Plant Soil (2024) 497:449–466
https://doi.org/10.1007/s11104-023-06406-8

RESEARCH ARTICLE

Enhancing *Phyllostachys edulis* seedling growth in phosphorus-deficient soil: complementing the role of phosphate-solubilizing microorganisms with arbuscular mycorrhizal fungi

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Received: 11 June 2023 / Accepted: 19 November 2023 / Published online: 1 December 2023

Abstract

Background and aims The addition of functional soil microbes to the soil can enhance the utilization of soil phosphorus (P) for plants, but the mechanisms underlying this process are not fully understood. The study aimed to investigate the effects of inoculating phosphate-solubilizing microorganisms (PSMs), with or without arbuscular mycorrhizal fungi (AMF), on *Phyllostachys edulis* seedling growth in soils with varying P levels.

Methods The pot experiment was conducted on *P. edulis* seedings with inoculation treatments of a mixture of 15 PSMs from the *P. edulis* rhizosphere alone, or PSMs in cooperation with AMF. Plant mass and nutrient concentrations, soil properties and soil microbial communities were assessed when the seedlings had completed their first-year growth spurt (T1) and second-year growth spurt (T2).

Results The application of PSMs, particularly in combination with AMF, resulted in significant increases in seedling mass and nutrient content under P-deficient conditions, accomplished through modifications in soil nutrient concentrations and enzyme activities related to nitrogen, carbon, and P metabolism. The beneficial outcomes were accompanied by alterations in the composition and interactions within the constituents of the rhizosphere microbial community. Remarkably, the co-inoculation of AMF and PSMs led to a higher abundance of microorganisms that promote plant growth within the rhizosphere.

Conclusion The utilization of PSMs, especially in combination with AMF, proves to be an effective strategy for enhancing *P. edulis* seedlings growth during their second-year growth, particularly in P-deficient soil. This approach modifies the soil microenvironment, offering a promising avenue for improving soil P utilization by woody plants.

Keywords Phosphorus deficiency · Functional soil microbes · Microbial inoculation · Rhizosphere microenvironment · Plant performance

Yijing Xing and Fucheng Wang contributed equally to this work.

Responsible Editor: Stéphane Compant.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-023-06406-8.

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Introduction

The low availability of phosphorus (P), one of the most crucial nutrient elements, often limits plant growth (Hinsinger et al. 2011). This limitation is particularly prevalent in tropical and subtropical regions, where the perennial rainy climate causes significant P source loss (Su et al. 2021; Wang et al. 2010). The presence of abundant metallic ions, such as Al^{3+}, Fe^{2+}, and Fe^{3+}, in acidic red soils can further worsen this problem by strongly immobilizing soluble P compounds (Gu et al. 2023).

A common strategy to increase P availability is to apply chemical P fertilizers in the processes of agricultural and forestry productions, but these can be expensive and their use can lead to soil hardening and water eutrophication (Lambers et al. 2013; Morales et al. 2011). Soil microorganisms play pivotal roles in ecosystem nutrient cycling (Wang et al. 2018). Certain microorganisms, like phosphate-solubilizing microorganisms (PSMs) and arbuscular mycorrhizal fungi (AMF), contribute significantly to the modification of nutrient availability (Aslani borj et al. 2022; Raymond et al. 2018). Utilizing these microorganisms as biofertilizers would be a more sustainable and ecological approach for enhancing P availability. AMF and PSMs play important roles in mediating P turnover and P acquisition by plants (Huang et al. 2021). AMF can colonize plant roots to promote the acquisition of mineral nutrients, releasing protons that mobilize insoluble soil P (Kalamulla et al. 2022). In fact, AMF can 'harvest' P from large soil volumes due to their extraradical mycelia (Gosling et al. 2006; Smith and Smith 2011). The development of extraradical mycelia from AMF can be promoted by other beneficial microbes and plant growth (Battini et al. 2017), which could affect the long-term effects of AMF at different stages of plant development. PSMs, primarily found in the rhizosphere soil, have the ability to secrete phosphatases, siderophores, and organic acids that enhance ion exchange, energy transfer, and mineral uptake by plant roots, so as to release available P from insoluble P forms (Gulati et al. 2010).

While the use of PSMs for enhancing the availability of P in soil has shown promise in laboratory settings, its effectiveness in field conditions can be inconsistent (Mpanga et al. 2019). To improve the reliability of this approach, researchers have explored inoculation with different PSM genera that can interact synergistically (Magallon-Servin et al. 2020; Xie et al. 2020), as well as co-inoculation of AMF and PSMs (Rezaei-Chiyaneh et al. 2021). These allow PSMs to convert insoluble soil P to available P, which is then transferred to host plants by extraradical mycelia of AMF (Zhang et al. 2011).

The precise mechanism by which PSMs and AMF enhance soil P availability remains unclear, and likely depends on soil nutrient conditions, especially the level of P (Xiao et al. 2019). Although the synergistic effects of PSMs, alone or with AMF, on P uptake by plants have been extensively studied in model plants such as *Arabidopsis* (Ryu et al. 2004) and in crops like rice and wheat (Mäder et al. 2011), their impact on woody plants, such as bamboo (*e.g.*, *Phyllostachys edulis*), have been less explored. *P. edulis* is a perennial monocot plant of the subfamily Bambusoideae of the Poaceae family (Han et al. 2009), and plays a vital role in the economy and ecology of its growing regions as the primary source for bamboo shoots and timber. It demonstrates fast growth, dynamic nutrient cycling, and boasts an expansive root system intricately linked with the soil microenvironment. This species predominantly flourishes in tropical and subtropical regions where available P is scarce (Guan et al. 2017; Song et al. 2011). While much research has centered on plant evolutionary strategies to accommodate P needs and plant reactions to functional microbe inoculation (Mora-Macías et al. 2017; Yue et al. 2023), scant attention has been given to exploring how functional microbes affect the soil microenvironment and their subsequent impact on the growth of woody plants.

We hypothesized that inoculating *P. edulis* soil with PSMs, either alone or in combination with AMF, can enhance the seedlings growth by altering the soil microenvironment, which was influenced by the changes in soil enzyme activities, nutrient concentrations and microbial community. This effect is expected to be particularly pronounced in the rhizosphere soil, as previous studies have shown (Allison and Vitousek 2005; Mayak et al. 2004; Naseby and Lynch 1997). In addition, inoculation of functional soil microbes would modify the survival and diversity of soil microbes in the rhizosphere by changing the carbon sources produced in root exudates (Iannucci et al. 2021; Sood 2003). Based on this deduction, we proposed that the inoculation of PSMs, either alone or in combination with AMF, has the potential

to regulate the abundance of specific functional microbes, thus contributing to the improved growth of plants.

To test these hypotheses, we conducted a study to examine the effects of inoculating *P. edulis*-derived PSMs, either alone or in combination with AMF, on the growth of *P. edulis* seedlings in soil with adequate or inadequate P concentrations. We also investigated whether any observed effects on growth were associated with changes in nutrient concentrations, enzyme activities, and microbial community composition in the rhizosphere soil.

Materials and methods

Plant culture, inoculants, and experimental design

The *P. edulis* seedlings were obtained in April 2020 from Hongya Nursery in Meishan, Sichuan, China (29°51′N, 103°28′E; average elevation, 416.9 m.a.s.l.). Seedlings with similar morphological attributes were selected for pot experiment, ensuring an initial healthy seedling height ranging from 11.9 to 23.2 cm and maintaining consistent root morphology. On 7 May 2020, the seedling roots were carefully cleaned and rinsed in running water for 1 hour, flushed with sterilized water for 5 seconds, and then transplanted into plastic containers (diameter: 22 cm; height: 21 cm; four seedlings per container). The containers were first sterilized in a 0.5% $KMnO_4$ solution for 15 minutes, and then filled with 5670 g of growth medium. The growth medium was also sterilized in an autoclave at 121 °C for 15 minutes prior to use. The growth medium consisted of a mixture of soil (passed through a 2.0-mm sieve) and perlite in a volumetric ratio of 3:1. The soil was collected from the Qingshan Bamboo Garden in Lin'an County, Hangzhou, Zhejiang, China (30°140′N, 119°51′E). It had a pH of 4.67, total nitrogen (TN) content of 1.9 $g{\cdot}kg^{-1}$, total phosphorus (TP) content of 0.26 $g{\cdot}kg^{-1}$, available phosphorus (AP) content of 4.03 $mg{\cdot}kg^{-1}$, and organic carbon (OC) content of 2.51 $g{\cdot}kg^{-1}$.

