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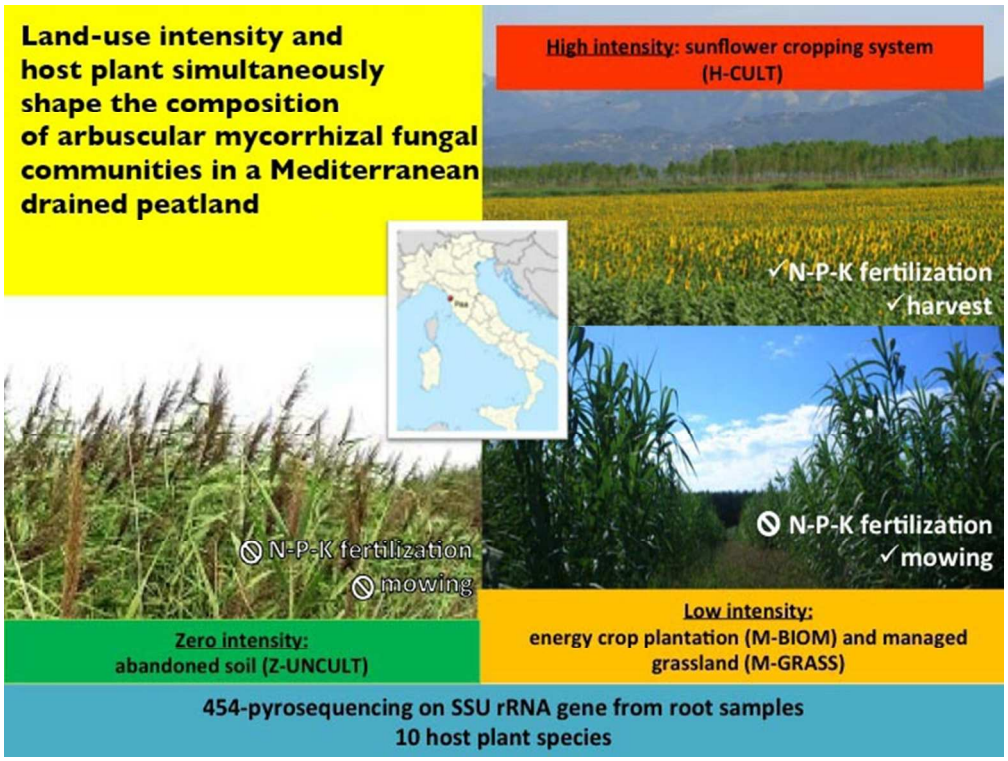

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LAND-USE INTENSITY AND HOST PLANT SIMULTANEOUSLY SHAPE THE COMPOSITION OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN A MEDITERRANEAN DRAINED PEATLAND

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Keywords: 454-pyrosequencing; arbuscular mycorrhizal fungal (AMF) diversity; community composition; host preference; land use; SSU rRNA gene

Running title: Land use as a driving factor of arbuscular mycorrhizal fungi

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ABSTRACT

Land-use change is known to be a major threat to biodiversity and ecosystem services in Mediterranean areas. However, the potential for different host plants to modulate the effect of land-use intensification on arbuscular mycorrhizal (AM) fungal community composition is still poorly understood. To test the hypothesis that low land-use intensity promotes AMF diversity at different taxonomic scales and to determine whether any response is dependent upon host plant species identity, we characterized AMF communities in the roots of ten plant species across four land use types of differing intensity in a Mediterranean peatland system. AMF were identified using 454-pyrosequencing. This revealed an overall low level of AMF richness in the peaty soils; lowest AMF richness in the intense cropping system at both Virtual Taxa (VT) and family level; strong modulation by the host plant of the impact of land-use intensification on AMF communities at the VT level; and a significant effect of land-use intensification on AMF communities at the family level. These findings have implications for understanding ecosystem stability and productivity and should be considered when developing soil-improvement strategies in fragile ecosystems, such as Mediterranean peatlands.

INTRODUCTION

Land-use change is known to be a major threat to plant and animal biodiversity and ecosystem services (Newbold *et al.*, 2015). It has been estimated that the conversion of natural habitats to human-impacted habitats, such as pasture, cropland, tree plantations and urban areas, has caused a global biodiversity decline of 8.1% in the last 500 years. This figure could increase by a further 3.4% in the next 100 years if conservative agricultural practices are not applied (McGill2015). As plant community composition and soil physico-chemical parameters shape soil microbial communities, land-use changes also strongly affect soil ecosystem functions and services, including plant growth, carbon (C) sequestration and regulation of nutrient availability and uptake by plants (Wardle *et al.*, 2004; Meyer *et al.*, 2013; Lange *et al.*, 2015).

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota; Schüßler, Schwarzott and Walker 2001) are an important soil microbial group that form one of the most common types of symbiosis globally, arbuscular mycorrhiza (Smith and Read 2008). AMF are associated with the roots of over 80% of terrestrial plants, including crops, and convey fundamental services, such as plant growth (Lekberg and Koide 2005), protection against pests and pathogens (Newsham, Fitter and Watkinson 1995), drought tolerance (Augé 2001) and nutrient uptake, in exchange for photosynthetically fixed C (Bago, Pfeffer and Shachar-Hill 2000; Hodge, Helgason and Fitter 2010). Moreover, AMF improve soil structure and aggregate stability thanks to the development of extraradical mycelia and the production of a coagulating glycoprotein, glomalin, that contributes to soil C and nitrogen (N) stocks (Rillig *et al*., 2001; Rillig and Mummey 2006; Bedini *et al.*, 2009).

In the last decade, the assumption of low host plant preference/specificity in AMF has been challenged by evidence of a host plant species effect on AMF diversity and community composition (Vandenkoornhuyse *et al.*, 2002; Sýkorová, Wiemken and Redecker 2007; Torrecillas, Alguacil and Roldán 2012). Other studies have identified an association between AMF and plant ecological groups (e.g., habitat generalists *vs* specialists) (Öpik *et al.*, 2009; Davison *et al.*, 2011) or

ecosystems (Veresoglou and Rillig 2014), rather than particular plant species. These associations were explained using a common framework that categorised both plants and AMF according to their life history strategies (i.e., competitor, stress tolerator and ruderal) (Chagnon *et al.,* 2013).

Changes in AMF community composition, with decreases in AMF species richness, have also been linked with land-use intensification both in soil (Lumini *et al.*, 2010; Gonzalez-Cortés *et al.*, 2011; Morris *et al.*, 2013; Xiang *et al.*, 2014) and roots (Helgason *et al.*, 1998; Moora *et al.*, 2014; Vályi, Rillig and Hempel 2015). Within arable systems, the level of intensification due to management practices, such as tillage, crop rotation, and fertilizer and biocide input, strongly impact upon AMF species richness and community composition, by promoting the dominance of particular taxa belonging to the family Glomeraceae (Helgason *et al.*, 1998, 2007; Jansa *et al.*, 2002; Mathimaran *et al.*, 2007; Borriello *et al.*, 2012).

In Mediterranean areas, high land-use intensification has been associated with low AMF richness in grasslands, pastures, vineyards, plantations and forests (e.g., Lumini et al., 2010; Pellegrino et al., 2011). Members of the AMF orders Glomerales and Diversisporales have largely been found in natural and high input land-uses, while the orders Paraglomerales and Archaesporales have been detected only in the less intense systems, such as pastures. At the same time, the impact of land-use intensification in wetlands and peatlands is poorly understood (Pellegrino et al., 2014; Ciccolini, Bonari and Pellegrino 2015).

Wetlands are important transitional ecosystems between terrestrial and aquatic ecosystems, covering only 6% of the global area, but playing crucial ecological roles in the balance and sequestration of C, N and phosphorus (P) and in the protection of biodiversity (Verhoeven and Setter 2009). In the past century, half of all wetlands globally have been lost due to conversion to agriculture (Zedler and Kercher 2005), and, consequently, the protection and restoration of wetlands, and in particular of peatlands, have become a priority. A lack of knowledge concerning the diversity and roles of microbes in peatland restoration is well recognised in the literature (e.g., Littlewood et al., 2010). Nevertheless, the positive outcome of restoration projects maybe

influenced by microbial activity (e.g., N_2 fixation, nutrient uptake, organic matter oxidation, methanotrophy), which itself may be modulated by management strategies, such as fertilization or manipulation of the water table or vegetation assemblages. In acidic systems, such as peatlands, fungi have been shown to be more abundant than bacteria, acting as major decomposers of complex carbon polymers in soil organic matter (Andersen, Chapman and Artz 2013). Changes in fungal community composition were mostly attributed to changes in litter type, whereas changes in AMF community were attributed also to changes in plant species assemblages (Thormann, Currah and Bayley 1999). Moreover, AMF were reported to be key components for the success of restoration programmes of disturbed peatlands by colonizing the roots of many wetland plant species and thus potentially improving the early growth of plant community (Turner et al., 2000; Tawaraya et al., 2003). In Mediterranean peatlands, where climatic conditions leave soil prone to degradation (Vallebona *et al*., 2015), studying the effects of some drivers of AMF community structure such as host plant preference/specificity, land-use intensification and their interaction may support the development of efficient strategies for peatland restoration and protection (i.e., less intensive agriculture, extensive grazing systems, rewetting).

In this study we aimed to investigate the effect on root AMF diversity of land-use intensification, host plant species and their interaction in a Mediterranean peatland drained for agricultural purposes. Four land uses with decreasing levels of intensity were studied to test the hypothesis that low land-use intensity promotes AMF diversity at different taxonomic scales and to determine whether any response is dependent upon host plant species identity

MATERIALS AND METHODS

Study site and experimental design

The experimental site is located in the southern part of the Massaciuccoli Lake basin (43°49'N, 10°19'E) (Pisa, Italy) (Pellegrino *et al*., 2014). The soil is classified as *Histosol* according to the USDA system (Soil Survey Staff, 1975) and defined as peaty soil (IPCC, 2006). The climate is Mediterranean (Csa) according to the Köppen classification, with dry and hot summers and rainfall mainly concentrated in autumn and spring (mean annual rainfall ca. 945 mm $year^{-1}$) and mean monthly air temperature ranging from 7°C in February to 30°C in August (yearly average 14.8°C).

The experiment consisted of a completely randomized design with a land use intensity treatment comprising three levels of agricultural intensification (high intensity-H; medium intensity-M; zero intensity-Z) and four land-use types, each represented by three field replicates (0.7 ha). In detail, prior to the start of the experiment (15 years ago), the site was intensively cultivated with sunflower and maize. At that time, we selected 12 field replicates and allowed a random number generator to allocate land use types to the different fields. Among the field replicates, three were assigned to high intensity-H, while nine were left to develop under natural successional vegetation, with no agricultural intervention (zero intensity-Z). Then, two years ago, six of these nine field replicates were randomly assigned to medium intensity-M. The specific land-use types were: (1) an intensive cropping system (H-Cult) based on sunflower (*Helianthus annuus* L.), carried out for the last 15 years. Plots were deeply ploughed (30-35 cm) and harrowed each year early in spring. Sunflower was sown in April at a rate of 6.7 plants m^2in rows spaced 75 cm apart and harvested at the beginning of September. Fertilizer was applied at sowing and mechanical weed control was applied post-emergence, while no pest control was performed; (2) a two-year-old plantation of perennial grasses for energy production (*Arundo donax* L. and *Miscanthus x giganteus* Greef et Deuter) (M-Biom), where no fertilizers or other agricultural practices were applied except for annual harvest in winter; (3) a two-year-old managed grassland of cool-season grasses (*Festuca arundinacea* L., *Lolium perenne* L.) and seashore paspalum (*Paspalum vaginatum* Swartz) (M-Grass). No fertilizers or other agricultural practices were applied, except for mowing when required; (4) an agricultural soil abandoned 15 years ago (Z-Uncult) and naturally colonised by indigenous grasses. The

dominant plant species were *Calystegia sepium* L. (18%), *Phragmites australis* (Cav.) Trin. ex Steud. (15%), *Arctium lappa* L. (14%) and *Bromus tectorum* L. (14%), respectively. Percentages represent the relative density of each plant species from a survey made in May 2013. No fertilizers, tillage or other agricultural practices were applied.

Sampling

To evaluate the effect on AMF community composition of land-use intensification and the interaction with host plant identity, two co-occurring plant species, *Poa* sp. and *C. sepium*, were sampled in every land-use type in May 2013 (Table 1). In addition, to test the main effect of host plant species, two further plant species unique to each land-use type were sampled from May to July 2013. For details of the ten sampled plant species see Table 1. In each replicate field, at least two individuals of each plant species were randomly collected, generating a total of 144 samples. Plants with entire root systems were excavated and placed in polyethylene bags for transport to the laboratory. Roots were rinsed, oven dried at 60 °C for 24 h and stored with silica gel at room temperature until analysis.

Molecular analyses

DNA was extracted from ca. 30 mg of dried roots from each plant individual using the PowerSoil-htp™ 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), following the modification to the protocol as in Davison *et al*. (2012). First, roots were milled to powder in 2 ml tubes with four 3 mm tungsten carbide beads per tube with Mixer Mill MM400 (Retsch GmbH, (Haan, Germany). Bead Solution (750 µL) was added to the tubes, mixed, and the slurry transferred to Bead Plates. To increase DNA yield, the Bead Plates were shaken at a high temperature (60°C following the manufacturer's suggestions) for 10 min at 150 rpm in a shaking incubator. Finally, in

order to increase DNA yield, the final elution was performed twice with 75 µL of Solution C6. Before PCR reaction DNA concentration was measured using the Appliskan fluorescence-based microplate reader (Thermo Scientific, MA, USA) and PicoGreen® dsDNA Quantitation Reagent (Quant-iTds DNA Broad Range Assay Kit, Invitrogen, Carlsbad, CA) in three replicates.

Glomeromycota nuclear small subunit (SSU) rRNA gene fragments were amplified using the primers NS31 and AML2 (Simon, Lalonde and Bruns, 1992; Lee, Lee and Young, 2008), linked to 454 sequencing primers A and B, respectively, and an 8 bp sample-distinguishing barcode (Davison *et al*., 2012). We targeted the SSUrRNA gene because the large and comprehensive AMF sequence database Maarj*AM* (Öpik*et al*., 2010) allows a reliable and fast identification of Glomeromycota and comparisons with other studies (Öpik *et al*., 2014). Polymerase chain reaction (PCR) was carried out in two sequential reactions of which the first was targeted PCR with region-specific primers, barcodes and partial sequencing adapters, and the second PCR was used to complete sequencing adapters, as in Davison *et al*. (2012). Three µL of stock DNA sample was used in the first PCR and 3µL of 10-fold dilution of the first PCR product was used in the second PCR reaction. The PCR mix contained 3 µL of template DNA, 0.2 µM of each primer and Smart-Taq Hot Red 2x PCR Mix (0.1 UμL^{-1} Smart Taq Hot Red Thermostable DNA Polymerase), 4 mM $MgCl_2$, 0.4 mM of each of the nucleotides; Naxo OÜ, Estonia) in a total volume of 30 µL. PCR reactions were performed in three replicates. The reactions were run on a Thermal cycler 2720 (Applied Biosystems, Foster City, CA, United States) following the conditions of Davison *et al*. (2012). PCR products were separated by electrophoresis through a 1.5% agarose gel in 0.5× TBE, and were purified with Agencourt® AMPure XP Kit® (Beckman Coulter Inc.) in plate. Samples were eluted in Buffer EB (10 mM Tris-Cl, pH 8.5; QIAGEN Inc.). The average concentration of amplicons in the pooled sample was 24.4 ngμL^{-1} (measured on a Qubit™ fluorometer in three replicates). Preparatory procedures for 454 sequencing (barcoded PCRs and PCR product purification) were performed by BiotaP LLC (Tallinn, Estonia). A total of 2.07 µg of the resulting

DNA mix was sequenced on a Genome Sequencer FLX System, using Titanium Series reagents (Roche Applied Science, Mannheim, Germany) at Microsynth AG (Balgach, Switzerland).

Bioinformatic analyses

Bioinformatic analysis was implemented following Davison *et al*. (2012). Only 454-sequencing reads that met all of the four following criteria (quality filtering) were included in subsequent analyses: (1) the read carried the correct bar-code; (2) the read carried the correct NS31 primer sequence; (3) the read was ≥ 170 bp (excluding bar-code and primer sequences) and (4) the read had an average quality score ≥ 25. As most reads were of approximately full amplicon length (between 500 and 550 bp long), we trimmed reads to 520 nucleotides to exclude reverse primer sequences. A total of 1205 potential chimeras were detected and removed using UCHIME (Edgar *et al*., 2011) in reference database mode using the default settings and the Maarj*AM* database. The analyses yielded a total of 514,457 reads.

For taxonomic identification of reads we used an open-reference operational taxonomic unit picking approach (Bik *et al*., 2012). After stripping the barcode and primer sequences, we used the Maarj*AM* database of published Glomeromycota SSU rRNA gene sequences to identify obtained reads. The Maarj*AM* database contains representative sequences from published environmental Glomeromycota sequence groups, so-called Virtual Taxa (VT; Öpik *et al*., 2010, 2014). Sequence reads were assigned to VT by conducting a BLAST search (soft masking with DUST) against the Maarj*AM* database that is based on environmental and cultured fungal sequences (status May 2014) with the following criteria required for a match: (a) sequence similarity ≥ 97%, (b) an alignment length > 95% of the length of the shorter of the query (pyrosequencing read) and subject (reference database sequence) sequence; (c) a BLAST e-value < 1 e^{-50}. These analyses yielded a total of 77,988 reads that matched with VT from the Maarj*AM* database.

Those reads that did not find a match in the Maarj*AM* database were identified by conducting a further BLAST search against the International Nucleotide Sequence Database (INSD), using slightly modified criteria: (a) sequence similarity ≥ 90% and (b) 90% alignment length.

Up to four sequences of each detected VT for each land-use type were picked and aligned together with the VT type sequences, representative AMF sequences (sequences included in the AMF species list of Schüßler and Walker2010; see open-access dataset http://sites.gooogle.com/site/restomedpeatland/microbiology) and previously recorded Glomeromycota sequences from the study site (Pellegrino *et al*., 2014; Ciccolini, Bonari and Pellegrino2015; 757 sequences in total). The alignment was performed using MAFFT version 7 multiple sequence alignment web service (Katoh and Standley 2013). Neighbour-joining (NJ) phylogenetic analysis was performed in MEGA5 (Tamura *et al*., 2011). Glomeromycota nomenclature follows Redecker *et al*. (2013). Representative sequences of VT detected in each land use type and plant species were deposited in the EMBL database under accession numbers from LT596223 to LT596539.

Chimera checking, primer and barcode sequence removal, parsing of BLAST output and selection of representative sequences were carried out using a series of Python and Java scripts developed at the Department of Botany, University of Tartu (Davison *et al*., 2012).

Statistical analyses

Root samples yielding < 10 sequences and singleton and doubleton VT were removed, leaving 96 samples and including at least two plant individuals of each host plant in each field replicate.

Diversity data matrices were built at the taxonomic levels of VT and family, with the relative abundance of taxonomic groups in samples estimated from the proportion of reads representing each group. To test the effect on AMF community composition of land-use intensification and its interaction with host plant species a matrix was compiled containing samples from the two co-

occurring host plant species (*Poa* sp. and *C. sepium* L.) in the four land-use types (48 samples; question 1) (Table 1). Then, to study the effect of host plant species on AMF community composition, a matrix was compiled containing samples from all ten plant species in all land use types (Table 1) (question 2).

Sequencing efficacy was assessed with rarefaction analysis, using the function rarefy() from the R package vegan (Oksanen *et al*., 2013). Because there was a high variability in the number of reads per sample (Fig. S1), sequencing depth per sample was standardized to the median number of reads across the samples in each data matrix (de Cárcer *et al*., 2011). Applying this approach, bias due to differences in sample size is reduced by randomly choosing in each sample a number of reads equal to the median number of reads across all samples. Samples that had fewer reads than the median were left unchanged.

To answer question 1, permutational analysis of variance (PERMANOVA; Anderson 2001) was used to test the effect of land-use type (H-Cult, M-Biom, M-Grass and Z-Uncult) and host plant species identity (*Poa* sp. and *C. sepium*) on VT/family relative abundance. Response data matrices were square-root transformed prior to analyses in order to down-weight the importance of dominant taxa and the Bray-Curtis index of dissimilarity was used to measure ecological distance. *P*-values were calculated using a Monte-Carlo test and residuals were permuted under a completely randomized model (Anderson and TerBraak 2003). To remove the effect of spatial variability of subsamples, the latitude and longitude of the plant individuals were used as covariates in the PERMANOVAs. Since PERMANOVA is sensitive to differences in multivariate location (average community composition of a group) and dispersion (within-group variability), analysis of homogeneity of multivariate dispersion (PERMDISP; Anderson 2006) was performed to check the homogeneity of multivariate dispersion between groups (beta-diversity) (Anderson, Ellingsen and McArdle 2006). When PERMANOVA and PERMDISP indicated a significant effect, principal coordinate analysis (PCO) was applied to the matrix of pairwise Bray-Curtis dissimilarities (Torgerson 1958) in order to visualize the most relevant patterns in the data. In each PCO biplot, we

displayed only the VT and families with a strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis. The circle in each plot, whose diameter is 1.0, allows the reader to understand the scale of the vectors in the vector plot. The output of the PCO analyses were utilized together with the indicator species analysis, according to Dufrene and Legendre (1997), to visualize the taxa that were most indicative of particular land-use and host plant categories. Classification and ordination analyses were performed using PRIMER 6 and PERMANOVA+ software (Clarke and Gorley 2006; Anderson *et al*., 2008), while indicator species analysis was performed using the indval function from the labdsv package for R (Roberts 2014). For each host plant species (*Poa* sp. and *C. sepium*), the standardized dataset was also used to generate Venn diagrams, representing VT and reads unique to each land use or shared among land uses. Venn diagrams were generated using Venny v. 2.0 software (Oliveros2015).

To answer question 2, PERMANOVA and PCO were applied to the 96 sample data matrix. In the PERMANOVA host plant species (n = 10) was used as a fixed factor, while land-use type and the spatial coordinates of plant individuals were included as covariates. The VT and families with strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis were displayed on PCO biplots.

PERMANOVAs were also performed using unstandardized datasets of relative abundance (i.e., VT and family based) per sample, to test whether data standardization produced changes in the patterns of AMF community composition (questions 1 and 2).

