https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Promoted phosphorus transformation by increasing soil microbial diversity and network complexity- A case of long-term mixed-species plantations of *Eucalyptus* with N-fixing tree species

Jiyin Li [a, 1], Yeming You [a, b, 1], Wen Zhang [c], Yi Wang [d], Yuying Liang [a], Haimei Huang [a], Hailun Ma [a], Qinxia He [a], Angang Ming [b, e], Xueman Huang [a, b, *]

[a] Guangxi Key Laboratory of Forest Ecology and Conservation, Guangxi Colleges and Universities Key Laboratory for Cultivation and Utilization of Subtropical Forest Plantation, College of Forestry, Guangxi University, Nanning, Guangxi 530004, China

[b] Guangxi Youyiguan Forest Ecosystem National Observation and Research Station, Youyiguan Forest Ecosystem Observation and Research Station of Guangxi, Pingxiang 532600, Guangxi, China

[c] Jinggangshan Institute of Red Soil, Jiangxi Academy of Agricultural Sciences, Ji'an Jiangxi 343016, China

[d] Institute of Resources and Environment, Key Laboratory of National Forestry and Grassland Administration/Beijing for Bamboo & Rattan Science and Technology, International Centre for Bamboo and Rattan, Beijing, 100102, China

[e] Experimental Centre of Tropical Forestry, Chinese Academy of Forestry, Pingxiang 532600, China

[*] Corresponding author. Tel.: +86 13667881541; E-mail address: huangxm168168@163.com (X. Huang)

[1] These authors contributed equally to this work.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Abstract

Increased nitrogen (N) availability influences soil phosphorus (P) cycling through multiple pathways. Soil microorganisms are essential facilitating a wide range of ecosystem functions. However, the impact of mixed plantations of *Eucalyptus* and N-fixing tree species affect P transformation and microbiota interactions remains unknown. Therefore, we conducted a 17-year field experiment in pure *Eucalyptus* plantations (PPs) to assess the effects of soil P transformation in mixed plantations (MPs) of *Eucalyptus* and N-fixing trees species. The results showed that α-diversity indices for bacteria ACE and Chao1 as well as Shannon indices index for both bacteria and fungi were significantly higher in MPs than in PPs. Significantly higher relative abundances in MPs than in PPs were determined for the bacterial phyla *Proteobacteria* (0–10 cm soil layer only), *Verrucomicrobia*, and *Rokubacteria* and for the fungal phyla *Mortierllomycota*, *Mucoromycota*, and *Rozellomycota*. By contrast, those of the bacterial phyla *Chloroflexi*, *Actinobacteria*, and *Planctomycetes* and fungal phylum *Ascomycota* were significantly lower in MPs than in PPs. Gene copy numbers for 16S rRNA, internal transcribed spacer (ITS), N functional genes [*nifH* (0–10 cm soil layer only), AOB-*amoA*, *narG*, *nirS*, and *nosZ* (0–10 cm soil layer only)] and P functional genes [*phoC*, *phoD* (0–10 cm soil layer only), *BPP*, and *pqqC*] were also significantly higher in MPs than in PPs. The findings indicated that MPs can enhance soil microbial diversity, network complexity, and the relative abundance of

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

functional genes associated with N and P cycling by optimizing soil nutrient levels and pH, thereby facilitating P transformation. Therefore, MPs of *Eucalyptus* and N-fixing tree species may represent a promising forest management strategy to improve ecosystem P benefits.

Keywords: Co-occurrence network; functional gene; mixed plantation; N-fixing species; phosphorous transformation

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

1. Introduction

Phosphorus (P) constitutes a crucial factor influencing the plant-microbe-soil interactions within forest ecosystems (Richardson and Simpson, 2011), while the availability of P serves as a key indicator of soil fertility and quality (Peng et al., 2021). In most ecosystems, P supply is limited because gaseous P compounds in the atmosphere are unavailable (Du et al., 2020), such that P cycling occurs mainly between plants, soil, and microorganisms. However, because the main source of P is derived from soil matrix weathering, large quantities of P tend to be masked by insoluble minerals, particularly iron (Fe) and aluminum (Al), or fixed in low-mobility inorganic and organic materials (Walker and Syers, 1976). These P reserves cannot be accessed directly by plants (Rodríguez and Fraga 1999; Fan et al., 2019). Consequently, it is essential to implement strategies for the sustainable management of soil P to enhance its utilization by plants, preserve soil quality, and mitigate the risk of P loss.

Microorganisms are critical for the processes of P mineralization, solubilization, and cycling, facilitating its transformation into bioavailable forms for plant uptake while also contributing to the maintenance of soil health (Pastore et al., 2020; Sun et al., 2022). Research on the genes associated with P uptake and utilization have highlighted the importance of microorganisms in enhancing these processes in plants (Dai et al., 2020). By functioning as both a reservoir and a source of phosphate ions, soil microorganisms significantly influence P availability and thus soil fertility (Bünemann et al., 2008; Zhou et al., 2018). The α-diversity, structure, and composition of soil bacterial and fungal communities are extremely sensitive to P

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

transformation (Jin et al., 2019).

Microbiome co-occurrence networks are prevalently employed to scrutinize the interrelationships within microbial communities, and network attributes (e.g., the mean degree, edge quantity, and node amount) can be utilized to appraise the reciprocal ties among these communities and their reactions to modifications in cultivation paradigms (Faust, 2021; Qiu et al., 2021). The complexity of microbial networks, defined as the strength of microbial interactions, predicts ecosystem function, including the transformation and cycling of soil nutrients such as P and nitrogen (N). Therefore, network complexity is closely linked to multifunctionality (Yang et al., 2023). Thus, understanding the interplay between microbial diversity and microbial networks is crucial for developing forest management strategies aimed at enhancing soil fertility and optimizing ecosystem functionality.

Functional gene markers are frequently used to examine the diversity and abundance of the microbial communities involved in elemental transformations. The P cycle cluster includes genes that stimulate the mineralization of organic P (e.g., *phoD*, *phoC*, and *BPP*) (Cao et al., 2022; Khan et al., 2023) and solubilization of inorganic P (e.g., *pqqC*) (Meyer et al., 2011). Additionally, the gene *phoD* encodes a non-specific alkaline phosphatase (ALP) that exhibits enhanced activity in acidic soil (Fraser et al., 2015). N is also an essential element for plant growth and development, typically coupled with P in biogeochemical cycles. The N cycle group consists of genes responsible for microbially driven nitrification (e.g., AOB-*amoA*), N fixation (e.g., *nifH*), and denitrification (e.g., *nirS*). Although increased N availability enhances

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

primary production, an optimal N: P ratio is needed to ensure P uptake and utilization, , thus maintaining the balanced nutrient availability that drives sustained growth (Tessier and Raynal 2003; Menge and Field, 2007). An increase in N content can influence soil pH, which subsequently alters the composition of soil microbial communities and impacts the abundance of phosphatase-coding genes (*phoC* and *phoD*) (Widdig et al., 2020). However, the presence of N-fixing plants also affects P uptake by enhancing litter decomposition rates and the release of organic acids from microbial biomass, thereby improving nutrient cycling and soil fertility (Perring et al., 2008; Li et al., 2021b). Therefore, an understanding of the impact of N availability on soil microbes is needed to assess P transformation in soil.

Eucalyptus are characterized by their straight trunks, well-developed horizontal root systems, and high adaptability. They are prevalent in subtropical and tropical regions, where they have significant economic and ecological value (Zhang and Wang, 2021b). However, monocultures and short-term rotation management of *Eucalyptus* plantation have led to soil degradation, reductions in soil nutrient effectiveness, and soil microbial function and diversity, as well as other adverse ecological effects. Planting *Eucalyptus* in a mixture with other trees has been demonstrated to enhance nutrient cycling efficiency, soil fertility, and overall productivity (Koutika and Mareschal, 2017; Epihov et al., 2021). *Acacia* are widely planted in South China because they require fewer exogenous N from the soil, due to their N-fixing capacity (Räsänen et al., 2001). Mixed plantations that include N-fixing trees such as *Acacia* can significantly boost productivity and enhance organic carbon sequestration,

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

thereby improving soil fertility and contributing to climate change mitigation (Marron and Epron, 2019; Zhang et al., 2023). Nevertheless, the mechanism through which microorganisms regulate P transformation after the long-term mixed planting of *Eucalyptus* and N-fixing tree remains unclear. We put forward the hypothesized that (1) the diversity and composition of soil microorganisms would be changed in the mixed plantations, that (2) mixed plantations intensify the response to the beneficial impacts of N-fixing tree, thereby strengthening the correlation between the genes associated with N and P cycling, and that (3) N-fixing tree would lead to higher diversity and network complexity in mixed plantations. The primary aims of this study (1) were to evaluate the changes in the structure, diversity, and stability of soil microbial communities after mixing *Eucalyptus* with N-fixing tree, and (2) to elucidate the mechanisms through which bacterial and fungal communities, along with genes associated with N and P cycling, regulate P transformation.

2. Materials and methods

2.1. Site description

The study was conducted in the Shaoping Experimental Field at the Experimental Center for Tropical Forestry, which is affiliated with the Chinese Academy of Forestry (106°56′E， 22°03′N). The area has a subtropical climate, with approximately 1,400 mm of rainfall annually and maintaining an average yearly temperature of 21.2°C. The landscape is characterized by low mountains and hills along with acidic red soil. Forests in this area are primarily composed of commercially managed plantations, as either pure or mixed stands.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

2.2. Plot design and sampling

In this study, the pure (monoculture) *Eucalyptus urophylla* plantations (PPs) and adjacent mixed plantations (MPs) of *Eucalyptus urophylla* and *Acacia mangium* (N-fixing tree) were established in 2004 on the logging tracks of *Pinus massoniana* plantations that were established in 1977. The MPs were planted at a 1: 1 mixing ratio with inter-row planting, consisting of one row of *Eucalyptus urophylla* and one row of *Acacia mangium*. In the first two consecutive years post-planting, both plantations were subjected to a similar stand management regime, which included practices such as weed control and fertilization, subsequently allowing them to proceed with their natural stand development. The experimental design is described in the study conducted by Huang et al. (2017). In 2021, taking into account the differences in plantation layout and topography, five 20 m × 20 m sample plots were randomly established in each stands (PPs and MPs), ensuring that adjacent plots maintained at a distance greater than 200 m to mitigate edge effects. The diameter at breast height, height, and stand density of every tree within each plot were assessed. Detailed information on the plantations is provided in Table S1.

Soil samples were carried out in early August 2021. Soil samples were gathered from eight different points within each plot, located at 5-m intervals from the center, along angles of 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315°. Soil samples were obtained from the depth intervals of 0−10 cm and 10−20 cm following the removal of extraneous materials such as little stones, and dead leaves. Eight undisturbed samples from each soil layer were amalgamated into a composite sample and transported to

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

the laboratory on ice. Each composite sample was partitioned into two aliquots: one designated for the analysis of physicochemical properties, and the other reserved for genomic DNA extraction.

