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Lack of complementary or synergistic benefits of arbuscular mycorrhizal fungi (AMF) and organic amendments but functional differentiation among AMF species in a maize–wheat rotation

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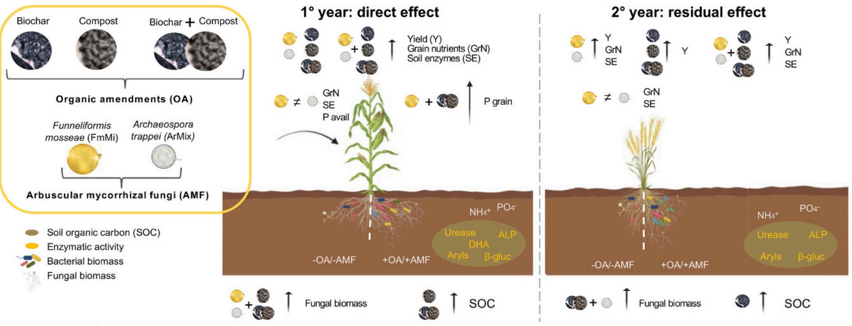
HIGHLIGHTS

- No complementary or synergic AMF-OA benefits on yield or soil were observed.
- *F. mosseae* and *A. trappei* varied in colonization and grain nutrient effects.
- *F. mosseae* with compost+biochar complementarily improved maize grain P.
- AMF-OA synergistically enhanced fungal biomass; effect persisted with *A. trappei*.
- AMF species shaped crop nutrient profiles and soil enzyme activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic amendments (OAs) and arbuscular mycorrhizal fungi (AMF) are promising tools for improving soil fertility and crop productivity in nutrient-poor soils. While AMF, biochar, and compost have shown complementary or synergistic effects under controlled conditions, their field-scale interactions remain poorly understood. In a two-year maize-wheat rotation, we applied OAs (biochar or compost) and AMF inocula (three isolates each of *Funneliformis mosseae*, FmMix, and *Archaeospora trappei*, ArMix) alone or combined with OAs. We tested whether AM inoculation enhances maize productivity and soil fertility, shows complementarities or synergisms with compost and/or biochar, whether effects persist in wheat, and if plant and soil responses differ by AM fungal species. Maize yield increased with AMF and OA, but the combined application did not yield a significant increase over the effect of each factor operating independently. A similar pattern was found for grain nutrients and soil (bio)chemical parameters, except for a complementary effect of FmMix with compost+biochar on maize P uptake. AMF applied with compost and biochar synergistically promoted fungal biomass, and the effect persisted in wheat with ArMix. Although yield did not differ between AM species, interspecies functional variability was observed in grain nutrients and soil enzymes. FmMix enhanced maize P and wheat K, Mg, and Fe, while ArMix increased maize Cu and Ca, and wheat P, Zn, and Cu. Our findings emphasize that the effectiveness of AMF and OAs is influenced by OA properties, soil conditions, crop and AM species. Although functional interspecies

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variability was observed, the comparable or superior agronomic benefits of *A. trappei* indicates that lesser-known AM taxa hold promise as efficient inoculants.

1. Introduction

Across crops and environmental conditions, high-quality soils, rich in soil organic matter (SOM), not only enhance crop yield (+1.7 %) but also reduce yield variability by 16 % compared to low-quality soils (Qiao et al., 2022). Management practices such as conservation tillage, cover cropping, mulching, and organic amendments (OAs) (e.g., manure, compost, biochar), have been shown to increase soil carbon (C) sequestration by 50–1000 kg C ha^{-1} $year^{-1}$ (Hoegh-Guldberg et al., 2018). In soils poor in SOC and nutrients (Tóth et al., 2014) such as those in the Mediterranean area, compost shows the highest C storage potential (Francaviglia et al., 2019). In addition to SOM increases, compost derived from manure or organic residues improves soil structure, microbial biomass and diversity, nutrient availability, and provides neutral to positive effects on arbuscular mycorrhizal fungi (AMF) (Cavagnaro, 2015). It also mitigates the environmental impact of manure disposal by several mechanisms such as the reduction of greenhouse gas emissions, water pollution by heavy metals and nitrogen (N) leaching, and pathogen spread (Eghball and Power, 1994).

Biochar, a stable C-rich amendment, increases SOC by up to 36 %, outperforming compost (+11 %) (Sánchez-Monedero et al., 2019). However, its microbial decomposition can temporarily limit plant N availability (Hunt et al., 2010) although reducing the risk of N leaching (Solaiman et al., 2020). On the other hand, biochar, owing to its relatively low N concentration, can stimulate biological N fixation, and, due of its physical and chemical properties, it increases the abundance and activity of microorganisms such as bacteria and AMF (Warnock et al., 2007; Agegnehu et al., 2017).

When biochar is co-applied or co-composted with organic residues, SOC storage increases further by up to 38 % and 150 %, respectively, compared to compost alone, with stronger effects when using high C/N biochar (Cooper et al., 2020). Over three years and across four cropping systems, biochar mixed with compost increased SOC by 20 % compared to untreated soil (Sánchez-Monedero et al., 2019). This combination also increased soil N and K by 37 % and 30 %, respectively, and stimulated microbial activity, as indicated by a 29 % rise in soil respiration (Sánchez-Monedero et al., 2019). The combination did not show complementary or synergistic effect on SOC and other soil parameters compared to either compost or biochar.

Integrating organic amendments with soil biological tools such as AMF could offer more consistent improvements in soil nutrient cycling and crop uptake. Arbuscular mycorrhizal fungi improve plant access to inorganic soil N and P by extending hyphal networks beyond root zones (Marschner and Dell, 1994), with notable benefits on crop, including cereals, under nutrient-poor soils (Lehmann et al., 2014; Pellegrino et al., 2015; Mason et al., 2025). They may also aid in accessing nutrients from organic sources, though they likely depend on saprotrophic microbes for mineralization (Hodge et al., 2001). The interaction AMF-OAs can shift microbial communities toward fungal dominance, promoting decomposition of recalcitrant substrates and enhancing nutrient cycling (Joergensen and Wichern, 2008). Therefore, irrespective of the involved mechanisms, AM fungal inoculation can help plants to acquire nutrients released from both compost and biochar, along with neutral or positive effects on AM fungal colonization and crop yield (Mason et al., 2025). The speed of release of nutrients from the organic sources and the AM fungal taxa used as inocula can affect the plant response to the AMF-OA co-application (Solaiman et al., 2019). Indeed, the slow release of nutrients from composts and biochar may establish a situation where available levels of nutrients are not so high to reduce AM colonization and mycorrhizal benefits, as observed in some AMF-compost and AMF-biochar co-applications (Cavagnaro, 2014). However, the underlying mechanisms driving the effects of these co-applications on crop productivity and soil fertility in agricultural systems remain poorly understood, likely involving complex interactions among nutrient dynamics, soil microbial communities, and plant physiological responses.

The effectiveness of the symbiosis plant/AMF depends on the combination of plant/variety and AM fungal taxon (Hart and Reader, 2002). High variability in plant-growth response to the presence of different AM fungal taxa can be a major determinant of the success or failure of the AM fungal inoculants in field conditions (Mensah et al., 2015; Säle et al., 2021). The magnitude and direction of these effects vary greatly across fungal orders (Marro et al., 2022), families (Arcidiacono et al., 2024; Hart and Reader, 2002), genera (Dodd et al., 2000), species, and even among isolates within the same species (Marro et al., 2022). Moreover, functional intraspecies variability resulted to be even higher than interspecies variability (Mensah et al., 2015; Marrassini et al., 2024a). Ancient AM fungal lineages are generally considered less effective in promoting plant growth (Säle et al., 2021). Taxa from the families Archaeosporaceae and Paraglomeraceae tend to confer lower benefits in terms of plant growth and nutrient uptake compared to those from Glomeraceae, Entrophosporaceae, and Diversisporaceae. The lower benefit of ancient taxa was associated to a lower AM fungal colonization and arbuscules occurrence in the roots of the host plant. Recently, isolates belonging to *Archaeospora trappei* were reported to be similarly efficient than isolates of *Funneliformis mosseae*, despite their lower root colonization ability (Marrassini et al., 2024a). These positive effects suggest that taxa belonging to Archaesporaceae can be taken into consideration while designing new inoculants, provided that their positive effect is confirmed in field conditions where competition with native AMF occurs. Therefore, evaluating the combined effects of biochar, compost, and AM fungal lineages under field conditions, particularly those differing in extraradical mycelium development and nutrient scavenging from organic amendments, is crucial to maximize the benefits of the co-application.

In this study, we evaluated the impact of AM inoculation on maize and wheat, and its interaction with organic amendments under field conditions. 2 AM fungal inoculants were applied to maize: one composed of three *F. mosseae* isolates and another composed of three *A. trappei* isolates. Inocula were applied either alone or with compost (olive pomace-derived) and/or biochar (wheat husk-derived) (Fig. S1). The organic amendments were chosen due to their different composition of C and nutrients and rate of degradation and nutrient release. Biochar has high C content and stable structure, with large cation exchange capacity and specific surface area, while compost has relatively high concentration of nutrients and high turnover of C. The AM fungal isolates were selected for their contrasting life-history strategies: *F. mosseae* (Glomeraceae) exhibits high infectivity, rapid root colonization, and a short life cycle. In contrast, *A. trappei*, typically found in lower spore abundance in arable soils, produces a greater volume of extraradical mycelium (Koch et al., 2017; Pan et al., 2021; Marrassini et al., 2024a). We tested three main hypotheses: (i) AM fungal inoculation enhances maize productivity and soil fertility, and show complementarities or synergisms with compost and/or biochar; (ii) the positive effect of inoculation is maintained in the following year on wheat, and this could be true especially for *A. trappei*, given its colonization behaviour; (iii) plant response differs depending on AM fungal taxa. Moreover, to identify the key factors influencing plant productivity under AM fungal inoculation and organic amendment application, a multivariate correlation model was developed by integrating data on root colonization, bacterial and total fungal abundance, and soil enzyme activities.

