



Positive response to inoculation with indigenous arbuscular mycorrhizal fungi as modulated by barley genotype

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Abstract

Climate change-driven extreme events are reducing barley productivity. The high use of mineral fertilizers, combined with low nutrient use efficiency, leads to environmental and economic concerns. Indigenous arbuscular mycorrhizal fungi (AMF) inoculants offer a sustainable alternative, especially in intensive farming systems where AM colonization and diversity are low. However, poor adaptation to local conditions limits inoculant success. Few studies have tested indigenous AMF inoculated on field crops, with limited research on barley. No research has yet explored how barley genotype and environment modulate field inoculation outcomes in terms of crop productivity. Key factors such as AM fungal abundance and community structure shifts remain unidentified. This study evaluated the agroecological effects of an indigenous AM fungal consortium on three barley varieties (Atlante, Atomo, and Concerto) over 2 years. In 2020, Atomo and Concerto responded positively to inoculation in terms of root colonization, with grain yield increases of 64% and 37%, respectively. In 2021, only Concerto showed enhanced root colonization, while grain yield increased by 78% in Concerto and 134% in Atlante. Multivariate analysis revealed a strong impact of environment on barley productivity, with a significant third-order interaction among AMF, genotype, and environment. Inoculation slightly altered AM composition but strongly influenced community structure, particularly at different plant growth stages. Root colonization was strongly correlated with barley productivity, with root length containing arbuscules being the best predictor. Changes in the AM community structure, rather than composition, drove barley response, with *Glomus* and *Septoglomus*, present in the inoculum, being main players. These findings support the use of indigenous AMF for sustainable biofertilization and highlight the importance of selecting genotypes with a stable AM response across environments. Our results disclose for the first time the role of barley genotype and plant growth stage on AM host preference with and without indigenous AM fungal inoculants.

Keywords Native arbuscular mycorrhizal fungi · Barley genotypes · Barley nutrient uptake · Mycorrhizal yield benefit · Molecular diversity · Field inoculation

1 Introduction

Barley (*Hordeum vulgare* L.) production has increased steadily for over 30 years, but it should improve by 50% or even more by 2050 to ensure global food security (Fischer et al. 2014). However, the high intensity and frequency of extreme events due to climate change have severely affected crop production (Mall et al. 2017; Meza et al. 2020; Trenberth et al. 2014). In addition, a decline of mineral concentration in grain under rising CO₂ has been reported (Gojon et al. 2023).

Moreover, the high use of mineral fertilizers in combination with low crop nutrient use efficiency results in severe environmental issues and economic losses for farmers (Chien et al. 2009; Mclaughlin et al. 1996). These issues can be addressed either through the implementation of management practices (Mhlanga et al. 2021; Pittelkow et al. 2015; Sharma et al. 2021) or by plant breeding (Lammerts van Bueren and Struik 2017). In such context, microbial inoculants could represent an efficient tool to support barley productivity, especially under abiotic stresses, and to reduce the use of mineral fertilizers (Larimer et al. 2010; Philippot et al. 2013; Schütz et al. 2018; Veresoglou and Menexes 2010).

Among beneficial microbes, arbuscular mycorrhizal fungi (AMF) (Glomeromycota; Tedersoo et al. 2018) have been reported to improve in field conditions wheat grain yield by

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20% (Pellegrino et al. 2015). This was further confirmed by Zhang et al. (2019) who estimated an increase of 16%. Mixed-species AM fungal inocula produced higher grain yield than single-species inocula, and the highest AM fungal contribution to grain yield was observed in cereals grown in soils with low N and P availability, as well as in sandy soils (Zhang et al. 2019). Wheat grain and straw P concentration were promoted, as well as grain N content and Zn concentration (Pellegrino et al. 2015).

Although barley is considered non-responsive to AMF (Grace 2008), colonization under controlled and field conditions has been consistently reported (e.g., Clarke and Mosse 1981; Jensen 1982; Masrahi et al. 2023; Powell et al. 1980). A neutral effect of AMF on yield was found by Zhang et al. (2019), but the authors based this result only on three experiments and highlighted the need for more studies. In field conditions, inoculation with *Gigaspora margarita* promoted plant barley growth by 92% (Powell et al. 1980). Under low P soil availability, ear fresh weight was doubled, irrespective of inoculated AM fungal species (Clarke and Mosse 1981). Inoculants composed of three exotic AM fungal species increased grain yield and P uptake by 27% and 35%, respectively, while no effect was reported with native AM fungal mixtures (Powell et al. 1981). By contrast, no changes were reported in grain yield and P uptake in barley inoculated with *Funneliformis mosseae* (Khaliq and Sanders 2000). Field inoculation with *F. mosseae* in not fumigated plots resulted in significant depressions of grain and straw yield (−20%) (Khaliq and Sanders 1998). Therefore, under field conditions, the outcome of the AM fungal inoculation has been reported to be variable. This can be due to several factors, such as the abundance of infective propagules in soil, plant-fungal compatibility, composition of the AM fungal inocula, availability of soil nutrients, and climatic parameters (Verbruggen et al. 2013).

Intensive agriculture practices, such as high P or N fertilization, plowing, and continuous monoculture, have been shown to negatively impact the abundance in terms of colonization, diversity, and functionality of AMF in soil (Peng et al. 2024; Verbruggen and Kiers 2010; Verbruggen et al. 2010). Intensified land use has altered the composition of AM fungal communities, leading to dominance by few taxa within the Glomerales order, which have limited functionality for crops (Oehl et al. 2010; Pellegrino et al. 2014). Therefore, it is evident that in soils with low biological fertility (Lal 2006; Nannipieri et al. 2003), modifying the abundance in terms of colonization and diversity of AMF through field application of AM fungal inocula, containing viable propagules, is crucial to maximizing benefits for crops (García de León et al. 2020; Kokkoris et al. 2019, 2020; Koziol et al. 2024; Salomon et al. 2022a, b; Vahter et al. 2023; Yang et al. 2014).

The variability in plant benefits received from AMF has been explained by the identity of plant host and fungus

(Klironomos 2003; Maherali and Klironomos 2007; Mensah et al. 2015; Munkvold et al. 2004), as well as by the type of inoculum (commercial *versus* laboratory grown fungi) (Koziol et al. 2024). Large differences were observed among genotypes/cultivars of wheat (Hetrick et al. 1993, 1996). By contrast, barley has been less investigated. Al Mutairi et al. (2020), testing the effect of inoculation of *Rhizophagus irregularis* in five barley cultivars, observed that genotype was a very strong driver for biomass, yield, and yield components. Therefore, the study of barley intraspecific response to AMF is of great importance. Moreover, since the interaction between cereals and AMF is modulated by soil and climatic factors (Grey 1991; Jerbi et al. 2020; Marrassini et al. 2024; Pellegrino et al. 2015) and by the season (Bertruti et al. 2018), there is the need to carry out field studies.

Commonly, commercial AM fungal inocula are composed of generalist single or few exotic AM species, having low genetic variability and not always offering efficiency and stability when applied (Salomon et al. 2022a, b). In a pot experiment, some commercial inoculants failed to form mycorrhizal associations, which may be caused by low adaptation to local edaphic conditions (Schreiner 2007). Moreover, since AMF are usually regarded as generalists and mutualists, there has been little concern regarding the potential negative consequences of their introduction. Nevertheless, the evidence that mycorrhizal function can range from mutualistic to parasitic (Johnson et al. 1997; Jones and Smith 2004; Klironomos 2003) led to take into account the potential agroecological concerns of exotic AM fungal introduction (Schwartz et al. 2006). Several studies were successfully carried out in the field with indigenous AMF, inoculated as single or mixture, in many field crops, and indigenous AMF were often reported to be more beneficial and less agroecologically harmful than exotic strains (Jansa et al. 2008; Oliveira et al. 2005; Pellegrino et al. 2011; Pellegrino and Bedini 2014). Recently, field inoculation of an indigenous AM fungal consortium did not modify the AM fungal composition in sunflower roots but increased root colonization and modified the structure of the root AM fungal community (Arcidiacono et al. 2024a). These modifications were associated with improvements in sunflower grain and oil yield, as well as seed nutritional value. Similarly, field inoculation of the same indigenous AMF in five wheat genotypes did not change the AM fungal community composition across the studied varieties, but it modified community structure over the plant growth cycle. This suggests that, under inoculation with indigenous AMF, host plant preference is more influenced by changes in structure than by composition (Marrassini et al. 2024). Furthermore, the changes in AM fungal structure and root colonization were significantly associated with increased wheat productivity. So far, no similar experiments were carried out on barley.

