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Research paper

Field inoculation with a local arbuscular mycorrhizal (AM) fungal consortium promotes sunflower agronomic traits without changing the composition of AM fungi coexisting inside the crop roots

Myriam Arcidiacono, Laura Ercoli, Gaia Piazza, Elisa Pellegrino *

Crop Science Research Center, Scuola Superiore Sant'Anna, Pisa, Italy

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ABSTRACT

Improving reliability and effectiveness of microbial inoculants in crops is a pressing necessity due to recent increases in price of synthetic fertilizers and environmental concerns related to their application. Usually, field inoculation of beneficial microbes, such as arbuscular mycorrhizal fungi (AMF), manipulates abundance and species composition, making it difficult to disentangle their independent effects. In this study, we investigated for the first time the mechanisms behind the agronomic performance of sunflower after field inoculation with a local AM fungal consortium under high and low soil fertility. The abundance of AMF in roots was promoted by inoculation more in low than high soil fertility. In both soil conditions, up to 68 % of the AM fungal taxa retrieved in roots were shared between the inoculated and control plants, confirming minor changes in AM fungal community composition. On the contrary, the structure of AM fungal community was modified by inoculation. Inoculation improved grain yield by 16 % in low soil fertility, oil yield up to 36 %, and enhanced grain content of nutrients under both soil conditions. The best predictor of agronomic performance of sunflower was percentage of AM fungal root colonization in high soil fertility and percentage of vesicles in low fertility. The structure of AM fungal community was not correlated with crop functional parameters under high soil fertility, while under low fertility the occurrence of *Rhizophagus* sp. VTX00105 in roots was the best predictor. Overall, our results demonstrated that local AM fungal inoculants do not affect root AM fungal composition, but increases abundance and modifies the structure of AM fungal community in roots. These modifications are associated with improvements in sunflower grain and oil yield, and in seed nutritional value, especially in low soil fertility. However, the mechanisms behind the functioning of field inoculum on crop performance were revealed to be context-dependent.

1. Introduction

The exploitation of microbial resources for the formulation of bioinoculants is currently considered a good alternative to chemical fertilizers due to their great potential to improve crop production and food safety (Chen et al., 2021; Mahanty et al., 2017). Microbial inoculants have the advantage of being ecofriendly, cost-effective, and of greatly improving soil fertility through their prolonged use (O'Callaghan et al., 2022; Singh et al., 2011). Among beneficial microbes, arbuscular mycorrhizal fungi (AMF) are widely used microbial inoculants in agriculture, together with rhizobia, free-living nitrogen fixers, and plant growth-promoting rhizobacteria (French et al., 2021). Arbuscular mycorrhizal fungi are obligate symbionts belonging to the phylum Glomeromycota (Tedersoo et al., 2018). They form a symbiosis with ca. 67 % of plant species in terrestrial environments (Bueno et al., 2019; Maherali et al., 2016), and they supply mineral nutrients to plants (mainly phosphorus, P) in exchange for photosynthetically fixed carbon (C) (Bago et al., 2000; Jiang et al., 2017; Luginbuehl et al., 2017). These fungi can improve plant growth and yield through increased uptake of P (Smith et al., 2009, 2015; Treseder, 2013; Zhang et al., 2019) and other nutrients, such as zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) (Lehmann et al., 2014; Lehmann and Rillig, 2015; Pellegrino et al., 2015), as well as they can improve plant tolerance to biotic and abiotic stresses (e.g., drought, salt) (Augé et al., 2015; Chandrasekaran et al., 2014; Marro et al., 2022; Pozo et al., 2015). The positive effects of AMF on plant water uptake were reported to be related to the increased surface area through the development of the mycelium in soil and the consequent improved nutrient uptake in dry conditions. However, the outcome of the symbiosis can greatly vary according to soil nutrient avail-

* Corresponding author.
E-mail address: elisa.pellegrino@santannapisa.it (E. Pellegrino).

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abilities, host identity, and environmental conditions (Duhamel and Vandenkoornhuyse, 2013).

Sunflower (*Helianthus annuus* L.) is an annual crop belonging to the family Asteraceae, mostly cultivated for edible oily seeds and usually grown during spring and summer (Yegorov et al., 2019). The expanded world production of sunflower in recent decades resulted primarily from the ability of this crop to adapt to different agro-environmental conditions and from the development of high-oil varieties and hybrids by breeders (Miladinović et al., 2019; Seiler and Jan, 2010). Although harvested primarily for oil production (Gupta, 2014), sunflower is used to produce birdseed, livestock feed and human food, as well as cosmetics, lubricants, biodiesel, and drugs (Park et al., 1997; Rocha-Filho et al., 2016). Due to its ability to withstand drought conditions, sunflower is largely cultivated in dryland and rainfed conditions and may become the oil crop of preference in the future, especially in the light of global climatic changes (Miladinović et al., 2019). However, water availability at anthesis and seed filling stages, together with adequate soil nutrient availability, are crucial for sunflower seed yield (Connor and Sadras, 1992; Flagella et al., 2002; Mukherjee et al., 2019; Rondanini et al., 2003, 2006). Nitrogen (N) and P soil availability affects crop growth and seed yield, while micronutrients may increase the percentage of flower fecundation (Grassini et al., 2009; Massignam et al., 2009; Ramulu et al., 2011).

In conventional agroecosystems, practices such as intensive tillage, monoculture, and the application of chemical fertilizers, pesticides, and herbicides can greatly impact the AMF occurring in soil and crop roots (Caggìa et al., 2023; Ciccolini et al., 2016; Jansa et al., 2006; Pellegrino et al., 2019; Rosner et al., 2018; Verbruggen et al., 2015). These practices can reduce the abundance and species richness of AMF and alter their community composition and structure, thus leaving the fields with AM fungal communities of reduced symbiotic quality. Therefore, the process of reestablishing a functional level of AM fungal abundance and diversity through field inoculation of AMF can represent a valid strategy to restore soil biological fertility and a promising alternative to conventional fertilization practices (Berruti et al., 2016). In fact, the implicit assumption of the inoculation in the field with selected AM fungal strains is that agricultural soils are limited by the abundance and functionality of resident AM fungal communities. So far, the degree of adoption of AM fungal inoculants remains limited. This is probably due to the variable crop response under different agro-environmental conditions, since the various mechanisms involved in crop growth promotion is host-, AM fungal strain- and site-specific (Verbruggen et al., 2013). Regarding sunflower, while experiments testing the multiple effects of AMF under controlled conditions are many (e.g., Bellido et al., 2021; Ibrahim, 2018; Kabir et al., 2020; Kavitha and Nelson, 2014; Yadav et al., 2015), field experiments are few and mainly focused on the effect of single exotic AM fungal isolates on crop productivity under drought stress (i.e., Gholamhoseini et al., 2013; Heidari and Karami, 2014; Langeroodi et al., 2021, 2022; Noroozi et al., 2023; Soleimanzadeh, 2010; Rosner et al., 2018). Under controlled conditions, experiments that applied both single and multiple species of AM fungal inoculants recorded significant increases in seedling germination, root and plant growth, shoot nutrient uptake, grain yield and quality. However, the benefits were variable across the type of inocula: generally mixed inocula performed better than single ones (Ibrahim, 2018, 2019; Kavitha and Nelson, 2014), and larger responses were observed under low soil P availability (Abobaker et al., 2018). Additionally, AMF have been reported to improve sunflower water status under drought stress, and its response to soil micronutrient deficiency (e.g., Fe), saline stress (Pereira et al., 2016; Ramzan et al., 2023), and root pathogens (Nafady et al., 2019; Rashad et al., 2020). Under field conditions, inoculation of sunflower with *Rhizophagus fasciculatus* at different levels of P increased total dry biomass, P content, and seed yield, but the positive effect decreased under high soil P levels, due to a lower root colonization and spore density (Chandrashekara et al., 1995). Langeroodi et al. (2021, 2022), studying the co-application of biochar and AMF to sunflower, found that AM fungal root colonization and mycorrhizal response at anthesis and physiological maturity increased with the highest biochar dose due to a higher crop water status under semi-arid conditions. Furthermore, Noroozi et al. (2023) highlighted, for the first time, a strong interaction between sunflower genotype and AM fungal inoculated species on the crop benefits. Recently, the biological processes of the symbiosis between AMF and sunflower were also deciphered by the changes in the transcriptome of plants inoculated with *Rhizophagus irregulare* and transcripts related to nutrient transport (e.g., P, N, Fe and Zn) were found to be overexpressed under AM fungal inoculation (Vangelisti et al., 2018, 2020). However, to the best of our knowledge, no studies have investigated in the field the changes in root AM fungal community induced by inoculation, which could be responsible for the agronomic response of sunflower.

Experiments in which AMF are applied to agricultural fields usually manipulate both their abundance and species composition/structure, making it difficult to disentangle the independent effects of factors. In our experiment, the tested inoculum was composed of many AM fungal species, isolated from soil located in the same agricultural area where the experiment was carried out. Under these conditions, plant responses to inoculation are likely to be driven by increases in AM fungal abundance and changes in AM fungal community structure, and not by modification of the AM fungal composition through the introduction of foreign taxa (Marrassini et al., 2024a, 2024b; Pellegrino et al., 2022).

Therefore, in this study, following field inoculation of sunflower with a local AM fungal consortium, we aimed to elucidate the effect of the potential increase of abundance of AMF in roots and of the change in intraradical community structure. Field inoculation was carried out in two consecutive years under two contrasting soil conditions, that is, high and low soil fertility. The mycorrhizal benefit of sunflower, known to be mycorrhizal dependent (Molla et al., 2010), was evaluated by assessing plant growth, nutrient uptake, seed and oil yield, while root abundance and community composition and structure of AMF were assessed using morphological and molecular tools, respectively. We hypothesized that under both soil fertility conditions, AM fungal community composition in roots would not be modified by the AM fungal inoculation. Moreover, we hypothesized that under low soil fertility conditions, the AM fungal root abundance and community structure would have been modified, but the increases in the AM fungal abundance in roots would be the main driver of the multiple beneficial effects on the crop (Fig. 1). On the contrary, under high fertility conditions, the main driver of crop agronomic benefits would have been the changes in community structure and not the increases in root AM fungal abundance. Finally, we hypothesized a larger crop mycorrhizal benefits under low soil fertility conditions.

