Plant Soil
https://doi.org/10.1007/s11104-025-07986-3

RESEARCH ARTICLE

Arbuscular mycorrhizal fungi inoculation enhances ^{15}N uptake by rice via modifying rhizosphere and hyphosphere soil bacterial and fungal community and soil enzyme activity

Pei Chen · Yu Cheng · Rui Chen · Ning Wang · Jianguang Yu · Lihong Xue · Linzhang Yang

Received: 31 March 2025 / Accepted: 5 October 2025

Abstract

Background and aims The interaction between arbuscular mycorrhizal fungi (AMF) and rice roots is essential for regulating nitrogen (N) uptake through roots and AM fungal hyphae. However, the effects of microbial community traits and activities in the rhizosphere and hyphosphere on plant N uptake under varying phosphorus (P) fertilization levels remain insufficiently understood.

Methods This study used ^{15}N-urea to assess N fertilizer uptake and retention, and measured soil chemical properties, soil enzyme activities, and the diversity, composition, and network complexity of bacterial and fungal communities within the root compartment (RC) and hyphae-only compartment (HC) for rice with and without AMF inoculation across high- and low-P fertilization levels.

Results AMF inoculation significantly enhanced urea-N accumulation in plants by 15.4–16.0% across both P levels, likely due to increased root biomass and AMF abundance. In RC soil, AMF inoculation elevated protease and cellulase activities, possibility by increasing the abundance of specific genera (e.g., *Clostridium sp.* and *Cystobasidiales*) and enhancing fungal community complexity, which could boost root biomass and ^{15}N uptake. Conversely, in HC soil, AMF inoculation elevated protease, β-glucosidase, and phosphatase activities, possibility by augmenting the abundance of specific genera (e.g., *Bacillus* and *GS16*), which could boost AMF abundance and ^{15}N uptake through AM fungal hyphae. In low-P input soil, AMF inoculation significantly increased the complexity of microbial communities and enhanced phosphatase and urease activities in HC soil, resulting increased AMF abundance and greater ^{15}N uptake through AM fungal hyphae. In contrast, in high-P input soil, root N uptake might be predominant due to increased root biomass facilitated by AMF inoculation.

Responsible Editor: Xinhua He.

Pei Chen and Yu Cheng contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-025-07986-3.

P. Chen · Y. Cheng · R. Chen · N. Wang (✉) · J. Yu · L. Xue · L. Yang
Key Laboratory of Agro-Environment in Downstream of Yangtze Plain, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China
e-mail: wang.ning4113@163.com

Y. Cheng · N. Wang
School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang 212001, Jiangsu, China

R. Chen · N. Wang
College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210014, China

Published online: 14 October 2025

Conclusion Our findings underscore the importance of rhizosphere and hyphosphere microbial communities and soil enzyme activities in optimizing N uptake via roots and AM fungal hyphae.

Keywords Arbuscular mycorrhizal fungi · N fertilizer uptake · Rhizosphere and hyphosphere microbial community · Soil enzyme activity

Introduction

Rice (*Oryza sativa L.*) is among the most essential cereal crops globally, serving as a staple food for over half of the world's population (Food and Agriculture Organization of the United Nations 2024). China's staple crop production is marked by excessive application of synthetic nitrogen (N) fertilizers and insufficient nitrogen use efficiency (NUE) (Liu et al. 2024). Approximately 80–84% of the total nitrogen input for staple crops in China is derived from synthetic fertilizers, which constitute 54% of the national synthetic nitrogen fertilizer usage (National Bureau of Statistics 2021; National Development and Reform Commission 2021; Sapkota et al. 2023). Notably, the NUE of staple crop production in China ranges from 0.20 to 0.24 (Sapkota et al. 2023), significantly below the global average of 0.42 (Zhang et al. 2015).The current application of large quantities of nitrogen (N) fertilizers in rice production has resulted in significant N losses through ammonia volatilization, nitrous oxide emissions, runoff, and leaching (Zhang et al. 2015). Arbuscular mycorrhizal fungi (AMF), a ubiquitous group of soil fungi, form symbiotic associations with the roots of various crops (Yimer et al. 2024; Li et al. 2025; Sato et al. 2025). Historically, the lower rates of AMF colonization and diversity were attributed to paddy fields, causing earlier studies to often overlook their diversity and ecological functions in these ecosystems (Ilag et al. 1987; Lumini et al. 2011). However, advancements in high-throughput sequencing technologies over the past decade have demonstrated that AMF can effectively thrive in paddy wetland environments (Jiang et al. 2020). In this mutualistic association, plants supply AMF with carbohydrates and lipids as carbon sources for their metabolic needs (Balestrini et al. 2020; Kakouridis et al. 2024). In return, AMF facilitate the absorption of water and nutrients, including N and phosphorus (P), from deeper soil strata through an extensive hyphal network, transporting them to the roots for plant uptake (Smith et al. 2004). Consequently, AMF plays a critical role as a biological fertilizer in promoting sustainable agriculture.

Nitrogen is a vital nutrient essential for plant growth and development. Roots and AM fungal hyphae represent two distinct strategies employed by plants for soil N foraging (Veresoglou et al. 2012; Hestrin et al. 2019; Wang et al. 2020). Increasing evidence underscored the critical role of AMF in plant N uptake, transport, and metabolism (Sun et al. 2024; Wang et al. 2025c), with studies indicating that the proportion of N supplied by AM fungal hyphae to plants can range from less than 10% to as much as 75% (Hui et al. 2022; Xie et al. 2022; Shi et al. 2023). Research on N foraging strategies has focused on either the root system (Wang et al. 2023c) or mycorrhizal fungi (Wu et al. 2021; Bicharanloo et al. 2023). However, relatively few studies have examined the combined role of roots and AM fungal hyphae in plant N acquisition (Zhang et al. 2019, 2022b; Yang et al. 2022).

The primary function of AMF has traditionally been recognized as enhancing P acquisition in host plants (Müller and Harrison 2019; Wang et al. 2023a). Recent studies have shown that AMF could recruit bacteria harboring phosphatase genes and alter the bacterial community composition to increase alkaline phosphatase (ALP) activity on their hyphal surfaces (Zhang et al. 2018). Additionally, AM fungal hyphae serves as "highways" for bacterial movement, facilitating access to soil organic P patches and improving the utilization of soil organic P (Jiang et al. 2021; Keyes et al. 2022). However, increased soil P availability could reduce plant dependence on AMF, shifting nutrient uptake from AM fungal hyphae to direct acquisition by the root system (Lambers et al. 2015; Werner and Kiers 2015), particularly when P is a limiting factor for plant growth in the soil environment. Consequently, the effects of AMF on N transformation and plant uptake might vary under different P fertilization levels (Liu et al. 2021; Thaqi et al. 2025). High levels of mineral P fertilizers may enhance N uptake by plant roots, whereas low levels of mineral P fertilizers may promote N uptake via AM fungal hyphae. However, the influence of P fertilizer levels on N uptake in AM-associated plants remains poorly understood.

Soil microorganisms are known to play a crucial role in soil nutrient cycling (Delgado-Baquerizo et al.

2016; Naylor et al. 2020). However, its activity and functionality are often limited by a lack of carbon (Demoling et al. 2007; Wan et al. 2015). This underscores the importance of soil zones rich in organic carbon compounds, such as the rhizosphere, which serve as critical hotspots for biogeochemical transformations involving microorganisms (Berendsen et al. 2012; Fan et al. 2025). Although AMF can deliver N to their plant hosts, a synergistic feedback through mycorrhizally-driven plant growth may also provide resources for additional photosynthate production, which can stimulate rhizosphere soil microbial communities and activities that support continued plant growth and nutrient uptake (Hestrin et al. 2019). Recent studies have demonstrated that the interactions between AMF and rice rhizosphere microbes significantly influenced rice growth and soil structural properties (Brahmaprakash et al. 2017; Mitra et al. 2021; Bao et al. 2022). Various microbial communities have been shown to interact with AMF in the rice rhizosphere, including bacteria and fungi (Mendes et al. 2013; Breidenbach et al. 2016). Compounds exuded from rice roots (e.g., organic acids, amino acids, polysaccharides, and hormones) and microbial secretions (e.g., volatile organic compounds [VOCs], 1-aminocyclopropane-1-carboxylate deaminase [ACCD], and polysaccharides) play a critical role in sustaining the symbiotic relationships among rice plants, AMF, and soil microorganisms (Glick 2014; Kai et al. 2016; Wang et al. 2025a).

Similar to the rhizosphere, AM fungal extraradical hyphae release carbon-rich exudates into the surrounding soil, stimulating microbial growth and recruiting distinct microbial communities to establish the hyphosphere microbiome, which facilitates nutrient uptake (Wang et al. 2023b). Recent in situ experiments have compared soil containing AM hyphae with soil where AM fungi were excluded. Using amplicon sequencing, these studies demonstrated significant differences in bacterial communities between soil with AM hyphae and bulk soil (Zhou et al. 2020a; Emmett et al. 2021; Zhang et al. 2022a). High-throughput stable isotope probing further revealed that specific bacterial phyla associated with AM hyphae assimilated the majority of carbon derived from AM fungi (Nuccio et al. 2022). Additionally, a recent study found that mycorrhiza-mediated recruitment of complete denitrifying Pseudomonas bacteria effectively reduced N_2O emissions from soil (Li et al. 2023). Collectively, these findings underscored the critical role of interactions between soil microbes and AMF in both the rhizosphere and hyphosphere in regulating N uptake by plant roots and AM hyphae (Duan et al. 2024).

Soil microorganisms do not exist in isolation but instead form intricate ecological interaction networks that sustain ecosystem functions (Wagg et al. 2019). Co-occurrence networks are commonly employed to evaluate interactions among microorganisms and their responses to environmental changes (Ma et al. 2020). Studies have shown that AMF inoculation can enhance the complexity and stability of soil microbial networks in agricultural systems (Huang et al. 2024). However, AMF inoculation may also reduce the complexity of microbial community networks due to competition between AMF and other microbes (Wang et al. 2024). The overall impact of AMF inoculation is shaped by factors such as environmental conditions, community composition, and the functional demands of the ecosystem. Nonetheless, it remains unclear whether microbial network responses to AMF inoculation are consistent in the rice rhizosphere and hyphosphere under varying P fertilization levels.

Soil enzyme activities, which reflect the level of soil microbial activity, are crucial for enhancing plant N uptake (Nannipieri et al. 2018). AMF could alter the structure and metabolic activity of soil microbial communities, thereby affecting soil enzyme activity (Ye et al. 2015). Research has demonstrated that AMF directly increased soil N availability by stimulating the activity of soil enzymes, thereby supporting plant growth and N uptake (Zhang et al. 2016; Qin et al. 2020a). However, in certain competitive environments, AMF may inhibit soil enzyme activity, potentially limiting nutrient cycling (Zhou et al. 2020b). Overall, AMF could regulate soil N uptake by influencing soil enzyme activity. Nonetheless, the effects of AMF on soil enzyme activities related to carbon, nitrogen, and phosphorus cycling are complex and multifaceted, encompassing both stimulatory and inhibitory impacts. Importantly, the role of rhizosphere and hyphosphere microbiomes, along with soil enzyme activities, in facilitating N uptake in mycorrhizal rice remains insufficiently explored.

To address these gaps, paddy soil was divided into a root compartment (RC) and a hyphae-only compartment (HC). Two P input levels were applied, and rice seedlings were cultivated with and without AMF

inoculation under greenhouse conditions. To evaluate N fertilizer uptake and retention in the rice-soil system, ^{15}N-urea was used. This study also examined the responses of soil chemical properties, soil enzyme activities, the diversity, composition, and network complexity of bacterial and fungal communities to AMF inoculation in RC and HC soils under varying P fertilizer input levels. The primary objective is to identify the factors contributing to N fertilizer uptake in RC and HC soils with AMF inoculation. We hypothesized that AMF inoculation may stimulate microbial growth and soil enzyme activities by increasing root exudates and carbon-rich exudates from AM fungal hyphae in the soil, thereby producing more nutrients to support plant growth in RC and AMF growth in HC soils, ultimately improving N uptake by roots and AM fungal hyphae.

Materials and methods

Soil collection and AMF inoculum

Soil samples were collected in May 2023, following the wheat harvest, from a typical rice–wheat rotation field in Changzhou, Jiangsu, China (31°46′48″ N, 119°57′0″ E). The soils belonged to yellow mud soil with high fertility, deriving from lacustrine sediment. After collection, the soil was air-dried, sieved through a 5-mm mesh, and homogenized for use in the microcosm. Soil properties were determined including pH (6.39, 1:2.5 of soil-to-water ratio), total organic carbon (TOC, 11.6 g kg^{-1}), total nitrogen (TN, 1.10 g kg^{-1}), alkali-hydro nitrogen (AN, 86.0 mg kg^{-1}), available phosphorus (AP, 27.5 mg kg^{-1}), and available potassium (AK, 131 mg kg^{-1}).

