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Optimal stimulation of rhizosphere nutrient mobilization and grassland plant growth by intermediate but not by high diversity of arbuscular mycorrhizal fungi[☆]

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are prevalent in grasslands and could aid in their restoration. It's still unclear whether increased AMF species diversity is universally beneficial for grassland plants. This study examined the impact of AMF diversity on plant growth using *Lolium perenne* L., *Dactylis glomerata* L., and *Trifolium repens* L in degraded grassland soil with the addition of five AMF species both individually and in various combinations. We found that a mix of three AMF species (3AMF) maximized plant growth, increasing shoot biomass by up to 58% compared to the non-mycorrhizal control, while a five-species mix (5AMF) showed a lesser effect. The 3AMF treatment enhanced soil nitrogen and phosphorus cycling, increased activities of key enzymes (e.g., acid phosphatase by ~40%), and favored three key bacterial genera (*Citrifermentans, Rhodococcus, Flavisolibacter*) positively correlated with nutrient cycles. In contrast, 5AMF intensified competition for plant-derived carbon, likely reducing the abundance of these functional bacteria. Synthesis: the study provides evidence that the benefits of AMF diversity follow a non-linear pattern, with an optimum at intermediate diversity, mediated by shifts in cooperation and competition with rhizosphere bacteria. This insight emphasizes the importance of optimizing rather than maximizing AMF diversity in grassland restoration strategies.

1. Introduction

Globally, grasslands account for approximately 50% of terrestrial ecosystems (Wilsey, 2018) and provide important ecosystem services and soil functions such as food production, biodiversity, cultural services, and carbon storage (Bai and Cotrufo, 2022). However, grasslands face severe threats due to degradation, which affects their ability to support biodiversity, ecosystem services, and human well-being (Bardgett et al., 2021). Therefore, the restoration of degraded grasslands has important ecological, economic, and social value. Arbuscular mycorrhizal fungi (AMF) coexist in symbiotic relationships with more than 80% of terrestrial plants (Brundrett and Tedersoo, 2018; He et al., 2024) and are particularly widely distributed in grassland ecosystems. These fungi can improve the absorption of water and mineral nutrients by the host plant from the soil, enhance net primary productivity, increase plant biomass, and improve soil quality and soil aggregate stability, thus promoting the stability of the plant–soil system (Rillig and Mummey, 2006). However, the ecological outcomes of AMF-mediated processes depend critically on fungal community composition, diversity, and interactions with native soil biota, factors that are often overlooked in restoration practices. While foundational studies have demonstrated that high AMF species richness can increase plant community productivity and biodiversity in simplified systems (Van der Heijden et al., 1998), the specific advantages of AMF diversity in grassland restoration contexts remain highly debated. In such settings, interventions are applied to soils containing pre-existing native microbial communities, and reported effects vary widely, ranging from positive to neutral or even negative outcomes (Jansa et al., 2008; Gosling

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et al., 2016; Horsch et al., 2023).

The relationship between AMF diversity and plant performance remains complex, with context-dependent outcomes observed across studies (Hartnett and Wilson, 2002; Horsch et al., 2023). In macrocosms characterized by a low-diversity background, plant biodiversity and productivity showed a linear increase with AMF species richness (Van der Heijden et al., 1998). Subsequent studies corroborate that diverse AMF communities often promote greater nutrient uptake and plant growth than fewer species inoculations do, partly due to functional complementation (Jansa et al., 2008; Blažková et al., 2021). For example, inoculation with a mixture of *Glomus claroideum* and *Glomus intraradices* provides additional P for the leek compared with either of the two AMF separately, indicating a synergistic effect of increasing AMF biodiversity (Jansa et al., 2008). Conversely, other studies report no consistent benefits of AMF diversity, with single-species inoculations occasionally outperforming mixtures (Gustafson and Casper, 2006; Jansa et al., 2008; Thonar et al., 2014; Gosling et al., 2016). This inconsistency primarily arises from interactions between introduced inoculants and resident AMF communities, which may lead to antagonism or functional redundancy (Horsch et al., 2023). Thus, moving beyond the general linear model, the mechanistic drivers of these context-dependent benefits, particularly the role of AMF diversity in modulating interactions with native rhizosphere bacteria under restoration scenarios, require deeper investigation to optimize management strategies.

Mechanistically, AMF influence plant–soil feedback by modulating rhizosphere microbial activity. Mycorrhizal hyphae exude carbohydrates derived from plants to the soil and serve as a carbon source attracting bacterial communities (Zhang et al., 2016). In some cases, exudates released by mycorrhizal hyphae serve as signaling molecules to stimulate bacterial function (Zhang et al., 2018a), such as P mobilization through decomposition or mineralization, or alternatively stimulate immobilization (Rozmoš et al., 2022; Zhang et al., 2022; Wang et al., 2023). For example, AMF-associated phosphate-solubilizing bacteria enhance organic phosphorus mobilization (Zhang et al., 2018b; Jiang et al., 2021). These interactions underscore the need to integrate microbial community dynamics when evaluating the effects of AMF on plant growth. However, most studies have focused narrowly on plant–AMF relationships, neglecting interactions with native soil biota that mediate ecosystem outcomes (Trivedi et al., 2020; Kang et al., 2021). Thus, to achieve a mechanistic understanding of the relationships between AMF and plant growth, it is essential to consider interactions with free-living soil microbes.

In this study, we investigated the impact of single and mixed-species AMF inoculation on grassland plants growth and explored the potential mechanisms involving rhizosphere bacterial interaction. We cultivated a representative grassland plant mixture (*Lolium perenne* L., *Dactylis glomerata* L. and *Trifolium repens* L.) inoculated with five AMF species applied individually or in defined combinations (1 to 5 species). The experimental treatments (see section 2.1) were designed to create a gradient of AMF diversity, ranging from suppression of the native community to introduction of high diversity, allowing us to assess effects against a realistic microbial background. Plant growth responses and soil enzyme activities related to nutrient cycling were quantified. Rhizosphere bacterial communities, including core genera associated with AMF symbiosis, were characterized via 16S rRNA gene sequencing. Building on ecological theory suggesting that biodiversity effects can be non-linear due to increasing competitive interactions, and on evidence for AMF-bacterial cross-feeding, we proposed the following mechanistic hypotheses: (1) Moderate AMF diversity (specifically 3-species mixtures) would optimize plant growth by balancing symbiotic resource exchange and fostering cooperative interactions with bacteria, whereas higher diversity (5-species mixtures) might induce competitive constraints among fungi and for host carbon; (2) The growth-promotion by optimal AMF diversity would be driven by enhanced phosphorus acquisition through synergistic upregulation of P-cycling enzymes and recruitment of specialized phosphate-solubilizing bacteria; (3) Higher AMF diversity (5-species) would intensify competition between AMF and rhizosphere bacteria for plant-derived carbon, thereby reducing bacterial functional contributions to nutrient mobilization. These hypotheses were tested to unravel the causal mechanisms underlying AMF diversity effects on plant performance, ultimately informing targeted grassland restoration strategies.

2. Material and methods

2.1. Experimental design and materials

The soil used in this study was superficial soil (0–20 cm depth) collected from a grassland in Zhaotong, Yunnan Province (27°40′N, 103°36′E), after the grass was removed. The soil type was classified as yellow–brown soil. The main characteristics of the soil were as follows: pH, 4.72; total carbon (C), 6.08%; total nitrogen (N), 0.47%; total P, 0.15%; total potassium, 1.89%; available phosphorus, 10.98 $mg \cdot kg^{-1}$; available potassium, 233.30 $mg \cdot kg^{-1}$; ammonium nitrogen, 16.52 $mg \cdot kg^{-1}$; nitrate nitrogen, 0.43 $mg \cdot kg^{-1}$; and water-soluble organic carbon, 39.65 $mg \cdot kg^{-1}$. To simulate natural grassland conditions as closely as possible, the soil used in this experiment was not sterilized. The native AMF community in the degraded grassland soil was characterized using AMF-specific SSU rRNA primers (Supplementary Table S1). The community was dominated by *Acaulospora*, with relatively low relative abundances of uncultivated *Glomeromycotina*, *Rhizophagus* sp., *Rhizophagus intraradices*, and *Entrophospora claroidea*. *Lolium perenne* L., *Dactylis glomerata* L., and *Trifolium repens* L. were the dominant plants in this grassland. Notably, each of these three plants forms an intimate symbiotic association with AMF (Gollotte et al., 2004; Hu et al., 2013; Legay et al., 2020; Wu et al., 2023). Thus, *L. perenne*, *D. glomerata*, and *T. repens* were used as host plants. The five most commonly used species of AMF (Xu et al., 2023a) were used in this study, namely, *Glomus intraradices*, *Glomus etunicatum*, *Glomus walkeri*, *Glomus mosseae*, and *Glomus versiforme*. These species are frequently employed in grassland restoration studies and are representative of key functional groups in such ecosystems.

To explore the effects of increasing AM fungal diversity on grassland plant growth, six treatments involving individual and mixed AMF species inoculation were implemented: (1) CK (mycorrhizal suppression via benomyl application), (2) NM (natural AMF community), (3) GM (addition of *G. mosseae*), (4) GV (addition of *G. versiforme*), (5) 3AMF (mixture of *G. mosseae*, *G. versiforme* and *G. intraradices)* and (6) 5AMF (mixture of *G. intraradices*, *C. etunicatum*, *C. walkeri*, *G. mosseae* and *G. versiforme*). This design was intended to create a gradient of AMF diversity, ranging from suppression of the native community (CK), through the baseline native community (NM) and single-species additions, to low (3AMF) and high (5AMF) diversity mixtures introduced against that native background. The specific species combinations were selected based on their common use in restoration research and to allow investigation of potential non-linear diversity effects under ecologically relevant conditions. Benomyl has been shown to reduce AM fungal colonization with minimal effects on plants and other soil microorganisms (Hartnett and Wilson, 1999; O'Connor et al., 2002), and benomyl application has been used successfully to test the functions of AM fungal communities in pot studies (Huangfu et al., 2019; Chen et al., 2020; Wang et al., 2022a). For the single-AMF inoculation treatment, 100 spores were added to each pot. For mixed AMF inoculation, each AM fungal spore was evenly added to a total of 100 spores. Each treatment consisted of 6 replicates with total of 36 pots (11 cm × 11 cm, height 20 cm). Each pot, containing 1.5 kg of soil (corresponding to a bulk density of approximately 1.3 g cm^{-3}, chosen to provide sufficient volume for root development without being overly limiting), was planted with mixtures of L. *perenne*, *D. glomerata*, and *T. repens*. Post-germination, thinning was conducted to adjust the number of each host species in all pots to 15 plants. This high planting density was employed to ensure rapid and uniform root exploration of the pot volume, to create a pronounced and consistent rhizosphere environment for microbial sampling, and to mimic the intense belowground competition often found in degraded grasslands. The seedlings were irrigated, and the level of soil moisture was maintained at 65–75% field capacity. Field capacity was determined as the water content retained after saturating a soil column and allowing free drainage for 48 h. The plants were grown in a greenhouse with 16 h of light at 28 °C and 8 h of darkness at 25 °C.