Therefore, in this work, we inoculated an indigenous AM fungal consortium on three barley varieties for 2 years

of cultivation to dissect the effect of the interaction among AMF, genotype, and environment on barley productivity (Fig. 1), removing the effect of potential changes in AM fungal composition previously found under inoculation with exotic AMF (Pellegrino et al. 2022). In the present work, the benefit of AM fungal inoculation was evaluated by assessing grain yield and nutrient concentration as described in Fig. S1. Abundance (i.e., AM fungal root colonization, arbuscules, vesicles) was evaluated by morphological tools, whereas the community composition and structure of AMF in roots (identity and relative abundance of the AM fungal taxa, respectively) were assessed using molecular tools. The indigenous inoculum was composed of many fungal species, isolated from soil located in the same agricultural area where the experiment was carried out. We tested the following hypotheses: (i) barley genotype exerts greater control over the response of the plant to AMF than the environment; (ii) changes of community structure but not composition by inoculation of indigenous AMF drive AM preferences of plant genotypes; (iii) plant growth stage has a role in modulating the preference of barley genotypes; (iv) increases in AM fungal colonization and changes in community structure and not in composition determine barley productivity.

2 Materials and methods

2.1 Fungal and plant material

The AMF used as an inoculant were a consortium of taxa originating from a local field. The AM fungal inoculant was composed of 14 species belonging to five families: *Acaulospora cavernata*, *Acaulospora spinosa*, *Acaulospora* sp., *Diversispora spurca*, *Funneliformis coronatum*, *Entrophospora etunicata* (syn. *Claroideoglossum etunicatum*),

Funneliformis geosporum, *Funneliformis mosseae*, *Glomus* sp., *Rhizophagus clarus*, *Rhizophagus irregularis*, *Scutellospora aurigloba*, *Scutellospora calospora*, and *Septoglossum viscosum*. Three barley varieties were tested: Atlante (six-row barley, intermediate growth habit), Atomo (two-row barley, winter growth habit), and Concerto (two-row barley, spring growth habit) (Limagrains Italia SpA, Fidenza, Parma, Italy). For details about barley varieties, see Table S1.

2.2 Experimental field site

The experiment was carried out in 2020 and 2021 at the “Società Cooperativa Rinnovamento Agricolo,” Santa Luce, Pisa, Tuscany (43° 26' 24"N–10° 29' 48"E; 37 m above sea level) in two adjacent fields. The soil of 2020 and 2021 showed differences in texture (clay loam and silty clay loam, respectively), but similar low nutrient availability (Table S2 and Supplementary Materials and Methods 1). We utilized adjacent fields for 2 consecutive years of experimentation to avoid the residual effect of the AM fungal inoculation. In addition, we followed the principle of crop rotation. Indeed, in sustainable agricultural systems, and especially in organic systems, the practice of avoiding sowing the same crop in the same field prevents soil fertility depletion and plant diseases (Berzsenyi et al. 2000; Congreves et al. 2015).

The climate of the site is cold and humid Mediterranean (Csa), according to the Köppen-Geiger climate classification (Kottek et al. 2006) with a 5-year average annual precipitation of 1033 mm and a 10-year average of annual maximum and minimum daily air temperature of 20.4 °C and 10.8 °C, respectively. During the barley cropping cycle in 2020 (January–July), mean maximum and minimum temperatures and total precipitation were 20.4 °C, 10.2 °C, and 373 mm, while in 2021 (March–July), 22.7 °C, 11.7 °C, and 170 mm, respectively (Fig. 2).

Fig. 1 Field experiment testing the effect of inoculation with arbuscular mycorrhizal fungi (AMF) (with AMF: +M; not-inoculated/control: -M) on three barley genotypes. Photo: Andrea Massini (La Pivota, Florence)



2.3 Experimental setup and sampling

A complete factorial experimental design with three factors was adopted with 2 years of cultivation (2020 and 2021), three barley varieties, and two AM fungal inoculation treatments (inoculated with the AM fungal consortium, +M; not-inoculated/control, -M) (Fig. 1). Hereafter, plants grown in inoculated soil are called inoculated plants. The experiment was arranged in a completely randomized design with three replicate plots (8 m × 42 m = 336 m² in 2020; 8 m × 20 m = 160 m² in 2021). The inoculum, produced as described by Pellegrino and Bedini (2014), was a micronized mixture of mycorrhizal roots of sorghum (*Sorghum halepense* L.), spores, hyphal fragments, and bentonite as a carrier. The inoculum was applied in 2020 and 2021 at sowing by manually coating seeds that had been previously moistened with water. The rate of the AM fungal inoculum was 0.8 g m² (8 kg ha⁻¹). The infectivity of the applied inoculum, measured by the mycorrhizal infection potential test (Pellegrino et al. 2011), was similar between years, and the soil samples collected in each study site did not show a large variability among replicates (data not shown). The mock inoculum (not-inoculated/control) consisted of the same dose of steam-sterilized AM fungal inoculum (121 °C for 25 min on 2 consecutive days). To ensure a common microflora, both inocula received 0.05 L kg⁻¹ of a filtrate obtained by filtering through a Whatman no. 1 filter paper the AM fungal consortium. The seed rate was 200 kg ha⁻¹, corresponding to approximately 350 viable seeds per m², distributed in rows 14 cm apart. Barley was seeded with a pneumatic seeding machine (Aguirre Bota) on 17 January 2020 and 1 March 2021. Before the experimental setup, the preceding crop was clover (*Trifolium alexandrinum* L.) in 2020 and faba bean (*Vicia faba* L. var. minor) in 2021. However, the long-term rotation sequence involved clover or faba bean, and the cereal. Soil tillage was carried out in autumn by moldboard plowing at a soil depth of 30 cm, and by harrowing at a soil depth of 15 cm, immediately before seeding. No organic/chemical fertilizer was applied. No weed and pest/pathogen

control treatments were applied. In both years of cultivation at the plant growth stage of four leaves unfolded (GS14) and at the physiological maturity (GS90) (Zadoks et al. 1974), ten plants, randomly selected in each replicate plot, were excavated with their root system to determine AM fungal abundance in terms of colonization and diversity. Barley was harvested on 22 July 2020 and 26 July 2021 in each replicate plot by a combine harvester (Laverda, Vicenza, Italy). Furthermore, in 2021, at GS14, shoots from the ten plants were also sampled.

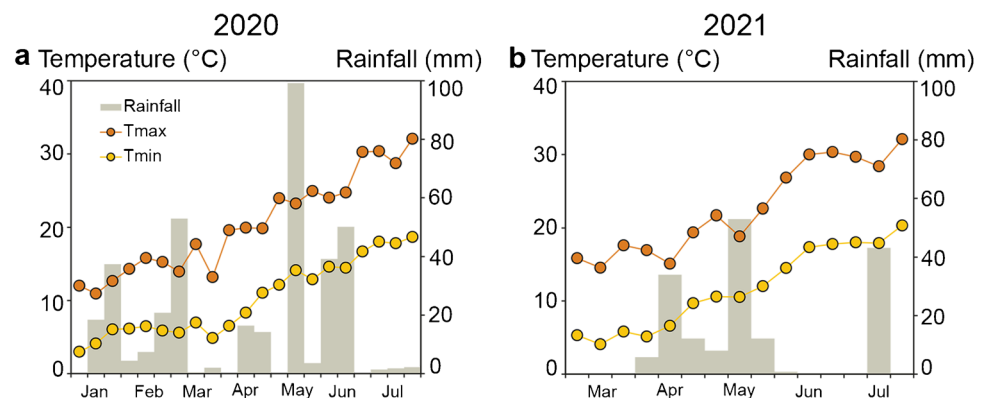
2.4 Mycorrhizal colonization traits in barley roots

At each sampling (GS14 and GS90 in both years of cultivation), fresh roots from each replicate plot were combined and cleaned from the attached soil by soft washing with tap water. Mycorrhizal colonization traits were measured by the percentage of root length containing arbuscules and vesicles and by the percentage of AM fungal root colonization (i.e., frequency of hyphae in the root system). The previous AM fungal root traits were evaluated under a light microscope (Leitz, Laboulux S, Wetzlar, Germany) after root clearing and staining (Phillips and Hayman 1970), and using the modified grid-line intersect method (McGonigle et al. 1990).

2.5 Grain yield and nutrient uptake

At physiological maturity (GS90), grain yield was determined by oven drying at 65 °C up to a constant weight. The concentration of N and P in the grains was determined by the Kjeldahl method (Jones 1991) and the ammonium-molybdophosphoric blue color method (Chapman and Pratt 1961), respectively. Furthermore, the grain concentration of K, Ca, Mg, Cu, Fe, Mn, and Zn was determined by a microwave-assisted acid digestion system (COOLPEX Smart Microwave Reaction System, Yiyao Instrument Technology Development Co., Ltd., Shanghai, China) and a microwave plasma atomic emission spectroscopy (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA). Host benefits

Fig. 2 Ten-daily rainfall data and air maximum and minimum temperature during the barley growth season 2020 and 2021



were calculated as $((\text{grain yield/nutrient concentration in inoculated plants} - \text{grain yield/nutrient concentration in not-inoculated plants})/\text{grain yield/nutrient concentration in not-inoculated plants}) \times 100$ (Avio et al. 2006). We calculated the effect size for the AM fungal colonization as the difference in colonization between the controls and +M treatment: $\Delta\text{AM} = \text{AM}_{+M} - \text{AM}_{-M}$ (AM, AM fungal root colonization) (Lekberg and Koide 2005). In addition, we calculated the mycorrhizal response ratio (MR) as the effect size to express the effects of increased colonization on yield and nutrient concentrations. For instance, the effect size for yield was calculated as follows: $\text{MR}_{\text{yield}} = \text{yield}_{+M} / \text{yield}_{-M}$ (Lekberg and Koide 2005). Moreover, in 2021, shoot samples at GS14 were analyzed for N and P concentrations (Jones 1991; Chapman and Pratt 1961).