AMF richness at the VT and family level was studied using standardized data matrices. The effects on richness of land-use type and its interaction with host plant species (question 1) were tested using analysis of covariance (ANCOVA), with land-use type and host plant species as fixed factors and the spatial coordinates of plant individuals as covariates. Similarly, the effect of host plant species on AMF richness (question 2) was studied using ANCOVA with host plant species as a fixed factor and land-use type and the spatial coordinates of the plant individuals as covariates.

Unless stated otherwise, analyses were performed in SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Pyrosequencing information

A total of 514,457 quality-filtered SSU rRNA gene sequences were obtained from 96 samples. After the BLAST against the Maarj*AM* database, we found 77,917 Glomeromycota reads, ranging from 10 to 3,865 reads per sample (length varying from 170 to 520 bp; mean length of 392 bp) that were assigned to a total of 48 Virtual Taxa (VT) (Table S1; Fig. S1). The remaining 465,137 reads were identified against the INSD. Plantae, fungi, bacteria and metazoa represented 73%, 11%, 9% and 2% of reads, respectively. Among fungi, the potential matches to Glomeromycota constituted less than 1% of qualifying reads and thus were not included in further analyses. The 48 VT belonged to the families Acaulosporaceae (2), Archaeosporaceae (2), Claroideoglomeraceae (4), Diversisporaceae (5), Glomeraceae (31) and Paraglomeraceae (4) (Table S1; Fig. S1). Rarefaction analysis suggested that the number of AMF reads per sample was generally sufficient to produce asymptotic estimates of VT richness per sample (Fig. S2). Since some samples had substantially lower sequencing depth than others, sequencing depth was standardized to the median number of reads per sample (154 pyrosequencing reads). After standardization of the data, a total of 11,167 reads belonging to 32 VT in six families were retained for subsequent analyses (Tables S1): Acaulosporaceae (1), Archaeosporaceae (2), Claroideoglomeraceae (4), Diversisporaceae (3), Glomeraceae (19) and Paraglomeraceae (3).

Land use effect on AMF diversity

The total numbers of VT identified in *Poa* sp. and *C. sepium* were 21 and 23, respectively, with both host plant species harbouring all the six families (Figs 1, S1; Table S1). In the roots of *Poa* sp., one VT (*Glomus* VT113) occurred in all four land uses (4.8% of VT; 6.3% of reads; Figs. 1 and S3, S4). In the roots of *C. sepium,* four VT (*Glomus* VT113; *Glomus*VT309, related to *Glomus* ORVIN GLO3E; *Claroideoglomus*VT193 and VT278, related to *C. claroideum, etunicatum, lamellosum, luteum* and to *C.* ORVIN GLO4, respectively (17.4% of total VT; 50.0% of total reads) occurred in all four land uses (Figs. 1, S3).

AMF richness per sample at VT and family level was affected by land-use intensification (P=0.020 and P=0.016, respectively) (Table S2). At the VT level, H-Cult and M-Grass exhibited a significantly lower number of VT per sample (mean ± SE: 2.80 ± 0.44 and 2.81 ± 0.40 VT, respectively) in comparison with Z-Uncult (4.80 ± 0.59 VT) (Fig. S4a), whereas M-Biom had an intermediate value (4.08 ± 0.61 VT). At the family level, H-Cult and M-Grass (1.70 ± 0.16 and 2.10 ± 0.38 families, respectively) exhibited a significantly lower number of families per sample in comparison with M-Biom (3.00 ± 0.32 families), whereas Z-Uncult had an intermediate value (2.40 ± 0.32 families) (Fig. S4b).

At the VT level, AMF community composition was affected by land-use type [P=0.001; Explained Variance (EV)=13%] and by its interaction with host plant species (P=0.002; EV=20%) (Table 2). PERMDISP indicated significant differences in AMF community dispersion among land uses (P=0.050; Table 2a) and specifically between M-Biom and M-Grass and between Z-Uncult and M-Grass (Table S3).

The interactive effect of land-use intensification and host plant species on AMF communities at VT level was visualised using PCO. ThePCO biplots in Fig. 2 show the differential host plant species effects on AMF communities within each land-use type. The same interaction from another perspective is shown in Fig. 3, where the differential effects of land-use intensity on AMF communities can be seen within each host plant species (Figs. 3, S6). Overall, 12 VT were shown to be highly correlated with AMF community responses to land-use type or indicative of one or more

land use types (Figs. 2, S5). Both H-Cult and Z-Uncult were characterized by *Glomus* (H-Cult: VT113, VT143, VT166, VT172, VT309; Z-Uncult: VT113, VT114, VT115, VT219, VT309) and *Claroideoglomus* (H-Cult: VT57, VT278; Z-Uncult: VT278) VT (Table 3). By contrast, land use types of medium intensity (i.e. M-Biom and M-Grass) were characterized by *Acaulospora* (VT30) and *Paraglomus* (VT281) VT, respectively, in addition to the common discriminant *Glomus* VT113 (Table 3). Specifically, within *Poa* sp.*, Glomus* VT characterized Z-Uncult (VT309) and M-Grass (VT113, VT115, VT143), whereas *Acaulospora* VT (VT30) characterized M-Biom (Fig. 3a, S6a). Within *C. sepium*, M-Biom was also characterized by *Archaeospora* VT245 in addition to *Acaulospora* VT30 and *Glomus* VT309 (Figs 3b, S6b). Indicator species analysis revealed *Acaulospora* VT30 to be a significant VT indicator for M-Biom both in *Poa* sp. and *C. sepium*, and *Archaeospora* VT245 only in *C. sepium* (Table 3). With regard to Z-Uncult, *Glomus* VT309 and *Claroideoglomus* VT57 were shown be indicator species in *Poa* sp. and *C. sepium*, respectively.

At the family level AMF, community composition was affected only by land-use type (P=0.001; EV=27%) (Table 2). PERMDISP confirmed differences in community dispersion among land uses (P=0.038; Table 2) and specifically between M-Biom and Z-Uncult and between M-Grass and Z-Uncult (Table S3). Acaulosporaceae were shown to be a representative family in M-Biom, while Glomeraceae and Claroideoglomeraceae were ubiquitous in the four landuses (Figs. 4, S7),

PERMANOVA and PERMDISP analyses performed using unstandardized data produced results similar to those generated using standardized data (Tables S4, S5).

Host effect on AMF diversity

Among the ten studied host plant species, AMF richness per sample differed at the family (ANCOVA, $F_{(9,78)}$=2.309, P = 0.023) but not at the VT level (ANCOVA, $F_{(9,78)}$=1.668, P = 0.111). The highest number of families per sample was observed in *Miscanthus* x *giganteus*, while the lowest number was observed in *B. tectorum*, *H. annuus* and *M. chamomilla* (Table S6). AMF

community composition was significantly affected by host plant species both at VT (P=0.001; EV=13.3%) and at family level (P=0.001; EV=22.4%) (Table 4; Fig 5a,b). The position of field replicates in the area had a significant effect on AMF community composition at both taxonomical levels (Table 4). PERMDISP confirmed significant differences between host plant species in AMF community dispersion at both taxonomical levels (Table S7). Three VT - *Acaulospora* VT30, prevalently detected in the roots of *A. donax* and *R. acris*; *Claroideoglomus* VT193, prevalently detected in the roots of *P. australis* and *Glomus* VT309 mostly retrieved in the roots of *M. chamomilla* (Fig. S8a) and three families - Acaulosporaceae largely detected within the roots of *A. donax* and *R. acris*; Claroideoglomeraceae and Glomeraceae most occurring in the roots of *P. australis* and *M. chamomilla/H. annuus* - were strongly correlated with the PCO axes (Fig. S8b). Indicator species analysis revealed six indicators for the ten host plant species: *Archaeospora*VT245 in *L. perenne*; *Claroideoglomus* VT193 in *P. australis*; *Rhizophagus* VT90 and VT264 in *H. annuus*; *Glomus*VT219 in *Miscanthus* x *giganteus* and *Rhizophagus* VT105 in *B. tectorum* (Table 3).

PERMANOVA and PERMDISP analyses performed using unstandardized data (Tables S8, S9) produced results similar to those performed using standardized data.

DISCUSSION

In this study, the effects on AMF communities of land-use intensification, host plant species and their interaction were evaluated at different taxonomic levels in a Mediterranean peatland drained for agricultural purposes. AMF diversity was measured in the roots of ten plant species across four land-use types with differing levels of land use intensity. Using 454-pyrosequencing of the SSU rRNA gene, we detected: (i) overall low AMF richness; (ii) lowest AMF richness in the high land-use intensity level both at Virtual Taxa (VT) and family level; (iii) an impact of land-use type on

AMF community composition at the VT level that was strongly modulated by host plant species identity; (iv) an effect of land-use type on AMF community compositionat the family level; and (vi) a host plant species effect on the richness and community composition of AMF at VT and family level.

Land-use effect on AMF diversity

The overall number of VT detected in this study (48) suggests that the AMF richness of Mediterranean peatland soils is low in comparison with other habitats across the Mediterranean basin, where up to 117 VT have been detected per site (Lumini *et al*., 2010; Varela-Cervero *et al*., 2015). However, the number of VT and families (six families) detected in the present study exceeds what has been recently recorded from the same site (ca. 15 VT and two families, of which nine VT and one family were also detected in the current study) from both root and soil samples (Pellegrino *et al*., 2014; Ciccolini, Bonari and Pellegrino2015). Specifically, *Funneliformis* VT67 and *Funneliformis* VT65 related to *F. mosseae* and *F. caledonium* were previously detected at low abundance (<15%) exclusively in the uncultivated system (Ciccolini, Bonari and Pellegrino 2015), whereas in this study both VT were detected in the roots from the uncultivated system, the biomass plantation and the grassland, albeit at lower abundance. Across the four land-use types, members of Archaeosporaceae, Acaulosporaceae, Diversisporaceae, Claroideoglomeraceae and Paraglomeraceae were detected here in addition to Glomeraceae. However, in comparison to Ciccolini, Bonari and Pellegrino (2015), Gigasporaceae was not be detected again. These differential diversity patterns may reflect the different sampling times (i.e., May *vs* July) and thus the different fungal life cycle stages represented at the times of sampling (i.e., intraradical development *vs* sporulation; Dumbrell *et al*., 2011),or differences in the molecular approach applied (i.e., primer pairs NS31/AM1 *vs* NS31/AML2).The former primer pair is known to not amplify some AMF families, such as Archaeosporaceae, Ambisporaceae and Paraglomeraceae (Lee, Lee

and Young 2008). Finally, the overall level of AMF richness retrieved in this study may have been also underestimated with respect to the whole pool of AMF, since the detection of root-colonizing AMF may not represent the total AMF species available in soil (Varela-Cervero *et al*., 2015).

Previous observations from the same study site (Ciccolini, Bonari and Pellegrino 2015) reported a low AMF richness both at VT and family levels across land-use types. These differences of AMF richness may be explained by the high sequencing depth of 454-pyrosequencing in comparison with the traditional cloning and Sanger sequencing method, allowing a more thorough characterization of AMF communities and detection of taxa even at very low abundances (Senés-Guerrero and Schüßler 2015; Nesme *et al*., 2016).

Nevertheless, the observed reduction of AMF richness in the intensive cropping system showing high soil total N and available P (13 and 70 mg kg^{-1}, respectively; Ciccolini, Bonari and Pellegrino 2015) may be explained by that the fact that plant species become less dependent on AMF for nutrient uptake in conditions of high soil nutrient availability (Camenzind *et al*., 2014).

Compared to other studies amplifying the 18 SSU rRNA region applying the same NGS technique (AMF sequence recovery ca. 47%) (Lumini *et al*., 2010; Valyi, Rillig and Hempel 2015; Varela-Cervero *et al*., 2015) our percentage of recovery of AMF sequences was lower (ca. 15%). The low recovery rate is likely to be due to the peat environment rather than to the inefficiency of the primer pair, since AMF establishment and growth are known to be highly affected by organic matter content (Gryndler *et al*., 2009).

We observed that the impact of land-use intensification on AMF community composition was strongly modulated by host plant species at VT level. AMF community composition in the roots of *Poa* sp. growing in grassland differed from those in the intensive cropping system andbiomass plantation. By contrast, AMF community composition in the roots of *C. sepium* did not differ according to land-use type. This difference between species was also reflected in the respective patterns of occurrence with respect to increasing management intensity (De Cauwer and Reheul 2009): *Poa* sp. mainly occurred in pastures with high intensity management, whereas *C.*

*sepium*occurred equally along the intensification gradient. These results support the idea that the diversification ofAMF communities within roots may confer a competitive advantage to the host plant species and drive plant community dynamics (van der Heijden *et al*., 1998; Zobel and Opik 2014). Our results allow a deeper insight into the poorly studied interactions between land use and other factors, such as plant species identity. So far, a single study has shown that land-use intensity and host plant species have interactive effects on AMF root assemblages in temperate grasslands (Valyi, Rillig and Hempel 2015), while interactions between land use and other factors, such as soil properties, has also been reported in temperate and Mediterranean areas (Jansa *et al*., 2014; Ciccolini, Bonari and Pellegrino 2015). Thus, future agroecological and monitoring studies on the effect of land-use intensification on AMF diversity should consider the potential interaction with host plant species.

At the family level, AMF communities differed between the zero level of land-use intensification and the medium intensive systems This is consistent with previous studies indicating that undisturbed habitats have fungal communities that are highly distinct from those found in anthropogenic areas, at both class and order level and at higher taxonomic resolution (in the Mediterranean area: Lumini *et al*., 2010; Ciccolini, Bonari and Pellegrino 2015; in different climatic zones: Moora *et al*., 2014; Xiang *et al*., 2014; Vályi, Rillig and Hempel 2015). In the intensive cropping system most obtained sequences belonged to Glomeraceae (ca. 80%), among which we found *Glomus* VT309 and VT172 (ca. 30%) and *Glomus* VT113 (ca. 15%) to be dominant, supporting the idea that arable lands favour the presence of this AMF family (Jansa *et al*., 2002). It should nonetheless be noted that additional genera (*Funneliformis* and *Septoglomus*) and families (Claroideoglomeraceae) also occurred in agricultural fields (Rosendahl *et al*., 2009; Lumini et al., 2010; Xiang *et al*., 2014). By contrast, uncultivated systems and the medium intensity systems were dominated by Glomeraceae (ca. 50%) and Claroideoglomeraceae (ca. 30%) and by Acaulosporaceae (ca. 50%) and Glomeraceae (ca. 50), respectively. For Z-Uncult one *Glomus* (VT309) and one *Claroideoglomus* (VT57) were identified as indicator species, whereas

*Acaulopora*VT30 and *Archaeospora* VT245 were found to be indicative for M-Biom. Specifically, *Acaulopora* VT30 was the most abundant VT in M-Biom, suggesting that plants with a long life cycle (i.e., perennial grasses) are more suitable for a symbiosis with AMF taxa that exhibit a slow growth rate and a high requirement for photosynthetically fixed C (Powell *et al*., 2009; Chagnon *et al*., 2013). We found that *Glomus* VT113 was the most dominant VT in the roots of *Poa* sp. and *C. sepium.* This VT is also the most abundantly recorded taxon in the Maarj*AM* database and is frequently the most abundant taxon in individual studies (Vályi *et al*. 2015). This result supports the observation of Davison *et al*. (2011) who reported VT113 as a generalist taxon and as the best indicator of habitat generalist plant species in the forest system studied by those authors, probably due its fast growth rate and the morphology of its propagules and mycelium (Gerdemann and Trappe 1974; Schenk and Smith 1982; Avio *et al*., 2006).

Host effect on AMF diversity

At the family level, highest AMF richness was found in the roots of the perennial grass *Miscanthus x giganteus*, while the lowest values were observed in annual species, namely *M. chamomilla*, *B. tectorum* and *H. annuus.* A similar pattern was recorded in semiarid soils by Alguacil *et al*. (2012), who found higher diversity in perennial compared with annual plants. This can be also explained by the fact that *Miscanthus x giganteus* is a C4 grass, which have been shown to have higher AMF root colonization than C3 grasses and benefit more in term of biomass and P uptake (Reinhart, Wilson and Rinella 2012; Treseder, 2013).

Host plant identity strongly shaped AMF communities within plant roots at both VT and family level. These results confirm those of previous studies showing that AMF in plant roots are not random assemblages, but that host plant identity plays a major role in the modulation of AMF community composition. Host plant modulation has been reported in several habitats, including in some Mediterranean areas (Sánchez-Castro, Ferrol and Barea 2012; Torrecillas, Alguacil and

Roldán 2012; Varela-Cervero *et al*., 2015), as well as in temperate grasslands (Vályi*et al*.2015), alpinesites (Beckli*net al*., 2012) and boreal forests (Öpik*et al.*, 2009; Davison *et al*., 2011).

Regarding AMF community composition, Acaulosporaceae, Claroideoglomeraceae and Glomeraceae were the families that differed most in abundance among host plant species. Glomeraceae were dominant in the roots of Asteraceae (i.e. *H. annuus* and *M. chamomilla*), in line with the results reported in Mediterranean areas by Torrecillas, Alguacil and Roldán (2012). A similar pattern was apparent when considering the VT level, since members of Glomeraceae (namely *Rhizophagus* VT90 and VT264) were found to be indicator species and preferential symbionts for *H. annuus*. By contrast, members of Claroideoglomeraceae were preferentially found in association with Poaceae (i.e. *P. australis* and *B. tectorum*). Along with the fact that members of Poaceae were recently shown to be good mycotrophic hosts (Pellegrino et al., 2015), these findings suggest that Poaceae may be important in shaping the community composition of AMF, contrary to the findings of Torrecillas, Alguacil and Roldán (2012). Finally, the abundance of Acaulosporaceae within the roots of *A. donax*, growing in peaty soils with low pH, is in agreement with the fact that Acaulosporaceae are widespread in acid soils (Clark, 1997) and that perennial grasses are suitable hosts for members of this family (Chagnon *et al*., 2013).

In conclusion, the VT level results demonstrated strong modulation by host plant species of the impact of land-use intensification on AMF community composition. Such a relationship has important implications for ecosystem stability and productivity since it may be expected to influence the composition and diversity of plant communities in the fact of environmental change. However, the fact that host modulation of land use effects is not evident when analysing AMF community composition at the family level indicates that the choosing suitable taxonomic resolution is important for appropriate monitoring of the impact of anthropogenic activities on plant communities. Overall this study shows that the planting of perennial grasses for energy production increased AMF diversity compared to an intensive arable cropping system and a grassland managed at medium intensity and, unexpectedly, also in comparison to an uncultivated system. Therefore,

effective soil-improvement strategies for Mediterranean drained peatland should include reduced soil disturbance and coverage of plant species that supply a large quantity of leaf and root litter able to boost the diversity of beneficial soil microorganisms, such as arbuscular mycorrhizal fungi.

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REFERENCES

Alguacil MM, Torrecillas E, Roldán A *et al*. Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*. *Soil Biol Biochem* 2012;**49**: 132-138.

Andersen R, Chapman SJ, Artz RRE. Microbial communities in natural and disturbed peatlands: a review. *Soil Biol Biochem* 2013;**57**:979-994.

Anderson MJ, Ellingsen KE and McArdle BH. Multivariate dispersion as a measure of beta diversity. *Ecol Lett* 2006;**9**:683-693.

Anderson MJ, Gorley RN, Clarke KR. *PERMANOVA+ for PRIMER*: *Guide to Software and Statistical Methods*. Plymouth, UK: PrimereE Ltd, 2008.

Anderson MJ, TerBraak CJF. Permutation tests for multi-factorial analysis of variance. *J Stat Comput Sim* 2003;**73**:85-113.

Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 2006;**62**:245-253.

Augé RM. Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza*2001;**1**:3-42.

Avio L, Pellegrino E, Bonari E *et al*., Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. *New Phytol* 2006;**172**:347-357.

Bago B, Pfeffer PE, Shachar-Hill Y. Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physol* 2000;**124**:949-958.

Becklin KM, Hertweck K and Jumpponen. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. *Microb Ecol* 2012;**63**:682-693.

Bedini S, Pellegrino E, Avio L, *et al*. Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. *Soil Biol Biochem* 2009;**4**1:1491-1496.

Bik HM, Porazinska DL, Creer S, *et al*. Sequencing our way towards understanding global eukaryotic biodiversity. *Trends Ecol Evol* 2012;**27**:233-243.

Borriello R, Lumini E, Girlanda M, *et al*. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biol Fert Soils* 2012;**48**:911-922.

Camenzind T, Hempel S, Homeier J, *et al*. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Glob Change Biol* 2014; **20**:3646-3659.

Chagnon PL, Bradley RL, Maherali H, *et al*. A trait-based framework to understand life history of mycorrhizal fungi. *Trends Plant Sci* 2013;**18**:484-491.

Ciccolini V, Bonari E, Pellegrino E. Land-use intensity and soil properties shape the composition of fungal communities in Mediterranean peaty soils drained for agricultural purposes. *Biol Fert Soils* 2015;**51**:719-731.

Ciccolini V, Ercoli L, Bonari E, *et al*. Phylogenetic and multivariate analyses to determine the effect of agricultural land-use intensification and soil physico-chemical properties on N-cycling communities in drained Mediterranean peaty soils. *Biol Fert Soils* 2016;**52:**811-824.

Clark RB. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant Soil* 1997;**192:**15-22.

Clarke KR, Gorley RN. *PRIMER v6: User Manual/Tutorial*. Plymouth, UK: PrimereE Ltd, 2006.

Davison J, Öpik M, Daniell TJ, *et al*. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiol Ecol* 2011;**78**:103-115.

Davison J, Öpik M, Zobel M, *et al*. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. *PLoS ONE* 2012;**7**:e41938.

DeCárcer DA, Denman SE, McSweeney C, *et al*. Evaluation of subsampling-based normalization strategies for tagged high-throughput sequencing data sets from gut microbiomes. *Appl Environ Microbiol* 2011;**77**:8795-8798.

De Cauwer B and Reheul D. Impact of land use on vegetation composition, diversity and potentially invasive, nitrophilous clonal species in a wetland region in Flanders. *Agron Sustainable Dev* 2009;**29**:277-285.

Deming JW. Sea ice bacteria and viruses. In: Thomas DN, Dieckmann GS (eds). *Sea Ice*. (2nd ed.), Oxford, UK: Wiley-Blackwell Publishing, 2010,247-82.

Dufrêne M, Legendre P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr* 1997;**67**:345-366.