2.2. Sample collection and analyses

A previous study revealed that plant growth is greatest 40 days after AMF inoculation (Xu et al., 2023b); thus, we collected plant and soil samples 40 days after inoculation with AMF. The rhizosphere soil was collected as previously described (MR et al., 2018). In summary, the loose soil attached to the roots was removed by gently shaking, and then the roots with rhizosphere soil were transported to precooled phosphate-buffered saline (pH 7.5). The abovementioned mixture was evenly mixed and then filtered through a sterile nylon 100 μm mesh filter, which was then centrifuged to remove the supernatant, and the soil of the rhizosphere was collected.

Shoot and root samples were harvested and dried in an oven at 105 °C for 30 min and then at 75 °C to a constant weight to measure dry weight (Gao et al., 2019). For the determination of nutrient concentrations, plant samples were first digested in H_2SO_4 with H_2O_2 as an additive. The dried plant samples were ground and passed through a 0.25 mm sieve to determine the C, N, and P concentrations. The total contents of N and P were measured using a CleverChem 380 discontinuous water quality analyzer (DeChem-Tech, Hamburg, Germany). Dissolving organic C (DOC) was extracted with distilled 2 M KCl (Jones and Willett, 2006). For total C, the plant samples were dried at 60 °C for 48 h to remove moisture and then ground into a fine powder. Approximately 5 mg of the ground plant material was weighed and placed into a tin capsule. DOC and total C were then quantified using an elemental analyzer Vario TOC analyzer (Vario TOC cube; Elementar, Hanau, Germany). A TOC analyzer was used to quantify the carbon content by combustion and subsequent detection of the evolved CO_2. The results are expressed as a percentage of the total dry weight of the plant material. Mycorrhizal growth responses (MGRs) were calculated using the following formula (Hetrick et al., 2011): $MGR = 100 \times (AM - NM)/NM$. AM refers to the total dry biomass of individual mycorrhizal plants, and NM refers to the average dry biomass of nonmycorrhizal plants (treated with the addition of benomyl). Similarly, we determined the effects of different AMF on the N, P, and C contents of plants. Microbial biomass C (MBC) and P (MBP) were measured by the chloroform fumigation extraction method as previously described (Brookes et al., 1985; Vance et al., 1987). Briefly, fresh soils were split into fumigated and non-fumigated groups, with fumigated samples subjected to 24 h of ethanol-free chloroform vapor treatment in a vacuum desiccator, followed by residual chloroform removal. Both groups were extracted with 0.5 M K_2SO_4 for MBC or 0.5 M $NaHCO_3$ (pH 8.5) for MBP via 30 min of shaking and centrifugation (4000 ×*g*, 20 min). MBC was quantified as the difference in fumigated/nonfumigated organic carbon (TOC analyzer) multiplied by 0.45 (Joergensen, 1996). MBP was determined colorimetrically (molybdenum blue method) using a 0.40 efficiency factor (Brookes et al., 1985). Triplicates and blank controls were included throughout. Root colonization by AMF was assessed for all treatments after staining with trypan blue (Xue et al., 2015), and the results of AMF colonization rates are presented in Fig. S1.

2.3. Soil enzyme activity

The activities of pyrroloquinoline quinone synthase (inorganic P transformation), phytase (organic P transformation), and acid phosphatases (organic P transformation) were assessed. Given the primary role of AMF in plant phosphorus nutrition and our initial plant data indicating phosphorus uptake as a key response variable, we focused our enzymatic analysis on processes central to the soil phosphorus cycle. Enzyme-linked immunosorbent assay kits (Mlbio) were used to determine the activities of these enzymes in the soil (Peng et al., 2021). The ELISA kits included a series of calibration standards to determine sample enzyme concentrations. The calibration and sample standards were analyzed simultaneously. The operator produced a standard optical density (O.D.) curve versus the enzyme concentration, with the enzyme activity determined by comparing the sample O.D. values to those of the standard curve.

2.4. 16S rRNA gene sequencing

Amplicon sequencing was performed on AMF mixture–inoculated rhizosphere soil samples, which included two groups (three types of AMF mixtures and five types of AMF mixtures), with four biological replicates of each group. 16S rRNA gene sequencing was performed by Sangon Biotech (Shanghai, China) on an Illumina MiSeq platform. The V3-V4 regions of the 16S rRNA gene were amplified with the universal primers 341F: CCTACGGGNGGCWGCAG and 805R: GACTACHVGGGTATCTAATCC (Defez et al., 2017). 16S rRNA gene sequencing was performed as a previous study described (Xu et al., 2023b). In summary, the DADA2 pipeline in R for inference of the amplicon sequence variant (ASV) was used to process the 16S rRNA gene sequencing data and the relative abundance values of each strain or taxon in a sample were calculated by proportional normalization of each sample by its sequencing depth. Alpha diversity was calculated using species richness, the Shannon index, the Chao1 index, and the ACE index. Furthermore, beta diversity was measured using Bray-Curtis metrics and nonmetric multidimensional scaling (NMDS) to investigate structural variation in bacterial communities across samples (Ramette, 2007). A neutral community model was used to test the effects of neutral processes on the assembly of the bacterial community as previously described (Sloan et al., 2006a).

2.5. Functional gene quantification

Total genomic soil DNA was extracted from the soil samples using a FastDNA SPIN kit (MP Biomedicals, France) following the manufacturer's instructions. The functional genes of bacteria associated with P cycling processes were assessed by quantitative PCR (qPCR). Standard curves were obtained using serial dilutions of a known amount of plasmid DNA containing specific gene fragments. Quantification was performed with an ABI QuantStudio 7 (Applied Biosystems, Germany) in a 20 μl reaction mixture that included 10 μl of 2× TB Green Premix Ex Taq II (Tli RNaseH Plus), 0.4 μl of 50× ROX reference dye II (TaKaRa Bio Inc., Dalian, China), 0.8 μl of each primer (10 μM; forward primer and reverse primer), 2 μl of template, and 6 μl of double-distilled water.

2.6. Genome analyses

We downloaded related bacterial genomes (*Flavisolibacter ginsengisoli* DSM 18119, *Citrifermentans bremense* and *Rhodococcus erythropolis*_pr4) from NCBI to explore the functions of the core bacteria. The genome sequences were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The draft genome of the core bacteria was subsequently drawn on the basis of the downloaded genome data. Genome assembly, circular genome mapping of draft genome sequences, and COG category annotation were conducted using CGView (Stothard and Wishart, 2005). Comprehensive and detailed functional annotation of the genome was performed using Bakta, an online platform for bacterial genome annotation.

2.7. Data analysis

Statistical analyses of plant growth and nutrient concentrations were performed using SPSS (SPSS26, Inc., Chicago, IL, USA), and statistically significant differences were determined by Student's *t*-test or two-way ANOVA among the different groups. A *p* value$<$0.05 was considered statistically significant. Sequencing data analysis, visualization and statistical calculations were performed using R (version 3.6.0). Alpha diversity was calculated using the 'vegan' package in R. For the sloan neutral model (Sloan et al., 2006b; Rezki et al., 2017), 95% binomial confidence intervals for the neutral model were constructed via the Wilson method using the 'Hmisc' package in R (Morris et al., 2013). A Sankey plot was created by ggplot2 in R. A Spearman correlation heatmap was constructed by the corrplot package (Wei and Simko, 2021) and the ggcorrplot package (Tian et al., 2021) in R. Other graphical representations, such as column charts and box plots, were created using Origin 2021. All the figures were refined for color matching and overall aesthetics using Adobe Illustrator 2019. The original R code used for data analysis and visualization has been uploaded to GitHub (https://github.com/xuyunjian1992/Intermediate-AMF-diversity-for-optimal-rhizosphere-stimulation.git).