Rhizophagus irregularis BGC BJ09, a model AMF species, was commercially supplied by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. *Rhizophagus irregularis* was propagated on maize and then the AMF inoculum consisted of cultured sands, colonized root fragments, spores and hyphae.

Experimental design

A microcosm experiment was conducted in the greenhouse at Jiangsu Academy of Agricultural Sciences (JAAS). Two factors were considered: two levels of AM fungal inoculation, either rice root inoculation or non-inoculation with *Rhizophagus irregularis*, and two P input levels, either 18.8 mg P kg^{-1} or 37.5 mg P kg^{-1}. All treatments were replicated three times. Initially, rice seeds (cv. *Nangeng 46*) were sterilized in 10% hydrogen peroxide (H_2O_2) for 10 min and then thoroughly rinsed with deionized water. Three days post-germination, 0.5 kg of seedlings (fresh weight) were mixed with 12.5 g of AMF inoculum (*Rhizophagus irregularis*, 70 spores g^{-1}), which included culture media with spores, hyphae, and colonized root pieces. Rice seedlings inoculated with sterilized AMF inoculum served as the control treatment without AMF inoculation.

The soil was then supplemented with potassium chloride (37.5 mg K kg^{-1}) and calcium phosphate monobasic monohydrate (either 37.5 mg P kg^{-1} or 18.8 mg P kg^{-1}). N fertilizer was applied as ^{15}N-urea at a rate of 100 mg N kg^{-1}. Subsequently, 3.0 kg dry weight (dw) of soil with fertilizer application was transferred into rhizo-bags (30 μm nylon mesh, diameter 10 cm × height 20 cm), which were placed in the center of polyvinyl chloride pots (diameter 15 cm × height 23 cm). An additional 9.0 kg dw of soil was added to fill the space outside the rhizo-bags. The two-compartment microcosms consisted of a root compartment (RC) and a hyphae-only compartment (HC), separated by rhizo-bags constructed from 30 μm nylon mesh (Fig. S1). Consequently, the application rate of fertilizers were consistent between the RC and HC soils. Finally, rice seedlings, with and without AMF inoculation, were transplanted, with three seedlings per pot. Following transplantation, rice was cultivated under alternating wet and dry conditions to provide an aerobic environment conducive to AMF growth.

At maturity, 132 days after transplantation, rice plants were removed from the rhizo-bags. Root samples were separated from shoots, washed, and weighed for root biomass analysis. Fine roots were randomly collected for root mycorrhizal colonization assessment. Remaining roots, shoots, and grains were dried, weighed, and analyzed for plant biomass, tissue N and P content, and ^{15}N abundance. The soils from RC and HC were sampled for analyses of soil chemical properties, soil enzyme activities, and bacterial and fungal community structure.

Mycorrhizal root colonization and AMF abundance in RC and HC soils analysis

To assess mycorrhizal colonization of rice roots, root fragments stored in 50% ethanol were cleared in 10% potassium hydroxide (KOH) and stained with 5% ink-vinegar, following the protocol described by Vierheilig et al. (1998). The percentage of total mycorrhizal colonization and the frequency of hyphae, arbuscules, and vesicles were determined using the magnified intersections method, examining 100 intersections per sample under a microscope at 200 × magnification (Mcgonigle et al. 1990).

AMF abundances in RC and HC soils were assessed by quantifying the copy numbers of the AMF gene using the primer sets AMV4.5NF/AMDGR (Lumini et al. 2010). The qPCR assays were conducted in triplicate using a real-time PCR system (ABI 7500, Applied Biosystems, USA). Gene standard curves were constructed based on gradient dilutions of standard plasmids with known copy numbers. To ensure accurate amplification, DNA extracts were highly diluted to eliminate inhibition. Negative controls without DNA templates were included in each batch of gene amplification to ensure no contamination during qPCR. Additionally, gene quantification for each sample was performed in three parallel real-time PCR reactions, with reaction efficiencies greater than 90% and correlation coefficients (r^2) greater than 0.99 being accepted. Target gene copy numbers for each sample were calculated from the standard curves and expressed on a soil dry weight basis (copies g^{-1} dw soil).

Plant biomass, plant N and P content, soil chemical properties, and soil enzyme activities analysis

Root, shoot, and grain samples were initially oven-dried at 105 °C for 30 min to halt plant metabolic activity, then dried to a constant weight at 65 °C. Subsequently, N and P concentrations in the root, shoot, and grain samples were determined using an elemental analyzer (Vario MICRO cube, Elementar, Germany) and the molybdenum-antimony anti-spectrophotometric method, respectively. N and P concentrations are listed in supporting information Fig. S2.

The pH of RC and HC soils were measured by mixing the soil with carbon dioxide-free water at a soil-to-water ratio of 1:5 (w/v) using a pH meter (XL60, Fisher Scientific, USA). Total organic carbon (TOC) was measured using a TOC analyzer. Total nitrogen (TN) was measured using an elemental analyzer (Vario MICRO cube, Elementar, Germany), while alkali-hydrolyzable nitrogen (AN) was measured using the alkali solution diffusion method (Lu 2000). Available potassium (AK) was measured using a flame photometer colorimetric method following extraction with an ammonium acetate solution (1 mol L^{-1}) (Lu 2000), whereas available phosphate (AP) was measured using a Mo-Sb colorimetric method following extraction with a sodium bicarbonate solution (0.5 mol L^{-1}) (Lu 2000). The ^{15}N abundances in root, shoot, grain, and RC and HC soils were determined using an isotope ratio mass spectrometer (Finnigan MAT251, Thermo Fisher, Waltham, MA).

To explore the effect of soil microbial activity on plant nitrogen fertilizer uptake, soil enzyme activities, including β−1,4-glucosidase (β-G), cellulase, total protease (t-Pro), β−1,4-N-acetylglucosaminidase (NAG), urease, nitrate reductase, and phosphatase, were measured using reagent kits (Elisa) from Shanghai Ruifan Biological Technology Co. Ltd. For these enzymes, β-G and cellulase primarily facilitate the degradation of organic carbon compounds (Liu et al. 2023), while t-Pro, NAG, urease, and nitrate reductase are essential for N cycling in soil. These enzymes perform distinct functions: t-Pro facilitates the decomposition and transformation of proteins and peptides; NAG hydrolyzes N-acetyl glucosaminides; urease catalyzes the hydrolysis of urea; and nitrate reductase enables the reduction of nitrate (NO_3^-) to nitrite as part of N transformation processes in the soil (Chen et al. 2023b). Phosphatase primarily functions to hydrolyze organic phosphorus into inorganic phosphorus, which can be readily absorbed by plants (Chen et al. 2025).

Illumina MiSeq sequencing and processing of sequence data

Genomic DNA from RC and HC soil samples was extracted using a FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA). The bacterial 16S rRNA gene and fungal 18S rRNA gene were amplified using the primer pairs 515F/907R and ITS1F/ITS2R, respectively (Walters et al. 2016). To differentiate soil samples, each primer pair was barcoded with a unique error-correcting eight-base barcode on both the

forward and reverse primers. The amplification conditions for the bacterial 16S rRNA gene and fungal 18S rRNA gene included: 25 μL SYBR® Premix Ex Taq™ II (2×), 0.5 μL BSA (20 mg mL^{-1}), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 21.5 μL ddH_2O, and 1 μL template DNA. The thermal profile consisted of an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s (denaturing), 60 °C for 30 s (annealing), 72 °C for 1 min (extension), and a final extension step for 5 min at 72 °C. Following amplification, PCR products were purified using a universal DNA Purification Kit (Qiagen, Germany) and DNA concentrations were determined using SpectraMax M5 (Molecular Devices) with PicoGreen. Equal amounts of PCR products from different samples were mixed, purified, quantified, and sent to Shanghai Meiji Biotech Limited Company (Shanghai, China) for Illumina sequencing.

Data processing and statistical analyses

The total urea-N (g N pot^{-1}) uptake by the plant for each pot was calculated for plant components (root, shoot, and grain) using the following equation:

$$\text{Nurea} = \text{con} \times \frac{(\text{ape} - \text{ape0})}{\text{ape(urea)}} \times \frac{\text{m}}{1000}$$

where "N_{urea}" (g N pot^{-1}) represents the mass of N derived from urea in root, shoot, and grain for each pot, "con" is the total N content (mg N g^{-1}) in root, shoot, and grain, "ape" (%) is the ^{15}N atom percentage excess for the N pool, "ape0" is the ^{15}N natural abundance for the N pool, and "ape (urea)" is the ^{15}N atom percentage excess for urea, and "m" is the biomass of root, shoot, and grain (g). Additionally, the total urea-N retention in soil for each pot was calculated similarly based on the weight of RC and HC soils.

Raw sequences were quality-screened and trimmed using the Quantitative Insights into Microbial Ecology (QIIME) package. After removing low-quality or ambiguous reads, the sequences were clustered into amplicon sequence variants (ASVs) using DEBLUR. The representative sequences, defined as the most abundant sequences, were assigned taxonomy using the SILVA database (v138.1) for the 16S rRNA gene and the UNITE database (v9.0) for the 18S rRNA gene. An ASV table was then generated to record the abundance and taxonomy of each ASV in each sample. An averaged, rounded, rarefied ASV table was created to minimize differences in sequencing depth across samples. Alpha diversity indices, including Shannon and Chao, were calculated using Mothur (v1.30.2) to assess the diversity of bacterial and fungal communities in soils with different treatments. Based on the ASV matrix, principal component analysis (PCoA) was performed to assess differences in overall bacterial and fungal community composition between RC and HC soils with different treatments. Significant differences in the major genera of microbial community composition in RC or HC soils between treatments with and without AMF inoculation were assessed using Welch's test with false discovery rate (FDR) correction.

We conducted a co-occurrence network analysis using "ggClusterNet" of R (version 4.2.0) based on Spearman's correlation matrices to evaluate the complexity of community co-occurrence networks (Wen et al. 2022). Genera present in at least three subsamples within each treatment were included to ensure accuracy. Spearman correlation scores were calculated, and only robust (Spearman's $r > 0.8$) and statistically significant ($p < 0.05$) correlations were kept. The Benjamini and Hochberg FDR was applied to adjust *p*-values. Network topological parameters, including nodes, edges, and degrees, were extracted using the igraph package. Network visualization was performed using Gephi (version 0.10.1, https://gephi.org/).

To investigate the differences in soil enzyme activities, the data were analyzed using Z-score normalization, calculated as (actual value—mean)/standard deviation, as described by Delgado-Baquerizo et al. (2016). A one-way analysis of variance (ANOVA) with the post hoc Duncan's multiple comparisons was conducted to test for significant differences ($p < 0.05$) in mycorrhizal root colonization, AMF abundance, plant properties (plant biomass and N content), soil chemical properties (pH, TOC, TN, AN, AP, and AK), and soil enzyme activities (β-G, cellulase, t-Pro, NAG, urease, nitrate reductase, and phosphatase), and bacterial and fungal alpha diversity.

To investigate the influence of plant and soil properties on plant nitrogen uptake (PNU), we conducted spearman correlation analyses to establish links among urea-N uptake of the total plant (PNU), urea-N retention in soil (SNR), plant biomass, soil

chemical properties, soil enzyme activities, and soil bacterial and fungal properties (alpha diversity based on the Chao and Shannon indices, beta diversity based on PCoA1, and network based on edges). Based on the results of the correlation analyses, the importance of factors influencing plant nitrogen fertilizer uptake (PNU) was determined using the "rfPermute" package in R (version 4.2.0) based on Random forest (RF) model (Chen et al. 2021). The randomness of a RF algorithm with cross-validation metrics is demonstrated by the random selection of training samples for each tree and the random determination of the split attribute set for each node within the tree. All statistical analyses were conducted using IBM SPSS Statistics version 23.0, and data processing was performed with Microsoft Excel. Diagrams were generated using Origin 2021 and R software.

