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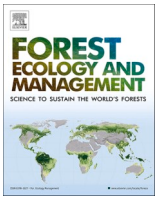

Interspecific plant-plant interactions increase the soil microbial network stability, shift keystone microbial taxa, and enhance their functions in mixed stands

Wanting Li [a,b], Qinghua Liu [a], Lulu Xie [a,b], Chunying Yin [a,*]

[a] CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, PR China
[b] University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, PR China

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ABSTRACT

Mixed forests can improve biomass productivity and soil quality compared with monospecific stands. Soil microbial communities are important for the functions and services in forests. However, the mechanisms by which mixed stands improve tree growth and soil nutrient availability remain unclear. Here, we assessed the soil microbial communities and soil characteristics of *Betula albosinensis* and *Picea asperata* growing alone (B and P), monospecific stands of *B. albosinensis* (BB) and *P. asperata* (PP), and a mixed stand of B. *albosinensis* with *P. asperata* (BP). Results showed that soil characteristics, composition and network of bacterial and fungal community differed significantly between tree species and stand types. Compared with monospecific stands, the mixed stand had 1) 65.36%, 57.51%, and 17.42% higher in SOC, TN, and SAP concentration, respectively; 2) higher diversity of soil bacterial and fungal communities; 3) better microbial networks and higher abundance of keystone bacterial and fungal taxa associated with nutrient decomposition and utilization. The results indicated that increasing of soil bacterial and fungal diversity, network stability and specialized keystone bacterial and fungal taxa by interspecific plant-plant interactions in mixed forests improve soil nutrient availability. This study highlights the importance of sustaining soil microbial taxa and ecological function in the soil nutrient cycling processes for better forest management.

1. Introduction

Mixed stands improve plant production, soil characteristics, and ecosystem services compared to monospecific stands (Ammer, 2019; Feng et al., 2022; Lu and Scheu, 2021). Mixed stands increase the biomass productivity via two mechanisms: (i) facilitation, one tree species benefits from the plant-plant interactions by ameliorating abiotic conditions (Vesterdal et al., 2013); and (ii) niche complementarity, tree species with distinct functional traits explore available resources in different ways (Xiang et al., 2021). Soil microbial community play vital roles in forest nutrient cycle and contribute to the high productivity in mixed stands (Garau et al., 2019; Lu and Scheu, 2021). However, the mechanism by which the soil microbial characteristics influence plant growth and soil properties in mixed stands is unclear.

Tree species and stand types potentially influence soil characteristics, and microbial composition and functions (Cremer et al., 2016; Likulunga et al., 2021). For instance, microbial communities utilize more soil carbon sources in broad-leaved stands than coniferous stands (Garau et al., 2019). In mixed stands, the bacterial community diversity and functions are strongly correlated with nitrogen cycles and litter quality (Pereira et al., 2019), while fungal abundance and composition are associated with soil carbon cycling (Asplund et al., 2018; Ji et al., 2021). Further, soil microbial taxa vary in their substrate preferences and nutrient acquisition strategies (Likulunga et al., 2021; Lu and Scheu, 2021). Indeed, certain microbial taxa in bacterial and fungal communities are better able to use resources under different nutrient conditions by enhancing their nutrient utilization capacity (Likulunga et al., 2021; Wang et al., 2020). Hence, differences in soil nutrient resources induce high variations in the soil microbial composition and function (Lucas Borja et al., 2012; Ye et al., 2022). The interactions between the tree species are more diverse and complex in mixed stands than in their monospecific stands, which influences the soil processes by increasing

* Corresponding author at: Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, PR China.
E-mail address: yincy@cib.ac.cn (C. Yin).

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the nutritional diversity (Cremer et al., 2016), thereby likely altering soil microbial taxa.

Soil microbial taxa and their networks significantly impact forest productivity and ecosystem stability (Banerjee et al., 2018a; Xue et al., 2022). Growing evidence has shown that certain microbial taxa are integral to microbial communities and considerably influence the ecological networks and functions in the soil (Lorenz and Thiele Bruhn, 2019). These microbial taxa that frequently co-exist with others are known as keystone taxa (Banerjee et al., 2018a). The interactions of these keystone taxa in co-occurrence networks can regulate the biogeochemical cycles, and hence are important for plant nutrient acquisition (Wagg et al., 2019). However, the mechanisms underlying the microbial networks, keystone microbial taxa, and their ecological functions in forest soils are still unclear. Soil bacterial and fungal community composition and functions are affected by nutrient availability in the forest soils (Xu et al., 2021). Mixed stands, especially with broadleaved and conifers, alter soil nitrogen (N), carbon (C) and phosphorus (P) availability, influence bacterial and fungal communities and enhance their activity and function (Ding et al., 2022; Pereira et al., 2019). Therefore, studying the association of the keystone bacterial and fungal taxa and their functions with soil characteristics contributes to better understand how soil biodiversity affect biogeochemical processes and forest productivity.

Subalpine forest ecosystems are the main part of the southwestern forests of China. Due to over-deforestation, natural forests have been degraded and replaced by secondary forests and shrubs in this region (Pang et al., 2011). Currently, *Betula albosinensis* and *Picea asperata* are mid-to-late successional species in the subalpine forests of western Sichuan (Wang, 2004). Mixed and monospecific stands of *B. albosinensis* and *P. asperata* are widespread in this region. The soil characteristics and microbial community could differ between monospecific and mixed stands due to root traits (i.e., root exudation and root debris) and litter inputs differences in *B. albosinensis* and *P. asperata* (Xu et al., 2015; Zhuang et al., 2018). Although tree species and stand types are known to potentially affect plant-plant interactions, soil microbes, and soil characteristics, their relationship remains unclear. Therefore, we conducted a field study here to explore the effects of keystone microbial taxa on soil nutrient cycling and forest productivity in different stand types, and aimed to analyze the underlying mechanisms. Our hypotheses were as follow: mixed stands (i) have higher soil nutrient availability and microbial diversity, (ii) have more stable microbial networks and shift the keystone microbial taxa, and (iii) enhance the functions of keystone taxa in nutrient cycling, thus contribute to high soil nutrient availabilities.

2. Materials and methods

2.1. Study site and sample collection

The study was conducted in the Miyaluo forest region of Lixian County (31°40′N–31°45′N, 102°45′E–102°50′E, altitude of 3300–3400 m), Sichuan Province, China, located in the eastern Tibetan Plateau (Fig. S1). This region has a monsoonal mountain climate with annual precipitation ranges from 600 to 1100 mm. The average temperatures in January and July are − 8 °C and 12.6 °C, respectively, and the annual accumulated temperature is 1200–1400 °C. In this region, the plant growing season is from early May to September. The dominant species in the area include trees (*Picea asperata*, *Abies squamata*, and *Betula albosinensis*, etc.), shrubs (*Rubus* spp., *Acer laxiflorum*, *Sinarundinaria nitida*, and *Quercus aguifolioides*, etc.), and herbs (*Epilobium laetum*, *Cacalia roborowskii*, *Cystopteris fragilis*, and multiple genera of Gramineae). According to Chinese soil taxonomy, the soil type at these study sites is mountain brown soil. Similar ages (30–40 years) of monospecific stands and mixed stands (Table S1) at altitude 3300–3400 m, with similar slope, aspect, mono-layered structure, fully stocked stands, and undisturbed soil were selected as materials. The area of each stand was>1 ha.

In order to explore effects of tree species and stand types on microbial communities and soil characteristics, we set five treatments in monospecific stands of *B. albosinensis*, *P. asperata*, and a mixed stand of *B. albosinensis* with *P. asperata*. 1) isolated *B. albosinensis* (B) and 2) isolated *P. asperata* (P) in gaps of monospecific stands; 3) *B. albosinensis*-*B. albosinensis* interaction (BB) in monospecific stands; 4) *P. asperata*-*P. asperata* interaction (PP) in monospecific stands; and 5) *B. albosinensis*-*P. asperata* interaction (BP) in mixed stands. Specifically, B and P were set as the controls, there was no other neighbor trees within 10 m. BB, PP, and BP were set as intraspecific and interspecific plant interaction treatments, depending on the actual situation of conspecific and heterospecific neighboring plants. In intra- and interspecific interaction treatments, the mean distance between tree individuals was about 3 m to avoid shading of the plants above-ground parts and ensure there is interaction of the below-ground parts. In August 2020, five plots (10 × 10 m) were established for each treatment, with a total 25 plots (5 plots each treatment × 5 treatments) (Fig. S1). The distance between plots was>20 m. Tree density of monospecific stands of *B. albosinensis*, *P. asperata*, and the mixed stand is about 685, 573, and 562 trees ha^{-2}, respectively.