2. Methods

2.1. Fungal and plant material

The inocula were composed of a mix of 3 AM fungal isolates belonging to two species, *Funneliformis mosseae* (FmMix) and *Archaeospora trappei* (ArMix). The FmMix was composed of W1–18, W1–20, and W1–27 isolates, while ArMix of W1–21, W2–32, and W2–36 (Marrassini et al., 2024a). In the first year of cultivation, maize (*Zea mays* L.) hybrid Lolita was grown. The genotype was described to be responsive to field AM fungal inoculation (unpublished data). Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) variety Anco Marzio was cultivated in the following year. Lolita (Pioneer Hi-bred Inc., Des Moines, IA) is a late-maturing hybrid (FAO class 600 = 130 days growth period) and necessitates rainfall of approximately 570 mm during the growing season.

2.2. Experimental field site

The field experiment was conducted in 2021 and 2022 at the Centro Interdipartimentale di Ricerche Agro-Ambientali "Enrico Avanzi" (San Piero a Grado, Pisa, Italy) (43°41′08"N 10°20′33″ E; 4 m a.s.l.). The soil had a silty loam texture (27.1 % sand, 58.3 % silt and 14.6 % clay), with 1.8 % SOM (medium organic C availability) (Walkley-Black) (Nelson and Sommers, 1982), pH (H_2O) of 8.2 (moderate alkaline) (deionized water 1:2.5 *w/v*; McLean, 1982), and the following soil nutrient concentrations: 1.2 g kg^{-1} total N (medium level) (Kjeldahl) (Bremner and Mulvaney, 1982), 23.2 mg kg^{-1} available P (very low level) (Olsen and Sommers, 1982), 22.9 mg kg^{-1} available Fe (diethylene-triamine-penta acetic acid method, DTPA; Lindsay and Norvell, 1978), and 1.7 mg kg^{-1} available Zn (DTPA method) (Lindsay and Norvell, 1978), indicating an optimum and medium level, respectively (Table S1). Furthermore, the C/N ratio was 8.7. The climate of the area is cold, humid Mediterranean according to the Köppen-Geiger climate classification (Kottek et al., 2006), with mean annual maximum and minimum air temperatures of 20.3 °C and 10.2 °C, respectively, and rainfall of 971 mm. During maize cropping cycle in 2021 (May–September), the mean maximum air temperature was 24.9 °C, while the mean minimum air temperature was 17.6 °C, and total rainfall was 96.4 mm (Fig. S2a). During wheat cropping cycle in 2022 (January–July), mean minimum and maximum temperatures were 9.6 °C and 18.5 °C, respectively, and total rainfall was 217 mm (Fig. S2b).

2.3. Experimental set-up and sampling

A full factorial experiment with four organic amendments (Amend) (biochar, Bio; compost, Co; biochar plus compost, Bio + Co; no amendments, NAm) and 3 AM fungal inoculation treatments (Inoc) (FmMix, ArMix, and a mock inoculated control, NM) was arranged in a completely randomized design with three replicate plots (30 m^2, 5 × 6 m^2). Biochar was derived from wheat husk through pyrolysis at a maximum temperature of 650 °C (Sonnenerde GmbH, Riedlingsdorf, Austria). Biochar was applied to plots at a rate of 20 t ha^{-1}, following recommendations from other studies (Streubel et al., 2011). Compost was derived from olive pomace (SimbioSoil®, BTM SIMBIOSIS, Manduria, Italy) and applied at a rate of 8 t ha^{-1}, comparable to the rates applied in other field studies (Diacono and Montemurro, 2011). Biochar and compost were incorporated into the top 15 cm of soil by two disk harrowing operations immediately before maize seeding. For the treatment Bio + Co, biochar and compost were applied at 20 and 8 t ha^{-1}. The physical and chemical characteristics of biochar and compost are reported in Table S2.

Arbuscular mycorrhizal fungal inoculation was performed by applying before maize seeding 11.7 g m^{-2} of crude inoculum (mycorrhizal roots, soil containing spores, and extraradical mycelium) produced as reported in Marrassini et al. (2024a). Soil tillage was performed in autumn 2020 and 2021 using moldboard plowing (30 cm soil depth) and disk harrowing (15 cm soil depth) before crop sowing. Maize was sown on 6 May 2021 at a density of 8.5 seeds m^{-2} (25,000 seeds ha^{-1}), with rows spaced 50 cm apart, while durum wheat was sown on 19 January 2022 at a density of 600 viable seeds m^{-2}, with rows spaced 13 cm apart in the same fields where maize was grown. Sowing was performed by a Pneumatic Precision Seeding Machine (Gaspardo, Padova, Italy). No mineral fertilizer was applied. Post-emergence weed control was performed twice on maize, in June with a disk harrow. On 5 July and 3 August 2021, 60 mm of irrigation (600 m^3 ha^{-1}) was applied on maize to prevent water stress. Insecticide DECIS® EVO (Bayer, Italy) (Deltamethrin 0.3 L ha^{-1}) was applied on 4 June 2021 to control *Agrotis* sp. (Lepidoptera: Noctuidae).

Roots and soil around the root system of maize were sampled at the stages of collar of 4th leaf visible (GS1; 3 June 2021) and pollen shedding (GS5; 29 July 2021), and in durum wheat at two leaves unfolded stage (GS12;17 March 2022) and anthesis half-way stage (GS64; 27 May 2022). At both stages, in 2021 and 2022, samples were taken randomly from the middle rows of each replicate plot. Ten plants per plot were excavated with their root system. Fresh root subsamples were used to assess AM fungal colonization. At the first sampling in each year, plants were also collected to determine shoot dry weight and nutrient concentration was measured on wheat grain at maturity. Soil subsamples from both stages and years were air-dried, passed through a 2 mm sieve, and stored at 6 °C for soil enzyme activity analyses, while soil subsamples from GS5 (maize) and GS64 (wheat) were stored for DNA extraction. At physiological maturity, maize and durum wheat plants were harvested by a plot harvester (14 September 2021 and 7 July 2022, respectively). In 2021, grain and stover (stalk plus leaves) from maize plants were collected, and shoot dry weight and grain yield were recorded, while in 2022, durum wheat grain yield was measured. Subsamples of grain were then stored at 6 °C for nutrient analyses.

2.4. Mycorrhizal colonization in maize and durum wheat roots

At each sampling, fresh root samples (10 combined subreplicates for each replicate plot) were gently washed to remove the attached soil using 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. These AM fungal root traits were assessed under an optical microscope following root clearing and staining and using a modified grid-line intersect method (McGonigle et al., 1990). Details are given in Supplementary Methods.

2.5. Plant growth, grain yield, and nutrient concentration

Grain yield and shoot dry weight of maize and wheat were determined by oven-drying samples at 65 °C until constant weight. Nitrogen (N) and phosphorus (P) concentrations in grain were determined using the Kjedahl method (Jones, 1991) and the ammonium-molybdophosphoric blue color method (Chapman and Pratt, 1961), respectively. Furthermore, the concentrations of potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), Fe, manganese (Mn), and Zn in grain were determined using a microwave-assisted acid digestion system (COOLPEX Smart Microwave Reaction System, Yiyao Instrument Technology Development Co., Ltd., Shanghai, China) followed by Microwave Plasma Atomic Emission Spectroscopy (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA). Details on sample preparation and the digestion process are given in Supplementary Methods. Additionally, the concentrations of N and P in maize shoot samples at GS1 were determined as described above.

2.6. Soil chemical and enzymatic analyses

At GS5(maize) in 2021 and at GS64 (wheat), soil samples (10 combined subreplicates for each replicate plot) were analysed for pH (1:2.5

w:v soil: distilled water) with a pH meter (XS Instruments, Carpi, MO, Italy), and inorganic C (IC) was determined using a Scheibler apparatus (Williams, 1948). The volume of CO_2 collected, barometric pressure, and temperature were recorded and used for the calculation of the percentage of $CaCO_3$ and the percentage of IC was obtained by multiplying by 0.12. Three technical replicates were analysed. Total C (TC) was determined by dry combustion method (Soon and Abboud, 1991) using a TruSpec Micro CHN elemental analyser (Leco CHN-600, St. Joseph, Michigan). Details on soil chemical analyses are given in Supplementary Methods. Total organic C (TOC) was estimated by subtracting the amount of IC from TC (TOC = TC-IC). The concentration of ammonium (NH_4^+-N) in soil was determined according to the method described by Keeney and Nelson (1982). Moreover, soil available P (P_{avail}) was assessed by colorimetry using a solution of sodium bicarbonate (Olsen and Sommers, 1982).

The following hydrolytic enzymes, involved in C, N, P, and sulfur (S) biogeochemical cycles, were analysed on soil at GS5 (maize) and at GS64 (wheat): β-glucosidase (β-gluc; EC 3.2.1.21), as key enzyme for cellulose, hemicellulose, and starch degradation; urea amidohydrolase = urease (Urease; EC 3.5.1.5), catalysing the hydrolysis of urea; alkaline phosphatase = phosphatase (ALP; EC 3.1.3.2) and arylsulphatase = sulphatase (Aryls; EC 3.1.6.1), catalysing the hydrolysis of organic phosphate esters and aryl sulphate ester, respectively; and dehydrogenase (DHA, alcohol dehydrogenase EC 1.1.1.1.) an indicator of overall microbial activity (Nannipieri et al., 2002). Details on the enzymatic analyses are given in Supplementary Methods.