Dumbrell AJ, Ashton PD, Aziz N, *et al*. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytol* 2011;**190**:794-804.

Edgar RC, Haas BJ, Clemente JC, *et al*. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;**26**:2194-2200.

Gerdemann JW and Trappe JM. The Endogonaceae in the Pacific North-West. *Mycol Mem* 1974;**5**: 1-76.

González-Cortés JC, Vega-Fraga M, Varela-Fregoso L, *et al*. Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. *Fungal Ecol* 2012;**5**:16-23.

Gryndler M, Hršelová H, Cajthaml T, Havránková M, Řezáčová V, Gryndlerová H, Larsen J. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza* 2009;**19**:255-266.

Helgason T, Daniell TJ, Husband R, *et al.* Ploughing up the wood-wide web? *Nature* 1998;**394**:431.

Helgason T, Merryweather JW, Young JPW, *et al*. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *J Ecol* 2007;**95**:623-630.

Hodge A, Helgason T, Fitter AH. Nutritional ecology of arbuscular mycorrhizal fungi. *Fung Ecol* 2010;**3**:267-273.

Hodge A, Storer K. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. *Plant Soil* 2015;**386**:1-19.

IPCC. Agriculture, Forestry and other land uses. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (eds) *Guidelines for National Greenhouse Gas Inventories* (Volume 4). Prepared by the National Greenhouse Gas Inventories Programme. Japan: IGES, 2006.

Jansa J, Mozafar A, Anken T, *et al*. Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza* 2002;**12**:225-234.

Jansa J, Erb A, Oberholzer HR *et al*. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Mol Ecol* 2014;**23**:2118-2135.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772-780.

Koorem K, Gazol A, Öpik M, *et al*. Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. *PLoS ONE* 2014;**9**:e91998.

Kohout P, Sudová R, Janoušková M, *et al*. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biol Biochem* 2014;**68**:482-493.

Lange M, Eisenhauer N, Sierra CA, *et al*. Plant diversity increases soil microbial activity and soil carbon storage. *Nature communications* 2015;6.

Lee J, Lee S, Young JPW. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* 2008;**65**:339-349.

Lekberg Y, Koide RT. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytol* 2005;**168**:189-204.

Littlewood N, Anderson P, Artz R *et al*. *Peatland biodiversity*. Edinburgh: IUCN UK Peatland Programme, 2010.

Lumini E, Orgiazzi A, Borriello R, *et al*. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environ Microbiol* 2010;**12**: 2165-2179.

Maherali H, Klironomos JN. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 2007;**316**:1746-1748.

Mathimaran N, Ruh R, Jama B, *et al*. Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agric Ecosyst Environ* 2007;**119**:22-32.

McGill B. Biodiversity: Land use matters. *Nature* 2015;**520**:38-39.

Meyer A, Focks A, Radl V, *et al*. Different land use intensities in grassland ecosystems drive ecology of microbial communities involved in nitrogen turnover in soil. *PLoS ONE* 2013:**8**;e73536.

Morris EK, Buscot F, Herbst C, *et al*. Land use and host neighbor identity effects on arbuscular mycorrhizal fungal community composition in focal plant rhizosphere. *Biodivers Conserv* 2013;**22**:2193-2205.

Nadimi M, Daubois L and Hijri M. Mitochondrial comparative genomics and phylogenetic signal assessment of mtDNA among arbuscular mycorrhizal fungi. *Mol Phylogenet Evol* 2016;**98:**74-83.

Nesme J, Achouak W, Agathos SN, *et al.* Back to the future of soil metagenomics. *Front Microbiol* 2016;**7:**73.

Newbold T, Hudson LN, Hill SL, *et al*. Global effects of land use on local terrestrial biodiversity. *Nature* 2015;**520**:45-50.

Newsham KK, Fitter AH, Watkinson AR. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecol Evol* 1995;**10**:407-411.

Oksanen J, Blanchet FG, Kindt R, *et al*. Vegan: community ecology package, ver.2.0–3. 2013, URL http://CRAN.Rproject.org/package=vegan.

Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams. 2015, URL http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Öpik M, Davison J, Moora M, *et al*. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. *Botany* 2014;**92**:135-147.

Öpik M, Metsis M, Daniell TJ *et al*. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytol* 2009;**184**:424-437.

Öpik M, Vanatoa A, Vanatoa E, *et al*. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* 2010;188:223-241.

Moora M, Davison J, Öpik M *et al.* Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. *FEMS Microbiol Ecol* 2014;**90**:609-621.

Pellegrino E, Bosco S, Ciccolini V, *et al.* Agricultural abandonment in Mediterranean reclaimed peaty soils: long-term effects on soil chemical properties, arbuscular mycorrhizas and CO_2 flux. *Agr Ecosyst Environ* 2014;**199**:164-175.

Pellegrino E, Öpik M, Bonari E, *et al*. Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. *Soil Biol Biochem* 2015;**84**:210-217.

Pellegrino E, Di Bene C, Tozzini C, *et al*. Impact on soil quality of a 10-year-old short-rotation coppice poplar stand compared with intensive agricultural and uncultivated systems in a Mediterranean area. *Agr Ecosyst Environ* 2011;**140**:245-254.

Powell JR, Parrent JL, Hart MM *et al*. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *P Roy Soc Lond B Bio* 2009;**276:**4237-4245.

Ravnskov S, Larsen J, Olsson PA, Jakobsen I. Effects of various organic compounds on growth and phosphorus uptakeof an arbuscular mycorrhizal fungus. *New Phytol* 1999;**141**:517-524.

Redecker D, Schüßler A, Stockinger H, *et al.* An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 2013;**23**:515-531.

Reinhart KO, Wilson GW and Rinella MJ. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. *Ecol Lett* 2012;**15**:689-695.

Rillig MC, Mummey DL. Mycorrhizas and soil structure. *New Phytol* 2006;**171**:41-53.

Rillig MC, Wright SF, Nichols KA, *et al.* Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* 2001;**233**:167-177.

Roberts DW. labdsv: ordination and multivariate analysis for ecology, package version 1.5-0. 2014, URL http://CRAN.R-project.org/package=labdsv.

Rosendahl S and Matzen HB. Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. *New Phytol* 2008;**179**:1154-1161.

Sánchez-Castro I, Ferrol N and Barea JM. Analyzing the community composition of arbuscular mycorrhizal fungi colonizing the roots of representative shrubland species in a Mediterranean ecosystem. *J Arid Environ* 2012;**80**:1-9.

Schenck NC and Smith GS Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia* 1982;77-92.

Schüßler A, Schwarzott D, Walker C. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 2001;**105**:1413-1421

Schüßler A. *The Glomeromycota: a species list with new families*. US: CreateSpace Independent Publishing Platform, 2010.

Senés-Guerrero C and Schüßler A. A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. *Fungal Divers* 2015;**77:**317-333.

Smith SE, Read DJ. *Mycorrhizal Symbiosis* (3rd ed.). New York: Academic Press, 2008.

Soil Survey Staff. *Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys* Washington DC: USDA-SCS Agric Handb 436 US Gov Print Office, 1975.

Sýkorová Z, Wiemken A, Redecker D. Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. *Appl Environ Microb* 2007;**17**:5426-5434.

Tamura K, Peterson D, Peterson N, *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;**28**:2731-2739.

Tawaraya K, Takaya Y, Turjaman M, *et al.* Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. *Forest Ecol Manag* 2003;**182:**381-386.

Thormann MN, Currah RS and Bayley SE. The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. *Wetlands* 1999;**19**:438-450.

Torgerson WS. *Theory and Methods of Scaling*. New York: John Wiley & Sons, 1958.

Torrecillas E, Alguacil MM, Roldán A. Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. *Appl Environ Microb* 2012;**78**:6180-6186.

Treseder KK. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant Soil* 2013;**371**:1-13.

Turner SD, Amon JP, Schneble RM, Friese CF. Mycorrhizal fungi associated with plants in ground-water fed wetlands. *Wetlands* 2000;**20**:200-204.

Vallebona C, Pellegrino E, Frumento P, *et al*. Temporal trends in extreme rainfall intensity and erosivity in the Mediterranean region: a case study in southern Tuscany, Italy. *Climatic Change* 2015;**128**:139-151.

Vályi K, Rillig MC, Hempel S. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. *New Phytol* 2015;**205**:1577-1586.

Van der Heijden MG, Klironomos JN, Ursic M, *et al*. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 1998;**396:**69-72.

Vandenkoornhuyse P, Husband R, Daniell TJ, *et al*. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Mol Ecol* 2002;**11**:1555-1564.

Veresoglou SD, Chen B, Rillig MC. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol Biochem* 2012;**46**:53-62.

Van der Heijden MG, Klironomos JN, Ursic M, *et al.* Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 1998;**396**:69-72.

Veresoglou SD, Rillig MC. Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant Soil* 2014;**377**:395-406.

Verhoeven JT and Setter TL. Agricultural use of wetlands: opportunities and limitations. *Ann Bot* 2009;**105**:155–163

Vogelsang KM, Reynolds HL, Bever JD. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytol* 2007;**172**:554-562.

Wardle DA, Bardgett RD, Klironomos JN, *et al*. Ecological linkages between aboveground and belowground biota. *Science* 2004;**304**:1629-1633.

Wright SF, Franke Snyder M, Morton JB, *et al*. Timecourse study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil* 1996;**181**:193-203.

Xiang D, Verbruggen E, Hu Y, Veresoglou SD, *et al*. Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China. *New Phytol* 2014; **204**:968-978.

Zedler JB, Kercher S. Wetland resources: Status, trends, ecosystem services, and restorability. *Annu Rev Environ Resour* 2005;**30**:39-74.

Zobel M, Öpik M. Plant and arbuscular mycorrhizal fungal (AMF) communities-which drives which? *J Veg Sci* 2014;**25**:1133-1140.

Table 1. Ten host plant species sampled for characterizing root-associating arbuscular mycorrhizal fungal communities in four land-use types.

Host plant species	Land-use types			
	H-Cult[a]	M-Biom	M-Grass	Z-Uncult
Calystegia sepium[b]	X	X	X	X
***Poa* sp.**	X	X	X	X
Helianthus annuus	X			
Matricaria chamomilla	X			
Arundo donax		X		
*Miscanthus*x *giganteus*		X		
Lolium perenne			X	
Ranunculus acris			X	
Bromus tectorum				X
Phragmites australis				X

[a] Four land-use types: an intensive cropping system based on sunflower (H-Cult); a two-year-old plantation of perennial grasses for energy production (M-Biom); a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult); [b] In bold co-occurring host plant species across land uses.

Table 2. PERMANOVA and PERMDISP analysis of the effect of land-use intensification (land-use type) and host plant species on arbuscular mycorrhyzal fungal (AMF) community composition at Virtual Taxa and family level within the roots of *Poa* sp. and *Calystegia sepium*.

Source of variation [a]	Total *df* [b]	SS	MS	Pseudo F	*P* (perm) [c]	Explained Variance (%)
(a) Virtual Taxa based PERMANOVA						
Land-use type (Land use)[d]	3	25029.0	8342.8	3.4	**0.001**	13.1
Host plant species (Host)[e]	1	3535.7	3535.7	1.4	0.171	1.2
Land-use × Host	3	20312.0	6770.7	2.7	**0.002**	20.3
Latitude	1	10447.0	10447.0	4.2	**0.001**	4.2
Longitude	1	2479.8	2479.8	1.0	0.436	0.0
Residuals	37	91864.0	2482.8			61.2
Total	46	153667.5				
PERMDISP						
Land use	3			42.6	**0.050**	
Total	43					
(b) Family based PERMANOVA						
Land-use type (Land use)[d]	3	22130.0	7376.8	6.1	**0.001**	27.0
Host plant species (Host)[e]	1	675.3	675.3	0.6	0.644	-1.2
Land-use × Host	3	5585.2	1861.7	1.5	0.172	6.0
Latitude	1	10218.0	10218.0	8.4	**0.001**	9.2
Longitude	1	1811.4	1811.4	1.5	0.250	0.6
Residuals	37	44850.0	1212.2			58.4
Total	46	85270.0				
PERMDISP						
Land use	3			42.8	**0.038**	
Total	43					

[a] Two-way PERMANOVA: land-use type and host plant species as fixed factors; spatial coordinates of plant indivduals were used as covariates. Response data were standardized to the median number of reads per sample; [b] Total df = total degrees of freedom, SS = Sum of squares, MS = Mean squares, Pseudo-F = F value by permutation, and *P* (perm) = *P* value by permutation.; [c] In bold statistically significant relationships ($P \leq 0.05$); [d] For land-use types see Table 1; [e] Two host plant species: *Poa* sp. and *Calystegia sepium.*

Table 3. Significant indicator Virtual Taxa (VT) of arbuscular mycorrhizal fungi (AMF) among four land use types and ten host plant species.

VT ID[a]	Land-use type[b]	Indicator value	Probability
Poa sp.			
Acaulospora VT30	M-Biom	0.704	0.023
Glomus VT309	Z-Uncult	0.749	0.001
C. sepium			
Acaulospora VT30	M-Biom	0.816	0.001
Archaeospora VT245	M-Biom	0.792	0.001
Claroideoglomus VT57	Z-Uncult	0.654	0.014

VT ID	Host plant species	Indicator value	Probability
Archaeospora VT245	*Lolium perenne*	0.442	0.021
Claroideoglomus VT193	*Phragmites australis*	0.429	0.006
Rhizophagus VT90	*Helianthus annuus*	0.664	0.001
Rhizophagus VT264	*Helianthus annuus*	0.418	0.006
Glomus VT219	*Miscanthus* x *giganteus*	0.307	0.041
Rhizophagus VT105	*Bromus tectorum*	0.499	0.002

[a] Taxonomic information about the VT observed in the roots of *Poa* sp. and *Calistegia sepium* can be found in Table S1; [b] For land-use types see Table 1.

Table 4. PERMANOVA and PERMDISP analysis of the effect of host plant species on arbuscular mycorrhyzal fungal (AMF) community compositon at Virtual Taxa and family level in the roots of ten plant species.

Source of variation [a]	Total *df* [b]	SS	MS	Pseudo-*F*	*P* (perm)[c]	Explained Variance (%)
(a) Virtual Taxa based						
PERMANOVA						
Host plant species[d]	9	21310.0	2367.7	2.4	**0.001**	13.3
Land-use type[d]	1	6577.8	6577.8	6.6	**0.001**	4.8
Latitude	1	4302.4	4302.4	4.3	**0.001**	2.9
Longitude	1	1321.7	1321.7	1.3	0.215	0.3
Residuals	78	78155.0	1002.0			78.6
Total	90	111670.0				
PERMDISP						
Host plant species	9			2.7	**0.050**	
Total	81					
(b) Family based						
PERMANOVA						
Host plant species[d]	9	15550.0	1727.8	3.8	**0.001**	22.4
Land-use type[d]	1	5058.0	5058.0	11.0	**0.001**	7.2
Latitude	1	3091.2	3091.2	6.7	**0.001**	4.2
Longitude	1	1007.6	1007.6	2.2	0.115	0.9
Residuals	78	35736.0	458.15			65.3
Total	90	60443.0				
PERMDISP						
Host plant species	9			3.49	**0.008**	
Total	81					

[a] One-way PERMANOVA: host plant species as fixed factor; land-use type and spatial coordinates of plant indivdual used as covariates. Data were standardized to the median number of reads per sample; [b] Total df = total degrees of freedom, SS = Sum of squares, MS = Mean squares, Pseudo-F = F value by permutation, and *P* (perm) = *P* value by permutation; [c] In bold statistically significant relationships ($P \leq 0.05$); [d] For host plant species and land-use types see Table1.

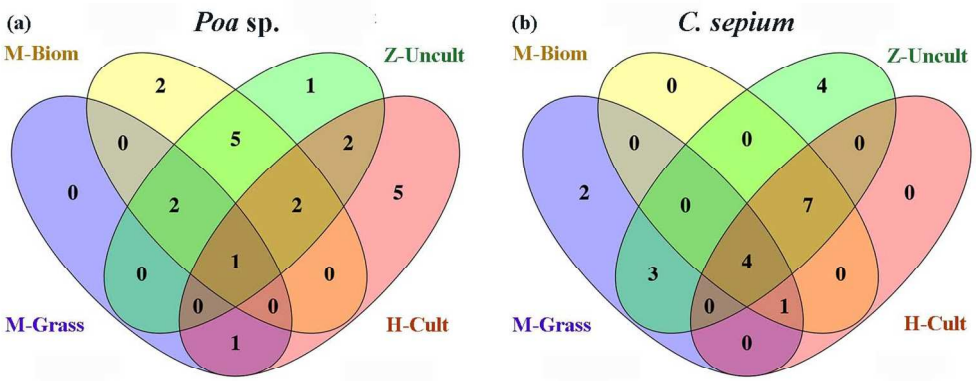


Figure 1. Venn diagrams showing the number of virtual taxa (VT) retrieved from the roots of (a) Poa sp. and (b) Calystegia sepium unique to and shared between different land-use types: an intensive continuous sunflower cropping system (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and agricultural soil left abandoned for 15 years (Z-Uncult). Results obtained after standardization of the data to the median number of reads per sample.

200x99mm (200 x 200 DPI)

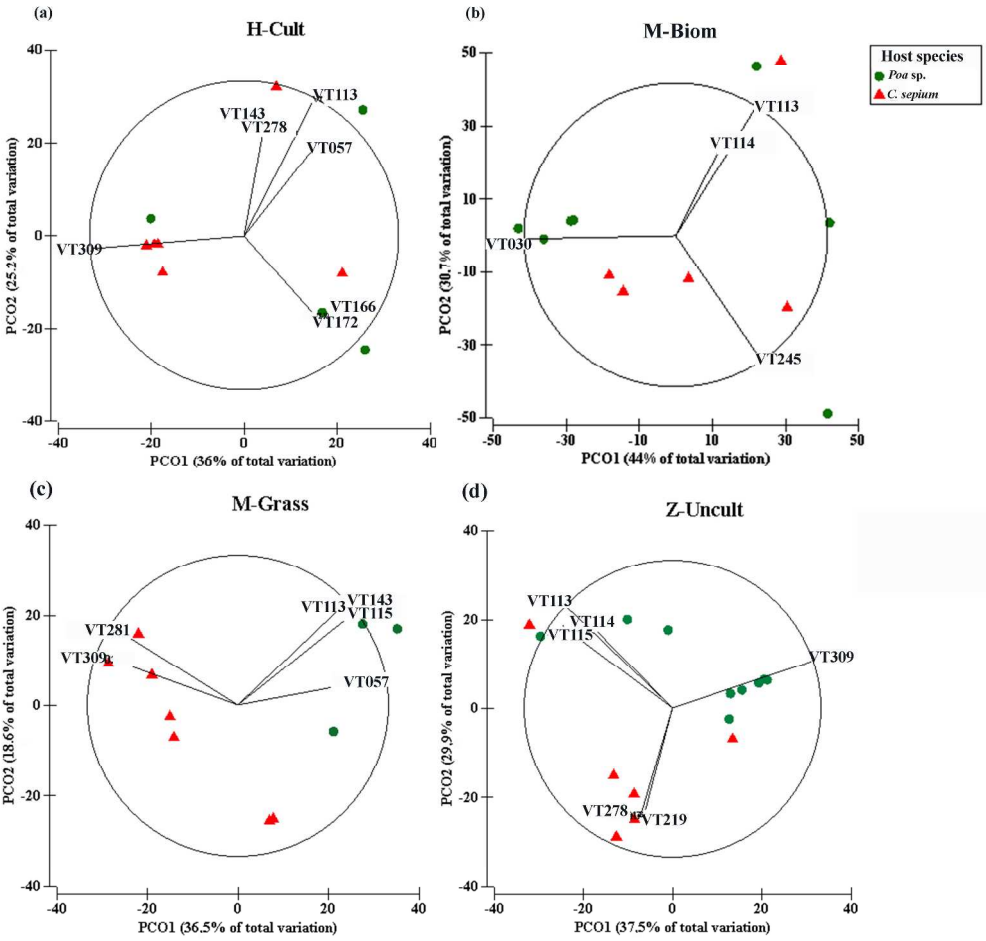


Figure 2. Principal coordinate analyses (PCO) biplots on the interaction between land-use intensity and host plant identity (Poa sp. and Calystegia sepium) on the arbuscular mycorrhizal fungal (AMF) communities within roots (based on virtual taxa, VT). Biplots show the differences of host plant effect on AMF communities in the same land-use intensity class: (a) an intensive continuous sunflower cropping system, H-Cult; (b) a two-year-old plantation of perennial grasses for energy production, M-Biom; (c) a two-year-old managed grassland, M-Grass; (d) an agricultural soil left abandoned for 15 years, Z-Uncult. The proportion of variance explained by each PCO axes is given in parentheses. Results shown are based on the data standardized to the median numbers of reads per sample.┼

399x399mm (250 x 250 DPI)

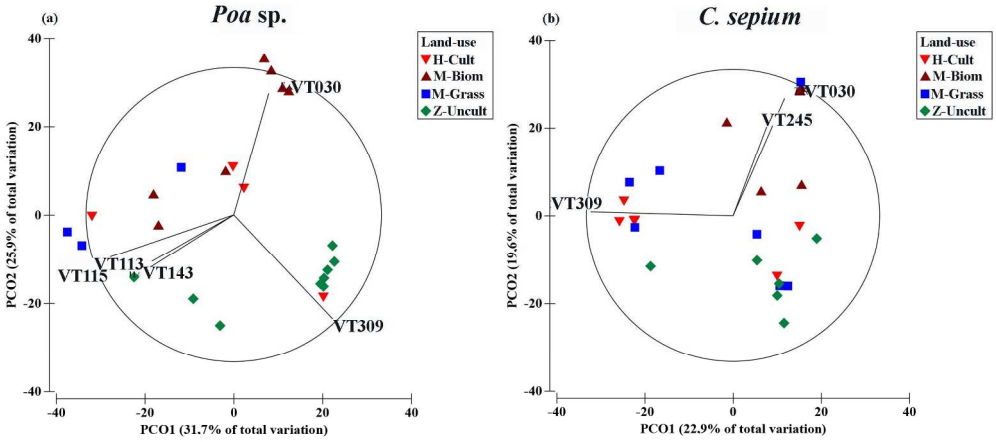


Figure 3. Principal coordinate analyses (PCO) biplots on the interaction between land-use intensity (an intensive continuous sunflower cropping system, H-Cult; a two-year-old plantation of perennial grasses for energy production, M-Biom; a two-year-old managed grassland, M-Grass; an agricultural soil left abandoned for 15 years, Z-Uncult) and host plant identity on the arbuscular mycorrhizal fungal (AMF) communities within roots (based on virtual taxa, VT). Biplots show the effect of land-use type on AMF communities in the roots of Poa sp. and C. sepium. The PCOs were calculated on the Bray-Curtis similarities based on AMF relative abundances clustered at "virtual taxa" (VT) level. Only the VT with a strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis are shown. The proportion of variance explained by the PCO axes is given in parentheses. Results shown are based on the data standardized to the median of the numbers of reads per sample.┼