In each plot, we identified the individual tree species, measured the tree diameters at breast height and tree heights (Table. S1), and estimated the aboveground biomass according to a previously described allometric equation (Liu et al., 2010). Neighbor effect index (NEI) was calculated to estimate the interaction intensity between the species using the following formula (Guo et al., 2019): NEI = (At–Ac)/(At + Ac), where At is the total aboveground biomass (g $plant^{-1}$) of the individual plants in three plant interaction treatments (BB, PP, and BP) and Ac is the total aboveground biomass of the plant in the control (B and P). NEI = 0 indicates that plant interaction did not significantly affect the plants, and NEI < 0 or > 0 indicates negative or positive effect, respectively.

Five soil cores, from four corners and the central point in each plot, were collected using a metal cylinder (5 cm in diameter) at a depth of 0–20 cm after removing the litter layer. Then, soil cores from the same plot were thoroughly mixed as one sample. After removing the rocks and roots, the soil samples were sieved (2 mm) and divided into three subsamples. One subsample was stored at 4 °C for the soil physical–chemical characteristics analysis, one was air-dried, and the other was immediately stored at − 80 °C for DNA extraction.

2.2. Soil characteristics

Soil pH was measured in deionized water (1:2.5 w/v). Soil ammonium (NH_4^+-N) and nitrate (NO_3^+-N) were extracted from moist soil using 2 M KCl solution (1:5 w/v) and analyzed using a continuous-flow autoanalyzer (SEAL Analytical, Germany). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted with 0.5 M K_2SO_4 (1:5 w/v) and determined with a total organic carbon TOC analyzer (Vario TOC, DKSH, China). The DON concentration was calculated as the difference between dissolved total nitrogen (DTN) and dissolved inorganic nitrogen (DIN, the sum of NH_4^+–N and NO_3^-–N). Microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) concentrations were determined by the chloroform fumigation extraction method and were calculated from the difference between soil extracted with 0.5 M K_2SO_4 in the fumigated and non-fumigated samples. Soil organic carbon (SOC) and total nitrogen (TN) concentrations were measured using the potassium dichromate oxidation and Kjeldahl methods, respectively. Soil available P (SAP) concentration was calculated using the ammonium molybdate ascorbic method with 0.5 M $NaHCO_3$.

In this study, we measured multiple soil enzymes to evaluate the ecosystem function, including enzymes related to the C (β-glucosidase (BG), phenol oxidase (Phox), and peroxidase (Per)), N (leucine amino peptidase (LAP) and β-1,4-*N*-acetyl-glucosaminidase (NAG)), and P (acid phosphatase (AP)) cycles. The Phox and Per activities were measured spectrophotometrically using L-3,4-dihydroxy-phenylalanine (DOPA) as the substrate in clear 96-well microplates (DeForest, 2009).

The other enzyme activities were analyzed using MUB-linked model substrate method (DeForest, 2009; Saiya-Cork et al., 2002).

The geometric mean of the enzyme activities (GMea) was calculated to illustrate the influence of the tree species and stand types on the soil carbon and nitrogen cycling (García-Ruiz et al., 2008; Luo et al., 2018) using the following formula: $GMeac = (BG \times Per \times Phox)^{1/3}$; $GMea_N = (NAG \times LAP)^{1/2}$. Where BG, Per, Phox, NAG, and LAP are the activities of the corresponding enzymes.

2.3. DNA extraction and high-throughput sequencing

The total DNA was extracted from the soil samples using the E.Z.N.A. ® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.A.). The final DNA concentration and purification were determined by a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, USA), and DNA quality was checked by 1 % agarose gel electrophoresis. The bacterial 16S rRNA genes were amplified using the primer sets 515F/806R (Shi et al., 2020), and the fungal DNA gene internal transcriber spacer (ITS) regions were amplified using the primer pairs ITS1F/ITS2R (Li et al., 2021b). The PCR conditions for the 16S rRNA gene were as follows: initial denaturation at 95 °C for 3 min; followed by 29 cycles of 95 °C for 30 *sec*, 30 *sec* at 53 °C, 68 °C for 45 *sec*, and a final extraction at 72 °C for 5 min. The PCR conditions for the ITS1 region were as follows: initial denaturation at 95 °C for 3 min; 37 cycles of 30 *sec* at 95 °C, 30 *sec* at 53 °C, and 45 *sec* at 72 °C; and a final extraction at 72 °C for 5 min. PCRs were performed in triplicate using a 20 μL mixture 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. The resulting PCR products were extracted from a 2 % agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) in accordance with the manufacturer's protocol. The 16S rRNA and ITS2 gene fragments were sequenced using the Illumina MiSeq PE300 platform. The raw amplicon sequences were deposited in the Sequence Read Archive and assigned the following BioProject accession number: PRJNA916853 (BioSamples SAMN18894937 - SAMN18894986).

The paired-end reads were assembled using FLASH v1.2.11 (https://www.cbcb.umd.edu/software/flash) (Magoč and Salzberg, 2011) to obtain raw tags. The resulting FASTQ files were generated using the DADA2 plugin in the QIIME 2 program (Bolyen et al., 2019). The DADA 2 denoised sequences are known as amplicon sequence variants (ASVs), and their taxonomic assignment was performed using the Vsearch consensus taxonomy classifier using QIIME 2. The SILVA reference database v.138 (Pruesse et al., 2007) was used for the taxonomic assignments of the bacterial full-length 16S rRNA sequences. Due to a lack of reference templates for the alignment of the fungal ITS sequences, we trimmed the raw sequences to 300 base pairs (bp) after removing the forward primer sequence. The fungal ITS sequences were classified using the Mothur-formatted ITS reference database UNITE 8 (Abarenkov et al., 2010) with the default bootstrapping algorithm. All the samples were rarified to the minimum number of sequences to enable comparison (43,666 for bacteria and 49,420 for fungi) (Kerdraon et al., 2019).

2.4. Network analyses and keystone taxa

Network analysis was conducted to determine the effect of the tree species and stand types on the microbiome complexity and to identify the potential keystone taxa. The network was constructed at the genus level to visualize the correlations among bacterial and fungal communities. To avoid spurious correlations, the top 450 and 300 dominant bacterial and fungal ASVs were selected, accounting for the top 53.63 % and 80.32 % of the relative bacterial and fungal abundance, respectively. This selection was also supported by the number of ASVs, as the total number of bacterial ASVs was ~ 1.5 times greater than the total number of fungal ASVs. The correlations with Spearman's correlation coefficients > 0.8 with a corresponding P value < 0.01 were considered statistically significant and used to construct networks. The network was analyzed and visualized with Gephi software v.0.9.2 (https://www.gephi.org/), using the undirected network and the Fruchterman-Reingold layout. Relevant topological parameters were obtained in the Gephi platform, representing the topology of the networks and node features, including the average of modularity, network diameter, path length, degree, clustering coefficient, graph density, betweenness, and closeness centrality. The microbial taxa (genera and ASVs) with the highest degree and closeness centrality, and the lowest betweenness centrality scores, were identified as the keystone taxa (Banerjee et al., 2018a; Zheng et al., 2021). Thus, we used high degree and high closeness centrality to statistically identify the keystone taxa in the network.

2.5. Data analysis

We examined the main effects of tree species and forest stands and their interaction on the soil characteristics, soil enzyme activities, and microbial alpha diversity using the two-way ANOVAs with IBM-SPSS 22.0 (IBM Company., Chicago, Illinois, USA) after testing the normality and variance homogeneity of the data. One-way ANOVA was used to examine the difference between NEI and zero. Multiple comparisons analysis among treatments for the same tree species was conducted using Tukey's HSD test ($P < 0.05$). Due to the soil properties are dependent of each other, P values were Bonferroni-corrected as < 0.004 to reduce type 1 error for the 12 soil properties in Table 1. Before further analysis, a collinearity analysis was conducted for all soil variables to avoid inter-correlations by excluding predictors with variance inflation factor > 5 (Oksanen et al., 2017). Thus, ten factors (pH, TN, SOC, SAP, NH_4^+-N, MBC/MBN, DOC, AP, $GMea_C$ and $GMea_N$) were used as explanatory variables.