The PSMs with higher P-solubilizing activity were derived from the rhizosphere soil of *P. edulis* forests and confirmed to solubilize 160.50–581.33 $mg{\cdot}L^{-1}$ of P from insoluble organic or inorganic P in laboratory tests (Xing et al. 2021; supplementary Table S1) The strains were identified at the genus level by analyzing the nucleotide sequences of the 18S rDNA gene for fungi and the 16S rDNA gene for bacteria. The obtained sequences were compared with those archived in GenBank using BLAST (National Biotechnology Information Center, USA). Bacterial strains were cultured in 250 ml Erlenmeyer flasks containing 100 ml of Luria-Bertani broth (5 $g{\cdot}L^{-1}$ yeast extract, 10 $g{\cdot}L^{-1}$ NaCl, 10 $g{\cdot}L^{-1}$ tryptone, pH 7.0), while fungal strains were cultured in 100 ml of potato dextrose broth (4 $g{\cdot}L^{-1}$ potato extract, 20 $g{\cdot}L^{-1}$ glucose, pH 5.6 ± 0.2). The cultures were agitated on a rotary shaker (180 rpm) at 28 °C for 24 hours, after which the microbial cells were harvested via centrifugation, washed three times with sterile water, and resuspended in sterile water to obtain a final density of 10^8 $cfu{\cdot}ml^{-1}$ (measured using an optical density at 625 nm = 0.1).

As AMF in our study, an isolate of *Glomus moseae* (BGC HK01; 1511C0001BGCAM0064) originating from rhizosphere soil of *P. edulis* forests, was obtained from the Institute of Plant Nutrition and Resources of Beijing Academy of Agriculture and Forestry Sciences. The AMF inoculum contained 1240 spores per 100 g of soil in addition to hyphae and zeolites.

Seedling growth experiments were conducted from the beginning of May 2020 to September 2021 under controlled conditions in a greenhouse (30°23′N, 119°72′E). The monthly average temperature was 19.5 °C during the experiments, while the relative humidity was 54.8%. To investigate the impact on seedling growth and soil characteristics, a full-factorial experimental design was employed to explore diverse levels of P and microbial treatments.

Soil P availability was set to deficient and sufficient levels (5 and 20 $mg{\cdot}kg^{-1}$ of soil available P content, respectively) based on the previous study conducted by He et al. (2023). The P-deficient condition (P5) was prepared using 0.004 g of KH_2PO_4 per kg of soil, and the P-sufficient condition (P20) was prepared using 0.070 g of KH_2PO_4 per kg of soil. These levels were achieved by watering the soil with 250 ml of 0.097 $g{\cdot}L^{-1}$ KH_2PO_4 (P5) and 1.589 $g{\cdot}L^{-1}$ (P20) solution per container, and if needed, 250 ml of 0.955 $g{\cdot}L^{-1}$ K_2SO_4 solution per container (0.042 g of K_2SO_4 per kg of soil) to balance the K added between treatments. Additionally, three inoculation treatments were examined: PSMs, PSMs+AMF, or no live inoculation (control). Before transplanting

the seedlings, 25 ml of PSMs and 25 g of inactivated *G. moseae* inoculum, 25 ml of PSMs with 25 g of *G. moseae* inoculum, or 25 ml of PSMs with 25 g of *G. moseae* inoculum that had been autoclaved at 121 °C for 15 minutes, were respectively injected into the rhizosphere soil of the three groups. Each treatment was replicated 28 times using a randomized design to assess their effects on seedling growth and soil characteristics.

Sample collection

On 28 September 2020, when the seedlings had completed their first-year growth spurt (T1), the rhizosphere soil, which consisted of the soil that remained attached to the roots after gentle shaking, was collected and preserved at −80 °C to assess microbial diversity and enzyme activity. On 7 September 2021, after the seedlings had completed their second-year growth spurt (T2), three seedlings from each treatment were harvested to determine their biomass, TN content and TP content. Rhizosphere soil was sampled and divided into halves: one half was preserved at −80 °C for subsequent soil enzyme activity analysis, and the other half was preserved at 4 °C to assess soil chemical properties. Bulk soil, which is defined as soil that falls from the roots with gentle shaking, was also sampled and maintained at 4 °C for soil chemical property analysis. The rhizosphere soil sample associated with each subject seedling was fashioned by amalgamating soil sourced from three arbitrarily chosen root segments. Similarly, the bulk soil sample was meticulously composed by blending soil from three distinct horizontal orientations within the identical stratum (namely, 10-20 cm—the primary expanse wherein seedling roots are prevalent).

Determination of plant mass and nutrient concentrations

Each sampled seedling of *P. edulis* was divided into shoot (leaves and stem) and root, and these materials were dried in a forced air oven at 105 °C for 30 minutes and subsequently kept at 65 °C until they attained a constant mass. Each of the organs was ground, and then passed through a 0.25-mm screen after which, it was wet digested using the H_2SO_4-H_2O_2 method (Ohyama et al. 1991). The TN and TP contents of the shoots and roots were determined according to the methods outlined in section 2.3.

Soil chemical and biological properties

Air-dried soil samples were resuspended in distilled water in a ratio of 1:2.5 (w:v) and the soil pH was measured using a pH electrode (PHS-3CW, Hangzhou Secco Instrument Company, Hangzhou, China). The P concentration in the rhizosphere soil and bulk soil was assessed through microwave digestion (Falciani et al. 2000) and subsequent analysis using a UV-visible spectrophotometer (model 8453, Agilent, Santa Clara, CA, USA). Meanwhile, the AP content was determined using colorimetry after extracting the soil with 0.05 M HCl-0.025 M H_2SO_4, following the method outlined by Murphy and Riley (1962). The soil N concentration was determined using standard Kjeldahl digestion with water distillation (Kjeltec 8400, FOSS, Denmark), while the soil OC concentration was measured by the dichromate oxidation method (Nelson and Sommers 1983). A commercial kit (ELISA kit; Meimian Industrial Co., Ltd., Jiangsu, China) was used to test the activities of the following enzymes: MM-2105O1 for α-glucosidase (αG), MM-2109O1 for β-1,4-glucosidase (βG), MM-90058O1 for β-1,4-N-acetylglucosaminidase (NAG), MM-1725O1 for acid phosphatase (ACP), MM-1735O1 for alkaline phosphatase (ALP), MM-91371O1 for cellobiohydrolase (CBH), MM-2121O1 for leucine aminopeptidase (LAP) and MM-1639O1 for urease (URE).

Analysis of soil microbial communities

Rhizosphere soils from all the tested treatments were sampled to evaluate the bacterial and fungal communities. DNA from microbial communities was extracted from 0.5 g of rhizosphere soil using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. To amplify bacterial 16S rRNA genes, PCR was conducted in a thermal cycler (GeneAmp 9700, ABI, USA) with forward primer 338 F (ACTCCTACGGGAGGCAGCAG) and reverse primer 806 R (GGACTACHVGGGTWTCTAAT). For amplifying 18S rRNA genes, forward primer ITS 3F (GCATCGATGAAGAACGCAGC) and reverse primer ITS 4R (TCCTCCGCTTATTGATATGC) were employed. PCR reactions

were carried out in triplicate for each sample with a total volume of 20 µl, containing 4 µl of 5×FastPfu buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.4 µl of FastPfu polymerase, and 10 ng of template DNA. Thermal cycling involved denaturation at 95 °C for 3 minutes, 28 cycles (16S rRNA) or 35 cycles (18S rRNA) of 30 seconds at 95 °C, 30 seconds at 55 °C for annealing, 45 seconds at 72 °C for elongation, and final extension at 72 °C for 10 minutes. The amplicons were purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), eluted with Tris-HCl (pH 8.0), quantified using the QuantiFluor™-ST system (Promega), and sequenced on the Miseq platform (Illumina, San Diego, CA, USA) at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China).