2.6 Mycorrhizal diversity in barley roots

We focused on the molecular characterization in 2021 when the barley genotypes were sown in March. Significant increases in heavy rainfall in inland Tuscany, including our experimental site, were reported in autumn and winter (Vallebona et al. 2015; Cammarano et al. 2019). This has determined a delay in the usual seeding period for cereals, including barley (November to March). The selection was further supported by the positive and significant grain yield response in the barley genotypes and by the low variability among replicate plots in 2021.

In 2021, at both growth stages (GS14 and GS90), a root subsample per each replicate plot was prepared for DNA extraction, employing a combination of washing and ultrasound treatments to simultaneously separate the rhizospheric fraction (1 mm root soil attached) from roots and the roots colonized by endophytes (Bulgarelli et al. 2015). Then, we analyzed only the roots colonized by endophytes. Genomic DNA was extracted from root samples (1 g of fresh weight) using the DNeasy Plant Mini Kit (Qiagen, Germany) (three barley genotypes \times three replicate plots \times two AMF inoculation levels \times two growth stages = a total of 36 samples). The extracted DNA was quantified by a spectrophotometer (NanoDrop Technology, Wilmington, DE), verified by Qubit fluorometric quantification (Thermo Fisher Scientific, USA), and then stored at -20°C until analysis. The small subunit ribosomal RNA (SSU) fragments were amplified using a nested PCR approach with two pairs of primers. The forward primer AML1 and the reverse primer AML2 were used in the first step, while the forward primer WANDA-ill and the reverse primer AML2-ill were used in the second step (in bold, the adaptors for the Illumina reaction) (Dumbrell et al. 2011; Mhlanga et al. 2022; Lee et al. 2008). PCR, purification, and quantification protocols are given in Supplementary Materials and Methods 2. The cleaned and quantified PCR products (a total of 18 per each growth stage) were

adjusted in an equimolar ratio ($10\text{ ng } \mu\text{L}^{-1}$) for the addition of dual-index barcodes using the Nextera® XT DNA library preparation kit (Illumina Inc., CA, USA). 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 12-pM final library concentration with 20% PhiX library spike-in (Illumina) and using an Illumina MiSeq V3 600 cycle sequencing kit.

2.7 Bioinformatics

Raw sequence data generated from the Illumina MiSeq sequencing run of the 36 samples (2021: 18 samples per growth stage) were processed and analyzed using the QIIME2 (2018.11) pipeline and plugins (Bolyen et al. 2019). Demultiplexed forward and reverse paired-end reads were joined using the “-fastq_mergepairs” of the USEARCH plugin (Edgar 2010). Details about bioinformatics are given in Supplementary Materials and Methods 3. The resulting operational taxonomic units (OTUs) were assigned to virtual taxa (VTXs) using the MaarjAM database (<https://maarjam.ut.ee>). All representative newly generated sequences were deposited in the NCBI Sequence Read (SRA) database as SUB14254691 (accession numbers from PP341529 to PP341554). Representative sequences were aligned with NCBI sequences of closely related AM fungal species, using the MAFFT online service (Katoh et al. 2019), and a neighbor-joining (NJ) tree was built using MEGA11 (Tamura et al. 2021), following the bootstrap test of phylogeny with 1000 bootstraps. The substitution model used was the Kimura 2-parameter with uniform rates among sites, pairwise deletion, and 7 threads. The NJ tree was edited using Adobe Illustrator 2022. Rarefaction curves were calculated and generated using iNEXT (iNterpolation and EXTrapolation) (<https://chao.shinyapps.io/iNEXTOnline/>), plotting diversity estimates with respect to sample size (sample-size-based or size-based R/E sampling curves) (Chao et al. 2014, 2016).

2.8 Statistical analysis

A three-way analysis of variance (ANOVA) was performed to test the effect of year of cultivation (Y), genotype (G), and AM fungal inoculation (Inoc) on the mycorrhizal abundance (i.e., AM fungal colonization, arbuscules, and vesicles) in barley roots, grain yield, and nutrient concentration. Genotype and Inoc were considered fixed factors and Y as a random factor. A three-way ANOVA was performed to test the effect of G, Inoc, and GS (fixed factors) on AM fungal richness (S) (number of VTXs per sample), Shannon index (H'), and Simpson index (λ), calculated from data collected in 2021 (Supplementary Materials and Methods 4). Data were transformed if necessary (e.g.,

log₁₀, arcsen). The Tukey-B procedure was used to test the differences among means. All univariate analyses were performed using the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA).

A multivariate approach based on permutational analysis of variance (PERMANOVA) was performed to test the effect of Y, G, and Inoc, and their interactions, on plant and AM fungal parameters (Anderson 2005). This approach was applied to test the effect of G, Inoc, and growth stage (GS) on the AM fungal community structures retrieved in 2021 within the roots (relative abundances of AM fungal VTXs). The explained variance was calculated and divided among the sources of the variation. For plant and AM fungal parameters, principal coordinate analysis (PCO) was performed to visualize the most relevant significant patterns (Gower 1966). For AM fungal communities, non-metric multidimensional scaling (nMDS) was applied for visualization (Kruskal 1964). 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). The dataset on AM fungal communities was used to generate Venn diagrams, representing the VTXs unique and shared to each treatment (i.e., AM fungal community composition: identity of AM fungal VTXs). The Venn diagrams were generated using InteractiVenn (Heberle et al. 2015) and edited by Adobe Illustrator 2022.

To understand the relationship between the AM fungal colonization in roots and plant parameters at GS90 in the 2 years of cultivation, a multivariate statistical approach (RELATE analysis) was applied to determine the strength of the correlation between the two matrices in rank-order patterns of dissimilarity (Clarke and Warwick 2001). In addition, the RELATE analysis was applied to understand the relationship between the composition and the structure of the AM fungal community and plant parameters detected in 2021 at GS90. The analysis was based on the Spearman rank and 999 permutations with ρ equal to 1 representing the perfect relationship. Then, the BEST analysis (Clarke et al. 2008) was used to identify the main AM fungal root traits and the taxa responsible for the plant functional changes. The BEST analysis was based on BioEnv methods (all combinations), Spearman rank, and 999 permutations. All multivariate analyses were performed using PRIMER 7 and PERMANOVA + software (Anderson 2008; Clarke and Gorley 2015). Details on the multivariate analyses are given in Supplementary Materials and Methods 5.

3 Results and discussion

3.1 Mycorrhizal colonization traits in barley roots

At physiological maturity (GS90), AM fungal root colonization, and percentage of root length containing arbuscules

were significantly affected by the interaction among year (Y), genotype (G), and AM fungal inoculation (Inoc) (Table S3). In 2020, Inoc increased the AM fungal root colonization of Atomo and Concerto up to an average of 53% (Fig. 3a). Compared with not-inoculated plants (-M), the increases were 27% in Atomo and 10% in Concerto, whereas in Atlante, there was a slightly, but not significant increase. Moreover, in 2020, the variety Atomo showed the highest rate of arbuscules in roots (14%), and compared with the other treatments, the increase was 12% (Fig. 3a, b). In 2021, a different pattern was reported, as AM fungal root colonization and arbuscules values were generally higher than in 2020 (76% vs 41% and 64% vs 4%), and only Concerto showed significant increases of both AM fungal root traits in comparison to control (+17% and +25%, respectively) (Fig. 3a, b). Thus, the pattern of response of Concerto was consistent across the years of cultivation that were characterized by differences in soil texture, total N, SOC, and pluviometric records (Table S2). Visualization of the AM fungal colonized root and arbuscules are in Fig. 3d, e.

Under field conditions, few experiments were carried out on barley for more than 1 year, and no one tested different genotypes (Beslemes et al. 2023; Clarke and Mosse 1981; Khaliq and Sanders 2000; Powell et al. 1980). Beslemes et al. (2023), in contrast with our results, did not find interactions between AM fungal inoculation and year of cultivation, but they recorded consistent increases in AM fungal colonization due to inoculation in the 2 years of cultivation (+M vs -M: 55% vs 49%).