Principal coordinate analyzes (PCoA) were performed using a Bray-Curtis distance matrix using the R package "phyloseq" to explore changes in the bacterial and fungal community structure (McMurdie and Holmes, 2013). Permutational multivariate analysis of variance (PERMANOVA) was conducted based on Bray-Curtis distance using the ANOSIM function of the R package "vegan" (Oksanen et al., 2017) to assess the effects of tree species and stand types on soil microbial composition. The multiple regression model (R package "stats") (Field et al., 2012) and variance decomposition analysis (R package "relaimpo") (Gromping, 2006) were used to estimate the influence of the soil properties on the top 15 keystone genera (Jiao et al., 2020). The relationship between soil properties and microbial community (at genus level) was determined by redundancy analysis (RDA) using the "vegan" package (Oksanen et al., 2017). FAPROTAX database was used to predict ecological functions of bacterial taxa (Louca et al., 2016; Xue et al., 2022). Fungal functional profiles were predicted using FUNGuild (Nguyen et al., 2016). The hierarchical cluster and bubble diagram of functional profiles prediction were constructed using R package "vegan" (Oksanen et al., 2017) and "ggplot2" (Wickham, 2016). STAMP was used to analyze the significant differences in the predicted ecological functions (Parks et al., 2014). Origin Version 9.1 was used to draw the figures of enzyme activities, and microbial alpha diversity including ACE index, Shannon index, and Chao index.

3. Results

3.1. Neighbour effect index

The NEI values varied with tree species and stand types. *P. asperata* suffered a significant negative effect (NEI = -0.46 ± 0.09, $P = 0.001$) from its neighbor in the monospecific stands. In mixed BP stands, the NEI was a marginally significant positive for *P. asperata* (NEI = 0.17 ± 0.08, $P = 0.076$), while no significant NEI was found for *B. albosinensis* in both monospecific and mixed stands (NEI = -0.10 ± 0.05 for BB stand and NEI = -0.12 ± 0.07 for BP stand, $P > 0.05$).

Table 1
Effects of tree species (S), stand types (ST) and their interactions (S × ST) on soil properties. B and P refer to isolated *Betula albosinensis* and isolated *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively. Different lowercase letters indicate significant differences for *B. albosinensis*, different uppercase letters indicate significant differences for *P. asperata*, at $P < 0.05$. Mean values ± standard errors (n = 5). The significant Bonferroni-corrected *P* values ($P < 0.004$) are in bold. SOC: soil organic carbon concentration (g kg^{-1}); TN: total nitrogen concentration (g kg^{-1}); SAP: soil availability phosphorus concentration (g kg^{-1}); NH 4^{+}-N: soil ammonium concentration (mg kg^{-1}); DON: dissolved organic nitrogen concentration (mg N kg^{-1}); DOC: dissolved organic carbon concentration (mg C kg^{-1}); DOC/DON: ratio of DOC to DON; NO_3^--N: soil nitrate concentration (mg kg^{-1}); MBC: microbial biomass C (mg kg^{-1}); MBN: microbial biomass N (mg kg^{-1}); MBC/MBN: ratio of MBC to MBN.

Variable	Treatment					S		ST		S × ST	
	B	BB	BP	PP	P	*F*	*P*	*F*	*P*	*F*	*P*
pH	5.14 ± 0.16b	5.15 ± 0.29ab	6.02 ± 0.29aA	5.81 ± 0.06A	5.77 ± 0.04A	5.834	0.024	4.25	0.026	1.461	0.252
SOC	69.21 ± 11.46b	97.12 ± 37.64b	240.04 ± 31.70aA	69.20 ± 9.65B	100.57 ± 11.11B	0.003	0.956	25.796	**<0.001**	0.699	0.507
TN	5.14 ± 0.78b	6.07 ± 1.62b	13.59 ± 1.73aA	5.48 ± 0.56B	8.00 ± 0.78B	0.507	0.483	21.944	**<0.001**	1.013	0.378
SAP	17.41 ± 0.33b	18.69 ± 0.63b	24.88 ± 0.54aA	22.86 ± 0.39A	28.63 ± 0.84A	121.264	**<0.001**	25.902	**<0.001**	49.345	**<0.001**
NH_4^+-N	16.05 ± 2.40a	14.14 ± 0.60ab	9.88 ± 0.77bA	7.04 ± 0.98A	7.13 ± 0.78A	29.002	**<0.001**	1.003	0.382	7.532	**0.003**
NO_3^--N	1.20 ± 0.60b	0.89 ± 0.83b	8.99 ± 3.00aA	12.64 ± 3.46A	7.56 ± 2.61A	8.666	0.007	1.689	0.206	2.742	0.085
DON	42.49 ± 3.63a	48.04 ± 1.44a	37.95 ± 4.41aA	34.07 ± 4.83A	44.87 ± 3.97A	1.441	0.242	1.059	0.362	2.512	0.102
DOC	866.01 ± 36.94a	917.19 ± 78.03a	782.47 ± 68.54aB	985.05 ± 23.73A	920.48 ± 18.28AB	0.843	0.368	4.966	0.016	0.218	0.805
DOC/DON	20.85 ± 1.49a	19.33 ± 2.17a	21.31 ± 2.44aA	31.84 ± 5.17A	21.10 ± 1.70A	3.367	0.079	1.641	0.215	3.167	0.060
MBC	491.16 ± 73.35b	641.60 ± 126.46ab	971.36 ± 72.90aA	641.87 ± 87.25A	937.79 ± 174.5A	2.851	0.104	5.136	0.014	2.845	0.078
MBN	141.84 ± 21.79a	157.92 ± 30.84a	234.01 ± 38.94Aa	165.65 ± 19.11A	246.38 ± 39.61A	1.972	0.173	2.457	0.107	1.593	0.224
MBC/MBN	3.48 ± 0.10a	4.07 ± 0.07a	4.48 ± 0.60aA	3.83 ± 0.14A	3.77 ± 0.15A	0.003	0.954	2.885	0.075	0.267	0.768

3.2. Soil characteristics

The SOC, TN, SAP and NH_4^+-N, concentrations varied significantly with the tree species or/and stand types (Table 1, Bonferroni-corrected $P < 0.004$). The SOC and TN concentrations were higher in mixed stands than in the monospecific stands. The concentration of SAP in mixed stands was similar to the monospecific PP stand but higher than in the monospecific BB stand. The SAP and NH_4^+-N concentrations displayed significant differences between isolated B and P (Table 1, Bonferroni-corrected $P < 0.004$). Specifically, SAP concentration was higher in P than B. Furthermore, the interaction between the species and stand types significantly affected the SAP and NH_4^+-N concentrations (Table 1, Bonferroni-corrected $P < 0.004$). No significant difference was observed in the pH, NO_3^--N, DON, MBC, and MBN concentrations and the ratios of DOC/DON and MBC/MBN across tree species and stand types (Table 1). The $GMea_C$ and $GMea_N$ were marginally affected by the stand types (Fig. S2, $P < 0.1$), with mixed stands showing the highest values.

3.3. Soil microbial community diversity and composition

The bacterial and fungal amplicon sequences clustered into 10,327 and 6734 ASVs, respectively. Soil bacterial and fungal alpha diversity was significantly affected only by stand types but not by tree species and their interactions (Fig. 1). The fungal alpha diversity indices, including Ace, Chao, and Shannon, were significantly greater in mixed stands than in the monospecific PP stand (Fig. 1).

Among the five treatments, the dominant bacterial phyla were Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobiota, and Chloroflexi (Fig. 2a). The dominant fungal phyla were Ascomycota, Basidiomycota, and Mortierellomycota (Fig. 2b). The bacterial and fungal genera varied with the tree species and stand type (Fig. S3). The PCoA plots separated based on the tree species and stand types showed that these factors significantly altered the bacterial and fungal community composition (Fig. 3 and Table 2, $P < 0.05$). The bacterial and fungal communities of isolated B were clearly separated from isolated P (Fig. 3 and Table 2, $P < 0.01$). The BB, BP, and PP stands were separated into three groups based on the bacterial and fungal communities (Fig. 3b and d). Specifically, the first two main PCoA components accounted for 53.18 % and 18.46 % of the bacterial community variations (Fig. 3b) and 23.55 % and 15.2 % of the fungal community variations (Fig. 3d), respectively.

3.4. Microbial co-occurring network, keystone taxa, and functional prediction

The microbial networks revealed markedly different microbial community structures for *B. albosinensis* and *P. asperata* under different treatments. The bacterial and fungal networks varied with the tree species and stand types. The effect of the stand types on the negative correlations of bacterial and fungal networks was similar: the mixed BP stands showed higher negative correlations in the bacterial and fungal networks than the isolated monospecific BB and PP stands (Fig. 4 and Table 3). Both the average clustering coefficient and modularity of bacterial (0.920 and 0.837, respectively) and fungal (0.919 and 0.898, respectively) networks were higher in mixed stands than the others (Table 3). Most bacterial and fungal network metrics varied with tree species (Table 3). The bacterial networks of isolated *B. albosinensis* were more complex than *P. asperata*, while more edges were observed in *B. albosinensis* (504 versus 205). Additionally, the average degree of the bacterial and fungal networks was greater in B than in P. Moreover, the negative edges between bacterial or fungal taxa were consistently stronger in *P. asperata* than in *B. albosinensis* (Table 3). The bacterial network structure was more complex than the fungal networks due to higher numbers of edges and nodes (Table 3).