Operational taxonomic units (OTUs) were defined as units that show ≥97% similarity between gene sequences in UPARSE (version 7.0.1090 http://drive5.com/uparse/). To determine the taxonomy of each 16S or 18S rDNA gene sequence, the Ribosomal Database Project Classifier (version 2.11 http://sourceforge.net/projects/rdp-classifier/) was utilized. The analysis was based on the Silva database (Release138 http://www.arb-silva.de) and the Unite algorithm (Release 8.0 http://unite.ut.ee/index.php), using a confidence threshold of 70%. The alpha diversity of the microbial communities was evaluated in Mothur (version v.1.30.2 https://mothur.org/wiki/calculators/) in terms of the Sobs, Chao, Shannon, and Good's coverage indices.

The potential associations among the top 60 bacterial or fungal genera were explored. Associations were assessed using the Spearman's rank correlation coefficient in the package 'psych' in R (R Core Team 2019). Associations were considered significant when $|r| > 0.5$ and $P < 0.05$. Microbial association networks were constructed using microbial genera as nodes, which were then connected by lines, called 'edges', indicating positive or negative associations between the genera. To analyze the topology of these microbial association networks in terms of modularity, number of edges, and number of nodes, the 'networkx' package in Python (https://networkx.org/) was utilized. Key microbial genera ('hub members') were identified as those showing the highest degree (number of nodes directly connected with it), degree centrality (node centrality and importance) and closeness centrality (close distance between nodes).

Statistical analysis

All statistical analyzes were conducted using SPSS 22.0 (IBM, Chicago, IL, USA). A two-way analysis of variance (ANOVA) was used to assess the effects of soil P condition and microbial treatment, as well as their interaction. Data were not transformed, as they exhibited a normal distribution according to the Shapiro-Wilk test and homogeneous variance according to Levene's test. Multiple comparisons of means were determined using a Duncan test at $\alpha = 0.05$. To identify variation in the composition of the microbial community, Principal Component Analysis (PCoA) was conducted using the 'vegan' package in R, based on the Bray-Curtis distances of OTUs.

Biomarkers that differed among soil treatments were identified based on the size of the effect in linear discriminant analysis (LEfSe). Groups were displayed in cladograms from the domain to the family level, with LEfSe confirming LDA scores of 2 or higher. The Kruskal-Wallis H test was used to assess the significance of differences in the relative abundance of bacterial or fungal genera among treatments.

All histograms were generated using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Bar graphs indicating the relative abundance of microbial phyla were created in R, and Gephi (version 0.9.2) was used to visualize the microbial networks.

Results

Effect of soil inoculation on seedling growth and nutrient content

The interaction between microbial inoculation and soil P conditions had a significant positive effect on the component mass of *P. edulis* seedlings ($P < 0.01$; Fig. 1a and d and supplementary Table S2). Under the P-deficient condition, the combined inoculation of PSMs and AMF resulted in the highest shoot and root mass. However, under the P-sufficient condition, the microbial inoculation treatment did not have a significant effect on shoot or root mass.

The concentration of N and P in the seedlings was significantly influenced by microbial inoculation, irrespective of whether the soil was P-deficient or P-sufficient ($P < 0.01$; Fig. 1b and c and supplementary Table S2).

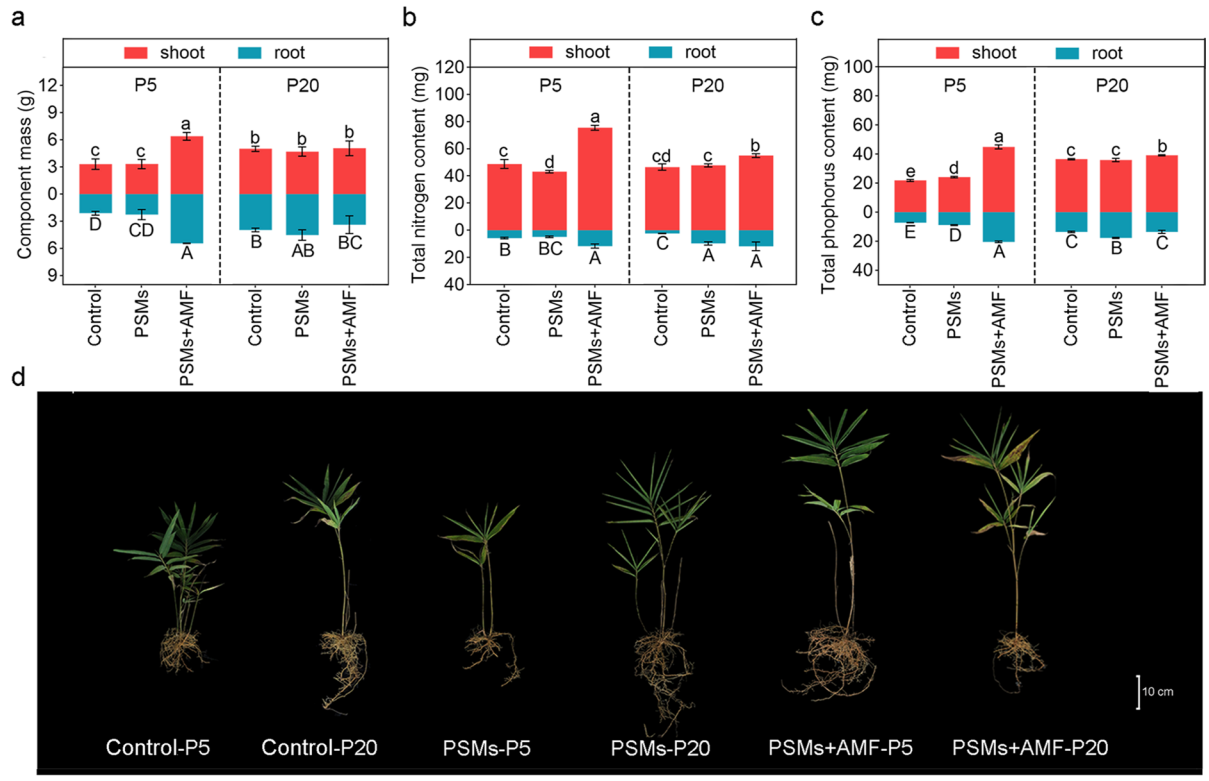


Fig. 1 Effects of inoculating P-deficient (P5) or P-sufficient (P20) soil with phosphate-solubilizing microorganisms (PSMs), alone or together with arbuscular mycorrhizal fungi (AMF), on the mass of shoots and roots (**a**), total nitrogen content (**b**), total phosphorus content (**c**) and morphology (**d**) of *Phyllostachys edulis* seedlings. The values plotted on the y-axis above the origin represent the plant shoots, while those below represent the plant roots. Values are mean ± SE ($n = 3$). The bars marked with different letters differ significantly (Duncan's test, $\alpha = 0.05$)

Under the P-sufficient condition, the PSMs significantly increased the total N content of the roots. The combined inoculation of PSMs and AMF resulted in the highest shoot N content in *P. edulis* seedlings, irrespective of whether the soil P levels were deficient or sufficient. Furthermore, this co-inoculation led to a peak in root N content under the P-deficient condition. Conversely, the root N content of seedlings subjected to combined PSMs and AMF inoculation, though similar to those inoculated solely with PSMs, exhibited a significant increase compared to the control in the P-sufficient soil (Fig. 1b).

Under the P-deficient condition, PSMs significantly increased the total P content in seedlings. Furthermore, a further increase was observed when PSMs were combined with AMF (Fig. 1c). Conversely, when soil P levels were sufficient, PSMs significantly increased the P content in plant roots. However, when PSMs and AMFs were combined, there was a significant increase in P content in the plant shoots.