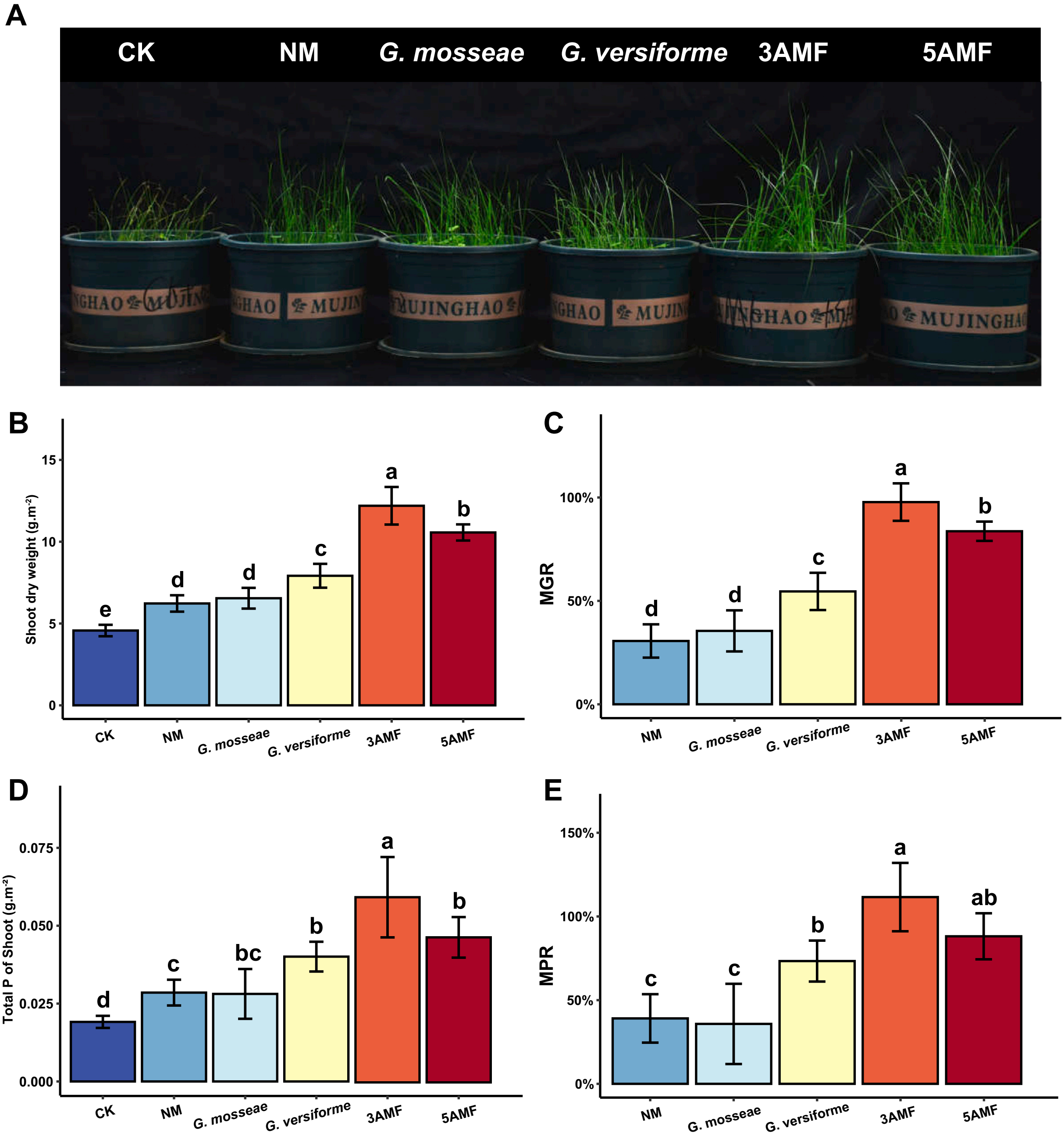

Fig. 1. Growth of plants under different AMF colonization conditions. (A) Phenotypes of plants inoculated with different AMF. (B) Shoot dry weight of plants inoculated with different AMF. (C) Mycorrhizal response to plant growth (MGR). (D) Total phosphorus concentrations of the shoots. (E) Mycorrhizal response to the phosphorus content (MPR). Three plant species (*Lolium perenne* L., *Trifolium repens* L., and *Dactylis glomerata* L.) were included. CK represents the application of benomyl to eliminate the influence of native AMF. NM represents native AMF in the soil. *G. mosseae* and *G. versiforme* represent single inoculations with *Glomus mosseae* or *Glomus versiforme* in the native soil. The 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. The 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*. The bars present the averages with standard errors. Different letters above the bars indicate significant differences ($P < 0.05$).

3. Results

3.1. Inoculation of three species of AMF had the most significant stimulatory effects on plants

The results revealed that both native AMF (NM) and the addition of additional AMF significantly promoted the growth of the three plants (*L. perenne* L., *T. repens* L., and *D. glomerata* L.) compared with the benomyl treatment (CK), whereas AMF addition had a greater effect than did NM addition (Fig. 1A, B and Fig. S2). All three grass species showed consistent growth responses after inoculation with different AMF. Both the height and shoot dry weight of the three pasture plants significantly increased after the various AMF inoculations (Fig. S2A, B). Interestingly, both inoculation with a single AMF species or coinoculation with five AMF species (5AMF) similarly increased the height and dry weight of each plant compared with those of the CK group. Compared with the addition of only three AMF species (3AMF), the addition of 5AMF had a smaller promoting effect on plant height and shoot dry weight (Fig. S2).

Compared with the NM treatment, single inoculation with *G. versiforme* resulted in a significantly greater MGR. The addition of 3AMF species further increased the MGR, which achieved the optimal effect, and the MGR after adding 5AMF was smaller (Fig. 1C). The response of the shoot dry weight of the three investigated plants aligned well with the MGR (Fig. S2B). Therefore, inoculation with AMF generally promoted grassland plant growth, but the response to increasing AMF diversity reached an optimum value at 3 added species.

3.2. Optimal AMF species diversity promotes soil phosphorus transformation and plant phosphorus uptake

Among the treatments, inoculation with 3AMF resulted in the highest total P content in the shoots, followed by inoculation with 5AMF or *G. versiforme* (Fig. 1D). The mycorrhizal P response (MPR) of the plants was consistent with the total P content of the shoot, in which 3AMF presented the highest MPR (Fig. 1E). Interestingly, the trends in shoot total C content and mycorrhizal C response (MCR) were consistent with the trends in shoot total P content and MPR response (Fig. S3 A, B). While *G. versiforme*, 3 AMF, 5AMF, and NM increased the total N in aboveground plant tissues, AMF inoculation induced significantly greater increases than did NM (Fig. S3C). The mycorrhizal nitrogen response (MNR) mirrored this pattern (Fig. S3D). Nevertheless, no significant differences were observed between the 3AMF and 5AMF treatments for either total N or MNR. Therefore, the superior ability of 3AMF to increase phosphorus uptake in plants may be the primary reason for the more pronounced growth-promoting effect of 3AMF relative to the other treatments.

To further elucidate whether the effects of AMF on plant P status are mediated by soil processes, enzymes involved in soil P turnover were quantified. AMF inoculation persistently and significantly increased the activities of pyrroloquinoline-quinone synthase, phytase and acid phosphatases (Fig. 2A-C). In terms of the activity of the three enzymes, we found a response to increasing AMF diversity similar to that observed for the MGR in that we observed the highest enzyme activity after inoculation with 3AMF species but not after inoculation with 5AMF species, indicating that the capacity of 5AMF to influence the transformation of available phosphorus in soil was weaker than that of the 3 AMF treatments. Similarly, the soil available P concentration and microbial biomass P were greater in the 3AMF treatments than in the 5AMF treatments (Fig. 2D, E).

3.3. Diversity and composition of the bacterial community in the rhizosphere

Taxonomic analysis revealed changes in the composition, richness and diversity of the bacterial community under different AMF treatments (Fig. 3, Figs. S4-S5). Richness and diversity were significantly greater in samples with AMF colonization compared to noncolonization based on the Shannon, Chao1, ACE, richness and Good's coverage indices (Fig. 3A and Fig. S4A). Among these parameters, the 5AMF

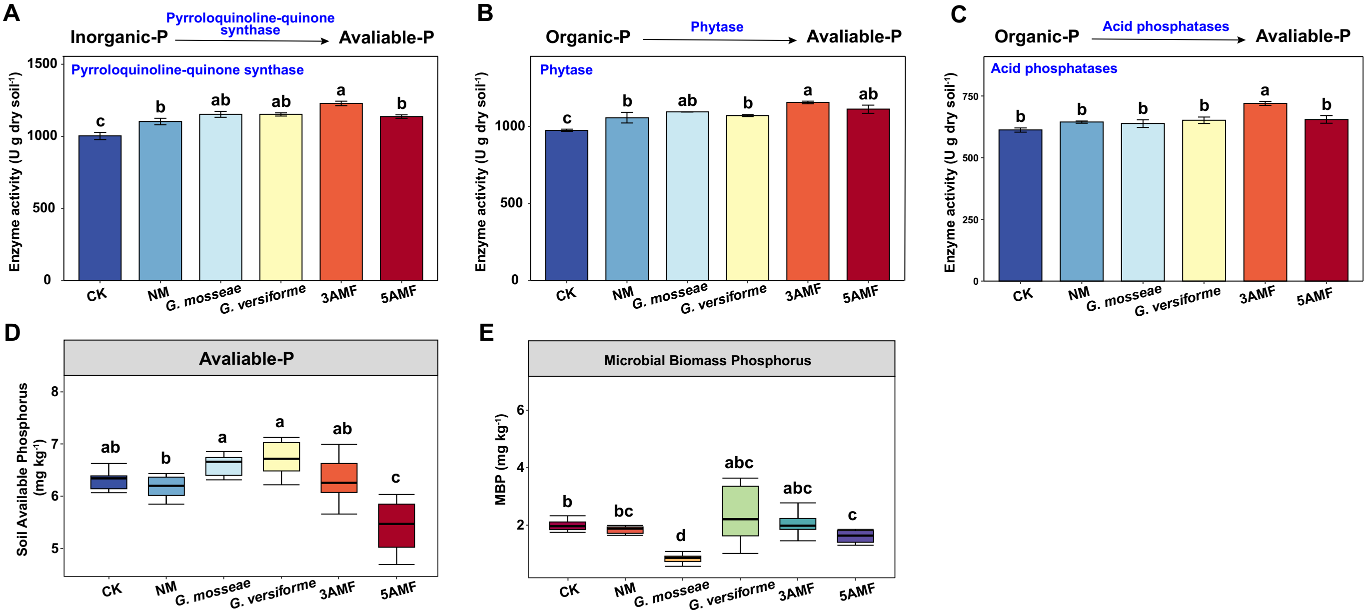

Fig. 2. Activities of enzymes related to phosphorus cycling in rhizosphere soil. (A) Activity of soil pyrroloquinone-quinone synthase. (B) Activity of soil phytase. (C) Activity of soil acid phosphatases. (D) Concentration of soil available phosphorus. (E) Microbial biomass phosphorus (MBP) in the rhizosphere soil. CK represents the application of benomyl to eliminate the influence of native AMF. NM represents native AMF in the soil. *G. mosseae* and *G. versiforme* represent single inoculations with *Glomus mosseae* or *G. versiforme* in the native soil. The 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*. The bars in (A), (B), and (C) present the averages with standard errors. In (D) and (E), the position of the upper whisker represents the highest score; the boxes represent the lower and higher quartiles; the position of the lower whisker represents the lowest score; and the line within each box marks the median. Different letters above the bars indicate significant differences ($P < 0.05$).

