectiveness), as well as, high soil phosphorus that inhibits the mycorrhizal colonization and plant responsiveness. Moreover, application of exogenous AMF strains may cause genetic replacement of indigenous fungal species and/or may provide only limited responses on plants. This would exclude range of classic commercial mycorrhizal products to be applied in some areas or situations. This case was posed in Mayotte island, under the frame of a reforestation program, in which no specific inoculum was available.

One strategy consists to generate a favorable plant metabolic context to improve mycorrhizal colonization and plant responses by the application of signaling (or inducing) molecules. The first step was to evaluate under greenhouse conditions the effects of oligosaccharides applied at low dose on the mycorrhizal development and mycorrhizal responses, under low and high P concentrations. In a second step, effects of oligosaccharides were monitored on native AMF species capacities during the production phase of tropical plant species at Mayotte.

Within several plant species, our data show that oligosaccharides, in particular glucose, can promote the mycorrhizal development of both exogenous and indigenous AMF species, can stimulate mycorrhizal responses, can counteract the P inhibition and can increase the inoculum quality (richness). Moreover, this new biotechnology represents a powerful tool to support plant growth prior transplantation on badlands areas.

Keywords: oligosaccharide biotechnology, phosphorus, mycorrhizal responses, mycorrhizal inoculum, reforestation program

POSTERS

P (ID 10)

Quantitative RT-PCR analysis of selected genes potentially associated with orchid mycorrhiza interaction in *Dendrobium burana* Charming

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Orchid mycorrhiza is a unique symbiotic relationship between an orchid and its fungal partner. To understand the molecular basis of orchid-mycorrhiza interaction, expression profiles of selected genes involving in water/photosynthate transport, selective protein degradation, and root symbiosis/plant defense were analyzed by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Primers of these genes were designed based on a publicly available suppression subtractive hybridization cDNA library prepared from symbiotic germinated seeds of Dendrobium catenatum. Total RNA was extracted from leaves and roots of micropropagated plantlets of *Dendrobium Burana* Charming cultivated in the presence or absence of a mycorrhizal fungus, Tulasnella deliquescens (Juel) Juel. Six reference genes, i.e., 185 rRNA, two elongation factor 1 (EF-1) genes, GAPDH, and two actin genes were tested for their expression stability under the studied conditions. Only the transcript levels of gene encoding EF- 1α were found to be stable between the tissue of mycorrhizal plants and non-mycorrhizal plants and thus this gene was used for data normalization. The gene encoding a class III chitinase was found to exhibit strongly upregulated expression not only in the roots (175-221 folds) but also in the leaves (6-4799 folds) of mycorrhizal orchids. Other five studied genes also exhibited either upregulated expression (i.e., gene encoding selenium-binding protein) or downregulated expression (i.e., genes encoding plasma membrane intrinsic protein, 121F-specific p53 inducible RNA, calcium dependent protein kinase 32, and trehalose-6phosphate synthase) in the roots of mycorrhizal orchids. Expression patterns of these differentially expressed genes and their possible roles in the orchid-mycorrhiza interaction are discussed.

Keywords: chitinase, differential gene expression, orchid mycorrhiza, *Tulasnella*, *Dendrobium*

P (ID 21)

High phosphorus tolerant mutants of *Gigaspora sp.* induced by Gamma rays and UV irradiation

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Gigaspora sp. is one of the Arbuscular Mycorrhiza fungi that are now widely used to improve plant growth and health. However, spore germination of this species will be inhibited in high phosphorous (P) environment. The aim of this study was to obtain Gigaspora sp. mutants which are tolerant to high P condition using Gamma rays and UV irradiation.

The spores of *Gigaspora* sp. were exposed to Gamma rays with different irradiation dose i.e. 0, 30, 100, 300, 1000, and 3000 gray. Some other spores were exposed to UV light in UV chamber (l=254nm) for 0, 5, 10, 20, 40, and 60 minutes. The treated spores were then put inside cell culture cluster consisted 96 wells which already contained 2000 ppm P solution. Each well was received single spore. The spores were then kept in incubator at temperature of 30°C for 4 weeks after which the number of germinated spore were counted for every treatment.

In this study, we obtained 17 mutants (nine from gamma rays and six from UV) which were able to germinate in 2000 ppm of P solution. None of the wild type were germinated in the mentioned above P concentration. This result indicated that this Gamma rays and UV irradiation can be used as useful tools to improve survival capability of *Gigaspora* sp. in high phosphorus environment.