Effect of microbial inoculation on soil nutrient content and enzyme activities

The nutrient status and OC concentration in both bulk and rhizosphere soils at the end of the experiment were generally unaffected by the interactions between microbial inoculation and soil P conditions. However, sometimes they significantly varied as the main effects of microbial inoculation and/or soil P conditions (supplementary Table S3). The effects of microbial inoculation treatments on N concentration in both bulk and rhizosphere soils remained non-significant under either P-deficient or P-sufficient conditions (Fig. 2a). However, the initial soil P condition did influence N concentration in bulk soil under specific inoculation treatment scenarios. Notably, in cases of the P-deficient condition, bulk soil subjected to combined PSMs and AMF co-inoculation exhibited a significant reduction in N concentration. The OC concentration in bulk soil

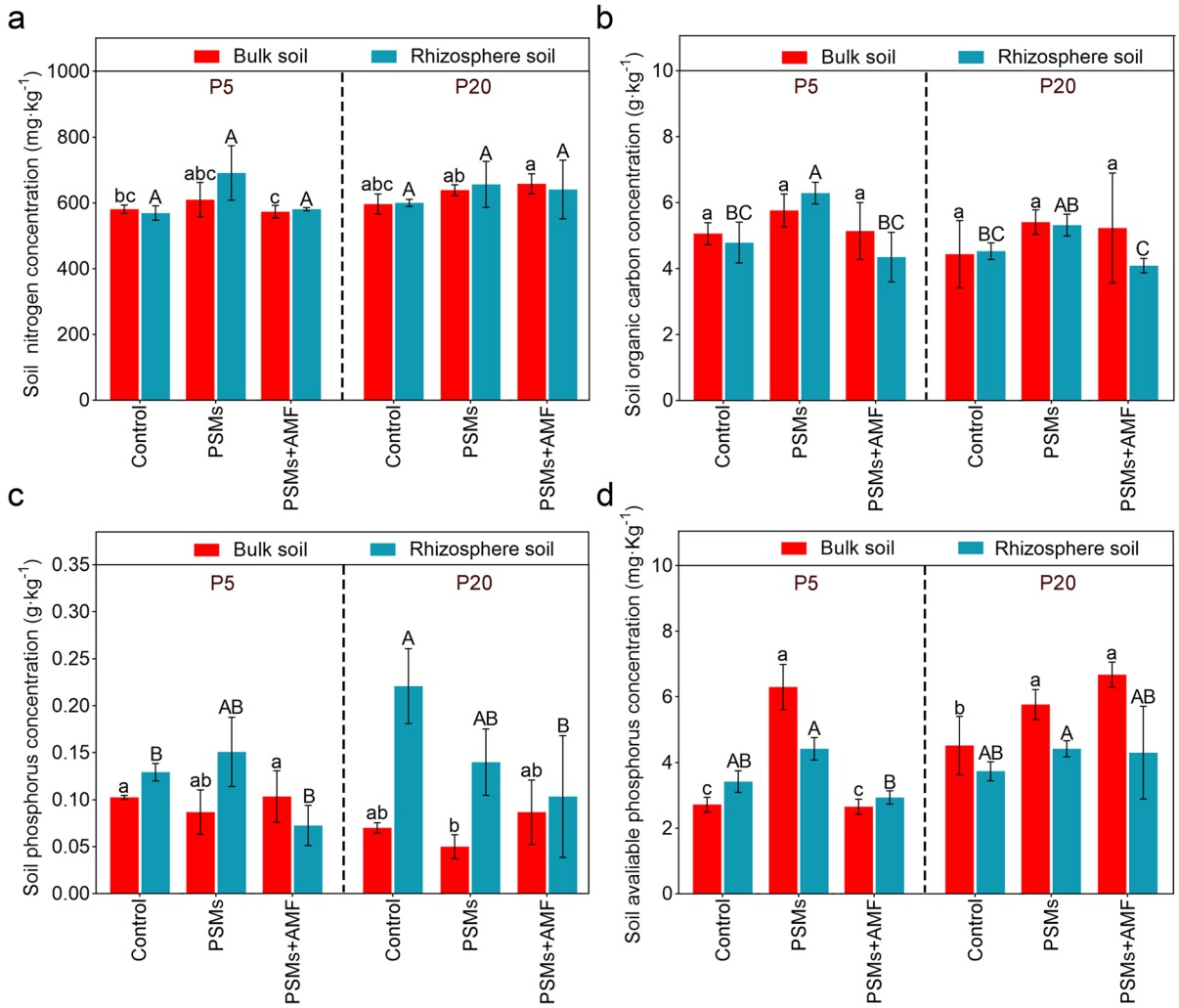


Fig. 2 Effects of inoculating P-deficient (P5) or P-sufficient (P20) soil with phosphate-solubilizing microorganisms (PSMs), alone or together with arbuscular mycorrhizal fungi (AMF), on nitrogen (**a**), organic carbon (**b**), phosphorus (**c**) and available phosphorus (**d**) in the bulk soil and the rhizosphere soil of *Phyllostachys edulis* seedlings. Values are mean ± SE ($n = 3$). The bars marked with different letters differ significantly (Duncan's test, $\alpha = 0.05$)

showed considerable similarity, revealing no significant differences among treatments. In contrast, within the rhizosphere soil, there was a distinct peak in soils inoculated with PSMs, regardless of the P-deficient or P-sufficient soil. Importantly, it is noteworthy that the discrepancy between the PSMs treatment and the control was not statistically significant in the P-sufficient soil (Fig. 2b). Meanwhile, PSMs and particularly PSMs+AMF led to a lower concentration of P in rhizosphere soil and a higher concentration of AP in bulk soil at the end of the experiment, although the effects of PSMs+AMF on the concentration of AP in the bulk and rhizosphere soil were less pronounced under the P-deficient condition (Fig. 2c and d).

The interaction between inoculation treatment and soil P conditions did not show any significant differences in the activities of soil enzymes at either sampling time point. However, the activities of soil enzymes in the rhizosphere showed significant variation due to soil microbial inoculation (supplementary Table S4). Inoculating with PSMs led to decreased activities of βG and CBH in the rhizosphere soil at T1, particularly under the P-deficient condition. Additionally, the activity of ACP was lower at T2 for

the PSMs-inoculated samples compared to those co-inoculated with PSMs+AMF or the non-inoculated control, irrespective of the soil's P status (Table 1).

Diversity and composition of the soil microbial community

The total sequences of 18 soil samples resulted in 958,860 bacterial sequence reads and 1,342,428 fungal sequence reads, which corresponded to >97% sequencing coverage. These sequences were used to assess the alpha diversity of soil microbes (Table 2). Under the P-deficient condition, the inoculation with PSMs yielded the highest values of Sobs, Shannon and Chao indices for fungal community within the rhizosphere soil, and simultaneously increased Sobs and Shannon indices for bacteria community, when compared to the scenarios of co-inoculation with PSMs+AMF or without live inoculation (control). Notably, the Chao index of the bacterial community

Table 1 Activities of enzyme in rhizosphere soil from *Phyllostachys edulis* seedlings inoculated in different ways

Enzyme	Timepoint	Activity ($IU \cdot g^{-1}$)					
		Control-P5	Control-P20	PSMs-P5	PSMs-P20	PSMs+AMF-P5	PSMs+AMF-P20
CBH	T1	3.71 ± 0.30 A	3.57 ± 0.54 AB	2.76 ± 0.34 BC	2.32 ± 0.14 C	2.93 ± 0.52 ABC	3.42 ± 0.28 AB
	T2	2.99 ± 0.81 A	3.38 ± 0.21 A	3.20 ± 0.48 A	2.45 ± 0.19 A	3.35 ± 0.23 A	2.78 ± 0.84 A
αG	T1	1.48 ± 0.17 A	1.49 ± 0.31 A	1.87 ± 0.13 A	1.87 ± 0.08 A	1.74 ± 0.18 A	1.72 ± 0.30 A
	T2	1.27 ± 0.03 B	1.75 ± 0.16 A	1.75 ± 0.13 A	1.65 ± 0.08 A	1.44 ± 0.18 AB	1.46 ± 0.31 AB
LAP	T1	1.31 ± 0.12 A	1.40 ± 0.24 A	1.14 ± 0.20 A	1.32 ± 0.30 A	1.41 ± 0.20 A	1.27 ± 0.27 A
	T2	1.57 ± 0.09 A	1.49 ± 0.24 A	1.46 ± 0.20 A	1.35 ± 0.27 A	1.25 ± 0.10 A	1.54 ± 0.26 A
NAG	T1	0.22 ± 0.03 A	0.17 ± 0.05 A	0.20 ± 0.04 A	0.23 ± 0.02 A	0.20 ± 0.02 A	0.22 ± 0.04 A
	T2	0.22 ± 0.02A	0.19 ± 0.05 A	0.22 ± 0.02 A	0.232 ± 0.03 A	0.23 ± 0.01 A	0.18 ± 0.02 A
βG	T1	0.35 ± 0.02 A	0.32 ± 10.01 AB	0.25 ± 0.05 C	0.26 ± 0.02 BC	0.30 ± 0.04 ABC	0.24 ± 0.02 C
	T2	0.27 ± 0.04 A	0.25 ± 0.03 A	0.23 ± 0.03 A	0.29 ± 0.07 A	0.27 ± 0.04 A	0.32 ± 0.04 A
ACP	T1	0.33 ± 0.04 A	0.32 ± 0.04 A	0.27 ± 0.07 A	0.26 ± 0.04 A	0.29 ± 0.05 A	0.27 ± 0.06 A
	T2	0.27 ± 0.02 AB	0.30 ± 0.01 AB	0.24 ± 0.03 B	0.23 ± 0.03 B	0.34 ± 0.03 A	0.31 ± 0.05 A
ALP	T1	0.59 ± 0.09 A	0.67 ± 0.10 A	0.61 ± 0.02 A	0.68 ± 0.06 A	0.59 ± 0.08 A	0.64 ± 0.04 A
	T2	0.65 ± 0.05 A	0.57 ± 0.01 A	0.65 ± 0.06 A	0.68 ± 0.11 A	0.61 ± 0.08 A	0.61 ± 0.02 A
URE	T1	5.70 ± 0.66 A	7.55 ± 0.65 A	7.32 ± 0.47 A	6.43 ± 1.32 A	6.51 ± 1.18 A	7.26 ± 1.50 A
	T2	6.09 ± 0.84 A	6.22 ± 1.38 A	8.39 ± 0.55 A	6.77 ± 1.40 A	6.58 ± 1.63 A	7.70 ± 0.72 A