199x99mm (300 x 300 DPI)

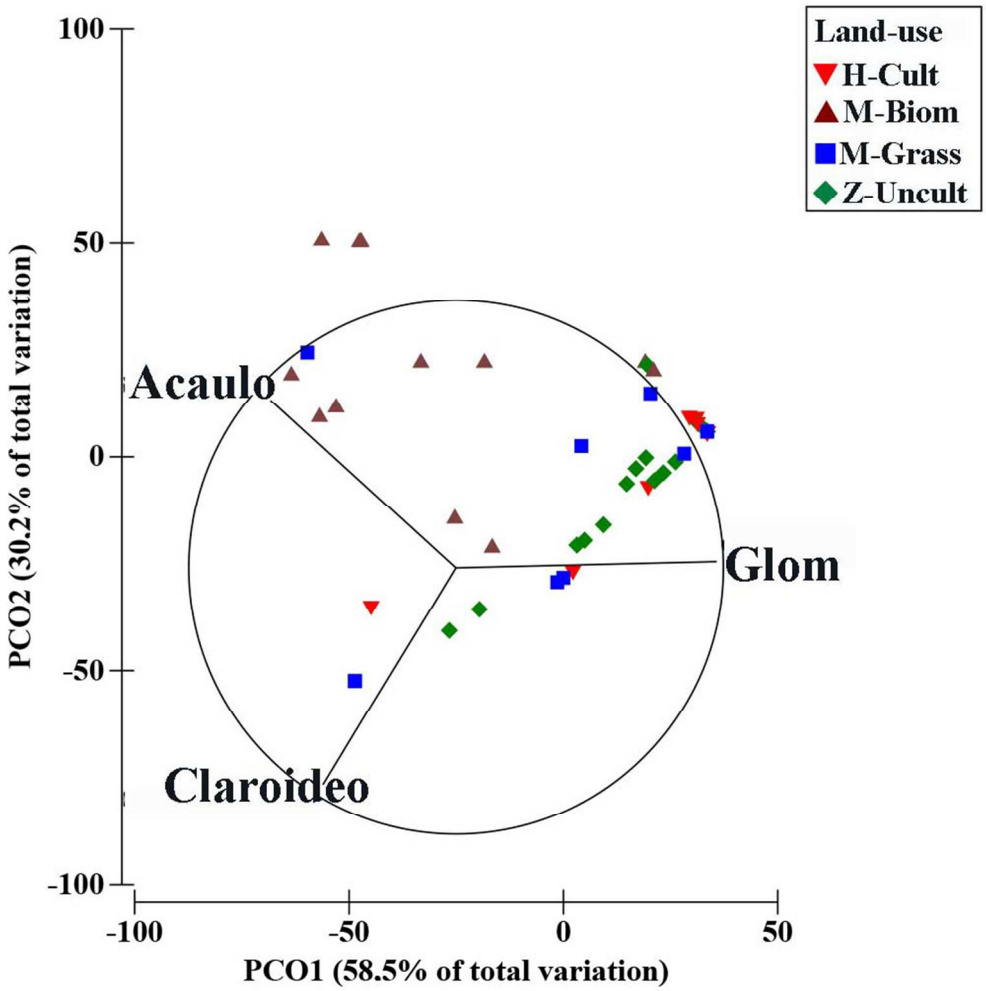


Figure 4. Principal coordinates analyses (PCO) biplot on the effect of land-use intensification on the arbuscular mycorrhizal fungal (AMF) communities within roots clustered at family level. Biplot shows the effect on AMF communities in the roots of Poa sp. and C. sepium of different land-use type: (a) an intensive continuous sunflower cropping system, H-Cult; (b) a two-year-old plantation of perennial grasses for energy production, M-Biom; (c) a two-year-old managed grassland, M-Grass; (d) an agricultural soil left abandoned for 15 years, Z-Uncult. Families are shown as: Acaulo = Acaulosporaceae, Claroideo = Claroideoglomeraceae, Glom = Glomeraceae. Spatial coordinates of plant individuals were used as covariates. Results shown are based on the data standardized to the median numbers of reads per sample.

86x88mm (300 x 300 DPI)

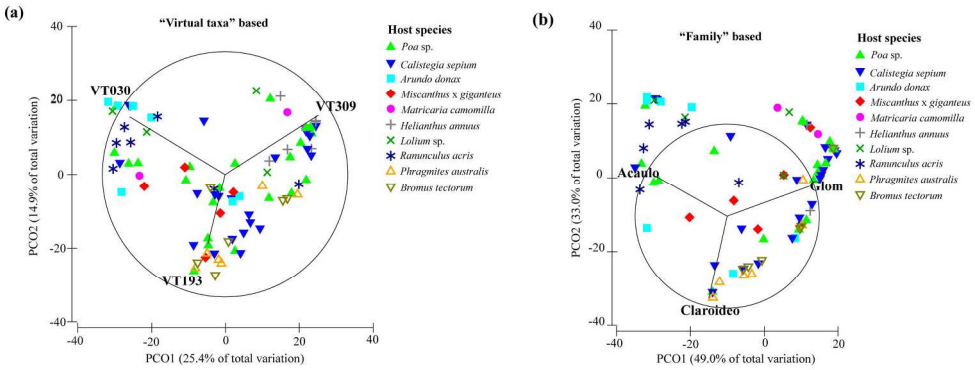


Figure 5. Principal coordinate analyses (PCO) biplots showing the effect of host plant species on root AMF communities (a) at Virtual Taxon level and (b) at family level. Land-use types and spatial coordinates of plant individuals were used as covariates. Results shown are based on the data standardized to the median numbers of reads per sample. ┼

401x148mm (150 x 150 DPI)

Table S1. Taxonomic information about the AMF Virtual Taxa (VT) found in this study. VT included in the reduced dataset after standardization to the median number of reads are shown in bold.

VT code[a]	Family	VT type sequence
VT4	Archaeosporaceae	*Archaeospora Wirsel OTU21*
VT28	Acaulosporaceae	*Acaulospora* Acau10
VT30	Acaulosporaceae	*Acaulospora* Acau2
VT54	Diversisporaceae	*Diversispora sp.*
VT56	Claroideoglomeraceae	*Claroideoglomus Douhan9*
VT57	Claroideoglomeraceae	*Claroideoglomus acna Glo7*
VT60	Diversisporaceae	*Diversispora MO-GC1*
VT62	Diversisporaceae	*Diversispora MO-GC2*
VT64	Glomeraceae	*Glomus MO-G18*
VT65	Glomeraceae	*Funneliformis caledonium, fragilistratum, geosporum, verruculosum*
VT67	Glomeraceae	*Funneliformis mosseae*
VT74	Glomeraceae	*Glomus* Glo3
VT76	Glomeraceae	*Glomus Franke A1*
VT90	Glomeraceae	*Rhizophagus manihotis*
VT92	Glomeraceae	*Glomus Yamato09 A1*
VT105	Glomeraceae	*Rhizophagus intraradices*
VT108	Glomeraceae	*Glomus Whitfield type 7*
VT113	Glomeraceae	*Glomus MO-G3*
VT114	Glomeraceae	*Glomus MO-G17*
VT115	Glomeraceae	*Glomus MO-G13*
VT132	Glomeraceae	*Glomus* Glo14b
VT143	Glomeraceae	*Glomus MO-G20*
VT160	Glomeraceae	*Glomus MO-G27*
VT163	Glomeraceae	*Glomus* MO-G25
VT166	Glomeraceae	*Glomus MO-G4*
VT172	Glomeraceae	*Glomus Winther07-B*
VT193	Claroideoglomeraceae	*Claroideoglomus lamellosum*
VT216	Glomeraceae	*Glomus* Glo54
VT219	Glomeraceae	*Glomus MO-G5*
VT238	Paraglomeraceae	*Paraglomus occultum*
VT239	Paraglomeraceae	*Paraglomus brasilianum*
VT245	Archaeosporaceae	*Archaeospora trappei*
VT247	Glomeraceae	*Glomus* Glo39
VT248	Glomeraceae	*Glomus* Yamato09 A2
VT264	Glomeraceae	*Rhizophagus clarus*
VT270	Glomeraceae	Glomus Yamato09 E
VT278	Claroideoglomeraceae	*Claroideoglomus* ORVIN GLO4
VT280	Glomeraceae	Glomus Glo2
VT281	Paraglomeraceae	*Paraglomus laccatum*
VT309	Glomeraceae	Glomus ORVIN GLO3E
VT310	Glomeraceae	Glomus ORVIN GLO3D
VT342	Glomeraceae	Glomus VeGlo18
VT375	Paraglomeraceae	*Paraglomus MO-P1*
VT403	Glomeraceae	*Glomus* sp.

[a]Source: Maarj*AM* database (status 18 April 2016), http://maarjam.botany.ut.ee/

Table S2 Two-way ANCOVA on the effect of land-use intensification (land-use type) and host preference (host plant species) on arbuscular mycorrhizal fungal (AMF) richness based on a classification at the Virtual Taxa and family level.

Parameter/Source of variation[a]	d.f.	Richness (S)	d.f.	Richness (S)
	Virtual Taxa based		*Family based*	
Land-use type (Land use)[b]	1	**0.020**[d]	1	**0.016**
Host plant species (Host)[c]	3	0.103	3	0.063
Land-use × Host	3	0.162	3	0.639
Latitude	1	0.515	1	0.582
Longitude	1	0.763	1	0.596
Residuals	37		37	
Total	47		47	

[a] Two-way ANCOVA: land-use type and host plant species as fixed factors; spatial coordinates of plant indivduals, used as covariates; [b]Four land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult); [c] Two host plant species: *Poa* sp. and *Calystegia sepium*; [d] Values in bold show $P \leq 0.05$. Data were standardized to the median numbers of reads.

Table S3. Analysis of multivariate dispersion of the community composition of arbuscular mycorrhizal fungi at Virtual Taxa and family level among four types of land use within *Poa* sp. and *Calystegia sepium* roots. Data were standardized to the median numbers of reads.

Pairwise comparisons	t	*P* (perm)[a]
(a) Virtual Taxa based		
M-Biom / H-Cult[b]	1.428	0.236
M-Biom / M-Grass	3.149	**0.005**
M-Biom / Z-Uncult	12.447	0.998
H-Cult/M-Grass	1.167	0.398
H-Cult / Z-Uncult	1.707	0.202
M-Grass/Z-Uncult	3.939	**0.002**
(b) Family based		
M-Biom / H-Cult	2.080	0.099
M-Biom / M-Grass	0.106	0.922
M-Biom / Z-Uncult	3.515	**0.005**
H-Cult/M-Grass	1.701	0.246
H-Cult / Z-Uncult	0.432	0.769
M-Grass/Z-Uncult	2.851	**0.023**

[a] PERMDISP analyses; 9999 permutations; *P* perm = value by permutation, in bold statistically significant relationships ($P \leq 0.05$); [b] Four land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult).

Table S4. PERMANOVA and PERMDISP analysis of the effect of land-use intensification (land-use type) and host plant species on arbuscular mycorrhyzal fungal (AMF) community compositon at Virtual Taxa and family level. Data were not standardized to the median number of reads per sample.

Source of variation [a]	Total *df* [b]	SS	MS	Pseudo F	*P* (perm) [c]	Explained Variance (%)
(a) Virtual Taxa based						
PERMANOVA						
Land-use type (Land use)[d]	3	21325.0	7108.2	3.2	**0.001**	12.5
Host plant species (Host)[e]	1	3301.7	3301.7	1.5	0.148	1.5
Land-use × Host	3	17935.0	5978.3	2.7	**0.001**	20.4
Latitude	1	8996.1	8996.1	4.1	**0.001**	4.1
Longitude	1	2137.5	2137.5	1.0	0.449	0.0
Residuals	37	81220.0	2195.1			61.6
Total	46	134915.3				
PERMDISP						
Land use	3			40.0	**0.050**	
Total	43					
(b) Family based						
PERMANOVA						
Land-use type (Land use)[d]	3	6627.0	2209.0	5.7	**0.001**	25.8
Host plant species (Host)[e]	1	290.4	290.4	0.7	0.517	-0.7
Land-use × Host	3	1626.8	542.3	1.4	0.212	4.6
Latitude	1	3117.6	3117.6	8.0	**0.001**	9.1
Longitude	1	587.4	587.4	1.5	0.241	0.7
Residuals	37	14393.0	389.0			60.7
Total	46	26642.0				
PERMDISP						
Land use	3			64.2	**0.010**	
Total	43					

[a] Two-way PERMANOVA: land-use type and host plant species as fixed factors; spatial coordinates of plant indivduals, used as covariates; [b] Total df = total degrees of freedom, SS = Sum of squares, MS = Mean squares, Pseudo-F = F value by permutation, and *P* (perm) = *P* value by permutation.; [c] In bold statistically significant relationships ($P \leq 0.05$); [d] Four land-use types: an intensive cropping system based on sunflower (H-Cult); a two-year-old plantation of perennial grasses for energy production (M-Biom); a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult); [e] Two host plant species: *Poa* sp. and *Calystegia sepium.*

Table S5. Analysis of multivariate dispersion of the community composition of arbuscular mycorrhizal fungi at virtual taxa and family level among four types of land use within the roots of *Poa* sp. and *Calystegia sepium*. Data were not standardized to the median numbers of reads.

Pairwise comparisons	t	*P* (perm)[a]
Virtual Taxa based		
M-Biom / H-Cult[b]	1.313	0.294
M-Biom / M-Grass	3.142	**0.008**
M-Biom / Z-Uncult	0.146	0.906
H-Cult/M-Grass	1.170	0.373
H-Cult / Z-Uncult	1.624	0.226
M-Grass/Z-Uncult	3.799	**0.001**
Family based		
M-Biom / H-Cult	2.643	0.083
M-Biom / M-Grass	0.359	0.754
M-Biom / Z-Uncult	4.470	**0.001**
H-Cult/M-Grass	2.014	0.137
H-Cult / Z-Uncult	0.395	0.806
M-Grass/Z-Uncult	3.394	**0.010**

[a] PERMDISP analyses; 9999 permutations; *P* perm = value by permutation, in bold statistically significant relationships ($P \leq 0.05$); [b] Four land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult).

Table S6. Effect of the host plant species on the richness of arbuscular mycorrhizal fungi (AMF) at Virtual Taxa and family level within the roots of ten host plant species.

Host plant species[a]	Richness[b]	
	Virtual Taxa based	*Family based*
Arundo donax	3.00	2.29 ab
Bromus tectorum	3.83	1.83 a
Calystegia sepium	4.08	2.46 ab
Helianthus annuus	3.67	1.5 a
Lolium perenne	3.00	3.00 ab
Matricaria chamomilla	2.00	1.5 a
Miscanthus x *giganteus*	6.00	3.60 b
Phragmites australis	4.67	2.67 ab
Poa sp.	3.41	2.18 ab
Ranunuculus acris	3.57	2.57 ab

[a] Values are means of at least three replicates for each host plant species. For each parameter, values in the same column followed by different letters are statistically different among host plant species according to the ANCOVAs and LSD test ($P < 0.05$). Data were standardized to the median numbers of reads per samples.

Table S7. Analysis of multivariate dispersion of the community composition of arbuscular mycorrhizal fungi at "virtual taxon" and family level among ten host plant species. Data were standardized to the median numbers of reads.

Host plant species[a]	t	*P* perm[b]
"Virtual taxon" based		
Poa - Hel	3.730	0.015*
Poa - Ran	2.340	0.050*
Cal - Aru	2.052	0.098**
Cal - Hel	4.197	0.009*
Cal - Mat	2.773	0.078**
Cal - Ran	2.810	0.044*
Hel - Bro	2.459	0.065**
Mis - Hel	2.755	0.082**
Family based		
Aru - Hel	3.633	0.006**
Aru - Mat	3.633	0.031**
Aru- Bro	2.195	0.089*
Cal-Bro	2.486	0.067*
Cal - Hel	3.735	0.002**
Cal - Mat	3.450	0.003**
Hel - Lol	4.968	0.013**
Hel -Phr	1.827	0.075*
Lol - Bro	2.889	0.037**
Mat - Lol	6.017	0.030**
Mat - Ran	2.490	0.097**
Mis - Bro	2.602	0.072*
Mis - Mat	5.443	0.011**
Mis - Hel	4.644	0.008**
Poa - Hel	3.131	0.045**
Poa - Mat	2.898	0.016**

[a] Aru = *Arundo donax*; Bro = *Bromus tectorum*; Cal = *Calystegia sepium*; Hel = *Helianthus annuus*; Lol = *Lolium perenne*; Mat = *Matricaria chamomilla*; Mis = *Miscanthus* x *giganteus;* Phr = *Phragmites australis*; Poa = *Poa* sp., Ran = *Ranunculus acris*, [b] PERMDISP analyses; 9999 permutations; P perm = value by permutation; statistically significant differences: $**P \leq 0.05$, $*P \leq 0.10$.

Table S8. PERMANOVA and PERMDISP analyses on the effect of host plant species on the arbuscular mycorrhyzal fungal (AMF) community compositon at "Virtual taxon" and family level within the roots of ten plant species. Data were not standardized to the median numbers of reads per sample.

Source of variation [a]	Total *df* [b]	SS	MS	Pseudo-*F*	*P* (perm) [c]	Explained Variance (%)
PERMANOVA			*"Virtual taxa" based*			
Host plant species[d]	9	52962.0	5884.5	2.2	**0.001**	13.9
Land-use type[e]	1	15485.0	15485.0	6.4	**0.001**	4.6
Latitude	1	10791.0	10791.0	4.5	**0.001**	3.1
Longitude	1	3023.5	3023.5	1.2	0.253	0.2
Residuals	78	189080.0	5884.7			78.2
Total	90	271340.0	2424.1			
PERMDISP						
Host plant species	9			2.6	**0.050**	
Total	81					
PERMANOVA				*Family based*		
Host plant species[d]	9	15566.0	1729.5	3.8	**0.001**	22.6
Land-use type[e]	1	4874.7	4874.7	10.7	**0.001**	7.0
Latitude	1	3076.7	3076.7	6.7	**0.001**	4.2
Longitude	1	1008.7	1008.7	2.2	0.113	0.9
Residuals	78	35581.0	456.2			65.4
Total	90	60107.0				
PERMDISP						
Host plant species	9			3.6	**0.007**	
Total	81					

[a] One-way PERMANOVA: host plant species as fixed factor; land-use type and spatial coordinates of plant indivduals used as covariates; [b] Total df = total degrees of freedom, SS = Sum of squares, MS = Mean squares, Pseudo-F = F value by permutation, and *P* (perm) = *P* value by permutation; [c] In bold statically significant relationships ($P \leq 0.10$); [d] Ten host plant species: *Arundo donax; Bromus tectorum; Calystegia sepium; Helianthus annuus; Lolium perenne; Matricaria chamomilla; Miscanthus x giganteus; Phragmites australis; Poa sp.; Ranunuculus acris*; [e] Four land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult).

Table S9. Analysis of multivariate dispersion in the community composition of arbuscular mycorrhizal fungi based on Virtual Taxa (VT) and family based classification of the in roots of different host plant species. Data were not standardized to the median numbers of reads.

Host plant species[a]	t	*P* perm[b]
Virtual Taxa based		
Poa - Hel	2.810	0.044*
Poa - Ran	4.197	0.009*
Cal -Aru	2.052	0.098**
Cal -Hel	4.197	0.009*
Cal - Mat	2.755	0.082**
Cal -Ran	2.340	0.050*
Hel - Bro	2.773	0.078**
Mis -Hel	2.052	0.098**
Family based		
Aru - Hel	3.450	0.003**
Aru - Mat	3.450	0.003**
Aru- Bro	2.486	0.067*
Cal-Bro	1.827	0.075*
Cal - Hel	6.017	0.030**
Cal - Mat	3.633	0.031**
Hel - Lol	2.889	0.037**
Hel -Phr	2.195	0.089*
Lol - Bro	4.968	0.013**
Mat - Lol	2.490	0.097**
Mat - Ran	4.968	0.013**
Mis - Bro	2.345	0.065*
Mis - Mat	3.131	0.045**
Mis - Hel	3.633	0.031**
Poa - Hel	2.898	0.016**
Poa - Mat	5.443	0.011**

[a] Aru = *A. donax;* Bro = *B. tectorum*; Cal = *C. sepium*; Hel = *H. annuus*; Lol = *L. perenne*; Mat = *M. chamomilla*; Mis = Miscanthus x giganteus; Phr = *P. australis*; Poa = *Poa* sp., Ran = *R. acris*; [b] PERMDISP analyses; 9999 permutations; P perm = value by permutation; statistically significant differences: $^{**}P \leq 0.05$, $^{*}P \leq 0.10$.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Neighbor-Joining tree showing phylogenetic relationships between arbuscular mycorrhizal fungal (AMF) sequences detected in four land use types [an intensive cropping system based on sunflower (H-Cult: Cult); a two-year-old plantation of perennial grasses for energy production (M-Biom: Biom); a two-year-old managed grassland (M-Grass: Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult: Uncult)] and within the roots of ten host plant species (A = *Poa* sp.; B = *Calystegia sepium*; C = *Matricaria chamomilla*; D = *Helianthus annuus*; E = *Arundo donax*; F = *Miscanthus x giganteus*; G = *Lolium perenne*; H = *Ranunuculu sacris;* I = *Phragmites australis*; L = *Bromus tectorum*). The NJ tree included 561 small subunit rRNA gene (NS31/AML2 fragment) sequences: 316 sequences detected in this study by 454-pyrosequencing (in bold: coded by land-use type and host plant species), 45 type sequences of AMF Virtual Taxa (VT) from the Maarj*AM* database of Glomeromycota (accession number and VT type); 60 sequences from an updated reference dataset of AMF morphotype sequences (Accession number and species) (Schüßler and Walker, 2010); 63 sequences already detected in the study site by Sanger sequencing (accession number and MOTU code based on the genus, see Ciccolini, Bonari and Pellegrino, 2015). Bootstrap values (based on 1,000 replicates) higher than 50% are shown. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Glomeromycota nomenclature follows the consensus classification of Redecker *et al*. (2013). The accession numbers of sequences from this study are followed by the name of land use type (e.g., uncult, see above), plant species (from A to L), field replicate plot (from 1 to 3), individual plant positions (from 1 to 3) and the VT AMF sequences type (i.e., uncultC_3_6_VT113). The 390 sequences represent up to four sequences of each detected VT for each land-use type across the plant species studied. Analyses were conducted in MEGA7. Details of the VT detected in this study are given in Tables S1.