2.7. Bacterial and fungal abundance in soil

At the stages GS5 (maize) and GS64 (wheat), DNA was extracted from a soil subsample (0.25 g dry soil per sample) from each replicate plot using the Dneasy PowerSoil Kit (QIAGEN, Venlo, Netherlands), following the manufacturer's instructions. To minimize bias in DNA extraction, DNA was extracted from three subreplicates from each replicate plots, and the DNA was then pooled before amplification. Relative abundance of fungi and bacteria in each replicate was quantified using a modification of the technique described by Fierer et al. (2005). Details on the quantitative PCR (qPCR) protocol are given in Supplementary Methods. A partial fragment of the 16S rRNA gene was used as bacterial target, while a fragment of the internal transcribed spacer 1 (ITS1) region was used as fungal target. The bacterial 16S rRNA gene was amplified using the primers Eub338/Eub518 (Eub338: 5′-ACT CCT ACG GGA GGC AGC AG-3′; Eub518: 5′-ATT ACC GCG GCT GCT GG-3′), and the fungal ITS1 was amplified using the primers ITS1f/5.8 s (ITS1f: 5′-TCC GTA GGT GAA CCT GCG G-3′; 5.8 s: 5′-CGC TGC GTT CTT CAT CG-3′) (Fierer et al., 2005). The efficiency of both primer pairs was similar (99.8 %). Each 96-well plate also contained reactions with tenfold serial dilutions of pure bacterial and fungal DNA from *Bacillus subtilis* and *Amanita rubescens* plasmids, respectively, to verify the linearity of the relationship between threshold cycle (Ct) and DNA concentration. Standard curves were generated using plasmid triplicates, ranging from 10^3 to 10^9 copies of the DNA template. Sample Ct values were divided by the mean slope of the standard curves across all runs to ensure that increases in fungal and bacterial abundance were equally weighted. Each sample was run in triplicate, and the mean Ct value was used for analysis. Gene copies were reported per g of dry soil. The fungal: bacterial ratio was calculated as the ratio of fungal ITS1 to bacterial 16S gene copy numbers and used as a predictor of organic residue decomposition and nutrient cycling (Delgado-Baquerizo et al., 2015).

2.8. Statistical analyses

A two-way analysis of variance (ANOVA) was performed to test the effect of organic amendment (Amend) and AM fungal inoculation (Inoc) (used as fixed factors) and their interaction on soil parameters, abundance of bacteria and fungi in soil, AM colonization traits, and plant parameters. Data were checked for normality of residuals using the Shapiro-Wilk test and for homogeneity of variances using the Levene's test. When necessary, data were transformed (e.g., $\log_{10}$ or arcsine) to meet these assumptions. Differences between means were determined using the post-hoc Tukey-B test. All analyses were performed through the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA).

A multivariate approach based on permutational analysis of variance (PERMANOVA) was used to test the effect of Amend, Inoc, and their interactions on soil (bio)chemical and biological parameters, and plant and AM fungal parameters. Data in the matrix were square root transformed and standardized, and Euclidean distance matrices were calculated. Principal coordinate analysis (PCoA) was conducted to visualize the most relevant patterns in the data. 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).

To understand the relationship between soil (bio)chemical and biological parameters, AM fungal traits (descriptors), and plant parameters (response variables) and to identify the main drivers responsible for plant productivity, a multivariate statistical approach (i.e., RELATE analysis) was applied. The RELATE analysis determined the strength of the correlation between the two matrices in a rank-order pattern of dissimilarity. The analysis was based on Spearman rank and 999 permutations, with ρ equal to 1 representing the perfect relationship. When the RELATE was significant, we displayed the graph. To identify the best descriptor of the relationship, the BEST analysis, based on BioEnv methods (all combinations), Spearman rank, and 999 permutations, was performed. All multivariate analyses were conducted using PRIMER 7 and PERMANOVA + software (Anderson, 2008; Clarke and Gorley, 2015).

3. Results

3.1. Effects on maize

3.1.1. Plant parameters

AM fungal inoculation (Inoc) significantly increased maize root colonization and the percentage of root length containing arbuscules and vesicles of maize at the early growth stage (GS1) (Table S3). Inoculation with *F. mosseae* mixture (FmMix) promoted AM colonization by 26 % and arbuscule abundance by 33 % compared to non-inoculated control (NM), whereas *A. trappei* mixture (ArMix) reduced both AM fungal colonization (51 %) and arbuscles occurrence (52 %) (Fig. 1a,b). Moreover, FmMix enhanced the percentage of root length containing vesicles compared to ArMix, while no difference was detected between FmMix and NM, which showed intermediate vesicle values (Fig. 1c). At the later growth stage (GS5), AM fungal colonization and percentage of vesicles were affected only by inoculation (Table S3). Notably, NM and ArMix exhibited higher colonization rates (80 %) than FmMix (67 %) (Fig. 1d). A similar trend was observed for vesicle abundance (Fig. 1e).

Maize shoot P concentration at GS1 was significantly promoted only by Inoc ($P = 0.001$) and no difference was found between AM fungal inocula (Fig. 1f). Inoculation influenced shoot dry weight at GS1 (Table S3), with both FmMix and ArMix yielding a mycorrhizal benefit of 3 % (Table S5). Conversely, shoot N concentration at GS1 was unaffected by treatments (Inoc: $P = 0.06$; Amend: $P = 0.805$; Amend x Inoc: $P = 0.789$).

Grain yield was affected by the interaction Amend x Inoc (Table S3). When organic amendments were not applied, inoculation with FmMix and ArMix increased grain yield, but differences were statistically significant only between not amended/not inoculated control and FmMix (Fig. 2a). Combining AMF with compost (Co), biochar (Bio), or both (Bio + Co) did not yield a significant increase over the effect of each factor operating independently (Fig. 2a). An image taken above the maize plots at GS5 is given in Fig. 2c.

The concentration of N, P, and Zn in maize grain was affected by the

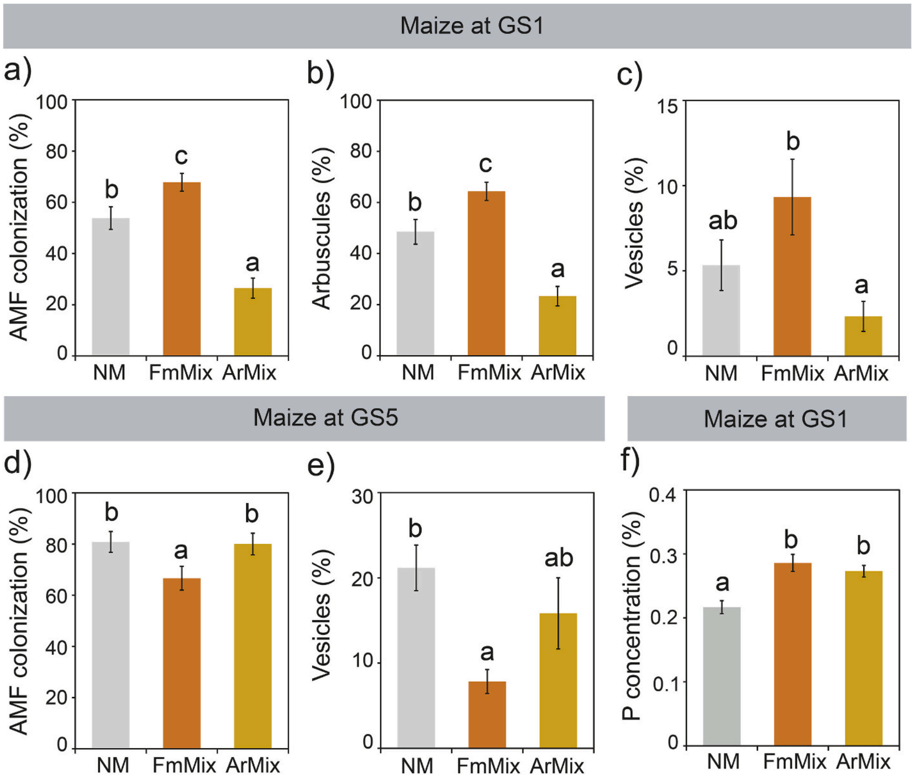

Fig. 1. Effect of inoculation with arbuscular mycorrhizal fungi (Inoc) on maize AM fungal root colonization (Col) (a), percentage of root length containing arbuscules (Arb) (b), percentage of root length containing vesicles (Ves) at collar of 4th leaf visible stage (GS1) (c), Col at pollen shedding (GS5) (d), Arb at GS5 (e), and P concentration in shoot at GS1 (f) ($n = 12$). Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S3).