Values are mean ± SE ($n = 3$). Values with different letters in the same row differ significantly ($\alpha = 0.05$)

T1 refers to the first-year growth spurt, while T2 refers to the second-year growth spurt

αG α-glucosidase, *βG* β-1,4-glucosidase, *ACP* acid phosphatase, *ALP* alkaline phosphatase, *AMF* arbuscular mycorrhizal fungi, *CBH* cellobiohydrolase, *LAP* leucine aminopeptidase, *NAG* β-1,4-N-acetylglucosaminidase, *PSMs* phosphate-solubilizing microorganisms, *URE* urease

Table 2 Coverage and diversity of bacterial and fungal communities in rhizosphere soil after different treatments

Treatment	Bacterial community				Fungal community			
	Sobs	Shannon	Chao	Coverage	Sobs	Shannon	Chao	Coverage
Control-P5	1097	4.91	1404.17	0.99	256	2.83	270.19	0.99
Control-P20	1118	4.63	1499.22	0.99	260	2.41	296.30	0.99
PSMs-P5	1511	5.33	1827.94	0.99	369	3.03	395.94	0.99
PSMs-P20	1288	5.13	1494.83	0.99	266	2.74	279.58	0.99
PSMs+AMF-P5	1434	4.76	1851.04	0.99	251	2.30	300.61	0.99
PSMs+AMF-P20	1604	5.21	2117.79	0.98	306	2.13	388.10	0.99

AMF arbuscular mycorrhizal fungi, *PSM* phosphate-solubilizing microorganism

in the rhizosphere soil, when solely inoculated with PSMs, was slightly lower than that observed in the rhizosphere soil co-inoculated with PSMs+AMF, although it remained higher than the control condition. Additionally, in cases where soil P was sufficient, the combination of PSMs+AMF resulted in a higher Sobs, Chao and Shannon indices for microbial community compared to other treatments, except for a lower Shannon index specifically observed for fungi.

Both microbial inoculation treatment and soil P condition significantly influenced the bacterial composition (R = 0.4008, $P = 0.001$; Fig. 3a) and the fungal composition (R = 0. 0.4696, $P = 0.001$; Fig. 3c) of the rhizosphere soil. These variables did not alter the dominant microbial phyla, although they did alter their relative proportions (Fig. 3b and d). The most abundant bacterial phyla were Proteobacteria, Actinobacteriota, Acidobacteriota and Bacteroidota, whose relative abundance together accounted for 68–82% of the total bacterial community (supplementary Table S5). The most abundant fungal phyla were Ascomycota and 'unclassified fungi', their relative abundance accounting for 55-90% of the overall fungal community. However, inoculation of PSMs induced an elevation in the relative abundance of Bacteroidota, whereas Actinobacteriota demonstrated the lowest relative abundance in soils with comparable P levels. Furthermore, the combinedly co-inoculation of PSMs+AMF led to an increased the relative abundance of Actinobacteriota and Ascomycota within the rhizosphere soil, while concurrently reducing the relative abundance of Bacteroidota and 'unclassified fungi', regardless of whether the initial soil P was deficient or sufficient (supplementary Table S5).

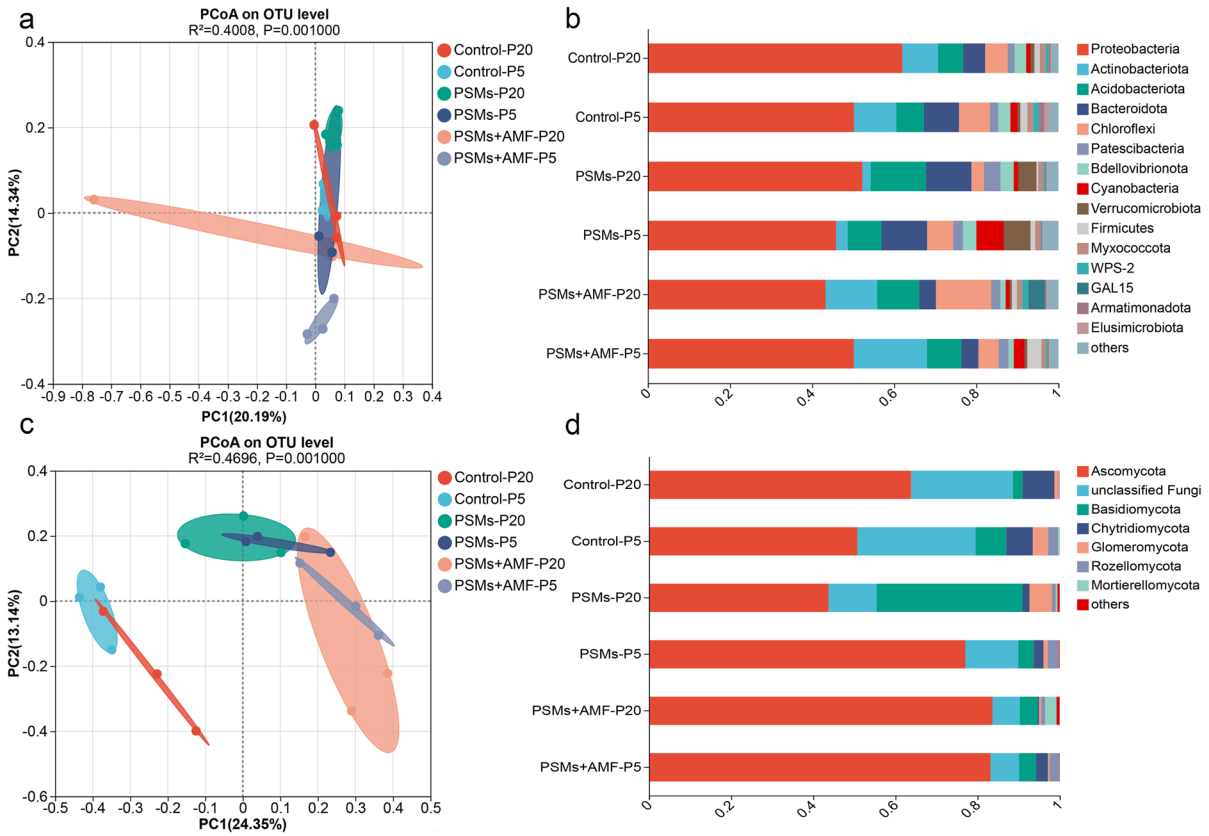


Fig. 3 Effects of inoculating P-deficient (P5) or P-sufficient (P20) soil with phosphate-solubilizing microorganisms (PSMs), alone or together with arbuscular mycorrhizal fungi (AMF), on the composition and abundance of microbial communities in the rhizosphere soil of *Phyllostachys edulis* seedlings. Principal component analysis (PCoA) on operational taxonomic unit (OTU) level and microbial composition on phylum level were determined for the bacterial community (**a**, **b**) and the fungal community (**c**, **d**)

In addition, the composition of bacterial and fungal genera was also influenced by soil P conditions and microbial inoculation treatments (supplementary Fig. S1). Under conditions of soil P deficiency, the inoculation treatments exhibited 62.4% shared bacterial genera and 39.21% shared fungal genera in comparison to no live inoculation (control). Similarly, there were 64.7% shared bacterial genera and 41.93% shared fungal genera relative to control in the P-sufficient soil. However, certain genera were exclusively shared with the inoculation treatments irrespective of soil P conditions. For instance, in the P-deficient soil, PSMs (PSMs+AMF) treatment specifically featured 70 (56) bacterial genera and 58 (39) fungal genera, respectively. Notably, the bacterial genera *Brevibacillus* and *Halobacillus* were exclusively found in the PSMs+AMF treatment, while the fungal genus *Arnium* was uniquely observed in the PSMs treatment.