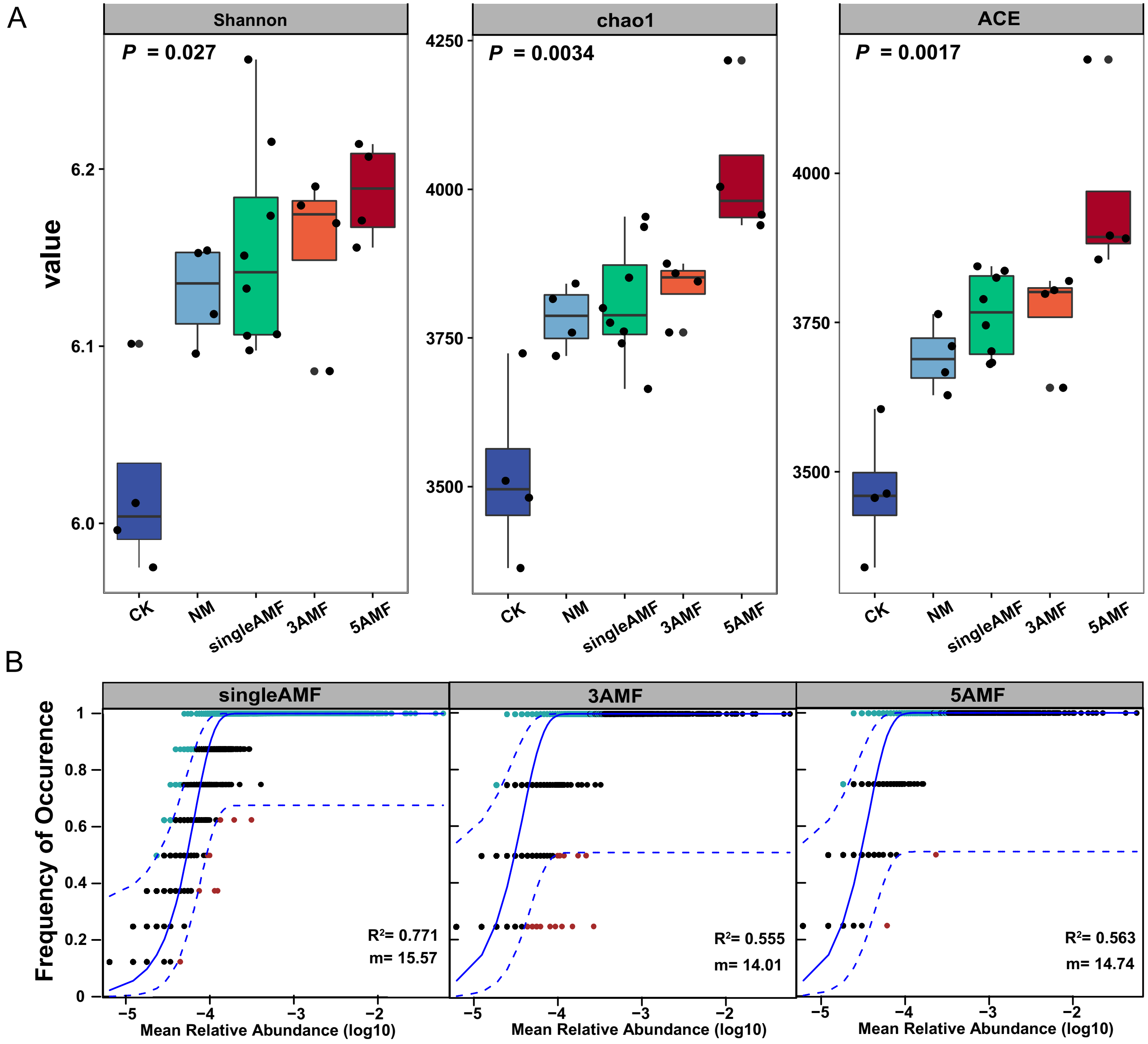

Fig. 3. Comparison of the bacterial community structure in rhizosphere soil under different AMF treatments on the basis of high-throughput sequencing of 16S rDNA amplicons. (A) Alpha diversity analysis, including the Shannon index, Chao1 index, and ACE index. (B) Sloan neutral model prediction of bacteria in rhizosphere soil inoculated with a single AMF, 3AMF or 5AMF. The R2 values indicate fit-to-neutral assembly, and the m values indicate the estimated migration rate. Data points represent operational taxonomic units (OTUs), and different colors represent the taxon fit above (blue-green), within (black), or below (brick red) the 95% confidence interval (dashed lines). The data for single AMF include *Glomus mosseae*- and *G. versiforme*-inoculated samples. CK represents the application of benomyl to eliminate the influence of native AMF. NM represents native AMF in the soil. 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment resulted in the greatest richness and diversity, followed by the 3AMF treatment, the single AMF treatment, the NM treatment, and the CK treatment. The structure of the bacterial community in each treatment was further compared using NMDS. As shown in Fig. S5, the clustering of samples was related to whether AMF was added and the type of addition.

Significant differences in the assembly of bacterial communities were observed among the different AMF addition groups on the basis of the Sloan neutral model. The assembly patterns of the rhizosphere bacterial community fit the neutral model under the different AMF treatments (Fig. 3B, Fig. S4B), and the deterministic process was essential for shaping the community assembly of bacteria related to mixed AMF inoculation. However, with the addition of AMF, the fitness of the neutral model to the bacterial community decreased, and the relative contribution of the stochastic process gradually decreased. The bacterial communities were initially affected by the stochastic process when a single AMF was added, but the deterministic process increased with the addition of 3AMF. However, when 5 AMF were added, the bacterial community tended to change to a stochastic process. The migration rates revealed that the addition of AMF limited the spread of bacteria, and the addition of 3AMF resulted in the lowest migration rates. Therefore, the bacterial community in a sample colonized with 3AMF was less affected by stochastic processes, whereas that colonized with 5 AMF tended to change to stochastic processes.

3.4. *Effects of AMF colonization on bacterial function*

The addition of AMF changed the structure of the bacterial community, thereby affecting its function. As the MPR and total P of plants, as well as enzymes related to the P cycle of the soil, were positively affected by AMF colonization (Fig. 1D, E and Fig. 2A-C). Therefore, genes of bacteria involved in the P cycle were assessed. The abundance of genes involved in the P cycle (*ndk*, *phoU*, *ppa*, *ppnk*, *ppk1*, *surE*) was significantly enriched in samples colonized by AMF, especially in the 3AMF treatments (Fig. 4A). Among them, *ndk*, *ppnk*, and *surE* are involved in polyphosphate degradation; *ppa* is involved in inorganic P solubilization; *ppk1* is involved in polyphosphate synthesis; and *phoU* is involved in regulation (Fig. 4B). The qPCR analysis revealed that the copy numbers of *phoC* and *pqqC* decreased in the 5AMF treatment group compared with those in the 3AMF treatment group (Fig. 4C, D). In summary, these findings further illustrate that the pronounced effects of the 3 AMF inoculations may have resulted from their ability to attract microorganisms associated with the soil P cycle.

3.5. *Screening and functional analysis of key bacteria*

To explore the underlying microbial interaction mechanisms that resulted in the difference in the growth-promoting effects of the addition of 5AMF and 3AMF, the bacterial composition of the rhizospheres of the 3AMF and 5AMF samples was analyzed. The abundances of *Flavisolibacter*, *Citrifermentans*, and *Rhodococcus* were markedly greater in the 3AMF-inoculated samples than in the 5AMF samples (Fig. 5A). To further assess whether the three bacteria affect the growth-promoting effect of AMF by regulating soil nutrient turnover, we analyzed their ability to utilize soil P at the genome level. First, the draft genomes of *Flavisolibacter*, *Citrifermentans*, and *Rhodococcus* with COG annotation of genes were obtained (Fig. S6). We found that *Flavisolibacter*, *Citrifermentans*, and *Rhodococcus* contained genes related to the P cycle. *Flavisolibacter* contained genes involved in polyphosphate degradation, polyphosphate synthesis, regulation, and transport (Fig. 5B); *Citrifermentans* contained genes involved in organic P mineralization, polyphosphate degradation, polyphosphate synthesis, regulation and transport (Fig. 5C); and *Rhodococcus* contained genes involved in inorganic P solubilization, organic P mineralization, polyphosphate degradation, polyphosphate synthesis, regulation and transport (Fig. 5D).

As the three bacterial genera were enriched in the rhizosphere of the 3AMF-inoculated samples but not in the 5AMF-inoculated samples, it was necessary to further study whether the soil P cycle was related to the three bacterial genera. Thus, we performed a correlation analysis between the abundance of the three bacterial genera and the P cycling genes (*ndk*, *phoU*, *ppa*, *ppnk*, *ppk1*, *surE*) that were significantly enriched in samples colonized by AMF (Fig. 6A). Nearly opposite results were observed for 3 AMF-inoculated samples and 5 AMF-inoculated samples. In samples inoculated with 3 AMF, *Flavisolibacter*, *Citrifermentans* and *Rhodococcus* were positively correlated with the genes *ndk*, *phoU*, *ppa*, *ppnk*, *ppk1*, and *surE*. Conversely, in the samples inoculated with 5 AMF, the three genera showed negative or no correlations with these P cycling genes, except for *Citrifermentans* and *ndk*, *Rhodococcus* and *ppnk*. Since plant-derived carbohydrates released into the soil serve as a carbon source to support microbial populations (Zhang et al., 2016), we hypothesized that changes in attracted microorganisms may result from changes in the amount of carbon released into the soil, as carbon is a key driver of microbial community assembly in the rhizosphere (Zhang et al., 2016). Thus, we assessed soil carbon and microbial biomass carbon. The results revealed that the amount of microbial biomass carbon in the 3AMF treatments was greater than that in the 5AMF treatments (Fig. 6B), whereas the amount of soil dissolved organic carbon tended to increase with increasing AMF species (Fig. 6C).