Results

Plant biomass and N fertilizer distribution in plant and soil

Compared to rice without AMF inoculation, AMF inoculation did not significantly affect the biomass of shoots and grains, but increased root biomass by 34.5%–41.7%, urea-N content in roots

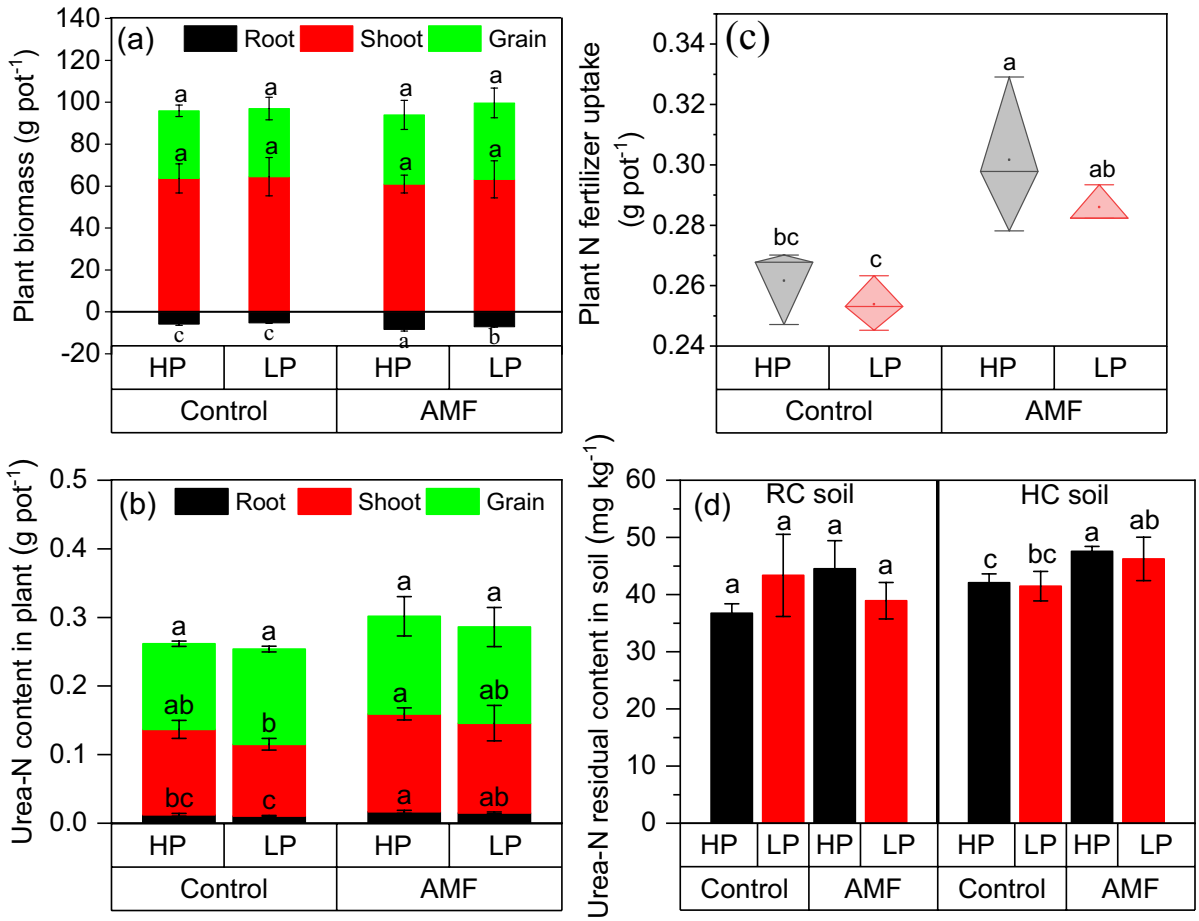

Fig. 1 Plant growth (**a**), urea-N distribution in root, shoot, grain (**b**), and N fertilizer uptake in the total plant (**c**), urea-N retention in RC and HC soils (**d**) under two phosphorus (P) fertilizer input levels for rice cultivation with and without arbuscular mycorrhizal fungi (AMF) inoculation at the rice maturity stage. Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: high-P fertilizer input; LP: low-P fertilizer input; RC: rhizosphere compartment; HC: hyphosphere compartment. Different lowercase letters indicated significant ($p < 0.05$) differences for plant (root, shoot and grain), or RC soil, or HC soils among various treatments

Table 1 Chemical characteristics of rhizosphere compartment (RC) and hyphosphere compartment (HC) soils under two phosphorus (P) fertilizer input levels for rice cultivation with and without arbuscular mycorrhizal fungi (AMF) inoculation at the rice maturity stage ($n = 3$)

Treatments			pH	Soil organic carbon (SOC) (g kg−1)	Total nitrogen (TN) (g kg−1)	Alkali-hydro nitrogen (AN) (mg kg−1)	Available phosphate (AP) (mg kg−1)	Available potassium (AK) (mg kg−1)
RC soil	Control	HP	5.77 ± 0.08^{a}	11.9 ± 0.18^{c}	1.09 ± 0.00^{b}	74.6 ± 1.05^{b}	22.8 ± 1.42^{ab}	115 ± 1.00^{a}
		LP	5.84 ± 0.09^{a}	12.2 ± 0.25^{bc}	1.09 ± 0.00^{b}	74.4 ± 2.65^{b}	20.4 ± 0.69^{bc}	110 ± 1.00^{c}
	AMF	HP	5.84 ± 0.03^{a}	12.6 ± 0.14^{b}	1.13 ± 0.00^{a}	83.8 ± 3.31^{a}	20.1 ± 1.51^{c}	112 ± 2.08^{ab}
		LP	5.89 ± 0.09^{a}	13.2 ± 0.40^{a}	1.12 ± 0.01^{a}	85.2 ± 3.59^{a}	23.5 ± 1.25^{a}	95.7 ± 2.52^{d}
HC soil	Control	HP	6.02 ± 0.09^{b}	11.2 ± 0.17^{a}	1.10 ± 0.01^{a}	78.2 ± 6.65^{a}	20.1 ± 1.37^{bc}	133 ± 4.04^{b}
		LP	6.12 ± 0.01^{ab}	11.4 ± 0.06^{a}	1.10 ± 0.01^{a}	62.8 ± 4.76^{b}	18.2 ± 1.26^{c}	125 ± 2.00^{c}
	AMF	HP	6.16 ± 0.08^{ab}	11.3 ± 0.16^{a}	1.10 ± 0.02^{a}	81.9 ± 5.73^{a}	21.6 ± 0.63^{b}	146 ± 2.50^{a}
		LP	6.17 ± 0.08^{a}	11.2 ± 0.20^{a}	1.08 ± 0.00^{a}	78.2 ± 5.95^{a}	29.9 ± 0.06^{a}	138 ± 3.21^{b}

Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: soil with high-P fertilizer input; LP: soil with low-P fertilizer input. Different lowercase letters indicated significant ($p < 0.05$) differences in RC/HC soils among various treatments

by 36.1%–44.2%, urea-N content in shoots by 14.4%–24.3%, and total plant urea-N uptake by 15.4%–16.0% in soils with two P input levels (Fig. 1-a, b and c). Additionally, AMF inoculation significantly increased urea-N retention in HC soil by 11.3%–12.8% for both P input levels, but had no significant effect in RC soil (Fig. 1-d).

Regardless of AMF inoculation, the urea-N content in roots, shoots, and whole plants, as well as the residual urea-N content in HC soil, was slightly higher in treatments with high-P fertilizer input compared to those with low-P fertilizer input (Fig. 1-b and c). However, no significant difference in root biomass was observed between the two P input levels for rice cultivation without AMF inoculation. After AMF inoculation, root biomass with high-P fertilizer input was significantly higher than that with low-P fertilizer input (Fig. 1-a). These results suggested that AMF inoculation enhanced plant N fertilizer uptake by increasing N uptake in roots and shoots and elevating urea-N retention in HC soil, with higher N uptake by plants under high-P fertilizer input compared to low-P fertilizer input.

AMF colonization and AMF abundance

Compared to rice cultivation without AMF inoculation, AMF inoculation significantly increased the AMF colonization rate from 13.1% to 30.6% (Fig. 2-a, c and d). However, AMF inoculation had no significant effect on AMF abundance in RC soil, and significantly increased AMF abundance by 35.8%–37.6% in HC soil across both P input levels (Fig. 2-b).

After rice with AMF inoculation, high-P fertilizer input slightly reduced AMF abundance in HC soil by 11.4% compared to low-P fertilizer input (Fig. 2-b). No significant difference in AMF colonization rate was observed between the two P fertilizer input levels for rice with AMF inoculation (Fig. 2-a). These results suggested that AMF inoculation could elevate AMF abundance in HC soil, with higher AMF abundance under low-P fertilizer input, potentially enhancing plant N fertilizer uptake via AM hyphae.

Soil chemical properties and soil enzyme activities

In RC soil, AMF inoculation significantly increased the concentrations of soil organic carbon (SOC), total nitrogen (TN), and alkali-hydrolyzed nitrogen (AN) for soils with both P fertilizer inputs, and available phosphate (AP) concentration for soil with low-P fertilizer input, but decreased AP concentration for soil with high-P fertilizer input and available potassium (AK) concentration for soils with both P fertilizer inputs (Table 1). In contrast, AMF inoculation significantly increased pH, AP, and AK

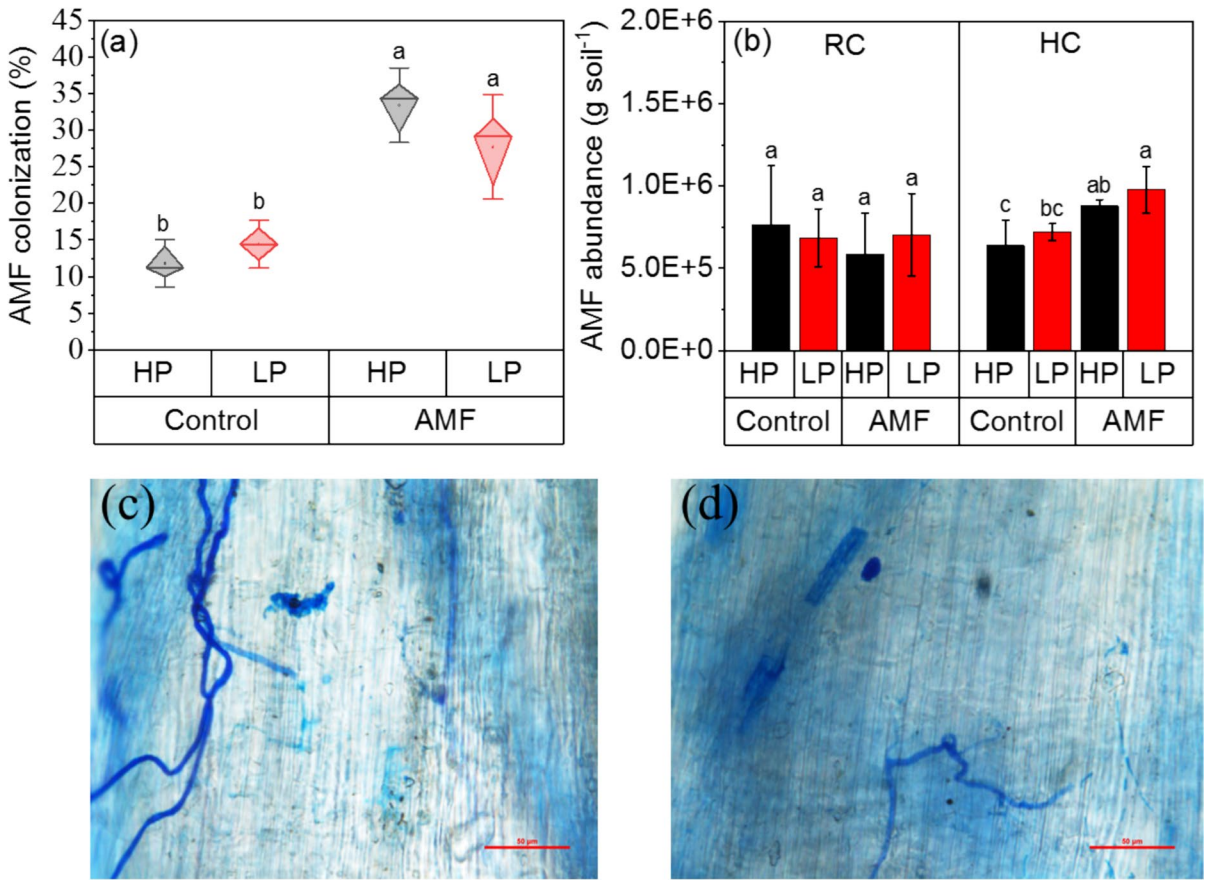

Fig. 2 Root morphology, and arbuscular mycorrhizal fungi (AMF) abundance in soils of RC and HC for rice cultivation without and with AMF inoculation at the rice maturity stage. a: root colonization by AMF; b, the copy numbers of the AMF gene in soil; c, d: AM fugal hyphae and vesicles at 200 × magnification in rice roots. Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: high-P fertilizer input; LP: low-P fertilizer input; RC: rhizosphere compartment; HC: hyphosphere compartment. Different lowercase letters indicated significant ($p < 0.05$) differences in rice root, or RC soil, or HC soils among various treatments

concentrations for HC soils with both P fertilizer inputs.