Keywords: *Gigaspora*, mutant, Gamma ray, UV, irradiation

P (ID 264)

Stimulation of plant growth and nutrition by arbuscular mycorrhizal fungal biomass – living or dead

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Arbuscular mycorrhizal fungi establish symbiotic association with most plant species on Earth. They directly interconnect root cortex with soil, transferring significant amounts of soil nutrients such as phosphorus and zinc to plants and receiving photosynthates in return. Functioning of this symbiosis is thus based on interaction of the two living partners complementing each other in acquisition/sharing of resources. During evolution, symbiotic dialogue involving various compounds and metabolic pathways established to allow partner recognition and root colonization by the symbiotic fungi without eliciting strong defense reaction of the plant. Yet the effects of the individual signaling compounds with a role in symbiosis establishment (e.g., strigolactones, myc-factors) on plant growth and/or nutrition usually remain limited. Here we applied either living or dead biomass (0.0005 weight %) of axenically produced arbuscular mycorrhizal fungus (Rhizophagus irregularis) to unsterile pots with Andropogon gerardii. We observed significant improvement of both growth and phosphorus acquisition of the plants in both of the mycorrhizal fungal biomass-amended treatments as compared to unamended control. By microscopy as well as by molecular analyses we confirmed that there was no mycorrhizal colonization in the roots developing in pots amended with dead mycorrhizal fungal biomass. It seems that the growth and nutritional stimulation of plants by dead mycorrhizal fungi could be either due persistent effects of symbiosis signalling compounds in the soil (i.e., the direct chemical effect) or through induced effect via soil microbiome – such as some members of Firmicutes, whose abundances were significantly elevated after addition of dead mycorrhizal fungal biomass.

Keywords: arbuscular mycorrhiza, dead biomass, phosphorus, signal, microbiota

P (ID 400)

The small secreted protein OmSSP1 is up-regulated in ericoid mycorrhiza and is involved in symbiosis

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Plant-colonizing fungi secrete "effector" molecules that can facilitate fungal invasion of plant tissues by manipulating the host cell metabolism. Symbiosis-induced small secreted proteins (SSPs) identified as fungal effectors have been characterized in ectomycorrhizal and arbuscular mycorrhizal fungi, whereas no information is available for ericoid mycorrhizal (ERM) fungi. ERM fungi are soil-born fungi, mostly belonging to Leotiomycetes (Ascomycetes), which form endomycorrhiza with plants in the family Ericaceae. The sequenced genome of the ERM model fungus *Oidiodendron maius* contains 445 *SSPs* genes. Aim of our work was to identify, among them, SSPs potentially involved in the molecular dialogue governing the ERM symbiosis.

RNA-Seq data revealed that 20% of *O. maius SSPs* are up-regulated during ERM symbiosis. The most highly symbiosis-induced *SSPs*, named OmSSP1, was functionally characterized through: a) gene expression validation; b) molecular characterization *in silico*; c) secretion assays; d) null-mutants generation; e) *in vitro* mycorrhizal synthesis with null-mutants.

OmSSP1 was confirmed to be strongly up-regulated during ERM symbiosis (FC>100). The protein shares some features with class I hydrophobins (i.e. cysteines pattern, hydrophobicity and length) but lacks the hydrophobin PFAM domain. OmSSP1 features a signal peptide and was experimentally confirmed to be secreted. The absence of a Nuclear Localization Signal suggests that OmSSP1 may interact with different plant cell compartments. A significant reduction in the degree of root mycorrhization was observed for three independent OmSSP1 null-mutants, suggesting that this protein is involved in the mycorrhization process.

Keywords: ericoid endomycorrhizal symbiosis, *Oidiodendron maius*, Small Secreted Proteins – SSPs, hydrophobins, homologous recombination

P (ID 412)

The role of auxin biosynthesis and efflux in the fungus *Laccaria bicolor* for ectomycorrhiza formation with Populus tree roots

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Ectomycorrhizal (ECM) symbiosis are mutualistic interactions occurring between tree root tips and soil fungi. In exchange for sugars from plants, fungi transfer nitrogen and phosphorous to the tree. ECM symbiosis are essential for forest ecosystems and promote tree growth and health. Currently our group is focusing on revealing the molecular mechanisms of ECM formation during Laccaria bicolor and poplar interaction. During the colonization process, fungal hyphae adhere to and surround root tips to form a mantle. Subsequently, hyphae penetrate between root cells to form the nutrient exchange structure, called Hartig net (1). Hartig net formation requires cell wall remodeling in both partners and auxin, a phytohormone secreted by the fungus has been hypothesized to favor this process through cell wall loosening. In order to better understand the role of fungal auxin for Hartig Net formation, we study the auxin biosynthesis and release pathways in L. bicolor. We have in silico identified genes involved in the auxin biosynthesis pathway. The transcript response of these genes to exogenously applied auxin biosynthesis intermediates as well as to ECM formation is under investigation. The enzyme activities will be functionally characterized in vitro. To reveal auxin efflux, the transport activity of putative ABCB and PIN/PILS-like transporters, in silico identified, will be addressed using a recently developed ratiometric auxin sensor system (2). Ultimately, functional analysis of L. bicolor mutant (3, 4) for functionally active auxin biosynthesis and efflux genes will be carried out to uncover the role of fungal auxin production and release for ECM formation.