The response of barley in terms of AM fungal root colonization was similar to values previously recorded in controlled sterile conditions with several spring cultivars and with many types of AM fungal inoculants (average AM fungal root colonization: 54%) (Baon et al. 1993; Coccina et al. 2019; Jensen 1983; Watts-Williams et al. 2020). Similar to our results, the cultivar affected AM fungal root colonization, from ca. 18 to 80%. Moreover, positive AM fungal colonization responses following inoculation were observed under low soil P availability (Brown et al. 2013; Jensen 1984). Noteworthy, similarly to 2021 where the crop received less water during the growth cycle, under water stress, the response in AM fungal colonization to inoculation was higher under water stress (Jerbi et al. 2022).

Furthermore, our data highlighted an opposite pattern between years of cultivation in terms of the occurrence of vesicles in roots: in 2020 when AM fungal colonization and arbuscules were low, AMF invested their resources in vesicles, whereas in 2021, when AM fungal colonization and arbuscules were high, low percentages of vesicles were recorded (Fig. 3c). This is consistent with classical studies in the subject (Johnson 1993).

Finally, we cannot confirm whether the introduced indigenous AMF were those that colonized the roots and

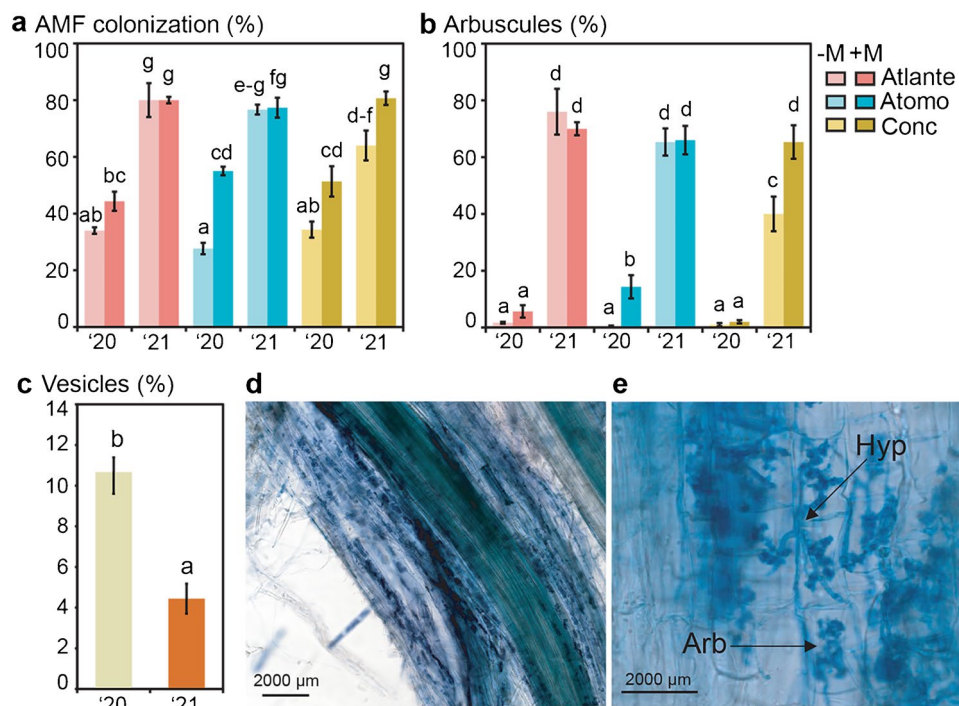


Fig. 3 Effect of the interaction year of cultivation (Y), barley genotype (G), and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on AMF root colonization (a) and percentage of root length containing arbuscules (b) (Table S3). Effect of Y on the percentage of root length containing vesicles (c); picture of the intraradical AMF root colonization (d). Picture of arbuscules in the root cells (e). The years of cultivation were 2020 ('20) and 2021 ('21). Three barley

genotypes: Atlante, Atomo, and Concerto (Table S1). The AMF inoculation treatments were inoculated with 14 AMF species originating from a local agricultural site (+M) and mock inoculum as control (-M). The AMF colonization in roots was measured at physiological maturity (GS90; Zadoks et al. 1974). Different letters highlight statistically significant differences according to the Tukey-B test

determined increases in intraradical colonization. The current molecular tools available do not allow the discrimination of multiple indigenous AM fungi at the isolate level in the field (Kolaříková et al. 2021). Illumina sequencing allows us to identify the AM fungal communities sometimes not even at the species level, due to the length of the reads. Approaches based on long and lower numbers of reads failed to characterize AMF at the isolate level, although they can well phylogenetically discriminate the AM fungal species. Another option could be to design specific primers, but taking into account the nature of our local consortium used as inoculum, this is quite challenging and would not overcome the problem of the detection of the native AMF. In addition, to assess mycorrhizal inoculation success and to determine whether field inoculation enhances ecosystem functions, a combination of high-throughput rDNA metabarcoding, RNA sequencing, and metaproteomics would be a suitable and robust approach (Martin and van der Heijden 2024).

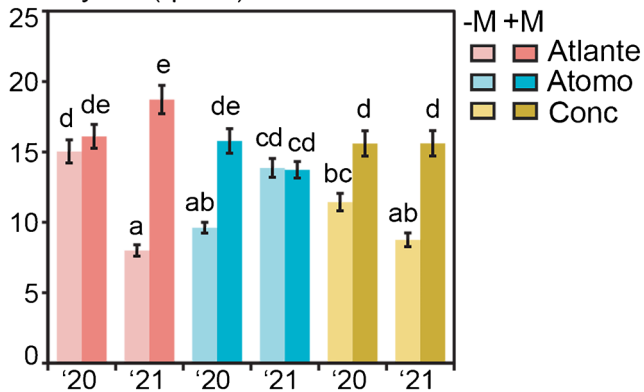
The increases in AM fungal colonization we detected in some varieties can be a direct result of the inoculum or an indirect effect through mechanisms of competitive interactions among AMF (Hart et al. 2001; Maherali and

Klironomos 2007). Moreover, we cannot exclude the effect of the preceding crop on AM fungal root colonization in the following main crop. While many studies suggest that preceding crops or cover crops can impact AM fungal root colonization and community diversity, this effect varies by plant species and may be diluted by family identity (Benitez et al. 2016; García-González et al. 2023; Hontoria et al. 2019). In our experiment, the influence of preceding crops, clover in 2020 and faba bean in 2021, both belonging to the Fabaceae family, was likely minimal. In addition, the rotation sequence involving clover or faba bean, and the cereal, may have further reduced this impact, as the crop sequence remained unchanged in the long term.

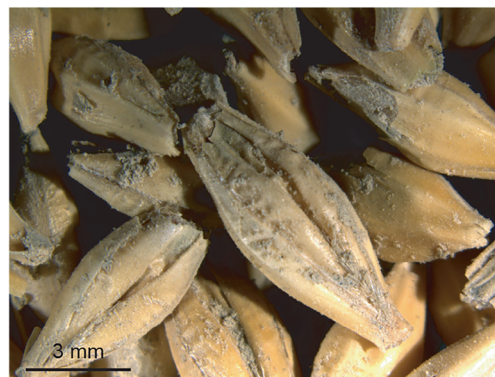
3.2 Effectiveness of AMF on barley grain yield

Barley varieties responded differently to AM fungal inoculation in the 2 years of cultivation in terms of grain yield (Fig. 4a; Table S3). In 2020, in line with the recorded increases in AM fungal root colonization and arbuscules, grain yield was significantly promoted by 64% and 37% in the inoculated varieties Atomo and Concerto, respectively. Furthermore in line with no changes in AM fungal

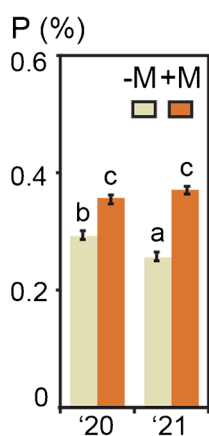
a Grain yield (q ha⁻¹)