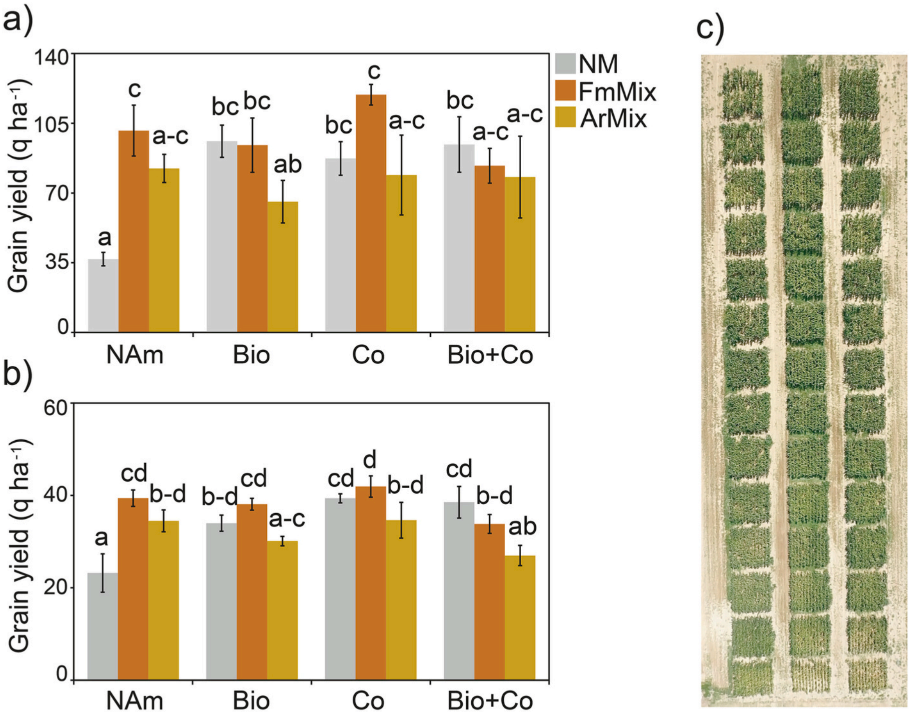

Fig. 2. Effect of the interaction between inoculation with arbuscular mycorrhizal fungi (Inoc) and organic amendment (Amend) on maize (a) and durum wheat (b) grain yield ($n = 3$). Picture of the experimental design in 2021 under maize cultivation (c). Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S3).

interaction Amend x Inoc while the concentration of Fe, Cu, and Ca was affected only by Inoc (Table S3). When organic amendments were not applied, inoculation with FmMix and ArMix increased N concentration in grain, while when Co and Bio + Co were applied, either alone or combined with AMF, N concentration was similar in all treatments (Fig. 3a). Finally, the application of Bio resulted in higher N concentration when inocula were not applied and in lower values when plants were treated with both inocula. Grain P concentration was increased by FmMix either applied alone or in combination with organic amendments, compared to not inoculated controls (Fig. 3c). Differences were higher and significant when FmMix was combined with Bio and Bio + Co. In contrast, ArMix either applied alone or in combination with all organic amendments did not modify grain P concentration. Grain Zn concentration was similar with all treatments, except for Co and Bio + Co, which nearly doubled Zn values (Fig. 3e). Compared to uninoculated control, grain Fe concentration was similarly increased by FmMix and ArMix, while Cu and Ca concentration were unaffected by FmMix and increased by ArMix (Fig. 3b,d,f).

3.1.2. Soil parameters

A significant effect of organic amendments on SOC was observed in the first year three months after application (Table S6). All organic amendments increased SOC compared to not amended control, but differences were statistically significant only for Bio and Bio + Co (Fig. 4a; Table S7). Soil ammonium concentration (NH_4^+-N) was significantly affected by the interaction Amend x Inoc (Table S6). When organic amendments were not applied, inoculation with FmMix and ArMix increased NH_4^+-N concentration compared with untreated control, whereas the combinations of AM fungal inocula and organic amendments produced similar increases respect to the individual treatments, except for the co-application of compost and ArMix, which reduced NH_4^+-N concentration compared with compost applied alone (Fig. 4b; Table S7). Soil available P (P_{avail}) was increased by both AM fungal inocula (Fig. 4d; Tables S6 and S7).

In maize at GS1, soil enzymes activities were differently affected by treatments: DHA was affected by the interaction Amend x Inoc, Urease and Aryls were affected by Amend and Inoc mean effects, while β-gluc and ALP were not modified by any treatment (Table S8). When amendments were not applied, DHA increased with both inoculants, but the increase was significant only with FmMix (Fig. 5a; Table S9). With the application of Bio, Co, and Bio + Co, the inoculants did not modify DHA, but values were higher with Co than with Bio. At the same plant growth stage, the highest value of Urease and Aryls were recorded with Co, intermediate values with Bio + Co and not amended control (Nam), and the lowest with Bio (Fig. 5b,d; Table S9). Urease and Aryls were also increased by FmMix compared to NAm and ArMix (Fig. 5c,e; Table S9).

At GS5, the application of amendment significantly affected DHA, β-gluc, Urease, and Aryls (Table S8). Compost resulted in the greatest Aryls and β-gluc activities, while NAm, Bio and Bio + Co produced lower and similar values (Fig. 5f,j; Table S9). Finally, DHA activity was highest with Co and Bio + Co and Urease with Bio + Co (Fig. 5g,h; Table S9). FmMix also increased β-gluc and ALP, while both AM fungal inocula

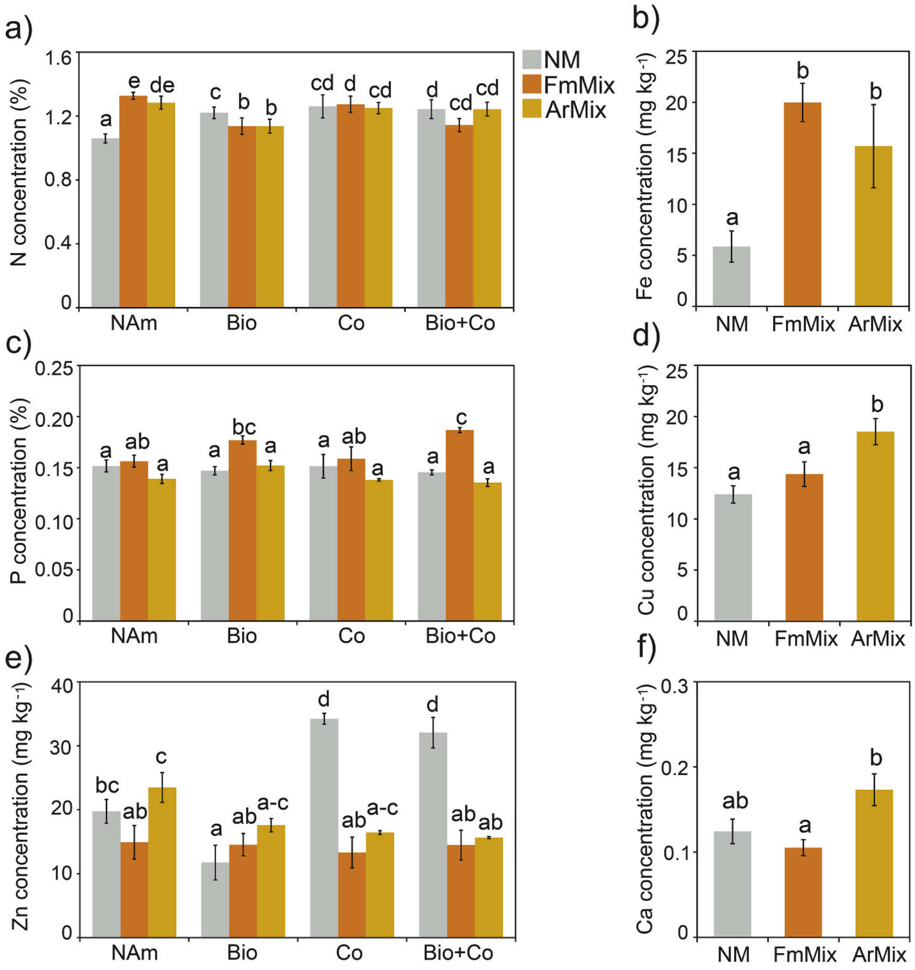

Fig. 3. Effect of the interaction between inoculation with arbuscular mycorrhizal fungi (Inoc) and organic amendment (Amend) on grain concentration of N (a), P (c), and Zn (e) in maize (n = 3). Effect of Inoc on grain concentration of Fe (b), Cu (d), Ca (f) in maize (n = 12). Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S3).

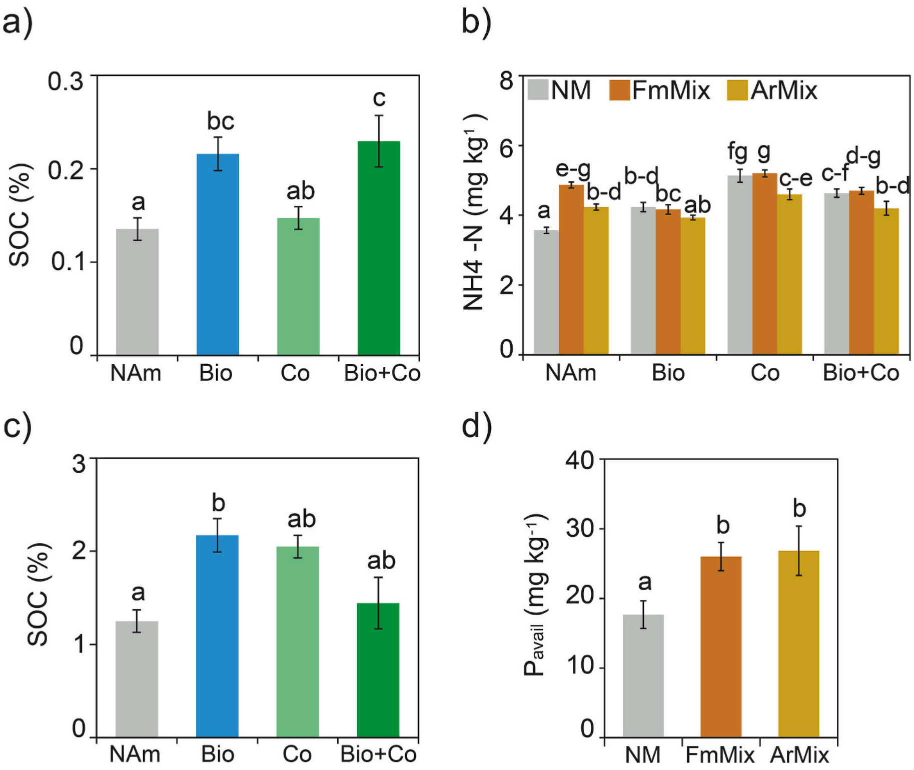

Fig. 4. Effect of organic amendments (Amend) on soil organic carbon (SOC) (a) under maize cultivation and on SOC (c) under durum wheat cultivation ($n = 9$). Effect of the interaction between inoculation with arbuscular mycorrhizal fungi (Inoc) and Amend on soil ammonium concentration (NH_4^+-N) (b) under maize cultivation ($n = 3$). Effect of Inoc on soil available phosphorus (P_{avail}) (d) under maize cultivation ($n = 12$). Soil samples were collected at the stage of pollen shedding (GS5) of maize. Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S6).

raised Urease compared to the non-inoculated control (Fig. 5i,k,l; Table S9).