For soil enzyme activity, AMF inoculation significantly increased the activities of β−1,4-glucosidase (β-G), cellulase, and total protease (t-Pro), but decreased nitrate reductase activity in RC soil with both P fertilizer inputs (Fig. 3-a). In HC soil, AMF inoculation increased the activities of β-G, t-Pro, and phosphatase for soils with both P fertilizer inputs, and the activities of urease and nitrate reductase for soil with low-P fertilizer input (Fig. 3-b).

After rice with AMF inoculation, low-P fertilizer input significantly elevated the concentrations of SOC and AP, and the activities of t-Pro and nitrate reductase, but decreased AK concentration, the activities of β-G and cellulase in RC soil compared to high-P fertilizer input. Conversely, low-P fertilizer input elevated HC soil pH, AP concentration, and phosphatase activity, but decreased AK concentration, and activities of β-G and t-Pro for rice with AMF inoculation.

Bacterial and fungal community diversity, composition, and co-occurrence network analysis

No significant differences in alpha-diversity, as measured by the Chao and Shannon indices, were observed in the bacterial and fungal communities across various treatments in RC and HC soils (Fig. S3). At the amplicon sequence variant (ASV) level, principal

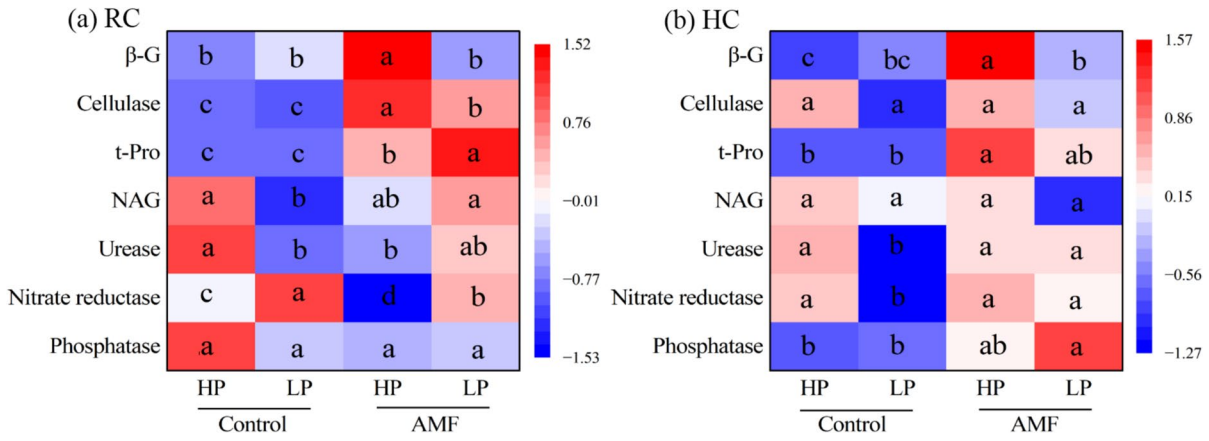

Fig. 3 The enzyme activity involved in C, N, and P cycling in paddy soils of RC (**a**) and HC (**b**) under two phosphorus (P) fertilizer input levels for rice cultivation with and without arbuscular mycorrhizal fungi (AMF) inoculation at the rice maturity stage. The data of soil enzyme activities were calculated as (actual value—mean)/standard deviation. Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: high-P fertilizer input; LP: low-P fertilizer input; RC: rhizosphere compartment; HC: hyphosphere compartment; t-Pro: total protease; β-G: β−1,4-glucosidase; NAG: β−1,4-N-acetylglucosaminidase. Different lowercase letters indicated significant ($p < 0.05$) differencesin RC/HC soil among various treatments

coordinates analysis (PCoA) indicated that bacterial and fungal communities in RC soil were significantly different from those in HC soil (Fig. S4). For bacteria, AMF inoculation caused a slight shift in community composition in both RC and HC soils across the two P fertilizer inputs, but these changes were not statistically significant ($p > 0.05$) (Fig. 4-a and b). However, significant differences in bacterial community composition were observed between high and low-P fertilizer input treatments in HC soils with AMF inoculation. For fungi, AMF inoculation significantly altered community compositions in both RC and HC soils, with clear separation between high and low P input treatments, regardless of AMF inoculation (Fig. 4-c and d).

At the phylum level, the predominant bacterial communities were composed of Firmicutes (19.5%−28.0%) and Chloroflexi (20.6%−23.9%), followed by Proteobacteria, Acidobacteriota, Bacteroidota, Actinobacteriota, Desulfobacterota, Myxococcota, and Planctomycetota (Fig. 4-e). The major fungal communities were dominated by Ascomycota (41.02%−73.52%), followed by Mortierellomycota, Fungi_phy_Incertae_sedis, Basidiomycota, and Rozellomycota (Fig. 4-f). These phyla include 573 bacterial genera and 293 fungal genera.

Compared to rice without AMF inoculation, AMF inoculation significantly altered the relative abundance of microbial genera in both RC and HC soils. Specifically, AMF inoculation increased the relative abundance of bacterial genera such as *Clostridium_sensu_stricto_9* in RC soil and *Bacillus* in HC soil, as well as fungal genera such as *Cystobasidium* in RC soil and *GS16* in HC soil. Conversely, it decreased the relative abundance of bacterial genera such as *BSV13* in RC soil and fungal genera such as *Sarocladium* in RC soil and *Scolecobasidium* in HC soil (Fig. S5). Furthermore, RC soil exhibited a higher relative abundance of bacterial genera such as *Anaerolinea* and *Anaeromyxobacter* and fungal genera such as *Penicillium* and *Stellatospora* compared to HC soil, while HC soil had a higher relative abundance of bacterial genera such as *Clostridium_sensu_stricto_10* and *Hydrogenispora* and fungal genera such as *Fungi_gen_Incertae_sedis* and *Condenascus* (Fig. S6).

To elucidate the impact of AMF on bacterial and fungal community structures, co-occurrence networks and their topological properties were constructed using Spearman correlations (Fig. 5). The results showed that AMF inoculation significantly decreased the average edge number of the bacterial community network and increased the average

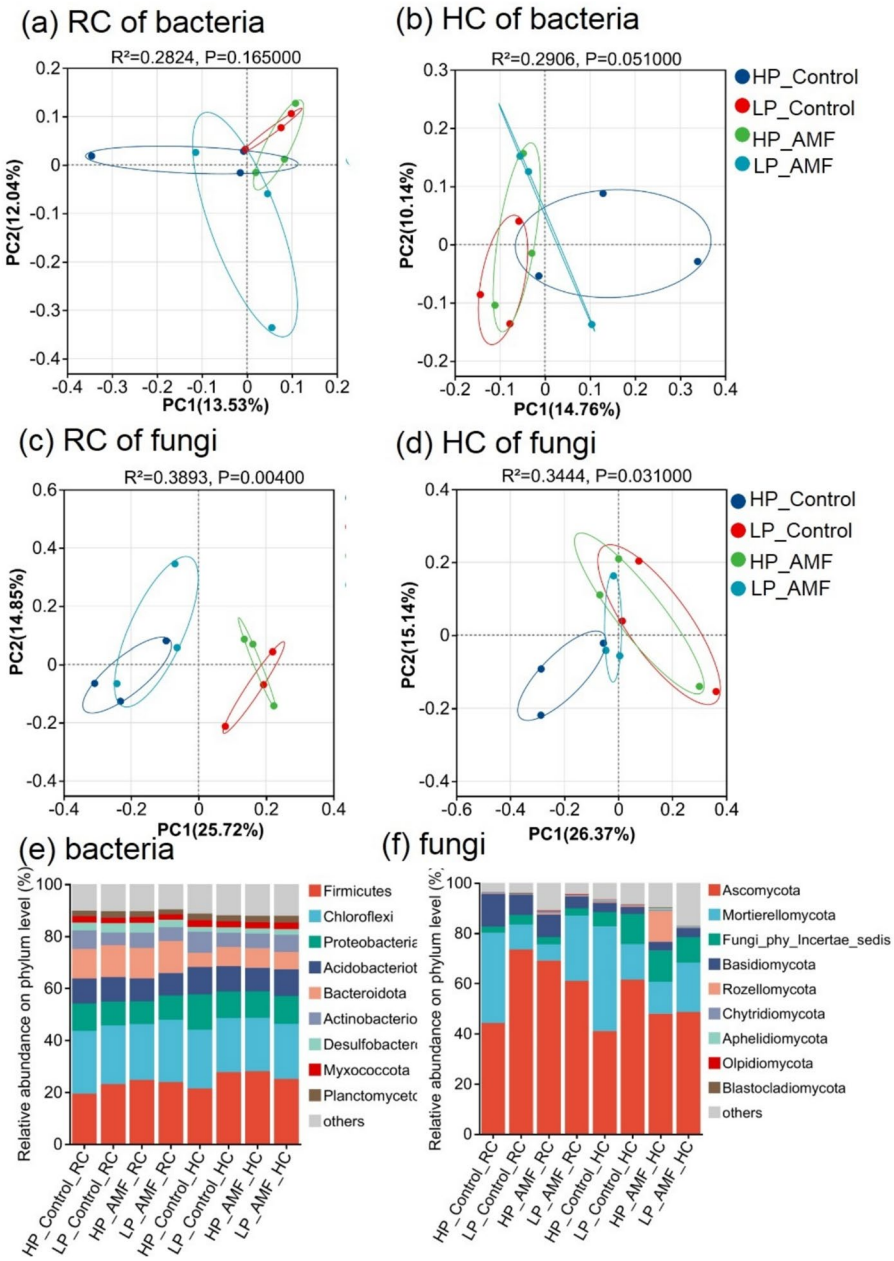

Fig. 4 Principal Component Analysis (PCoA) of bacterial (**a**, **b**) and fungal (**c**, **d**) community at the ASV level, and the community compositions of bacterial (**e**) and fungal (**f**) at the phylum level in soils of rhizosphere compartment (RC) and hyphosphere compartment (HC) under two phosphorus (P) fertilizer input levels for rice cultivation with and without arbuscular mycorrhizal fungi (AMF) inoculation. Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: high-P fertilizer input; LP: low-P fertilizer input

edge number of the fungal community network in RC soils across both P fertilizer input levels compared to rice without AMF inoculation (Fig. 5-a; c), indicating the reduced network complexity of the bacterial community and the increased network complexity of the fungal community. In HC soils, the response of microbial network complexity to AMF inoculation varied with P fertilizer input levels (Fig. 5-b), indicating a significant decrease in network complexity of both bacterial and fungal communities under high-P fertilizer input, but an increase under low-P fertilizer input.