2.3. Soil properties and soil enzyme activity

Soil pH was measured using a 1:2.5 soil-to-water ratio method, and soil organic carbon (SOC) was quantified using the $K_2Cr_2O_7$-H_2SO_4 oxidation method. The total nitrogen (TN) content of soil was determined using an Auto Analyzer III in an extract obtained by digestion of the sample with H_2SO_4 and a catalyst ($CuSO_4$: H_2SO_4 = 10: 1). The levels of nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) were determined by $CaCl_2$ extraction, followed by quantitative analysis using an AutoAnalyzer III. Total P (TP) and available P (AP) were quantified using the molybdenum blue colorimetric method following extraction of the samples with $HClO_4$-H_2SO_4 and HCl-H_2SO_4, respectively.

N and P metabolismed by soil extracellular enzyme activity (EEA), i.e., β-1,4-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) activity for N and acid phosphomonoesterase (ACP) activity for P, were quantified in a fluorescence assay conducted in a 96-well microplate. Soil EEA was calculated from the fluorescence readings of the enzyme after its reaction with the appropriate substrate. The assay was conducted using 200 µL of a soil suspension prepared by weighing 1.25 g of fresh soil to which sodium acetate buffer (pH 4.5) was added, and stirred for 1 min to ensure consistent extraction conditions and effective solubilization of the soil constituents. Eight replicates per sample were tested. The samples were

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

incubated in darkness at 25°C for 3 h, after which the reaction was terminated by adding NaOH. Fluorescence was then immediately measured within the wavelength range of 365–450 nm by using a fluorescence microplate reader. Information on the substrates of the three soil extracellular enzymes can be found in Table S2.

2.4. Soil DNA extraction and sequencing

Microbial genomic DNA was obtained from soil samples utilizing the PowerSoil DNA isolation kit (MN NucleoSpin 96 Soi) for subsequent analysis and measurements. The primers employed were 338F (5′-ACTCCTACGGAGCGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for the amplification of the V3–V4 hypervariable region of the 16S rRNA gene (Mori et al., 2014; Parada et al., 2016), additionally, ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were utilized to amplify the ITS1 region of fungal rRNA gene loci (Adams et al., 2013; Dong et al., 2021). Sequencing data were processed by filtering the raw reads using Trimmomatic v0.33, removing the primers using Cutadapt v1.9.1, assembling the clean reads by overlap with Usearch v10, and removing chimeras with UCHIME v4.2 to ensure data validity. After the removal of potential chimeras, 1,600,678 and 1,550,033 high-quality bacterial and fungal reads were obtained, respectively.

The genetic potential of the soil microorganisms was assessed by real-time fluorescence quantitative PCR (qPCR) to quantitatively determine the gene copy numbers of bacteria (16S) and fungi (ITS). To evaluate the ability of genes to support N and P cycle processes, we analyzed the abundances of the functional genes *nifH*,

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

AOB-*amoA*, *narG*, *nirS*, *nirK*, and *nosZ* as representatives of the N cycle. Similarly, we analyzed the genetic potential of *phoC*, *phoD*, *BPP*, and *pqqC* as representatives of the P cycle. These functional genes are well-established biomarkers of the biochemical pathways essential for nutrient cycling in various ecosystems. The qPCR amplification efficiencies ranged from 90% to 110%. The primers and references for the functional genes are reported in Table S3.

2.5. Network construction

Networks for bacteria and fungi were constructed by dividing the 20 samples into four groups, consisting of two soil layers for PPs and MPs, respectively. First, sample operational taxonomic units (OTUs) were filtered, discarding those that appeared in fewer than three samples within each group (3 out of 5 replicates) (Hu et al., 2023). OTUs with a relative abundance exceeding 1% in the bacterial and fungal communities were selected for further correlation analysis (Fan et al., 2018). The network was built according to thresholds of Spearman's correlation coefficient > 0.6 and $P < 0.05$, assessed using the *Hmisc* package in R v4.0.5. We adjusted the *P* values according to the Hochberg false discovery rate test (Benjamini et al., 2006), with a cut-off of adjusted $P < 0.05$. Network properties were computed utilizing the *igraph* R package, and visualized using Gephi (https://gephi.org/). In all figures, bacterial and fungal phyla exhibiting a relative abundance greater than 1% within the network are represented by distinct colors.

Keystone species were identified by utilizing the connectivity within modules (Zi) and between modules (Pi). Microorganisms were classified into four categories

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

depending on intra-module degree (z score) and participation coefficient (c score) thresholds, into network hubs, module hubs, connectors, and peripherals (Poudel et al., 2016). Network hubs refer to nodes with a high degree of connectivity both globally and within individual modules; module hubs are nodes with significant connectivity restricted to a single module; connectors are nodes that facilitate strong connections between different modules, and peripheral nodes are those with few connections to other nodes (Poudel et al., 2016). Network hubs, module hubs, and connectors occupy critical positions within the network and are classified as keystone topological features. These characteristics are essential for sustaining the stability of microbial communities (Delmas et al., 2019). Consequently, OTUs associated with these nodes were designated as keystone species.

2.6. Data analyses

Alpha index analyses including ACE, Chao1 and Shannon indices were performed using Mothur (v 1.30.2) software (Schloss et al., 2009). Soil physicochemical properties, microbial community indices, such as the ACE and Shannon and Chao1 indices, as well as functional genes and enzyme activity, were analyzed in independent samples t-tests using SPSS v24.0. This statistical approach was applied to evaluate differences attributable to stand type (monoculture or mixed). Differences in soil microorganisms across stand types and soil layers were analyzed using non-metric multidimensional scaling (NMDS) with Bray–Curtis dissimilarity and analysis of similarity (ANOSIM), implemented using the *vegan* package in R (Oksanen et al., 2013; Knowles et al., 2019). Pearson correlation coefficients were

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

used to analyze the relationships among soil characteristics, microbial characteristics, genes associated with N and P cycling, N and P transformation, with the results visualized in a heat map generated in Origin 2024. A redundancy analysis (RDA) was employed to explore the multivariate associations between soil physicochemical characteristics and microorganisms. The most important soil physicochemical properties affecting bacterial and fungal phyla were identified in the RDA and visualized using CANOCO v5. A partial least squares path model (PLS-PM) was constructed using R software to assess the direct and indirect effects of mixed planting of *Eucalyptus* and *Acacia* on P transformation. A PLS-PM can reveal causal connections between observed and latent variables, and its superiority for small sample sizes has been demonstrated in simulation studies, in which path modeling estimation was shown to be reliable (Monecke and Leisch, 2012; Sanchez, 2013). The goodness-of-fit statistic was used to assess the adequacy of the PLS-PM fit, with a value > 0.7 indicating good model fit (Henseler and Sarstedt, 2013).

3. Results

3.1. Soil properties

Significant ($P < 0.05$) increases in SOC, TN, NO_3^--N, C :P, N :P, and pH were determined in both soil layers of the MPs and PPs (Table 1); however, TP (10–20 cm soil layer) was significantly lower in MPs than in PPs ($P < 0.05$, Table 1).

Table 1 Soil physicochemical properties in both 0−10 cm and 10−20 cm soil layers in PPs and MPs.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Soil layer (cm)	Stand type	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	C: N	C: P	N: P	pH
0−10	PPs	12.98± 0.90b	1.15± 0.04b	18.92± 1.49a	4.86± 0.06b	0.31± 0.02a	11.38± 0.96a	42.04± 3.18b	3.74± 0.25b	4.28± 0.04b
	MPs	21.18± 1.10a	2.17± 0.15a	15.14± 2.25a	13.90± 0.67a	0.30± 0.02a	9.82± 0.39a	72.75± 5.35a	7.37± 0.44a	5.09± 0.11a
10−20	PPs	10.31± 0.79b	0.83± 0.02b	13.84± 0.83a	3.05± 0.05b	0.32± 0.03a	12.37± 0.89a	32.73± 2.47b	2.67± 0.17b	4.21± 0.05b
	MPs	14.45± 0.59a	1.33± 0.09a	11.71± 0.44a	5.39± 0.05a	0.22± 0.01b	10.98± 0.76a	64.63± 2.62a	6.00± 0.52a	5.04± 0.13a

SOC: Soil Organic Carbon; TN: Total Nitrogen; NH_4^+-N: Ammonium Nitrogen; NO_3^--N: Nitrate Nitrogen; TP: Total Phosphorus; C: N: Carbon: Nitrogen ratio; C: P:Carbon: Phosphorus ratio; N: P: Nitrogen: Phosphorus ratio; pH: Soil pH Value; Different lowercase letters in the table represent significant differences between PPs and MPs (P<0.05), the same below.

3.2. Bacterial and fungal community diversity and composition

In both soil layers, the bacterial ACE, Chao1, and Shannon indices of α-diversity were significantly higher in MPs than in PPs (P < 0.05, Fig. 1a–c). Fungal Shannon index in the 0–10 cm soil layer was also significantly higher in MPs than in PPs (P < 0.05, Fig. 1f). The composition of bacterial and fungal community exhibited significant differences between the two plantation types and soil layers, except for the fungal communities in PPs, which did not differ between the surface and deeper soil layers (P < 0.05, ANOSIM: R^2 = 0.85, P = 0.01, stress = 0.03 and R^2 = 0.73, P = 0.01, stress = 0.05, respectively, Fig. S1).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

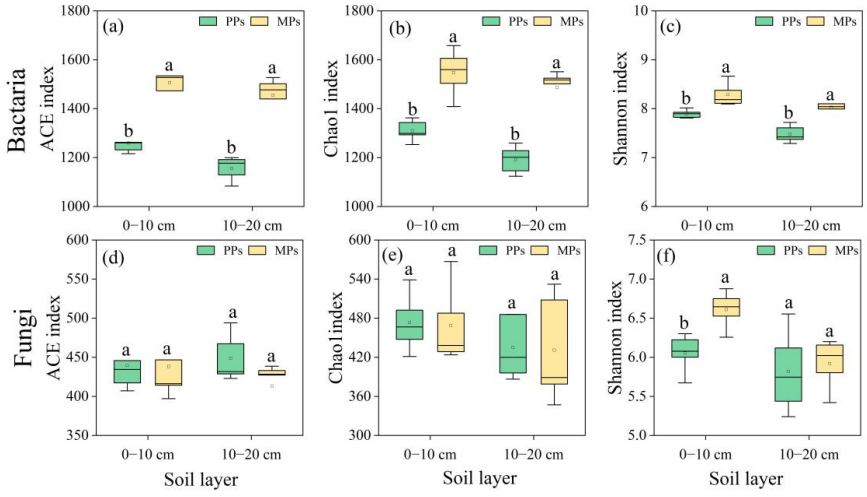

Fig. 1 Comparisions of (a-c) bacterial and (d-f) fungal community, by α diversity index in two soil layers in PPs and MPs.