The keystone bacterial and fungal taxa in the networks were different among tree species and stand types (Fig. 4 and Table S3). At the phylum level, Ascomycota, Basidiomycota, and Mortierellaceae were the dominant keystone taxa in fungal networks (Fig. 4), while those in bacterial networks were Acidobacteriota, Proteobacteria, Actinobacteriota, Verrucomicrobiota, Firmicutes, Chloroflexi, Myxococcota, and Bacteroidota (Fig. 4). There are more keystone bacterial taxa than fungal taxa (Table S3). The number of keystone bacterial taxa was higher in the monospecific PP (21) and the mixed BP stands (19) than in the monospecific BB stand (10). The number of keystone fungal taxa was in the following order: BB > PP > BP (Fig. 4 and Table S3). Most keystone fungal taxa belonged to Ascomycota and Basidiomycota, suggesting that these taxa are important for network structure.

FAPROTAX and FUNGuild were used to predict the ecological

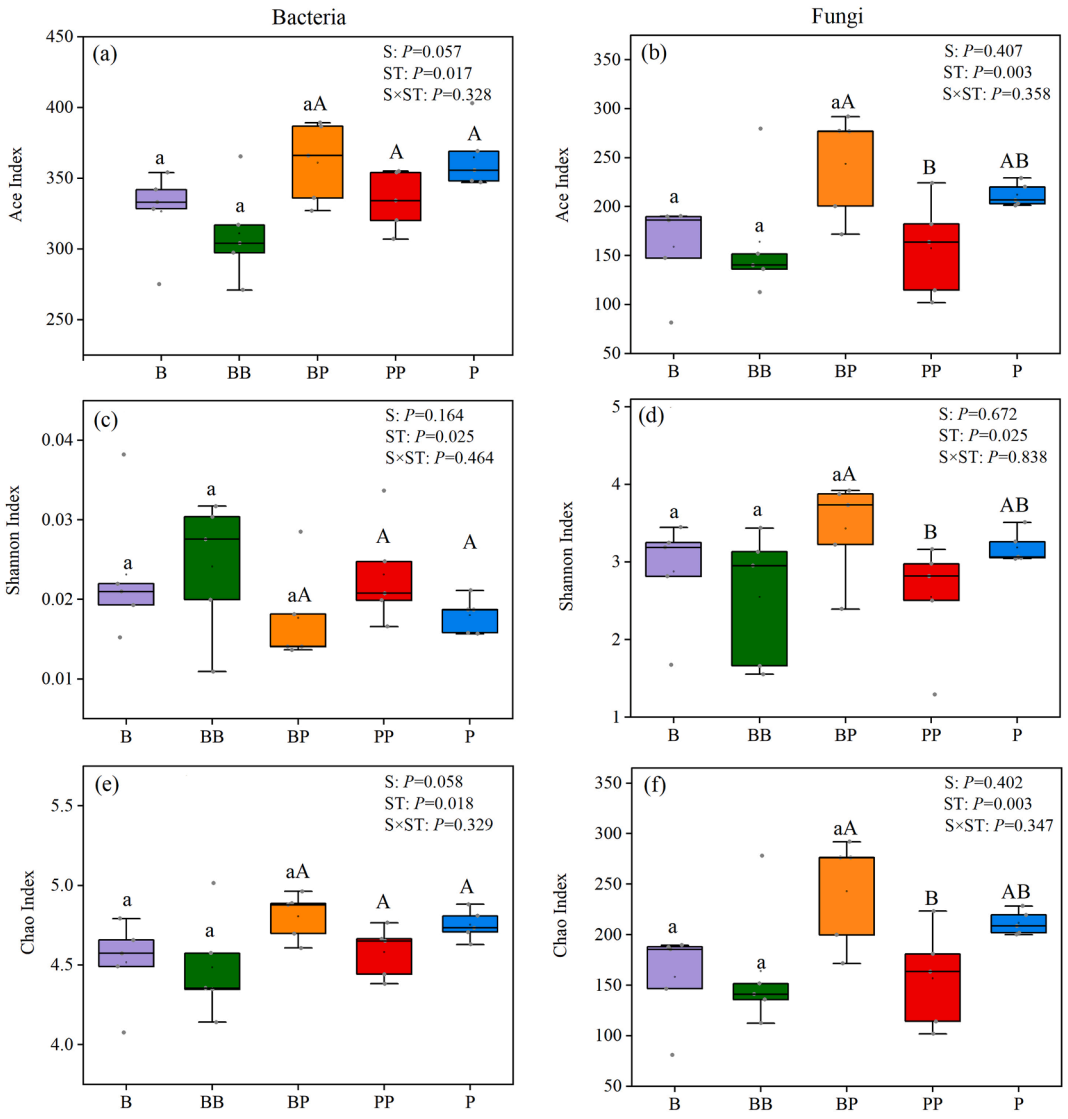

Fig. 1. Alpha diversity index for bacterial and fungal communities. B and P refer to isolated *Betula albosinensis* and *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively. Effects of tree species (S), stand types (ST), and their interactions (S × ST) were shown. Different lowercase letters indicate significant differences for *B. albosinensis*, different uppercase letters indicate significant differences for *P. asperata*, at $P < 0.05$. Mean values ± standard errors (n = 5).

functions of the keystone taxa across the tree species and stand types (Fig. 5). There were clear differences in the ecological functions of the keystone bacterial functional profiles across the monospecific BB and PP, and the mixed BP stands. The keystone bacterial taxa in the mixed BP stands were related to N cycles in soil, including complete, nitrite, and nitrous oxide denitrification, nitrite and nitrate respiration, nitrate reduction, nitrogen respiration, and ureolysis (Fig. 5a). The N cycling functional assignments mainly were significantly affected by the tree species and stand type (Fig. 5c, $P < 0.05$). The proportion of bacterial taxa responsible for N cycling in the mixed BP stands was higher than in monospecific BB and PP. *B. albosinensis* exhibited higher cellulolysis and lower ureolysis functional assignment than *P. asperata* (Fig. 5a, $P < 0.05$). Cellulolysis, ureolysis, and aromatic compound degradation, chemoheterotrophy functional assignments were affected by tree species. Aromatic compound degradation, chemoheterotrophy, and ureolysis were significantly higher in P than in B (Fig. 5c, $P < 0.05$). The keystone fungal functional profiles differed among the tree species and stand types (Fig. 5b and d, $P < 0.05$). The wood saprotroph and ectomycorrhizal functional assignments were highest in the mixed BP stands, followed by the monospecific PP stands (Fig. 5d, $P < 0.05$) and the BB stand. However, the ectomycorrhizal assignments were lower in the PP stands than in the BB stands (Fig. 5d, $P < 0.05$).

3.5. Relationship of soil characteristics and microbial keystone taxa

Redundancy analysis (RDA) showed that the first two axes explained 49.91 % and 29.38 % of the variance in keystone bacterial and fungal community compositions influenced by soil characteristics (Fig. 6). The concentrations of SOC, TN, SAP, NH_4^+-N, and pH ($P < 0.05$) primarily affected the bacterial community, while TN, SAP, NH_4^+-N concentrations, $GMea_N$, and pH were the five most influential factors ($P < 0.05$) contributing to the changes in the fungal community. Among the five treatments, the soil characteristics mainly affect the keystone bacterial and fungal taxa. The keystone bacterial taxa are more strongly associated with the soil characteristics than the keystone fungal taxa (Fig. 7). Soil TN, SOC, SAP concentrations, and pH were strong predictors of dissimilarities for most keystone bacterial taxa (Fig. 7a). The specific soil characteristics that are correlated to the relative abundance of the corresponding bacterial taxa is shown below: 1) TN concentration: *Acidibacter*, *Roseiarcus*, *Conexibacter*. 2) SOC concentration: *Candidatus*