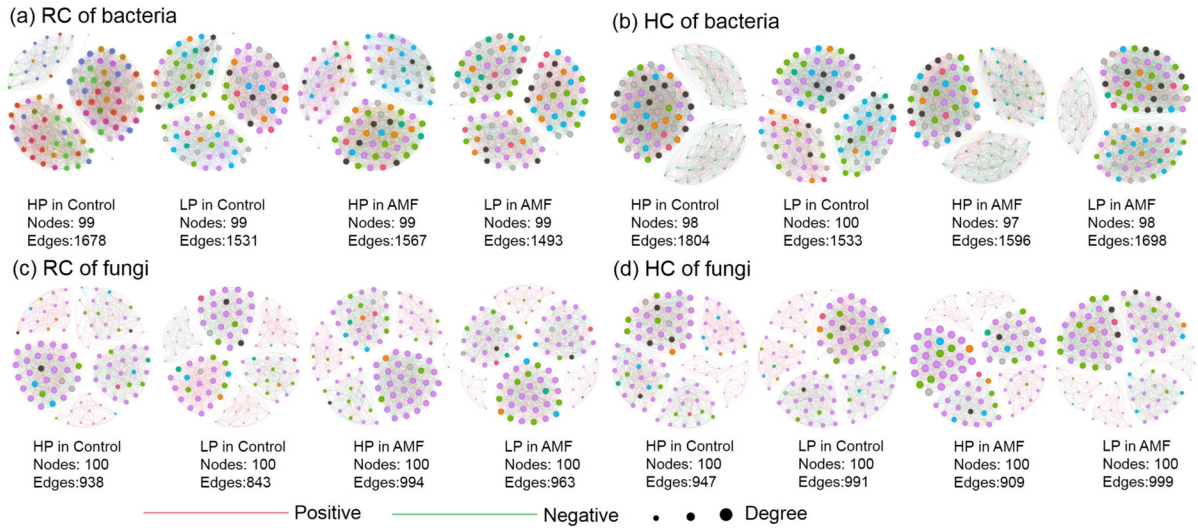

Fig. 5 Microbial co-occurrence network at the genus level of bacteria (**a**, **b**) and fungi (**c**, **d**) in soils of rhizosphere compartment (RC) and hyphosphere compartment (HC) under two phosphorus (P) fertilizer input levels for rice cultivation with and without arbuscular mycorrhizal fungi (AMF) inoculation. Control: rice root without AMF inoculation; AMF: rice root with AMF inoculation; HP: high-P fertilizer input; LP: low-P fertilizer input

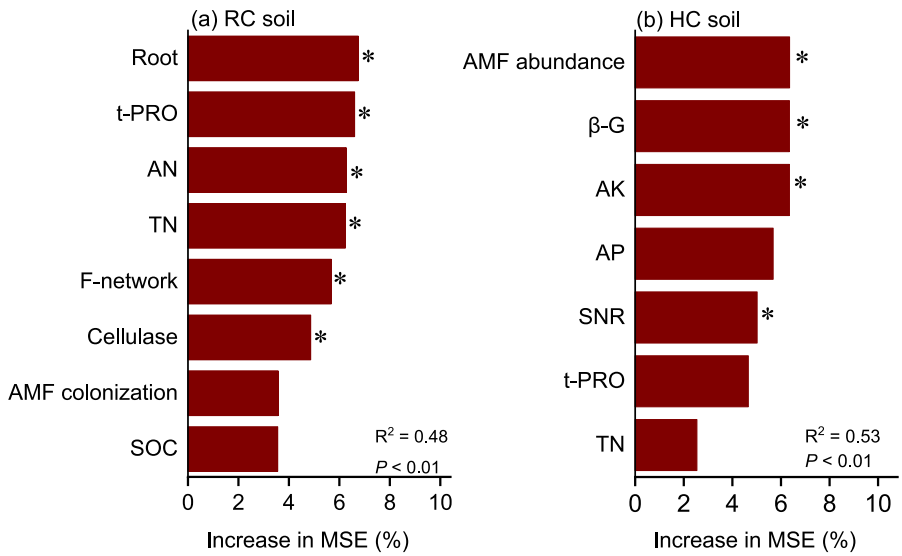

Fig. 6 Relative importance of plant properties, soil chemical properties, soil enzyme activities and microbial properties in impacting plant N fertilizer uptake. t-Pro: total protease; TN: total nitrogen; AN: Alkali-hydro nitrogen; F-network: network complexity of Fungal community based on the edge; SOC: soil organic carbon; β-G: β−1,4-glucosidase; AP: available phosphate; AK: Available potassium; SNR: N fertilizer retention in soil. The significant statistical results were labeled (*$p < 0.05$, **$p < 0.01$)

Relationships among plant properties, soil chemical properties, soil enzyme activities, and soil microbial properties

The spearman correlation analyses showed that PNU was significantly positively correlated with TN, SOC, AN, t-Pro, cellulose, fungal network (F-network), and root AMF colonization in RC soils ($p < 0.05$) (Fig. S7-a). In HC soils, PNU was significantly positively correlated with soil nitrogen retention (SNR), AP, AK, t-Pro, β-G, and AMF abundance ($p < 0.05$) (Fig. S7-b).

Based on the correlation results, a random forest algorithm was employed to rank the importance of factors affecting nitrogen fertilizer uptake (Fig. 6). We found that root biomass was the most important factor influencing N fertilizer uptake in RC soil, followed by t-Pro, AN, TN, AN, fungal network,

and cellulose (Fig. 6-a). In HC soil, AMF abundance was the most important factor affecting N uptake, followed by β-G, AK, and SNR (Fig. 6-b).

Discussion

Our results showed that AMF inoculation enhanced plant N fertilizer uptake by increasing N uptake in roots and shoots and elevating urea-N retention in HC soil, with higher N uptake by plants and urea-N retention in HC soil with high-P fertilizer input compared to low-P fertilizer input (Fig. 1). Moreover, root biomass in RC soil and AMF abundance in HC soil were the most important factors influencing N fertilizer uptake (Fig. 6). In addition, our findings suggested that soil microbial communities and enzyme activities in the rhizosphere and hyphosphere played a vital role in N uptake via roots and AM fungal hyphae.

The role of root and AM fungal hyphae on plant N fertilizer uptake

Plant roots and their associated AMF are two primary pathways for nutrient acquisition from soil (Sun et al. 2024). Enhanced plant N acquisition may occur through direct root uptake or, in the case of AM plants, through a combination of direct root uptake and AM fungal hyphal uptake and transfer to plants (Yang et al. 2022). In this study, AMF inoculation significantly increased urea-N uptake in the rice-soil system (Fig. 1-c), possibly due to the combined effects of direct root uptake and AM fungal hyphal uptake. Our observation that the root systems of rice with AMF inoculation were consistently larger than those without AMF inoculation (Fig. 1-a), in line with previous studies (Hestrin et al. 2019), suggested that a more extensive root network facilitates the uptake of mineralized nutrients. In RC soil, we also found a positive relationship between root biomass and N fertilizer uptake, but no significant difference in AMF abundance between rice with and without AMF inoculation (Fig. S7-a), indicating that roots might be the primary pathway for plant N acquisition in the rhizosphere with limited N sources, which might not support AMF growth. The random forest model identified root biomass as the most important factor influencing N fertilizer uptake in RC soil (Fig. 7-a), further emphasizing the role of roots in N fertilizer uptake in the rhizosphere.

AM fungal hyphae can acquire and transfer N from soils to plants, serving as another crucial pathway for N fertilizer uptake (Xue et al. 2024; Wang et al. 2025c). In HC soil, a positive relationship between AMF abundance and N fertilizer uptake (Fig. S7-b) suggested that increased AMF abundance may enhance rice N acquisition. The random forest model also identified AMF abundance as the most important factor influencing urea N uptake in HC soil (Fig. 6-b), further underscoring the role of AM fungal hyphae in plant N acquisition. AMF growth could increase N fertilizer uptake via direct transfer of more N to the plant (Hodge et al. 2010), but AMF could also assimilate N fertilizer into their biomass (Rillig and Steinberg 2002). For rice with AMF inoculation, the higher N fertilizer retention in HC soil might be attributed to AMF assimilation, which might incorporate soil N into their biomass to prevent urea-N loss, and the subsequent degradation of microbial biomass can release assimilated N for plant uptake (Zhou et al. 2002), thereby indirectly enhancing N fertilizer uptake by AM fungal hyphae. Our study observed that urea N retention in HC soil was also an important factor influencing N fertilizer uptake (Fig. 6-b), supporting our hypothesis.

However, for rice without AMF, root AMF colonization rates remained between 11.8% and 14.4%, likely due to the presence of native AMF in paddy soil. In flooded paddy fields, the waterlogged environment and high fertilizer application rates can restrict the growth of native AMF (Lumini et al. 2011), thereby possibly reducing their contribution to nutrient uptake. In contrast, exogenous AMF inoculation during the rice seedling stage significantly increased root colonization rates (Fig. 2-a), possibly because the exogenous AMF species *Rhizophagus irregularis* can rapidly colonize rice root systems earlier than native AMF, ultimately enhancing N fertilizer uptake by plants. After mycorrhizal rice is transplanted into paddy soil, native AMF and exogenous AMF can establish synergistic interactions that expand the hyphal network and enhance nutrient absorption in rice (Yao et al. 2008). However, they may also compete for root space and nutrient resources, which can reduce the colonization efficiency of exogenous AMF and diminish the contribution of mycorrhizae to N uptake in crops (Herrera et al. 1993; Klironomos

et al. 2003). The effects of exogenous AMF inoculation on N fertilizer uptake in the presence of native AMF are influenced by the colonization ability, functional traits of the exogenous AMF, and their interactions with native AMF (Yao et al. 2008; Martignoni et al. 2021). In general, the use of multiple AMF species is more suitable for agricultural applications, as it offers broader ecological adaptability and functionality, particularly in complex environments or long-term agricultural production systems (Shu et al. 2022). However, under specific conditions or for targeted applications, a single AMF species may exhibit greater efficiency (Shu et al. 2022). Consequently, the selection of an appropriate AMF inoculation strategy should be carefully tailored to farmland conditions, crop requirements, and desired outcomes. Further field studies involving diverse AMF species are necessary to validate these findings. Moreover, the functional experiments involving the inoculation of exogenous AMF in the absence of native AMF can more clearly distinguish the contributions of native and exogenous AMF to plant N uptake.

The role of soil microorganisms and soil enzyme activities on plant N fertilizer uptake

Interactions between plant–microbe and microbe-microbe are common phenomena in agricultural ecosystems, playing a crucial role in promoting nutrient uptake (Duan et al. 2024; Ge et al. 2025). A synergistic feedback loop through mycorrhizally-driven plant growth can provide resources for additional photosynthate production, which in turn stimulates rhizosphere soil microbial communities and soil enzyme activities that support continued plant growth (Hestrin et al. 2019; Qin et al. 2020a; Alsherif et al. 2024). In rhizosphere soil, we found that AMF inoculation slightly altered the bacterial community composition and significantly increased the relative abundance of *Clostridium* species (Fig. S5-a), which are known to facilitate the degradation of soil organic matter (Shanh et al. 2014). Furthermore, AMF inoculation substantially modified the fungal community composition and enhanced the complexity of fungal networks in RC soil (Fig. 4-f; 5-c), potentially by increasing the abundance of specific fungal genera, such as *Cystobasidium* (Fig. S5-c), which are associated with promoting plant growth (Ramos-Garza et al. 2016). These changes in bacterial and fungal communities, along with the increased complexity of fungal networks in RC soil, may enhance the activities of protease and cellulase (Fig. 3-a), which could lead to greater N availability to support plant growth, potentially improving root uptake of N fertilizer. Additionally, a random forest model identified protease activity, cellulase activity, and fungal network complexity in RC soil as key factors influencing N fertilizer uptake, underscoring the critical role of rhizosphere microbial communities and soil enzyme activities in N acquisition.

However, AMF inoculation was also found to decrease the relative abundance of certain bacterial genera and reduce the complexity of the bacterial community network in RC soil (Fig. 5-a; Fig. S5-a). This reduction may be attributed to increased competition for nutrients between AMF and soil bacteria, which can adversely affect bacterial communities and diminish microbial activity in rhizosphere soil (Zhang et al. 2022b). Furthermore, the observed decrease in nitrate reductase activity in RC soil (Fig. 3) may be closely linked to the reduced relative abundance of specific bacterial genera and the simplified bacterial community network. However, this reduction in nitrate reductase activity could potentially lower nitrogen loss through denitrification in rhizosphere soil (Bardon et al. 2016), thereby indirectly enhancing nitrogen fertilizer efficiency.

Similar to the microbiome in the rhizosphere, the hyphosphere hosts a distinct microbiome that plays a critical role in nutrient acquisition through the mycorrhizal pathway (Wang et al. 2023b; 2025b). In HC soil, AMF inoculation significantly increased the relative abundance of specific bacterial and fungal genera, such as *Bacillus* and *GS16* (Fig. S5-b and d). This enhancement could boost soil enzyme production and the decomposition of soil organic matter, thereby supporting AMF growth and increasing AMF abundance, ultimately improving N fertilizer retention and uptake by AM fungal hyphae. Additionally, the observed increase in the activities of β-G, protease, and phosphatase in HC soil for rice with AMF inoculation (Fig. 3-b) further supported the hypothesis that AMF inoculation could enhance soil enzyme activities by altering soil microbial communities. Furthermore, a random forest model identified β-G activity in HC soil as a key factor influencing nitrogen fertilizer uptake (Fig. 6-b), emphasizing the importance

of soil enzyme activities in nitrogen acquisition by AM fungal hyphae.