The cladograms illustrated distinct groups, and LEfSe confirmed LDA scores of 2 or greater (Fig. 4a and b). In the P-sufficient soil, the PSMs treatment exhibited significant enrichment in seven bacterial groups and one fungal group. These mainly included Verrucomicrobiae (specifically within the Pedosphaerales order and Terrimicrobiaceae family) and Acidobacteriae (class to family), along with Pezizomycotina (class to family). Similarly, the PSMs+AMF treatment showed enrichment in six bacterial groups and one fungal group, mainly encompassing Acidimicrobiia (class to family except for Microtrichales order), Thermoleophilia (class to family), and Trichocomaceae family. In contrast, the PSMs treatment led to the enrichment of ten bacterial groups and one fungal group in the P-deficient soil. These mainly included Nitrospirota (phylum to family) and Spirochaetota (phylum to family), as well as Trechisporales order. In the same scenario, the PSMs+AMF treatment resulted in significant enrichment of five bacterial groups and one fungal group. These mainly involved Actinobacteriota (within the Actinobacteria class and specifically Micrococcales order to Micrococcaceae family), Dehalococcoidia (class to family), Paenibacillales (order to family), and Stachybotryaceae family.

At the microbial genus level, the co-inoculation of PSMs+AMF led to a significantly higher relative abundance of certain bacterial genera, such as unclassified_f_Micrococcaceae and *Paenibacillus*, as well as fungal genera like *Talaromyces*, *Aspergillus* and *Fusicolla*. This trend was observed across different soil P levels (Fig. 4c and d). Additionally, the inoculation of PSMs resulted in a significantly higher relative abundance of bacterial genera including *Bryobacter*, norank_f_Microscillaceae and norank_f_Pedosphaeraceae, along with fungal genera *Ciliophora*, irrespective of soil P status. In the P-deficient soil, the inoculation of PSMs significantly increased the relative abundance of bacterial genera norank_f_Gemmatimonadaceae and of fungal genera *Shiraia* and unclassified_f_Olpidiaceae; conversely, the co-inoculation of PSMs+AMF significantly elevated the relative abundance of fungal genera *Penicillium* and *Neocosmospora*.

The configuration of bacterial and fungal networks within the rhizosphere soil was affected by both the inoculation treatments and soil P conditions at the end of the experiment (Fig. 5). Across all scenarios, the bacterial and fungal networks exhibited the highest count of nodes and links in response to soil P deficiency following PSMs treatment. Conversely, the lowest modularity was observed within bacterial genera treated with PSMs and fungal genera treated with PSMs+AMF (Fig. 5b, h, and i). In the P-sufficient soil, the bacterial and fungal networks showed the highest number of links and the lowest modularity after co-inoculating with PSMs+AMF (Fig. 5f and l).

Moreover, the microbial inoculation treatments and soil P conditions significantly affected the hub members within bacterial networks. Notably, 48.3% hub bacterial genera and 71.9% hub fungal genera were exclusive to a single treatment (supplementary Table S6 and Table S7). The highest number of specific hub bacterial genera was observed in P-deficient soil treated with PSMs+AMF, while for fungal networks, it was in P-sufficient soil treated with PSMs+AMF. Specifically, in the P-deficient soil, the bacterial genera *Pseudomonas*, *Paenibacillus* and *Bacillus*, as well as fungal genera *Aspergillus* were exclusively found as hub genera in the PSMs+AMF treatment (Fig. 5c and i; supplementary Table S7). Additionally, the hub fungal genus *Penicillium* was detected in the fungal network of P-deficient soil inoculated with PSMs or co-inoculated with PSMs and AMF (Fig. 5h and i).

Discussion

Functional microorganisms in the soil, such as PSMs and AMF, play critical roles in enhancing plant nutrient acquisition efficiency (Hansen et al. 2020). In