Figure S2. Rarefaction analyses of arbuscular mycorrhizal fungi associated with ten plant species from four land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production(M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult). The dashed line shows the median number of reads recovered from samples (154 reads). A = *Poa* sp.; B = *Calystegia sepium*; C = *Matricaria chamomilla*; D = *Helianthus annuus*; E = *Arundodonax*; F = *Miscanthus x giganteus*; G = *Lolium perenne*; H = *Ranunuculus acris;* I = *Phragmites australis*; L = *Bromus tectorum*. For analysis of community patterns, data were standardized to the median number of reads per sample.

Figure S3. Venn diagrams showing the number of reads recorded in the roots of (a) *Poa* sp. and (b) *Calystegia sepium* unique to and shared between different land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland(M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult). Data were standardized to the median number of reads per sample.

Figure S4. Number of AMF Virtual Taxa (a) and families (b) per sample in relation to land-use type. Data were standardized to the median number of reads per sample. Boxplots represent the median and 1st and 3rd quartile.

Figure S5. Bar plots showing the relative abundances of the 12 AMF Virtual Taxa (VT) in roots of *Poa* sp. and *Calystegia sepium* shown to be highly discriminant of the AMF communities in different land-use types or representative of one or more land use. The VT shown are those with a strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis in Fig. 2. Values are means ± SE of at least three replicate for each host plant species. Data were standardized to the median

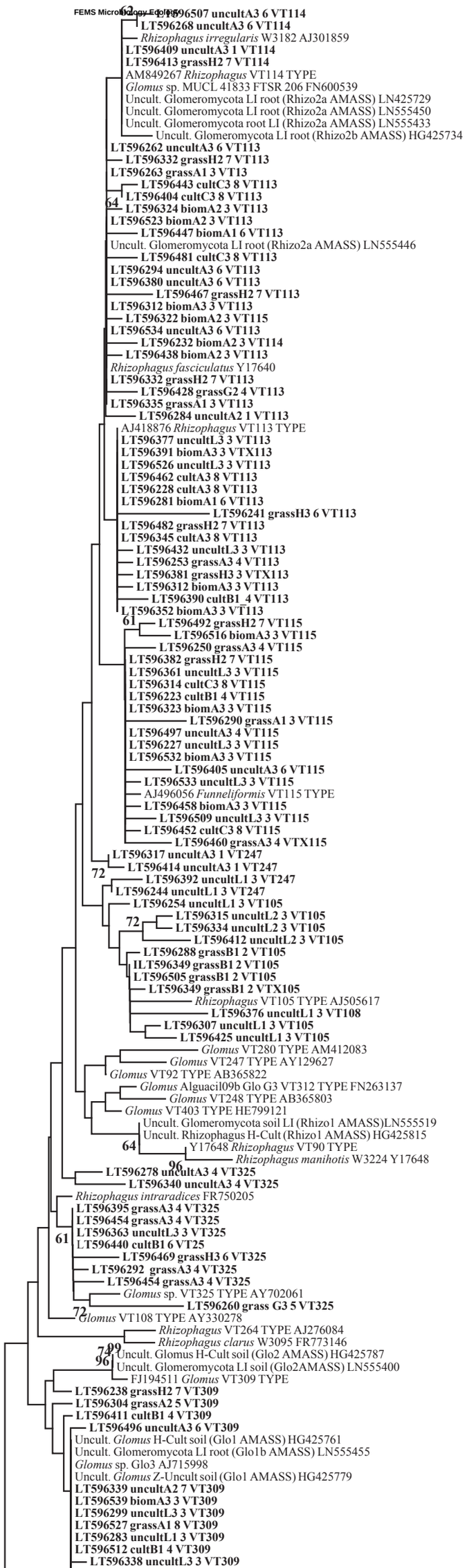
number of reads per sample.. H-Cult = an intensive cropping system based on sunflower; M-Biom = a two-year-old plantation of perennial grasses for energy production, M-Grass = a two-year-old managed grassland; Z-Uncult = an agricultural soil abandoned 15 years ago.

Figure S6. Bar plots showing the relative abundance of arbuscular mycorrhizal fungal (AMF) Virtual Taxa (VT) in roots of (a) *Poa* sp. and (b) *Calystegia sepium* from different land-use types: an intensive cropping system based on sunflower(H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago(Z-Uncult). The VT shown are those with a strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis in Fig. 3. Values are means ± SE of at least three replicate for each host plant species. Data were standardized to the median number of reads per sample.

Figure S7. Bar plots showing the relative abundance of arbuscular mycorrhizal fungal (AMF) families in the roots of *Poa* sp. and *Calystegia sepium* from different land-use types: an intensive cropping system based on sunflower (H-Cult), a two-year-old plantation of perennial grasses for energy production (M-Biom), a two-year-old managed grassland (M-Grass) and an agricultural soil abandoned 15 years ago (Z-Uncult). Values are means ± SE of at least three replicate for each host plant species. Data were standardized to the median number of reads per sample.

Figure S8. Bar plots showing the relative abundance of the three AMF Virtual Taxa (a) and family (b) shown to be highly discriminant of the AMF community composition in the roots of the ten host plant species sampled: Aru = *Arundo donax;* Bro = *Bromus tectorum;* Cal = *Calystegia sepium*; Hel = *Helianthus annuus*; Lol = *Lolium perenne*; Mat = *Matricaria chamomilla*; Mis = *Miscanthus x giganteus*; Poa = *Poa* sp.;Phr= *Phragmites australis*; Ran = *Ranunuculus acris.* The VT and families showed are those with a strong correlation ($r \geq 0.60$) with the ordination scores on each

PCO axis of Fig.5. Values are means ± SE of at least three replicate for each host plant species. Bar plots marked with different letters are statistically different according to the ANCOVAs and LSD test ($P < 0.05$). Data were standardized to the median number of reads per sample.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LT596409 uncultA3 1 VT114
LT596413 grassH2 7 VT114
AM849267 Rhizophagus VT114 TYPE
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Uncult. Glomeromycota LI root (Rhizo2a AMASS) LN555450
Uncult. Glomeromycota root LI (Rhizo2a AMASS) LN555433
Uncult. Glomeromycota LI root (Rhizo2b AMASS) HG425734
LT596262 uncultA3 6 VT113
LT596332 grassH2 7 VT113
LT596263 grassA1 3 VT13
LT596443 cultC3 8 VT113
LT596404 cultC3 8 VT113
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LT596324 biomA2 3 VT113
LT596523 biomA2 3 VT113
LT596447 biomA1 6 VT113
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LT596294 uncultA3 6 VT113
LT596380 uncultA3 6 VT113
LT596467 grassH2 7 VT113
LT596312 biomA3 3 VT113
LT596322 biomA2 3 VT115
LT596534 uncultA3 6 VT113
LT596232 biomA2 3 VT114
LT596438 biomA2 3 VT113
Rhizophagus fasciculatus Y17640
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LT596428 grassG2 4 VT113
LT596335 grassA1 3 VT113
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LT596462 cultA3 8 VT113
LT596228 cultA3 8 VT113
LT596281 biomA1 6 VT113
LT596241 grassH3 6 VT113
LT596482 grassH2 7 VT113
LT596345 cultA3 8 VT113
LT596432 uncultL3 3 VT113
LT596253 grassA3 4 VT113
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LT596390 cultB1_4 VT113
LT596352 biomA3 3 VT113
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AJ496056 Funneliformis VT115 TYPE
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LT596460 grassA3 4 VTX115
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Glomus VT280 TYPE AM412083
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Glomus VT92 TYPE AB365822
Glomus Alguacil09b Glo G3 VT312 TYPE FN263137
Glomus VT248 TYPE AB365803
Glomus VT403 TYPE HE799121
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Uncult. Rhizophagus H-Cult (Rhizo1 AMASS) HG425815
64
Y17648 Rhizophagus VT90 TYPE
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96
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Glomus VT108 TYPE AY330278
Rhizophagus VT264 TYPE AJ276084
Rhizophagus clarus W3095 FR773146
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96
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FJ194511 Glomus VT309 TYPE
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Uncult. Glomeromycota LI root (Glo1b AMASS) LN555455
Glomus sp. Glo3 AJ715998
Uncult. Glomus Z-Uncult soil (Glo1 AMASS) HG425779
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LT596539 biomA3 3 VT309
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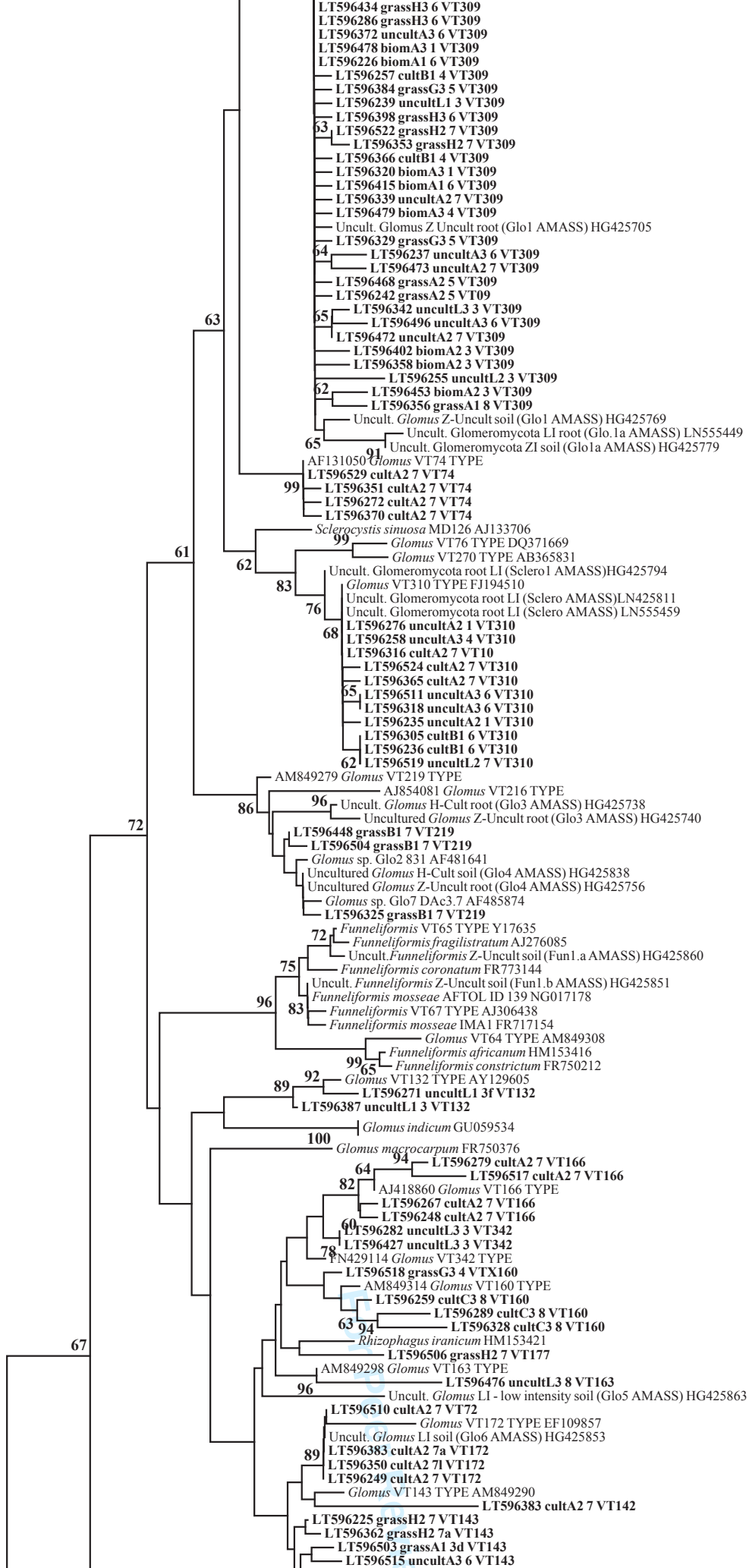
LT596527 grassA1 8 VT309
LT596283 uncultL1 3 VT309
LT596512 cultB1 4 VT309
LT596338 uncultL3 3 VT309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LT596453 biomA2 3 VT309
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Uncult. Glomus Z-Uncult soil (Glo1 AMASS) HG425769
Uncult. Glomeromycota LI root (Glo.1a AMASS) LN555449
65
91
Uncult. Glomeromycota ZI soil (Glo1a AMASS) HG425779
AF131050 Glomus VT74 TYPE
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Glomus VT76 TYPE DQ371669
Glomus VT270 TYPE AB365831
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AM849279 Glomus VT219 TYPE
AJ854081 Glomus VT216 TYPE
86
96
Uncult. Glomus H-Cult root (Glo3 AMASS) HG425738
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72
LT596448 grassB1 7 VT219
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Glomus sp. Glo2 831 AF481641
Uncultured Glomus H-Cult soil (Glo4 AMASS) HG425838
Uncultured Glomus Z-Uncult root (Glo4 AMASS) HG425756
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Funneliformis VT65 TYPE Y17635
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Funneliformis coronatum FR773144
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Glomus VT64 TYPE AM849308
Funneliformis africanum HM153416
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Funneliformis constrictum FR750212
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Glomus VT132 TYPE AY129605
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Glomus macrocarpum FR750376
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FN429114 Glomus VT342 TYPE
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AM849314 Glomus VT160 TYPE
LT596259 cultC3 8 VT160
LT596289 cultC3 8 VT160
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LT596328 cultC3 8 VT160
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LT596506 grassH2 7 VT177
AM849298 Glomus VT163 TYPE
LT596476 uncultL3 8 VT163
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Uncult. Glomus LI - low intensity soil (Glo5 AMASS) HG425863
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Glomus VT172 TYPE EF109857
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LT596503 grassA1 3d VT143
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LT596430 grassH2 7d VT143
LT596514 grassH2 7c VT43
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Diversispora trimurales FR686955
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Otospora barae O barae Dol Z2 FR865463
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Diversispora VT62 TYPE AM849266
Diversispora sp. VT306 FM875900 TYPE
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Gigaspora gigantea NC1 50 AJ852602
Racocetra fulgida W2993 AJ306435
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Pacispora scintillans W3793 AJ619940
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Acaulospora cavernata AJ306442
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LT596378 grassA2 5 VT278
LT596421 grassB3 4 V278
76
LT596437 uncultA2 5 VT278
LT596291 grassB3 4c VT278
86
64
Claroideoglomus VT278 TYPE FJ194513
Claroideoglomus VT56 TYPE AY916419
LT596285 uncultA2 5 VT56
99
LT596457 uncultA2 5b VT56
LT596346 uncultL2 3a VT56
LT596256 uncultL2 3 VT56
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LT596459 uncultL2 3b VT56
88
LT596485 uncultL2 3c VT56
62
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Claroideoglomus claroideum AJ276080
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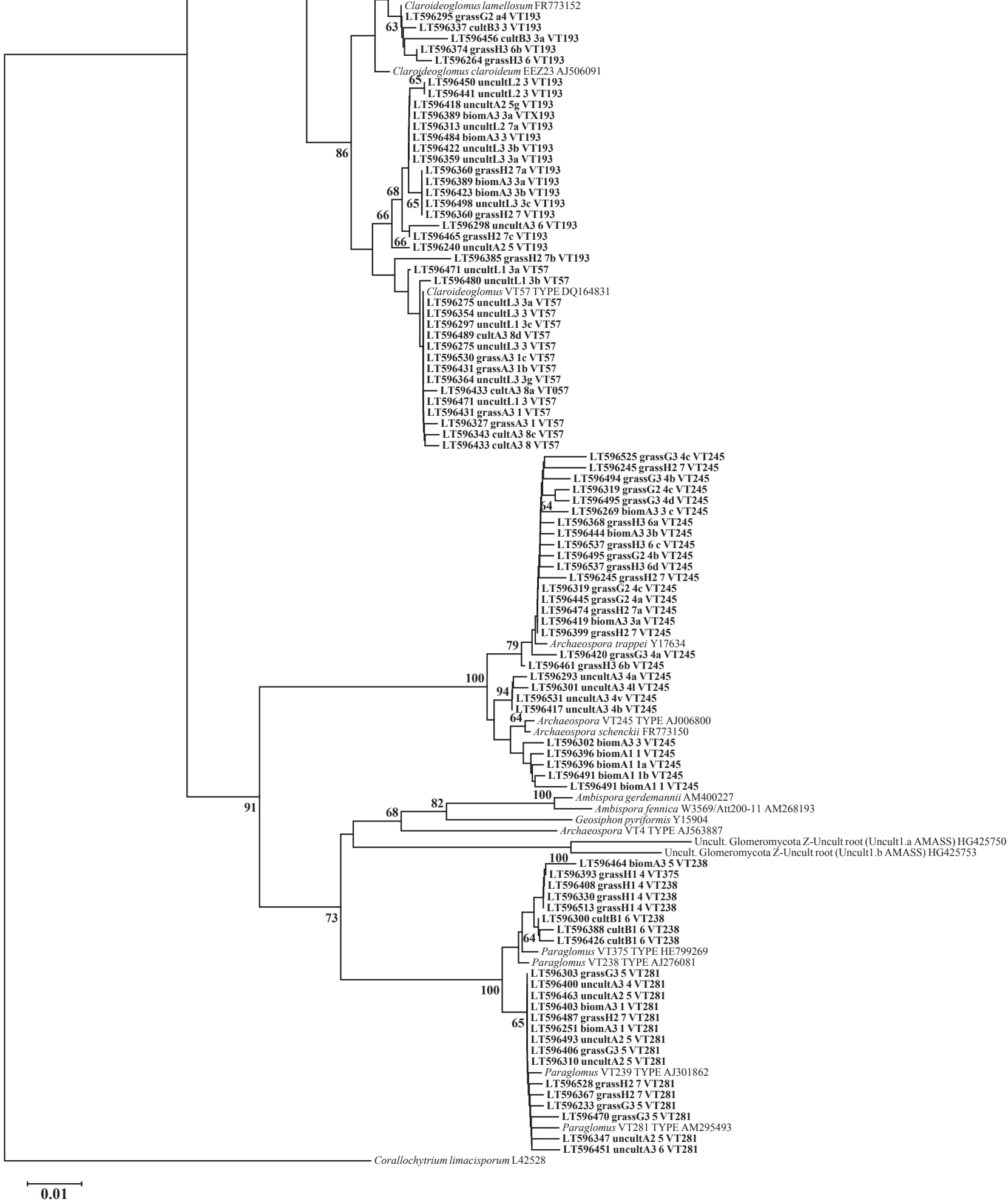

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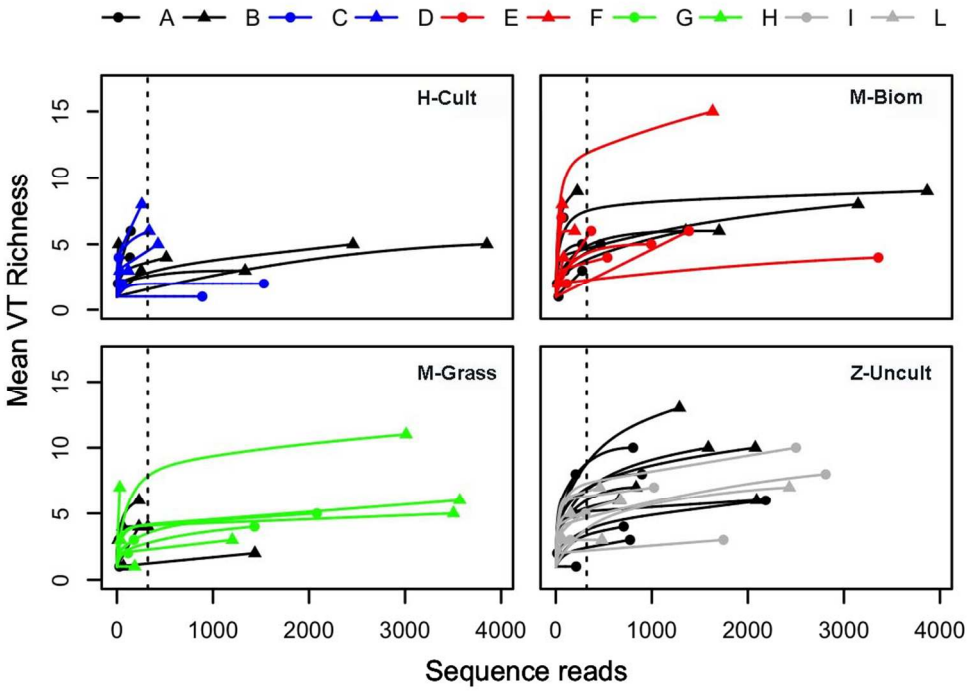

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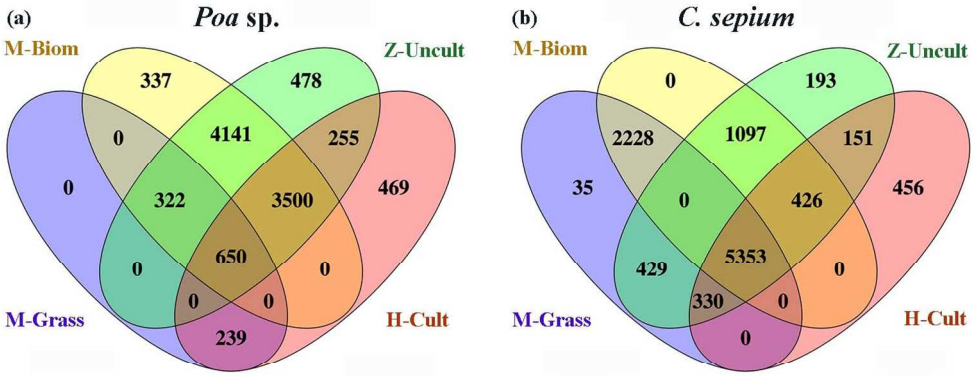