The complementary functions of rhizosphere and hyphosphere microbiomes work synergistically to support nutrient uptake in mycorrhizal plants (Bao et al. 2022; Duan et al. 2024). Rhizosphere microbiomes primarily interact directly with plant roots, whereas hyphosphere microbiomes collaborate with mycorrhizal fungi to facilitate nutrient absorption (Andrade et al. 1997; Zhou et al. 2020a; Qin et al. 2022; Johnson et al. 2023). In this study, the bacterial and fungal community compositions in RC soil differed significantly from those in HC soil following AMF inoculation in rice cultivation (Fig. S4). The relative abundance of key bacterial genera (*Anaerolinea* and *Anaeromyxobacter*), which are involved in organic matter decomposition (Chen et al. 2023a; Xu et al. 2023), and major fungal genera (*Penicillium*), known for promoting plant growth (Yang et al. 2025), were significantly higher in RC soil compared to HC soil (Fig. S6). These findings suggested that AMF inoculation could enhance soil organic matter decomposition and plant growth in RC soil, thereby improving N uptake by plants. Moreover, AMF inoculation might primarily enhance the decomposition of soil organic matter (Xiao et al. 2025) in HC soil by increasing the relative abundance of key bacterial genera (*Clostridium* sensu stricto *10* and *Hydrogenispora*) (Fig. S6-a), which are known to play critical roles in organic matter decomposition (Zhou et al. 2021, 2022). This process could support nutrient availability for AMF growth in HC soil and improved N uptake by AM fungal hyphae. Consequently, the combined functions of rhizosphere and hyphosphere microbiomes may enhance N uptake in rice cultivation with AMF inoculation.

This study successfully demonstrated the impact of soil microbiomes and enzyme activities in RC and HC soils on N fertilizer uptake in rice cultivation with AMF inoculation using a two-compartment system. However, the potential cross-talk between mobile microbes or soluble exudates in RC and HC, particularly in flooded soils, cannot be excluded. Microbiomes in the root chamber and hyphal chamber can establish synergistic interactions through the exchange of metabolic products (Bonfante et al. 2009; Smith 2010). However, the exchange of substances between the chambers may also intensify competition among certain microorganisms (Joanne et al. 2011). A more recent study has implemented buffer zones or air gaps within compartments to reduce the exchange of substances between RC and HC (Jiang et al. 2020; Wang et al. 2023b). Future experiments should include these new technologies to more effectively study microbial cooperation and competition between both compartments. Additionally, the microcosm experiment in this study was constructed with field-collected soils and their corresponding microbial communities, but the greenhouse conditions cannot fully simulate the complex ecological environment and dynamic processes of the field. The realistic environmental conditions are crucial for capturing the emergent properties of mycorrhizal collectives. Therefore, whenever possible, further research should be conducted in the field to investigate the mechanisms by which AMF inoculation influences N uptake in rice, using on-site field trials (Wang et al. 2023b).

Effect of P fertilizer input level on plant N uptake

Previous studies have indicated that high levels of P fertilizer input can reduce AMF colonization in roots, thereby negatively impacting nutrient uptake (Qin et al. 2020b). Contrary to previous findings, our study found no significant difference in AMF colonization rates between high- and low-P fertilizer input levels in rice with AMF inoculation (Fig. 2-a). This discrepancy may be attributed to the flooded environment, which plays a critical role in shaping AMF colonization in rice fields (Vallino et al. 2014). AMF are aerobic organisms that depend on oxygen for their growth and functionality. However, the waterlogged conditions typical of paddy fields significantly reduce soil oxygen levels, creating anaerobic environments. These anaerobic conditions likely constrain AMF activity and proliferation (Lumini et al. 2011), thereby diminishing their capacity to respond to varying phosphorus fertilizer application rates. Additionally, higher AMF abundance was observed in HC soil under low-P fertilizer input compared to high-P fertilizer input (Fig. 2-b), suggesting that the flooded environment may not significantly impact the growth of AM fungal hyphae in soil. Following AMF inoculation, the noticeably lower root biomass under low-P fertilizer input could potentially reduce plant N uptake. However, compared to high-P fertilizer input, there was no significant decrease in plant urea-N

uptake under low-P fertilizer input, which might be attributed to the higher AMF abundance in HC soil, enhancing N uptake by AM fungal hyphae. Furthermore, higher total plant P content was observed under low-P fertilizer input compared to high-P fertilizer input (Fig. S2), further supporting the enhanced role of AM fungal hyphae in nutrient uptake under low P input conditions.

Interestingly, rice plants inoculated with AMF under high-P fertilizer input exhibited slightly higher accumulation of urea-N compared to those under low-P fertilizer input (Fig. 2-c), potentially due to increased root biomass. For rice with AMF inoculation, roots appear to remain a critical pathway for N uptake. Recent studies have found that the percentage contribution of AMF to rice ^{15}N uptake was approximately 40% under N-NO_3^- supply conditions (Wang et al. 2020), indicating that the mycorrhizal pathway contributes less than 50% to plant N acquisition. These findings further emphasized that roots played a more dominant role in N uptake in AMF-inoculated plants. Compared to low-P fertilizer input, higher β-G and cellulose activities, along with lower nitrate reductase activity in RC soil under high P input, suggested increased N availability and reduced N loss (Abdelmagid et al. 1987; Nevins et al. 2020), which could provide more N sources for rice root growth and enhance root N fertilizer uptake. Consequently, the relative contributions of root uptake and AM fungal hyphal uptake to plant N acquisition in soils after rice cultivation with AMF inoculation may differ between the two P input levels. However, this study did not quantify the relative contributions of root uptake and AM fungal hyphal uptake to rice N acquisition. Moreover, the carbon (C) supply from the host plant is considered a key factor triggering N uptake in the symbiosis (Sun et al. 2024). Future studies should quantify AMF-mediated N uptake while simultaneously accounting for their C consumption by the host plant. This can be achieved by tracing the flow of C from plants into soil using ^{13}C stable isotope labeling (e.g., $^{13}CO_2$) and identifying key microbial consumers of plant-derived C through stable isotope probing (SIP)-enabled metagenomic sequencing (Kakouridis et al. 2024).

Additionally, we observed that the complexity of the microbial network in HC soil varied between high-and low-P fertilizer input levels. The reduced complexity of the bacterial and fungal community network in HC soil under high-P fertilizer input might be attributed to the decreased dependency of plants on AMF, which weakens AMF activity and symbiotic relationships (Mitra et al. 2021), thereby diminishing their facilitation of other microorganisms. In contrast, under low P conditions, plants rely more heavily on AMF for nutrient acquisition, which enhances AMF activity and interactions with other microorganisms (Kahiluoto et al. 2001), thereby increasing the complexity of the bacterial and fungal community network in HC soil. This increased complexity under low-P fertilizer input might promote the activities of phosphatase and urease (Fig. 3-b), facilitating the production of nutrients such as available phosphorus to support AMF growth and potentially increasing N uptake by AM fungal hyphae.

However, the reduced complexity of microbial community networks can negatively impact ecosystem functions and weaken resistance to disturbances (Chen et al. 2022). In this study, AMF inoculation reduced the complexity of bacterial community networks in RC soil and decreased the complexity of bacterial and fungal community networks in HC soil under high P input (Fig. 5). The potential benefits or drawbacks of reduced microbial network complexity on paddy field ecosystems warrant further investigation. This experiment, conducted in glasshouse pots, was limited by sampling at only one time, which restricted the sample size. We acknowledge that relying solely on network correlation analysis can lead to spurious associations, particularly with low replicate numbers. Furthermore, the relationship between enzyme activity, microbial community shifts, and ecosystem functions is complex (Zak et al. 2003). Increases in protease or β-glucosidase activity do not necessarily indicate enhanced mineralization or improved nutrient uptake by AMF. In our study, enzyme activity and microbial community shifts serve as indirect indicators of potential soil nutrient cycling and changes in microbial ecological processes. We recognize that these indicators should be supplemented with direct evidence, such as measurements of mineralization rates or actual nutrient uptake by AMF, to more comprehensively elucidate their ecological functions. Future studies should increase the sample size and employ structural equation modeling (SEM) or path analysis to test causal relationships among microbial traits, enzyme activities, AMF colonization, and nitrogen uptake. Additionally, these findings

should be validated through functional experiments or experimental manipulations using stable isotope tracing techniques or by directly quantifying AMF nutrient uptake efficiency. This approach will more clearly validate the contributions of enzyme activity and microbial shifts to ecosystem functions.

Conclusions

This study demonstrated the effects of AMF on N fertilizer translocation and uptake, as well as the microbial process involved in the rice-soil system following AMF inoculation under different P fertilizer input levels. These results suggested that AMF inoculation was an effective strategy for increasing rice N uptake through both root and AM hyphal pathways by enhancing root biomass and AMF abundance of HC soil. Under AMF inoculation condition, high-P fertilizer input might increase N uptake via root biomass enhancement, while low-P fertilizer input could promote N uptake through AM fungal hyphae by stimulating AMF growth. Changes in the microbial community and increases in soil enzyme activities were key factors in promoting N uptake of root and AM fungal hyphae. These findings enrich our understanding of the interactions among AMF, roots, and the microbiome in enhancing N fertilizer utilization and reducing N loss.

Acknowledgements This study was supported by the National Key Research and Development Project of China (2021YFD1700800) and Key Research and Development Project of Jiangsu Province (D21YFD17008).

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Pei Chen, Yu Cheng, Rui Chen and Ning Wang. The first draft of the manuscript was written by Pei Chen and Ning Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest The authors declare that there is no competing interest.

References

Abdelmagid HM, Tabatabai MA (1987) Nitrate reductase activity of soils. Soil Biol Biochem 19:421–427. https://doi.org/10.1016/0038-0717(87)90033-2

Alsherif EA, Sonbol H, Abdelgawad H, Ramadan A, Korany SM, Crecchio C (2024) Arbuscular mycorrhizal fungi improve tolerance of wheat plants under soil europium contamination. Plant Soil 505:881–895. https://doi.org/10.1007/s11104-024-06936-9

Andrade G, Mihara K, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79. https://doi.org/10.1023/A:1004249629643

Balestrini R, Brunetti C, Chitarra W, Nerva L (2020) Photosynthetic traits and nitrogen uptake in crops: which is the role of arbuscular mycorrhizal fungi? Plants 9:1105. https://doi.org/10.3390/plants9091105

Bao XZ, Zou JX, Zhang B, Wu LM, Yang TT (2022) Arbuscular mycorrhizal fungi and microbes interaction in rice mycorrhizosphere. Agronomy 12:1277. https://doi.org/10.3390/agronomy12061277

Bardon C, Poly F, Piola F, Pancton M, Comte G, Meiffren G, Haichar FEZ (2016) Mechanism of biological denitrification inhibition: procyanidins induce an allosteric transition of the membrane-bound nitrate reductase through membrane alteration. FEMS Microbiol Ecol 92(5):fiw034. https://doi.org/10.1093/femsec/fiw034

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Bicharanloo B, Salomon MJ, Cavagnaro TR, Keitel C, Brien C, Jewell N, Berger B, Lines T, Dijkstra FA (2023) Arbuscular mycorrhizae are important for phosphorus uptake and root biomass, and exudation for nitrogen uptake in tomato plants grown under variable water conditions. Plant Soil 490:325–342. https://doi.org/10.1007/s11104-023-06078-4

Bonfante P, Anca IA (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol 63:363–383

Brahmaprakash G.P, Sahu PK, Lavanya G, Nair SS, Gangaraddi VK, Gupta A (2017) Microbial functions of the rhizosphere. Plant-Microbe Interactions in Agro-Ecological Perspectives: 177–210. https://doi.org/10.1007/978-981-10-5813-4_10

Breidenbach B, Pump J, Dumont MG (2016) Microbial community structure in the rhizosphere of rice plants. Front Microbiol 6:1537. https://doi.org/10.3389/fmicb.2015.01537

Chen BB, Xiong W, Qi JJ, Pan HB, Chen S, Peng ZH, Gao H, Zhang LM, Jiang YJ, Wei GH, Jiao S (2021) Trophic interrelationships drive the biogeography of protistan community in agricultural ecosystems. Soil Biol Biochem 163:108445. https://doi.org/10.1016/j.soilbio.2021.108445

Chen WQ, Wang JY, Chen X, Meng ZX, Xu R, Duoji DZ, Zhang JH, He J, Wang ZG, Chen J, Liu KX, Hu TM, Zhang YJ (2022) Soil microbial network complexity

predicts ecosystem function along elevation gradients on the Tibetan Plateau. Soil Biol Biochem 172:108766. https://doi.org/10.1016/j.soilbio.2022.108766

Chen MM, Zhang YL, Gao CW, Gao CW, Zhang SR, Lu L, Wu LP, Li YY, Ding XD (2023a) Mineral-microbial interactions in nine-year organic fertilization field experiment: a mechanism for carbon storage in saline-alkaline paddy soil. Plant Soil 489:465–481. https://doi.org/10.1007/s11104-023-06032-4