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Keywords: ectomycorrhiza, auxin, Hartig net, cell wall remodeling

P (ID 451)

Fatty acid biosynthesis partially induced by LjERM transcription factors is presumed to regulate arbuscule development

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Arbuscules are the sites of nutrient exchange between plants and arbuscular mycorrhizal (AM) fungi. The formation of arbuscules is suppressed within several hours after highphosphate (Pi) application in rice roots¹. During the suppression by high-Pi, the sub-set of genes required for arbuscule development is thought to be synchronously suppressed. In this study, we performed RNA-seq analysis using AM roots of Lotus japonicus applied with a high-Pi solution to obtain a comprehensive understanding of a mechanism underlying arbuscule development. Arbuscule density in mature AM roots was rapidly decreased by high-Pi application. During the reduction of arbuscule density, 504 genes of the host plant were significantly down-regulated, which included a set of genes related to fatty acid biosynthesis and modification (e.g., acetyl-CoA carboxylase, FatM and RAM2). We found that AP2/ERF transcription factor LiERMs that comprise a sister clade to the fatty acid biosynthesis regulator WRIKLED were also repressed during high-Pi exposure. These genes highly expressed in arbuscule-containing cortical cells. In RNAi lines of LiERMs, the development of arbuscules was severely impaired. Several genes related to fatty acid biosynthesis were repressed in AM roots of the LiERM-RNAi lines at the equal levels to non-mycorrhizal roots of wild type. These results suggest that de novo fatty acid biosynthesis in arbuscule-containing cortical cells plays a crucial role in arbuscule development.

References:

¹Kobae et al. (2016) Plant Physiology 171: 566-579

Keywords: arbuscule development, RNA-seq, lipidome, fatty acid biosynthesis, AP2/ERF transcription factor

P (ID 465)

The transcriptional landscape of *Laccaria bicolor*: Ectomycorrhiza formation, reproduction via sporocarp and impact of nutrition

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Ectomycorrhizal (ECM) fungi are essential for forest ecosystems. In exchange for carbohydrates, ECM fungi offer an improved mineral supply to trees.

Proper functioning of ECM implies differentiation as well as communication and nutrition exchange within the fungal colony. Fungal hyphae surround root tips of host trees and penetrate between their root cells to form a symbiotic structure dedicated to nutrient exchange. Hyphae extending from mycorrhizal roots, the extramatricular mycelium, explore the soil for essential elements such as N and P and transfer them to the host. Eventually sporocarps, both nutrient and carbon sinks, develop to assure last stages of sexual reproduction and sporulation.

The aim of this study is to understand how an ECM fungus uses its gene repertoire to support functional differentiation and development of specialized morphological structures. We performed RNAseq analyses on different developmental stages of *Laccaria bicolor – Populus tremula x alba* ECM and associated extramatricular mycelium, sporocarps

and free-living mycelium under various conditions of external N, P and C supply. We extracted biologically meaningful patterns from the high-dimensional data using the integrative omics platform, Self-organizing map Harboring Informative Nodes with Gene Ontology (SHIN+GO).

Genes specifically upregulated in ECM with a putative role in plant-fungal interaction could be identified and distinguished from genes with rather a function in hyphal aggregation or in N-P and carbon homeostasis. The results of this study direct future functional genetics research in order to better understand how ECM fungi fulfill their important role in forest ecosystems.