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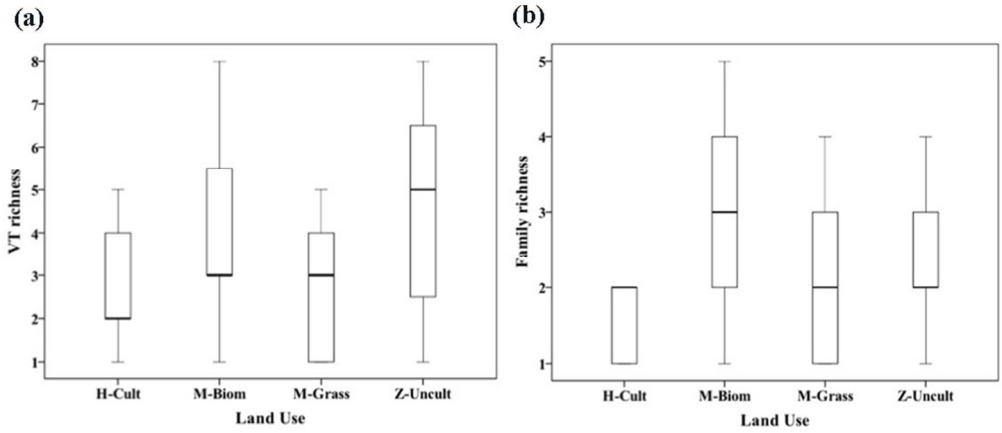

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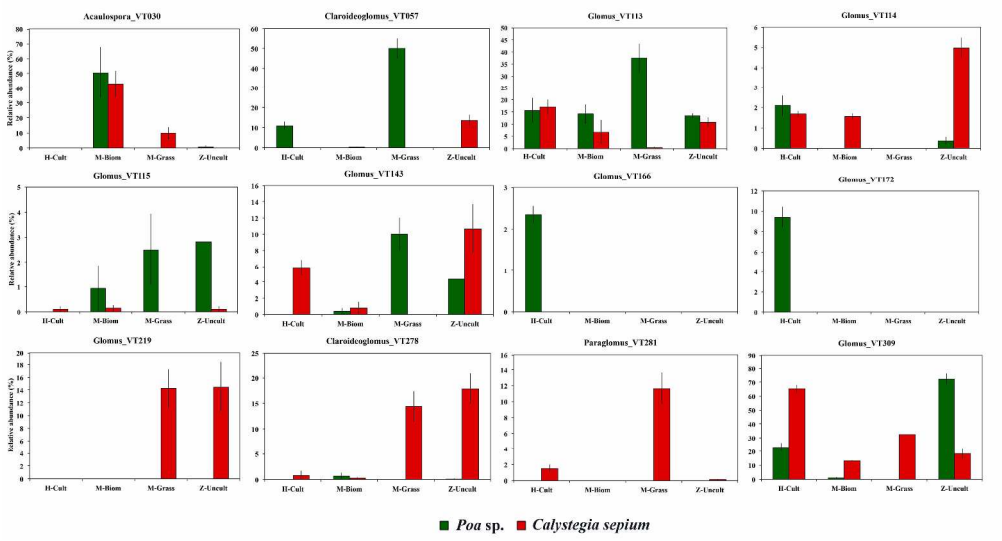

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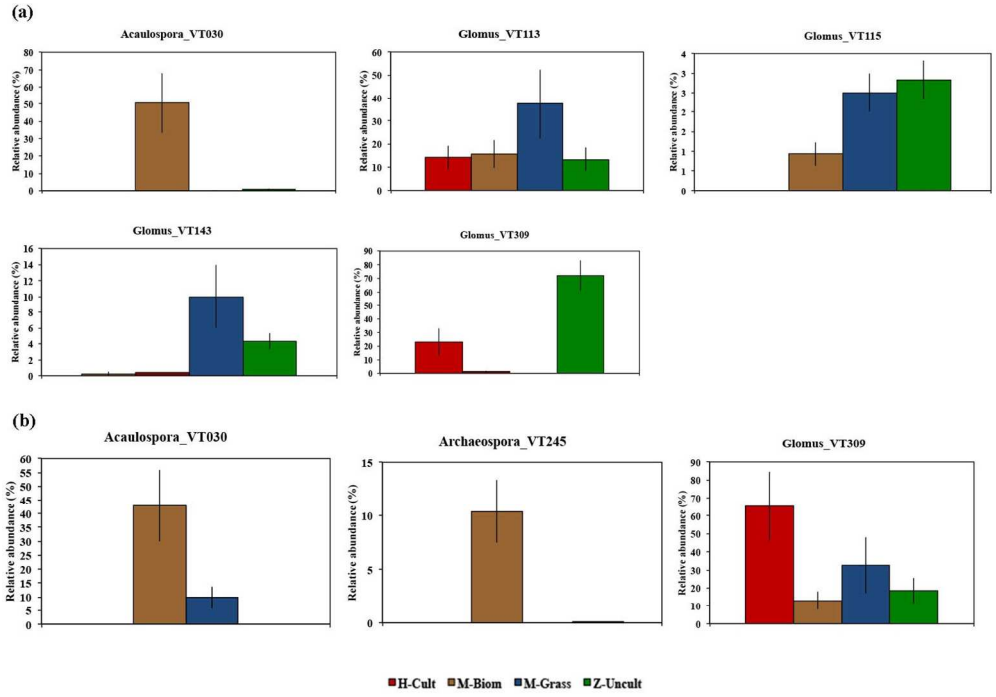

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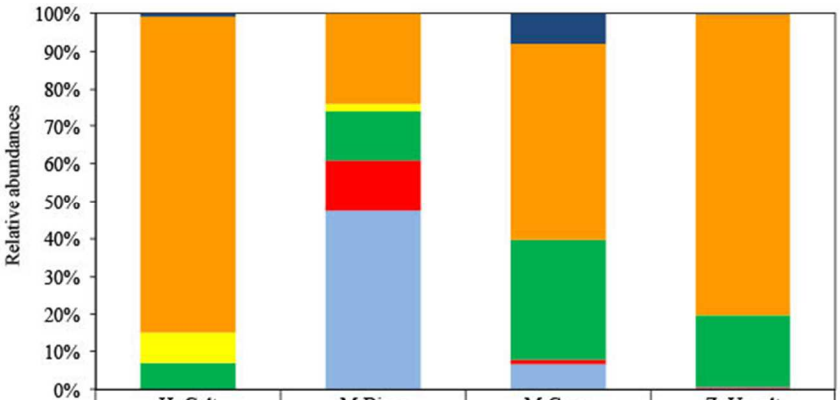

	H -Cult	M-Biom	M-Grass	Z -Uncult
Paraglomeraceae	0.01	0.00	0.08	0.00
Glomeraceae	0.84	0.24	0.52	0.80
Diversisporaceae	0.08	0.02	0.00	0.00
Claroideoglomeraceae	0.07	0.13	0.32	0.19
Archaeosporaceae	0.00	0.14	0.01	0.00
Acaulosporaceae	0.00	0.47	0.07	0.00

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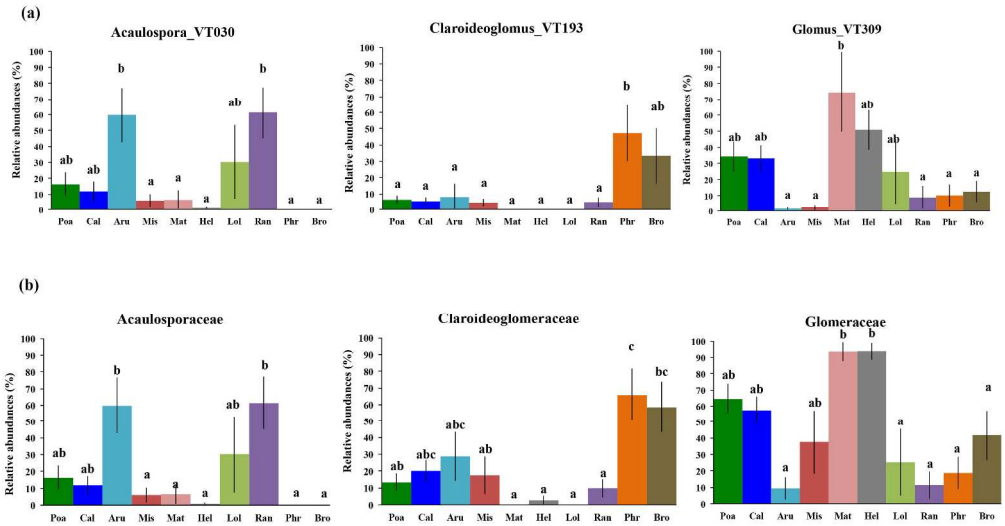

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LAND-USE INTENSITY AND HOST PLANT SIMULTANEOUSLY SHAPE THE COMPOSITION OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN A MEDITERRANEAN DRAINED PEATLAND

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Keywords: 454-pyrosequencing; arbuscular mycorrhizal fungal (AMF) diversity; community composition; host preference; land use; SSU rRNA gene

Running title: Land use as a driving factor of arbuscular mycorrhizal fungi

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ABSTRACT

Land-use change is known to be a major threat to biodiversity and ecosystem services in Mediterranean areas. However, the potential for different host plants to modulate the effect of land-use intensification on arbuscular mycorrhizal (AM) fungal community composition is still poorly understood. To test the hypothesis that low land-use intensity promotes AMF diversity at different taxonomic scales and to determine whether any response is dependent upon host plant species identity, we characterized AMF communities in the roots of ten plant species across four land use types of differing intensity in a Mediterranean peatland system. AMF were identified using 454-pyrosequencing. This revealed an overall low level of AMF richness in the peaty soils; lowest AMF richness in the intense cropping system at both Virtual Taxa (VT) and family level; strong modulation by the host plant of the impact of land-use intensification on AMF communities at the VT level; and a significant effect of land-use intensification on AMF communities at the family level. These findings have implications for understanding ecosystem stability and productivity and should be considered when developing soil-improvement strategies in fragile ecosystems, such as Mediterranean peatlands.

INTRODUCTION

Land-use change is known to be a major threat to plant and animal biodiversity and ecosystem services (Newbold *et al.*, 2015). It has been estimated that the conversion of natural habitats to human-impacted habitats, such as pasture, cropland, tree plantations and urban areas, has caused a global biodiversity decline of 8.1% in the last 500 years. This figure could increase by a further 3.4% in the next 100 years if conservative agricultural practices are not applied (McGill2015). As plant community composition and soil physico-chemical parameters shape soil microbial communities, land-use changes also strongly affect soil ecosystem functions and services, including plant growth, carbon (C) sequestration and regulation of nutrient availability and uptake by plants (Wardle *et al.*, 2004; Meyer *et al.*, 2013; Lange *et al.*, 2015).

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota; Schüßler, Schwarzott and Walker 2001) are an important soil microbial group that form one of the most common types of symbiosis globally, arbuscular mycorrhiza (Smith and Read 2008). AMF are associated with the roots of over 80% of terrestrial plants, including crops, and convey fundamental services, such as plant growth (Lekberg and Koide 2005), protection against pests and pathogens (Newsham, Fitter and Watkinson 1995), drought tolerance (Augé 2001) and nutrient uptake, in exchange for photosynthetically fixed C (Bago, Pfeffer and Shachar-Hill 2000; Hodge, Helgason and Fitter 2010). Moreover, AMF improve soil structure and aggregate stability thanks to the development of extraradical mycelia and the production of a coagulating glycoprotein, glomalin, that contributes to soil C and nitrogen (N) stocks (Rillig *et al*., 2001; Rillig and Mummey2006; Bedini *et al.*, 2009).

In the last decade, the assumption of low host plant preference/specificity in AMF has been challenged by evidence of a host plant species effect on AMF diversity and community composition (Vandenkoornhuyse *et al.*, 2002; Sýkorová, Wiemken and Redecker 2007; Torrecillas, Alguacil and Roldán 2012). Other studies have identified an association between AMF and plant ecological groups (e.g., habitat generalists *vs* specialists) (Öpik *et al.*, 2009; Davison *et al.*, 2011) or

ecosystems (Veresoglou and Rillig 2014), rather than particular plant species. These associations were explained using a common framework that categorised both plants and AMF according to their life history strategies (i.e., competitor, stress tolerator and ruderal) (Chagnon *et al.,* 2013).

Changes in AMF community composition, with decreases in AMF species richness, have also been linked with land-use intensification both in soil (Lumini *et al.*, 2010; Gonzalez-Cortés *et al.*, 2011; Morris *et al.*, 2013; Xiang *et al.*, 2014) and roots (Helgason *et al.*, 1998; Moora *et al.*, 2014; Vályi, Rillig and Hempel 2015). Within arable systems, the level of intensification due to management practices, such as tillage, crop rotation, and fertilizer and biocide input, strongly impact upon AMF species richness and community composition, by promoting the dominance of particular taxa belonging to the family Glomeraceae (Helgason *et al.*, 1998, 2007; Jansa *et al.*, 2002; Mathimaran *et al.*, 2007; Borriello *et al.*, 2012).

In Mediterranean areas, high land-use intensification has been associated with low AMF richness in grasslands, pastures, vineyards, plantations and forests (e.g., Lumini et al., 2010; Pellegrino et al., 2011). Members of the AMF orders Glomerales and Diversisporales have largely been found in natural and high input land-uses, while the orders Paraglomerales and Archaesporales have been detected only in the less intense systems, such as pastures. At the same time, the impact of land-use intensification in wetlands and peatlands is poorly understood (Pellegrino et al., 2014; Ciccolini, Bonari and Pellegrino 2015).

Wetlands are important transitional ecosystems between terrestrial and aquatic ecosystems, covering only 6% of the global area, but playing crucial ecological roles in the balance and sequestration of C, N and phosphorus (P) and in the protection of biodiversity (Verhoeven and Setter 2009). In the past century, half of all wetlands globally have been lost due to conversion to agriculture (Zedler and Kercher 2005), and, consequently, the protection and restoration of wetlands, and in particular of peatlands, have become a priority. A lack of knowledge concerning the diversity and roles of microbes in peatland restoration is well recognised in the literature (e.g., Littlewood et al., 2010). Nevertheless, the positive outcome of restoration projects maybe

influenced by microbial activity (e.g., N_2 fixation, nutrient uptake, organic matter oxidation, methanotrophy), which itself may be modulated by management strategies, such as fertilization or manipulation of the water table or vegetation assemblages. In acidic systems, such as peatlands, fungi have been shown to be more abundant than bacteria, acting as major decomposers of complex carbon polymers in soil organic matter (Andersen, Chapman and Artz 2013). Changes in fungal community composition ~~of the fungal community are thus~~ were mostly attribut~~able~~ed to changes in litter type, ~~but~~whereas ~~in the case of AMF~~ changes in AMF community were attributed ~~even~~also to changes in ~~vegetation~~plant species assemblages ~~have been shown an effect on this symbiotic community~~ (Thormann, Currah and Bayley 1999). Moreover, ~~it is actually growing the awareness that importance of mycorrhizae~~AMF were reported to be ~~to the~~key components for the success of restoration programmes ~~of~~of disturbed peatlands by colonizing the roots of many wetland plant species and thus potentially improving the early growth ~~-~~of plant ~~species~~ community (Turner et al., 2000; Tawaraya et al., 2003). ~~In peat swamp forests, Tawaraya et al. (2003) suggested that inoculation of AMF can improve the early growth of some tree species becoming a key technology to rehabilitate such disturbed peatlands. Similarly, Turner et al. (2000) suggested that projects to restore temperate peatlands may have limited success unless inoculation wih mycorrhizae is considered in the revegetation process.~~

~~Among fungi, shifts from mycorrhizal to saprotrophic fungal communities have been shown to occur during litter degradation (Peltoniemi et al., 2012), but the high spore density and root colonization (up to 60%) of AMF observed at the beginning of litter decomposition suggests that these fungi may play a role in conferring competitive advantages to host plants through direct nutrient absorption (Thormann, Currah and Bayley 1999; Turner et al., 2000; Fuchs and Haselwandter, 2004).~~ In Mediterranean peatlands, where climatic conditions leave soil prone to degradation (Vallebona *et al*., 2015), studying the effects of some drivers of AMF community structure such as host plant preference/specificity, land-use intensification and their interaction ~~on~~

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~~AMF community composition~~ may support the development of efficient strategies for peatland~~their~~ restoration and protection (i.e., less intensive agriculture, extensive grazing systems, rewetting).

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In this study we aimed to investigate the effect on root AMF diversity of land-use intensification, host plant species and their interaction in a Mediterranean peatland drained for agricultural purposes. Four land uses with decreasing levels of intensity were studied to test the hypothesis that low land-use intensity promotes AMF diversity at different taxonomic scales and to determine whether any response is dependent upon host plant species identity

MATERIALS AND METHODS

Study site and experimental design

The experimental site is located in the southern part of the Massaciuccoli Lake basin (43°49'N, 10°19'E) (Pisa, Italy) (Pellegrino *et al*., 2014). The soil is classified as *Histosol* according to the USDA system (Soil Survey Staff, 1975) and defined as peaty soil (IPCC, 2006). The climate is Mediterranean (Csa) according to the Köppen classification, with dry and hot summers and rainfall mainly concentrated in autumn and spring (mean annual rainfall ca. 945 mm $year^{-1}$) and mean monthly air temperature ranging from 7°C in February to 30°C in August (yearly average 14.8°C).

The experiment consisted of a completely randomized design with a land use intensity treatment comprising three levels of agricultural intensification (high intensity-H; medium intensity-M; zero intensity-Z) and four land-use types, each represented by three field replicates (0.7 ha). In detail, prior to the start of the experiment (15 years ago), the site was intensively cultivated with sunflower and maize. At that time, we selected 12 field replicates and allowed a random number generator to allocate land use types to the different fields. Among the field replicates, three were assigned to high intensity-H, while nine were left to develop under natural successional vegetation, with no agricultural intervention (zero intensity-Z). Then, two years ago, six of these nine field replicates

were randomly assigned to medium intensity-M. The specific land-use types were: (1) an intensive cropping system (H-Cult) based on sunflower (*Helianthus annuus* L.), carried out for the last 15 years. Plots were deeply ploughed (30-35 cm) and harrowed each year early in spring. Sunflower was sown in April at a rate of 6.7 plants m^2in rows spaced 75 cm apart and harvested at the beginning of September. Fertilizer was applied at sowing and mechanical weed control was applied post-emergence, while no pest control was performed; (2) a two-year-old plantation of perennial grasses for energy production (*Arundo donax* L. and *Miscanthus x giganteus* Greef et Deuter) (M-Biom), where no fertilizers or other agricultural practices were applied except for annual harvest in winter; (3) a two-year-old managed grassland of cool-season grasses (*Festuca arundinacea* L., *Lolium perenne* L.) and seashore paspalum (*Paspalum vaginatum* Swartz) (M-Grass). No fertilizers or other agricultural practices were applied, except for mowing when required; (4) an agricultural soil abandoned 15 years ago (Z-Uncult) and naturally colonised by indigenous grasses. The dominant plant species were *Calystegia sepium* L. (18%), *Phragmites australis* (Cav.) Trin. ex Steud. (15%), *Arctium lappa* L. (14%) and *Bromus tectorum*L. (14%), respectively. Percentages represent the relative density of each plant species from a survey made in May 2013. No fertilizers, tillage or other agricultural practices were applied.

Sampling

To evaluate the effect on AMF community composition of land-use intensification and the interaction with host plant identity, two co-occurring plant species, *Poa* sp. and *C. sepium*, were sampled in every land-use type in May 2013 (Table 1). In addition, to test the main effect of host plant species, two further plant species unique to each land-use type were sampled from May to July 2013. For details of the ten sampled plant species see Table 1. In each replicate field, at least two individuals of each plant species were randomly collected, generating a total of 144 samples. Plants with entire root systems were excavated and placed in polyethylene bags for transport to the

laboratory. Roots were rinsed, oven dried at 60 °C for 24 h and stored with silica gel at room temperature until analysis.

Molecular analyses

DNA was extracted from ca. 30 mg of dried roots from each plant individual using the PowerSoil-htp™ 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), following the modification to the protocol as in Davison *et al*. (2012). First, roots were milled to powder in 2 ml tubes with four 3 mm tungsten carbide beads per tube with Mixer Mill MM400 (Retsch GmbH, (Haan, Germany). Bead Solution (750 µL) was added to the tubes, mixed, and the slurry transferred to Bead Plates. To increase DNA yield, the Bead Plates were shaken at a high temperature (60°C following the manufacturer's suggestions) for 10 min at 150 rpm in a shaking incubator. Finally, in order to increase DNA yield, the final elution was performed twice with 75 µL of Solution C6. Before PCR reaction DNA concentration was measured using the Appliskan fluorescence-based microplate reader (Thermo Scientific, MA, USA) and PicoGreen® dsDNA Quantitation Reagent (Quant-iTds DNA Broad Range Assay Kit, Invitrogen, Carlsbad, CA) in three replicates.

Glomeromycota nuclear small subunit (SSU) rRNA gene fragments were amplified using the primers NS31 and AML2 (Simon, Lalonde and Bruns, 1992; Lee, Lee and Young, 2008), linked to 454 sequencing primers A and B, respectively, and an 8 bp sample-distinguishing barcode (Davison *et al*., 2012). We targeted the SSUrRNA gene because the large and comprehensive AMF sequence database Maarj*AM* (Öpik*et al*., 2010) allows a reliable and fast identification of Glomeromycota and comparisons with other studies (Öpik *et al*., 2014). Polymerase chain reaction (PCR) was carried out in two sequential reactions of which the first was targeted PCR with region-specific primers, barcodes and partial sequencing adapters, and the second PCR was used to complete sequencing adapters, as in Davison *et al*. (2012). Three µL of stock DNA sample was used in the

first PCR and 3µL of 10-fold dilution of the first PCR product was used in the second PCR reaction. The PCR mix contained 3 µL of template DNA, 0.2 µM of each primer and Smart-Taq Hot Red 2x PCR Mix (0.1 $UµL^{-1}$ Smart Taq Hot Red Thermostable DNA Polymerase), 4 mM $MgCl_2$, 0.4 mM of each of the nucleotides; Naxo OÜ, Estonia) in a total volume of 30 µL. PCR reactions were performed in three replicates. The reactions were run on a Thermal cycler 2720 (Applied Biosystems, Foster City, CA, United States) following the conditions of Davison *et al*. (2012). PCR products were separated by electrophoresis through a 1.5% agarose gel in 0.5× TBE, and were purified with Agencourt® AMPure XP Kit® (Beckman Coulter Inc.) in plate. Samples were eluted in Buffer EB (10 mMTris-Cl, pH 8.5; QIAGEN Inc.). The average concentration of amplicons in the pooled sample was24.4 $ngµL^{-1}$(measured on a Qubit™ fluorometer in three replicates). Preparatory procedures for 454 sequencing (barcoded PCRs and PCR product purification) were performed by BiotaP LLC (Tallinn, Estonia). A total of 2.07 µg of the resulting DNA mix was sequenced on a Genome Sequencer FLX System, using Titanium Series reagents (Roche Applied Science, Mannheim, Germany) at Microsynth AG (Balgach, Switzerland).