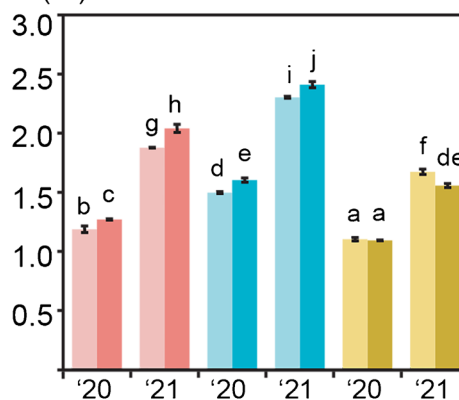
b



c



d N (%)



e

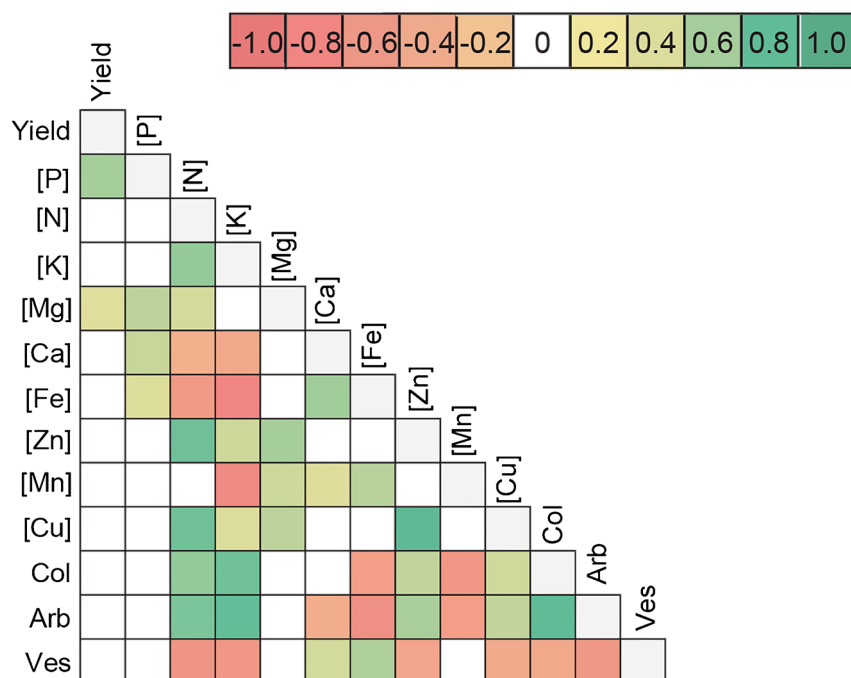


Fig. 4 Effect of the interaction among year of cultivation (Y), barley genotype (G), and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on grain yield (a) and N concentration in grain (d) (Table S3). Effect of Y and Inoc on P concentration in grain (c) at the physiological maturity (GS90; Zadoks et al. 1974). Picture of AMF-coated seeds (b). Pearson (r) correlation map among AMF root colonization traits and plant functional parameters (e). The years of cultivation were 2020 ('20) and 2021 ('21). Three barley genotypes: Atlante, Atomo, and Concerto (Table S1). The AMF inoculation treatments were inoculated with 14 AMF species originating from a local agricultural site (+M) and mock inoculum as control (-M). Different letters highlight statistically significant differences according to the Tukey-B test

root traits, a slight but not significant enhancement was reported in Atlante (+7%) (Fig. 4a). In 2021, Concerto showed a consistently positive response in grain yield (+78%) (Fig. 3a, b and Fig. 4a). This was also supported by the recorded promotion of root colonization and arbuscules. Moreover, while Atlante showed strong increases in grain yield under inoculation (+134%), Atomo did not show any change. Thus, the variety Concerto had a more stable response to inoculation with the indigenous AM fungal consortium and can be a good candidate for AM fungal inoculation, irrespective of soil nutrient availability and drought stress. Seeds coated by the AM fungal indigenous consortium are shown in Fig. 4b.

Previously, in a meta-analysis on the effect of AM fungal inoculation on cereal grain yields, no effect was reported on barley both in controlled and field conditions (Zhang et al. 2019). However, they pointed out the lack of experiments on barley and the importance of studying the effect of breeding and environment to validate the pattern. Under low soil nutrient availability, yield grain increases were reported in field inoculation with a mixture of AMF (Beslemes et al. 2023; Masrahi et al. 2023). In accordance with the general relationship found in crop plants between ΔAM and MR_{yield} (Lekberg and Koide 2005; Pellegrino et al. 2015), in 2020, we found a significant relationship ($R^2 = 0.71$; $P = 0.004$). By contrast, no relationship was found in 2021 ($R^2 = 0.024$; $P = 0.691$). This is in contrast with our expectations that higher AM fungal colonization would have a greater driven grain yield under drought stress. However, our results support that soil nutrient availability is a key driver of the interaction between AM fungal inoculation and crop yield (Zhang et al. 2019).

The pattern of yield in not-inoculated plants may be explained by the interaction between genotype and environment (i.e., climate and soil) (Schneider et al. 2024). Our experiment is a 2-year field experiment, and not a multi-year field experiment (3–5 years or more). According to many authors, 2-year field experiments are valuable in providing short-term insights for testing hypotheses, whereas multi-year experiments would better suit the detection of inter-annual variability, long-term trends, and cumulative effects

(Deytieux et al. 2016; Lutz et al. 2023). However, in studies of AM fungal inoculation, we have to take into account the biological nature of inocula whose composition and infectivity can vary along the time of storage. In this regard, Arcidicono et al. (2024a) applied the same indigenous AM fungal inoculum we applied in our experiment and found no differences between the 2 years in its infectivity.

3.3 Effectiveness of AMF on barley nutrient uptake

Inoculation determined in both years an increase of P in grain of all varieties (Fig. 4c; Table S3). However, the relative increase in 2021 (42%), characterized by soil with low P availability and drought stress, was larger than in 2020 (24%) when soil had a very low P availability and no drought stress. This indicates that under low and very low soil P availabilities, AM fungal inoculation in field conditions strongly promotes the concentration of P in barley grains. Moreover, the symbiosis may be more efficient under drought conditions. Nevertheless, the ΔAM and mycorrhizal P response ratio were not related to each other in both years ($R^2 = 194$; $P = 0.236$; $R^2 = 0.361$; $P = 0.087$). Furthermore, the positive and significant relationship we found between grain yield and P concentration (Fig. 4e) does not support the agronomic phenomenon in which nutrient concentration in grain decreases as yield increases (i.e., the dilution effect). Our results are in agreement with the increases in P content (+20%) found in wheat grains (Pellegrino et al. 2015). Moreover, the variability we observed between years is in accordance with Porcel and Ruiz-Lozano (2004) who reported that AMF enable host plants to grow and uptake P more efficiently under drought stress, through plant osmotic adjustment (Harrier 2001).

As regards N concentration in grain, at both years of cultivation, Atomo and Atlante were reported to be positively affected by AM fungal inoculation (+6% and +8%, respectively), while Concerto was not affected in 2020 and negatively affected in 2021 (Fig. 4d; Table S3). Moreover, grain yield and N concentration were not significantly related (Fig. 4e). In field experiments, no change in N grain concentration, but increases in N content (+31%) were reported in wheat (Pellegrino et al. 2015). However, the number of meta-analyzed field trials was low, pointing out that the field response of cereals to AMF deserves more study. Recently, it was observed that *Glomus* sp. and *Gigaspora* sp. and a multiple-species AM fungal field inoculum promoted the uptake of N in the grain of two cultivars of barley (Beslemes et al. 2023; Masrahi et al. 2023). The increases of N grain concentration in Atomo and Atlante cannot be explained by significant relationships between ΔAM and mycorrhizal N response ratio (data not shown), but by changes of AM fungal communities in the roots induced by inoculation, as found by Marrassini et al. (2024) on wheat.

In 2021, the concentration of P was also promoted in the shoots of Concerto sampled at the four-leaves unfolded stage (GS14) (+18%) (Fig. S2a). This pattern of response to the AM fungal inoculation can support the success of the inoculation and the high responsiveness of this variety. This response is in line with the increase of AM fungal root colonization in the roots of Concerto and with its grain yield response. In addition, averaged over genotypes, N shoot concentration was increased in inoculated treatments (Fig. S2b), further supporting the success of inoculation at early plant growth stages. The effects of Y, G, and Inoc on K, Ca, Mg, Cu, Fe, Mn, and Zn grain concentrations are reported in Supplementary Results and Discussion 1 and in Fig. S3. In addition, details on the relationships of these elements among each other and with P and N are reported in Fig. 4e and Results and Discussion 1.

Finally, PERMANOVA highlighted significant interactions between Y, G, and Inoc (Table S4). The PCO biplot (Fig. 5) showed that all genotypes differently responded to inoculation in the 2 years of cultivation. Although the year of cultivation very strongly affected the pattern of responses, with an explained variance of 65%, the third-order interaction explained 7%. Looking at the PCO biplot, it showed a clear difference between years. In 2021, the agronomic response was less variable among replicate plots, and this encouraged a further investigation on root AM fungal communities. Our results are in contrast with our first hypothesis that genotype exerts greater control over the response of barley to AM fungal inoculation than the environment. Indeed, our hypothesis was based on recent findings that the wheat

genotype inoculated in the field with the same indigenous consortium was a major driver of the agronomic response (Marrassini et al. 2024).