After clustering at a 97.0% similarity level, a total of 1,869 OTUs were obtained for bacteria, which revealed 21 phyla, 64 classes, 140 orders, 201 families, and 311 genera. For fungi, a total of 1,128 OTUs were obtained, showing 8 phyla, 24 classes, 62 orders, 104 families, and 157 genera (Table S4). The most abundant bacterial phyla (relative abundance > 1%) in both PPs and MPs were *Acidobacteria* (26.83%), *Proteobacteria* (22.46%), *Chloroflexi* (13.95%), *Actinobacteria* (13.62%), *Verrucomicrobia* (11.16%), *Planctomycetes* (5.6%), and *Rokubacteria* (3.5%), which represented 94.08% of the total bacterial community in the 0–10 cm layer (Figs. 2a, b and S2a). The most abundant fungal phyla (relative abundance >1%) in both PPs and MPs were *Ascomycota* (63.25%), *Basidiomycota* (28.14%), *Mortierellomycota* (1.77%), *Mucoromycota* (1.18%), and *Rozellomycota* (1.06%), which represented 95.40% of the total fungal community (Figs. 2c, d and S2b). The introduction of

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

N-fixing tree resulted in changes in the relative abundance and composition of these microbial communities, although these changes were not always statistically significant (Fig. 2).

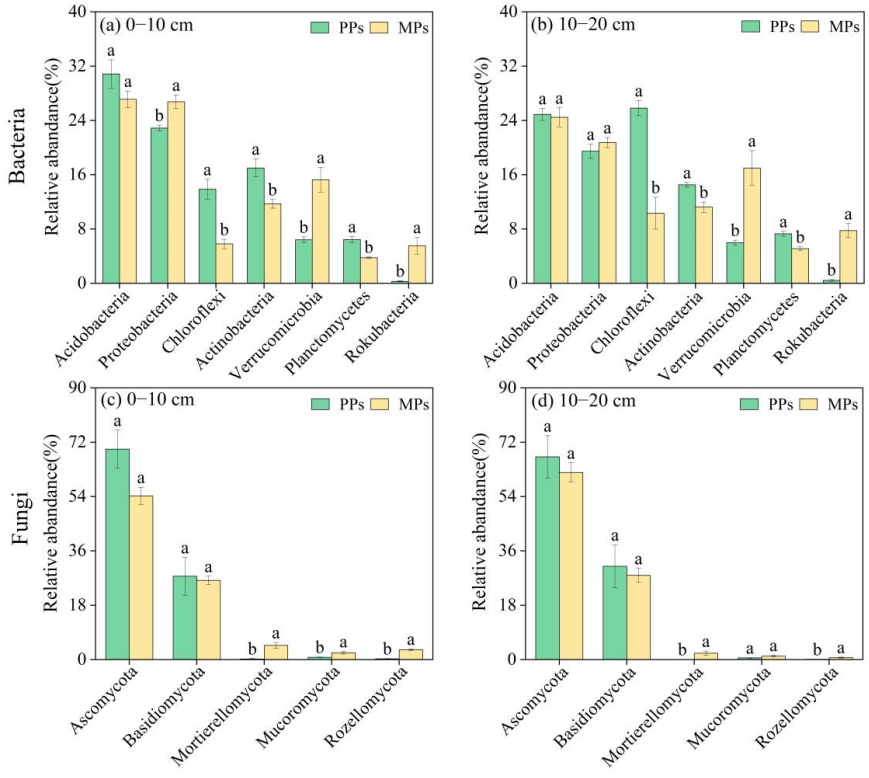

Fig. 2 Abundance difference of (a-b) bacterial and (c-d) fungal and based on relative abundance > 1% at phylum level.

The first two RDA axes explained 66.76% and 14.11% of the total variation in bacterial communities, with pH, TN, and SOC as the major drivers (Fig. 3a). For the fungal communities, the first two RDA axes explained 38.05% and 9.7% of the total variation, with pH as the most important regulator (Fig. 3b).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

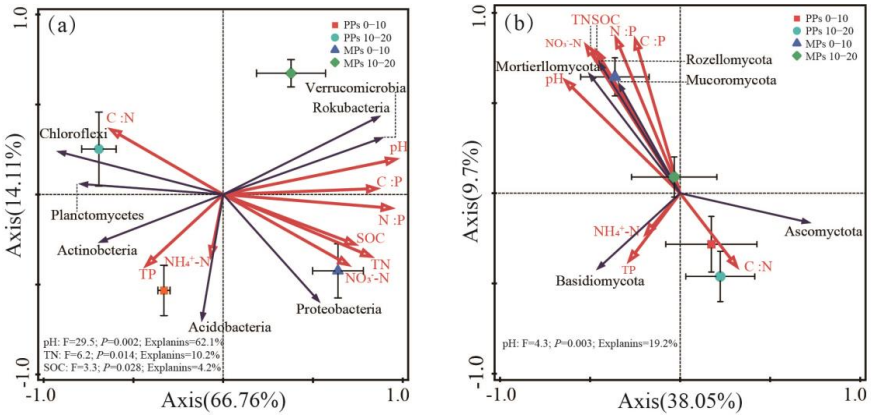

Fig. 3 RDA plot showing significant factors affecting bacterial (a)and fungal(b) communities.

3.3. Microbial network complexity and stability

Microbial species with an average abundance of at least 1% in the 0–10 and 10–20 cm soil layers of PPs and MPs were selected for network analysis. Significant differences in microbial network structure were found between PPs and MPs in both soil layers (Fig. 4a, b). In the bacterial and fungal networks, there were significantly more nodes in MPs than in PPs (Table 2). Therefore, compared to PPs, MPs significantly stimulated the complexity of the co-occurrence network, particularly in the 0–10 cm soil layer. Positive correlations (bacterial networks: ranging = 0.665–0.712, fungal networks: ranging = 0.754–0.849) were determined for both PPs and MPs (Table 2). Compared with PPs, the average path lengths in MPs were shorter (except for the fungal network in the 10–20 cm soil layer) and the network diameter was smaller (except for the bacterial network in the 10–20 cm soil layer) and had a higher average degree for both the bacterial and the fungal networks in both soil layers (Table 2).

The Zi–Pi plot showed that network hubs were absent from the bacterial and

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

fungal networks, with keystone species instead concentrated in connectors and module hubs (Fig. 4c, d). Bacterial keystone OTUs were primarily found in the top three phyla, Proteobacteria, Acidobacteriota, and Actinobacteriota (Fig. 4c). Fungal keystone OTUs were likewise concentrated in the top three phyla, Ascomycota, Basidiomycota, and Mucoromycota (Fig. 4d).

Table 2 Co-occurrence network parameters of bacterial and fungal community at OTU level

Species type	Soil layer (cm)	Stand type	Number of nodes	Number of edges	positive edges	negative edges	Average path length	Network diameter	Average degree
Bactaria	0−10	PPs	529	2498	1661	837	13.58	38	9.44
		MPs	667	7930	5403	2527	7.79	26	23.67
	10−20	PPs	447	2509	1786	723	9.41	27	11.23
		MPs	581	6342	4257	2085	8.51	30	21.83
Fungi	0−10	PPs	298	642	484	158	6.47	22	4.31
		MPs	344	859	722	137	5.80	20	4.99
	10−20	PPs	260	511	421	90	3.00	12	3.93
		MPs	304	779	661	118	5.04	15	5.13

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

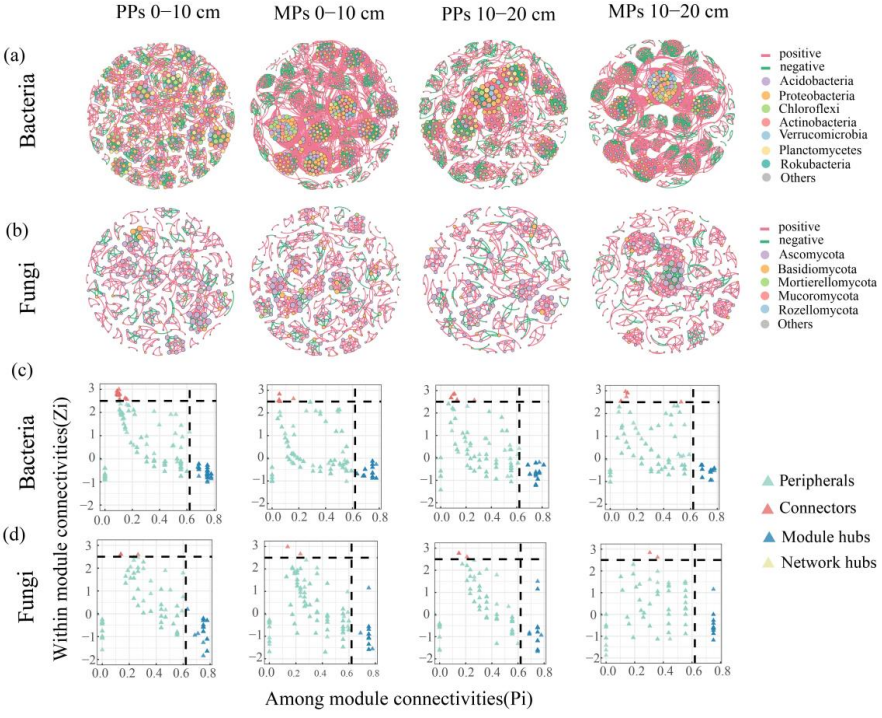

Fig. 4 Co-occurrence network characteristics of (a) bacterial and (b) fungal communities. The node color node size represent the relative abundance >1% phyla and degree, respectively. The Zi-Pi plot (c-d) predicts keystone OTUs in (c) bacterial and (d) fungal networks.

3.4. Microbial functional genes involved in N and P transformation and enzyme activity

Introducing *Acacia mangium* into the *Eucalyptus urophylla* plantation increased the abundances of functional genes involved in N and P transformation (Figs. 5 and 6). Specifically, the abundances of the N-related functional genes *nifH*, AOB-*amoA*, *narG*, *nirS*, and *nosZ* in the 0–10 cm soil layer and of AOB-*amoA*, *narG*, and *nirS* in the 10–20 cm soil layer were significantly higher in MPs than in PPs ($P < 0.05$, Fig. 5a–f).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

The abundances of the P functional genes *phoC*, *phoD*, *BPP*, and *pqqC* in both soil layers were significantly higher in MPs than in PPs ($P < 0.05$), with the exception of *phoD* in the 10–20 soil layer (Fig. 6).