Keywords: ectomycorrhiza, *Laccaria bicolor*, transcriptome, sporocarp, nutrition

P (ID 472)

Impact of arbuscular mycorrhizal communities on the biomass potential of prairie cordgrass – a potential bioenergy crop

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Native perennial grasses, such as prairie cordgrass (PCG, Spartina pectinata), and switchgrass (Panicum virgatum) have a great potential as bioenergy crops, because they require fewer inputs, produce more energy, and reduce greenhouse gas emissions in comparison to annual cropping systems such as corn and soybean. Switchgrass has been selected by the U.S. Department of Energy for development as bioenergy crop, but studies have shown that PCG can outcompete switchgrass in terms of biomass production. In order to examine the impact of arbuscular mycorrhizal (AM) communities on the biomass production of PCG, we analyzed the diversity of AM communities of natively grown PCG plants in South Dakota, studied the mycorrhizal responsiveness of different PCG genotypes, and compared the PCG transcriptome in response to AM fungal colonization and different nutrient supply conditions to the transcriptome of the important grass model Brachypodium distachyon. The metagenome analysis revealed that the AM communities of PCG plants in SD were dominated by Glomus hoi, Rhizophagus intraradices/irregularis, and Septoglomus contrictum, and we examined the effect of Rhizophagus intraradices and Glomus agaregatum on the biomass potential of different prairie cordgrass genotypes and found a high genotypic variability in the biomass potential under different nutrient supply conditions and in the mycorrhizal responsiveness of different PCG genotypes. Mycorrhizal benefits were correlated to an improved phosphate but not nitrogen nutrition of the plants. We identified hundreds of genes that were differentially expressed in mycorrhizal and in non-mycorrhizal plants, and discuss the function of these genes for different metabolic pathways.

Keywords: bioenergy, perennial grasses, arbuscular mycorrhizal communities, transcriptome, genotypic differences

P (ID 478)

Re-examination of symbiotic phenotype in Lotus japonicus ME966 mutant

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The common symbiosis signaling pathway (CSSP) is essential for the establishment of both arbuscular mycorrhiza (AM) and root nodule (RN) symbioses in plants. The activation of this CSSP leads to the generation of calcium spiking in and around the nucleus and decoding the calcium spiking by the calcium-calmodulin dependent kinase CCaMK. Downstream of the CSSP, several AM-specific transcription factors including RAM1 are upregulated, which are required for AM fungal accommodation in roots. However, knowledge of AM-specific signal transduction is limited. We isolated an AM-defective mutant, ME966, from Lotus japonicus¹. In this study, we examined phenotypic characterization of this mutant in detail and attempted the identification of the causative gene. Calcium spiking did not generate in epidermal cells of ME966 mutant introducing yellow chameleon YC2.60-NLS after the application of either *Mesorhizobium loti* Nod factor, Myc-lipochitooligosaccharide or chitin mix. This observation was unexpected because ME966 line was originally isolated as a Nod⁺ Myc⁻ mutant under semi-water-logging conditions¹. Then, we re-examined the phenotype of nodulation under semi-water-logging and dry conditions. We confirmed mature nodules in ME966 mutant under semi-waterlogging conditions. However, the number of nodules in ME966 mutant significantly decreased compared to that in wild type under dry conditions, although a few mature nodules were observed. The causative gene of ME966 mutant was mapped in a gap region on chromosome 2 where there are no known CSSP genes. These results suggest the ME966 mutant is a novel symbiotic mutant involved in the CSSP.

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¹Kojima, T., Saito, K., Oba, H., Yoshida, Y., Terasawa, J., Umehara, Y., Suganuma, N., Kawaguchi, M. and Ohtomo, R. (2014) Isolation and phenotypic characterization of *Lotus japonicus* mutants specifically defective in arbuscular mycorrhizal formation. Plant and Cell Physiology 55, 928-941.

Keywords: Lotus japonicus, symbiotic mutant, nodulation

P (ID 508)

AMF antagonism against *Arabidopsis* is unaffected by phytohormone-regulated growthdefense tradeoffs

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Host-supported AMF can colonize the non-host plant Arabidopsis without a symbiotic phenotype [1]. Non-symbiotic colonization generally antagonizes the non-host plants [1-3], but the underlying mechanism of this antagonism remains elusive. The hypotheses of nutrient depletion or fungal allelochemicals were unsupported in previous studies [2, 3]. A new emergent hypothesis proposes that the continuous contact of AM fungi with non-host roots activates costly plant defenses that leads to growth-defense tradeoffs [1], which are regulated in plant by several phytohormones [4]. To test this hypothesis, we studied growth responses to AMF in Arabidopsis mutants impaired in the defense phytohormone SA, JA or ethylene, under the hypothesis that impaired plant defense signaling leads to less AMF antagonism against Arabidopsis. Then, we studied Arabidopsis mutant or overexpression lines impaired in the growth phytohormone auxin, GAs, cytokinins or brassinosteroids, under the hypothesis that an impairment in specific plant growth signals augments the AMF antagonism. Our observations confirmed earlier findings that host-supported AMF antagonizes Arabidopsis (with a plant growth reduction of 24% on average). Compared with wild-type Col-0, neither defense nor growth phytohormonerelated Arabidopsis lines affected the AMF antagonism as predicted. Although we cannot fully exclude that genetic redundancy may mask crosstalk effects, our data from several important phytohormone pathways do not support the growth-defense tradeoff hypothesis. This result combined with previous studies [1-3] suggests that AMF antagonism against non-host plants may depend on a more specialized mechanism yet uncharted.