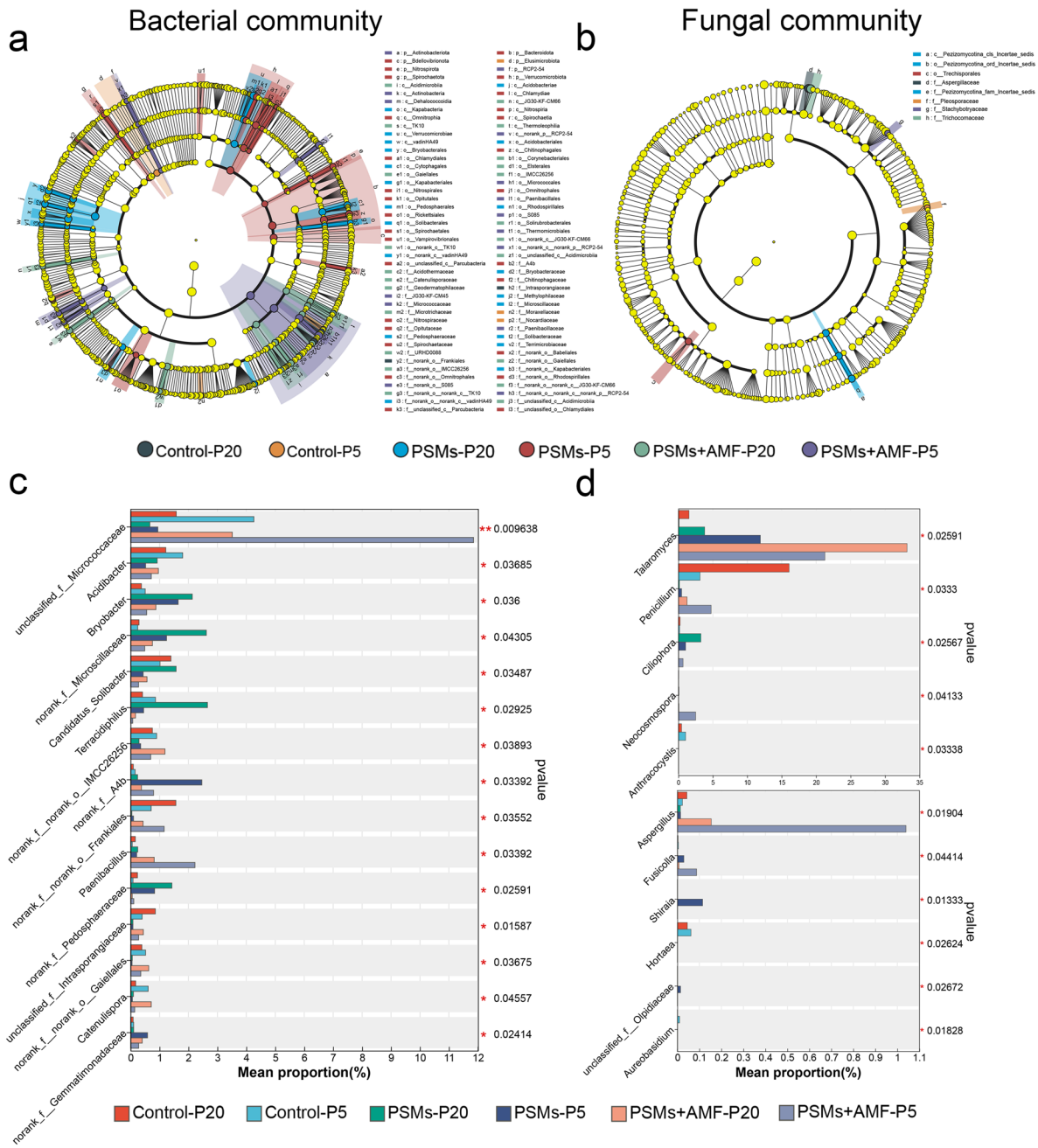
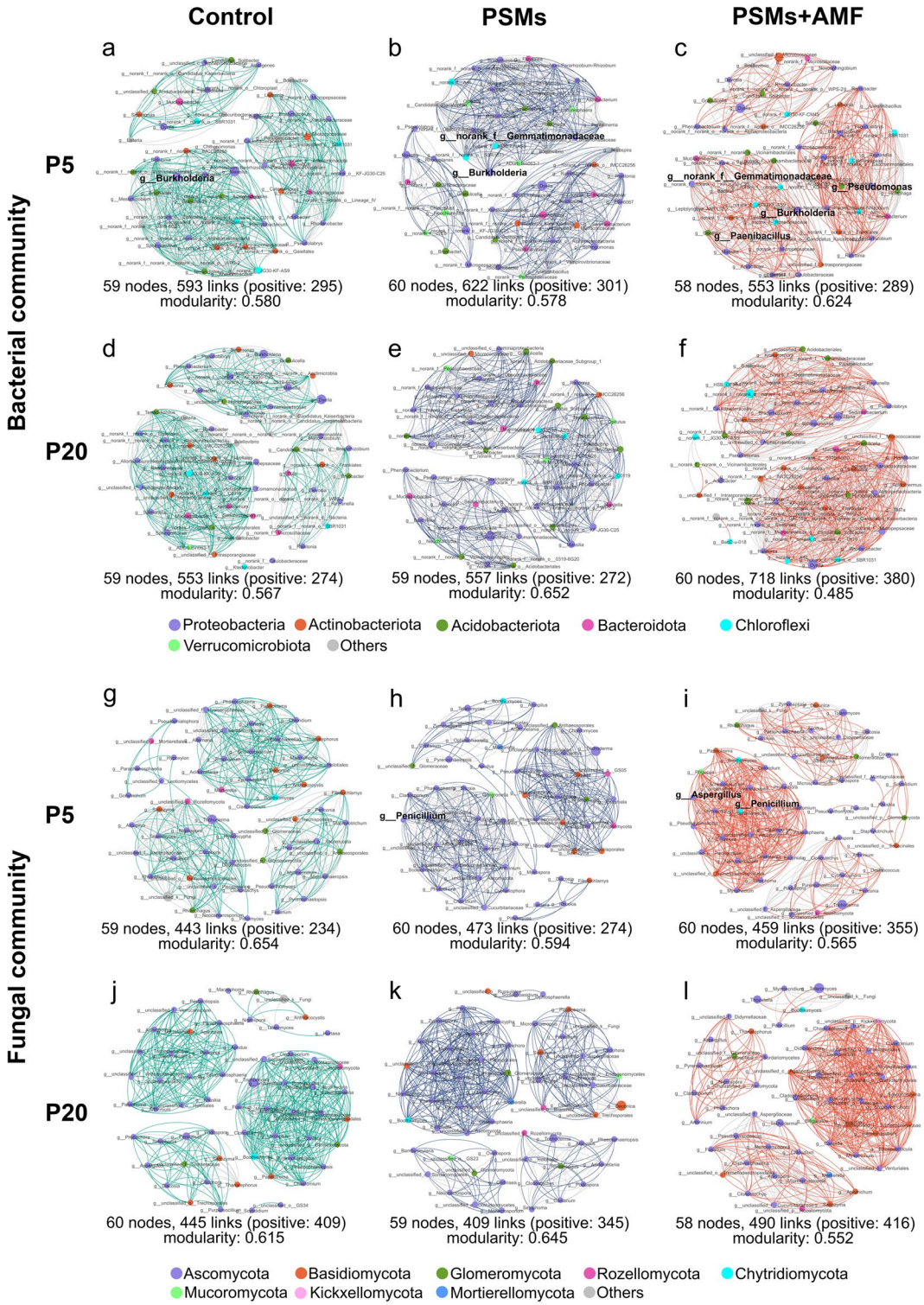


Fig. 4 Effects of inoculating P-deficient (P5) or P-sufficient (P20) soil with the combination of phosphate-solubilizing microorganisms (PSMs) and arbuscular mycorrhizal fungi (AMF) on microbes in the rhizosphere soil of *Phyllostachys edulis* seedlings. Treatments are color-coded as defined at the bottom of the figure. (**a** and **b**) Cladogram showing the phylogenetic distribution of the bacterial (**a**) and fungal (**b**) lineages. The circles indicate phylogenetic levels from domain to family, and the diameter of the circle is proportional to group abundance. Distinctly colored nodes represent microbial groups that exhibit significant enrichment in their corresponding treatment and significantly contribute to the differences observed between treatments. Conversely, pale yellow nodes indicate the opposite scenario, where there is no significant enrichment or contribution to inter-treatment distinctions. (**c** and **d**) Relative abundances of bacterial (**c**) and fungal (**d**) genera whose abundance differed significantly among treatments. The top 15 genera with the mean abundance totals were displayed. *P*-values were calculated using the Kruskal-Wallis H test


Control
PSMs
PSMs+AMF
Bacterial community
Fungal community
P5
P20
a
b
c
d
e
f
g
h
i
j
k
l
g__Burkholderia
g__norank_f__Gemmatimonadaceae
g__Pseudomonas
g__Paenibacillus
g__Penicillium
g__Aspergillus
59 nodes, 593 links (positive: 295)
modularity: 0.580
60 nodes, 622 links (positive: 301)
modularity: 0.578
58 nodes, 553 links (positive: 289)
modularity: 0.624
59 nodes, 553 links (positive: 274)
modularity: 0.567
59 nodes, 557 links (positive: 272)
modularity: 0.652
60 nodes, 718 links (positive: 380)
modularity: 0.485
Proteobacteria
Actinobacteriota
Acidobacteriota
Bacteroidota
Chloroflexi
Verrucomicrobiota
Others
59 nodes, 443 links (positive: 234)
modularity: 0.654
60 nodes, 473 links (positive: 274)
modularity: 0.594
60 nodes, 459 links (positive: 355)
modularity: 0.565
60 nodes, 445 links (positive: 409)
modularity: 0.615
59 nodes, 409 links (positive: 345)
modularity: 0.645
58 nodes, 490 links (positive: 416)
modularity: 0.552
Ascomycota
Basidiomycota
Glomeromycota
Rozellomycota
Chytridiomycota
Mucoromycota
Kickxellomycota
Mortierellomycota
Others

◂**Fig. 5** Networks of associations within microbial communities in the rhizosphere soil of *Phyllostachys edulis* seedlings at the genus level. Networks for bacterial communities (*top two rows*) and fungal communities (*bottom two rows*) are shown for the treatments indicated across the top of the figure. The bacterial and fungal phyla in different microbial communities are color-coded as indicated at the bottom of the figure. The nodes are colored according to the genus. Edges in green, blue or red indicate positive interactions; those in grey indicate negative interactions. The hub genera, whose abundance differed significantly between the microbial inoculation treatments and control samples, or that were part of the original inoculum, are written in bold

the present study, we found that PSMs significantly increased the TP content in plant roots, while when we combined PSMs and AMF and added them to the soil with low levels of P, even greater increases in nutrient content and growth of plant shoots and roots were observed. This phenomenon can be attributed to the establishment of mycorrhizal network by AMF, which aids in the uptake of crucial mineral nutrients including P, nitrogen, copper, zinc, and others by plants. Additionally, the mycelia of AMF contribute to this symbiotic relationship by releasing carbon compounds that provide essential support for the growth of PSMs (Cope et al. 2022; Singh and Kapoor 1999). As a result, the presence of AMF provides an increased supply of carbon sources to PSMs, thereby enhancing their energy resources. This, in turn, empowers PSMs to efficiently convert insoluble P from the surrounding environment into available forms that can be efficiently utilized by plants and mycorrhizal networks (Herman et al. 2012).