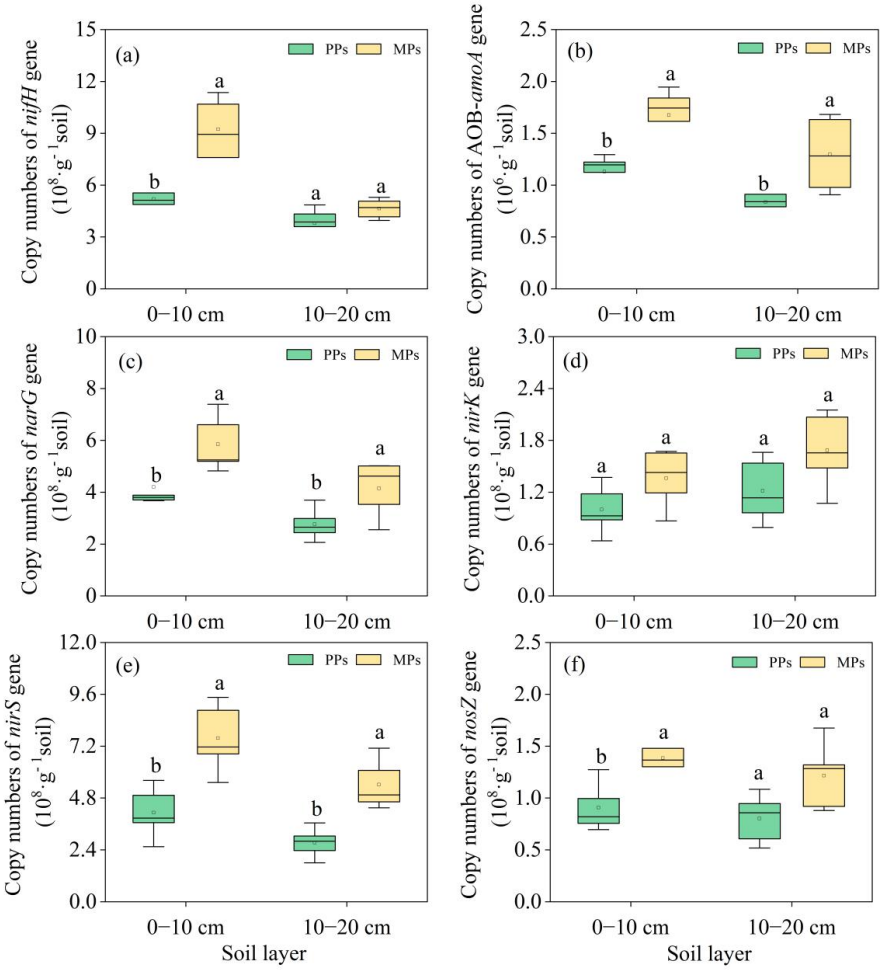

Fig. 5 Comparisions nitrogen cycle functional genes of (a) *nifH*, (b) AOB-*amoA*, (c) *narG*, (d) *narK*, (e) *nirS*, and (f) *nosZ* in two soil layers in PPs and MPs.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

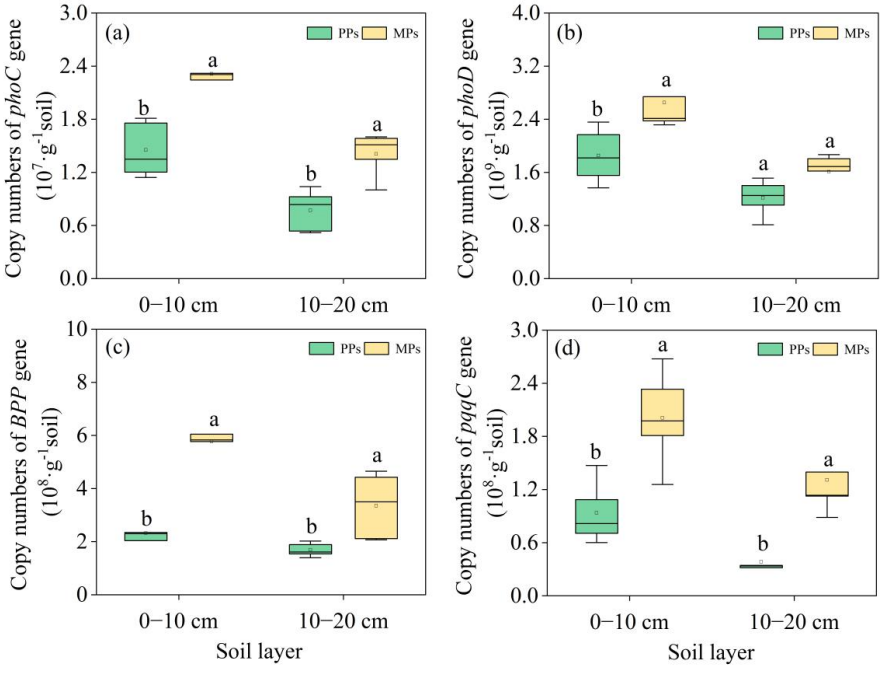

Fig. 6 Comparisions phosphorus cycle functional genes of (a) *phoC*, (b) *phoD*, (c) *BPP*, and (d) pqqC in two layers in PPs and MPs.

The EEA analysis results showed that NAG, LAP, and ACP in the 0–10 cm soil layer were significantly higher in MPs than in PPs ($P < 0.05$), by 97.31%, 31.72%, and 64.35% respectively (Fig. 7). In the 10–20 cm soil layer, NAG, LAP, and ACP were also significantly higher in MPs than in PPs ($P < 0.05$), by 24.02%, 88.54%, 39.83%, and 47.72%, respectively (Fig. 7). The qPCR results showed significantly higher levels of 16S rRNA and ITS in MPs than in PPs ($P < 0.05$, Fig. S3).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

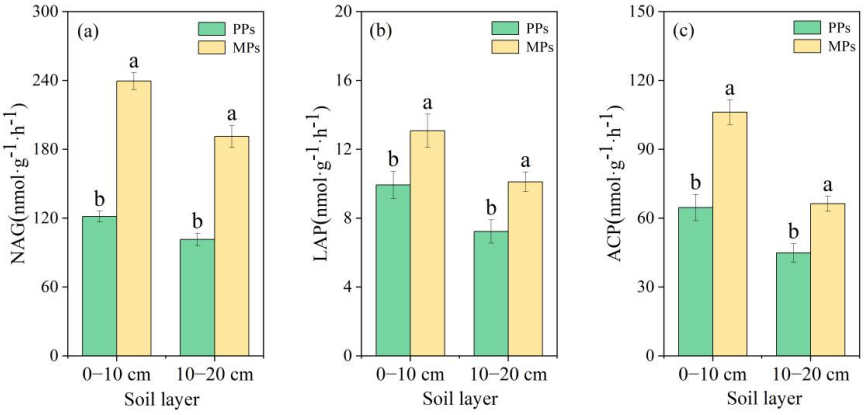

Fig.7 Comparisions extracellular soil enzyme activity of (a) NAG, β-1,4-N-acetylglucosaminidase; (b) LAP, Leucine aminopeptidase; and (c) ACP, Acid phosphatase two soil layers in PPs and MPs.

3.5. Integrating variation in microbial diversity and network complexity with P transformation

The Spearman correlation analysis results (Fig. 8) showed significant positive correlations for NAG, LAP, and ACP with SOC, TN, NO_3^--N, C :P, N :P, and pH; for the three enzymes with 16S rRNA, $ACE_{bacteria}$, $Chao1_{bacteria}$, $Shannon_{bacteria}$, $nodes_{bacteria}$, $edges_{bacteria}$, and average $degree_{bacteria}$ ($P < 0.05$); for NAG, LAP, and ACP with ITS, $Shannon_{fungi}$, $edges_{fungi}$, and average $degree_{fungi}$; for LAP and ACP with $nodes_{fungi}$; for NAG, LAP, and ACP with *nifH*, *AOB-amoA*, *narG*, and *nirS*; for NAG and LAP with *nosZ*; and for NAG, LAP, and ACP with *phoC*, *phoD*, *BPP*, and *pqqC* (all $P < 0.05$). In addition, NAG was significantly negatively correlated with average path $length_{bacteria}$ ($P < 0.05$).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

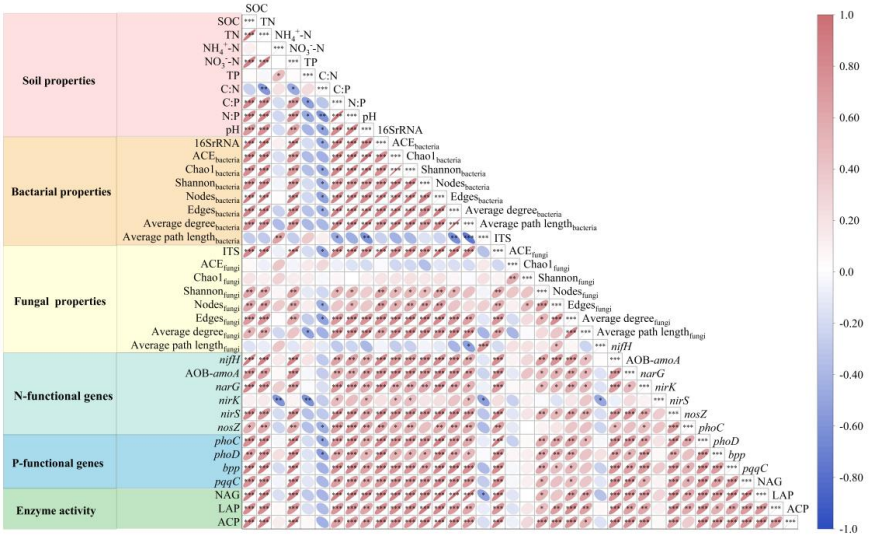

Fig. 8 Pearson correlations of enzyme activity, soil, bacteria and fungi properties, nitrogen (N) and phosphorus (P) cycle functional genes.

According to the PLS-PM analysis results (Fig. 9a), soil properties bacterial and fungal properties, N and P functional genes, and N transformation strongly impacted P transformation, together accounting for 78.2% of the variance, with a high goodness of fit. N transformation and P functional genes (*phoC*, *phoD*, and *BPP*) had a strong direct influence on P transformation, with path coefficients of 0.283 and 0.605, respectively ($P < 0.01$. The diversity and complexity of the network also had favorable effects on N and P functional genes, exerting a substantial influence on P transformation. The overall influence of each factor on P transformation in soil followed the order: soil properties > P functional genes > bacterial properties > N functional genes > fungal properties > N transformation (Fig. 9b). Overall, the mixture of *Eucalyptus* with N-fixing tree directly induces alterations in soil properties, which subsequently influence soil microbial characteristics, functional genes involved

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

in N and P cycling, as well as P transformation, ultimately regulating P transformation.