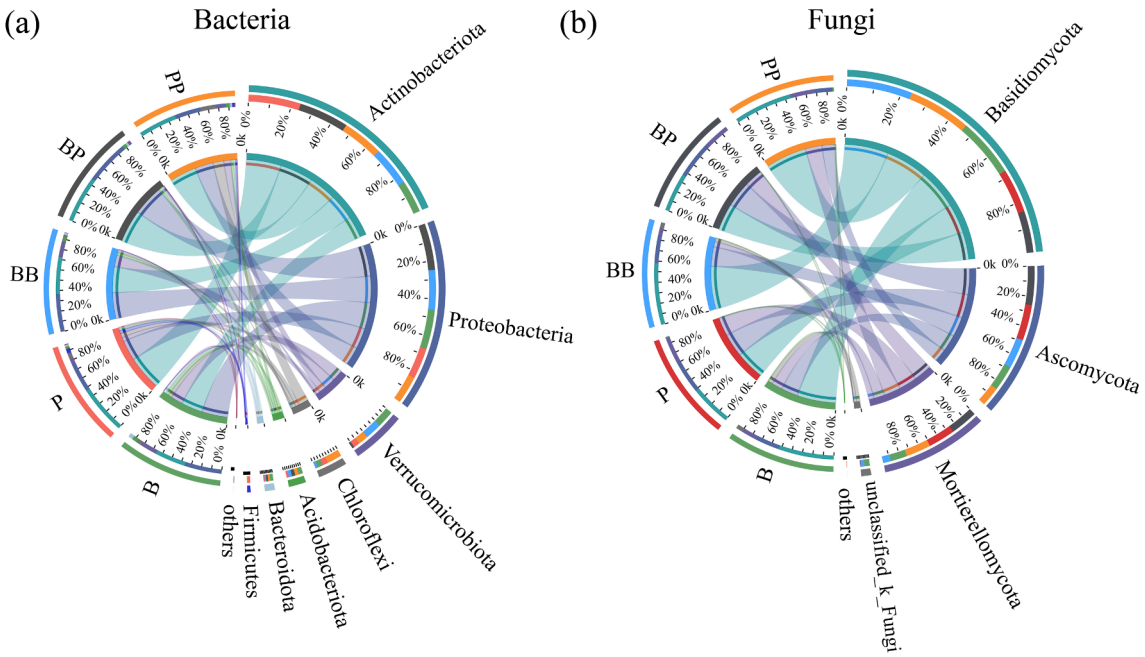

Fig. 2. Taxonomic composition of soil bacterial (a) and fungal (b) communities at the phylum level. B and P refer to isolated *Betula albosinensis* and *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively.

Xiphinematobacter, *Nakamurella*, *Nocardiodes*, *Rhodoplanes*. 3) SAP concentration: *Candidatus soilbacter*. 4) pH: *Acidothermus*, *Nakamurella*. For the keystone fungal taxa, the soil concentrations of TN, SAP, SOC, and pH were predictive for some keystone fungal taxa (Fig. 7b) as follows: 1) Soil TN, SAP, SOC concentrations and pH: *Holtermanniella*. 2) SOC, SAP, and TN concentrations: *Hebeloma*. 3) SOC concentration: *Hydnodontaceae*, *Hebeloma*, *Holtermanniella*. 4) TN concentration: *Hebeloma*, *Holtermanniella*, *Trichophaea*, *Thelephoraceae*.

4. Discussion

Soil microbial community and soil characteristics differed as a function of stand types and tree species. In mixed stands, positive interspecific interactions between *B. albosinensis* and *P. asperata* resulted in better growth of *P. asperata* than in monospecific stands of *P. asperata*. The probable explanation was that the mixed BP stands had better soil nutrient availability, and higher enzyme activities, which had more stable microbial co-occurrence networks compared with monospecific stands. The current study provides novel insights for understanding the enhanced ecosystem function of mixed stands.

4.1. Soil characteristics

Several soil properties differed among stands. SOC, TN, SAP, and MBC concentrations were higher in mixed stands than in the monospecific stands (Table 1). These results agree with other studies demonstrating that mixed stands typically increase soil nutrient availability (Chodak et al., 2022; Likulunga et al., 2021) and partially support our first hypothesis. Generally, mixed stands can increase soil nutrient availability and improve soil nutrient cycle (Liu et al., 2017). Moreover, C and *N*-related enzyme activities, including $GMea_C$ and $GMea_N$, are higher in mixed stands than in monospecific stands (Fig. S2 and Table S2), consistent with previous studies (Zhang et al., 2021a) showing that mixed stand had higher nutrient resources and microbial activities in soil.

4.2. Microbial community diversity and composition

Soil bacterial and fungal community diversity and composition are known to be significantly different among stand types (Hartman et al., 2018; Likulunga et al., 2021; Zhang et al., 2021c). In this study, mixed BP stands had significant higher bacterial and fungal diversity than monospecific stands (Fig. 1), supporting our first hypothesis. Soil nutrient availability are closely related to soil bacterial and fungal diversity (Pereira et al., 2019; Rachid et al., 2013). The bacterial and fungal diversity were positively correlated with TN, SOC, SAP, and NH_4^+-N (Fig. S4). The mixed BP stands had higher soil nutrients than monospecific stands, with high SOC, TN, and SAP (Table 1). Therefore, our results suggest that mixed BP stands provided microhabitats conducive to microbial activity and growth by facilitating soil nutrients, which increased soil bacterial and fungal diversity.

In this study, soil bacterial and fungal communities were separated by tree species and stand types (Fig. 3 and Table 2). Our results demonstrate that Proteobacteria, Actinobacteriota, Acidobacteria, Verrucomicrobiota, and Chloroflexi were the dominant bacterial phyla in the three stands (Fig. 2). The relative abundance of Proteobacteria and Actinobacteria in mixed BP stands was intermediate between the monospecific BB and PP stands, while a lower relative abundance of Acidobacteria was observed in mixed BP stands (Fig. 2). Proteobacteria can proliferate in soil with sufficiently labile C substrates (Zhang et al., 2016), while Actinobacteria can decompose recalcitrant organic matter by penetrating their hypha and producing extracellular enzymes (Dang et al., 2017). Acidobacteria prefers nutrient-poor (low-C sources) soil environments (Fierer et al., 2007). Compared with monospecific stands, interspecific plant-plant interaction between coniferous species (*P. asperata*) and broadleaved species (*B. albosinensis*) provide higher SOC, TN, and SAP concentrations (Table 1) in mixed BP stands, which is are suitable for Proteobacteria, Actinobacteria, and Acidobacteria growth.

For soil fungal community, Basidiomycota, Ascomycota, and Morticrellomycota were the predominant phyla in three stand types (Fig. 2). The abundance of Ascomycota and Morticrellomycota were higher but the abundance of Basidiomycota was lower in mixed BP stands compared with monospecific stands. Ascomycota and

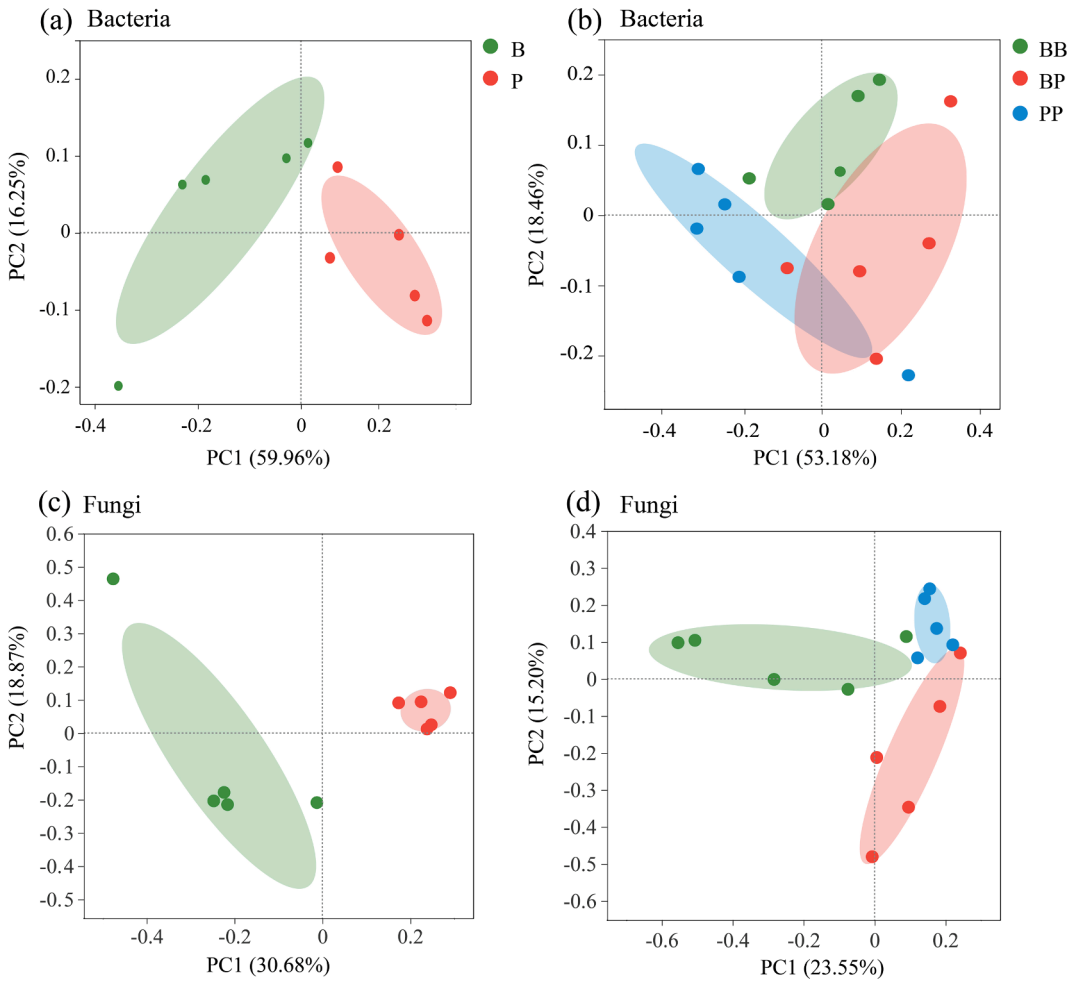

Fig. 3. Principal coordinate analysis (PCoA) ordination of bacterial (a) and fungal (c) community composition in different tree species, and bacterial (b) and fungal (d) community composition in different stand types, based on Bray-Curtis distance. B and P refer to isolated *Betula albosinensis* and *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively.