We found that inoculating P-deficient soil with PSMs led to higher OC and AP content in the rhizosphere soil compared to co-inoculation with PSMs+AMF. This suggests that P deficiency can stimulate the activities of PSMs, leading to increased secretion of organic compounds such as organic acids that can dissolves insoluble soil P (Guiñazú et al. 2010; Kaur et al. 2016). However, it is important to recognize that different species of bacteria rarely work together, so the negative interactions of PSMs could predominate (Palmer and Foster 2022). This would result in the mass proliferation of PSMs to compete for available P for microbial biomass, leaving less for plants to absorb (Richardson and Simpson 2011). Thus, the interactions of PSMs could also be critical for their function in P activation, which needs to be further explored and analyzed. Moreover, in the absence of mycorrhizal hyphae, most of the available P generated by PSMs may remain in the soil rather than be taken up by plant roots (Burke et al. 2018). In contrast, the presence of AMF may result in lower levels of OC in the soil with PSMs+AMF, which can enhance AMF-driven decomposition and improve N uptake by seedlings (Hodge and Fitter 2010).

Conversely, we observed that inoculating P-sufficient soil with either PSMs or PSMs+AMF resulted in a decrease in P concentration in the rhizosphere soil. It can be contributed to that under P-sufficient conditions, the PSMs or PSMs+AMF do not substantially activate P sources for plant absorption, but instead, these microbes may assimilate a portion of the soil P for their own growth and metabolism (Achat et al. 2010; Chen and Xiao 2023).

As expected, the application of the microbial inoculation treatment exhibited a more pronounced influence on nutrient concentration in the rhizosphere soil when compared to the bulk soil. This observation aligns with the principle that the rhizosphere constitutes the primary zone of interaction among microorganisms, soil, and plants (Gamalero et al. 2003). Microbes in the rhizosphere soil of seedlings, especially functional soil microorganisms such as AMF and plant growth-promoting microorganism (*e.g.*, phosphate-solubilizing bacteria, phosphate-solubilizing fungi, nitrogen-fixing bacteria, etc.), can communicate with plants by exchanging signals and/or materials (Andrino et al. 2021; Khan et al. 2013), indirectly or directly affecting the physicochemical properties of the rhizosphere soil (Chaudhary et al. 2020; Lynch and Ho 2005). Our results showed that microbial inoculation treatments significantly affected enzymes activities in the rhizosphere soil that metabolize C and N at T1 (supplementary Table S4). In the absence of live inoculation, βG and CBH activities in the rhizosphere soil were higher when the soil P was deficient than when it was sufficient, likely reflecting the increased root production of organic acids, amino acids, and carbohydrates under P-deficient conditions (Carvalhais et al. 2011). Building upon previous researches, it becomes evident that a P-deficient soil triggers plants to enhance their nutrient and energy uptake mechanisms, leading to the up-regulation of these specific enzymes (López-Arredondo et al. 2014; Olander and Vitousek 2000).

However, when compared to the no live inoculation control in the current study, the inoculation with

PSMs significantly down-regulated these enzymes in the rhizosphere soil at T1 under the P-deficient condition. This suggests that PSMs inoculation could potentially contributed to mitigating P stress within plant, which could reduce substrates for enzymatic hydrolysis in root exudates and hence, soil enzyme activities. In contrast, inoculating with PSMs+AMF gradually improved the efficiency of soil P activation through plant-microbe interactions that continue, resulting in the highest ACP activity in the rhizosphere soil and the highest TP content of *P. edulis* seedlings at T2. This finding is contrast to a study that reported a peak in soil ACP activity in the *Artemisia annua* rhizosphere 70 days after transplantation, followed by a steady decrease (Ma et al. 2021). This contrast may indicate that inoculating the soil with functional microbes may improve P absorption and utilization primarily during the first year of growth in the case of annual plants, but mainly during the second year in the case of woody plants. The beneficial effects of AMF may take longer due to the time needed for the formation and development of the mycelial network that can harvest AP from a larger soil volume, as well as the time needed for other beneficial microbes to accumulate in the rhizosphere (Ferrol et al. 2019; Xavier and Germida 2003). Our experiments support our first hypothesis that PSMs, especially when co-inoculated with AMF and particularly under the P-deficient condition, can improve nutrient uptake by *P. edulis* seedlings by changing enzyme activities and nutrient status in rhizosphere soil.

The modifications observed in the microbial communities within rhizosphere soils were closely associated with the beneficial impacts of microbial inoculation, especially when AMF was co-inoculated with PSMs (Vázquez et al. 2000). In this study, the application of microbial inoculation significantly influenced the diversity and composition of the microbial community (Fig. 3), resulting notably in a substantial increase in the abundance of beneficial microbial genera. Specifically, inoculating the soil with PSMs resulted in a significantly higher recruitment of the bacterial genera "norank_f_ Gemmatimonadaceae" under the P-deficient condition. In contrast, co-inoculation with PSMs and AMF led to a significant recruitment increase of the bacterial genera *Paenibacillus*, as well as the fungal genera *Talaromyces* and *Aspergillus*, in the rhizosphere soil of *P. edulis* seedlings, regardless of whether the soil P was deficient or not. These genera have been recognized for their ability to promote plant growth by producing antibiotics, siderophores, or enzymes (Doilom et al. 2020; Jin et al. 2022; Khan et al. 2008). Furthermore, several specific bacterial and fungal genera were exclusively present in the microbial inoculation treatments (supplemental Fig. S1). The co-inoculation with PSMs and AMF recruited a total of 56 bacterial genera exclusively found in the rhizosphere soil, with plant growth-promoting bacteria *Brevibacillus* and *Halobacillus* accounting for 27.25% of these genera (Tiwari et al. 2019). These findings align with our second hypothesis that PSMs, particularly co-inoculation with AMF, possesses the capacity to enhance the recruitment of plant growth-promoting microorganisms into the rhizosphere. These microorganisms, in turn, provide C sources and energy for AMF, allowing them to interact with AMF and plants (Lioussanne et al. 2010; Marschner et al. 1997).

Among the hub members of the microbial association networks in rhizosphere microbial communities, some were growth-promoting microorganism (*e.g.*, *Paenibacillus*, *Bacillus* and *Aspergillus*) exclusively recruited into the rhizosphere in microbial inoculation treatments, particularly in the PSMs+AMF treatment under P-deficient conditions, while some were bacterial genera already in the original inoculum, such as *Burkholderia*, *Pseudomonas* and *Penicillium.* This may imply that the composition of the original inoculum and recruited growth-promoting microorganism could influence microbe-microbe interactions, leading to the expression of complementary traits that alleviate stress to P for plants (Paredes et al. 2018).

Conclusion

Phosphate-solubilizing microorganisms, especially in combination with AMF, demonstrate the potential to enhance nutrient uptake in *P. edulis* seedlings by inducing changes in enzyme activities and nutrient status within the rhizosphere soil. These effects appears to be more pronounced during the second year of *P. edulis* seedling growth and involve alterations in the composition and interactions among bacterial and fungal members of the rhizosphere microbial community. Notably, the co-inoculation with PSMs and AMF appears to recruit a greater number of microorganisms that promote plant growth, leading to positive impacts on enzyme activities and nutrient status. Consequently, this co-inoculation strategy benefits *P. edulis* seedlings growth,

particularly when they are cultivated in P-deficient soil. Our findings offer insights into the mechanisms through which functional soil microbes regulate P uptake and underscore the potential of employing PSMs and AMF for sustainable agriculture and forestry practices. Future research endeavors could delve into the exchange of substances in addition to mineral nutrient elements between *P. edulis* and PSMs or PSMs+AMF to optimize sustainable agricultural practices.

Acknowledgments We gratefully acknowledge the executive editor and anonymous reviewers for their insightful comments. We express our sincere gratitude to Dr. Chapin Rodriguez for his valuable help in English editing, and we would like to thank Majorbio Bio-Pharm Technology (Shanghai, China) for their technical assistance with DNA sequencing.

Author contributions WS and YX designed the study and co-wrote the manuscript. YX and FW performed the experiments, analyzed the data and commented on previous versions of the manuscript. SY and YZ participated in data collection and analysis. YY and WS supervised the study and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the National Natural Science Foundation of China (32271971, 32171879), the Natural Science Foundation of Zhejiang Province (LY22C160004) and the Cooperation Project between Zhejiang Province and the Chinese Academy of Forestry (2020SY07).

Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Competing interests 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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