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Keywords: *Arabidopsis,* non-host plants, mycorrhizal fungal antagonism, phytohormones, growth-defense tradeoffs

PLENARY SESSION: Genomics for understanding mycorrhizal evolution and ecology

Chair: Ian R. Sanders

KL (ID 332)

Getting to the Root of Mycorrhizal Symbioses: Impact of Fungal Genomics on Mycorrhizal Research

Francis M. Martin (Inra, Unité Mixte de Recherche 1136 Interactions Arbres/Microorganismes, Laboratoire d'excellence ARBRE, Centre INRA-Lorraine, Champenoux, France)

Within the plant microbiota, mycorrhizal fungi are striking examples of microorganisms playing crucial roles in nutrient acquisition. They have coevolved with their hosts since the raise of land plants and the recent development of calibrated phylogenies, linked with the growing understanding of fungal genomes, provides remarkable insights into the evolutionary histories of mycorrhizal symbioses. The comparison of 100+ genomes from mycorrhizal fungi, wood decayers, soil decomposers has revealed several independent lifestyle transitions from saprotrophism to mutualism in fungal lineages. In addition to reconstructing the evolution of mycorrhizal symbioses, our growing ability to use reference genomes for profiling differentially-expressed transcripts, for instance, is helping us to identify hundreds of symbiosis-related genes. Mycorrhizal genomics has thus chalked up some sweet victories. But when it comes to how genes shape the traits that matter most to us — from host specificity and nutrient efficiency to plant fitness and plant adaptation — the Age of Genomics underdelivers on actionable knowledge. We have an idea as to some of the genetic underpinnings in plants that render them susceptible to mutualistic fungi, but the molecular mechanisms that underlie the development and physiology of mycorrhizal symbioses have yet to be elucidated. Indeed, a molecular definition of the mycorrhizal symbioses remains a major challenge for the field. We now know what mycorrhizal fungi are present in a specific environment, we have 100+ of ecologically-relevant mycorrhizal reference genomes at our disposal, but are these data useful in furthering our goal of understanding how the mycorrhizal fungal community as a whole contributes toward ecosystem function?

The challenges facing our community include determining the mechanisms that regulate nutrient trade dynamics, as well as characterizing how perturbations of the recognition and accommodation systems influence plant responses, how metabolic responses are regulated in multiple symbiotic interactions and how the signaling pathways triggered by multiple microbial interactions are integrated by host cells. Using synthetic ecosystems constructed using specifically chosen plant and microbial combinations that are easily manipulated and for which we have genomic and molecular data is likely a promising avenue. We must work together as a community to set new research priorities to make meaningful advances in the coming years taking benefit from the Age of Genomics.

Supported by the 'ARBRE' Laboratory of Excellence and the U.S. Department of Energy (ORNL Plant Microbe Interfaces project and the Joint Genome Institute). Thanks to J. M. Plett for insightful discussions.

Keywords: ectomycorrhizae, genomics, evolution

IL (ID 50)

Origin and evolution of genetic diversity within and between the AMF mycelia

Nicolas Corradi (Biology, University of Ottawa, Ottawa, Canada)

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts with a perpetually multinucleated mycelium, and a very controversial biology. Major controversies in AMF research have centered on (i) the genetic organization of co-existing AMF nuclei, which was suggested to be either highly heterokaryotic or homokaryotic, and (ii) on their long-term asexual evolution. Here, I provide evidence the model AMF *Rhizophagus* either homokaryotic (co-existing nuclei harbor one MAT-locus) or "dikaryotic" (co-existing nuclei harbor one of two divergent MAT-loci), and will show that this nuclear organization supports the existence of mating processes in AMF.

Recent data on the structure and annotation of six *R. irregularis* genomes will also be shown. In particular, I will discuss the identification of large inter-isolate genome diversity we recently found in these isolates, and its potential consequences for AMF biology and evolution. The extent of inter-isolate functional and structural genomic divergence also raises intriguing questions regarding their conspecificity, and warrants caution in the interpretation of past "population-based" analyses of these symbionts based on one genome reference.