Fungal biomass detected in soil of maize at GS5 was significantly affected by the interaction Amend x Inoc, while neither bacterial biomass nor fungal:bacteria ratio was affected by treatments (Table S6). When amendments were not applied or with Bio, fungal biomass was very low and unaffected by AM fungal inoculation, while with both Co and Bio + Co, inoculated or not with AMF, fungal biomass was significantly promoted (Fig. 6a; Table S7). When AM fungal inocula were co-applied with Bio + Co, fungal biomass was significantly increased compared with the Bio + Co.

3.2. *Effects on wheat*

3.2.1. *Plant parameters*

Wheat colonization traits were not affected either by inoculants and amendments or by their interaction (Table S3). Root colonization was almost at saturation at GS64, reaching 90 % across all treatments (Table S10).

Grain yield of wheat was affected by the interaction Amend x Inoc (Table S3). The pattern of response was similar to the one observed on maize, but differences among treatments were smaller (Fig. 2b). Both AM fungal inocula enhanced wheat grain yield compared to untreated control, reaching levels similar to those observed by OAs. However, combining AMF with Co, Bio, or both did not further increase yields beyond the individual treatments, except for ArMix combined with Bio + Co, which reduced grain yield compared to the uninoculated Bio + Co treatment. Wheat grain nutrient concentration was significantly affect by Inoc, while N and Ca were not affected by treatments (Table S3). FmMix increased grain K, Mg, and Fe concentrations (Fig. 7b,c,e), whereas ArMix enhanced P, Zn, and Cu (Fig. 7a,d,f).

3.2.2. *Soil parameters*

Soil organic C was significantly increased only by organic amendments (Table S6), but significant differences were observed only with Bio (Fig. 4c). By contrast, NH_4^+-N and P_{avail} in soil were not affected by any treatment.

At both stages of the growth cycle of wheat, the application of amendments to maize did not affect the activities of the tested enzymes, with the only exception of ALP at GS12 (Table S8). Conversely, inoculation significantly affected the activities of β-gluc, Urease, ALP, and Aryls. DHA was not influenced by any treatment. At GS12, ALP activity was greater with Co than with the other treatments (Fig. 8c; Table S9). At GS12 and GS64, FmMix promoted the highest activities of β-gluc, Urease, ALP, and Aryls, while uninoculated control and ArMix produced similar and lower values (Fig. 8a,b,d-i; Table S9).

Fungal biomass in soil collected at GS64 was significantly affected by the interaction Amend x Inoc, while neither bacterial biomass nor fungal:bacteria ratio was affected by treatments (Table S6). When amendments were not applied and with Bio or Co application, fungal biomass was very low and unaffected by AM fungal inoculation, while it was significantly increased when ArMix was applied with Bio + Co (Fig. 6b; Table S7).

3.3. *Mechanisms driving maize-wheat productivity*

PERMANOVA indicated significant direct (maize) and residual (wheat) effects of both Amend and Inoc, while the interaction of treatments was not significant (Tables S11 and S12). In the first year on maize, Amend and Inoc accounted for 30 % and 18 % of total variance, respectively, while their contributions declined in the second year on wheat (9 % and 19 %). In maize, Co and Bio + Co improved all measured parameters compared to other treatments (Fig. 9a). Inoculum with FmMix increased maize productivity traits, AM fungal and soil

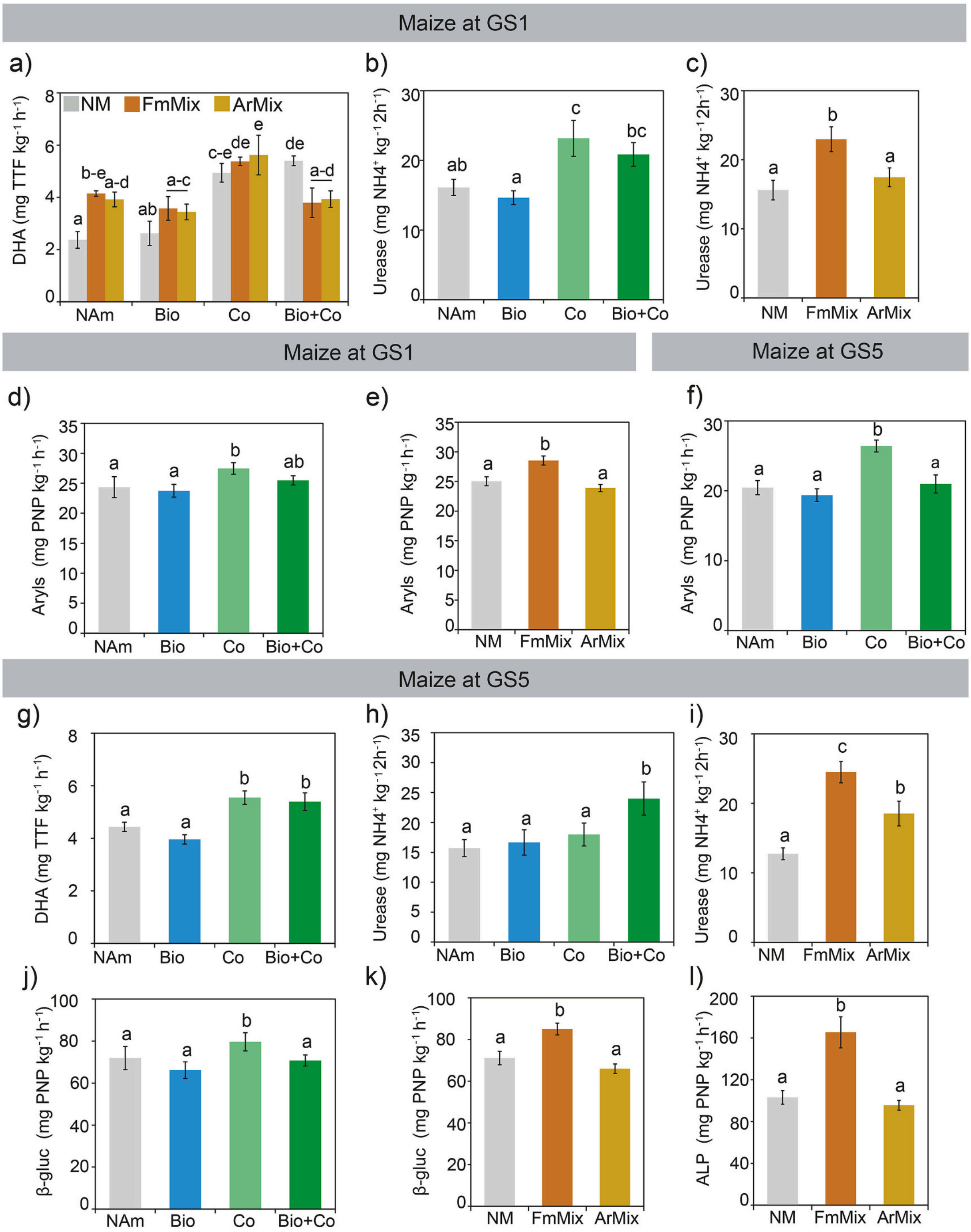

Fig. 5. Effect of the interaction between inoculation with arbuscular mycorrhizal fungi (Inoc) and organic amendment (Amend) on soil dehydrogenase (DHA) (a) at GS1 of maize ($n = 3$). Effect of Amend on urease (Urease) (b) and arylsulphatase (Aryls) (d) at GS1 of maize ($n = 9$). Effect of Inoc on Urease (c) and Aryls (e) at GS1 of maize ($n = 12$). Effect of Amend on Aryls (f), DHA (g), Urease (h) and on β-glucosidase (β-glu) (j) at maize GS5 ($n = 9$). Effect of Inoc on Urease (i), β-glu (k), and alkaline phosphatase (ALP) at GS5 (l) ($n = 12$). Soil samples were collected at the stage of pollen shedding (GS5) of maize. Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S8).

biochemical parameters relative to non-inoculated control, while ArMix was associated with higher Ca in grain and soil pH (Fig. 9b). In the second year on wheat, Co alone or with Bio was positively associated to grain yield and shoot dry weigh, and Aryls at GS1, while non-amended control and Bio were associated with AM fungal colonization traits at GS1 and N in grain (Fig. 9c). Furthermore, FmMix was associated to wheat productivity traits, Aryls at GS1, ALP at GS5, and AM colonization at early crop growth stage, while ArMix was related to higher Urease at GS1, microbial biomass (bacteria and fungi) at GS5, and DHA at GS1 (Fig. 9d). The control treatment had a pattern similar to FmMix. Moreover, when assessing the effects of Amend and Inoc on wheat, within-treatment variability exceeded the variability among treatments (see *PERDISP* values in Table S11). Finally, the plant-soil-fungal parameters associated with significant treatment differences were more numerous in the first year on maize than in the second on wheat (Fig. 9).