2. Materials and methods

2.1. Fungal material

A locally sourced consortium consisting of AMF originating from a local field site was used as inoculum (Pellegrino and Bedini, 2014). In detail, the AM fungal inoculum was obtained by setting up an AM fungal trap culture with local soil and maize (*Zea mays* L.). The AM fungal inoculum was composed by 14 AM fungal species: *Acaulospora cavernata*, *Acaulospora spinosa*, *Acaulospora* spp., *Diversispora spurca*, *Funneliformis coronatus*, *Entrophospora etunicata* (syn. *Glomus etunicatum*), *Funneliformis geosporus* (syn. *Glomus geosporum*), *Funneliformis mosseae*, *Glomus* spp., *Rhizophagus clarus*, *Rhizophagus irregularis*, *Scutellospora aurigloba*, *Scutellospora calospora* and *Septoglomus viscosum* (http://www.amf-phylogeny.com). The crude inoculum applied to the field consisted of a micronized mixture of mycorrhized roots, spores, hyphal fragments, and bentonite as the carrier.

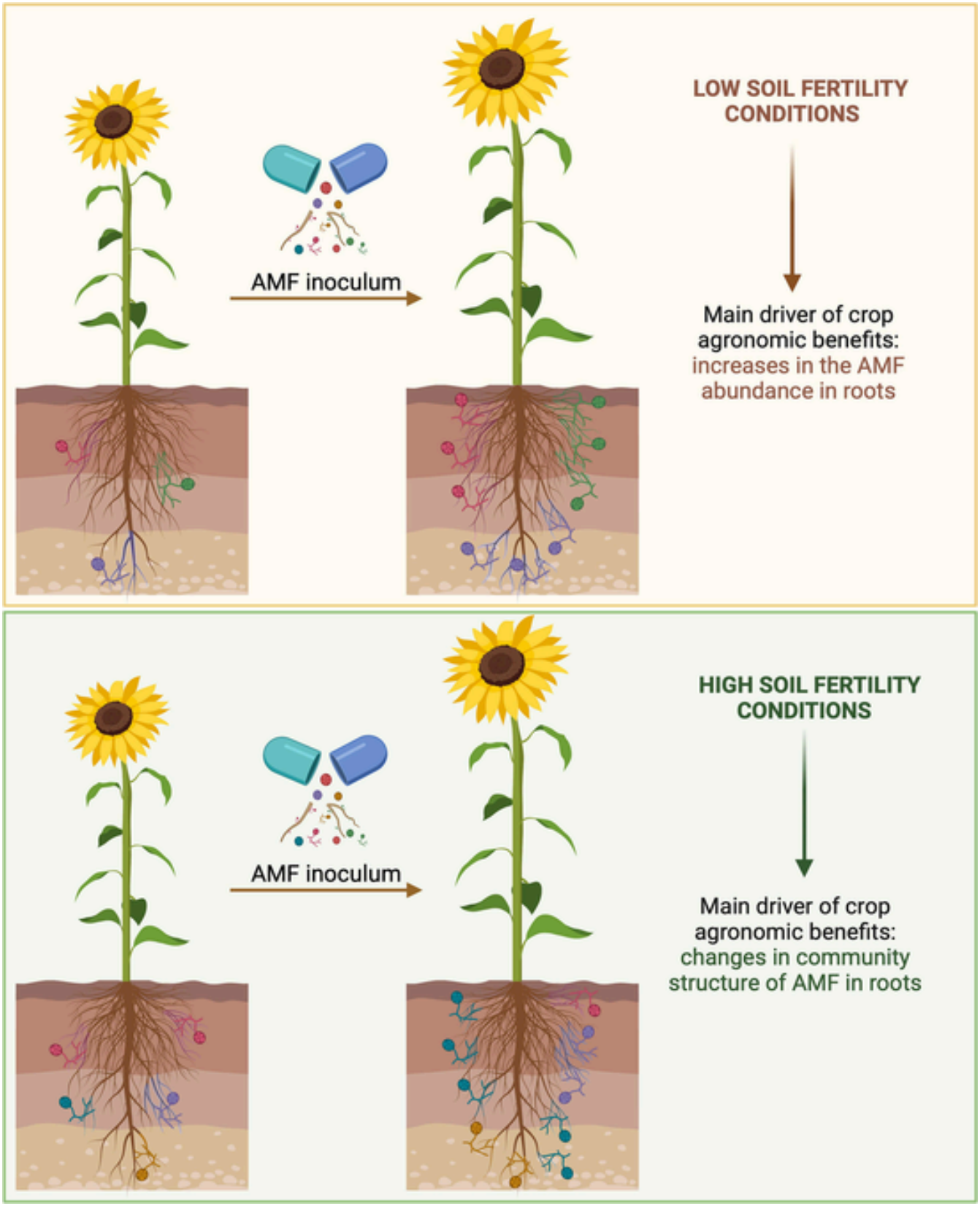


Fig. 1. Research hypotheses of the study on sunflower inoculated with a local AM fungal consortium and mock inoculated in low and high soil fertility conditions. The image was created with BioRender.com.

2.2. Experimental field site and climatic data

The experiment was carried out in two consecutive years (2019 and 2020) at the farm "Azienda Agricola Musu Giuseppe e Francesco" Pisa, Italy (43°39′ N, 10°31′E, 5 m above sea level and 0.8 % slope), in two distinct fields. In 2019 the soil was sandy loam (53.8 % sand, 35.4 % silt, and 10.7 % clay) with 75.02 g kg^{-1} soil organic carbon (SOC) (very high content) (Walkley-Black; Nelson and Sommers, 1982), 7.5 pH (subalkaline) (deionized water 1:2.5 w/v; McLean, 1982), 4.4 g kg^{-1} total N (high content) (Kjeldahl; Bremner and Mulvaney, 1982), 1.83 g kg^{-1} total P, 64.60 mg kg^{-1} available P (Olsen) (low availability) (Olsen and Sommers, 1982) and C/N equal to 18.1 (referred as high-fertility site) (Table S1). The soil in 2020 was silt loam (23.4 % sand, 54.7 % silt and 21.9 % clay) with 9.6 g kg^{-1} SOC (medium content) (Walkley-Black), 8.0 pH(H_2O) (subalkaline), 1.13 g kg^{-1} total N (medium content) (Kjeldahl), 18.2 g kg^{-1} total P and 22.4 mg kg^{-1} available P (Olsen) (very low availability) and C/N equal to 8.4 (referred as low-fertility site). In the experimental area, the climate was classified as cold and humid Mediterranean (Csa) according to the Köppen-Geiger climate classification (Kottek et al., 2006). Averaged over 1990–2020, mean annual maximum and minimum air temperature were 20.7 and 9.9 °C, respectively, and mean annual precipitation was 744 mm. During the field experiment (May to September), temperature was similar to the 30-year average, as mean maximum and minimum temperature were 29.5 and 15.7 °C, respectively, in 2019, and 27.6 and 15.7 °C, respectively, in 2020. Rainfall varied over the two cropping cycles: in 2019 it was 187 mm and in 2020 it was 244 mm. Furthermore, rainfall in 2020 was well distributed through the sunflower cycle and therefore was more favorable for crop growth and development. The preceding crop of sunflower was bread wheat (*Triticum aestivum* L.) in both years. Rainfall and temperature (mean, minimum and maximum daily temperature) for the sunflower growth cycle are given in Fig. S1.

2.3. Experimental set-up

The research was set up with sunflower (*Helianthus annuus* L.) cv. Talento in 2019 and 2020. In both years, the layout of the experiment was a completely randomized design with AM fungal inoculation treatment (local AM fungal consortium: +M; mock inoculum as control: −M) as factor and four and three replicate plots in 2019 and 2020, respectively. Each replicate plot in 2019 had a size of 260 m length x 15 m width (3900 m^2), while in 2020 they had a size of 220 m length x 25 m width (5500 m^2). The plots were moldboard ploughed (40 cm depth), disked (15 cm depth) and harrowed (5 cm depth) in early spring and then sown on 29 May 2019 and 9 May 2020, with 6 kg seed ha^{-1} to obtain a plant density of 5–6 plants m^{-2} (75 cm between rows) by a pneumatic precision seeding machine (MaterMacc Srl, Pordenone, Italy). Field inoculation with AMF was performed, before harrowing, by manually applying it to the soil at a rate of 150 kg ha^{-1} of inoculum (ca. 45,000 spores ha^{-1}). The control mock inoculum consisted of the same amount of sterilized inoculum combined with bentonite as a carrier, applied to the soil. Additionally, a filtrate obtained by sieving the AM fungal inoculum through a 50 μm pore diameter sieve was added to the mock inoculum to ensure consistent microflora across the treatments (5 L ha^{-1}). Basal fertilization was carried out by applying, before planting, an organic fertilizer at a dose of 350 kg ha^{-1} (3:3:3 N:P:K; Bioletamix, Agrovitaly) and triple mineral phosphate [$Ca(H_2PO_4)_2{\cdot}H_2O$] and potassium sulphate (K_2SO_4) at rates of 70 kg ha^{-1} P and 60 kg ha^{-1} K. Nitrogen was applied topdressing as urea at a rate of 75 kg N ha^{-1} at V4 growth stage (Schneiter and Miller, 1981). Weed control was achieved with a preemergence application of 1 L per ha^{-1} of Dual Gold® (S-metolachlor, Syngenta, China), postemergence application of 1 L per ha^{-1} of Beyond® (Imazamox, BASF, Germany), and of 2 L ha^{-1} of Leopard® (quizalofop-P-ethyl, ADAMA, Syngenta, China). No fungicides or insecticides were applied. Sunflower was harvested on September 16, 2019, and September 15, 2020.

2.4. Mycorrhizal infection potential of the experimental soil and the AM fungal inoculum

The infectivity of the experimental field soil and the AM fungal inoculum was evaluated using a modified version of the mycorrhizal infection potential (MIP) test of Pellegrino et al. (2011). The test was set up in a growth chamber (24 °C day and 18 °C night temperature; 12:12 h light:dark cycle, 420 μmol m^{-2} s^{-1}). Three seeds of sorghum (*Sorghum vulgare* L.) were sown in 50 mL sterile plastic tubes filled with 25 mL of soil or AM fungal inoculum, and 25 mL of sterile quartz grit. Soil was obtained by randomly collecting five samples from each field (high- and low-fertility sites) before sunflower sowing. Soil samples were collected at a depth of 30 cm using a soil corer (8-cm in diameter) and then they were air-dried. Three technical replicates in plastic tubes were used for each soil sample (five soil replicates per site; n = 30) and for the AM fungal inoculum (three biological replicates; n = 9). After plant emergence, the sorghum plants were thinned to one. The plants were harvested after four weeks of growth and the root systems were cleared and stained, using lactic acid instead of phenol (Phillips and Hayman, 1970). The roots were then mounted on microscope slides and examined under an optical microscope (Leitz Laborlux S, Wetzlar, Germany) to assess AM fungal root parameters (i.e., percentage of root length containing arbuscules and vesicles and percentage of AM fungal root colonization) (McGonigle et al., 1990).