Table 2
PERMANOVA analysis of the factors affecting bacterial and fungal communities based on Bray-Curtis distance matrix. B and P refer to isolated *Betula albosinensis* and isolated *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively. The effects of tree species were analyzed using B and P stands, and the effects of stand types were analyzed using BB, PP and BP stands, respectively. The significant P values ($P < 0.05$) are in bold.

Taxonomy	Dissimilarity Group	ANOSIM *F*	R	*P*
Bacteria	**Tree species**	5.830	0.422	**0.009**
	Stand types	3.310	0.356	**0.012**
	BB vs BP	3.892	0.327	**0.031**
	BB vs PP	3.240	0.288	**0.028**
	PP vs BP	2.650	0.249	**0.035**
Fungi	**Tree species**	3.103	0.279	**0.009**
	Stand types	2.346	0.281	**0.002**
	BB vs BP	2.580	0.244	**0.022**
	BB vs PP	2.432	0.233	**0.010**
	PP vs BP	1.216	0.132	0.245

R statistics represent difference of mean ranks between the two groups.

Morticrellomycota participate in many degradation and transformation processes, such as decomposition of lignin and cellulose and mineralization and utilization of phosphorus in soil (Li et al., 2021a). Mixed BP stands have higher nutrient concentrations and diversity of fungal community (Asplund et al., 2018; Pereira et al., 2019), which may explain the relative abundance of Ascomycota and Morticrellomycota increased in mixed BP stands. Fungal communities differed significantly between mixed BP stands and monospecific BB stands, and the monospecific PP stands had similar fungal communities to the mixed BP stands (Table 2). Soil pH was an important driver for the separation of fungal communities in soil (Fierer et al., 2007). Compared to monospecific BB stands, monospecific PP stands and mixed BP stands have similar soil pH microenvironments (Table 1), which may shape similar fungal communities in soil.

4.3. Co-occurrence networks and keystone taxa

Microbial co-occurrence networks often vary with soil microbial community composition under different stand types (Hartman et al., 2018; Zheng et al., 2021). In this study, stand types affected the soil bacterial and fungal networks, including the number of edges and nodes, diameter, and graph density of the co-occurrence network (Fig. 4 and Table 3), which was consistent with Lan et al. (2022). Bacterial and fungal communities maintain network stability through different strategies (Banerjee et al., 2018b; Wang et al., 2020). Mixed BP stands increased the negative links in bacterial taxa networks compared with the monospecific stands (Fig. 4 and Table 3), indicating that increased competitive interactions in bacterial networks. In contrast, fungal networks had more positive links than bacterial networks (Fig. 4 and Table 3), suggesting that more cooperation between different fungal taxa in soil. Higher soil nutrient concentrations in mixed BP stands

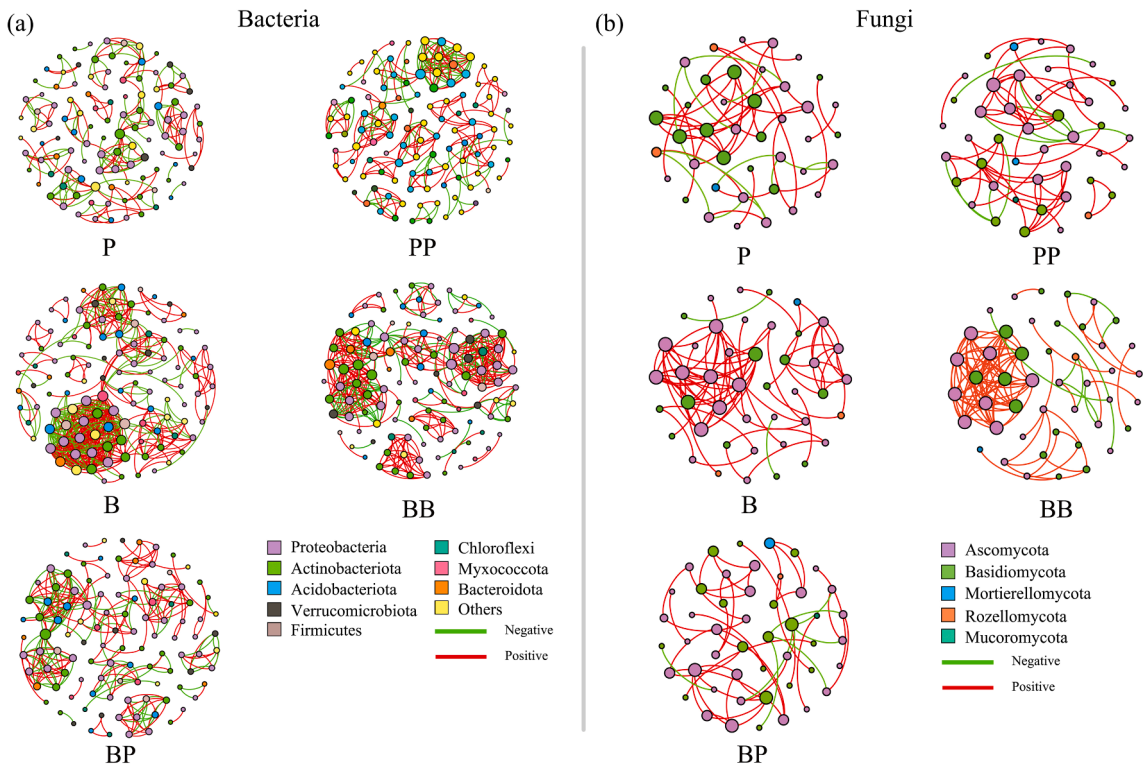

Fig. 4. Network co-occurrence analysis of bacterial (a) and fungal (b) communities basing on the phylum level. Connection stand for strong (Spearman's $\rho > 0.8$) and significant ($P < 0.01$) correlations. For each panel, the size of each node is proportional to the number of connections (i.e. degree). Red edges indicate positive interactions, and green edges indicate negative interactions. The co-occurrence networks are colored by phylum. B and P refer to isolated *Betula albosinensis* and *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively.

Table 3
Topological properties of bacterial and fungal networks. B and P refer to isolated *Betula albosinensis* and isolated *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively.

Network metrics	**Bacterial network**					**Fungal network**				
	B	P	BB	BP	PP	B	P	BB	BP	PP
Number of phylotypes involved	450	450	450	450	450	300	300	300	300	300
Number of nodes	117	111	117	110	112	43	41	46	48	45
Number of edges	504	205	401	259	263	82	50	107	50	69
Positive edges (%)	64.57	56.61	70.12	57.67	63.50	97.57	76.00	92.52	84.00	84.52
Negative edges (%)	35.43	43.39	29.88	42.33	36.50	2.43	24.00	7.48	16.00	15.48
Modularity	0.615	0.880	0.722	0.837	0.961	0.653	0.840	0.456	0.898	0.761
Network diameter	12	8	12	7	5	1.21	3	4	2	5
Average path length	3.521	3.409	3.496	2.145	1.532	1.050	1.140	1.113	1.138	1.606
Average degree	8.615	3.694	6.855	4.709	4.696	3.814	2.439	4.652	2.083	3.067
Average clustering coefficient	0.867	0.910	0.884	0.920	0.890	0.989	0.927	0.925	0.919	0.907
Graph density	0.074	0.034	0.059	0.043	0.042	0.091	0.061	0.103	0.044	0.070

provide more resources (i.e., SOC and TN) (Table 1) and ecological niches for certain microbial taxa growth, which increases soil microbial diversity and thus enhances the stability of bacterial and fungal networks. Furthermore, soil bacterial and fungal networks are influenced by tree species (Banerjee et al., 2018b; Lan et al., 2022). Our results showed that *B. albosinensis* and *P. asperata* have different bacterial and fungal networks. *B. albosinensis* had more positive links than *P. asperata* (Fig. 4 and Table 3), indicating that *B. albosinensis* had more potential cooperation interactions for bacterial and fungal taxa compared to *P. asperata*. This can be explained by the specificity of tree species, *B. albosinensis* stand has high litter decomposition rate than *P. asperata* stand (Xu et al., 2015; Zhuang et al., 2018), suggesting bacterial and fungal taxa with *B. albosinensis* may adopt mutually cooperative strategy to better exploit soil resources.