Significant relationships between key determinants and response variables were identified in both years of cultivation (maize: $\rho = 0.155$, $P = 0.029$; wheat: $\rho = 0.162$, $P = 0.039$) (Fig. S3a,b). However, the most

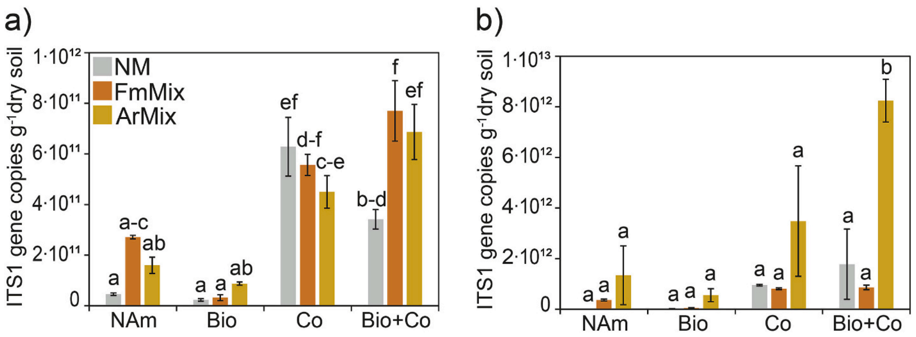

Fig. 6. Effect of the interaction between inoculation with arbuscular mycorrhizal fungi (Inoc) and organic amendment (Amend) on the abundance of fungi in soil under maize cultivation (a) and under durum wheat cultivation (b) (n = 3). Soil samples were collected at the stage of pollen shedding (GS5) of maize and at the stage of anthesis half-way (GS64) of wheat. Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S6).

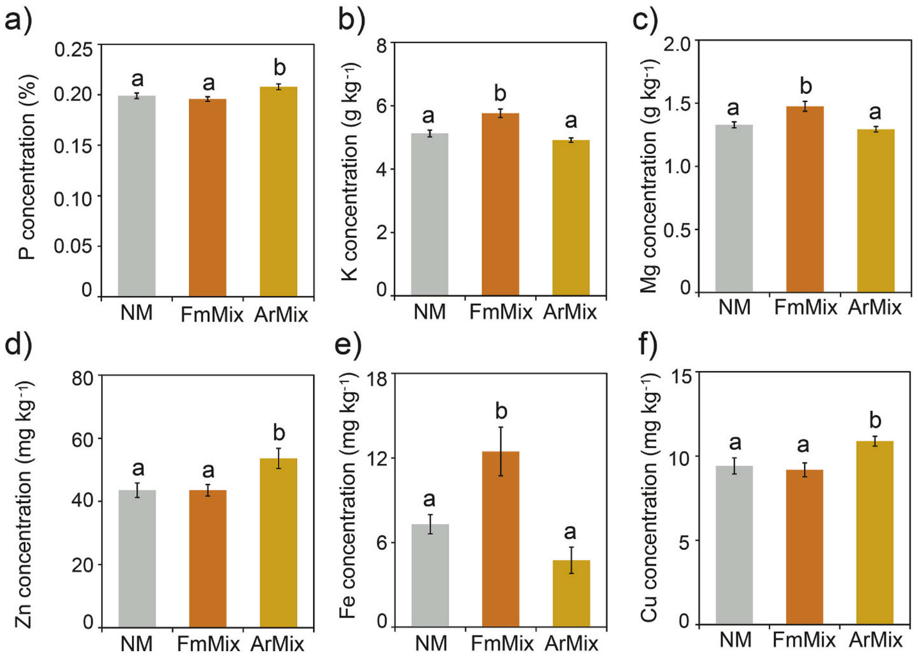

Fig. 7. Effect of inoculation with arbuscular mycorrhizal fungi (Inoc) on grain concentration of P (a), K (b), Mg (c), Zn (d), Fe (e), Cu (f) in durum wheat (n = 12). Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S3).

effective predictors of crop response were identified only for maize. The percentage of arbuscules at growth stage GS1 and soil enzymatic activity at both plant growth stages emerged as the strongest predictors ($\rho = 0.355$, $P = 0.016$) (Fig. S3c).

4. Discussion

4.1. Direct effect of AM fungal inoculation, organic amendments and their interaction on root colonization and productivity of maize, and soil fertility

Inoculation significantly increased maize root colonization traits at early growth stages (Fig. 1a,b). However, the co-application of biochar and/or compost with AMF did not affect mycorrhizal colonization, likely due to the low nutrient content of the amendments. Specifically, the olive pomace compost and wheat husk-derived biochar used in this study were characterized by low N and P content, which may explain their neutral effect on AM colonization, consistent with previous studies using similar materials (Warnock et al., 2007, 2010). Previous reports indicate that biochar may promote the development of AM fungal hyphae in soil and roots, depending largely on its physical properties, such as pore structure, surface area, and heterogeneity (Hammer et al., 2015). In our case, the biochar was classified as having medium specific surface area (SSA), which is generally less favourable for supporting AM fungal hyphal growth and microbial colonization compared to high SSA biochar. The latter provides greater surface complexity, which enhances soil aggregation and microbial microhabitats (Warnock et al., 2007, 2010).

The application of organic amendments and AMF improved grain yield but the effect was not additive (Fig. 2a). The lack of a significant yield effect from the combined application of AMF and organic amendment over each factor operating independently (i.e., complementarity) or over the sum of their effects (i.e., synergism) (Fig. 2a) is in line with our findings on AM fungal colonization and soil chemical parameters (Fig. 1a and Fig. 4a,b,d), and it is consistent with previous multi-year studies on compost and biochar co-application in the Mediterranean area (D'Hose et al., 2020). Notably, a complementary effect on grain P concentration was detected on maize, following the co-application of FmMix with compost and/or biochar (Fig. 4c). This effect may be attributed to enhanced root colonization at early growth stages (Fig. 1a) which likely improved P uptake and accumulation in maize grain, as previously reported by Mhlanga et al. (2022).

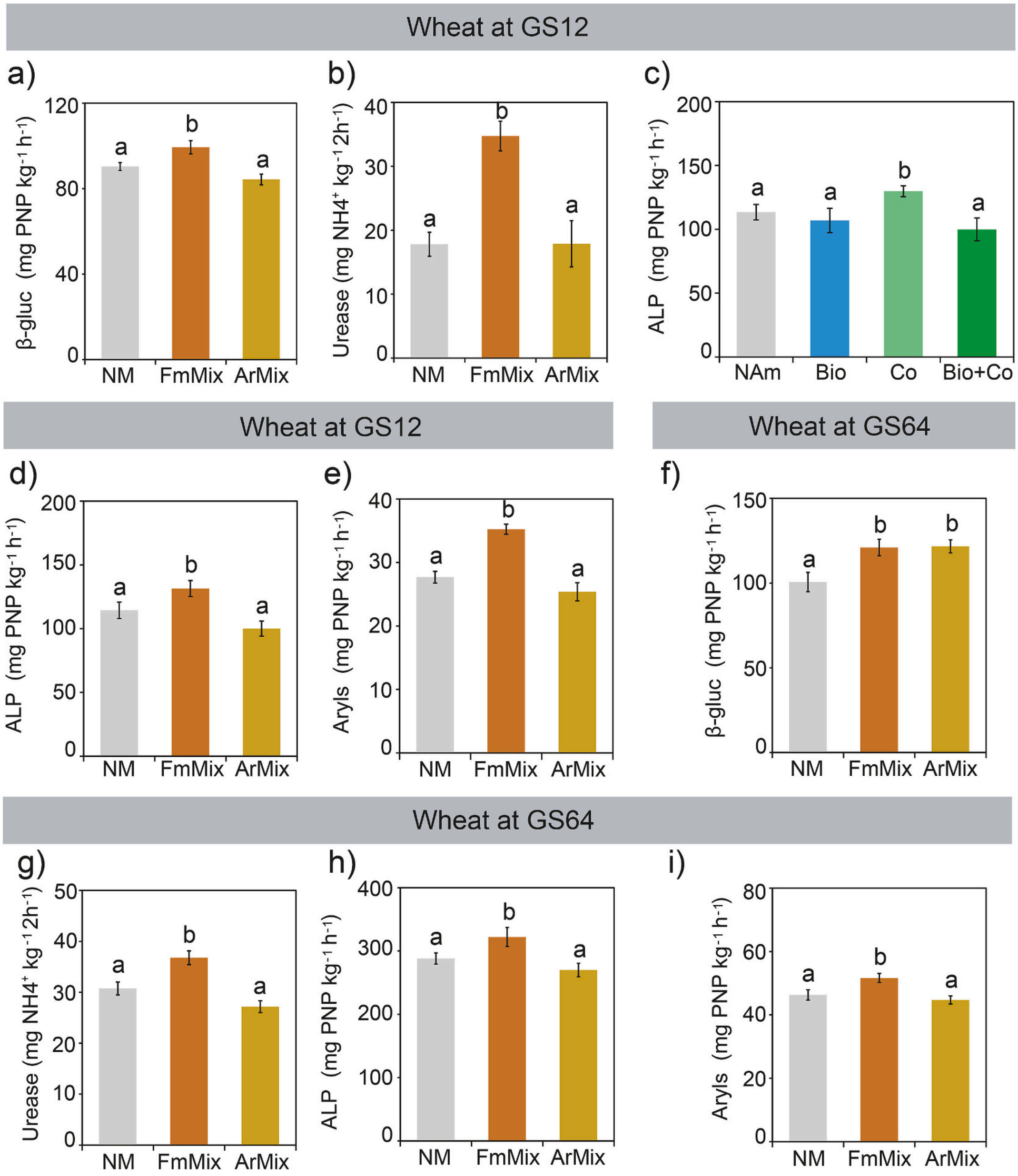

Fig. 8. Effect of inoculation with arbuscular mycorrhizal fungi (Inoc) on soil β-glucosidase (β-glu) (a), urease (Urease) (b), alkaline phosphatase (ALP) (d), and arylsulphatase (Aryls) (e) at GS12 of wheat (n = 12). Effect of Amend on ALP (c) at GS12. Effect of Inoc on β-glu (f), Urease (g) ALP (h), and Aryls (i) at anthesis half-way stage (GS64) of wheat (n = 9). Different letters show statistically significant differences according to the Tukey-B post-hoc test (Table S8).