2.5. Mycorrhizal abundance in sunflower roots

At the beginning of anthesis (growth stage R5.5; Schneiter and Miller, 1981), root samples were collected by sampling a turf (20 cm depth) from three areas (1 m^2) per each replicate plot (a total of 24 samples at the high-fertility site and 18 samples at the low-fertility site). In the laboratory, the roots were separated from the soil by gently washing with tap water. Fresh roots were utilized for the assessment of mycorrhizal abundance by measuring the AM fungal colonization traits (i.e., percentage of root length containing arbuscules and vesicles, and percentage of AM fungal root colonization), following the method previously described.

2.6. Mycorrhizal diversity in sunflower roots

DNA was extracted from 0.02 g of root samples collected at stage R5.5. as described above (a total of 24 samples for the high-fertility site and of 18 samples for the low-fertility site). DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA), following the manufacturer instructions. The extracted DNA was quantified by a spectrophotometer (NanoDrop Technology, Wilmington, DE) and then stored at −20 °C for further analyses. DNA was amplified using an amplicon-specific polymerase chain reaction (PCR). The small subunit ribosomal RNA (SSU) fragments were amplified using a two-step nested PCR approach with two primer pairs. The forward primer AML1 (5′-ATC AAC TTT CGA TGG TAG GAT AGA-3′) and the reverse primer AML2 (5′-GAA CCC AAA CAC TTT GGT TTCC-3′) were used in the first step, while the forward primer WANDA-ill (5′-**TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG ANN NHN NNW NNN HGC** AGC CGC GGT AAT TCC AGCT-3′) (Dumbrell et al., 2011) and the reverse primer AML2- ill (5′-**GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G**GA ACC CAA ACA CTT TGG TTT CC-3′) (Lee et al., 2008) were used in the second step (in bold the adaptors for the Illumina reaction). PCR protocol, amplicon purification and quantification methods are given Supplementary Material and Methods 1. A total of 13 amplicons per site were cleaned and quantified (high-fertility site: −M n = 6, +M n = 7; high-fertility site: −M n = 5, +M n = 8), and

adjusted in an equimolar ratio (10 ng/μL) for dual-index barcodes addition using the Nextera® XT DNA library preparation kit (Illumina Inc., CA, United States), and the generated metabarcoding libraries were sequenced on an Illumina MiSeq sequencer (2 × 300 bp paired-end reads) at the University of York (UK), loading a final library concentration of 12-pM with 20 % PhiX library spike-in (Illumina) and using an Illumina MiSeq V3 600 cycle sequencing kit. Species richness (S) was calculated. Moreover, Shannon index (H') was calculated as $H' =$ -SUM (Pi*Log(Pi)) using an exponential log base, while Gini-Simpson index (λ) was calculated as $1\text{-}\lambda' = 1\text{-SUM(Ni-1)/N*(N-1)}$.

2.7. Sunflower plant growth, grain and oil yield, and nutrient uptake

At both sites, during stage R5.5, three areas of 1 m^2 were sampled for each replicate plot by manually cutting plants at ground level (a total of 24 at the high-fertility site and 18 samples at the low-fertility site). Then, after oven drying at 65 °C up to constant weight, shoot dry weight (SDW) was determined and the samples were stored for mineral element analysis. Additionally, within each square meter, two plants were excavated with their root system (a total of 48 and 36 plants at the high- and low-fertility sites, respectively). The excavation was carried out at a depth of approximately 40 cm. The roots were separated from the soil by gently washing with tap water and oven dried at 65 °C up to constant weight to determine root dry weight per plant (RDW). The SDW and RDW were expressed per square meter (RDW). Furthermore, at physiological maturity (stage R9), three areas of 1 m^2 were randomly identified in each replicate plot and plant height (cm), head diameter (cm), root system radius (cm), and tap root length (cm) were assessed on two plants from each sampling area (a total of 48 and 36 plants at the high- and low-fertility sites, respectively). Additionally, grains were harvested from each 1 m^2 area (a total of 24 and 18 samples at the high- and low-fertility sites, respectively) to assess grain oil concentration.

Grain yield was determined at stage R9 by harvesting the whole plot using a plot combine harvester. Grain oil concentration was determined on dried achene samples (4 g) obtained from both experiments. The achens were previously ground in a laboratory mill, and then grain oil concentration was determined by Soxhlet extraction using 150 mL of petroleum ether as solvent for 3 h (Pomeranz et al., 1994). Oil yield was calculated by multiplying grain yield per unit area by grain oil concentration.

In addition, shoot and grain samples were ground to fine powder prior to the analysis of mineral nutrients. Approximately 0.3 g of sunflower shoots and seeds, respectively, were digested using the COOLPEX Smart Microwave Reaction System (Yiyao Instrument Technology Development Co., Ltd., Shanghai, China) after the addition of 8.0 mL of nitric acid (65 %). The solution was diluted with Milli-Q water and analyzed. The concentration of P, potassium (K), calcium (Ca), magnesium (Mg), Fe, Zn, Cu and Mn was determined using a microwave plasma atomic emission spectroscopy instrument (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA) (Liberato et al., 2017), while the concentration of N was determined using the Kjedahl method. Nutrient uptake (NU) of shoot and grain was calculated by multiplying shoot and grain yield by the respective nutrient concentration.

2.8. Bioinformatic analysis

Raw sequence data were processed and analyzed using the QIIME2 pipeline (2018.11) and the modules (Bolyen et al., 2019). Demultiplexed forward and reverse paired-end reads were joined using the '-fastq_mergepairs' of the USEARCH plugin (Edgar, 2010). From the high-fertility site, MiSeq sequencing, out of the 18,864 reads exposed to merging, 84 % (15,828 reads) were successfully merged and 17 % (3181 reads) were aligned with zero differences. The primer sequences were trimmed from the sequences using the cutadapt plugin 1.18 with Python 3.5.5 (Martin, 2011), and 15,810 valid sequences were obtained after optimization. From the low-fertility site, MiSeq sequencing, out of the 12,702 reads exposed to merging, 87 % (11,029 reads) were successfully merged and 19 % (2392 reads) were aligned with zero differences. Primer sequences were trimmed off from the sequences, and 11,017 valid sequences were obtained after optimization. The average read length was approximately 300 base pairs (bp) based on the maximum expected error (MaxEE). The command USEARCH 'fastq_eestats2' was used to check the quality of the sequence and based on the MaxEE percentage, the reads were truncated at the drop-off point of 260 bp using the USEARCH 'fastq_filter' command. Quality-filtered reads were de-replicated using the USEARCH 'fastx_uniques' command and operational taxonomic units (OTUs) were generated by clustering reads at a similarity threshold of 95.3 % and 98.5 % using the USEARCH 'cluster_otus' command. During the process, chimeric sequences and singletons were also removed. The resulting OTUs were assigned to virtual taxa (VTX) using the MaarjAM database (https://maarjam.ut.ee/). All representative newly generated sequences (a total of 22 and 15 for the high- and low-fertility sites, respectively) were deposited in the NCBI Sequence Read (SRA) database as SUB14264070 (accession numbers from PP378456 to PP378477) and SUB14264075 (accession numbers from PP378490 to PP378504). The representative sequences were aligned, together with NCBI sequences of closely related AM fungal species, using the MAFFT online service (Katoh et al., 2019) and a neighbour-joining (NJ) tree was built using MEGA11 (Tamura et al., 2021), following the bootstrap test of phylogeny with 1000 bootstraps (Figs. S2 and S3). The substitution model used was the Kimura 2-parameter model with uniform rates among sites, pairwise deletion, and 7 threads. The NJ tree was edited using Adobe Illustrator 2022.

2.9. Statistical analysis

Since the environment (i.e., climate and physico-chemical and biological parameters of the soil) was markedly different between sites (Fig. S1 and Table S1), they were analyzed separately. To test the effect of inoculation on mycorrhizal abundance in sunflower roots, plant growth, grain and oil yield, nutrient uptake AM fungal richness (S), Shannon (H') and Simpson (λ) indices, an univariate ANOVA was performed with AM fungal inoculation (+M and –M) as fixed factor, and subreplicates, when present, as nested factor within the main factor. The data were transformed if necessary (e.g., log10, arcsen). Differences between means were determined using the Tukey-B test. All analyses were performed with the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA).

A permutational analysis of variance (PERMANOVA) was performed to test the effect of inoculation on the AM fungal community structure (VTX), separately for the high- and low-fertility sites. Reads in the AM fungal VTX matrix were normalized to the median number of reads across the samples and the relative abundances were calculated. The response data were then transformed as fourth-root and the Bray-Curtis matrices of similarity were calculated. The P value in PERMANOVA [P(MC)] was calculated using the Monte Carlo test (999 permutations) (Anderson and Braak, 2003). Moreover, PERMANOVAs were also performed to test the effect of inoculation on fungal traits, i.e. AM fungal root colonization, arbuscules, vesicles, richness, H' and λ in roots, and on plant functional parameters, i.e. plant growth, grain and oil yield, shoot and grain nutrient content. Data in the matrices were standardized, square-root transformed, and Euclidean distances were calculated. The analysis of homogeneity of multivariate dispersion (PERMDISP; Anderson, 2006) was performed to check the homogeneity of dispersion among groups (beta-diversity) (Anderson et al., 2006). If PERMANOVA was statistically significant, principal coordinate analysis (PCO) was performed (Gower, 1966). The data sets were also used to generate Venn diagrams, representing the unique and shared OTUs for each treatment at the high- and low-fertility sites (i.e., composition;

data in Venn diagrams are expressed as percentages). Venn diagrams were generated using InteractiVenn (Heberle et al., 2015) and edited by Adobe Illustrator 2022.