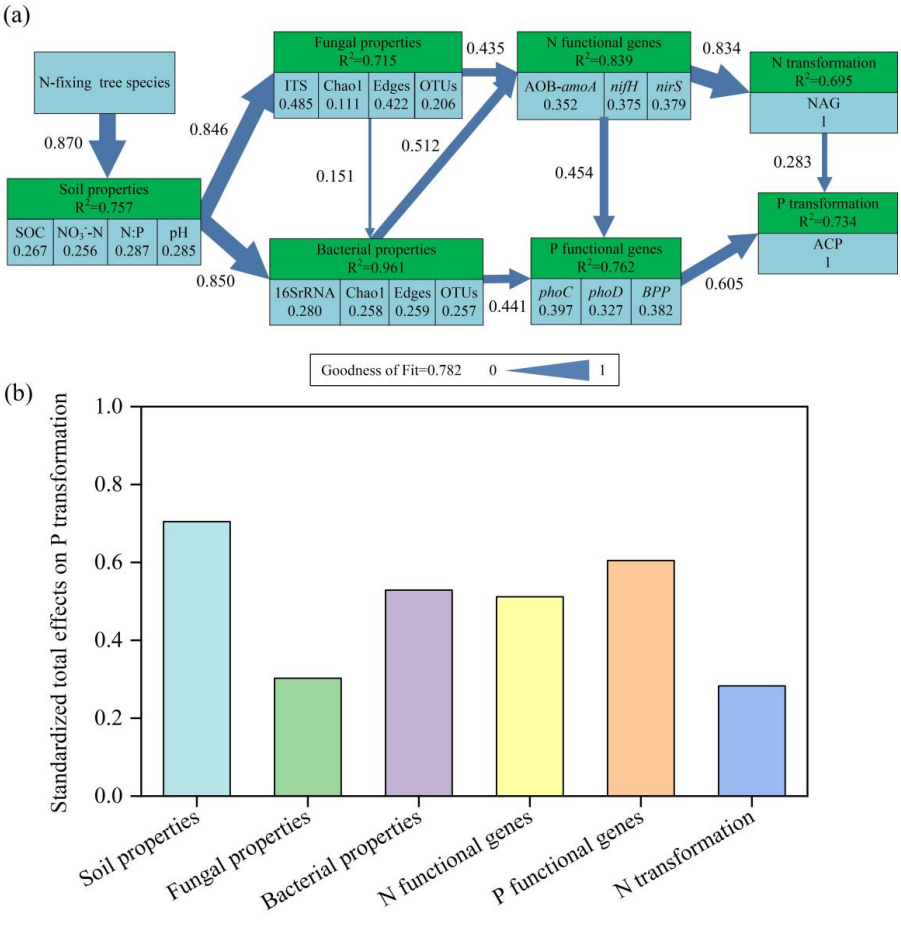

Fig. 9 The light blue represents the observation variable, the light green represents the latent variable, the number under the observation variable represents the contribution weight of the observation variable to the latent variable, the number and the width of the arrow on the arrow represent the standardized path coefficient between the latent variables, and R^2 represents the explanation rate of the model to the latent variable, the same below.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

4. Discussion

4.1 Soil microbial diversity and network response in a mixed plantation of Eucalyptus and N-fixing tree species

The complexity and diversity of microbial communities in soil are fundamental to ecosystem persistence and resilience, as they both reinforce ecological functions and offer a robust defense against external disruptions (Guo et al., 2021). Our study demonstrated that mixed plantations improved the α- and β-diversity of soil bacteria and fungi (Figs. 1 and S1). These findings align with those of a previous study, which demonstrated that the incorporation of *Eucalyptus* with N-fixing tree increased the abundance and diversity of microorganisms, while also revealing variability in community structure across different stands (Li et al., 2023). In our study, *Eucalyptus* mixed with N-fixing *Acacia mangium* resulted in increased SOC, TN, and NO_3^--N content as well as soil C: P and N :P ratios, while decreasing the soil C :N ratio. Soil properties are key in influencing the composition of microbial communities, which serves as a vital indicator of soil health (Xia et al., 2020). The presence of N-fixing trees leads to an increase in litter production, resulting in higher exogenous nutrient inputs (Huang et al., 2014; Zhang et al., 2022), which enhance bacterial and fungal α-diversity (Guo et al., 2019). The composition and diversity of soil microbial communities are primarily driven by C: N: P ratios (Delgado-Baquerizo et al., 2017). The availability of essential nutrients such as N, P, and Fe is controlled by the soil C supply, which subsequently influences the structure of the microbial communities and their co-occurrence patterns (Yuste et al., 2011; Qiu et al., 2021).

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

In natural habitats, soil microbial communities form intricate arrays and robustly structured networks that allow adaptation to shifting environments (de Vries et al., 2018). The complexity of the topological structure and connectivity between nodes influence the overall stability of microbial networks and their resilience to environmental disturbances (Yuan et al., 2021). The overwhelming predominance of positive over negative correlations indicated microbial adaptation to similar ecological niches through co-operation (Gao et al., 2022). Networks characterized by higher connectivity and larger numbers of interrelationships are better equipped to withstand environmental changes, thereby preserving the functional stability of the ecosystem (Cornell et al., 2023). Our study showed more complex bacterial and fungal networks in MPs than in PPs (Fig. 4), demonstrated by the higher number of nodes and edges and the predominance of positive over negative associations, which suggested stronger competition between microorganisms in MPs (Ma et al., 2020; Niraula, 2021). Correlation heat maps also revealed a robust positive association between the number of nodes and the diversity of fungal and bacterial species expressing enzymes responsible for N and P transformation (Fig. 8). These results align with our hypothesis, suggesting that *Eucalyptus* mixed with N-fixing tree increases the complexity of microbial networks (Guo and Gong, 2024).

The relative abundances of *Proteobacteria*, *Verrucomicrobia*, and *Rokubacteria* in the bacterial community were also higher in MPs than in PPs (particularly in the 0–10 cm soil layer), as were the relative abundances of *Mortierllomycota*, *Mucoromycota*, and *Rozellomycota* in the fungal community. Several edaphic factors

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

collectively influenced the structure of both communities, among which pH was the most important. These findings are in line with earlier research, which demonstrated that soil pH was a key determinant in shaping the structure and composition of microbial communities (Siciliano et al., 2014; Cheng et al., 2020). *Verrucomicrobia* is associated with N fixation (Wertz et al., 2012) and serves as an indicator of chemical changes associated with increased soil fertility (Navarrete et al., 2015). According to our Zi–Pi plots, the keystone species of the bacterial community were members of phyla *Proteobacteria*, *Acidobacteriota*, and *Actinobacteria*, and those of the fungal community belonged to *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. The ability of leguminous plant species to establish symbiotic associations with root nodule bacteria, commonly referred to as rhizobia, is well established (e.g., Stougaard, 2000; Yang et al., 2022). Rhizobia are gram-negative bacteria within the extensive and significant phylum *Proteobacteria*. *Proteobacteria* and *Actinobacteria* include N-fixing for plants (Sprent and Platzmann, 2001) and both are critical contributors to maintaining the complexity and stability of microbial networks (Fu et al., 2022). Among fungi, *Ascomycota* is the dominant phylum in soil worldwide (Egidi et al., 2019). However, in the present study, although the relative abundance of Ascomycetes showed dominance in both PPs and MPs, its relative abundance diminished in MPs. Although keystone taxa may not always abundant, they play a vital role in shaping microbial communities and maintaining their ecological functions, through specific regulatory pathways that affect community structure and function (Banerjee et al., 2018; Liu et al., 2022). For example, a prior study demonstrated that keystone taxa

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

played a critical role in increasing the complexity of microbial networks, enhancing plant health and biomass, and promoting the hydrolysis of organophosphorus compounds through enzymatic activity (Qiao et al., 2024; Zeng et al., 2024).

4.2 Association of microbial diversity and networks with P transformation and key environmental drivers

P is crucial for maintaining plant health and ecological balance in terrestrial ecosystems (Du et al., 2020). Soil microorganisms possess numerous different genes involved in N and P transformation, which enable them to regulate N and P cycling by synthesizing and secreting extracellular enzymes (Dai et al., 2020). Our study showed that the abundance of functional genes related to N and P cycles significantly increases after intercropping with N-fixing trees, which supports our second hypothesis (Fig. 5 and 6). In contrast to this finding, Qin et al. (2024) reported that although planting N-fixing tree with *Eucalyptus* enhanced the complexity and stability of N and P functional gene networks, it reduced the abundances of these genes. This discrepancy can be explained by shifts in soil microbial communities related to N and P cycles, which consequently affect the microbial functions that respond to environmental changes (Graham et al., 2016; Zhang et al., 2021a). A previous study also found that the microbial community associated with a mixed plantation of *Eurograndis* and *Amangium* differed from that associated with monocultures of either species, attributable to positive effects of the mixture on soil P and nitrate levels, which enhanced the abundances of N and P functional genes (Rachid et al., 2013).

Biological N fixation is a fundamental ecosystem process that involves the

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

conversion of atmospheric N into a form usable by plants, which, facilitated by a highly diverse group of microorganisms, significantly enhances soil fertility and promoting plant growth (Burns and Hardy, 2012; Soumare et al., 2020). All N-fixing microorganisms carry functional *nifH* genes that encode a component of nitrogenase and act as markers of the abundance and diversity of N-fixing microorganisms across various environmental contexts (Wang et al., 2018). Furthermore, our results suggested high abundances of the P functional genes *phoC*, *BPP*, and *ppqC* in both soil layers and significantly higher abundance of *phoD* in the 0–10 cm soil layer in MPs than in PPs. Both *phoC* and *phoD* are functional genes that encode phosphatase activity needed for P solubilization and mineralization and are thus critically involved in promoting soil P availability (Tian et al., 2021; Cao et al., 2022). The P cycling gene *pqqC*, which encodes the P-mobilizing enzyme pyrroloquinoline quinone synthase, is a marker of phosphate-mobilizing bacteria (Meyer et al., 2011). The predominant bacteria containing *phoD* and *pqqC* are primarily members of the *Proteobacteria* and *Actinobacteria* (Tan et al., 2013; Hu et al., 2018), whose community structure was shown to remain unchanged with an increase in soil P pools (Ragot et al., 2015). In line with our results, a higher abundance and diversity of *phoD*-, *phoC*-, and *pqqC*-bearing soil microorganisms; higher abundances of these genes in soil were correlated with higher soil SOC and TN contents (Luo et al., 2019; Cao et al., 2022). Our study also identified significantly positive correlations between most N and P functional genes and 16S rRNA as well as the ACE, Chao1, and Shannon indexes in bacterial communities, whereas a significant positive correlation

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

was determined only between the ITS region and the Shannon index in fungal communities (Fig. 8). This variation can be attributed to the significant positive impact that high levels of available nutrients have on the development of bacterial communities in the soil (Ming et al., 2016).