This occurred because the rhizosphere's attraction to microbes is partly driven by the release of carbon sources. Plants import 20% of their carbon from photosynthesis into the soil through their roots (Pausch and Kuzyakov, 2017). Therefore, we propose the following conceptual model: compared with 3AMF, 5AMF increases competition for carbon with rhizosphere bacteria, reducing the enrichment of functional bacterial species related to phosphorus transformation, including

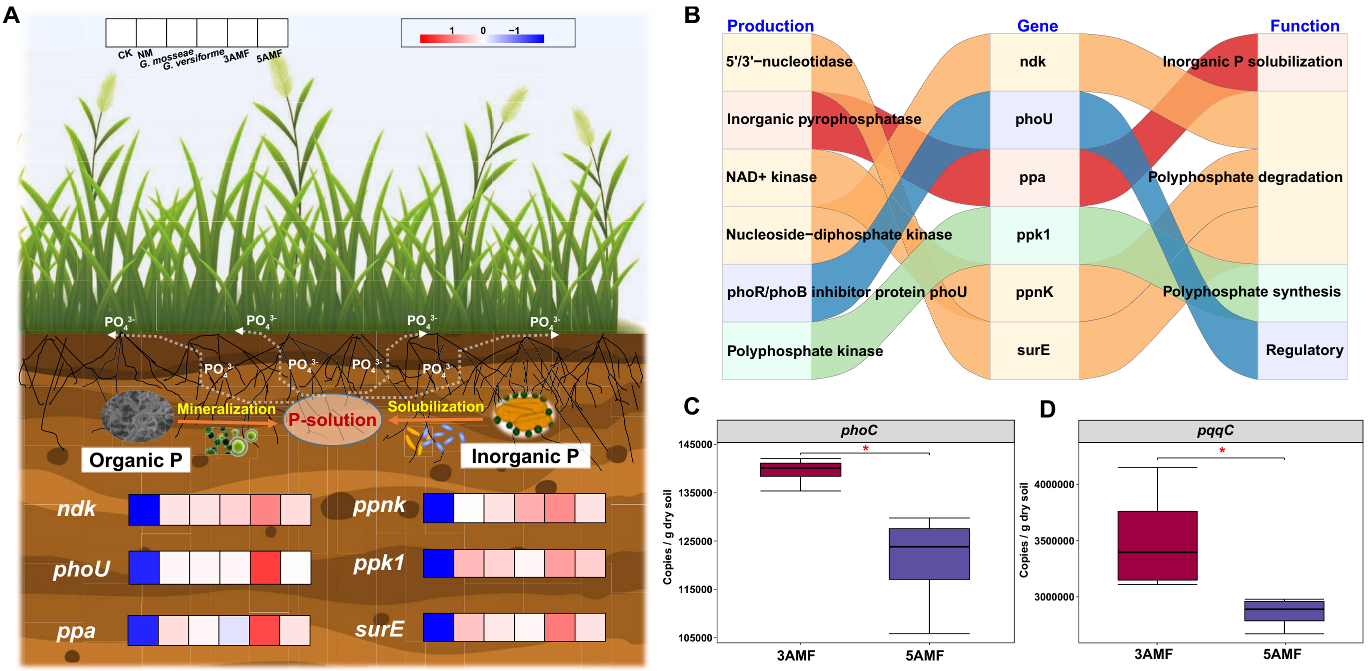

Fig. 4. Relative abundances of genes involved in the phosphorus cycle under different AMF addition treatments. (A) Relative abundances of genes involved in the phosphorus cycle. (B) Sankey plot showing the correlations among P-cycle-related genes, products and functions. Connections indicate associations with each other. (C) The *phoD* gene copy numbers in soil. (D) *pqqC* gene copy numbers in soil. The data for single AMF include *G. mosseae*- and *G. versiforme*-inoculated samples. CK represents the application of benomyl to eliminate the influence of native AMF. NM represents native AMF in the soil. 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*.

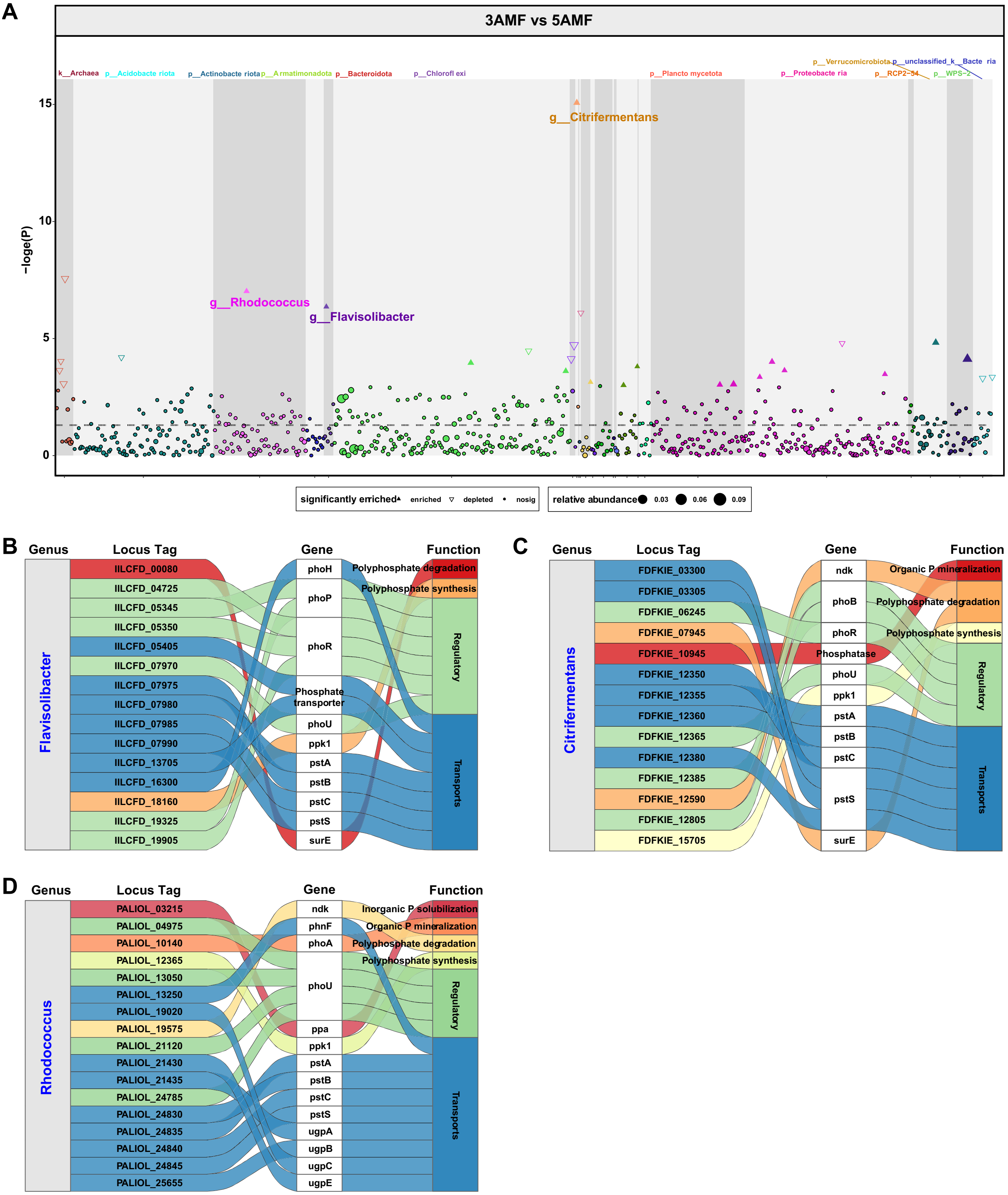

Fig. 5. Screening of key bacteria associated with AMF diversity. (A) Differences in the abundances of bacterial genera between the 3AMF group and the 5AMF group according to the Manhattan diagram. Differences between the two groups are shown in a point shape, indicating that the ASV was enriched, depleted or not significant; the point size indicates the abundance of the ASV. Sankey plot showing the correlations of the locus tag in *Flavisolibacter* (B), *Citrifermentans* (C), *Rhodococcus* (D) with P-cycle-related genes and functions. Connections indicate associations with each other.