To understand the relationship between fungal traits and plant functional parameters and between AM fungal community and plant functional parameters at the two sites, and to understand which were the main responsible fungal traits or VTX, a multivariate statistical approach was applied. The relationships were determined by a RELATE analysis (Clarke and Warwick, 2001). The analysis was based on Spearman rank and 999 permutations with ρ equal to 1 representing a perfect relationship. To find the best descriptor(s) of the relationships, a BEST analysis, based on BioEnv methods (all combinations), Spearman rank and 999 permutations, was applied (Clarke et al., 2008). Finally, the analysis of the distance-based linear method (DistLM), using a stepwise selection and Akaike's information criterion (AICc), was applied to measure the significance and variance explained by the best descriptor/s (Knorr et al., 2000), and the distance-based redundancy analysis was used to plot the first and second axes of the DistLM (Legendre and Anderson, 1999). All multivariate analyses were performed using the PRIMER 7 and PERMANOVA + software (Anderson et al., 2008; Clarke and Gorley, 2015)

3. Results

3.1. Mycorrhizal infection potential of the experimental soil and the AM fungal inoculum

The results of the mycorrhizal infection potential (MIP) test showed that the infectivity of the experimental field soils was markedly different between the two sites (Tables 1 and S2). The percentages of root length that contained arbuscules and vesicles, and AM fungal root colonization at the high-fertility site were significantly higher than those at the low-fertility site (i.e., +236 %, +427 %, and +128 %, respectively). Furthermore, the MIP test showed that the infectivity of the AM fungal inoculum was high: the percentages of root length containing arbuscules and vesicles, and the colonization of the root by AMF were 15.7 ± 3.5, 6.9 ± 2.4, and 38 ± 6.4 (results not shown).

Table 1
Infectivity of the experimental field soil at high- and low-fertility sites (High-fert and Low-fert, respectively), assessed by the mycorrhizal infection potential (MIP) test, measuring the percentage of root length containing arbuscules and vesicles, and the percentage of arbuscular mycorrhizal fungal (AMF) colonization in roots of *Sorghum vulgare* L. AMF abundance in sunflower roots sampled at the beginning of anthesis at High-fert and Low-fert. Nutrient content in shoots of sunflower (*Helianthus annuus* L. cv. Talento) inoculated with a local AMF consortium. The sampling was performed at R5.5 growth stage (beginning of anthesis) at both sites.

		Arbuscules	Vesicles	AMF colonization
		%		
MIP				
High-fert[a]		13.0 ± 1.9 b[b]	1.9 ± 0.7 b	28.6 ± 3.6 b
Low-fert		3.9 ± 2.2 a	0.4 ± 0.3 a	12.6 ± 3.1 a
AMF abundance[c]				
High-fert[b]	−M[c]	48.7 ± 5.0[d]	10.0 ± 1.9 a	72.0 ± 1.9 a
	+M	59.5 ± 11.5	32.5 ± 7.8 b	84.3 ± 7.9 b
Low-fert[b]	−M	48.4 ± 7.4[d]	2.5 ± 1.2 a	60.1 ± 5.7 a
	+M	60.6 ± 7.7	12.9 ± 1.5 b	86.5 ± 1.8 b

[a] Means ± SE of five replicate per year. Three technical replicates for each soil sample.
[b] Values within columns with different letters are significantly different between years or treatments within a year, according to the Tukey-B test ($P \leq 0.05$) (Table S2).
[c] +M: inoculation treatment; −M: mock-inoculated treatment (control).
[d] Means ± SE of four and three replicate plot per treatment at High-fert and Low-fert, respectively. Three technical replicates for each plot (a total of 24 at High-fert and 18 samples at Low-fert) (Table S2).

3.2. Mycorrhizal abundance and diversity in sunflower roots

Under both high and low soil fertility conditions, AM fungal inoculation increased the percentage of root length containing vesicles in sunflower roots by more than twofold and fourfold, respectively (Tables 1 and S2). Similarly, AM fungal inoculation increased AM fungal colonization by 17 % and 44 % in high- and low-fertility soils, respectively (Tables 1 and S2).

A total of 26 AM fungal VTX were retrieved in AM fungal inoculated (+M) and mock inoculated (−M) sunflower roots considering both sites (Fig. S4). Details on AM fungal community composition in the high- and low soil fertility sites are given in Supplementary Results 1. At the high-fertility site, 15 VTX were shared between +M and −M (Fig. 2c) and among these, *Rhizophagus* sp. VTX00363, *Rhizophagus* sp. VTX00113, *Rhizophagus* sp. VTX00105 and *Glomus* sp. VTX00342 were the most abundant. These VTX accounted for 79 % of total abundance in +M and for 86 % in −M. Moreover, five VTX were uniquely found in +M (i.e., *Acaulospora* sp. VTX00024, *Glomus* sp. VTX000143, *Glomus* sp. VTX00130, *Ambispora* sp. VTX00283 and *Acaulospora* sp. VTX00030), whereas two in −M (i.e., *Paraglomus* sp. VTX00001, *Scutellospora* sp. VTX00041). The indices of AM fungal diversity, i.e. fungal richness (S), Shannon index (H') and Simpson index (λ) were not affected by AM fungal inoculation (Fig. 2a and Table S3). Finally, PERMANOVA showed that at the high-fertility site the structure of root AM fungal community was significantly affected by AM fungal inoculation and the variance explained was 27.3 % (Table S4). In the PCO biplot it is evident that most AM fungal VTX characterized the inoculated plants (+M), whereas *Rhizophagus* sp. VTX00363, *Scutellospora* sp. VTX00041 and *Paraglomus* sp. VTX00001 characterized the mock inoculated ones (−M) (Fig. 2e).

At the low-fertility site, a total of 15 AM fungal VTX were recovered from +M and −M sunflower roots (Fig. S4b). From the Venn diagram, it can be seen that 9 AM fungal VTX were shared between +M and −M (Fig. 2d), and among these, *Rhizophagus* sp. VTX00105, *Rhizophagus* sp. VTX00363, and *Rhizophagus* sp. VTX00113 were the most abundant, accounting for 89 % of total abundance in +M and 80 % in −M (Fig. S4b). Furthermore, 5 AM fungal VTX were uniquely recovered in +M (i.e., *Entrophospora* sp. VTX00056, *Glomus* sp. VTX00098, *Glomus* sp. VTX00155, *Septoglomus* sp. VTX00063 and *Glomus* sp. VTX000153), whereas there was only one VTX in −M (*Glomus* sp. VTX000143). Inoculation with AMF reduced the indices of AM fungal diversity, i.e., S, H' and λ by 29 %, 35 %, and 31 %, respectively (Fig. 2b and Table S3). Furthermore, PERMANOVA showed that the community structure of AMF within the roots of sunflower was significantly affected by inoculation and the variance explained was 29.2 % (Table S4). In the PCO biplot, some AM fungal VTX characterized the +M treatments (e.g., *Septoglomus* sp. VTX00063; *Entrophospora* sp. VTX00057; *Rhizophagus* sp. VTX00105), while others were linked to −M treatment (e.g., *Funneliformis* sp. VTX00067; *Rhizophagus* sp. VTX00363) (Fig. 2f). However, in both sites, the distances of the group objects from their centroids were significantly different, according to the PERMDISP (Table S4). Indeed, under both soil fertility conditions, the samples belonging to +M showed a higher alpha diversity than the samples belonging to −M.

PERMANOVA showed that the fungal traits (i.e., AM fungal root colonization, arbuscules, vesicles, richness, Shannon and Simpson indices in roots) were significantly affected by inoculation at both sites and the explained variance was 15 % and 78 % at the high- and low-fertility sites, respectively (Table S5). In the PCO biplot of the fungal traits in the high fertility-site, the inoculated and non-inoculated samples (+M and −M) were well separated along the second axis and the AM fungal abundance traits were more discriminant than the diversity indices (Fig. 3b). Furthermore, in the PCO biplot of the fungal traits at the low-

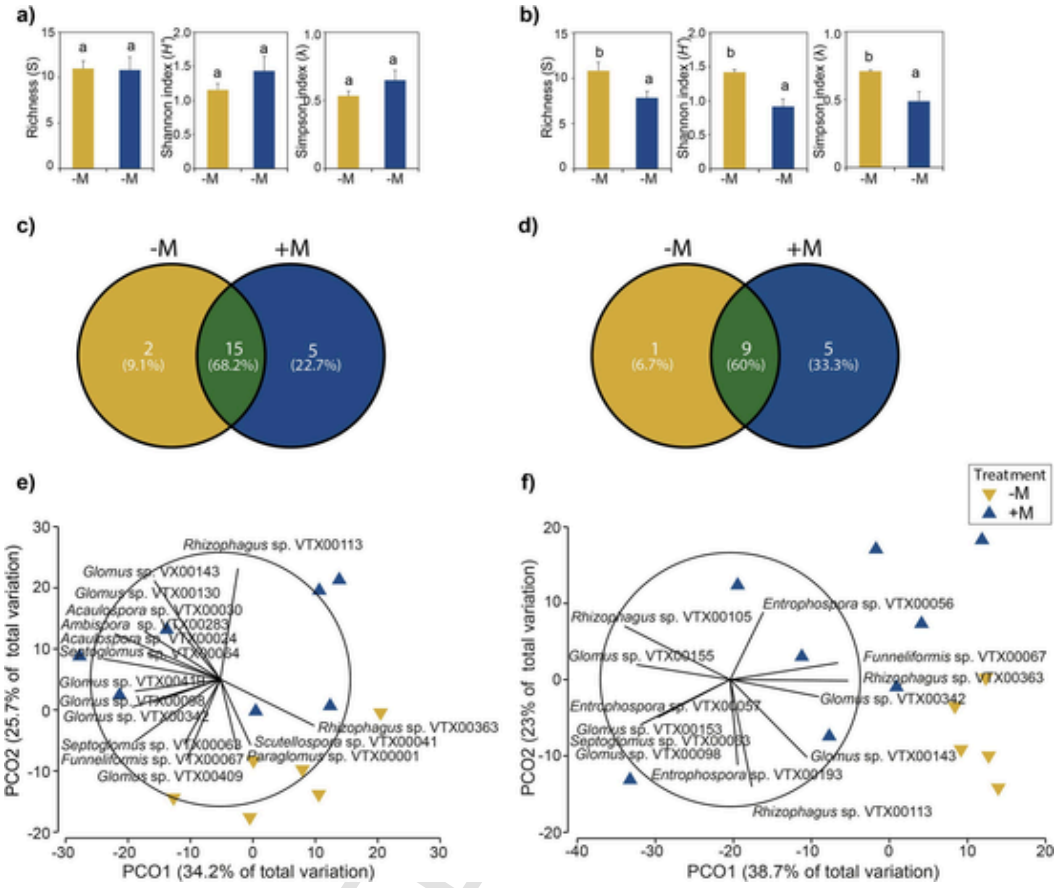


Fig. 2. Effect of arbuscular mycorrhizal fungal (AMF) inoculation on richness (S), Shannon (H') and Gini-Simpson ($\lambda = 1$) indices in roots of sunflower (*Helianthus annuus* L.) at high -fertility (a) and low-fertility (b) sites (High-fert and Low-fert, respectively). Figure reports results of one-way ANOVAs testing AMF inoculation as fixed factor (−M: mock inoculation, control; +M: inoculation by a local AMF consortium) and subreplicates as nested factor within the main factor (Table S3). Means ± SE of four replicates at High-fert and three at Low-fert. Up to three technical replicates per each replicate plot were analyzed (−M n = 6, +M n = 7 at High-fert; −M: n = 5, +M: n = 8 at Low-fert). Venn diagrams showing the number of AM fungal virtual taxa (VTX) retrieved in roots of sunflower (*Helianthus annuus* L.) unique to and shared among treatments −M and +M at High-fert (c) and Low-fert (d). Significant effect of AMF inoculation (−M and +M) on AM fungal community (VTX relative abundances) at High-fert (e) and Low-fert (f) (Table S4). Arbuscular mycorrhizal fungal VTX are reported. The community structures were visualized by Principal Coordinates Analysis (PCO). In each PCO biplot, we displayed the AM fungal VTX with a minimum correlation coefficient (r) of 0.60 with the ordination scores on each PCO axis.

fertility site, +M and −M were well separated along the first axis, and both AM fungal abundance and diversity played a major role in discriminating them.