Bioinformatic analyses

Bioinformatic analysis was implemented following Davison *et al*. (2012). Only 454-sequencing reads that met all of the four following criteria (quality filtering) were included in subsequent analyses: (1) the read carried the correct bar-code; (2) the read carried the correct NS31 primer sequence; (3) the read was ≥ 170 bp (excluding bar-code and primer sequences) and (4) the read had an average quality score ≥ 25. As most reads were of approximately full amplicon length (between 500 and 550 bp long), we trimmed reads to 520 nucleotides to exclude reverse primer sequences. A total of 1205 potential chimeras were detected and removed using UCHIME (Edgar *et*

al., 2011) in reference database mode using the default settings and the Maarj*AM* database. The analyses yielded a total of 514,457 reads.

For taxonomic identification of reads we used an open-reference operational taxonomic unit picking approach (Bik *et al*., 2012). After stripping the barcode and primer sequences, we used the Maarj*AM* database of published Glomeromycota SSU rRNA gene sequences to identify obtained reads. The Maarj*AM* database contains representative sequences from published environmental Glomeromycota sequence groups, so-called Virtual Taxa (VT; Öpik *et al*., 2010, 2014). Sequence reads were assigned to VT by conducting a BLAST search (soft masking with DUST) against the Maarj*AM* database that is based on environmental and cultured fungal sequences (status May 2014) with the following criteria required for a match: (a) sequence similarity ≥ 97%, (b) an alignment length>95% of the length of the shorter of the query (pyrosequencing read) and subject (reference database sequence) sequence; (c) a BLAST e-value < 1 e^{-50}. These analyses yielded a total of 77,988 reads that matched with VT from the Maarj*AM* database.

Those reads that did not find a match in the Maarj*AM* database were identified by conducting a further BLAST search against the International Nucleotide Sequence Database (INSD), using slightly modified criteria: (a) sequence similarity ≥ 90% and (b) 90% alignment length.

Up to four sequences of each detected VT for each land-use type were picked and aligned together with the VT type sequences, representative AMF sequences (sequences included in the AMF species list of Schüßler and Walker2010; see open-access dataset http://sites.gooogle.com/site/restomedpeatland/microbiology) and previously recorded Glomeromycota sequences from the study site (Pellegrino *et al*., 2014; Ciccolini, Bonari and Pellegrino2015; 757 sequences in total). The alignment was performed using MAFFT version 7 multiple sequence alignment web service (Katoh and Standley 2013). Neighbour-joining (NJ) phylogenetic analysis was performed in MEGA5 (Tamura *et al*., 2011). Glomeromycota nomenclature follows Redecker *et al*. (2013). Representative sequences of VT detected in each land use type and plant species were deposited in the EMBL database under accession numbers from

LT596223 to LT596539.

Chimera checking, primer and barcode sequence removal, parsing of BLAST output and selection of representative sequences were carried out using a series of Python and Java scripts developed at the Department of Botany, University of Tartu (Davison *et al*., 2012).

Statistical analyses

Root samples yielding < 10 sequences and singleton and doubleton VT were removed, leaving 96 samples and including at least two plant individuals of each host plant in each field replicate.

Diversity data matrices were built at the taxonomic levels of VT and family, with the relative abundance of taxonomic groups in samples estimated from the proportion of reads representing each group. To test the effect on AMF community composition of land-use intensification and its interaction with host plant species a matrix was compiled containing samples from the two co-occurring host plant species (*Poa* sp. and *C. sepium* L.) in the four land-use types (48 samples; question 1) (Table 1). Then, to study the effect of host plant species on AMF community composition, a matrix was compiled containing samples from all ten plant species in all land use types (Table 1) (question 2).

Sequencing efficacy was assessed with rarefaction analysis, using the function rarefy() from the R package vegan (Oksanen *et al*., 2013). Because there was a high variability in the number of reads per sample (Fig. S1), sequencing depth per sample was standardized to the median number of reads across the samples in each data matrix (de Cárcer *et al*., 2011). Applying this approach, bias due to differences in sample size is reduced by randomly choosing in each sample a number of reads equal to the median number of reads across all samples. Samples that had fewer reads than the median were left unchanged.

To answer question 1, permutational analysis of variance (PERMANOVA; Anderson 2001) was used to test the effect of land-use type (H-Cult, M-Biom, M-Grass and Z-Uncult) and host plant

species identity (*Poa* sp. and *C. sepium*) on VT/family relative abundance. Response data matrices were square-root transformed prior to analyses in order to down-weight the importance of dominant taxa and the Bray-Curtis index of dissimilarity was used to measure ecological distance. *P*-values were calculated using a Monte-Carlo test and residuals were permuted under a completely randomized model (Anderson and TerBraak2003). To remove the effect of spatial variability of subsamples, the latitude and longitude of the plant individuals were used as covariates in the PERMANOVAs. Since PERMANOVA is sensitive to differences in multivariate location (average community composition of a group) and dispersion (within-group variability), analysis of homogeneity of multivariate dispersion (PERMDISP; Anderson2006) was performed to check the homogeneity of multivariate dispersion between groups (beta-diversity) (Anderson, Ellingsen and McArdle2006). When PERMANOVA and PERMDISP indicated a significant effect, principal coordinate analysis (PCO) was applied to the matrix of pairwise Bray-Curtis dissimilarities (Torgerson1958) in order to visualize the most relevant patterns in the data. In each PCO biplot, we displayed only the VT and families with a strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis. The circle in each plot, whose diameter is 1.0, allows the reader to understand the scale of the vectors in the vector plot. The output of the PCO analyses were utilized together with the indicator species analysis, according to Dufrene and Legendre (1997), to ~~identify~~ visualize the taxa that were most indicative of particular land-use and host plant categories. Classification and ordination analyses were performed using PRIMER 6 and PERMANOVA+ software (Clarke and Gorley 2006; Anderson *et al*., 2008), while indicator species analysis was performed using the indval function from the labdsv package for R (Roberts 2014). For each host plant species (*Poa* sp. and *C. sepium*), the standardized dataset was also used to generate Venn diagrams, representing VT and reads unique to each land use or shared among land uses. Venn diagrams were generated using Venny v. 2.0 software (Oliveros2015).

To answer question 2, PERMANOVA and PCO were applied to the 96 sample data matrix. In the PERMANOVA host plant species (n = 10) was used as a fixed factor, while land-use type and the

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spatial coordinates of plant individuals were included as covariates. The VT and families with strong correlation ($r \geq 0.60$) with the ordination scores on each PCO axis were displayed on PCO biplots.

PERMANOVAs were also performed using unstandardized datasets of relative abundance (i.e., VT and family based) per sample, to test whether data standardization produced changes in the patterns of AMF community composition (questions 1 and 2).

AMF richness at the VT and family level was studied using standardized data matrices. The effects on richness of land-use type and its interaction with host plant species (question 1) were tested using analysis of covariance (ANCOVA), with land-use type and host plant species as fixed factors and the spatial coordinates of plant individuals as covariates. Similarly, the effect of host plant species on AMF richness (question 2) was studied using ANCOVA with host plant species as a fixed factor and land-use type and the spatial coordinates of the plant individuals as covariates. Unless stated otherwise, analyses were performed in SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Pyrosequencing information

A total of 514,457 quality-filtered SSU rRNA gene sequences were obtained from 96 samples. After the BLAST against the Maarj*AM* database, we found 77,917 Glomeromycota reads, ranging from 10 to 3,865 reads per sample (length varying from 170 to 520 bp; mean length of 392 bp) that were assigned to a total of 48 Virtual Taxa (VT) (Table S1; Fig. S1). The remaining 465,137 reads were identified against the INSD. Plantae, fungi, bacteria and metazoa represented 73%, 11%, 9% and 2% of reads, respectively. Among fungi, the potential matches to Glomeromycota constituted less than 1% of qualifying reads and thus were not included in further analyses. The 48 VT

belonged to the families Acaulosporaceae (2), Archaeosporaceae (2), Claroideoglomeraceae (4), Diversisporaceae (5), Glomeraceae (31) and Paraglomeraceae (4) (Table S1; Fig. S1). Rarefaction analysis suggested that the number of AMF reads per sample was generally sufficient to produce asymptotic estimates of VT richness per sample (Fig. S2). Since some samples had substantially lower sequencing depth than others, sequencing depth was standardized to the median number of reads per sample (154 pyrosequencing reads). After standardization of the data, a total of 11,167 reads belonging to 32 VT in six families were retained for subsequent analyses (Tables S1): Acaulosporaceae (1), Archaeosporaceae (2), Claroideoglomeraceae (4), Diversisporaceae (3), Glomeraceae (19) and Paraglomeraceae (3).

Land use effect on AMF diversity

The total numbers of VT identified in *Poa* sp. and *C. sepium* were 21 and 23, respectively, with both host plant species harbouring all the six families (Figs 1, S1; Table S1). In the roots of *Poa* sp., one VT (*Glomus* VT113) occurred in all four land uses (4.8% of VT; 6.3% of reads; Figs. 1 and S3, S4). In the roots of *C. sepium,* four VT (*Glomus* VT113; *Glomus*VT309, related to *Glomus* ORVIN GLO3E; *Claroideoglomus*VT193 and VT278, related to *C. claroideum, etunicatum, lamellosum, luteum* and to *C.* ORVIN GLO4, respectively (17.4% of total VT; 50.0% of total reads) occurred in all four land uses (Figs. 1, S3).

AMF richness per sample at VT and family level was affected by land-use intensification (P=0.020 and P=0.016, respectively) (Table S2). At the VT level, H-Cult and M-Grass exhibited a significantly lower number of VT per sample (mean ± SE: 2.80 ± 0.44 and 2.81 ± 0.40 VT, respectively) in comparison with Z-Uncult (4.80 ± 0.59 VT) (Fig. S4a), whereas M-Biom had an intermediate value (4.08 ± 0.61 VT). At the family level, H-Cult and M-Grass (1.70 ± 0.16 and 2.10 ± 0.38 families, respectively) exhibited a significantly lower number of families per sample in comparison with M-Biom (3.00 ± 0.32 families), whereas Z-Uncult had an intermediate value (2.40

± 0.32 families) (Fig. S4b).

At the VT level, AMF community composition was affected by land-use type [*P*=0.001; Explained Variance (EV)=13%] and by its interaction with host plant species (*P*=0.002; EV=20%) (Table 2). PERMDISP indicated significant differences in AMF community dispersion among land uses (*P*=0.050; Table 2a) and specifically between M-Biom and M-Grass and between Z-Uncult and M-Grass (Table S3).

The interactive effect of land-use intensification and host plant species on AMF communities at VT level was visualised using PCO. The PCO biplots in Fig. 2 show the differential host plant species effects on AMF communities within each land-use type. The same interaction from another perspective is shown in Fig. 3, where the differential effects of land-use intensity on AMF communities can be seen within each host plant species (Figs. 3, S6). Overall, 12 VT were shown to be highly correlated with AMF community responses to land-use type or indicative of one or more land use types (Figs. 2, S5). Both H-Cult and Z-Uncult were characterized by *Glomus* (H-Cult: VT113, VT143, VT166, VT172, VT309; Z-Uncult: VT113, VT114, VT115, VT219, VT309) and *Claroideoglomus* (H-Cult: VT57, VT278; Z-Uncult: VT278) VT (Table 3). By contrast, land use types of medium intensity (i.e. M-Biom and M-Grass) were characterized by *Acaulospora* (VT30) and *Paraglomus* (VT281) VT, respectively, in addition to the common discriminant *Glomus* VT113 (Table 3). Specifically, within *Poa* sp., *Glomus* VT characterized Z-Uncult (VT309) and M-Grass (VT113, VT115, VT143), whereas *Acaulospora* VT (VT30) characterized M-Biom (Fig. 3a, S6a). Within *C. sepium*, M-Biom was also characterized by *Archaeospora* VT245 in addition to *Acaulospora* VT30 and *Glomus* VT309 (Figs 3b, S6b). Indicator species analysis revealed *Acaulospora* VT30 to be a significant VT indicator for M-Biom both in *Poa* sp. and *C. sepium*, and *Archaeospora* VT245 only in *C. sepium* (Table 3). With regard to Z-Uncult, *Glomus* VT309 and *Claroideoglomus* VT57 were shown be indicator species in *Poa* sp. and *C. sepium*, respectively.

At the family level AMF, community composition was affected only by land-use type (*P*=0.001; EV=27%) (Table 2). PERMDISP confirmed differences in community dispersion among land uses

(*P*=0.038; Table 2) and specifically between M-Biom and Z-Uncult and between M-Grass and Z-Uncult (Table S3). Acaulosporaceae were shown to be a representative family in M-Biom, while Glomeraceae and Claroideoglomeraceae were ubiquitous in the four land uses (Figs. 4, S7), PERMANOVA and PERMDISP analyses performed using unstandardized data produced results similar to those generated using standardized data (Tables S4, S5).

Host effect on AMF diversity

Among the ten studied host plant species, AMF richness per sample differed at the family (ANCOVA, $F_{(9,78)}$=2.309, P = 0.023) but not at the VT level (ANCOVA, $F_{(9,78)}$=1.668, P = 0.111). The highest number of families per sample was observed in *Miscanthus* x *giganteus*, while the lowest number was observed in *B. tectorum*, *H. annuus* and *M. chamomilla*(Table S6). AMF community composition was significantly affected by host plant species both at VT (*P*=0.001; EV=13.3%) and at family level (*P*=0.001; EV=22.4%) (Table 4; Fig 5a,b). The position of field replicates in the area had a significant effect on AMF community composition at both taxonomical levels (Table 4). PERMDISP confirmed significant differences between host plant species in AMF community dispersion at both taxonomical levels (Table S7). Three VT - *Acaulospora* VT30, prevalently detected in the roots of *A. donax* and *R. acris*; *Claroideoglomus* VT193, prevalently detected in the roots of *P. australis* and *Glomus* VT309 mostly retrieved in the roots of *M. chamomilla* (Fig. S8a) and three families - Acaulosporaceae largely detected within the roots of *A. donax* and *R. acris*; Claroideoglomeraceae and Glomeraceae most occurring in the roots of *P. australis* and *M. chamomilla/H. annuus* - were strongly correlated with the PCO axes (Fig. S8b). Indicator species analysis revealed six indicators for the ten host plant species: *Archaeospora*VT245 in *L. perenne*; *Claroideoglomus* VT193 in *P. australis*; *Rhizophagus* VT90 and VT264 in *H. annuus*; *Glomus*VT219 in *Miscanthus* x *giganteus* and *Rhizophagus* VT105 in *B. tectorum* (Table 3).

PERMANOVA and PERMDISP analyses performed using unstandardized data (Tables S8, S9) produced results similar to those performed using standardized data.

DISCUSSION

In this study, the effects on AMF communities of land-use intensification, host plant species and their interaction were evaluated at different taxonomic levels in a Mediterranean peatland drained for agricultural purposes. AMF diversity was measured in the roots of ten plant species across four land-use types with differing levels of land use intensity. Using 454-pyrosequencing of the SSU rRNA gene, we detected: (i) overall low AMF richness; (ii) lowest AMF richness in the high land-use intensity level both at Virtual Taxa (VT) and family level; (iii) an impact of land-use type on AMF community composition at the VT level that was strongly modulated by host plant species identity; (iv) an effect of land-use type on AMF community composition at the family level; and (vi) a host plant species effect on the richness and community composition of AMF at VT and family level.

Land-use effect on AMF diversity

The overall number of VT detected in this study (48) suggests that the AMF richness of Mediterranean peatland soils is low in comparison with other habitats across the Mediterranean basin, where up to 117 VT have been detected per site (Lumini *et al*., 2010; Varela-Cervero *et al*., 2015). However, the number of VT and families (six families) detected in the present study exceeds what has been recently recorded from the same site (ca. 15 VT and two families, of which nine VT and one family were also detected in the current study) from both root and soil samples (Pellegrino *et al*., 2014; Ciccolini, Bonari and Pellegrino2015). Specifically, *Funneliformis* VT67 and *Funneliformis* VT65 related to *F. mosseae* and *F. caledonium* were previously detected at low

abundance (<15%) exclusively in the uncultivated system (Ciccolini, Bonari and Pellegrino 2015), whereas in this study both VT were detected in the roots from the uncultivated system, the biomass plantation and the grassland, albeit at lower abundance. Across the four land-use types, members of Archaeosporaceae, Acaulosporaceae, Diversisporaceae, Claroideoglomeraceae and Paraglomeraceae were detected here in addition to Glomeraceae. However, in comparison to Ciccolini, Bonari and Pellegrino (2015), Gigasporaceae was not be detected again. These differential diversity patterns may reflect the different sampling times (i.e., May *vs* July) and thus the different fungal life cycle stages represented at the times of sampling (i.e., intraradical development *vs* sporulation; Dumbrell *et al*., 2011),or differences in the molecular approach applied (i.e., primer pairs NS31/AM1 *vs* NS31/AML2).The former primer pair is known to not amplify some AMF families, such as Archaeosporaceae, Ambisporaceae and Paraglomeraceae (Lee, Lee and Young 2008). Finally, the overall level of AMF richness retrieved in this study may have been also underestimated with respect to the whole pool of AMF, since the detection of root-colonizing AMF may not represent the total AMF species available in soil (Varela-Cervero *et al*., 2015).

Previous observations from the same study site (Ciccolini, Bonari and Pellegrino 2015) reported a low AMF richness both at VT and family levels across land-use types. These differences of AMF richness may be explained by the high sequencing depth of 454-pyrosequencing in comparison with the traditional cloning and Sanger sequencing method, allowing a more thorough characterization of AMF communities and detection of taxa even at very low abundances (Senés-Guerrero and Schüßler 2015; Nesme *et al*., 2016).

Nevertheless, the observed reduction of AMF richness in the intensive cropping system showing high soil total N and available P (13 and 70 mg kg^{-1}, respectively; Ciccolini, Bonari and Pellegrino 2015) may be explained by that the fact that plant species become less dependent on AMF for nutrient uptake in conditions of high soil nutrient availability (Camenzind *et al*., 2014).

Compared to other studies amplifying the 18 SSU rRNA region applying the same NGS technique (AMF sequence recovery ca. 47%) (Lumini *et al*., 2010; Valyi, Rillig and Hempel 2015; Varela-

Cervero *et al*., 2015) our percentage of recovery of AMF sequences was lower (ca. 15%). The low recovery rate is likely to be due to the peat environment rather than to the inefficiency of the primer pair, since AMF establishment and growth are known to be highly affected by organic matter content (Gryndler *et al*., 2009).

We observed that the impact of land-use intensification on AMF community composition was strongly modulated by host plant species at VT level. AMF community composition in the roots of *Poa* sp. growing in grassland differed from those in the intensive cropping system and biomass plantation. By contrast, AMF community composition in the roots of *C. sepium* did not differ according to land-use type. This difference between species was also reflected in the respective patterns of occurrence with respect to increasing management intensity (De Cauwer and Reheul 2009): *Poa* sp. mainly occurred in pastures with high intensity management, whereas *C. sepium* occurred equally along the intensification gradient. These results support the idea that the diversification of AMF communities within roots may confer a competitive advantage to the host plant species and drive plant community dynamics (van der Heijden *et al*., 1998; Zobel and Opik 2014). Our results allow a deeper insight into the poorly studied interactions between land use and other factors, such as plant species identity. So far, a single study has shown that land-use intensity and host plant species have interactive effects on AMF root assemblages in temperate grasslands (Valyi, Rillig and Hempel 2015), while interactions between land use and other factors, such as soil properties, has also been reported in temperate and Mediterranean areas (Jansa *et al*., 2014; Ciccolini, Bonari and Pellegrino 2015). Thus, future agroecological and monitoring studies on the effect of land-use intensification on AMF diversity should consider the potential interaction with host plant species.

At the family level, AMF communities differed between the zero level of land-use intensification and the medium intensive systems This is consistent with previous studies indicating that undisturbed habitats have fungal communities that are highly distinct from those found in anthropogenic areas, at both class and order level and at higher taxonomic resolution (in the

Mediterranean area: Lumini *et al*., 2010; Ciccolini, Bonari and Pellegrino 2015; in different climatic zones: Moora *et al*., 2014; Xiang *et al*., 2014; Vályi, Rillig and Hempel 2015). In the intensive cropping system most obtained sequences belonged to Glomeraceae (ca. 80%), among which we found *Glomus* VT309 and VT172 (ca. 30%) and *Glomus* VT113 (ca. 15%) to be dominant, supporting the idea that arable lands favour the presence of this AMF family (Jansa *et al*., 2002). It should nonetheless be noted that additional genera (*Funneliformis* and *Septoglomus*) and families (Claroideoglomeraceae) also occurred in agricultural fields (Rosendahl *et al*., 2009; Lumini et al., 2010; Xiang *et al*., 2014). By contrast, uncultivated systems and the medium intensity systems were dominated by Glomeraceae (ca. 50%) and Claroideoglomeraceae (ca. 30%) and by Acaulosporaceae (ca. 50%) and Glomeraceae (ca. 50), respectively. For Z-Uncult one *Glomus* (VT309) and one *Claroideoglomus* (VT57) were identified as indicator species, whereas *Acaulopora*VT30 and *Archaeospora* VT245 were found to be indicative for M-Biom. Specifically, *Acaulopora* VT30 was the most abundant VT in M-Biom, suggesting that plants with a long life cycle (i.e., perennial grasses) are more suitable for a symbiosis with AMF taxa that exhibit a slow growth rate and a high requirement for photosynthetically fixed C (Powell *et al*., 2009; Chagnon *et al*., 2013). We found that *Glomus* VT113 was the most dominant VT in the roots of *Poa* sp. and *C. sepium*. This VT is also the most abundantly recorded taxon in the Maarj*AM* database and is frequently the most abundant taxon in individual studies (Vályi *et al*. 2015). This result supports the observation of Davison *et al*. (2011) who reported VT113 as a generalist taxon and as the best indicator of habitat generalist plant species in the forest system studied by those authors, probably due its fast growth rate and the morphology of its propagules and mycelium (Gerdemann and Trappe 1974; Schenk and Smith 1982; Avio *et al*., 2006).