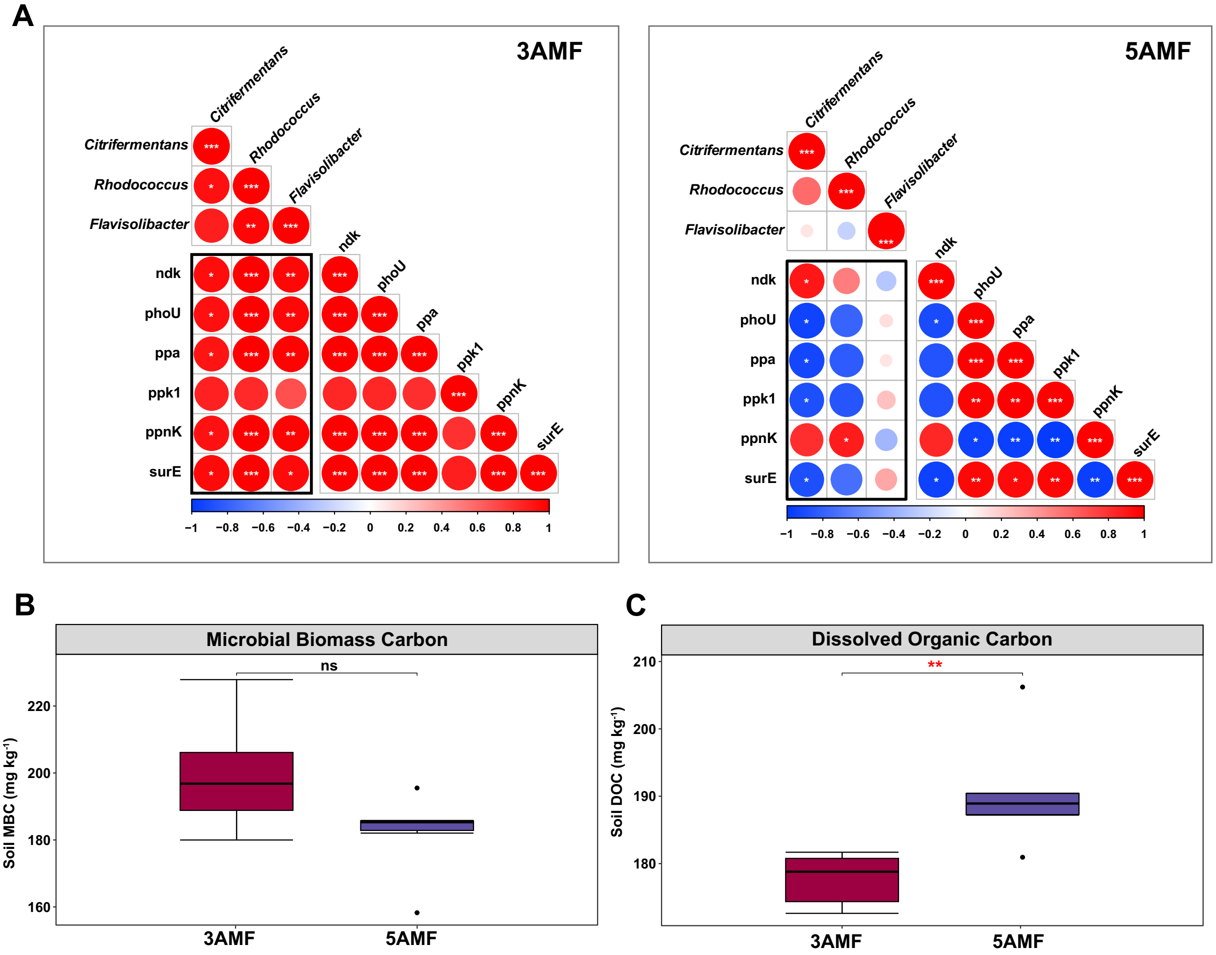

Fig. 6. Comparative analysis of phosphorus cycle-related genes associated with three core genera, microbial biomass carbon analysis, and dissolved organic carbon content analysis. (A) Pairwise comparisons of *Flavisolibacte*, *Citrifermentans*, *Rhodococcus*, and phosphorus cycle-related genes with a color gradient denoting Spearman's correlation coefficient. Stars indicate significant correlations after multiple testing (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (B) Microbial biomass carbon in the soil. (C) Dissolved organic carbon in the soil. 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*.

Rhodococcus, *Flavisolibacter*, and *Citrifermentans*, and as a result, reduces the growth-promoting effect of mycorrhizae (Fig. 7).

4. Discussion

Our results support and extend our initial hypotheses. Our study provides novel insights into the complex relationship between AMF diversity and plant performance in grassland ecosystems. In contrast to the common assumption that increased AMF species diversity invariably enhances plant growth and nutrient uptake, and consistent with our first hypothesis, our findings suggest that the benefits of AMF diversity are not linear and may reach an optimal point. Specifically, inoculation with a mixture of 3AMF appeared to be more advantageous. The 3AMF treatment increased the plant phosphorus content and soil phosphorus cycling enzyme activity, which are critical for plant nutrition and growth. In line with our second and third hypotheses, the observed decrease in growth-promoting effects with 5AMF could be due to increased competition for resources, particularly carbon, with rhizosphere bacteria, which in turn may reduce the abundance of bacteria involved in phosphorus transformation. These findings underscore the importance of considering AMF community composition and diversity in grassland restoration efforts, as optimizing AMF diversity could significantly influence plant health and ecosystem functioning.

4.1. Promotion of growth does not always increase with increasing number of AMF species

The symbiosis of AMF promoted the growth of the grassland plants, which is consistent with the findings of most studies on the effects of AMF on different plants (Jansa et al., 2008; Shao et al., 2023). However, previous studies have shown inconsistent results concerning whether an increase in the number of AMF species further increases the growth-promoting impact on plants. Whereas a positive linear relationship between AMF richness and productivity was reported in a system with minimal background AMF (van der Heijden et al., 1998), our investigation in soils containing a resident native community revealed an optimum at intermediate diversity. One possibility is that increasing the number of AMF species does not increase the growth-promoting effect compared with a single inoculation. In one study, the biomass of plants inoculated with a mixture of AMF never exceeded the range of growth promoted by the respective single-species inoculations (Jansa et al., 2008). However, there is a contrasting view that the growth-promoting effect of plants increases with the diversity of AMF inoculation (Bennett and Bever, 2007; Maherali and Klironomos, 2007). We note that our experimental design tested specific species combinations at each diversity level. Therefore, the observed ‘diversity effect’ is conflated with species identity effects, and the optimal effect of the 3AMF mix may be specific to the particular species combination used. Future studies with replicated compositions at each diversity level are needed to isolate the

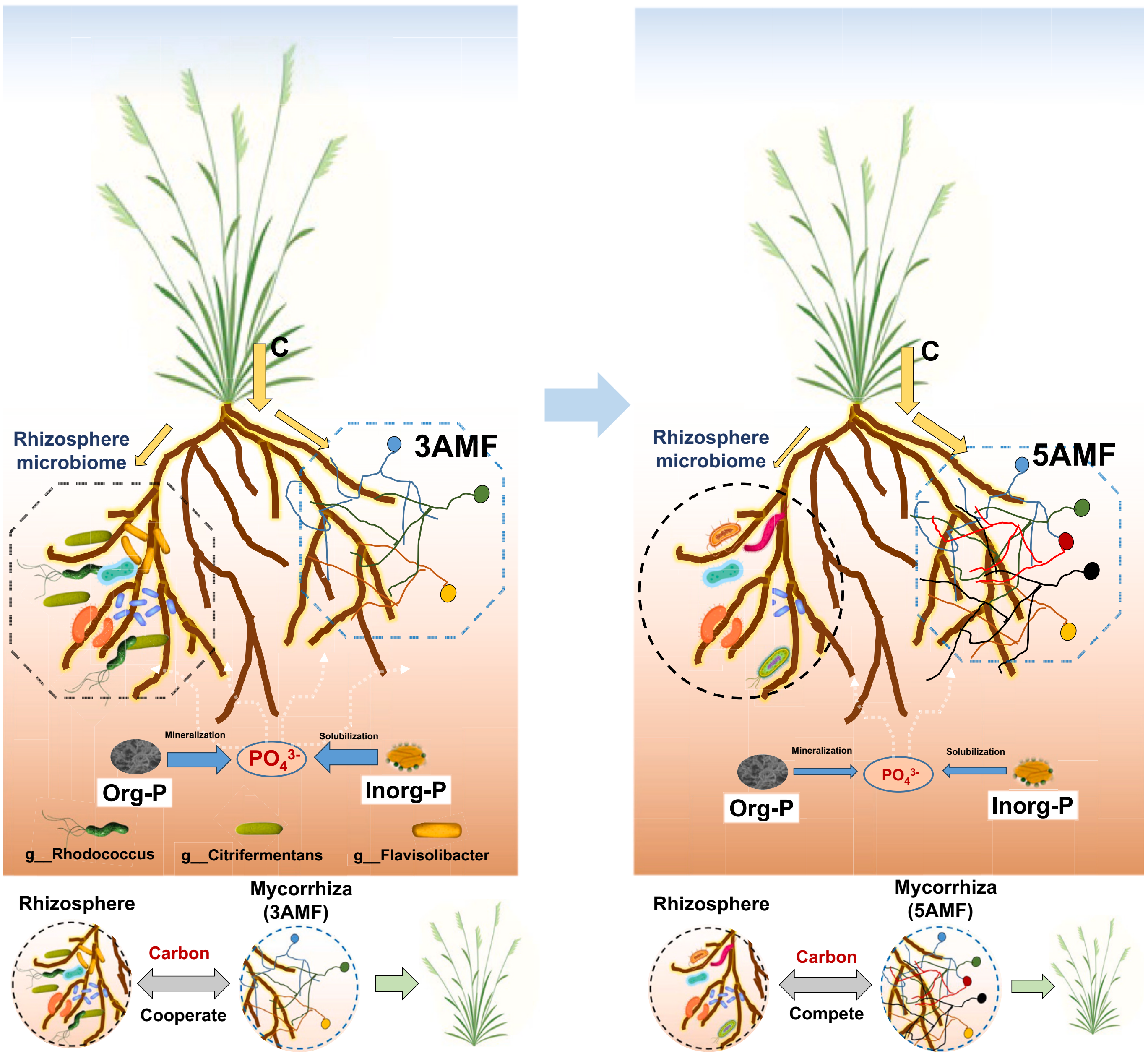

Fig. 7. Mechanism of the increase in AMF diversity (5AMF) with a decrease of growth-promoting effects. 3AMF represents coinoculation with *G. mosseae*, *G. versiforme*, and *G. intraradices*. 5AMF represents coinoculation with *G. mosseae*, *G. versiforme*, *G. intraradices*, *G. walker*, and *G. etunicatum*.

pure effect of richness. Nonetheless, our study identifies a clear non-linear pattern and a plausible mechanistic explanation for why benefits might diminish at higher diversity in certain contexts. In this context, our study demonstrated that the 3AMF inoculum had the strongest growth-promoting effect on plants, with a lesser effect with 5AMF inoculation.

Similar to growth promotion, inoculation with 3AMF led to higher phosphorus concentrations in plants than did the 5AMF treatment. The role of AMF in plant nutrition is to provide mineral nutrients to plants (Berger and Gutjahr, 2021; You et al., 2023) and thereby promote plant growth. Study has revealed that the mixture of *G. claroideum* and *G. intraradices* provided more P for Leek than either of the two AMFs did separately (Jansa et al., 2008). However, unlike phosphorus, the 3AMF treatment had no significant effect on the total nitrogen or carbon content relative to the 5AMF treatment. The role of AMF in plant nutrition involves the exchange of mineral nutrients with carbohydrates and lipids (Berger and Gutjahr, 2021; You et al., 2023). The reduced efficacy of 5AMF in promoting plant growth may thus primarily arise from its diminished capacity to facilitate phosphorus uptake.

These multifaceted phosphorus-acquisition mechanisms involve both physical and molecular adaptations (Guo et al., 2016; Wang et al., 2023): AMF increase phosphorus availability by forming extensive hyphal networks that expand root absorption surfaces and reduce nutrient transport distances. Concurrently, AMF mobilize soil phosphorus through phosphatase secretion, organic acid exudation, and rhizosphere acidification while recruiting beneficial microbes and upregulating phosphate transporter genes (Liu et al., 2021; Lu et al., 2023). Under the 3AMF treatment, the synergistic interactions among *G. mosseae*, *G. versiforme*, and *G. intraradices* likely amplify these processes: *G. mosseae* optimizes root architecture (Chen et al., 2017; Liu et al., 2017; Peng et al., 2021); *G. versiforme* and *G. intraradices* stimulate phosphorus-transport protein synthesis (Yang et al., 2012; Guo et al., 2016); and *G. intraradices* increase phosphorus solubility by modifying rhizosphere microenvironments (Onyeaka et al., 2024). High-affinity

Pi/H^+ transporters, conserved across these species (Harrison and van Buuren, 1995; Benedetto et al., 2005), further drive phosphorus uptake from the soil. The phosphorus uptake process is driven primarily by high-affinity Pi/H^+ transporters identified across multiple AMF species, including *G. mosseae*, *G. versiforme*, and *G. intraradices*. This complementarity, ranging from physical network expansion to molecular-level regulation, likely creates synergistic effects that substantially improve phosphorus-use efficiency. Nevertheless, the functional contributions of individual AMF species within this consortium require further experimental validation.