The significant positive correlations detected for the N enzymes NAG and LAP with *nifH*, AOB-*amoA*, and the denitrification genes *narG*, *nirS*, and *nosZ* determined in our study suggest that, after the introduction of N-fixing *Acacia*, the microbial community facilitated soil N transformation by increasing the abundance of N cycling genes. Both correlation heat maps and PLS-PM analyses indicated that P transformation reflected the interaction of biological and non-biological factors in ecological processes influenced by the introduction of N-fixing tree (Figs. 8 and 9). Complex interactions between bacteria, fungi, and P cycle genes have been shown to promote microbial community stability while facilitating P cycle transformation processes (Pereira et al., 2021; Liu et al., 2024). *Eucalyptus* mixed with N-fixing tree also increased soil TN and the NH_4^+-N content, which increased ACP activity and thus soil organic P mineralization. The higher soil pH in MPs than in PPs was likely driven by exchange interactions involving Fe/Al hydroxide minerals and functional groups (Table 1), which enhanced the conversion of potentially labile inorganic P into plant-available P via competitive adsorption (Hinsinger, 2001; Kang et al., 2021).

Together, these results indicate that forest management practices that *Eucalyptus* mixed with N-fixing tree will improve soil physicochemical properties, microbial community diversity, and correlations between microbial N and P cycling

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

genes, thereby promoting soil P transformation.

5. Conclusions

This study suggests the benefits of incorporating mixed N-fixing tree species with Eucalyptus, specifically highlighting their positive effects on P transformation. The presence of *Acacia* was shown to alter soil physicochemical properties, improved soil bacterial and fungal community diversity, network complexity, and the abundance of N and P cycling functional genes, ultimately driving P transformation. Increases in soil nutrient content, particularly SOC, TN, and NO_3^--N, as well as the increase in pH that occurred in MPs influenced soil microbial diversity. PLS-PM analysis revealed that mixed plantations have significantly enhanced correlations between P transformation and microbial functional genes that mediate N and P cycling. Our findings offer fresh insights into the predictive capacity of potential shifts in the belowground microbial communities for soil functionality within mixed plantation ecosystems involving N-fixing tree and *Eucalyptus*.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Acknowledgments

This research was funded by grants from the National Natural Science Foundation of China (Nos. 32171755, 32101500, and 31960240), and the scientific research capacity building project for Youyiguan Forest Ecosystem Observation and Research Station of Guangxi under Grant (No. 2203513003).

References

Adams, R. I., Miletto, M., Taylor, J. W., Bruns, T. D.: Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances, ISME J., 7(7), 1262-1273, https://doi.org/10.1038/ismej.2013.28, 2013.

Banerjee, S., Schlaeppi, K., van der Heijden, M. G.: Keystone taxa as drivers of microbiome structure and functioning, Nat. Rev. Microbiol, 16(9), 567-576, https://doi.org/10.1038/s41579-018-0024-1, 2018.

Benjamini, Y., Krieger, A. M., Yekutieli, D.: Adaptive linear step-up procedures that control the false discovery rate, Biometrika 93(3), 491-507, https://doi.org/10.1093/biomet/93.3.491, 2006.

Bünemann, E. K., Smernik, R. J., Marschner, P., McNeill, A. M.: Microbial synthesis of organic and condensed forms of phosphorus in acid and calcareous soils, Soil Biol. Biochem., 40(4), 932-946, https://doi.org/10.1016/j.soilbio.2007.11.012, 2008.

Burns, R. C., Hardy, R. W.: Nitrogen fixation in bacteria and higher plants, 2012.

Cao, N., Zhi, M., Zhao, W., Pang, J., Hu, W., Zhou, Z., Meng, Y.: Straw retention combined with phosphorus fertilizer promotes soil phosphorus availability by enhancing soil P-related enzymes and the abundance of *phoC* and *phoD* genes, Soil Till. Res., 220, 105390, https://doi.org/10.1016/j.still.2022.105390, 2022.

Cheng, J., Zhao, M., Cong, J., Qi, Q., Xiao, Y., Cong, W., Deng, Y., Zhou, J., Zhang, Y.: Soil pH exerts stronger impacts than vegetation type and plant diversity on soil bacterial community composition in subtropical broad-leaved forests, Plant Soil, 450, 273-286, https://doi.org/10.1007/s11104-020-04507-2, 2020.

Cornell, C. R., Zhang, Y., Ning, D., Xiao, N., Wagle, P., Xiao, X., Zhou, J.: Land use conversion

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

increases network complexity and stability of soil microbial communities in a temperate grassland, ISME J., 17(12), 2210-2220, https://doi.org/10.1038/s41396-023-01521-x, 2023.

Dai, Z., Liu, G., Chen, H., Chen, C., Wang, J., Ai, S., Wei, D., Li, D. Ma, B., Tang, C., Brookes, P. C., Xu, J.: Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems, ISME J., 14(3), 757-770, https://doi.org/10.1038/s41396-019-0567-9, 2020.

de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., Hallin, S., Kaisermann, A., Keith, A. M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J. I., Thion, C., Thomson, B., Bardgett, R. D.: Soil bacterial networks are less stable under drought than fungal networks, Nat. Commun., 9(1), 3033, https://doi.org/10.1038/s41467-018-05516-7, 2018.

Delgado-Baquerizo, M., Reich, P. B., Khachane, A. N., Campbell, C. D., Thomas, N., Freitag, T. E., Al-Soud, W. A., Sørensen, S., Bardgett, R. D., Singh, B. K.: It is elemental: soil nutrient stoichiometry drives bacterial diversity, Environ. Microbiol., 19(3), 1176-1188, https://doi.org/10.1111/1462-2920.13642, 2017.

Delmas, E., Besson, M., Brice, M. H., Burkle, L. A., Dalla Riva, G. V., Fortin, M. J., Gravel, D., Guimarães, P. R., Guimarães Jr., P. R., Hembry, D. H., Newman, E. A., Olesen, J. M., Pires, M. M., Yeakel, J. D., Poisot, T.: Analysing ecological networks of species interactions, Biol. Rev., 94(1), 16-36, https://doi.org/10.1111/brv.12433, 2019.

Dong, H., Ge, J., Sun, K., Wang, B., Xue, J., Wakelin, S. A., Wu, J., Sheng, W., Xu, Q., Jiang, P., Chen, J., Qin, H.: Change in root-associated fungal communities affects soil enzymatic activities during *Pinus massoniana* forest development in subtropical China, Forest Ecol. Manag., 482, 118817, https://doi.org/10.1016/j.foreco.2020.118817, 2021.

Du, E., Terrer, C., Pellegrini, A. F., Ahlström, A., van Lissa, C. J., Zhao, X., X, N., Wu, X., Jackson, R. B.: Global patterns of terrestrial nitrogen and phosphorus limitation, Nat. Geosc., 13(3), 221-226, https://doi.org/10.1038/s41561-019-0530-4, 2020.

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K.: A few Ascomycota taxa dominate soil fungal communities worldwide, Nat. Commun., 10(1), 2369, https://doi.org/10.1038/s41467-019-10373-z, 2019.

Epihov, D. Z., Saltonstall, K., Batterman, S. A., Hedin, L. O., Hall, J. S., van Breugel, M., Leake, J.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

R., Beerling, D. J.: Legume–microbiome interactions unlock mineral nutrients in regrowing tropical forests, P Natl. Acad. Sci., 118(11), e2022241118, https://doi.org/10.1073/pnas.2022241118, 2021.

Fan, K., Weisenhorn, P., Gilbert, J. A., Chu, H.: Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil, Soil Biol. and Biochem., 125, 251-260, https://doi.org/10.1016/j.soilbio.2018.07.022, 2018.

Fan, Y., Zhong, X., Lin, F., Liu, C., Yang, L., Wang, M., Chen, G., Chen, Y., Yang, Y.: Responses of soil phosphorus fractions after nitrogen addition in a subtropical forest ecosystem: Insights from decreased Fe and Al oxides and increased plant roots, Geoderma, 337, 246-255, https://doi.org/10.1016/j.geoderma.2018.09.028, 2019.

Faust, K.: Open challenges for microbial network construction and analysis, ISME J., 15(11), 3111-3118, https://doi.org/10.1038/s41396-021-01027-4, 2021.

Fraser, T., Lynch, D. H., Entz, M. H., Dunfield, K. E.: Linking alkaline phosphatase activity with bacterial *phoD* gene abundance in soil from a long-term management trial, Geoderma, 257, 115-122, https://doi.org/10.1016/j.geoderma.2014.10.016, 2015.

Fu, L., Yan, Y., Li, X., Liu, Y., Lu, X.: Rhizosphere soil microbial community and its response to different utilization patterns in the semi-arid alpine grassland of northern Tibet, Front. Microbiol, 13, 931795, https://doi.org/10.3389/fmicb.2022.931795, 2022.

Gao, C., Xu, L., Montoya, L., Madera, M., Hollingsworth, J., Chen, L., Purdom, E., Singan, V., Vogel, J., Hutmacher, R. B., Dahlberg, J. A., Coleman-Derr, D., Lemaux, P. G., Taylor, J. W.: Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities, Nat. Commun., 13(1), 3867, https://doi.org/10.1038/s41467-022-31343-y, 2022.

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier1, A., Yuste, J. C., Glanville, H. C., Jones, D. L., Angel, R., Salminen, J., Newton, R. J., Bürgmann, H., Ingram, L. J., Hamer, U., Siljanen, H. M. P., Peltoniemi, K., Potthast, K., Bañeras, L., Hartmann, M., Banerjee, S., Yu, R. Q., Nogaro, G., Richter, A., Koranda, M., Castle, S. C., Goberna, M., Song, B., Chatterjee, A., Nunes, O. C., Lopes, A. R., Cao, Y., Kaisermann, A., Hallin, S., Strickland, M. S., Garcia-Pausas, J., Barba, J., Kang, H., Isobe, K., Papaspyrou, S., Pastorelli, R.,

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Lagomarsino, A., Lindström, E. S., Basiliko, N., Nemergut1, D. R.: Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes?, Front. Microbial, 7, 214, https://doi.org/10.3389/fmicb.2016.00214, 2016.

Guo, Q., Gong, L.: Compared with pure forest, mixed forest alters microbial diversity and increases the complexity of interdomain networks in arid areas, Microbiology Spectrum, 12(1), e02642-23, https://doi.org/10.1128/spectrum.02642-23, 2024.

Guo, Y., Hou, L., Zhang, Z., Zhang, J., Cheng, J., Wei, G., Lin, Y.: Soil microbial diversity during 30 years of grassland restoration on the Loess Plateau, China: Tight linkages with plant diversity, Land Degrad. Dev., 30(10), 1172-1182, https://doi.org/10.1002/ldr.3300, 2019.