3.3. Sunflower plant growth, yield, and nutrient uptake

At the high-fertility site, at the beginning of anthesis (R5.5 growth stage), AM fungal inoculation increased root and shoot dry weight (DW) (+65 % and +19 %, respectively) (Fig. 5a, b and Table S6). Similarly, at the low-fertility site, root and shoot DW were increased under inoculation by 22 % and 24 %, respectively (Fig. 5i, j and Table S6). At the high-fertility csite, at full physiological maturity, the root system radius, tap root length and head diameter were 22 %, 8 % and 6 % higher in +M than in −M (Fig. 3e, f, h), while plant height was not affected by inoculation (Fig. 5g and Table S6). Furthermore, inoculation did not affect grain yield (Fig. 5c and Table S6). At the low-fertility site, tap root length, plant height, and grain yield increased under inoculation by +23.5 %, +6.6 % and 16 %, respectively (Fig. 5k, n, o and Table S6).

In high soil fertility, at the beginning of anthesis (R5.5), inoculation did not affect the content of nutrients in sunflower shoots, except for P and K, which increased by 25 % and 21 % (Fig. S5a, b, Tables S7–S8). On the contrary, inoculation significantly increased the content of nutrients in grains (Fig. 6a–I and Table S9). In detail, N, P, K, Ca and Mg increased by 26 %, 19 %, 31 %, 103 % and 28 %, respectively (Fig. 6a–e), while Fe, Zn, Cu and Mn by 39 %, 21 %, 22 % and 24 %, respectively (Fig. 6f–i). Similarly, in low soil fertility, at R5.5, the nutrient content in shoots was not affected by inoculation, except for K, which increased by 139 % (Fig. S5c, Tables S7–S8). Furthemore, similar to the high-fertility site, inoculation increased the content of nutrients in grains, with the exception of Zn (Fig. 6j–r and Table S9). In detail, N, P, K, Ca, and Mg increased by 7 %, 9 %, 15 %, 33 % and 9 %, respectively (Fig. 6j–n). Additionally, inoculation increased the content of Fe, Cu and Mg by 10 %, 15 % and 14 %, respectively (Fig. 6o, q–r). Finally, oil yield was improved by inoculation at both sites, with a host benefit of 36 % and 20 %, respectively (Fig. 5d, l and Table S6).

PERMANOVA showed that plant functional parameters (i.e., plant growth, grain and oil yield, shoot and grain nutrient content) were significantly affected by inoculation in both soil fertility conditions and the explained variance was 26 % and 29 %, in the high- and low-fertility sites, respectively (Table S5). In high soil fertility, the inoculated and mock-inoculated samples (+M and −M) were well separated along the first axis in the PCO biplot and all parameters were promoted in +M, except for plant height (PH) (Fig. 3a). Furthermore, in low fertility conditions, +M and −M were well separated along the first axis of

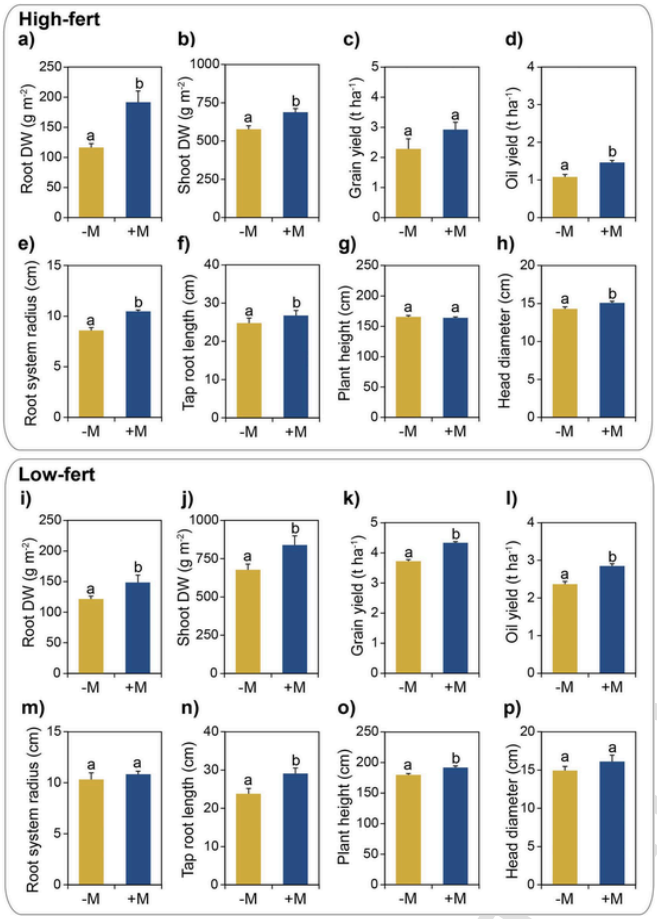


Fig. 3. Effect of arbuscular mycorrhizal fungal (AMF) inoculation on: root dry weight (DW) (a, i) and shoot DW (b, j) of sunflower (*Helianthus annuus* L.) at R5.5 growth stage (beginning of anthesis) at high- and low-fertility sites, respectively (High-fert and Low-fert, respectively); grain yield (c, k) and oil yield (d, l) in High-fert and Low-fert, respectively; root system radius (e, m), tap root length (f, n), plant height (g, o) and head diameter (h, p), at full maturity at High-fert and Low-fert, respectively. Figure reports results of one-way ANOVA testing AMF inoculation as fixed factor (−M: mock inoculation, control; +M: inoculation by a local AMF consortium) and subreplicates as nested factor within the main factor (Table S5). Means ± SE of four and three replicate plot per treatment at High-fert and Low-fert, respectively. Different letters represent significant differences between −M and +M, according to the Tukey-B test ($P \leq 0.05$).

the biplot, and all parameters were promoted in +M, except for Mn in shoots (Fig. 4a). Furthermore, PERMDISP analyses did not report differences in both sites (Table S5).

3.4. Relationship between AMF and sunflower agronomic performance

A the high-fertility site, the AM fungal traits were significantly related to plant functional parameters, as supported by the RELATE analysis ($\rho = 0.191$; $P = 0.05$) (data not shown). This relationship is detectable from Fig. 3b and a. Overall, arbuscules and AM fungal root colonization were positively and strongly correlated with all plant functional parameters. Furthermore, the BEST analysis highlighted that AM fungal root colonization, arbuscules and vesicles were the best predictors of sunflower agronomic performance (Figs. 3c and S6a), and the DistLM analysis supported that the AM fungal root colonization was the main determinant of this pattern (Fig. 3d). In low soil fertility, the AM fungal traits were significantly related to the plant functional parameters as supported by the RELATE analysis ($\rho = 0.470$; $P = 0.004$) (data not shown). This relationship can be seen by the patterns in PCO biplots (Fig. 4b and a). Overall, the traits of AM fungal abundance were positively correlated with all plant functional parameters, with the only exception of Mn in shoots that was positively correlated with the indices of diversity. Furthermore, the BEST analysis highlighted that vesicles and Simpson index (λ) were the best predictors of the agronomic sunflower performace (Figs. 4c and S6b), and the DistLM analysis supported the main role played by vesicles in determining this pattern (Fig. 4d).

In high soil fertility conditions, the RELATE analysis did not highlight a significant relationship between root AM fungal community structure and plant functional parameters (ρ: -0,088; $P = 0.712$) (data not shown). On the contrary, in low soil fertility, the RELATE analysis highlighted a significant relationship between root AM fungal community structure and plant functional parameters (ρ: 0.239; $P = 0.05$) (data not shown). Moreover, the BEST analysis highlighted that *Rhizophagus* sp. VTX00105, *Glomus* sp. VTX00098, *Septoglomus* sp. VTX00063, *Glomus* sp. VTX00153, and *Glomus* sp. VTX00143 were the best predictors of sunflower agronomic performance (Figs. 4e and S6c), and the DistLM analysis supported the main role played by the occurrence of *Rhizophagus* sp. VTX00105 in determining the pattern of plant functionality (Fig. 4f).

4. Discussion

4.1. Inoculation affects mycorrhizal abundance and diversity in sunflower roots

The mycorrhizal infection potential (MIP), often used as a proxy of soil biological fertility due to the beneficial roles played by AMF for soil health and crop productivity, was significantly higher at the high-fertility site than that at the low-fertility site. Generally, a higher MIP indicates an environment that supports more beneficial biological processes, leading to improved crop nutrient cycling and health. Therefore, our results suggest that the biological fertility of the soil aligns with its chemical fertility. These results are in disagreement with the meta-analysis of Han et al. (2020), which reported that under high soil fertility conditions due to N fertilization, the abundance of AMF in soil and roots is significantly reduced. Similarly, mycorrhizal abundance (i.e., AM fungal root colonization and spore density) was reported to decrease by 15 % under high soil N availability and by 32 % under high P availability (Treseder, 2004). However, in addition to soil chemical fertility, soil texture can also affect AM fungal abundance, with sandy texture supporting higher AM fungal development and propagation (Land and Schönbeck, 1991). According to this, the soil at the high-fertility site was sandy loam, thus likely more favorable for MIP.