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IL (ID 94)

Comparative genomics and transcriptomics for understanding ericoid mycorrhizal fungi ecology

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Some soil fungi in the Leotiomycetes form ericoid mycorrhiza (ERM) with ericaceous plants. ERM plants occur in harsh habitats, where they rely on their fungal partners both for nutrients mobilisation from soil organic matter (SOM) and for detoxification of toxic metals. Characterization of the fungal genetic machinery underpinning symbiotic lifestyle and SOM degradation is needed to understand ERM functioning and evolution, and its impact on soil C turnover. We therefore sequenced the genomes of four taxonomically distinct ERM fungi (Meliniomyces bicolor, M. variabilis, Oidiodendron maius and Rhizoscyphus ericae) and compared their gene repertoires to those of 56 other fungi with different lifestyles (ecto- and orchid mycorrhiza, endophytes, saprotrophs, pathogens). Fungal transcript profiling was performed during symbiosis and also in the presence of cadmium. Gene contents for polysaccharide-degrading enzymes, lipases, proteases, and secondary metabolism enzymes place ERM fungi closer to saprotrophs and pathogens than to ectomycorrhizal symbionts. Among the most highly symbiosis-upregulated genes in ERM fungi were cell wall degrading enzymes (CWDE), lipases, proteases, but also transporters and mycorrhiza-induced small secreted proteins (MiSSPs). The most highly symbiosis-induced MiSSP in O. maius was further characterized. Overall, the predicted gene repertoire of ERM fungi reveals a dual saprotrophic and biotrophic lifestyle. This may reflect an incomplete transition from saprotrophy to the mycorrhizal habit. Indeed, our phylogenomic reconstruction provides the first experimental evidence that ERM is the youngest of all mycorrhizal symbioses. Alternatively, this genetic machinery may mirror a more versatile life strategy of ERM fungi, similar to fungal endophytes.

IL (ID 196)

Streamlining the genomes of wood decay fungi for the evolution of ectomycorrhizal symbioses

Eniko Kiss (Institute of Biochemistry, Biological Research Center, HAS, Szeged, Hungary), Marisol Sanchez-Garcia (Clark University, Worcester, USA), Alan Kuo (Joint Genome Institute, Walnut Creek, USA), Zsofia Keri (Evolutionary Genomics Research Group, Eotvos Lorand University, Budapest, Hungary), Gergely Szollosi (Evolutionary Genomics Research Group, Eotvos Lorand University, Budapest, Hungary), Igor V. Grigoriev (Joint Genome Institute, Walnut Creek, USA), David S. Hibbett (Clark University, Worcester, USA), Francis M. Martin (INRA-Nancy, Nancy, France), Laszlo G. Nagy (Institute of Biochemistry, Biological Research Center, HAS, Szeged, Hungary)

Ectomycorrhizae evolved convergently in many major fungal lineages, mostly in the Basidio- but also in the Ascomycota. The evolution of ECM fungi coincided with significant changes in genome composition and architecture, including the emergence of several short lineage-specific protein families and the loss of much of the ancestral plant cell wall degrading enzyme (PCWDE) gene complement. Emerging genome datasets, however, challenge this paradigm. For example, several families of PCWDEs have been found in the genomes of Cortinarius and Tricholoma, suggesting that independently evolved ECM lineages show significant variability in their PCW decomposing abilities. To uncover conserved and lineage-specific patterns of gene duplication and loss in ECM lineages, we sequenced and analyzed the genomes of 10 new ECM Basidiomycota. These species, combined with previously sequenced ECM fungi comprise 8 independently evolved clades of ectomycorrhizal agarics. We reconstructed gene duplications and losses in genome-wide collections of gene trees and mapped the origins of inferred orthogroups onto the phylogenetic tree connecting these taxa. We analyzed PCWDE genes in particular detail: consistent with previous studies, we observed significant loss of lignolytic and cellulolytic gene families, but also the selective retention of lineage specific complements of PCWDE genes. These results suggest that although genes related to plesiomorphic wood-decay capabilities became largely dispensable for ECM fungi, different lineages retain different abilities to degrade plant cell wall materials. Thus, it is likely that fungi currently classified as ECM cover a functionally and nutritionally diverse array of species that fulfill different roles in forest ecosystems.