Guo, Y., Xu, T., Cheng, J., Wei, G., Lin, Y.: Above-and belowground biodiversity drives soil multifunctionality along a long-term grassland restoration chronosequence, Sci. Total Environ., 772, 145010, https://doi.org/10.1016/j.scitotenv.2021.145010, 2021.

Henseler, J., Sarstedt, M.: Goodness-of-fit indices for partial least squares path modeling. Computation, Stat, 28, 565-580, https://doi.org/10.1007/s00180-012-0317-1, 2013.

Hinsinger, P.: Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review, Plant soil, 237(2), 173-195, https://doi.org/10.1023/A:1013351617532, 2001.

Hu, J. P., Zhang, M. X., Lü, Z. L., He, Y. Y., Yang, X. X., Khan, A., Xiong, Y. C., Fang, X. L., Dong, Q. M., Zhang, J. L.: Grazing practices affect phyllosphere and rhizosphere bacterial communities of Kobresia humilis by altering their network stability, Sci. Total Environ., 900, 165814, https://doi.org/10.1016/j.scitotenv.2023.165814, 2023.

Hu, Y., Xia, Y., Sun, Q. I., Liu, K., Chen, X., Ge, T., Zhu, B., Zhu, Z., Zhang, Z., Su, Y.: Effects of long-term fertilization on *phoD*-harboring bacterial community in Karst soils, Sci. Total Environ., 628, 53-63, https://doi.org/10.1016/j.scitotenv.2018.01.314, 2018.

Huang, X., Liu, S., Wang, H., Hu, Z., Li, Z., You, Y.: Changes of soil microbial biomass carbon and community composition through mixing nitrogen-fixing species with *Eucalyptus urophylla* in subtropical China, Soil Biol. Biochem., 73, 42-48, https://doi.org/10.1016/j.soilbio.2014.01.021, 2014.

Huang, X., Liu, S., You, Y., Wen, Y., Wang, H., Wang, J.: Microbial community and associated enzymes activity influence soil carbon chemical composition in *Eucalyptus urophylla*

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

plantation with mixing N_2-fixing species in subtropical China, Plant and Soil, 414, 199-212, https://doi.org/10.1007/s11104-016-3117-5, 2017.

Jin, X., Liu, Y., Hu, W., Wang, G., Kong, Z., Wu, L., Ge, G.: Soil bacterial and fungal communities and the associated nutrient cycling responses to forest conversion after selective logging in a subtropical forest of China, Forest Ecol. Manag., 444, 308-317, https://doi.org/10.1016/j.foreco.2019.04.032, 2019.

Kang, L., Zhang, G., Chu, G.: Split delivering phosphorus via fertigation to a calcareous soil increased P availability and maize yield (Zea mays L.) by reducing P fixation, J. Soil. Sediment., 21, 2287-2300, https://doi.org/10.1007/s11368-021-02914-1, 2021.

Khan, A., Zhang, G., Li, T., He, B.: Fertilization and cultivation management promotes soil phosphorus availability by enhancing soil P-cycling enzymes and the phosphatase encoding genes in bulk and rhizosphere soil of a maize crop in sloping cropland, Ecotox. Environ., Safe, 264, 115441, https://doi.org/10.1016/j.ecoenv.2023.115441, 2023.

Knowles, S. C. L., Eccles, R. M., Baltrūnaitė, L.: Species identity dominates over environment in shaping the microbiota of small mammals, Ecol. Lett., 22(5), 826-837, https://doi.org/10.1111/ele.13240, 2019.

Koutika, L. S., Mareschal, L.: Acacia and eucalypt change P, N and C concentrations in POM of Arenosols in the Congolese coastal plains, Geoderma Reg., 11, 37-43, https://doi.org/10.1016/j.geodrs.2017.07.009, 2017.

Li, C., Xu, Y., Wang, Z., Zhu, W., Du, A.: Mixing planting with native tree species reshapes soil fungal community diversity and structure in multi-generational eucalypt plantations in southern China. Front. Microbiol, Fro., 14, 1132875, https://doi.org/10.3389/fmicb.2023.1132875, 2023.

Li, Q., Lv, J., Peng, C., Xiang, W., Xiao, W., Song, X.: Nitrogen-addition accelerates phosphorus cycling and changes phosphorus use strategy in a subtropical Moso bamboo forest, Environ. Res. Lett., 16(2), 024023, https://doi.org/10.1088/1748-9326/abd5e1, 2021b.

Liu, S., Li, H., Xie, X., Chen, Y., Lang, M., Chen, X.: Long-term moderate fertilization increases the complexity of soil microbial community and promotes regulation of phosphorus cycling genes to improve the availability of phosphorus in acid soil, Appl. Soil Ecol., 194, 105178, https://doi.org/10.1016/j.apsoil.2023.105178, 2024.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

689 Liu, S., Yu, H., Yu, Y., Huang, J., Zhou, Z., Zeng, J., Chen, P., Xiao, F., He, Z., Yan, Q.: Ecological
690 stability of microbial communities in Lake Donghu regulated by keystone taxa, Ecol. Indic.,
691 136, 108695, https://doi.org/10.1016/j.ecolind.2022.108695, 2022.
692 Luo, G., Sun, B., Li, L., Li, M., Liu, M., Zhu, Y., Guo, S., Ling, N., Shen, Q.: Understanding how
693 long-term organic amendments increase soil phosphatase activities: insight into *phoD*-and
694 *phoC*-harboring functional microbial populations, Soil Biol. Biochem., 139, 107632,
695 https://doi.org/10.1016/j.soilbio.2019.107632, 2019.
696 Ma, B., Wang, Y., Ye, S., Liu, S., Stirling, E., Gilbert, J. A., Faust, K., Knight, R., Jansson, J. K.,
697 Cardona, C., Röttjers, L., Xu, J.: Earth microbial co-occurrence network reveals
698 interconnection pattern across microbiomes, Microbiome, 8(1), 1-12,
699 https://doi.org/10.1186/s40168-020-00857-2, 2020.
700 Marron, N., Epron, D.: Are mixed-tree plantations including a nitrogen-fixing species more
701 productive than monocultures?, Forest Ecol. Manag., 441, 242-252,
702 https://doi.org/10.1016/j.foreco.2019.03.052, 2019.
703 Menge, D. N., Field, C. B.: Simulated global changes alter phosphorus demand in annual
704 grassland, Global Change Biol., 13(12), 2582-2591,
705 https://doi.org/10.1111/j.1365-2486.2007.01456.x. 2007.
706 Meyer, J. B., Frapolli, M., Keel, C., Maurhofer, M.: Pyrroloquinoline quinone biosynthesis gene
707 *pqqC*, a novel molecular marker for studying the phylogeny and diversity of
708 phosphate-solubilizing pseudomonads, Appl. Environ. Microb., 77(20), 7345-7354,
709 https://doi.org/10.1128/AEM.05434-11, 2011.
710 Ming, L. I., Ming, L. I. U., Li, Z. P., Jiang, C. Y., Meng, W. U.: Soil N transformation and
711 microbial community structure as affected by adding biochar to a paddy soil of subtropical
712 China, Journal of Integrative Agriculture, 15(1), 209-219,
713 https://doi.org/10.1016/S2095-3119(15)61136-4, 2016.
714 Monecke, A., Leisch, F.: semPLS: structural equation modeling using partial least squares, J Stat.
715 Softw., 48, 1-32, https://doi.org/10.18637/jss.v048.i03, 2012.
716 Mori, H., Maruyama, F., Kato, H., Toyoda, A., Dozono, A., Ohtsubo, Y., Nagata, Y., Fujiyama, A.,
717 Tsuda, M., Kurokawa, K.: Design and experimental application of a novel non-degenerate
718 universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

719 amplify eukaryotic rRNA genes, DNA Res., 21(2), 217-227,
720 https://doi.org/10.1093/dnares/dst052, 2014.
721 Navarrete, A. A., Soares, T., Rossetto, R., van Veen, J. A., Tsai, S. M., Kuramae, E. E.:
722 Verrucomicrobial community structure and abundance as indicators for changes in chemical
723 factors linked to soil fertility, Anton. Leeuw. Int. J. G., 108, 741-752,
724 https://doi.org/10.1007/s10482-015-0530-3, 2015.
725 Niraula, S.: Effects of a N_2-Fixing Biofertilizer on the Rhizosphere Microbiome and the Influence
726 of Biochar on Soil Fertility and Microbial Communities, The University of Texas at
727 Arlington, 2021.
728 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Solymos, P.,
729 Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker , B., Borcard,
730 D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., Evangelista, H. B. A., FitzJohn, R.,
731 Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M.
732 H., Cunha, E. R., Smith, T., Stier, A., Braak, C. J. K. T., Weedon, J.: Package 'vegan'.
733 Community ecology package, version, 2(9), 1-295, 2013.
734 Parada, A. E., Needham, D. M., Fuhrman, J. A.: Every base matters: assessing small subunit rRNA
735 primers for marine microbiomes with mock communities, time series and global field
736 samples, Environ. Microbiol., 18(5), 1403-1414, https://doi.org/10.1111/1462-2920.13023,
737 2016.
738 Pastore, G., Kaiser, K., Kernchen, S., Spohn, M.: Microbial release of apatite-and goethite-bound
739 phosphate in acidic forest soils, Geoderma, 370, 114360,
740 https://doi.org/10.1016/j.geoderma.2020.114360, 2020.
741 Peng, Y., Duan, Y., Huo, W., Xu, M., Yang, X., Wang, X., Wang, B., Blackwell, M. S. A., Feng, G.:
742 Soil microbial biomass phosphorus can serve as an index to reflect soil phosphorus fertility,
743 Biol. Fert. Soils, 57, 657-669, https://doi.org/10.1007/s00374-021-01559-z, 2021.
744 Pereira, A. P. D. A., Santana, M. C., Zagatto, M. R., Brandani, C. B., Wang, J. T., Verma, J. P.,
745 Singh, B. K., Cardoso, E. J.: Nitrogen-fixing trees in mixed forest systems regulate the
746 ecology of fungal community and phosphorus cycling, Sci. Total Environ., 758, 143711,
747 https://doi.org/10.1016/j.scitotenv.2020.143711, 2021.
748 Perring, M. P., Hedin, L. O., Levin, S. A., McGroddy, M., De Mazancourt, C.: Increased plant

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

growth from nitrogen addition should conserve phosphorus in terrestrial ecosystems, P. Natl. A. Sci. India., 105(6), 1971-1976, https://doi.org/10.1073/pnas.0711618105, 2008.

Poudel, R., Jumpponen, A., Schlatter, D. C., Paulitz, T. C., Gardener, B. M., Kinkel, L. L., Garrett, K. A.: Microbiome networks: a systems framework for identifying candidate microbial assemblages for disease management, Phytopathology, 106(10), 1083-1096, https://doi.org/10.1094/PHYTO-02-16-0058-FI, 2016.