4.2. Bacteria related to phosphorus mobilization are attracted to the rhizosphere

The symbiosis of AMF can affect the soil bacterial community (Xu et al., 2023b; Xu et al., 2023a). Consistent with previous studies, we found that the soil bacterial communities present in samples with or without AMF inoculation are markedly distinct. A recent study revealed that AMF chemical exudates recruit bacteria that further influence AMF fitness by mediating nutrient cycling, particularly organic nutrient mobilization (Zhang et al., 2022). The colonization of 3AMF increased the deterministic process of the assembled bacterial community, recruiting rhizosphere bacterial groups that are related to P mobilization. This function was further supported by the results of the soil enzyme activities, which revealed that 3AMF increased the activities of pyrroloquinoline-quinone synthase, phytase and acid phosphatases, whereas 5AMF inoculation weakened this effect. Soil enzymes are secreted by microorganisms, and their activities are strongly related to soil microbial characteristics, reflecting how the microbial community invests in nutrient acquisition (Gong et al., 2019). The decreased P uptake observed with the 5AMF treatment compared with the 3AMF treatment may be due to alterations in the rhizosphere bacterial communities associated with each group.

4.3. Key genera of bacteria cooperate with AMF to improve phosphorus absorption

The symbiosis of AMF can attract specific bacteria to improve their ability to obtain and/or transport soil nutrients, and the core bacteria that are specifically associated with multiple AMF promote their function (Zhang et al., 2018a; Wang et al., 2022b). Our study also supported the aforementioned findings; that is, *R. irregularis* MUCL 43194 recruited a specific rhizosphere bacterial community with a positive correlation with alkaline phosphatase activity (Wang et al., 2022a). In this study, *Citrifermentans*, *Rhodococcus*, and *Flavisolibacter* were markedly enriched in the rhizosphere with 3AMF added compared with those in the rhizosphere with 5AMF. The identified bacterial genera harbored many P cycle-related genes and were positively correlated with soil P cycling. This shift in the microbial communities responsible for soil phosphorus transformation in the rhizospheres of the 5AMF treatments compared with those in the 3AMF treatments may be due to changes in carbon source availability. Considering that the core microbiome is functional and contains genes that encode specific functional characteristics (Lemanceau et al., 2017), *Citrifermentans*, *Rhodococcus*, and *Flavisolibacter* can be identified as the key bacterial genera. Overall, our results indicate that 3AMF inoculation may increase the growth-promoting effect of plants by cooperating with the key bacterial genera related to the P cycle.

During symbiosis with AMF, the rhizosphere and mycorrhizal pathways, are formed to promote nutrient absorption in plants, particularly P (Shi et al., 2023). The rhizosphere pathway relies on functional bacteria containing genes for organic compound conversion, nitrogen fixation, and phosphorus mineralization (Zhang et al., 2016; Jiang et al., 2021; Ling et al., 2022), whereas the mycorrhizal pathway primarily facilitates nutrient transport. Importantly, different AMF species may exert distinct effects on rhizosphere microbial communities through species-specific interactions, such as altering root exudate composition or modifying biochemical signals (Wang et al., 2021; Mythili and Ramalakshmi, 2022; Xu et al., 2023b; Yang et al., 2024). In AM symbiosis systems, plants transfer a portion of their photosynthetic products to the soil (Pausch and Kuzyakov, 2017), which partially sustains rhizosphere microbial communities (Zhang et al., 2016). When plants are inoculated with fewer AMF species, more carbon is allocated to rhizosphere microorganisms, whereas increasing AMF diversity (for example, the 5AMF treatment) intensifies carbon competition between AMF and other microbes. This shift in carbon allocation, combined with potential AMF species-specific effects on root exudation patterns, could jointly drive the observed changes in the microbial communities responsible for phosphorus transformation. Specifically, the reduced microbial growth under high AMF diversity may result not only from carbon source limitation but also from differential modulation of microbial niches by specific AMF species through biochemical interactions.

In conclusion, a cooperative or competitive relationship exists between AMF and rhizosphere bacteria, which may depend on the status of underground carbon provided by plants. In the presence of lower AMF diversity, the competition for carbon is weaker, and cooperation prevails. However, it has been hypothesized that increasing AMF diversity may intensify competition for carbon resources, which could potentially reduce the growth-promoting effects of mycorrhizae. In this study, when inoculated with 3AMF, the three bacterial genera were enriched in the rhizosphere, which played a role in growth promotion in cooperation with AMF by improving soil P turnover; however, when inoculated with 5AMF, the three bacteria did not accumulate, thus reducing the release of nutrients and leading to a weakened growth-promoting effect. Nevertheless, understanding how AMF-mediated resource allocation and facilitation of plant coexistence occur is crucial for comprehending ecosystem processes. Although our current study did not explicitly explore these interactions, they are ecological significant and will be prioritized in our future research.

Availability of data and materials

The raw sequencing data generated in this study are available in the NCBI SRA database under accession number PRJNA983437. Processed data, statistical results, and supplementary materials are included within the manuscript and its additional files. All data supporting this study are fully available without restriction.

Author contribution

Yunjian Xu and Junhua Liu conducted the experiments. Zhe Chen and Yunjian Xu designed the study. Zhe Chen, Yunjian Xu, and Michael Dannenmann analyzed the data and wrote the manuscript. All authors reviewed and approved the final version.

CRediT authorship contribution statement

Yunjian Xu: Writing – original draft, Resources, Data curation, Conceptualization. **Junhua Liu:** Resources, Investigation, Data curation. **Zhe Chen:** Writing – review & editing, Funding acquisition. **Michael Dannenmann:** Writing – review & editing.

Consent for publication

Not applicable. The manuscript does not contain data from any individual person.

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, animals, or clinical trials.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2026.114749.

Data availability

The raw sequencing data generated in this study are available in the NCBI SRA database under accession number PRJNA983437.

References

Bai, Y., Cotrufo, M.F., 2022. Grassland soil carbon sequestration: current understanding, challenges, and solutions. Science 377 (6606), 603–608.

Bardgett, R.D., Bullock, J.M., Lavorel, S., Manning, P., Schaffner, U., Ostle, N., Chomel, M., Durigan, G., L. Fry, E., Johnson, D., et al., 2021. Combatting global grassland degradation. Nat. Rev. Earth Environ. 2 (10), 720–735.

Benedetto, A., Magurno, F., Bonfante, P., Lanfranco, L., 2005. Expression profiles of a phosphate transporter gene (*GmosPT*) from the endomycorrhizal fungus Glomus mosseae. Mycorrhiza 15 (8), 620–627.

Bennett, A.E., Bever, J.D., 2007. Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88 (1), 210–218.

Berger, F., Gutjahr, C., 2021. Factors affecting plant responsiveness to arbuscular mycorrhiza. Curr. Opin. Plant Biol. 59, 101994.

Blažková, A., Jansa, J., Püschel, D., Vosátka, M., Janoušková, M., 2021. Is mycorrhiza functioning influenced by the quantitative composition of the mycorrhizal fungal community? Soil Biol. Biochem. 157, 108249.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17 (6), 837–842.

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220 (4), 1108–1115.

Chen, M., Yang, G., Sheng, Y., Li, P., Qiu, H., Zhou, X., Huang, L., Chao, Z., 2017. *Glomus mosseae inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of liquorice under nutrient stress*. Front. Plant Sci. 8.

Chen, E., Liao, H., Chen, B., Peng, S., 2020. Arbuscular mycorrhizal fungi are a double-edged sword in plant invasion controlled by phosphorus concentration. New Phytol. 226 (2), 295–300.

Defez, R., Andreozzi, A., Bianco, C., 2017. The overproduction of Indole-3-acetic acid (IAA) in endophytes upregulates nitrogen fixation in both bacterial cultures and inoculated rice plants. Microb. Ecol. 74 (2), 441–452.

Gao, Z., Wang, Y., Chen, G., Zhang, A., Yang, S., Shang, L., Wang, D., Ruan, B., Liu, C., Jiang, H., et al., 2019. The indica nitrate reductase gene *OsNR2* allele enhances rice yield potential and nitrogen use efficiency. Nat. Commun. 10 (1), 5207.

Gollotte, A., van Tuinen, D., Atkinson, D., 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14 (2), 111–117.

Gong, X., Liu, C., Li, J., Luo, Y., Yang, Q., Zhang, W., Yang, P., Feng, B., 2019. Responses of rhizosphere soil properties, enzyme activities and microbial diversity to intercropping patterns on the loess plateau of China. Soil Tillage Res. 195, 104355.

Gosling, P., Jones, J., Bending, G.D., 2016. Evidence for functional redundancy in arbuscular mycorrhizal fungi and implications for agroecosystem management. Mycorrhiza 26 (1), 77–83.

Guo, Y.-e., Li, F., Li, Y., Duan, T., 2016. Progress in the elucidation of the mechanisms of arbuscular mycorrhizal fungi in promotion of phosphorus uptake and utilization by plants. Pratacultural Science 33 (12), 2379–2390.

Gustafson, D.J., Casper, B.B., 2006. Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating co-occurring Glomus species. Plant Ecol. 183 (2), 257–263.

Harrison, M.J., van Buuren, M.L., 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378 (6557), 626–629.

Hartnett, D.C., Wilson, G.W.T., 1999. Mycorrhizal influence plant community structure and diversity in tallgrass prairie. Ecology 80 (4), 1187–1195.

Hartnett, D.C., Wilson, G.W.T., 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant Soil 244 (1), 319–331.

He, J., Dai, H., Zhang, X., Wang, E., 2024. Mycorrhizal signals promote root development dependent on LysM-receptor like kinases in rice. New Crops 1, 100009.

Hetrick, B., Wilson, G., Cox, T., 2011. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. 70, 2032–2040.