The PERMANOVA pairwise comparisons, utilized to dissect this interaction, highlighted consistent differences among the inoculated and not-inoculated groups of varieties in 2020 and 2021 (Table S5). Concerto was consistently and positively affected by AM fungal inoculation in both years, while Atomo and Atlante only in 2020 and 2021, respectively. This supports a robust and stable response of Concerto to inoculation across years and demonstrates its less susceptibility to pedo-climatic variability. Therefore, the modern crossbreed variety Concerto, developed in the UK, showed high mycorrhizal responsiveness to the indigenous AMF, indicating good adaptation of the genotype to both soil and AMF, regardless of their origin. Furthermore, the year was consistently found to significantly affect the pattern of plant and AM fungal parameters in both inoculated and not-inoculated groups of barley varieties, underlining the expected role of the environment in shaping plant response under the same agronomic practice. Finally, PERMDISP showed significant differences among barley genotypes and no differences between inoculated and not-inoculated plants or between years of cultivation (Table S4). A higher variable pattern of response was observed in Atomo, whereas less variable patterns were reported in Concerto and Atlante (Fig. 5). Therefore, irrespective of inoculation and year of cultivation, the response of Concerto and Atlante is more stable.

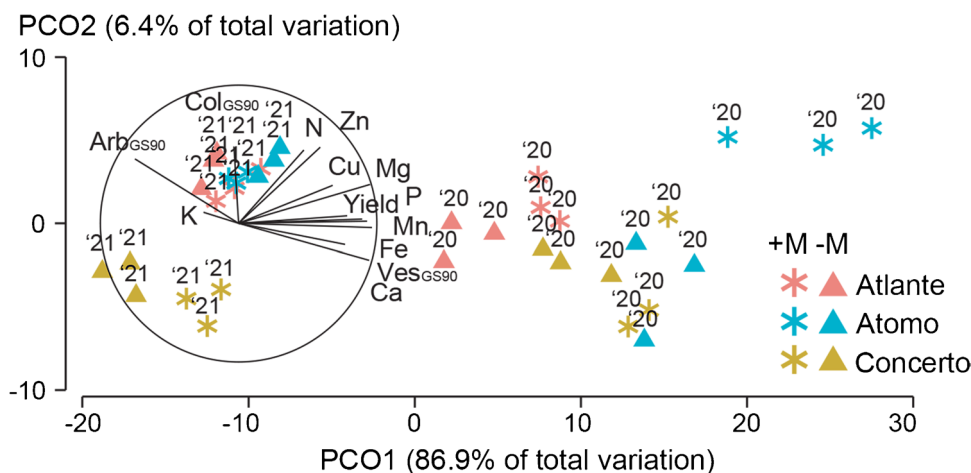


Fig. 5 Principal coordinate analysis (PCO) biplot on the significant effect of the interaction among year of cultivation, barley genotype, and arbuscular mycorrhizal fungal (AMF) inoculation on plant and AMF functional parameters (Table S4). The data matrix is built using yield and nutrient concentration in grain (i.e., N, P, K, Mg, Ca, Zn, Fe, Mn, Cu) ($n = 36$). The AMF functional parameters were AMF root colonization (Col), percentage of root length containing arbuscules (Arb), and percentage of root length containing vesicles (Ves)

at physiological maturity (GS90; Zadoks et al. 1974). The years of cultivation were 2020 ('20) and 2021 ('21). Three barley genotypes: Atlante, Atomo, and Concerto (Table S1). The AMF inoculation treatments were inoculated with 14 AMF species originating from a local agricultural site (+M) and mock inoculum as control (-M). In the plot, the overlay of vectors is reported and only the parameters with a strong correlation ($r > 0.6$) are displayed

3.4 Diversity of AMF in the roots of barley

To test our hypothesis that changes in root community structure but not composition by inoculation of indigenous AMF drive AM preferences of plant genotypes, we investigated the AM fungal community diversity in the roots of the three barley genotypes. We focused the investigation on root samples collected in 2021. This allowed us to study the effect of AM fungal root diversity on crop productivity. Moreover, we investigated the host plant preference by AMF under no inoculation and the role of the plant growth stage in modulating the host plant preference.

3.4.1 Illumina sequencing output

Before the curation of the AM fungal sequences, the number of paired reads was 42,716. After curation, 34,907 reads, ranging from 395 to 1292 reads per sample, were retrieved and assigned to 26 VTXs (Fig. S4; Table S6). The 26 AMF VTXs belonged to three orders (i.e., Diversisporales, Entrophosporales, Glomerales) and four families (i.e., Diversisporaceae, Gigasporaceae, Entrophosporaceae, Glomeraceae). The accumulation curves and rarefaction analyses of AMF confirmed that the Illumina sequencing effort was sufficient for the analysis (Fig. S5).

3.4.2 AM fungal richness and diversity indices in barley roots

Barley genotype and inoculation differently affected AM fungal richness (S) and the diversity indices, H' and λ , in the two growth stages (G12 and GS90) (Table S7). *Atlante* showed a higher diversity (S and H') than the other genotypes (+51% and +22%, respectively) (Table S8). Previously, the barley AM fungal diversity was only studied in single varieties utilizing the taxonomic-based assessment of spores in soil and the molecular characterization in roots (Aguilera et al. 2017; Kaidzu et al. 2020). Therefore, our study highlights for the first time a host plant preference in AMF across barley genotypes. This is in accordance with the results obtained among genotypes of other crops (Kavadia et al. 2020; Mao et al. 2014; Parvin et al. 2021). Indeed, root trait variability among varieties of cereals, including barley, can be large (Nakhforrosh et al. 2014; Robinson et al. 2018; Sendek et al. 2019). Therefore, although we did not measure root morphology, we can hypothesize that differences in root architecture could have determined the observed variable pattern of AM fungal diversity.

However, *Inoc* increased AM fungal richness and H' only in *Atlante* at GS14 (+133% and +75%, respectively), while λ was promoted in *Atlante* and *Atomo* only at GS14 (+54% and +68%). In addition, AM fungal richness was higher at GS90 than at GS14 (+23%). Since AM fungal diversity in

roots enhances plant productivity (van der Heijden et al. 1998; Vogelsang et al. 2006), inoculation that promotes AM fungal diversity (e.g., *Atlante* and *Atomo*), can be expected to boost productivity.

3.4.3 Community composition and structure of AMF in barley roots

Sixty-five percent of AM fungal taxa (VTXs) (65%) were shared among genotypes, irrespective of AM fungal inoculation and plant growth stage (GS) (Fig. S6a). Similarly, barley genotypes shared 50% and 50% VTXs in inoculated conditions and control, irrespective of GS (Fig. S6b). Moreover, at GS14, the percentage of VTXs shared in inoculated conditions was similar to the one in the controls (32% and 37%) (Fig. S6c), while at GS90, it reached 65% in inoculated plants and 40% in controls (Fig. S6d). These results support our hypothesis of host preference in AMF across barley genotypes in both inoculation treatments. However, further field studies on multiple sampling points over time and space would provide a more comprehensive understanding of this trait. This was also previously observed in wheat by molecular approaches (Marassini et al. 2024; Pellegrino et al. 2020). Thus, also in barley, there is a core composition of AMF that can be considered generalist, but there are several putative specialist taxa uniquely retrieved in the roots of each genotype. Moreover, since inoculation with indigenous AMF did not modify the rate of shared taxa at early stages, we can hypothesize that locally sourced inocula did not change the AM fungal recruitment of the crop. By contrast, the increase of shared VTXs observed at GS90 due to inoculation allows us to highlight that indigenous AM fungal inoculants can reduce the variability in host preference among genotypes. Similarly, inoculating the same AM fungal consortium in wheat, the percentage of shared VTXs at maturity was higher than in the controls (46% vs 35%) (Marrassini et al. 2024). This may suggest that indigenous taxa contained in the inoculum, during crop growth, colonize a larger number of plant genotypes and that our consortium was composed of high-compatible taxa.

Focusing on the pattern of AM fungal composition in inoculated and not-inoculated barley genotypes, the average percentage of shared VTXs was 51% and 65% at GS14 and GS90, while the percentage of VTXs uniquely retrieved in inoculated plants was 23% and 18%, respectively (Fig. S7a). Thus, we cannot fully confirm our hypothesis that inoculation with an indigenous AM fungal consortium did not modify the composition of the AM fungal communities. However, we can suppose that when indigenous inoculants are applied, they start to compete with closely related AM fungal species present in the soil at very low abundance, and thus not detectable by the molecular tools. Indeed, when individuals are rare

in a microbial community, Illumina sequencing has some limitations in the accuracy of detection (Cheng et al. 2023; Egan et al. 2018). Moreover, the VTXs uniquely found in the inoculated barley genotypes had a very low abundance (0.6%).