Keystone taxa contribute to the soil biogeochemical process by regulating nutrient cycling and transformation (Banerjee et al., 2018b; Shi et al., 2020). This study found that the stand types shift the keystone taxa in the soil bacterial and fungal communities. In mixed BP stands, Ascomycota, Basidiomycota, Actinobacteriota, Acidobacteriota, Proteobacteria, Chloroflexi, and Bacteroidota were the keystone microbial taxa (Table S3). The variation patterns of soil bacterial and fungal communities at the genera level across stand types were generally consistent with phylum level (Fig. 3 and S3). At the genus level, there are more keystone bacterial taxa but fewer keystone fungal taxa in mixed BP stands than in monospecific stands (Table S3). Most keystone bacterial and fungal taxa are related to soil carbon and nitrogen metabolism, decomposing organic compounds, soil fertility, and pathogenicity. Among the keystone bacterial genera, *Rhodoplanes* synergistically interact with other bacteria taxa and participate in C and energy flows; *Acidothermus* are cellulolytic bacteria with endocellulase activity; *Candidatus Xiphinematobacter* participates in soil nutrient turnover (Zhang et al., 2020); *Nakamurella* and *Nocardioides,* belonging to Actinobacteria,

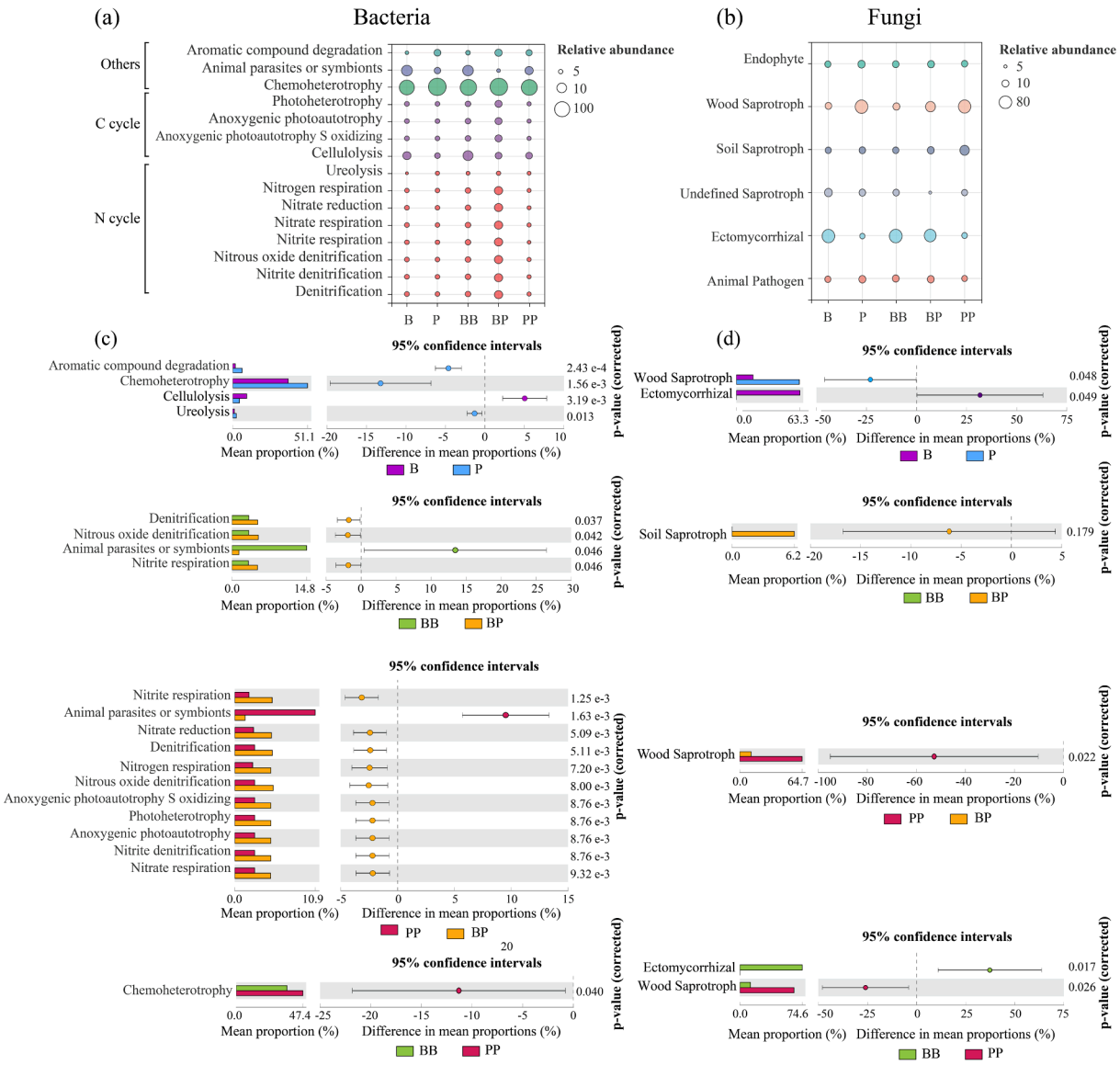

Fig. 5. Variation of keystone taxa for bacterial (a) and fungal (b) ecological functions for different species and stand types based on FAPROTAX and FUNGuild, respectively. Keystone of bacterial (c) and fungal (d) taxa annotated with ecological function that exhibited significant differences ($P < 0.05$) under different species and stand types. The ecological function with significant differences between the groups and their proportions were shown on the left. B and P refer to isolated *Betula albosinensis* and isolated *Picea asperata*, respectively. BB, PP and BP refer to monospecific stand of *B. albosinensis*, *P. asperata*, and the mixed stand of *B. albosinensis* with *P. asperata*, respectively.

contribute to soil nutrient mineralization and cycling (Buresova et al., 2019). Among the keystone fungal genera, *Holtermanniella* can assimilate C from various sources and have extracellular enzymes (Mozzachiodi et al., 2022); *Thelonectria* is classified as Sordariomycetes, which are plant pathogens (Koberl et al., 2020); and *Trichophaea* facilitates nutrients acquisition (Li et al., 2021b). The enrichment of these keystone soil bacterial and fungal taxa with nutrient functions might affect microbial networks (Morriën et al., 2017), change nutrient cycling and transformation in soil, and finally, promote plants nutrients absorption and utilization in mixed BP stands.

4.4. *Relationship of soil characteristics, microbial diversity and networks, and keystone taxa and their functions*

Previous studies showed that stand types could altered keystone bacterial and fungal taxa and its functions (Zhang et al., 2021b). In our study, tree species and stand type significantly influenced keystone bacterial and fungal taxa and their ecological functions (Fig. 5), and these taxa closely associated with soil nutrient concentrations and soil pH (Fig. 7). The relative abundance of bacterial taxa with ecological functions including chemoheterotrophy, aromatic compound degradation, and ureolysis were higher in *P. asperata* than in *B. albosinensis* (Fig. 5a). Chemoheterotrophy and aromatic compound degradation are important pathways for C flow in microbial communities. The bacterial taxa involved in this process are important for organic matter cycling in the ecosystems (Gu et al., 2019). These results showed that the relative abundance of keystone bacterial taxa related to C and N cycling was higher in mixed BP stands than in monospecific stands (Fig. 5), resulting in a better nutrient supply in mixed stands (Fig. 5 and Table 1). Additionally, a higher abundance of keystone bacterial taxa related to N and C cycling might be due to the high demand for C and N sources between trees and microbes (Chodak et al., 2022; Siefert et al., 2018).