Host effect on AMF diversity

At the family level, highest AMF richness was found in the roots of the perennial grass *Miscanthus x giganteus*, while the lowest values were observed in annual species, namely *M. chamomilla*, *B. tectorum* and *H. annuus.* A similar pattern was recorded in semiarid soils by Alguacil *et al*. (2012), who found higher diversity in perennial compared with annual plants. This can be also explained by the fact that *Miscanthus x giganteus* is a C4 grass, which have been shown to have higher AMF root colonization than C3 grasses and benefit more in term of biomass and P uptake (Reinhart, Wilson and Rinella 2012; Treseder, 2013).

Host plant identity strongly shaped AMF communities within plant roots at both VT and family level. These results confirm those of previous studies showing that AMF in plant roots are not random assemblages, but that host plant identity plays a major role in the modulation of AMF community composition. Host plant modulation has been reported in several habitats, including in some Mediterranean areas (Sánchez-Castro, Ferrol and Barea 2012; Torrecillas, Alguacil and Roldán 2012; Varela-Cervero *et al*., 2015), as well as in temperate grasslands (Vályi *et al*.2015), alpinesites (Beckline*t al*., 2012) and boreal forests (Öpik *et al.*, 2009; Davison *et al*., 2011).

Regarding AMF community composition, Acaulosporaceae, Claroideoglomeraceae and Glomeraceae were the families that differed most in abundance among host plant species. Glomeraceae were dominant in the roots of Asteraceae (i.e. *H. annuus* and *M. chamomilla*), in line with the results reported in Mediterranean areas by Torrecillas, Alguacil and Roldán (2012). A similar pattern was apparent when considering the VT level, since members of Glomeraceae (namely *Rhizophagus* VT90 and VT264) were found to be indicator species and preferential symbionts for *H. annuus*. By contrast, members of Claroideoglomeraceae were preferentially found in association with Poaceae (i.e. *P. australis* and *B. tectorum*). Along with the fact that members of Poaceae were recently shown to be good mycotrophic hosts (Pellegrino et al., 2015), these findings suggest that Poaceae may be important in shaping the community composition of AMF, contrary to the findings of Torrecillas, Alguacil and Roldán (2012). Finally, the abundance of Acaulosporaceae within the roots of *A. donax*, growing in peaty soils with low pH, is in agreement with the fact that

Acaulosporaceae are widespread in acid soils (Clark, 1997) and that perennial grasses are suitable hosts for members of this family (Chagnon *et al*., 2013).

In conclusion, the VT level results demonstrated strong modulation by host plant species of the impact of land-use intensification on AMF community composition. Such a relationship has important implications for ecosystem stability and productivity since it may be expected to influence the composition and diversity of plant communities in the fact of environmental change. However, the fact that host modulation of land use effects is not evident when analysing AMF community composition at the family level indicates that the choosing suitable taxonomic resolution is important for appropriate monitoring of the impact of anthropogenic activities on plant communities. Overall this study shows that the planting of perennial grasses for energy production increased AMF diversity compared to an intensive arable cropping system and a grassland managed at medium intensity and, unexpectedly, also in comparison to an uncultivated system. Therefore, effective soil-improvement strategies for Mediterranean drained peatland should include reduced soil disturbance and coverage of plant species that supply a large quantity of leaf and root litter able to boost the diversity of beneficial soil microorganisms, such as arbuscular mycorrhizal fungi.

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REFERENCES

Alguacil MM, Torrecillas E, Roldán A *et al*. Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*. *Soil Biol Biochem* 2012;**49**: 132-138.

Andersen R, Chapman SJ, Artz RRE. Microbial communities in natural and disturbed peatlands: a review. *Soil Biol Biochem* 2013;**57**:979-994.

Anderson MJ, Ellingsen KE and McArdle BH. Multivariate dispersion as a measure of beta diversity. *Ecol Lett* 2006;**9**:683-693.

Anderson MJ, Gorley RN, Clarke KR. *PERMANOVA+ for PRIMER*: *Guide to Software and Statistical Methods*. Plymouth, UK: PrimereE Ltd, 2008.

Anderson MJ, TerBraak CJF. Permutation tests for multi-factorial analysis of variance. *J Stat Comput Sim* 2003;**73**:85-113.

Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 2006;**62**:245-253.

Augé RM. Water relations, drought and VA mycorrhizal symbiosis. *Mycorrhiza*2001;**1**:3-42.

Avio L, Pellegrino E, Bonari E *et al*., Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. *New Phytol* 2006;**172**:347-357.

Bago B, Pfeffer PE, Shachar-Hill Y. Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physol* 2000;**124**:949-958.

Becklin KM, Hertweck K and Jumpponen. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. *Microb Ecol* 2012;**63**:682-693.

Bedini S, Pellegrino E, Avio L, *et al*. Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. *Soil Biol Biochem* 2009;**41**:1491-1496.

Bik HM, Porazinska DL, Creer S, *et al*. Sequencing our way towards understanding global eukaryotic biodiversity. *Trends Ecol Evol* 2012*;***27**:233-243.

Borriello R, Lumini E, Girlanda M, *et al*. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biol Fert Soils* 2012;**48***:*911-922.

Camenzind T, Hempel S, Homeier J, *et al*. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. *Glob Change Biol* 2014; **20**:3646-3659.

Chagnon PL, Bradley RL, Maherali H, *et al*. A trait-based framework to understand life history of mycorrhizal fungi. *Trends Plant Sci* 2013;**18**:484-491.

Ciccolini V, Bonari E, Pellegrino E. Land-use intensity and soil properties shape the composition of fungal communities in Mediterranean peaty soils drained for agricultural purposes. *Biol Fert Soils* 2015;**51**:719-731.

Ciccolini V, Ercoli L, Bonari E, *et al*. Phylogenetic and multivariate analyses to determine the effect of agricultural land-use intensification and soil physico-chemical properties on N-cycling communities in drained Mediterranean peaty soils. *Biol Fert Soils* 2016;**52:**811-824.

Clark RB. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant Soil* 1997;**192:**15-22.

Clarke KR, Gorley RN. *PRIMER v6: User Manual/Tutorial*. Plymouth, UK: PrimereE Ltd, 2006.

Davison J, Öpik M, Daniell TJ, *et al*. Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiol Ecol* 2011;**78**:103-115.

Davison J, Öpik M, Zobel M, *et al*. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. *PLoS ONE* 2012;**7**:e41938.

DeCárcer DA, Denman SE, McSweeney C, *et al*. Evaluation of subsampling-based normalization strategies for tagged high-throughput sequencing data sets from gut microbiomes. *Appl Environ Microbiol* 2011;**77**:8795-8798.

De Cauwer B and Reheul D. Impact of land use on vegetation composition, diversity and potentially invasive, nitrophilous clonal species in a wetland region in Flanders. *Agron Sustainable Dev* 2009;**29**:277-285.

Deming JW. Sea ice bacteria and viruses. In: Thomas DN, Dieckmann GS (eds). *Sea Ice*. (2nd ed.), Oxford, UK: Wiley-Blackwell Publishing, 2010,247-82.

Dufrêne M, Legendre P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr* 1997;**67**:345-366.

Dumbrell AJ, Ashton PD, Aziz N, *et al*. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytol* 2011;**190**:794-804.

Edgar RC, Haas BJ, Clemente JC, *et al*. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;**26**:2194-2200.

Gerdemann JW and Trappe JM. The Endogonaceae in the Pacific North-West. *Mycol Mem* 1974;**5**: 1-76.

González-Cortés JC, Vega-Fraga M, Varela-Fregoso L, *et al*. Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. *Fungal Ecol* 2012;**5**:16-23.

Gryndler M, Hršelová H, Cajthaml T, Havránková M, Řezáčová V, Gryndlerová H, Larsen J. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza* 2009;**19**:255-266.

Helgason T, Daniell TJ, Husband R, *et al.* Ploughing up the wood-wide web? *Nature* 1998;**394**:431.

Helgason T, Merryweather JW, Young JPW, *et al*. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *J Ecol* 2007;**95**:623-630.

Hodge A, Helgason T, Fitter AH. Nutritional ecology of arbuscular mycorrhizal fungi. *Fung Ecol* 2010;**3**:267-273.

Hodge A, Storer K. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. *Plant Soil* 2015;**386**:1-19.

IPCC. Agriculture, Forestry and other land uses. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (eds) *Guidelines for National Greenhouse Gas Inventories* (Volume 4). Prepared by the National Greenhouse Gas Inventories Programme. Japan: IGES, 2006.

Jansa J, Mozafar A, Anken T, *et al*. Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza* 2002;**12**:225-234.

Jansa J, Erb A, Oberholzer HR *et al*. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Mol Ecol* 2014;**23**:2118-2135.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772-780.

Koorem K, Gazol A, Öpik M, *et al*. Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. *PLoS ONE* 2014;**9**:e91998.

Kohout P, Sudová R, Janoušková M, *et al*. Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution? *Soil Biol Biochem* 2014;**68**:482-493.

Lange M, Eisenhauer N, Sierra CA, *et al*. Plant diversity increases soil microbial activity and soil carbon storage. *Nature communications* 2015;6.

Lee J, Lee S, Young JPW. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* 2008;**65**:339-349.

Lekberg Y, Koide RT. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytol* 2005;**168**:189-204.

Littlewood N, Anderson P, Artz R *et al*. *Peatland biodiversity*. Edinburgh: IUCN UK Peatland Programme, 2010.

Lumini E, Orgiazzi A, Borriello R, *et al*. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environ Microbiol* 2010;**12**: 2165-2179.

Maherali H, Klironomos JN. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 2007;**316**:1746-1748.

Mathimaran N, Ruh R, Jama B, *et al*. Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agric Ecosyst Environ* 2007;**119**:22-32.

McGill B. Biodiversity: Land use matters. *Nature* 2015;**520**:38-39.

Meyer A, Focks A, Radl V, *et al*. Different land use intensities in grassland ecosystems drive ecology of microbial communities involved in nitrogen turnover in soil. *PLoS ONE* 2013:**8**;e73536.

Morris EK, Buscot F, Herbst C, *et al*. Land use and host neighbor identity effects on arbuscular mycorrhizal fungal community composition in focal plant rhizosphere. *Biodivers Conserv* 2013;**22**:2193-2205.

Nadimi M, Daubois L and Hijri M. Mitochondrial comparative genomics and phylogenetic signal assessment of mtDNA among arbuscular mycorrhizal fungi. *Mol Phylogenet Evol* 2016;**98:**74-83.

Nesme J, Achouak W, Agathos SN, *et al.* Back to the future of soil metagenomics. *Front Microbiol* 2016;**7:**73.

Newbold T, Hudson LN, Hill SL, *et al*. Global effects of land use on local terrestrial biodiversity. *Nature* 2015;**520**:45-50.

Newsham KK, Fitter AH, Watkinson AR. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecol Evol* 1995;**10**:407-411.

Oksanen J, Blanchet FG, Kindt R, *et al*. Vegan: community ecology package, ver.2.0–3. 2013, URL http://CRAN.Rproject.org/package=vegan.

Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams. 2015, URL http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Öpik M, Davison J, Moora M, *et al*. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. *Botany* 2014;**92**:135-147.

Öpik M, Metsis M, Daniell TJ *et al*. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytol* 2009;**184**:424-437.

Öpik M, Vanatoa A, Vanatoa E, *et al*. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* 2010;188:223-241.

Moora M, Davison J, Öpik M *et al.* Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. *FEMS Microbiol Ecol* 2014;**90**:609-621.

Pellegrino E, Bosco S, Ciccolini V, *et al.* Agricultural abandonment in Mediterranean reclaimed peaty soils: long-term effects on soil chemical properties, arbuscular mycorrhizas and CO_2 flux. *Agr Ecosyst Environ* 2014;**199**:164-175.

Pellegrino E, Öpik M, Bonari E, *et al*. Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. *Soil Biol Biochem* 2015;**84**:210-217.

Pellegrino E, Di Bene C, Tozzini C, *et al*. Impact on soil quality of a 10-year-old short-rotation coppice poplar stand compared with intensive agricultural and uncultivated systems in a Mediterranean area. *Agr Ecosyst Environ* 2011;**140**:245-254.

Powell JR, Parrent JL, Hart MM *et al*. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *P Roy Soc Lond B Bio* 2009;**276:**4237-4245.

Ravnskov S, Larsen J, Olsson PA, Jakobsen I. Effects of various organic compounds on growth and phosphorus uptakeof an arbuscular mycorrhizal fungus. *New Phytol* 1999;**141**:517-524.

Redecker D, Schüßler A, Stockinger H, *et al.* An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 2013;**23**:515-531.

Reinhart KO, Wilson GW and Rinella MJ. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. *Ecol Lett* 2012;**15**:689-695.

Rillig MC, Mummey DL. Mycorrhizas and soil structure. *New Phytol* 2006;**171**:41-53.

Rillig MC, Wright SF, Nichols KA, *et al.* Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* 2001;**233**:167-177.

Roberts DW. labdsv: ordination and multivariate analysis for ecology, package version 1.5-0. 2014, URL http://CRAN.R-project.org/package=labdsv.

Rosendahl S and Matzen HB. Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. *New Phytol* 2008;**179**:1154-1161.

Sánchez-Castro I, Ferrol N and Barea JM. Analyzing the community composition of arbuscular mycorrhizal fungi colonizing the roots of representative shrubland species in a Mediterranean ecosystem. *J Arid Environ* 2012;**80**:1-9.

Schenck NC and Smith GS Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia* 1982;77-92.

Schüßler A, Schwarzott D, Walker C. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol Res* 2001;**105**:1413-1421

Schüßler A. *The Glomeromycota: a species list with new families*. US: CreateSpace Independent Publishing Platform, 2010.

Senés-Guerrero C and Schüßler A. A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. *Fungal Divers* 2015;**77:**317-333.

Smith SE, Read DJ. *Mycorrhizal Symbiosis* (3rd ed.). New York: Academic Press, 2008.

Soil Survey Staff. *Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys* Washington DC: USDA-SCS Agric Handb 436 US Gov Print Office, 1975.

Sýkorová Z, Wiemken A, Redecker D. Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. *Appl Environ Microb* 2007;**17**:5426-5434.

Tamura K, Peterson D, Peterson N, *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;**28**:2731-2739.

Tawaraya K, Takaya Y, Turjaman M, *et al.* Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. *Forest Ecol Manag* 2003;**182:**381-386.

Thormann MN, Currah RS and Bayley SE. The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. *Wetlands* 1999;**19**:438-450.

Torgerson WS. *Theory and Methods of Scaling*. New York: John Wiley & Sons, 1958.

Torrecillas E, Alguacil MM, Roldán A. Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. *Appl Environ Microb* 2012;**78**:6180-6186.

Treseder KK. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant Soil* 2013;**371**:1-13.

Turner SD, Amon JP, Schneble RM, Friese CF. Mycorrhizal fungi associated with plants in ground-water fed wetlands. *Wetlands* 2000;**20**:200-204.

Vallebona C, Pellegrino E, Frumento P, *et al*. Temporal trends in extreme rainfall intensity and erosivity in the Mediterranean region: a case study in southern Tuscany, Italy. *Climatic Change* 2015;**128**:139-151.

Vályi K, Rillig MC, Hempel S. Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. *New Phytol* 2015;**205**:1577-1586.

Van der Heijden MG, Klironomos JN, Ursic M, *et al*. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 1998;**396:**69-72.

Vandenkoornhuyse P, Husband R, Daniell TJ, *et al*. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Mol Ecol* 2002;**11**:1555-1564.

Veresoglou SD, Chen B, Rillig MC. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol Biochem* 2012;**46**:53-62.

Van der Heijden MG, Klironomos JN, Ursic M, *et al.* Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 1998;**396**:69-72.

Veresoglou SD, Rillig MC. Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant Soil* 2014;**377**:395-406.

Verhoeven JT and Setter TL. Agricultural use of wetlands: opportunities and limitations. *Ann Bot* 2009;**105**:155–163

Vogelsang KM, Reynolds HL, Bever JD. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytol* 2007;**172**:554-562.

Wardle DA, Bardgett RD, Klironomos JN, *et al*. Ecological linkages between aboveground and belowground biota. *Science* 2004;**304**:1629-1633.

Wright SF, Franke Snyder M, Morton JB, *et al*. Timecourse study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant Soil* 1996;**181**:193-203.

Xiang D, Verbruggen E, Hu Y, Veresoglou SD, *et al*. Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China. *New Phytol* 2014; **204**:968-978.

Zedler JB, Kercher S. Wetland resources: Status, trends, ecosystem services, and restorability. *Annu Rev Environ Resour* 2005;**30**:39-74.

782 Zobel M, Öpik M. Plant and arbuscular mycorrhizal fungal (AMF) communities-which drives

783 which? *J Veg Sci* 2014;**25**:1133-1140.

Reply to reviewers

FEMSEC-16-04-0249
Title: **Land-use intensity and host plant simultaneously shape the composition of arbuscular mycorrhizal fungal communities in a Mediterranean drained peatland**.
Authors: Ciccolini, Valentina; Ercoli, Laura; Davison, John; Vasar, Martti; Opik, Maarja; Pellegrino, Elisa. *FEMS Microbiology Ecology*

Responses follow the order given by the reviewers. Reviewers' comments are shown in italics, our responses in normal font.

Reviewer 1

The paper of Ciccolini et al. is a resubmission. The authors provide point-to-point response to comments of both reviewers and improved the manuscript substantially. However, I still have several minor comments: I did not see a direct link between the new paragraph on l. 99-106 to your work: you mean acidic systems, such as peatlands? Where is the connection between litter degradation and your work (the next sentence from this part)? I think there is still a lack of information about AMF specifically in peatlands in your introduction.

Reply: we improved the introduction section stressing the importance of AMF in restoration projects of peatlands. See lines 98-106:
"In acidic systems, such as peatlands, fungi have been shown to be more abundant than bacteria, acting as major decomposers of complex carbon polymers in soil organic matter (Andersen, Chapman and Artz 2013). Changes in fungal community composition were mostly attributed to changes in litter type, whereas changes in AMF community were attributed also to changes in plant species assemblages (Thormann, Currah and Bayley 1999). Moreover, AMF were reported to be key components for the success of restoration programmes of disturbed peatlands by colonizing the roots of many wetland plant species and thus potentially improving the early growth of plant community (Turner *et al*., 2000; Tawaraya *et al*., 2003). In Mediterranean peatlands, where climatic conditions leave soil prone to degradation (Vallebona *et al*., 2015), studying the effects of some drivers of AMF community structure such as host plant preference/specificity, land-use intensification and their interaction may support the development of efficient strategies for peatland restoration and protection (i.e., less intensive agriculture, extensive grazing systems, rewetting)".

Further, I still have questions concerning the PCO biplots: you displayed the VT with strong correlation with the ordination scores on each PCO axis. But the axes do not correlate to your environmental factors (host plant or management), do they? So how can you interpret the VTs as you write on l. 277-279 as most indicative of particular land use or host plant? Maybe another type of multivariate analysis such as RDA or CCA, where the environmental factors would be included, may be used for this purpose? I am not expert on statistics, but the PCO biplots, as they are presented now, I see more as a display of similarity of your samples based on their AMF communities than a clear picture of VT/families indicative for certain land uses/host plants and I would put these figures into supplementary.

Reply: It is important to specify that to identify differences due to land use and host plant species on AMF communities we applied the PERMANOVA and PERMDISP analyses and land use and its interaction with host were shown to be significant (Table 2). We applied an unconstrained ordination such as the Principal Coordinate analysis (PCO) to visualize the results, since it can represent, unlike PCA and CA, not linear or unimodal relationships between original variables and

PCO axes. Indeed, PCO was applied with the aim to visualize the (dis)similarities among samples and, moreover, which VT determined these (dis)similarities. Since the interaction between Land use and Host was shown to be significant, these environmental variables are correlated to the axes. The high correlation of the VT plotted is confirmed by the relative abundances presented in the supplementary materials. The indicator species analyses was then applied to identify the taxa that were most indicative of particular land-use and host plant categories: the goodness of our approach was confirmed by the fact that the output of the indicator species analyses overlaps in the most part of the cases to the output of the PCO. For such a reason, we believe that these figures need to be presented in the main text in order to better understand the PERMANOVA analyses.

Tab 2: Add to the heading that it is only for the two host plant species.
Reply: The heading of the table was changed as suggested:
"Table 2. PERMANOVA and PERMDISP analysis of the effect of land-use intensification (land-use type) and host plant species on arbuscular mycorrhyzal fungal (AMF) community composition at Virtual Taxa and family level within the roots of *Poa* sp. and *Calystegia sepium*."

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Pisa, 30th August 2016

Subject: submission of revised manuscript FEMSEC-16-04-0249

Dear Editor,

Enclosed please find the reply to the reviewer and the revised version of the manuscript entitled **Land-use intensity and host plant simultaneously shape the composition of arbuscular mycorrhizal fungal communities in a Mediterranean drained peatland"** for publication as a regular paper in FEMS Microbiology Ecology in the special issue "Ecology of microorganisms in soils" related to the conference 'Ecology of Soil Microorganisms – Microbes as Important Drivers of Soil Processes' (Prague, 2015).

A detailed reply to reviewer's minor comments was uploaded in the section "View and respond to decision letter", the new revised version of the manuscript was uploaded as main document (file n°2) while the new revised manuscript with track changes was uploaded as supplementary file (file n°19).
We also upload the revised version of tables (file n°3 and n°9).
All authors and I are grateful to the anonymous reviewers for their helpful suggestions.

We also state that we uploaded as Supporting Information nine tables and eight figures for eventual online application.

We hope that our manuscript can be accepted for publication in FEMS Microbiology Ecology. We thank you for receiving and considering it for review.

Yours sincerely

Valentina Ciccolini