Chen X, Chen J, Cao JJ (2023b) Intercropping increases soil N-targeting enzyme activities: a meta-analysis. Rhizosphere 26:100686. https://doi.org/10.1016/j.rhisph.2023.100686

Chen DM, Zang LP, Zhang GQ, Liu QF, Sui MZ, Liu YR (2025) Organic-inorganic fertilization optimizes phodharboring microbial communities and increases phosphorus availability in paddy soils under intensive rice cropping. Appl Soil Ecol 211:106129. https://doi.org/10.1016/j.apsoil.2025.106129

Delgado-Baquerizo M, Maestre FT, Reic PB, Jeffries TC, Gaitan JJ, Encinar D, Berdugo M, Campbell CD, Singh BK (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun 7:10541. https://doi.org/10.1038/ncomms10541

Demoling F, Figueroa D, Baath E (2007) Comparison of factors limiting bacterial growth in different soils. Soil Biol Biochem 39:2485–2495. https://doi.org/10.1016/j.soilbio.2007.05.002

Duan S, Feng G, Limpens E, Bonfante P, Xie X, Zhang L (2024) Cross-kingdom nutrient exchange in the plant-arbuscular mycorrhizal fungus-bacterium continuum. Nat Rev Microbiol 12:773–790. https://doi.org/10.1038/s41579-024-01073-7

Emmett BD, Lévesque-Tremblay V, Harrison MJ (2021) Conserved and reproducible bacterial communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. ISME J 15:2276–2288. https://doi.org/10.1038/s41396-021-00920-2

Fan XY, Ge AH, Qi SS, Guan YF, Wang R, Yu N, Wang ET (2025) Root exudates and microbial metabolites: signals and nutrients in plant-microbe interactions. Sci China Life Sci. https://doi.org/10.1007/s11427-024-2876-0

Food and Agriculture Organization of the United Nations (2024) FAO Data Lab. https://www.fao.org/datalab/zh

Ge AH, Wang ET (2025) Exploring the plant microbiome: a pathway to climate-smart crops. Cell 188:1469–1485. https://doi.org/10.1016/j.cell.2025.01.035

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169(1):30–39. https://doi.org/10.1016/j.micres.2013.09.009

Herrera MA, Salamanca CP, Barea JM (1993) Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified mediterranean ecosystems. Appl Environ Microbiol 59:129–133

Hestrin R, Hammer EC, Mueller CW, Lehmann J (2019) Synergies between mycorrhizal fungi and soil microbial communities increase plant nitrogen acquisition. Commun Biol 2:233. https://doi.org/10.1038/s42003-019-0481-8

Hodge A, Helgason T, Fitter AH (2010) Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecol 3:267–273. https://doi.org/10.1016/j.funeco.2010.02.002

Huang T, Xie KL, Zhang ZH, Zhang Q, Li YY, Lin S, Zhou J, Chen J, Li X (2024) The colonization of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* affects the diversity and network structure of root endophytic bacteria in maize. Sci Hortic 326:112774. https://doi.org/10.1016/j.scienta.2023.112774

Hui J, An X, Li ZB, Neuhäuser B, Ludewig U, Wu X, Schulze WX, Chen FJ, Feng G, Lambers H, Zhang FU, Yuan LX (2022) The mycorrhiza- speciffc ammonium transporter ZmAMT3;1 mediates mycorrhiza-dependent nitrogen uptake in maize roots. Plant Cell 34:4066–4087. https://doi.org/10.1093/plcell/koac225

Ilag LL, Rosales AM, Elazegui FA, Mew TW (1987) Changes in the population of infective endomycorrhizal fungi in a rice-based cropping system. Plant Soil 103:67–73. https://doi.org/10.1007/BF02370669

Jiang YJ, Luan L, Hu KJ, Liu MQ, Chen ZY, Geisen S, Chen XY, Li HX, Xu QS, Bonkowski M, Sun B (2020) Trophic interactions as determinants of the arbuscular mycorrhizal fungal community with cascading plant-promoting consequences. Microbiome 8:142. https://doi.org/10.1186/s40168-020-00918-6

Jiang FY, Zhang L, Zhou JC, George TS, Feng G (2021) Arbuscular mycorrhizal fungi enhance mineralization of organic P by carrying bacteria along their extraradical hyphae. New Phytol 230:304–315. https://doi.org/10.1111/nph.17081

Joanne L, Fitter AH, Angela H (2011) Growth and symbiotic effectiveness of an arbuscular mycorrhizal fungus in organic matter in competition with soil bacteria. FEMS Microbiol Ecol 3:428–438

Johnson NC, Marín C (2023) Microbial villages in the geography of arbuscular mycorrhizal symbioses. New Phytol 238:461–463. https://doi.org/10.1111/nph.18803

Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001) Promotion of am utilization through reduced P fertilization. Field Studies Plant Soil 231:65–79. https://doi.org/10.1023/A:1010366400009

Kai M, Effmert U, Piechulla B (2016) Bacterial-plant-interactions: approaches to unravel the biological function of bacterial volatiles in the rhizosphere. Front Microbiol 7:108. https://doi.org/10.3389/fmicb.2016.00108

Kakouridis A, Yuan MT, Nuccio EE, Hagen JA, Fossum CA, Moore ML, Estera-Molina KY, Nico PS, Weber PK, Pett-Ridge J, Firestone MK (2024) Arbuscular mycorrhiza convey significant plant carbon to a diverse hyphosphere microbial food web and mineral-associated organic matter. New Phytol 242:1661–1675. https://doi.org/10.1111/nph.19560

Keyes S, Veelen AV, Fletcher DM, Scotson C, Koebernick N, Petroselli C, Williams K, Ruiz S, Cooper L, Mayon R, Duncan S, Dumont M, Jakobsen I, Oldroyd G, Tkacz A, Poole P, Mosselmans F, Borca C, Huthwelker T, Jones DL, Roose T (2022) Multimodal correlative imaging and modelling of phosphorus uptake from soil by hyphae of mycorrhizal fungi. New Phytol 234:688–703. https://doi.org/10.1111/nph.17980

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301. https://doi.org/10.1890/02-0413

Lambers H, Martinoia E, Renton M (2015) Plant adaptations to severely phosphorusimpoverished soils. Curr Opin Plant Biol 25:23–31. https://doi.org/10.1016/j.pbi.2015.04.002

Li X, Zhao RT, Li DD, Wang GZ, Bei SK, Ju X, An R, Li L, Kuyper TW, Christie P, Bender FS, Veen C, van der Heijden MGA, van der Putten WH, Zhang F, Butterbach-Bahl K, Zhang J (2023) Mycorrhiza-mediated recruitment of complete denitrifying *Pseudomonas* reduces N_2O emissions from soil. Microbiome 11:45. https://doi.org/10.1186/s40168-023-01466-5

Li X, Wu Y, Huang C, Rahman MA, Argaman E, Xiao Y (2025) Inoculation with arbuscular mycorrhizal fungi in the field promotes plant colonization rate and yield. Eur J Agron 164:127503. https://doi.org/10.1016/j.eja.2024.127503

Liu HF, Wu Y, Xu HW, Ai ZM, Zhang JY, Liu GB, Xue S (2021) Mechanistic understanding of interspecific interaction between a C4 grass and a C3 legume via arbuscular mycorrhizal fungi, as influenced by soil phosphorus availability using a ^{13}C and ^{15}N dual-labelled organic patch. Plant J 108:183–196. https://doi.org/10.1111/tpj.15432

Liu X, Chen Q, Zhang HC, Zhang JE, Chen YT, Yao FC, Chen YT (2023) Effects of exogenous organic matter addition on agricultural soil microbial communities and relevant enzyme activities in southern China. Sci Rep 13:8045. https://doi.org/10.1038/s41598-023-33498-0

Liu YZ, Zhuang MH, Liang X, Lam SK, Chen DL, Malik A, Li MY, Lenzen M, Zhang LY, Zhang R, Zhang LX, Hao Y (2024) Localized nitrogen management strategies can halve fertilizer use in Chinese staple crop production. Nat Food 5(10):825–835. https://doi.org/10.1038/s43016-024-01057-z

Lu R (2000) Analysis Methods of Soil Agricultural Chemistry. Agricultural Science and Technology Publishing House, China ((**In Chinese**))

Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V (2010) Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environ Microbiol 12:2165–2179

Lumini E, Vallino M, Alguacil MM, Romani M, Bianciotto V (2011) Different farming and water regimes in Italian rice fields affect arbuscular mycorrhizal fungal soil communities. Ecol Appl 21:1696–1707

Ma B, Wang YL, Ye SD, Liu S, Stirling E, Gilbert JA, Faust K, Knight R, Jansson JK, Cardona C, Röttjers L, Xu JM (2020) Earth microbial co-occurrence network reveals interconnection pattern across microbiomes. Microbiome 8:82. https://doi.org/10.1186/s40168-020-00857-2

Martignoni M, Garnier J, Zhang X, Rosa D, Kokkoris V, Tyson R, Hart M (2021) Co-inoculation with arbuscular mycorrhizal fungi differing in carbon sink strength induces a synergistic effect in plant growth. J Theor Biol 531:110859. https://doi.org/10.1016/j.jtbi.2021.110859

Mcgonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol 1153:495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663

Mitra D, Guerra Sierra BE, Khoshru B, De Los Santos Villalobos S, Belz C, Chaudhary P, Shahri FN, Djebaili R, AdeyemiIcon NO, El-Ballat EM, El-Esawi MA, Moradi S, Mondal R, Senapati A, Panneerselvam P, Das Mohapatra PK (2021) Impacts of arbuscular mycorrhizal fungi on rice growth, development, and stress management with a particular emphasis on strigolactone effects on root development. Commun Soil Sci Plant Anal 52:1591–1621.https://doi.org/10.1080/00103624.2021.1892728

Müller LM, Harrison MJ (2019) Phytohormones, miRNAs, and peptide signals integrate plant phosphorus status with arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 50:132–139. https://doi.org/10.1016/j.pbi.2019.05.004

Nannipieri P, Trasar-Cepeda C, Dick RP (2018) Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. Biol Fertil Soils 54:11–19. https://doi.org/10.1007/s00374-017-1245-6

National Bureau of Statistics (2021) China Rural Statistical Yearbook [in Chinese]. China Statistics Press, Beijing. https://www.stats.gov.cn/zs/tjwh/tjkw/tjzl/202302/t20230215_1907997.html

National Development and Reform Commission (2021) National Agricultural Product Cost-Benefit Data Compilation [in Chinese]. China Statistics Press, Beijing. https://www.ndrc.gov.cn/fgsj/

Naylor D, Sadle N, Bhattacharjee A, Graham EB, Anderton CR, McClure R, Lipton M, Hofmockel KS, Jansson JK (2020) Soil microbiomes under climate change and implications for carbon cycling. Annu Rev Env Res 45:29–59. https://doi.org/10.1146/annurev-environ-012320-082720

Nevins CJ, Lacey C, Armstrong S (2020) The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil till Res 197:104518. https://doi.org/10.1016/j.still.2019.104518

Nuccio EE, Blazewicz SJ, Lafer M, Campbell AN, Kakouridis A, Kimbrel JA, Wollard J, Vyshenska D, Riley R, Tomatsu A, Hestrin R, Malmstrom RR, Firestone M, Pett-Ridge J (2022) HT-SIP: a semi-automated stable isotope probing pipeline identifies cross-kingdom interactions in the hyphosphere of arbuscular mycorrhizal fungi. Microbiome 10:1–20. https://doi.org/10.1186/s40168-022-01391-z

Qin MS, Zhang Q, Pan JB, Jiang SJ, Liu YJ, Bahadur A, Peng ZL, Yang Y, Feng HY (2020a) Effect of arbuscular mycorrhizal fungi on soil enzyme activity is coupled with increased plant biomass. Eur J Soil Sci 71:84–92. https://doi.org/10.1111/ejss.12815

Qin ZF, Zhang HY, Feng G, Christie P, Zhang JL, Li XL, Cai JP (2020b) Soil phosphorus availability modifies the relationship between AM fungal diversity and mycorrhizal

benefits to maize in an agricultural soil. Soil Biol Biochem 144:107790. https://doi.org/10.1016/j.soilbio.2020.107790

Qin YQ, Zhang W, Feng ZW, Feng GD, Zhu HH, Qing Y (2022) Arbuscular mycorrhizal fungus differentially regulates P mobilizing bacterial community and abundance in rhizosphere and hyphosphere. Appl Soil Ecol 170:104294. https://doi.org/10.1016/j.apsoil.2021.104294