Horsch, C.C.A., Antunes, P.M., Fahey, C., Grandy, A.S., Kallenbach, C.M., 2023. Trait-based assembly of arbuscular mycorrhizal fungal communities determines soil carbon formation and retention. New Phytol. 239 (1), 311–324.

Hu, J., Wu, S., Wu, F., Leung, H.M., Lin, X., Wong, M.H., 2013. Arbuscular mycorrhizal fungi enhance both absorption and stabilization of cd by Alfred stonecrop (*Sedum alfredii* Hance) and perennial ryegrass (*Lolium perenne* L.) in a cd-contaminated acidic soil. Chemosphere 93 (7), 1359–1365.

Huangfu, C., Li, K., Hui, D., 2019. Influences of plant interspecific competition and arbuscular mycorrhizal fungi on nitrogen form preference of an invasive plant. Biogeochemistry 145 (3), 295–313.

Jansa, J., Smith, F.A., Smith, S.E., 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol. 177 (3), 779–789.

Jiang, F., Zhang, L., Zhou, J., George, T.S., Feng, G., 2021. Arbuscular mycorrhizal fungi enhance mineralisation of organic phosphorus by carrying bacteria along their extraradical hyphae. New Phytol. 230 (1), 304–315.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the K_{EC} value. Soil Biol. Biochem. 28 (1), 25–31.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38 (5), 991–999.

Kang, H., Yu, W., Dutta, S., Gao, H., 2021. Soil microbial community composition and function are closely associated with soil organic matter chemistry along a latitudinal gradient. Geoderma 383, 114744.

Legay, N., Clément, J.C., Grassein, F., Lavorel, S., Lemauviel-Lavenant, S., Personeni, E., Poly, F., Pommier, T., Robson, T.M., Mouhamadou, B., et al., 2020. Plant growth drives soil nitrogen cycling and N-related microbial activity through changing root traits. Fungal Ecol. 44, 100910.

Lemanceau, P., Blouin, M., Muller, D., Moënne-Loccoz, Y., 2017. Let the core microbiota be functional. Trends Plant Sci. 22 (7), 583–595.

Ling, N., Wang, T., Kuzyakov, Y., 2022. Rhizosphere bacteriome structure and functions. Nat. Commun. 13 (1), 836.

Liu, C., Wu, Q., Zou, Y., 2017. Effects of arbuscular mycorrhizal fungi on phosphorous uptake and phosphatase release in trifoliate orange seedlings. Mycosystema 36 (7), 942–949.

Liu, H., Wu, Y., Xu, H., Ai, Z., Zhang, J., Liu, G., 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 13C and 15N dual-labelled organic patch. Plant J. 108, 183–196.

Lu, Y., Yan, Y., Qin, J., Ou, L., Yang, X., Liu, F., Xu, Y., 2023. Arbuscular mycorrhizal fungi enhance phosphate uptake and alter bacterial communities in maize rhizosphere soil. Front. Plant Sci. 14, 1206870.

Maherali, H., Klironomos, J.N., 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316 (5832), 1746–1748.

Morris, A., Beck, J.M., Schloss, P.D., Campbell, T.B., Crothers, K., Curtis, J.L., Flores, S.C., Fontenot, A.P., Ghedin, E., Huang, L., et al., 2013. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Am. J. Respir. Crit. Care Med. 187 (10), 1067–1075.

MR, McPherson, Wang, P., Marsh, E.L., Mitchell, R.B., Schachtman, D.P., 2018. Isolation and analysis of microbial communities in soil, rhizosphere, and roots in perennial grass experiments. J. Vis. Exp. 137, 57932.

Mythili, M., Ramalakshmi, A., 2022. Unraveling the distribution of AMF communities and their metabolites associated with soils of minor millets. Rhizosphere 21, 100473.

O'Connor, P.J., Smith, S.E., Smith, F.A., 2002. Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. New Phytol. 154 (1), 209–218.

Onyeaka, H.N., Akinsemolu, A.A., Siyanbola, K.F., Adetunji, V.A., 2024. Green microbe profile: *Rhizophagus intraradices*—a review of benevolent fungi promoting plant health and sustainability. Microbiol. Res. 15 (2), 1028–1049.

Pausch, J., Kuzyakov, Y., 2017. Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Glob. Chang. Biol. 24.

Peng, Q., He, H., Zhang, X., 2021. Mechanisms of increasing alfalfa growth and phosphorus uptake by inoculation with arbuscular mycorrhizal fungal under low phosphorus application level. Journal of Plant Nutrition and Fertilizers 27 (2), 293–300.

Ramette, A., 2007. Multivariate analyses in microbial ecology. FEMS Microbiol. Ecol. 62 (2), 142–160.

Rezki, S., Campion, C., Simoneau, P., Jacques, M.-A., Shade, A., Barret, M., 2017. Assembly of seed-associated microbial communities within and across successive plant generations. Plant Soil 422 (1–2), 67–79.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytol. 171 (1), 41–53.

Rozmoš, M., Bukovská, P., Hršelová, H., Kotianová, M., Dudáš, M., Gančarčíková, K., Jansa, J., 2022. Organic nitrogen utilisation by an arbuscular mycorrhizal fungus is mediated by specific soil bacteria and a protist. ISME J. 16 (3), 676–685.

Shao, Y., Jiang, S., Peng, H., Li, H., Li, P., Jiang, R., Fang, W., Chen, T., Jiang, G., Yang, T., et al., 2023. Indigenous and commercial isolates of arbuscular mycorrhizal fungi display differential effects in *Pyrus betulaefolia* roots and elicit divergent transcriptomic and metabolomic responses. Front. Plant Sci. 13.

Shi, J., Wang, X., Wang, E., 2023. Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. Annu. Rev. Plant Biol. 74 (1), 569–607.

Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006a. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. 8 (4), 732–740.

Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006b. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. 8 (4), 732–740.

Stothard, P., Wishart, D.S., 2005. Circular genome visualization and exploration using CGView. Bioinformatics 21 (4), 537–539.

Thonar, C., Frossard, E., Šmilauer, P., Jansa, J., 2014. Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. Mol. Ecol. 23 (3), 733–746.

Tian, W., Xiang, X., Wang, H., 2021. Differential impacts of water table and temperature on bacterial communities in pore water from a subalpine peatland, Central China. Front. Microbiol. 12, 649981.

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18 (11), 607–621.

Van der Heijden, M., Klironomos, J., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.

Wang, X., Feng, H., Wang, Y., Wang, M., Xie, X., Chang, H., Wang, L., Qu, J., Sun, K., He, W., et al., 2021. Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia–legume symbiosis. Mol. Plant 14 (3), 503–516.

Wang, G., George, T.S., Pan, Q., Feng, G., Zhang, L., 2022a. Two isolates of *Rhizophagus irregularis* select different strategies for improving plants phosphorus uptake at moderate soil P availability. Geoderma 421, 115910.

Wang, B., Huangfu, C., Jia, X., Hui, D., 2022b. Mycorrhizal suppression decouples the coordination of plant functional traits that mediate nitrogen acquisition under different soil water contents in a subtropical wetland ecosystem. Appl. Soil Ecol. 175, 104441.

Wang, G., Jin, Z., George, T.S., Feng, G., Zhang, L., 2023. Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. New Phytol. 238 (6), 2578–2593.

Wei, T., Simko, V., 2021. R Package 'Corrplot': Visualization of a Correlation Matrix (version 0.84).

Wilsey, B.J., 2018. The Biology of Grasslands. Oxford University Press.

Wu, W.J., Zou, Y.N., Hashem, A., Avila-Quezada, G.D., Abd_Allah, E.F., Wu, Q.S., 2023. *Rhizoglomus intraradices* is more prominent in improving soil aggregate distribution and stability than in improving plant physiological activities. Agronomy 13 (5), 1427.

Xu, Y., Chen, Z., Li, X., Tan, J., Liu, F., Wu, J., 2023a. The mechanism of promoting rhizosphere nutrient turnover for arbuscular mycorrhizal fungi attributes to recruited functional bacterial assembly. Mol. Ecol. 32 (9), 2335–2350.

Xu, Y., Chen, Z., Li, X., Tan, J., Liu, F., Wu, J., 2023b. Mycorrhizal fungi alter root exudation to cultivate a beneficial microbiome for plant growth. Funct. Ecol. 37 (3), 664–675.

Xue, L., Cui, H.T., Buer, B., Vijayakumar, V., Delaux, P.M., Junkermann, S., Bucher, M., 2015. Network of GRAS transcription factors involved in the control of arbuscule development in Lotus japonicus. Plant Physiol. 167 (3), 854.

Yang, S.Y., Grønlund, M., Jakobsen, I., Grotemeyer, M.S., Rentsch, D., Miyao, A., Hirochika, H., Kumar, C.S., Sundaresan, V., Salamin, N., et al., 2012. Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the PHOSPHATE TRANSPORTER1 gene family. Plant Cell 24 (10), 4236–4251.

Yang, M., Song, Y., Ma, H., Li, Z., Ding, J., Yin, T., Niu, K., Sun, S., Qi, J., Lu, G., et al., 2024. Unveiling the hidden world: how arbuscular mycorrhizal fungi and its regulated core fungi modify the composition and metabolism of soybean rhizosphere microbiome. Environmental Microbiome 19 (1), 78.

You, Y., Ray, R., Halitschke, R., Baldwin, G., Baldwin, I.T., 2023. Arbuscular mycorrhizal fungi-indicative blumenol-C-glucosides predict lipid accumulations and fitness in plants grown without competitors. New Phytol. 238 (5), 2159–2174.

Zhang, L., Xu, M., Liu, Y., Zhang, F., 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 (3), 1022–1032.

Zhang, L., Feng, G., Declerck, S., 2018a. Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate solubilizing bacterium. ISME J. 12 (10), 2339–2351.

Zhang, L., Shi, N., Fan, J., Wang, F., George, T.S., Feng, G., 2018b. Arbuscular mycorrhizal fungi stimulate organic phosphate mobilization associated with changing bacterial community structure under field conditions. Environ. Microbiol. 20 (7), 2639–2651.

Zhang, L., Zhou, J., George, T.S., Limpens, E., Feng, G., 2022. Arbuscular mycorrhizal fungi conducting the hyphosphere bacterial orchestra. Trends Plant Sci. 27 (4), 402–411.