Inoculation with the local consortium of AMF promoted AM fungal colonization and vesicles in roots of sunflower regardless the level of soil fertility, but the increase was more pronounced in the low-fertility site. This aligns with our hypothesis that low-fertility soils, which are less rich in native AMF, favor the “extra” root colonization following field inoculation. This is likely due to the lower initial colonization rates and reduced competition between the inoculated AMF and the locally occurring AMF. Similarly, Soleimanzadeh (2010), in a field study on the effects of inoculation with a mixture of AMF (i.e., *E. etunicata*, *F. mosseae*, and *Rhizophagus intraradices*) on sunflower at different levels of P, reported that AM fungal root colonization was promoted by the application of P. However, this effect decreased as the P rate increased. Gholamhoseini et al. (2013), studying the effect of field inoculation of sunflower with *F. mosseae* and *Glomus hoi* under drought stress, found that under very low soil mycorrhizal infectivity, root colonization rates in non-inoculated plots remained consistently low (5 %), regardless of the stress. However, in inoculated plants, similarly with our results, root colonization rates increased significantly, reaching up to 60 %.

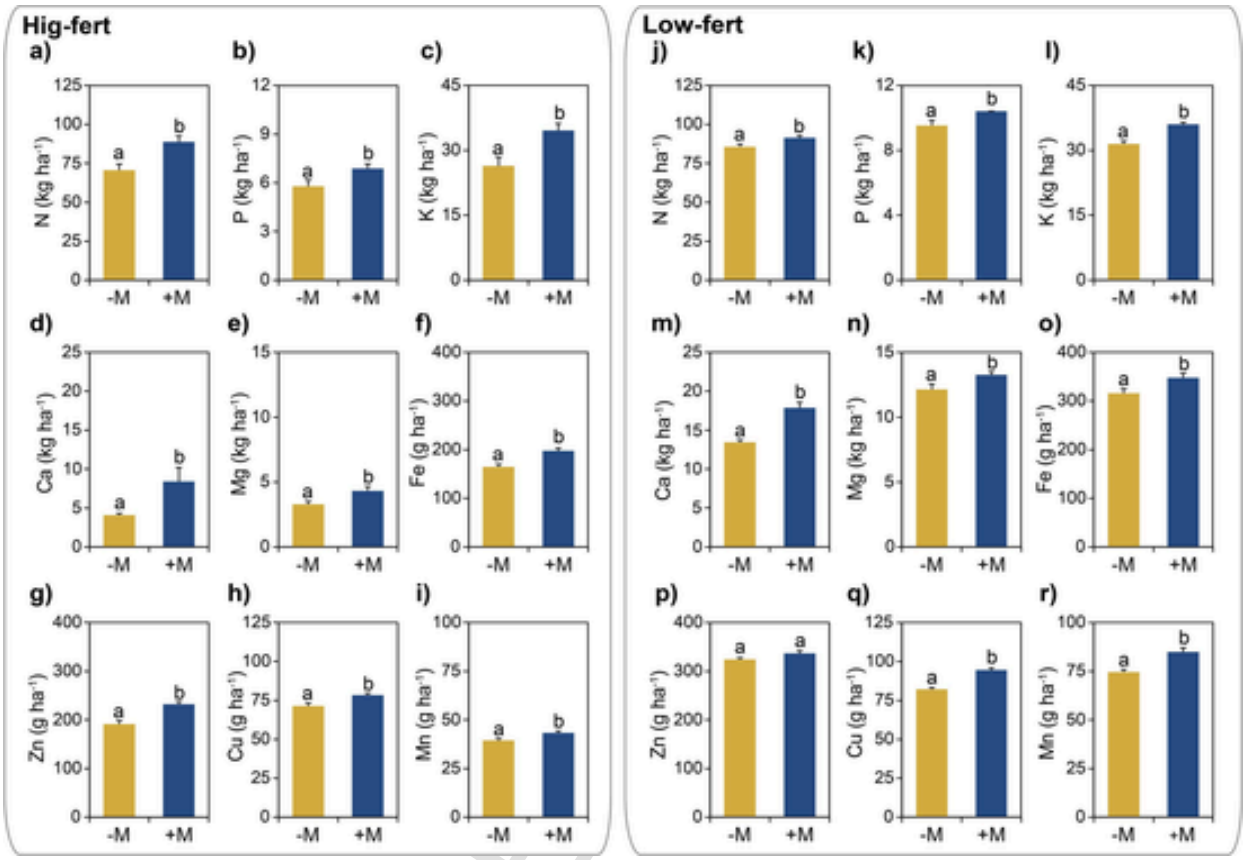


Fig. 4. Effect of arbuscular mycorrhizal fungal (AMF) inoculation on nutrient content in grain of sunflower (*Helianthus annuus* L.): N (a), P (b), K (c), Ca (d), Mg (e), Fe (f), Zn (g), Cu (h), Mn (i) at high-fertility site (High-fert), and N (j), P (k), K (l), Ca (m), Mg (n), Fe (o), Zn (p), Cu (q) and Mn (r) at low-fertility site (Low-fert). Figure reports results of one-way ANOVA testing AMF inoculation as fixed factor (−M: mock inoculation, control; +M: inoculation by a local AMF consortium) and subreplicates as nested factor within the main factor (Table S8). Means ± SE of four and three replicate plot per treatment at High-fert and Low-fert, respectively.

The high values of AM fungal root colonization found at the beginning of anthesis in both inoculated and non-inoculated plants in both years of cultivation pointed to a good responsiveness of the modern cv. Talento to the presence of AMF in the soil. Indeed, the AM fungal root colonization ranged from 72 % to 84 % at the high-soil-fertility site and from 60 % to 87 % at the low-fertility site, in control and inoculated plants, respectively. Our results are supported by the work of Leff et al. (2017) who reported, among all fungal endophytes, a great proportion of Glomeromycota in modern sunflower cultivars. In contrast, in the work of Soleimanzadeh (2010), AM fungal colonization of the modern cultivar Azargol was very low under not inoculated conditions (3 %) and reached a maximum of 22 % under AM fungal application. Furthermore, Gholamhoseini et al. (2013) found very low AM fungal root colonization rates in the modern cultivar Alestar under not inoculated conditions (4 %) along a gradient of drought stress.

The larger presence of vesicles we found, at the beginning of anthesis, in the roots of the inoculated sunflower compared to the control might be explained by the major occurrence of species able to form vesicles in the local AM fungal consortium used as field inoculant (https://i.invam.wvu.edu/the-fungi/species-descriptions.html). Indeed, the AM fungal genera that form vesicles within the roots, such as *Funneliformis*, *Diversispora*, *Glomus* and *Septoglomus*, were well represented in the inoculum, while the taxa belonging to Gigasporaceae that do not form vesicles were less abundant. Higher occurrence of vesicles in roots can be considered advantageous, as it was demonstrated that their presence in pieces of roots in crude inocula increases the inoculum potential, and that they can also act as source of inoculum per se (Biermann and Linderman, 1983). In addition, an increased number of vesicles may indicate that the fungus is actively foraging and also investing in these structures, and this is consistent with the concept of the mutualism-parasitism continuum, modulated by soil nutrient availability and plant C costs (Johnson et al., 1997).

The absence of strong changes in the AM fungal composition in inoculated and control plants is in line with our hypothesis that, using a local AM fungal consortium, few changes would have occurred in the mycorrhizal composition of roots. On the contrary, using the local AM fungal consortium under low fertility conditions, AM fungal diversity (i.e., S, H' and λ indices) in the roots of sunflower was reduced by inoculation, while under high fertility conditions no changes were detected. Thus, the observed reduction of diversity at the low-fertile site supports a consequent change in AM fungal communities and a higher susceptibility of the community to inoculation. This was expected, since the low mycorrhizal infection potential observed at the low-fertility site likely reflects a limited capacity of the native AM fungal community to effectively compete with the introduced inoculum, even though the inoculum itself consisted of native AM fungal taxa. Such competitive dynamics between native and introduced AM fungal communities have been well-documented, with studies highlighting that environmental condition, particularly soil fertility, strongly influence the competitive success and establishment of inoculated AMF (Johnson et al., 1997; Verbruggen et al., 2013). Moreover, low soil fertility has been shown to favor the colonization potential of inoculated AMF, as native communities in such environments often exhibit reduced infectivity and lower abundance (Lekberg and Koide, 2005). However, the community structures of AMF in sunflower roots were similarly modified by inoculation under low and high soil fertility conditions. This is evident from the