The observed positive effect of AM fungal inoculation on maize grain nutrient content (Fig. 3b,d,f), coupled with the lack of additive or synergistic interaction with biochar and/or compost (Fig. 3a), suggests that AMF do not enhance the degradation of organic amendments or the uptake of nutrients released from compost and biochar, either directly or through interactions with other soil microorganisms (Hodge et al., 2001). This aligns with previous field studies reporting neutral effects of co-applying biochar and compost on crops such as peanut, sorghum, and maize (Agegnehu et al., 2015). The positive effect of AM fungal inoculation on grain N content, combined with the absence of an additive response when co-applied with organic amendments (Fig. 3a), supports recent field-based evidence that AMF preferentially absorb inorganic rather than organic forms of N (Wang et al., 2024). In similar field conditions, we previously observed increased grain N concentration following AM fungal inoculation, with the magnitude of the response influenced by crop or variety identity and soil N availability (Ercoli et al., 2017; Marrassini et al., 2024b, 2025). The increase in Zn content in maize grain following the application of compost and compost plus biochar, and its reduction when AMF were co-applied (Fig. 3e), can be explained by the high Zn release from compost, as previously reported in *Lolium perenne* treated with compost derived from olive oil extraction (Alburquerque et al., 2007). In contrast, AMF may limit Zn accumulation in plant tissues through a protective mechanism that regulates uptake under high soil Zn availability (Cavagnaro, 2008). This mechanism may also explain the lack of a significant effect on grain Zn concentration when AMF were applied alone, aligning with previous studies reporting no impact of AMF on Zn uptake in shoots under soils with non-deficient Zn availability (Lehmann et al., 2014).

Contrary to our hypothesis, the effect of organic amendments on soil chemical fertility was not enhanced by AM fungal inoculation (Fig. 4a,b, d). This is consistent with the unchanged fungal:bacterial ratio observed across all treatments (Table S7), indicating that AM fungal inoculation did not modify the balance of major microbial groups in the soil. Co-application of compost and/or biochar with AMF did not increase soil

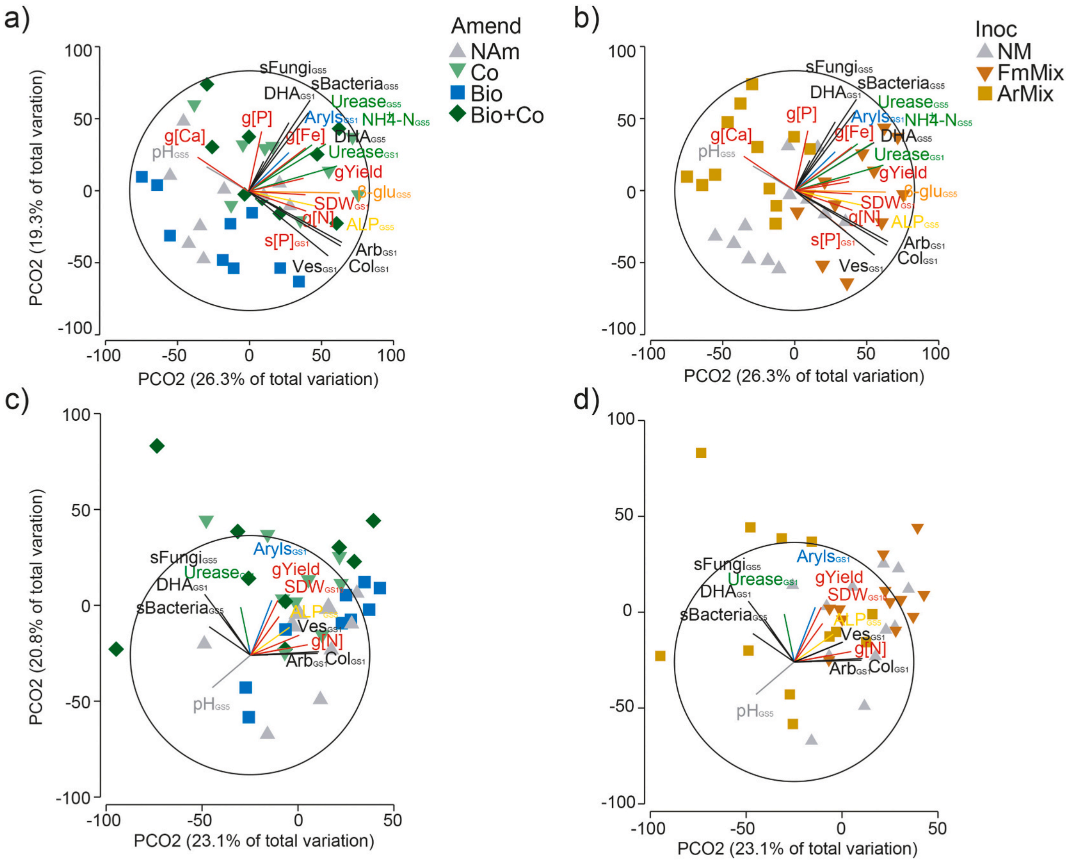

Fig. 9. Principal coordinate analysis (PCoA) biplot on the effect of inoculation with arbuscular mycorrhizal fungi (Inoc) on plant and soil biological and (bio) chemical parameters under maize (a) and durum wheat cultivation (c). PCoA biplot on the effect of organic amendments (Amend) on plant and soil parameters under maize (b) and durum wheat cultivation (d). The PCoA is based on the Euclidian distance matrix calculated on the square-root and standardized plant, AMF, and soil parameters measured at the stage of pollen shedding (GS5) of maize and at the stage of anthesis half-way (GS64) of wheat. In the plot, the overlay of vectors is reported and only the parameters with a good correlation ($r > 0.4$) are displayed. Plant parameters are shown in red: shoot dry weight (SDW), grain yield (gYield), harvest index (HI), and nutrient concentration in grain and shoot (e.g., g[P], s[P]). Biological and soil biochemical parameters linked to the microbial activity are shown in black: AM fungal root colonization (Col), percentage of root length containing arbuscules (Arb), percentage of root length containing vesicles (Ves); abundance of bacteria (sBacteria) and fungi (sFungi); soil dehydrogenase (DHA). Soil biochemical parameter linked to the C cycle is shown in orange: β-glucosidase (β-glu). Soil (bio)chemical parameters linked to the N cycle are shown in orange: urease (Urease) and ammonium concentration (NH_4^+-N). Soil biochemical parameters linked to the P and S cycle are shown in yellow and light blue, respectively: alkaline phosphatase (ALP) and arylsulphatase (Aryls).

C accumulation (Fig. 4a). This finding contrasts with the results of Mason et al. (2025) who reported that, in wheat mesocosms, the addition of AMF to biochar influenced soil C dynamics, reducing C mineralization and easily extractable C, and increasing soil C concentration compared to biochar alone. Our results provide further support for the idea that biochar with medium specific surface area (SSA), such as the one used in our study, may be less favourable for fungal hyphal network development. Moreover, similar to our results, the sole AM fungal inoculation of wheat and barley did not increase SOC.

Both AM fungal inocula and organic amendments increased soil NH_4^+-N levels (Fig. 4b). However, contrary to our expectations and previous results (Hodge et al., 2001; Cavagnaro, 2015), no additive effect was observed when they were applied in combination. This results, along with the increase in soil P availability detected under inoculation with both AM fungal mixtures, further supports the role of AMF in enhancing P acquisition and contributing to N cycling (Pellegrino et al., 2015). In our study, this effect is associated to similar and high AM fungal colonization levels at GS5 (Table S4) and unchanged bacterial biomass (Tables S6 and S7). The significant and positive effect of inoculation with AMF on crop yield (Fig. 2a,b) and N and P grain concentration (Fig. 3a and Fig. 7a) is also associated with enhanced multiple soil enzyme activities (Figs. 5 and 8). These effects align with meta-analyses showing AM fungal stimulation of soil enzymes involved in C, N, and P cycling, particularly in low and very low P soil availability (Qin et al., 2020), similar to those of our study (Table S1). However, AMF co-applied with compost and/or biochar did not further enhance soil enzymatic activity compared to single treatments (Fig. 5 and Table S8). This may be due to reduced plant dependence on AMF under soil amended conditions (Hammer et al., 2015), leading to similar root colonization across treatments (Table S4).