Qiao, Y., Wang, T., Huang, Q., Guo, H., Zhang, H., Xu, Q., Shen, Q., Ling, N.: Core species impact plant health by enhancing soil microbial cooperation and network complexity during community coalescence, Soil Biol. and Biochem., 188, 109231, https://doi.org/10.1016/j.soilbio.2023.109231, 2024.

Qin, F., Yang, F., Ming, A., Jia, H., Zhou, B., Xiong, J., Lu, J.: Mixture enhances microbial network complexity of soil carbon, nitrogen and phosphorus cycling in *Eucalyptus* plantations, Forest Ecol. Manag., 553, 121632, https://doi.org/10.1016/j.foreco.2023.121632, 2024.

Qiu, L., Zhang, Q., Zhu, H., Reich, P. B., Banerjee, S., van der Heijden, M. G., Sadowsky, M. J., Ishii, S., Jia, X., Shao, M., Liu, B., Jiao, H., Li, H., Wei, X.: Erosion reduces soil microbial diversity, network complexity and multifunctionality, ISME J., 15(8), 2474-2489, https://doi.org/10.1038/s41396-021-00913-1, 2021.

Rachid, C. T., Balieiro, F. D. C., Peixoto, R. S., Pinheiro, Y. A. S., Piccolo, M. D. C., Chaer, G. M., Rosado, A. S.: Mixed plantations can promote microbial integration and soil nitrate increases with changes in the N cycling genes, Soil Biol. Biochem., 66, 146-153, https://doi.org/10.1016/j.soilbio.2013.07.005, 2013.

Ragot, S. A., Kertesz, M. A., Bünemann, E. K.: phoD alkaline phosphatase gene diversity in soil, Appl. Environ. Microb., 81(20), 7281-7289, https://doi.org/10.1128/AEM.01823-15, 2015.

Räsänen, L. A., Sprent, J. I., Lindström, K.: Symbiotic properties of sinorhizobia isolated from Acacia and Prosopis nodules in Sudan and Senegal, Plant and Soil, 235, 193-210, https://doi.org/10.1023/A:1011901706936, 2001.

Richardson, A. E., Simpson, R. J.: Soil microorganisms mediating phosphorus availability update on microbial phosphorus, Plant physiol., 156(3), 989-996, https://doi.org/10.1104/pp.111.175448, 2011.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

779 Rodríguez, H., Fraga, R.: Phosphate solubilizing bacteria and their role in plant growth promotion,
780 Biotechnol. Adv., 17(4-5), 319-339, https://doi.org/10.1016/S0734-9750(99)00014-2, 1999.
781 Sanchez, G.: PLS path modeling with R. Trowchez Editions, 2013.
782 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski,
783 R. A., Oakley, B. B., Parks,D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G.,
784 Horn, D. J. V., Weber, C. F.: Introducing mothur: open-source, platform-independent,
785 community-supported software for describing and comparing microbial communities, Appl.
786 Environ. Microb., 75(23), 7537-7541, https://doi.org/10.1128/AEM.01541-09, 2009.
787 Siciliano, S. D., Palmer, A. S., Winsley, T., Lamb, E., Bissett, A., Brown, M. V., van Dorst, J., Ji,
788 M., Ferrari, B. C., Grogan, P., Chu, H., Snape, I.: Soil fertility is associated with fungal and
789 bacterial richness, whereas pH is associated with community composition in polar soil
790 microbial communities, Soil Biol. Biochem., 78, 10-20,
791 https://doi.org/10.1016/j.soilbio.2014.07.005, 2014.
792 Soumare, A., Diedhiou, A. G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., Kouisni,
793 L.: Exploiting biological nitrogen fixation: a route towards a sustainable agriculture, Plants,
794 9(8), 1011, https://doi.org/10.3390/plants9081011, 2020.
795 Sprent, J. I., Platzmann, J.: Nodulation in legumes (p. 146p), Kew: Royal Botanic Gardens, 2001.
796 Stougaard, J.: Regulators and regulation of legume root nodule development, Plant Physiol.,
797 124(2), 531-540, https://doi.org/10.1104/pp.124.2.531, 2000.
798 Sun, H., Wu, Y., Zhou, J., Yu, D., Chen, Y.: Microorganisms drive stabilization and accumulation
799 of organic phosphorus: An incubation experiment, Soil Biol. Biochem., 172, 108750,
800 https://doi.org/10.1016/j.soilbio.2022.108750, 2022.
801 Tan, H., Barret, M., Mooij, M. J., Rice, O., Morrissey, J. P., Dobson, A., Griffiths, B., O'Gara, F.:
802 Long-term phosphorus fertilisation increased the diversity of the total bacterial community
803 and the *phoD* phosphorus mineraliser group in pasture soils, Biol. Fert. Soils, 49, 661-672,
804 https://doi.org/10.1007/s00374-012-0755-5, 2013.
805 Tessier, J. T., Raynal, D. J.: Use of nitrogen to phosphorus ratios in plant tissue as an indicator of
806 nutrient limitation and nitrogen saturation, J. Appl. Ecol., 40(3), 523-534,
807 https://doi.org/10.1046/j.1365-2664.2003.00820.x, 2003.
808 Tian, J., Ge, F., Zhang, D., Deng, S., Liu, X.: Roles of phosphate solubilizing microorganisms

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

from managing soil phosphorus deficiency to mediating biogeochemical P cycle, Biology, 10(2), 158, https://doi.org/10.3390/biology10020158, 2021.

Walker, T. W., Syers, J. K.: The fate of phosphorus during pedogenesis, Geoderma, 15(1), 1-19, https://doi.org/10.1016/0016-7061(76)90066-5, 1976.

Wang, Q., Wang, J., Li, Y., Chen, D., Ao, J., Zhou, W., Shen, D., Li, Q., Huang, Z., Jiang, Y.: Influence of nitrogen and phosphorus additions on N_2-fixation activity, abundance, and composition of diazotrophic communities in a Chinese fir plantation, Sci. Total Environ., 619, 1530-1537, https://doi.org/10.1016/j.scitotenv.2017.10.064, 2018.

Wertz, J. T., Kim, E., Breznak, J. A., Schmidt, T. M., Rodrigues, J. L.: Genomic and physiological characterization of the *Verrucomicrobia* isolate Diplosphaera colitermitum gen. nov., sp. nov., reveals microaerophily and nitrogen fixation genes, Appl. Environ. Microb., 78(5), 1544-1555, https://doi.org/10.1128/AEM.06466-11, 2012.

Widdig, M., Heintz-Buschart, A., Schleuss, P. M., Guhr, A., Borer, E. T., Seabloom, E. W., Spohn, M.: Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil, Soil Biol. Biochem, 151, 108041, https://doi.org/10.1016/j.soilbio.2020.108041, 2020.

Xia, Q., Rufty, T., Shi, W.: Soil microbial diversity and composition: Links to soil texture and associated properties, Soil Biol. Biochem., 149, 107953, https://doi.org/10.1016/j.soilbio.2020.107953, 2020.

Yang, J., Lan, L., Jin, Y., Yu, N., Wang, D., Wang, E.: Mechanisms underlying legume–rhizobium symbioses, J. Integr. Plant Biol., 64(2), 244-267, https://doi.org/10.1111/jipb.13207, 2022.

Yang, Y., Chai, Y., Xie, H., Zhang, L., Zhang, Z., Yang, X., Hao, S., Gai, J., Chen, Y.: Responses of soil microbial diversity, network complexity and multifunctionality to three land-use changes, Sci Total Environ., 859, 160255, https://doi.org/10.1016/j.scitotenv.2022.160255, 2023.

Yuan, M. M., Guo, X., Wu, L., Zhang, Y. A., Xiao, N., Ning, D., Shi, Z., Zhou, X., Wu, L., Yang, Y., Tiedje, J. M., Zhou, J.: Climate warming enhances microbial network complexity and stability, Nat. Clim. Change, 11(4), 343-348, https://doi.org/10.1038/s41558-021-00989-9, 2021.

Yuste, J. C., Penuelas, J., Estiarte, M., GARCIA-MAS, J., Mattana, S., Ogaya, R., PUJOL, M.,

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Sardans, J.: Drought-resistant fungi control soil organic matter decomposition and its response to temperature, Global Change Biol., 17(3), 1475-1486, https://doi.org/10.1111/j.1365-2486.2010.02300.x, 2011.

Zeng, Q., Peñuelas, J., Sardans, J., Zhang, Q., Zhou, J., Yue, K., Chen, C., Yang, Y., Fan, Y.: Keystone bacterial functional module activates P-mineralizing genes to enhance enzymatic hydrolysis of organic P in a subtropical forest soil with 5-year N addition, Soil Biol. and Biochem., 192, 109383, https://doi.org/10.1016/j.soilbio.2024.109383, 2024.

Zhang, M., O'Connor, P. J., Zhang, J., Ye, X.: Linking soil nutrient cycling and microbial community with vegetation cover in riparian zone, Geoderma, 384, 114801, https://doi.org/10.1016/j.geoderma.2020.114801, 2021a.

Zhang, W., You, Y., Su, X., Yan, J., Gao, G., Ming, A., Shen, W., Huang, X.: Introducing N_2-fixing tree species into *Eucalyptus* plantations promotes soil organic carbon sequestration in aggregates by increasing microbial carbon use efficiency, Catena, 231, 107321, https://doi.org/10.1016/j.catena.2023.107321, 2023.

Zhang, X., Wang, L., Zhou, W., Hu, W., Hu, J., Hu, M.: Changes in litter traits induced by vegetation restoration accelerate litter decomposition in Robinia pseudoacacia plantations, Land Degrad. Dev., 33(1), 179-192, https://doi.org/10.1002/ldr.4136, 2022.

Zhang, Y., Wang, X.: Geographical spatial distribution and productivity dynamic change of eucalyptus plantations in China, Sci. Rep.-UK, 11(1), 1-15, https://doi.org/10.1038/s41598-021-97089-7, 2021b.

Zhou, Y., Boutton, T. W., Wu, X. B.: Soil phosphorus does not keep pace with soil carbon and nitrogen accumulation following woody encroachment, Global Change Biol., 24(5), 1992-2007, https://doi.org/10.1111/gcb.14048, 2018.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Data availability

The data that support the findings of this study are available on request from the corresponding author, [Xueman Huang], upon reasonable request.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Author contributions

JL, XH, and YY conceived and designed of the study. JL, XH, YY, and WZ processed and analyzed data acquisition of field experiments. JL, WZ, YL, HH, HM, and QH conducted the fieldwork. JL and WZ performed laboratory analysis. JL completed the analysis of the data and prepared the original draft of the manuscript, XH, YY, YW, and AM helped to review and edit the manuscript. All the authors gave approval for the final manuscript.

https://doi.org/10.5194/egusphere-2024-3456
Preprint. Discussion started: 20 November 2024

Declaration of Interest Statement

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.