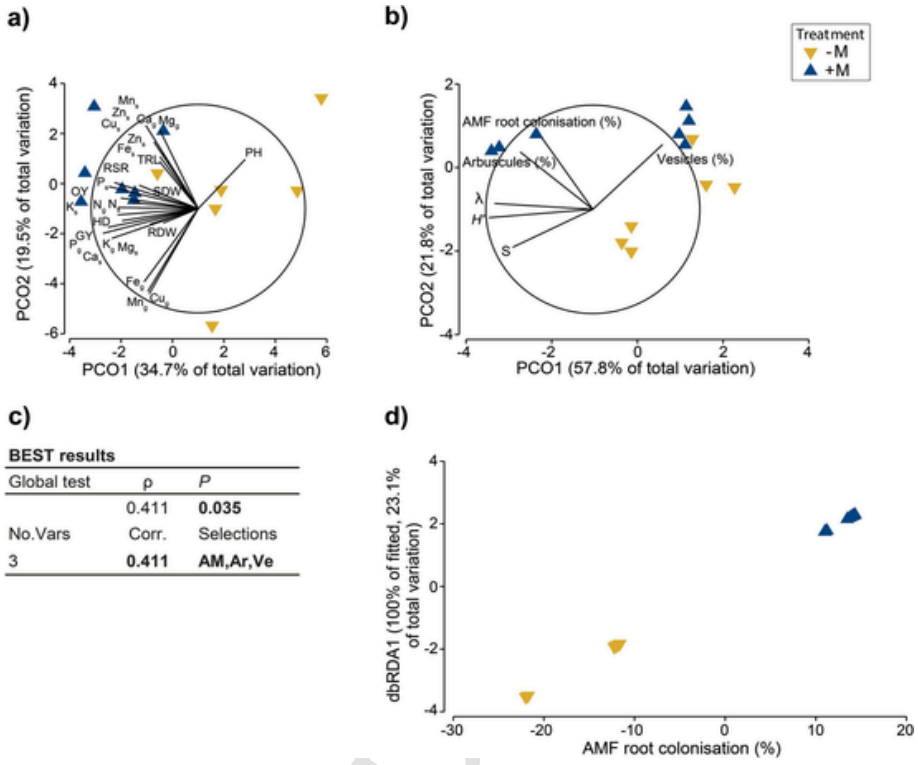


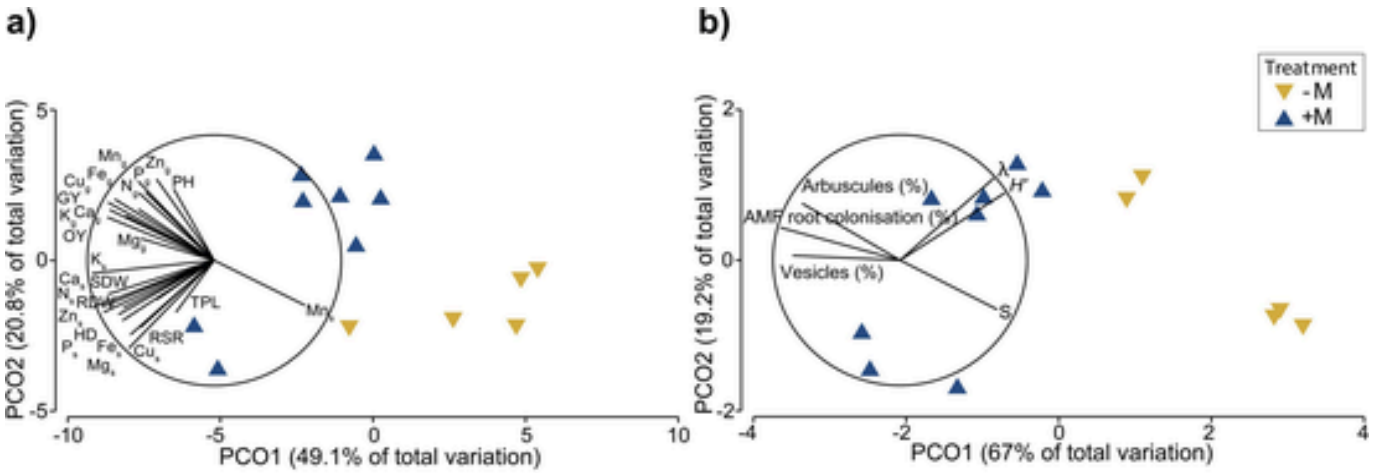
Fig. 5. Principal Coordinates Analysis (PCO) biplots on the significant effect of arbuscular mycorrhizal fungal (AMF) inoculation on plant functional traits (i.e., plant growth, grain and oil yield, shoot and grain nutrient contents) (a) and on fungal traits (i.e., AMF root colonization, arbuscules, vesicles, richness, Shannon and Simpson indices in roots) (b) of sunflower (*Helianthus annuus* L.) at high-fertility site (High-fert) 2019 (Table S9). Treatments are: −M (mock inoculation, control) and +M (inoculation by a local AMF consortium). Output of the BEST analysis used to find the best descriptor(s) of the relationships between fungal and plant functional traits at High-fert: global test and model showing the highest ρ (c). Distance-based redundancy analysis (dbRDA) plot used to visualize the first and second axes of the Distance-based linear method (DistLM) analysis applied to measure the significance and the variance explained by the best descriptor among the fungal traits at High-fert (d).

high variance explained by the factor inoculation in both sites and supports our hypothesis that field inoculation with a native consortium of AMF would have modified the community structure of AMF under both soil fertility conditions. However, the low fertile soil is more affected by inoculation through changes in both the abundance and diversity of AMF. On the contrary, the high fertile soil is more affected by inoculation through changes in AM fungal abundance. This partially disagrees with our original hypothesis. Previously, pre-inoculation of seedlings of oxeye daisy with single exotic AMF strongly influenced the AM fungal community assemblages within roots, although some differences were observed among AM fungal taxa (Mummey et al., 2009). In agreement, inoculation with an exotic isolate of *R. irregularis* of a legume and a grass species suppressed root colonization of native AMF and modified the community structure of AMF in the host roots (Janoušková et al., 2017). Additionally, in microcosms under varying soil P availability and abundance of native AMF, inoculation with an exotic taxon (i.e., *R. irregularis*) increased its abundance in roots and modified native communities (Köhl et al., 2016). By contrast, in a recent study on the inoculation of corn, wheat, and soybean with *R. irregularis* DAOM 197198, no effect was detected on the relative abundance of *R. irregularis*, and neither on the AM fungal alpha-diversity, community composition, and structure (Renaut et al., 2020). These authors suggested that the lack of effect could be due to the fact that this species was already naturally present in significant amounts in the experimental fields. Furthermore, in field sites under similar climatic conditions, inoculants composed of exotic single and multiple species AMF persisted for up to two years and significantly modified the local AM fungal communities (Pellegrino et al., 2012, 2022). However, under inoculation with a local AM fungal consortium, fewer changes in the native AM fungal communities were recorded (Pellegrino et al., 2022), supporting its lower environmental impact.

4.2. Inoculation enhances sunflower plant growth, yield, and nutrient uptake

In this work, field inoculation with the local AM fungal consortium was highly effective in promoting sunflower productivity. Although some experimental works demonstrated that foreign AMF can outperform local strains (Pellegrino et al., 2011, 2022), others demonstrated that field-sourced native AMF, ecologically and genetically more adapted to local environments, performed better (Frew, 2020; Jerbi et al., 2022; Klironomos, 2003; Pellegrino and Bedini, 2014). In this context, questions have been raised about potential AM fungal invasion and, if any, about the positive or negative consequences (Hart et al., 2018). Therefore, strategies have been suggested that reduce overdependence on introduced inoculants, such as the adoption of management practices that promote the richness and functional diversity of native AMF and the development of propagules (Basiru and Hijri, 2022).

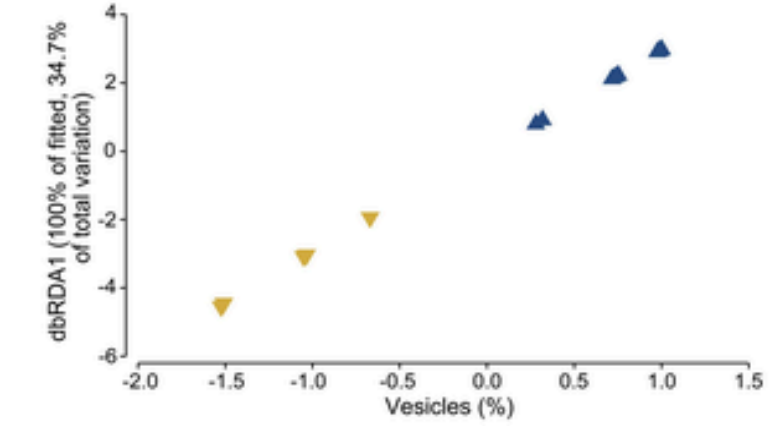
Our results showed that in low soil fertility conditions, the response to inoculation in terms of AM fungal root colonization was greater, while the magnitude of benefits on sunflower growth parameters and yield was variable between sites. At the high-fertility site, unfavorable climatic conditions (higher temperature and lower rainfall) resulted in a drought stress, but the development of AMF in roots in inoculated treatments supported the benefits in terms of root dry weight. This is in agreement with previous results on the positive effect of AM fungal application under water stress conditions on crop growth in terms of root development (+20 %) (Jayne and Quigley, 2014). On the contrary, at



c)

BEST results

Global test	ρ	*P*
	0.626	**0.002**
No.Vars	Corr.	Selections
2	**0.626**	**Ve,λ**



e)

BEST results

Global test	ρ	*P*
	0.564	**0.018**
No.Vars	Corr.	Selections
5	**0.564**	**VTX00105,VTX0009,VTX00063, VTX00153,VTX0014**

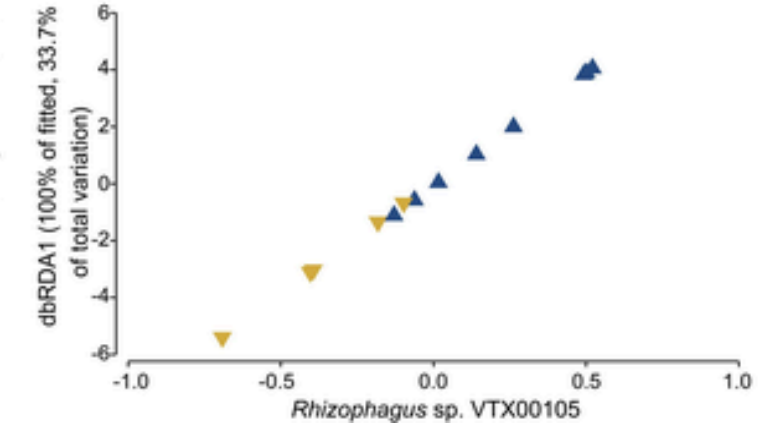


Fig. 6. Principal Coordinates Analysis (PCO) biplots on the significant effect of arbuscular mycorrhizal fungal (AMF) inoculation on plant functional traits (i.e., plant growth, grain and oil yield, shoot and grain nutrient contents) (a) and on fungal traits (i.e., AMF root colonization, arbuscules, vesicles, richness, Shannon and Simpson indices in roots) (b) of sunflower (*Helianthus annuus* L.) at low-fertility site (Low-fert) (Table S9). Treatments are: −M (mock inoculation, control) and +M (inoculation by a local AMF consortium). Output of the BEST analysis used to find the best descriptor(s) of the relationships between fungal and plant functional traits at Low-fert 2020: global test and model showing the highest ρ (c). Distance-based redundancy analysis (dbRDA) plot used to visualize the first and second axes of the Distance-based linear method (DistLM) analysis applied to measure the significance and the variance explained by the best descriptor among the fungal traits at Low-fert 2020 (d). Output of the BEST analysis used to find the best descriptor(s) of the significant relationships between AM fungal community structure and plant functional traits at Low-fert: global test and model showing the highest ρ (e). The dbRDA plot used to visualize the first and second axes of the DistLM analysis applied to measure the significance and the variance explained by the best descriptor among the AM fungal virtual taxa at Low-fert (f).

the low-fertility site, a larger increase in AM fungal root colonization was observed following inoculation, but the benefit in root dry weight was smaller. Furthermore, in high soil fertility conditions, the prolongation of drought during grain filling did not allow full yield potential to be reached and consequently grain yield was low and not affected by inoculation, although most of plant growth parameters, such as head diameter, root system radius, and root system depth, were positively related to inoculation. Furthermore, the absence of a response in grain yield in the high-fertility site could be explained by the sandy soil texture that promoted, during the plant growth cycle, a higher development of infective AM fungal propagules that supports the growth of intraradical AMF and increased nutrient uptake by plants in both inoculated and non-inoculated conditions (Lekberg and Koide, 2005; Pellegrino et al., 2015, 2020). On the contrary, in the low-fertility site, the mycorrhizal benefit in terms of grain yield was supported by the high AM fungal root colonization in inoculated plants, and by lower presence of native infective propagules also during the plant growth cycle likely related to the less favorable soil texture. The benefits we observed were similar to those detected in field conditions on three sunflower cultivars treated with a single exotic AM fungal taxa for two years of cultivation (from 7 % to 26 % depending on the strains) (Noroozi et al., 2023). In other studies of field inoculation with single exotic strains (Gholamhoseini et al., 2013; Soleimanzadeh, 2010), similar benefits were recorded in grain yield of modern sunflower cultivars, although lower increases were observed at high soil P availability and a variable response was detected between years of cultivation and AM fungal strains. However, in the study of Noroozi et al. (2023), a greater effect of the inoculation was reported under drought stress conditions, whereas Heidari and Karami (2014) found no mycorrhizal benefit in grain yield under a range of water stress.