Ramos-Garza J, Bustamante-Brito R, Ángeles de Paz G, Medina-Canales MG, Vásquez-Murrieta MS, Wang ET, Rodríguez-Tovar AV (2016) Isolation and characterization of yeasts associated with plants growing in heavy-metal- and arsenic-contaminated soils. Can J Microbiol 62:307–319. https://doi.org/10.1139/cjm-2015-0226

Rillig MC, Steinberg PD (2002) Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? Soil Biol Biochem 34:1371–1374. https://doi.org/10.1016/S0038-0717(02)00060-3

Sapkota TB, Bijay S, Takele R (2023) Digital agriculture for sustainable crop production. In: Sparks DL (ed) Advances in agronomy (vol 178, pp 195–239). Academic Press. https://doi.org/10.1016/bs.agron.2023.05.003

Sato T, Sato T, Saito M, Suzuki T, Fukunaga A, Cheng WG, Tawaraya K (2025) Nursery inoculation with arbuscular mycorrhizal fungi improves yield of *Allium fistulosum* during a 3-year field trial. Soil Sci Plant Nutr 71(4):398–404. https://doi.org/10.1080/00380768.2025.2491693

Shanh M, Lin CC, Kukkadapu R, Engelhard MH, Zhao X, Wang YP, Barkey T, Yee N (2014) Syntrophic effects in a subsurface clostridial consortium on Fe(III)-(oxyhydr) oxide reduction and secondary mineralization. Geomicrobiol J 31(2):101–115. https://doi.org/10.1080/01490451.2013.806610

Shi JC, Wang XL, Wang ET (2023) Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. Annu Rev Plant Biol 74:569–607. https://doi.org/10.1146/annurev-arplant-061722-090342

Shu Q, Xia D, Ma YY, Zhang Y, Luo T, Ma JX, Liu F, Yan SX, Liu DX (2022) Response of physiological characteristics of ecological restoration plants to substrate cement content under exogenous arbuscular mycorrhizal fungal inoculation. Front Plant Sci 13:1028553. https://doi.org/10.3389/fpls.2022.1028553

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic Press, London, pp 117–144

Smith SE, Andrew Smith F, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytol 162:511–524. https://doi.org/10.1111/j.1469-8137.2004.01039.x

Sun JH, Tang Y, Chen KY, Ren SJ, Shi HL, Dong Q, Dong JF, Zhang L, Cui XY, Wang YF, Ji BM, Zhang J (2024) Nitrogen level determines arbuscular mycorrhizal fungi nitrogen uptake rate of *stipa purpurea* in alpine steppe. Plant Soil. https://doi.org/10.1007/s11104-024-07106-7

Thaqi SK, Siani R, Chiba A, Vitow N, Baum C, Leinweber P, Panten K, Schloter M, Schulz S (2025) Effects of novel P fertilizers on microbial abundance related to N and P cycling in two on-farm systems. Agr Ecosyst Environ 385:109565. https://doi.org/10.1016/j.agee.2025.109565

Vallino M, Fiorilli V, Bonfante P (2014) Rice flooding negatively impacts root branching and arbuscular mycorrhizal colonization, but not fungal viability. Plant Cell Environ 37:557–572. https://doi.org/10.1111/pce.12177

Veresoglou SD, Chen BD, Rillig MC (2012) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62. https://doi.org/10.1016/j.soilbio.2011.11.018

Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007. https://doi.org/10.1128/AEM.64.12.5004-5007.1998

Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat Commun 10:4841. https://doi.org/10.1038/s41467-019-12798-y

Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R (2016) Improved bacterial 16S rRNA gene (V4 and V4–5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1:e00009–15

Wan XH, Huang ZQ, He ZM, Yu ZP, Wang MH, Davis MR, Yang YS (2015) Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. Plant Soil 387:103–116. https://doi.org/10.1007/s11104-014-2277-4

Wang SS, Chen AQ, Xie K, Yang XF, Luo ZZ, Chen JD, Zeng DC, Ren YH, Yang CF, Wang LG, Feng HM, López-Arredondo DL, Herrera-Estrella LR, Xu GH (2020) Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen acquisition in plants. Proc Natl Acad Sci U S A 117:16649–16659. https://doi.org/10.1073/pnas.2000926117

Wang GW, Jin ZX, George TS, Feng G, Zhang L (2023a) Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. New Phytol 238:2578–2593. https://doi.org/10.1111/nph.18772

Wang LT, Zhang L, George TS, Feng G (2023b) A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. New Phytol 238:859–873. https://doi.org/10.1111/nph.18642

Wang YL, Niu GX, Wang RZ, Rousk K, Li A, Hasi M, Wang CH, Xue JG, Yang GJ, Lü XT, Jiang Y, Han XG, Huang JH (2023c) Enhanced foliar ^{15}N enrichment with increasing nitrogen addition rates: role of plant species and nitrogen compounds. Glob Chang Biol 29:1591–1605. https://doi.org/10.1111/gcb.16555

Wang BY, Xiao QC, Geng XW, Lin KQ, Li ZS, Li YY, Chen J, Li XY (2024) Arbuscular mycorrhizal fungi alter rhizosphere bacterial diversity, network stability and function of lettuce in barren soil. Sci Hortic 323:112533. https://doi.org/10.1016/j.scienta.2023.112533

Wang H, Lu JY, Dijkstra FA, Sun LJ, Yin LM, Wang P, Cheng WX (2025a) Rhizosphere priming effects and trade-offs among root traits, exudation and mycorrhizal symbioses. Soil Biol Biochem 202:109690. https://doi.org/10.1016/j.soilbio.2024.109690

Wang LT, Zhang L, George TS, Feng G (2025b) Hyphosphere core taxa link plant-arbuscular mycorrhizal fungi combinations to soil organic phosphorus mineralization. Soil Biol Biochem 201:109647. https://doi.org/10.1016/j.soilbio.2024.109647

Wang SS, Ye HH, Yang CF, Zhang Y, Pu JW, Ren YH, Xie K, Wang LX, Zeng DC, He HQ, Ji HY, Herrera-estrella LR, Xu GH, Chen AQ (2025c) OsNLP3 and OsPHR2 orchestrate direct and mycorrhizal pathways for nitrate uptake by regulating NAR2.1–NRT2s complexes in rice. Proc Natl Acad Sci U S A 122:e2416345122

Wen T, Xie P, Yang S, Niu G, Liu X, Ding Z, Xue C, Liu YX, Shen Q, Yuan J (2022) Ggclusternet: an R package for microbiome network analysis and modularity-based multiple network layouts. iMeta 1:e32

Werner GDA, Kiers ET (2015) Partner selection in the mycorrhizal mutualism. N New Phytol 205(4):1437–1442. https://doi.org/10.1111/nph.13113

Wu YJ, Chen CJ, Li JZ, Wang GA (2021) Effects of arbuscular mycorrhizal fungi on maize nitrogen uptake strategy under different soil water conditions. Plant Soil 464:441–452. https://doi.org/10.1007/s11104-021-04972-3

Xiao JT, Kao-Kniffin J, Zhu B (2025) Arbuscular mycorrhizal fungi enhance nitrogen acquisition from, but not carbon loss of, organic matter in soil. New Phytol 247:1415–1425

Xie K, Ren YH, Chen AK, Yang CF, Zheng QS, Chen J, Wang DS, Li YT, Hu SJ, Xu GH (2022) Plant nitrogen nutrition: the roles of arbuscular mycorrhizal fungi. J Plant Physiol 269:153591. https://doi.org/10.1016/j.jplph.2021.153591

Xu YG, Peng ZP, Tu YT, Huang JC (2023) Combining organic and inorganic fertilization increases rice yield and soil nitrogen and carbon: dissolved organic matter chemodiversity and soil microbial communities. Plant Soil 492:557–571. https://doi.org/10.1007/s11104-023-06203-3

Xue J, Guo L, Li L, Zhang Z, Huang M, Cai J, Wang X, Zhang Y, Dai T, Jiang D (2024) Effects of arbuscular mycorrhizal fungi on uptake, partitioning and use efficiency of nitrogen in wheat. Field Crop Res 306:13

Yang JF, Zang WS, Chen J, Lu DY, Li RT, Li CY, Chen YH, Liu Q, Niu XL (2025) Genomic analysis of *Penicillium griseofulvum* CF3 reveals potential for plant growth promotion and disease resistance. J Fungi 11(2):153. https://doi.org/10.3390/jof11020153

Yang HS, Fang C, Li YF, Wu YC, Fransson P, Rillig MC., Zhai SL, JJ Xie JJ, Tong ZY, Zhang Q, Sheteiwy MS, Li F, Weih M (2022) Temporal complementarity between roots and mycorrhizal fungi drives wheat nitrogen use efficiency. New Phytol 236: 1168–1181https://doi.org/10.1111/nph.18419

Yao Q, Zhu HH, Hu YL, Liu LQ (2008) Differential influence of native and introduced arbuscular mycorrhizal fungi on growth of dominant and subordinate plants. Plant Ecol 196:261–268. https://doi.org/10.1007/s11258-007-9350-5

Ye SP, Yang YJ, Xin GR, Wang YT, Ruan L, Ye GR (2015) Studies of the Italian ryegrass–rice rotation system in southern China: Arbuscular mycorrhizal symbiosis affects soil microorganisms and enzyme activities in the *Lolium mutiflorum* L. rhizosphere. Appl Soil Ecol 90:26–34. https://doi.org/10.1016/j.apsoil.2015.01.017

Yimer T, Abera G, Beyene S, Bono B, Rasche F (2024) Combined inoculation of arbuscular mycorrhiza fungi with *Meso-rhizobium* improves nutrient uptake, growth performance, and moisture stress tolerance of chickpea (*Cicer arietinum* L.). Agrosyst Geosci Environ 7:e20562. https://doi.org/10.1002/agg2.20562

Zak DR, Holmes WE, White DC, Peacock AD, Tilman D (2003) Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84(8):2042–2050. https://doi.org/10.1890/02-0433

Zhang X, Davidson EA, Mauzerall DL, Searchinger TD (2015) Managing nitrogen for sustainable development. Nature 528:51–59. https://doi.org/10.1038/nature15743

Zhang L, Xu MG, Liu Y, Zhang FS, Hodge A, Feng G (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytol 210:1022–1032. https://doi.org/10.1111/nph.13838

Zhang L, Shi N, Fan JQ, Wang F, George TS, Feng G (2018) Arbuscular mycorrhizal fungi stimulate organic phosphate mobilization associated with changing bacterial community structure under field conditions. Environ Microbiol 20:2639–2651. https://doi.org/10.1111/1462-2920.14289

Zhang J, Wang P, Xue K, Hao YB, Wang YF, Cui XY (2019) Trait complementarity between fine roots of *Stipa purpurea* and their associated arbuscular mycorrhizal fungi along a precipitation gradient in Tibetan alpine steppe. J Mt Sci 16:542–547. https://doi.org/10.1007/s11629-018-5311-9

Zhang L, Zhou JC, George TS, Limpens E, Feng G (2022a) Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. Trends Plant Sci 27:402–411. https://doi.org/10.1016/j.tplants.2021.10.008

Zhang XL, Qiu YP, Gilliam FS, Gillespie CJ, Tu C, Reberg-Horton SC, Hu SJ (2022b) Arbuscular mycorrhizae shift community composition of N-cycling microbes and suppress soil N_2O emission. Environ Sci Technol 56:13461–13472

Zhou JB, Li SX, Chen ZJ (2002) Soil microbial biomass nitrogen and its relationship to uptake of nitrogen by plants. Pedosphere 12:251–256

Zhou J, Zang HD, Loeppmann S, Gube M, Kuzyakov Y, Pausch J (2020b) Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. Soil Biol Biochem 140:107641. https://doi.org/10.1016/j.soilbio.2019.107641

Zhou GP, Gao SJ, Chang DN, Rees RM, Cao WD (2021) Using milk vetch (*astragalus sinicus* L.) to promote rice straw decomposition by regulating enzyme activity and bacterial community. Bioresour Technol 319:124215. https://doi.org/10.1016/j.biortech.2020.124215

Zhou Q, Sun HM, Jia LX, Wu WZ (2022) Simultaneously advanced removal of nitrogen and phosphorus in a biofilter packed with ZVI/PHBV/sawdust composite: deciphering the succession of dominant bacteria and keystone species. Bioresour Technol. https://doi.org/10.1016/j.biortech.2022.126724

Zhou J, Chai XF, Zhang L, George TS, Wang F, Feng G, Chu HY(2020a) Different arbuscular mycorrhizal fungi co-colonizing on a single plant root system recruit distinct microbiomes. mSystems 5: e00929–20. https://doi.org/10.1128/msystems.00929-20