IL (ID 507)

Comparison of symbiotic features in two arbuscular mycorrhizal (AM) fungi

Christophe Roux (Laboratory of Plant Sciences, University of Toulouse, Castanet-Tolosan, France)

Genetic and functional studies on AM symbiosis are mainly investigated on the plant partner. Recent publications on fatty acid plant-fungus transfers in AM symbiosis illustrate it perfectly. The reasons explaining such situation are well known: the efficiency of reverse and forward genetics on plants, the heritage of genetic in Legume-root nodule symbiosis to investigate the common symbiotic signalling pathway, and above all the absence of genetic tools suitable for AM fungi. But we should not neglect the incidence of "our" anthropocentric interests on plants and their uses.

For five years now, next-genetration sequencing technologies have had a tremendous impact on our knowledge on the biology of AM fungi as more transcriptomic/genomic data are available. Associated to HIGS/V(H)IGS strategies, great opportunities are now open to investigate the physiology of the fungal partner in AM symbiosis. Following first genomic resources obtained on *Rhizophagus irregularis* (Kohler et al., 2011, Tisserant et al, 2013, Lin et al, 2014), we developed genomics on *Gigaspora rosea*. This species, endobacteria-free, is phylogenetically distant from *R. irregularis* among Glomeromycota and presents a narrower host spectrum. We performed comparative transcriptome profiling of these two AM fungi on several hosts. Specific and shared features will be presented on fungal primary metabolism and fungal strategies to control host immunity. The identification of common features as general traits of symbiotic physiology of AM fungi will be discussed.

Keywords: arbuscular mycorrhizal fungi, Gigaspora rosea, genomics

IL (ID 331)

Ectomycorrhizal ecology is imprinted in the genome of the dominant and only known mycorrhizal Dothideomycete *Cenococcum geophilum*

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The most frequently encountered ectomycorrhizal (ECM) symbiont is *Cenococcum geophilum*, being often the dominant ECM fungus on tree roots, particularly under stressed conditions. *C. geophilum* is the only ECM species within the largest fungal class Dothideomycetes that harbors many plant pathogens.

To identify the genomic innovations underlying adaptation to the mutualistic lifestyle in *C. geophilum*, we sequenced the genomes of this and two closely related saprotrophic species and compared them with other fungi having various lifestyles. We also studied the transcriptome of *C. geophilum* in symbiosis and free-living condition.

The genome of *C. geophilum* reveals the same signs of mycorrhizal evolution as ECM basidiomycetes showing reduced numbers of plant cell wall degrading and lineage-specific, symbiosis-induced genes. *C. geophilum* still holds a significant set of genes known to be involved in pathogenesis and the large, transposable elements enriched genome shows signs of a two-speed evolution as in some pathogens. The symbiotic transcriptome is characterized by the striking upregulation of genes coding for membrane transporters, particularly water channels. Plant water and nutrient transporters were more highly expressed in mycorrhizas of *Pinus sylvestris* interacting with *C. geophilum* compared to other ECM fungi, but also genes involved in pathogen resistance were upregulated, some of them known to prime interlinked pathways of abiotic stress tolerance. These findings support a possible role of *C. geophilum* in drought tolerance of host trees. The genome of *C. geophilum* is an important resource to understand the genetic basis of fungal ecology transitions and the functioning of mycorrhizal symbiosis.

Keywords: comparative genomics, Dothideomycetes, aquaporin, mycorrhiza-induced small secreted proteins (MiSSPs), transposable elements

IL (ID 515)

Comparative analysis of genomic data to understand the extent of variation among and within isolates of *Rhizophagus irregularis*

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The symbiosis between arbuscular mycorrhizal fungi (AMF) and plant roots can confer growth advantages to plants. In our group, we have evidence that variation within the AMF species *Rhizophagus irregularis* leads to large differences in plant growth. For this reason, understanding variation in this fungus is important. Several studies reported different levels of genomic variation among and within *R. irregularis* isolates. We undertook a comparative analysis of genomic data available for *R. irregularis* isolates to investigate the types of variation that exist and, where possible, to compare which are consistent among studies. We used RAD-seq, whole genome sequencing data and RNA-seq to identify genetic variants (SNPs) and structural genomic variants within and among isolates of *R. irregularis*. We did this within an *R. irregularis* population and also tried to obtain a more global view from data on *R. irregularis* isolates across continents. We aim to provide a synthetic and unified view of the genomic organisation found in the *R. irregularis* "complex" and discuss how relevant this is to overall generalizations about AMF genomes.