The benefits observed in the grain nutrient content at both sites are in agreement with the observed general pattern of response in leaves and fruits (i.e., P, Zn, Fe, Ca, Mg, Cu, K and N) (Arcidiacono et al., 2024; Lehmann et al., 2014; Lehmann and Rillig, 2015; Watts-Williams and Gilbert, 2021; Yang et al., 2023). Specifically, in sunflower, field inoculation with single exotic strains promoted P uptake in shoots (Chandrashekara et al., 1995; Soleimanzadeh, 2010). Under well-water conditions, the benefits ranged from 23 % to 85 %, while under drought stress, they ranged from 23 % to 91 %. Furthermore, at low availability of P, benefits reached 72 % at anthesis and 43 % at crop maturity, and strongly decreased under high soil P availability (Chandrashekara et al., 1995). Furthermore, mycorrhizal N benefits were previously observed on sunflower grains (up to 12 %) and leaves (up to 20 %) (Gholamhoseini et al., 2013). Accordingly, in a field experiment under semi-arid conditions shoot N, P, K and Mg uptake was promoted in sunflower inoculated with a single exotic strain (Langeroodi et al., 2021). It is interesting to note that in our work, K content was increased by inoculation in shoots and grain in both soil fertility conditions. In this context, it is noteworthy that K plays a pivotal role in plant metabolism by activating a variety of enzymes (Evelin et al., 2009). Additionally, K is crucial for regulating stomatal movements and facilitating protein synthesis. Consistent with our findings, field AM fungal inoculation significantly increased K absorption in sunflower seeds up to 18 % (Gholamhoseini et al., 2013).

In both soil fertility conditions, inoculation increased oil yield. Our findings are in agreement with the results of Heidari and Karami (2014), who reported that the application of two single exotic strains in the field increased the amount of oil content in sunflower grain up to 6 %. Accordingly, other researches showed that field inoculation of sunflower with single exotic AM fungal strains improved oil yield from 11 % to 36 % (Gholamhoseini et al., 2013; Langeroodi et al., 2021; Soleimanzadeh, 2010).

The multivariate analysis on the effect of the local AM fungal consortium allowed to point out that the changes in the agronomic performance of sunflower were consistent in both soil fertility conditions, suggesting that AMF can contribute to food nutrition by boosting not only crop yield but also the qualitative traits such as grain nutrients. The strength and robustness of our findings could have been further enhanced by conducting both experiments within the same year to minimize temporal variability or by replicating the experiments across multiple years to account for potential environmental fluctuations. However, this limitation was primarily due to constraints in the quantity of crude inoculum that could be produced annually and the availability of field space. Therefore, AMF can be considered effective agronomic tools for the biofortification of crops.

4.3. Relationship between AMF and sunflower agronomic performance

Our results highlighted that AM fungal traits (i.e., abundance and diversity indices) at anthesis were associated with sunflower agronomic performance in high and low soil fertility conditions. However, the magnitude of the relationship was higher in the low fertility soil. The best predictor in the high fertility soil was the percentage of AM fungal root colonization, while in the low fertile soil the best predictor was the percentage of vesicles. The association between increased AM fungal root colonization and increased sunflower growth is consistent with the work of Treseder (2013), in which a higher percentage of root length colonized by AMF was associated with greater plant growth and P uptake in various functional groups of plants, grown under laboratory and field conditions. Similarly, other meta-analyses found positive relationships between changes in AM fungal colonization and the yield of numerous crops under field conditions (Lekberg and Koide, 2005; McGonigle, 1988; Pellegrino et al., 2015). What is not clear is why vesicles are the best predictors of sunflower agronomic performance under low soil fertility conditions. Indeed, the function of these mycorrhizal structures has been little studied and is still uncertain. Vesicles are generally regarded as storage structures for lipids and other essential nutrients (Gerdemann, 1968; Mosse, 1973), and there are studies reporting that they can concentrate K, Fe, Mn, Cu, and some heavy metals, suggesting that they could reduce metal toxicity in the host (Weiersbye et al., 1999; Whitfield et al., 2004). However, the higher presence of vesicles in roots suggests a change in the composition of taxa toward a higher presence of species producing many vesicles. Therefore, we can assume that the pattern of functional traits observed in sunflower in low soil fertility conditions is likely due to the identity of the AM fungal species developing in the roots, indirectly reflected by the different development of vesicles between inoculated plants and control. In addition, vesicles can be regarded as indicators of a well-established symbiosis, as the stored lipids may be utilized by the fungus under adverse environmental conditions (Graham, 2000). This idea is further supported by the vesicle-to- arbuscule ratio, which is higher in inoculated and high soil fertility conditions (−M: 0.21, +M: 0.55 in 2019; −M: 0.05, +M: 0.21 in 2020). However, we cannot demonstrate a causal relationship between vesicles and plant functional traits, as vesicles may be associated with other microbial traits responsible for host benefits.

Furthermore, our results highlighted that the AM fungal root community structure was associated with the agronomic performance of sunflower only under low soil fertility conditions. Therefore, under low soil fertility, both the occurrence of vesicles and diversity of AMF can be considered good predictors of the agronomic performance of sunflower, and this was confirmed by the magnitudes of the relationships. However, the fact that under high soil fertility conditions, the AM fungal root colonization and not the structure of the community was associated with host benefit, is not consistent with our original hypothesis. Therefore, in soils where mycorrhizal propagules are abundant, the effect of locally sourced inoculants on AM fungal development in roots had a greater impact on the agronomic productivity than shifts in the AM fungal communities. The fact that the AM fungal community structure is not a predictor of host benefits under high soil fertility suggests that the plant does not favor the AM fungal species providing greater

benefits, and that the communities in roots are mainly determined by the presence of fast colonizer species, which are likely less effective in providing host benefits. Consistent with this concept, Johnson (1993) found that AMF from fertilized soil exerted a higher net C cost on their host compared to AMF from unfertilized soil, implying that high nutrient status in soils selects AMF that could be inferior symbionts. Similarly, the high presence of native AMF at the high-fertility site (i.e., high mycorrhizal potential of the soil) could have determined a redirection of resources to competitive interactions after inoculation (Janoušková et al., 2013).

In contrast, in low fertility conditions, the occurrence of *Rhizophagus* sp. VTX00105 in the roots of sunflower was the best predictor of crop functional traits. *Rhizophagus* sp. VTX00105 was recovered in both the roots of inoculated and control plants. However, inoculation with the AM fungal consortium increased around three times the relative abundance of this native taxon, ultimately affecting the outcome of the symbiosis. This is in agreement with recent findings obtained by relating the AM fungal assemblages within alfalfa roots and the corresponding plant traits (Pellegrino et al., 2022). In this work, the change in abundance of a local isolate of *R. irregularis* induced by all exotic and local inoculants was sufficient to describe the agronomic performance of alfalfa. Moreover, recently, it was demonstrated that in tomato var. Rio Grande inoculated with single-species exotic inocula, some native taxa (i.e., *R. fasciculatus*, *Rhizophagus* sp. and *Archeospora* sp.) were the best predictors of the pattern of fruit quality (Pellegrino et al., 2024). Therefore, the improved host benefits were associated with increases in the roots of the abundance of some native AM fungal taxa highly competitive with the inoculated AMF.

The relationship between the AM fungal community in roots and the agronomic performance of sunflower at the low-fertility site could be thus highly related to the low soil mycorrhizal potential. Indeed, a low occurrence of native AMF in soil indicates the availability of more unoccupied niches (Verbruggen et al., 2013) and, therefore, a possible lower competition between inoculants and native AMF. In our study, the low presence of native AMF, together with the low availability of nutrients in soil, could have determined optimal conditions for the best functioning of the symbiosis with multiple beneficial effects for the host, in line with our original hypothesis. However, our results at the high-fertility site prove that inoculation with a local AM fungal consortium can still be beneficial, even under conditions typical of intensive agricultural systems.

5. Conclusions

Improving the reliability and responses of AM fungal inoculants in crops is a pressing necessity due to recent increases in the price of synthetic fertilizers and environmental concerns related to their application. The results of this study showed that the use of local AMF as mixed inoculants can be very effective in improving sunflower grain and oil yield, as well as seed nutritional value. Additionally, multivariate analyses on the AM fungal diversity within sunflower roots confirmed our hypothesis that the use of an AM fungal inoculum composed of native species affects AM fungal abundance (i.e., root colonization, vesicles) and community structure, but not mycorrhizal composition. However, the mechanisms behind the functioning of the field inoculum on crop performance are context-dependent. Under low soil fertility conditions, both root AM fungal abundance and community changes affected sunflower productivity, while under high soil fertility only the AM fungal abundance was predictive for crop productivity. Therefore, the use of local AM fungal consortia produced on farm with mycotrophic plants species could represent a convenient alternative to commercial inocula, offering important economic and ecological advantages for sustainable cropping systems.

CRediT authorship contribution statement

Myriam Arcidiacono: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Laura Ercoli:** Writing – review & editing, Supervision, Funding acquisition. **Gaia Piazza:** Data curation. **Elisa Pellegrino:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization, Investigation, Validation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Molecular data are deposited in NCBI Sequence Read (SRA) database as SUB14264070 (accession numbers from PP378456 to PP378477) and SUB14264075 (accession numbers from PP378490 to PP378504). All other data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105830.

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