Our findings are in accordance with the high percentage of AM fungal VTX shared between inoculated and not-inoculated genotypes of wheat and sunflower (Arcidiacono et al. 2024a; Marrassini et al. 2024). These results were achieved using the same indigenous AM fungal consortium under similar climatic conditions, indicating a low environmental and ecological impact of indigenous inocula. However, the indigenous inoculum was not molecularly characterized, and the metabarcoding approach used to analyze the AM fungal communities in the roots relied on amplifying a short SSU fragment, which is not specific enough for identifying AM fungal isolates (Piazza et al. 2019). Therefore, the molecular method applied does not confirm whether the introduced indigenous AMF were the ones that colonized the roots. It is also possible that competitive interactions occurred among AM fungal isolates of the same species and/or among different species (Hart et al. 2001; Jansa et al. 2008; Maherali and Klironomos 2007; Thonar et al. 2014), adding another limitation to the interpretation of our results. Nevertheless, given that the AM fungal infectivity (mycorrhizal infection potential) measured in soil samples from each study site showed little variability among replicates, we believe it is reasonable to attribute the observed effect on shared and uniquely AM fungal root VTX in both inoculated and not-inoculated plants primarily to the direct effect of inoculation.

PERMANOVA indicated that a high percentage of variance (24%) in the root AM fungal community structures was due to the interaction among G, Inoc, and GS (Table S9). This pattern is also visualized in the PCO and shade plots (Fig. 6a, b) and in the bar chart of the relative abundance of AM fungal VTXs (Fig. S7b). Overall, at GS14, no differences were recorded among not-inoculated barley genotypes, while significant differences were recorded between inoculated Atlante and Atomo/Concerto (Table S10). At GS90, no differences were recorded among not-inoculated barley genotypes and neither among inoculated genotypes. This intra-annual variation is also supported by a previous study on wheat that indicated a strong effect of the season in determining the AM fungal structure in roots (Berruti et al. 2018).

Therefore, at early and late plant development and under no inoculation, host preference in AMF is more discriminated by the pattern of composition (% of unique AM fungal VTX at GS14: Atlante 5%, Concerto 32%; -M at GS90: Atlante and Concerto had a significantly different AM fungal community composition, $P(\text{MC}) = 0.025$, according to PERMANOVA and pairwise tests) than by the pattern of the community structure (Table S10). By contrast, at early plant development (GS14), in inoculated conditions, host

preference is more discriminated by the AM fungal community structure (Table S10) than by composition (data not shown), while at plant maturity, no differences were reported in both parameters. These findings highlight that to study the host preference of AMF in field crops, it is important to take into account these two aspects of community diversity. Moreover, we should take into account, during the interpretation of the results of the different plant growth stages, the time that AM fungal propagules, including resting spores, need for germination, and colonization of the host plants (Giovannetti and Avio 2002).

Moreover, inoculation caused changes in the AM fungal community structure in the Atlante genotype at GS14, while at GS90, changes were observed in both Atlante and Atomo (Table S10). These results support the high variability in AM fungal community structure among wheat genotypes (Mao et al. 2014; Marrassini et al. 2024; Pellegrino et al. 2020) and give new insights into the mechanisms by which diversity within AM fungal populations is enhanced or maintained in roots. Indeed, a genotype like Concerto whose AM fungal community structure was similar in inoculated and not-inoculated treatments and AM fungal abundance and yield were promoted can be considered a suitable variety in biofertilization programs. Previously, other factors, such as soil phosphate availability and host species, were identified as main determinants of root AM fungal community structure (Cavagnaro et al. 2005; Ehinger et al. 2009; Eom et al. 2000; Helgason et al. 2002; Vanderkoornhuysen et al. 2003). Finally, the fact that two barley genotypes, Atomo and Atlante, in not-inoculated and inoculated conditions, respectively, showed significant differences in the AM fungal community structure between growth stages, is in accordance with a previous work reporting differences along the plant cycle in bread wheat, oat, and barley, inoculated and not with AMF (Aguilera et al. 2021). The importance of the phenological stage in shaping AM fungal community structure in roots of wheat was also reported in field conditions (Marrassini et al. 2024) and perennial and annual grasses and non-grasses species (Lingfei et al. 2005). The dispersion among plant growth stage groups, GS14 and GS90, was not significantly homogeneous, as shown by the PERMDISP (Table S9). The higher variability at the early development of barley (GS14) can be attributed to mechanisms of competition among AMF within a community (Chagnon et al. 2013; Jansa et al. 2008) or to the fact that the symbiosis with the introduced indigenous AMF has not been established yet in some barley genotypes (Fig. S7).

3.5 Modeling barley productivity against AM fungal colonization and structure in roots

Our results showed that there was a significant relationship between the AM fungal root traits and barley productivity

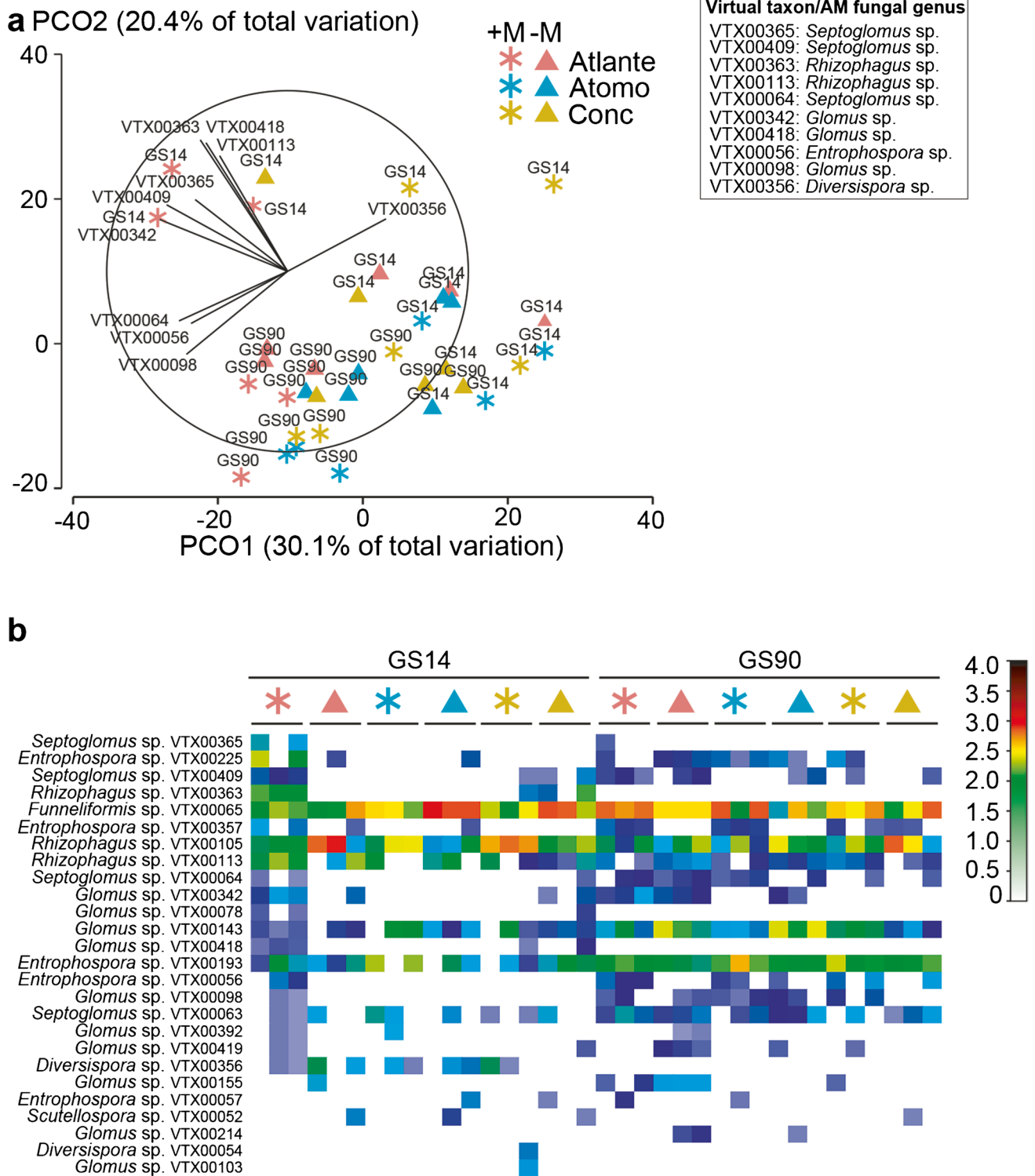


Fig. 6 Principal coordinate analysis (PCO) biplot (a) and shade plot (b) on the significant effect of barley genotype, arbuscular mycorrhizal fungal (AMF) inoculation, and plant growth stage on the AMF community structure in the roots of barley. The data matrix is built using the abundances of the AMF virtual taxa (VTX) found at the four-leaves unfolded stage and at physiological maturity (GS14 and

GS90; Zadoks et al. 1974) in 2021 ($n = 36$). The three barley genotypes are Atlante, Atomo, and Concerto (Table S1). The AMF inoculation treatments were inoculated with 14 AMF species originating from a local agricultural site (+M), and mock inoculum as control (-M)

(RELATE: $\rho = 0.587$; $P = 0.001$) (Fig. 7a), and the BEST analysis identified the percentage of root length containing arbuscules as the best predictor of barley productivity (Fig. 7b). Therefore, under low soil fertility, characterizing the 2 years of cultivation, AM fungal colonization traits, such as the percentage of arbuscules, are likely key factors of barley productivity. Similarly, in field conditions, arbuscules were positively and strongly correlated with all functional traits of sunflower grown at low and high soil fertility (Arcidiacono et al. 2024a). The association between increased AM fungal root colonization and increased barley productivity is consistent with the results of meta-analytic works (Lekberg and Koide 2005; McGonigle 1988; Pellegrino et al. 2015; Treseder 2013). However, measurements on soil AM fungal hyphae, by which AMF explore the soil to mobilize resources and

transfer them to the plant, may be useful for understanding the whole system, since they contribute significantly to AM fungal functionality. Indeed, there are two categories of AMF: the first predominantly explores roots, and the second is more active in the soil exploration (Antunes et al. 2024; Arcidiacono et al. 2024b; Djotan et al. 2023).