For fungal taxa, the relative abundance of ectomycorrhizal, soil saprotroph, and wood saprotroph in mixed BP stands was intermediate that in monospecific BB and PP stands (Fig. 5), which might be related to the distinct colonization strategies of fungal community. Soil

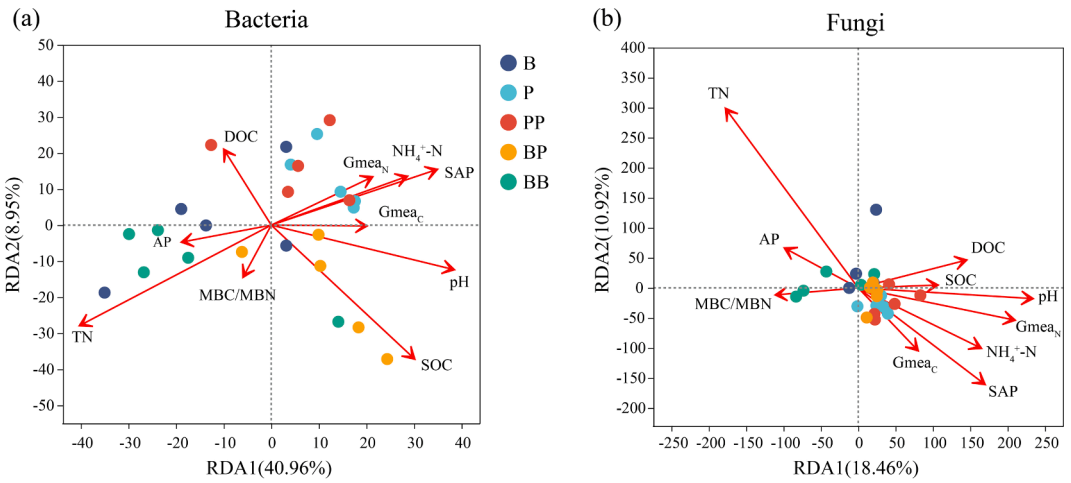

Fig. 6. Redundancy analysis illustrating relationships between soil bacterial community (a), fungal community (b) and soil characteristics across tree species and stand types. SOC: soil organic carbon concentration (g kg^{-1}); SAP: soil availability phosphorus concentration (g kg^{-1}); TN: total nitrogen concentration (g kg^{-1}); NH_4^+-N: soil ammonium concentration (mg kg^{-1}); DOC: dissolved organic carbon concentration (mg C kg^{-1}); MBC/MBN: ratio of MBC to MBN; AP: acid phosphatase (nmol/h g^{-1}); $GMea_C$: geometric mean of the assayed carbon enzyme activities; $GMea_N$: geometric mean of the assayed nitrogen enzyme activities.

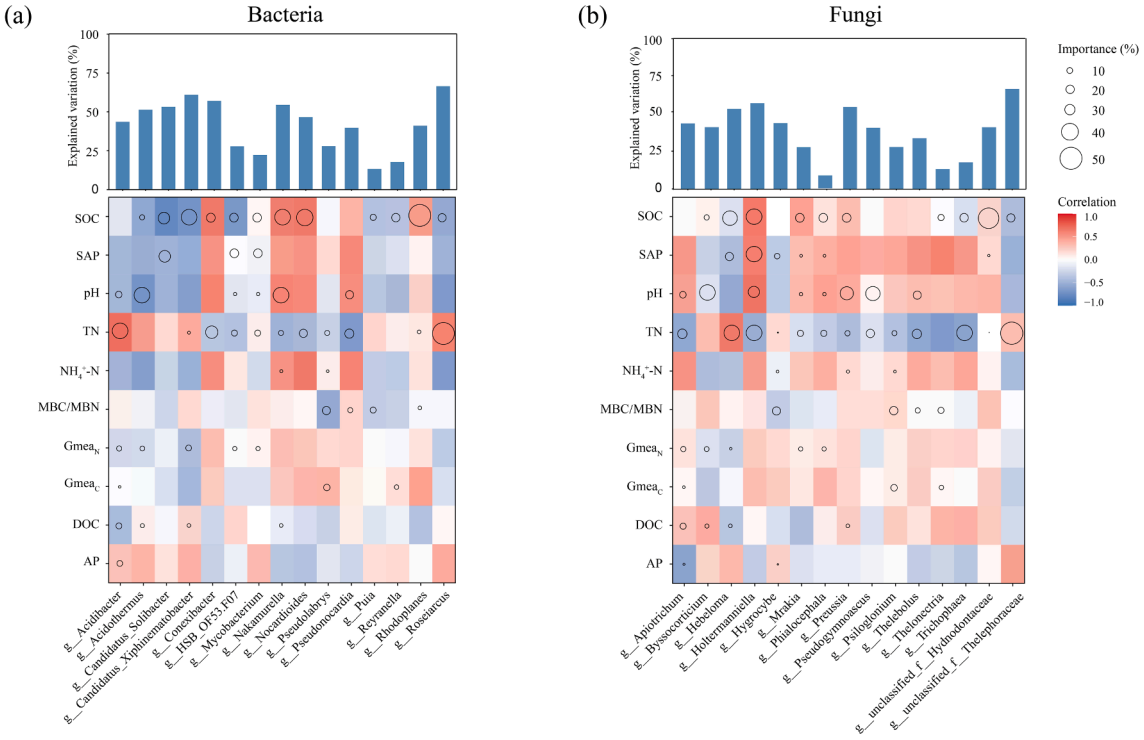

Fig. 7. Contributions of soil characteristics to the differences in the top 15 bacterial (a) and fungal (b) keystone taxa based on correlation and multiple regression model. Circle size represents the variable importance, that is, proportion of explained variability calculated via multiple regression modeling and variance decomposition analysis. Colors represent Spearman correlations. Continuous and dashed arrows indicate significant and nonsignificant relationships, respectively. SOC: soil organic carbon concentration (g kg^{-1}); SAP: soil availability phosphorus concentration (g kg^{-1}); TN: total nitrogen concentration (g kg^{-1}); NH_4^+-N: soil ammonium concentration (mg kg^{-1}); DOC: dissolved organic carbon concentration (mg C kg^{-1}); MBC/MBN: ratio of MBC to MBN; AP: acid phosphatase (nmol/h g^{-1}); $GMea_C$: geometric mean of the assayed carbon enzyme activities; $GMea_N$: geometric mean of the assayed nitrogen enzyme activities.

availability of carbon resources and substrate quality affect certain fungal taxa (Xu et al., 2021), such as the abundance of ectomycorrhizal fungi and saprotrophs fungi were significantly positively correlated with soil nutrient availability. Competition between saprophytic fungi and ectomycorrhizal fungi for soil resources is influenced by tree species (Asplund et al., 2018). Soil planted coniferous tree species contains high cellulose and low lignin contents (Likulunga et al., 2021), which favour the decomposer communities (Asplund et al., 2018). This may explain the lower relative abundance of ectomycorrhizal fungi and higher relative abundance of saprophytic fungi observed in PP than BB stands and mixed stands (Fig. 6). The increase of ectomycorrhizal fungi and saprotrophs fungi relative abundances were accompanied by decreased soil TN and SOC (Table 1 and Fig. 5), suggesting that monospecific stands may adapt to the low available nutrients by different fungal taxa. Thus, mixed BP stands can modulate the contribution of keystone soil microbial taxa to microbiome stability and facilitated soil nutrients and microenvironment, which could then be improved forest ecosystem services.

In this study, we focused on the changes in soil microbial communities and soil nutrients under plant-plant interactions, hence other soil properties were not considered. Except for soil nutrient properties, soil physical properties, such as soil hydrology, soil temperature, soil structure and so on, also play important roles in soil microbial community composition (Augusto et al., 2015; Lorenz and Thiele-Bruhn., 2019). As for sampling, the soil was sampled in August, the mid-growth season for trees. Some studies showed that soil microbial communities are most active and vigorous, and have a closer connection during this period than other periods (Ji et al., 2021; Xie and Yin, 2022). Although there are some limitations of one-time samplings indeed, we sampled soil only in August of one unique year similar with Garau et al. (2019) and Kooch and Noghre (2020). To reveal the underlying mechanisms of mixed stand maintain high forest productivity and soil nutrient cycling, more possible influence factors and multiple sampling need be considered in further studies.

5. Conclusions

The tree species and stand types affected the soil characteristics, microbial community composition and diversity, and the keystone microbial taxa. Mixed BP stands significantly improved the soil enzyme activity ($GMea_C$ and $GMea_N$) and nutrient availability (i.e., SOC, TN), increased the microbial community diversity compared with the monospecific PP, and BB stands. Mixed stands enhanced the soil and fungal network stability and reduced the bacterial and fungal network complexity. Moreover, these stands enriched the keystone bacterial and fungal taxa, which are involved in decomposing complex organic matter and transforming nutrients. This, in turn, improves nutrient availability, and promotes plant growth in mixed stands. In conclusion, tree species and stand types can directly or indirectly influence plant growth by impacting soil microbial diversity, networks and keystone taxa, and soil characteristics. This study highlights the importance of sustaining soil microbial taxa and ecological function in the soil nutrient cycling processes for better forest management.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.120851.

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