IL (ID 100)

A Genome Wide Association Study to disentangle legume-specific root responses to phosphate

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Phosphate is an essential plant macronutrient and its low soil availability leads farmers to massive application of phosphate-based fertilizer. Being a key regulator of Arbuscular Mycorrhizal symbiosis, a better understanding of plant genetic and molecular bases of phosphate absorption and utilization is needed. Much progress in that area has been made over the past years, but most approaches focused on Arabidopsis thaliana. However, it is unclear which of the discovered mechanisms are conserved and which ones are specific to it. Here, we approach this problem by using a combination of high throughput root phenotyping and IC-based phosphate quantification of natural populations of three different plant species, Arabidopsis thaliana and the legumes Lotus japonicus and Medicago truncatula. This allowed us to identify multiple candidate genes regulating phosphate metabolism and root growth responses to varying phosphate levels. We could determine multiple orthologs whose variants are significantly associated with these phosphate related traits in both plant species. We are currently testing the involvement of high confidence candidate genes in phosphate homeostasis, root growth responses upon phosphate levels in both species and also the mutant capacity to form Arbuscular Mycorrhizal symbiosis at different phosphate regimes.

Keywords: genome wide association study, root system architecture, phosphate, plant nutrition

PLENARY SESSION: Perspectives

Chair: Lynette Abbott

IL (ID 203)

Learning from the past: John Laker Harley (1911–1990)

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John Laker (Jack) Harley (1911–1990) was a pioneer "mycorrhizast". Much of his research concerned various aspects of the physiology of "ectotrophic" mycorrhizas, now known as ectomycorrhizas, particularly those of beech (*Fagus sylvatica*). His lasting contributions to our field, however, transcend physiology and continue to influence research in ecology, community biology and ecosystem science. His ability to contribute meaningfully to our field did not occur as a consequence of a high H-index or because he published in prestigious journals. It stemmed from 1) his view of mycorrhizas in a very broad biological context, 2) his ability to piece together seemingly disparate bits of information into insightful syntheses, and 3) his fearless and unselfish desire to share his ideas in order to stimulate further research, mainly through his books. Our purpose in this contribution is to illustrate just a few of many examples of Harley's insights foreshadowing important research threads decades later. We highlight the importance of recognizing the progress made by our scientific ancestors if we are not to pursue paths that lead nowhere or waste valuable resources reinventing wheels that already exist.

Keywords: physiology, ecology, community biology, ecosystem science, history

IL (ID 248)

Beyond ICOM8: Perspectives on Advances in Mycorrhizal Research from 2015 to 2017

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Over 500 mycorrhizal scientists attended ICOM8, held in 2015 in the USA. The program included more than 400 presentations on thirteen broad topics with a theme of Mycorrhizal Integration Across Continents and Scales. In this perspective presentation, we review the recent literature to describe the "hot topics" in mycorrhizal research between ICOM8 and ICOM9. We highlight major advancements both within and across levels of biological organization and describe areas where greater integration has led to unique insights. Particularly active areas of research include the mycorrhizal microbiome, its development, maintenance and function, comparisons among types of mycorrhizas from molecular to ecosystem scales, and enhanced understanding of the role of mycorrhizas in carbon dynamics from local to global scales. The popular top-tier scientific journals have acknowledged mycorrhizas to be complex adaptive systems that play key roles in the development of communities and ecosystem processes. Understanding the mechanisms driving these large-scale effects requires effective integration of knowledge across scales of organization. This integration remains a significant challenge, although advances in technical and analytical methods are rapidly moving the field forward.

IL (ID 514)

Perspectives: Bringing different disciplines together to answer the "BIG questions" in mycorrhizal research

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Biologists have had access to much more powerful and sophisticated tools allowing them to investigate the mycorrhizal symbiosis in a way that was unimaginable a short time ago. This is particularly true of "omics" tools for understanding molecular interactions between plants and fungal symbionts; a field that has seen rapid, exciting advances over the past years. High-throughput molecular and bioinformatics tools also allowed researchers to rapidly uncover fascinating details on ecology of mycorrhizal fungi. However, mycorrhizal research in plant molecular biology, fungal molecular biology, mycorrhizal fungal ecology, plant physiology, signalling, agriculture has largely been conducted independently. It is impossible to predict the breakthrough technologies that will be available in the next 5 to 10 years. However, irrespective of future technological developments, an interdisciplinary approach sharing existing knowledge, data, approaches and technology to answer "bigger" questions on the contribution of the mycorrhizal symbiosis to important problems at the global level should be possible. I will highlight some key advances in mycorrhizal research. Coupled with other findings from several disciplines this could be used to address globally important problems to which the mycorrhizal symbiosis might be important. I argue that research groups acting alone, or within their own discipline, cannot solve such problems and that a key to advance will be the appreciation of the value of different disciplines.

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