The observed synergistic increase of soil fungal biomass following the co-application of AMF with biochar and compost to maize (Fig. 6a) aligns with previous findings that reported positive shifts in the fungal component of soil microbial biomass after the application of four types of AM fungal inocula (i.e., single-species inoculants) in two tomato varieties grown under similar climatic conditions (Arcidiacono et al., 2024). Our result supports the successful development of the inoculated AMF or/and of saprotrophic fungi when organic amendments were co-applied. In contrast to Arcidiacono et al. (2024), where AM fungal inoculation led to increases in both bacterial biomass and fungal-to-bacterial ratio, no changes of these parameters were observed here in any treatment (Table S6). Contrary to our hypothesis, the application of organic amendments, either alone or in combination with AMF, did not influence the turnover of labile substrates, associated with bacterial activity, nor the decomposition of more recalcitrant organic material, linked to fungal activity.

The lack of interaction between AM fungal inoculation and organic amendment application is also confirmed by the multivariate analysis

(Table S11). This lack of interaction may be due, in addition to site-specific soil conditions, to the low quality of the applied organic amendments.

4.2. Residual effect of AM fungal inoculation, organic amendments, and their interaction on root colonization and productivity of wheat, and soil fertility

Neither AM fungal inoculation nor the application of organic amendments significantly affected wheat root colonization, which was high (ca. 80 %) (Table S3). This aligns with the saturation levels observed at physiological maturity across several wheat genotypes inoculated in the same environment (i.e., low fertility conditions) (Marrassini et al., 2024b). The absence of a measurable difference in root colonization between inoculated and control treatments could be due to a generally enhanced AM fungal propagule bank from the previous year's treatments, and not necessarily to the persistence of the inoculated taxa. However, it is also possible that inoculated AMF colonized wheat roots more than native strains although such differences are not detectable using the applied morphological identification techniques (Bender et al., 2019).

Our findings are also consistent with studies showing that mycorrhizal host crops, such as maize, can enhance the soil density of AMF, thereby improving root colonization in subsequent crops (Vestberg et al., 2005). Notably, the positive effects of AM fungal inoculation without organic amendments on wheat grain yield and nutrient uptake (Figs. 2b and 7), as well as on soil enzymatic activity (Fig. 8), support the persistence of the inoculated AMF across the crop rotation.

The lack of effect of AM inoculation on SOC accumulation and the absence of interaction between AMF and organic amendments (Fig. 4c), despite contrasting with our hypothesis and recent findings by Mason et al. (2025), are in agreement with our first-year observations (Fig. 4a).

A clear synergistic effect was observed on the soil fungal biomass only with ArMix combined with biochar and compost (Fig. 6b). This singular synergistic effect is in line with the results recorded on maize (Fig. 6a) and is likely to be due to the higher extraradical hyphal volume produced by members of Archaeosporaceae family (Koch et al., 2017; Pan et al., 2021).

Despite the demonstrated ability of AM fungal inoculants to enhance agricultural productivity under specific conditions (Pellegrino et al., 2015), their widespread adoption at the farm scale remains limited due to the high cost of AM inoculum production and application (Mason et al., 2025). Nonetheless, the persistent general benefits observed on wheat following the inoculation on maize, suggests that AMF can have long-lasting effects, mirroring earlier findings on maize following inoculated Egyptian clover (Pellegrino et al., 2011), This indicates that annual inoculation may not be necessary, offering a potential strategy for cost reduction over time.

4.3. Plant and soil responses differences between AM fungal taxa

The higher rate of mycorrhizal colonization detected at the early crop growth stage of maize inoculated with *F. mosseae*, compared to *A. trappei* (Fig. 1a-c), highlights interspecific differences in infectivity, consistent with previous findings under controlled conditions using the same isolates (Marrassini et al., 2024a). This supports the well-established ability of AMF from the Glomeraceae family (i.e., *F. mosseae*) to rapidly and extensively colonize host roots, in contrast to the lower infectivity of ancient Archeosporaceae family (Hart and Reader, 2002; Säle et al., 2021). However, because the study was conducted under field conditions, the native AM fungal community may have influenced the observed interspecific variability. The superior performance of the *F. mosseae* mixture compared to *A. trappei* suggests that *F. mosseae* is more competitive than *A. trappei* against the native AMF.

The early colonization of maize roots by *F. mosseae* was likely facilitated by the specific soil conditions of our site, characterized by very low soil P availability, moderately alkaline pH, and low N level (Table S1), along with a putatively low abundance of native AMF. These findings align with previous studies showing that native AM fungal abundance is reduced under P and N fertilization and tends to be lower in acidic soils respect to alkaline ones (Bender et al., 2019).

At the later growth stage, *A. trappei* outperformed *F. mosseae* in both root colonization rate and vesicle formation (Fig. 1d,e), suggesting that its slower initial development was offset by greater infectivity at later stages. However, the differential response between inoculated AM fungal taxa is also attributable to a combination of the inoculated strains and the stimulated native AM fungal community. To our knowledge, this is the first field study investigating inoculation with members of Archaeoporaceae, contributing to a deeper understanding of the infectivity dynamics of this less-studied fungal group in competition with native AMF.

Nonetheless, the higher AM fungal colonization rate observed in maize roots inoculated with *A. trappei* mixture at the later stage, compared to *F. mosseae* (Fig. 1d) is supported by the colonization levels similar to not-inoculated maize (Fig. 1d), as well as by the consistent AM colonization rates observed across treatments in wheat. It is important to note that morphological assessments does not differentiate among AM fungal taxa colonizing roots. To distinguish the inoculated isolates from native AMF, molecular approaches such as PCR-based identification, sequencing, or qPCR targeting specific AM fungal taxa would be required (Alkan et al., 2006).

Interspecies variability was also observed in crop nutrient uptake, with ArMix outperforming FmMix for Ca and Cu in maize, and P, Zn, Cu and soil fungal biomass in wheat (Figs. 3, 6, 7), and FmMix outperforming ArMix for K, Mg, and Fe in wheat. However, no significant variability between AM fungal species was found in grain yield of either crop (Fig. 2). Therefore, our data do not support the higher efficiency of *F. mosseae* compared to the members of the Archaeosporaceae family, as reported in the comprehensive study by Säle et al. (2021), nor do they align with previous findings using the same isolates inoculated on leek under controlled conditions (Marrassini et al., 2024a). In that study, although the magnitude of the effect largely varied among isolates of the same species (Marrassini et al., 2024a), no interspecies variability was detected. The higher efficiency of ArMix we found in increasing certain nutrient levels may be attributed to greater extraradical hyphal development relative to root colonization compared with Glomeraceae (Koch et al., 2017; Pan et al., 2021).

Enzyme activity stimulation varied between treatments, with FmMix generally performed better than ArMix (Figs. 5 and 8). This differential response between AM fungal species may be attributed to differences in hyphal development, which can create distinct niches that influence microbial communities and soil enzymatic activity (Veresoglou et al., 2012).

Although both AM fungal inocula improved grain yield and nutrient uptake in both years of cultivation, the superior performance of ArMix in certain parameters (Fig. 9) highlights its potential for field inoculation in Mediterranean soils, which are typically low in nutrient availability and biological fertility. However, the observed differences between *F. mosseae* and *A. trappei*, whether due to direct or indirect effects, do not justify a clear preference for one taxon over the other.

The observed relationship between early-stage arbuscule formation and maize productivity (Fig. S2) highlights the essential role of arbuscules as sites of nutrient exchange, particular for P (Wipf et al., 2019). Pi transporters expressed in both the extraradical mycelium and the arbuscules facilitate efficient P delivery to the host plant (Wipf et al., 2019). Similarly, soil enzyme activity emerged as a key parameter associated with crop productivity (Fig. S3). For example, stimulation of urease activity was attributed to AMF-induced promotion of urease-producing microbial taxa (Innangi et al., 2017). However, the simultaneous increases in microbial biomass and urease activity under AM fungal inoculation suggest that this effect is not solely indirect. Instead, it points to a complex interaction between AMF, microbial communities, and nutrient cycling processes (Veresoglou et al., 2012; Innangi et al., 2017).

5. Conclusions

This study highlights the complexity of interactions among AMF, compost, and biochar in a maize-wheat rotation system under Mediterranean conditions. While both organic amendments and AM fungal inoculation individually enhanced crop performance and improved soil (bio)chemical properties, their combined application often led to neutral effects. This indicate that their interactions may not always be complementary or synergistic and instead could involve antagonistic or competitive dynamics depending on specific context-factors. Overall, our findings emphasize that the effectiveness of AMF and OAs is strongly influenced by multiple variables, including the physicochemical properties of the amendments, soil conditions, crop species and AM fungal identity. Although functional variability between AM fungal taxa was observed, the superior agronomic performance of *A. trappei* for certain nutrients compared with the commonly used *F. mosseae* indicates that lesser-known AM fungal taxa hold promise as effective inoculants. These results highlight the need to broaden the range of AM fungal species considered for inoculum development, especially for low-fertility soils typical of Mediterranean regions. Furthermore, the inclusion of a highly mycotrophic crop like maize in the rotation may help sustain or enhance AM fungal propagule density, potentially benefiting subsequent crops.

CRediT authorship contribution statement

Valentina Marrassini: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Laura Ercoli:** Writing – review & editing, Resources. **Roberto Cardelli:** Writing – review & editing. **Valentina Cantini:** Writing – review & editing. **Elisa Pellegrino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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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 influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.181292.

Data availability

DATASET is available as supplementary Material

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