Moreover, the agronomic response of barley was driven also by changes in the structure of the AM fungal community, as highlighted in Fig. S8, and by the significance of RELATE analysis ($\rho = 0.514$; $P = 0.003$) (Fig. 8a), and not by changes of composition ($\rho = 0.084$; $P = 0.167$; data not shown). This relationship is clear comparing the pattern of AM fungal community structure in roots of barley genotypes at GS90 displayed in the nMDS plot (Fig. S8a) and the pattern of yield and nutrient uptake displayed in the PCO plot (Fig. S8b). Therefore, we confirmed our hypothesis that changes in AM

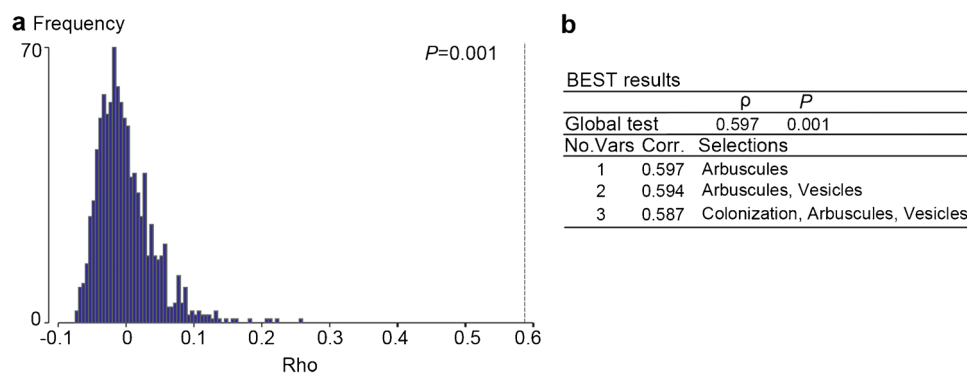


Fig. 7 RELATE analysis (a) based on Spearman rank and 999 permutations for testing the significance of the relationship between the two matrices: AMF colonization in roots and plant functional parameters ($\rho = 0.587$; $P = 0.001$) (Clarke and Warwick 2001). Results of

the BEST analysis (b) based on BioEnv methods (all combinations), Spearman rank, and 999 permutations: ρ and P values of the Global Test and BEST descriptor(s) of the relationship together with the correlation values (Clarke et al. 2008)

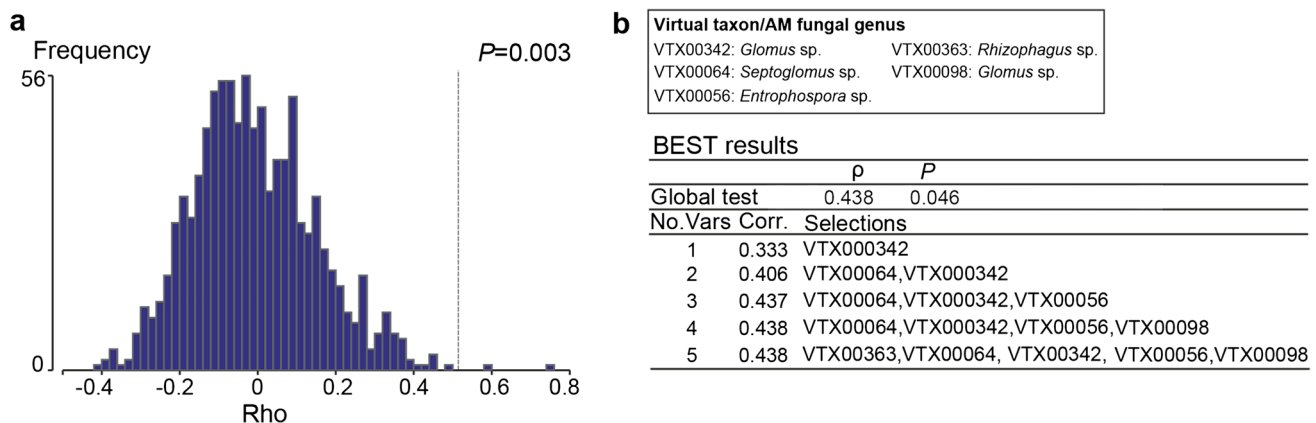


Fig. 8 RELATE analysis (a) based on Spearman rank and 999 permutations for testing the significance of the relationship between the two matrices: AMF community structure and plant functional parameters ($\rho = 0.514$; $P = 0.003$) (Clarke and Warwick 2001) at GS90. Results of the BEST analysis (b) based on BioEnv methods (all com-

binations), Spearman rank, and 999 permutations: ρ and P values of the Global Test and BEST descriptor(s) of the relationship together with the correlation values (Clarke et al. 2008). In the PCO plot, the overlay of vectors is reported, and only the AMF taxa with a strong correlation ($r > 0.6$) are displayed

fungal community structures and not in composition determine barley productivity. Moreover, the BEST analysis highlighted the best predictors of barley productivity ($\rho = 0.44$, $P = 0.046$) which was *Glomus* sp. VTX00342, considering one descriptor ($r = 0.333$), or the taxa VTX00342 with *Septoglomus* sp. VTX00064, considering two descriptors ($r = 0.406$) (Fig. 8b). These BEST predictors were also highlighted in the nMDS plot (Fig. S8a). Indeed, *Glomus* sp. VTX00342 and *Septoglomus* sp. VTX00064, putative members of the indigenous AM fungal inoculum, were more present in inoculated than in not-inoculated plots (+46% and +56%, respectively) (Fig. S7b). Previously, indigenous AM fungal communities were little changed by divergent abiotic conditions (Janoušková et al. 2023), and changes in AM fungal community structure, and not in composition, were identified as the main driver of crop productivity (Arcidiacono et al. 2024a; Marrassini et al. 2024; Pellegrino et al. 2022). Indeed, the inoculated indigenous strains, highly compatible with all barley genotypes, as well as with the local environmental conditions, are preferentially recruited by the roots.

4 Conclusions

The general positive outcome in barley productivity supports the use of indigenous AMF for building future inoculant generations and their wide inclusion in sustainable agriculture. Overall, our data support the fact that AMF can contribute to increasing the nutritional values of barley. This is particularly important under the current context where most minerals are significantly decreased in grain due to the dilution effects caused by the intensification of agricultural management and high-yielding plant breeding. Contrary to our expectation, environment rather than barley genotype exerts a greater control over the response of the plant to AMF. However, the detected significant third-order interaction showed that Concerto was consistently and positively affected by AM fungal inoculation in both years, while Atomo and Atlante only in 2020 and 2021, respectively. This supports a robust and stable response of Concerto to inoculation across years and demonstrates its less susceptibility to pedo-climatic variability. The effect of the environment in modulating the response of barley demonstrated in this study can help in the selection of genotypes with stable AM fungal response in specific climatic conditions. This is of key importance given the current challenges related to climate change. Moreover, we cannot fully confirm our hypothesis that inoculation with an indigenous AM fungal consortium did not modify the composition of the AM fungal communities. Our results disclose the role of barley genotype and plant growth stage on AM fungal host preference with and without the addition of indigenous AM fungal inoculants. Finally, we demonstrated for the first time that changes in AM fungal community structures and

not in composition determine barley productivity. Therefore, we suggest the propagation of AM fungal consortia adapted to specific climatic contexts to boost AM fungal inoculum production and to reduce the potential ecological risks of using exotic AM fungal inoculants.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13593-025-01016-3>.

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Authors contributions Conceptualization, supervision, methodology, resources, and funding acquisition: LE and EP; methodology, investigation, formal analysis: VM; data curation, result interpretation, visualization, writing original draft, review, and editing: VM, EP; review and editing: AVAP, LE. All authors gave their final approval for publication.

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Data availability Data will be made available on request. Furthermore, DNA sequences are available in the NCBI Sequence Read (SRA) database as SUB14254691.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All the authors whose names appeared on the submission approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that the questions related to the accuracy of integrity of any part of the work were appropriately investigated and resolved.

Conflict of interest The authors declare no competing interests.

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