

Changes in Soil Microbial Community Structure and Functional Genes Following with Different Functional Traits Species Afforestation

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Abstract

Aim Soil microbial community structure and functional genes are critical to the cycling of carbon and nutrients in forest soils. As afforestation practices increasingly promote different functional traits tree species, it becomes critical to understand how they influences soil microbial community structures and functional genes, which directly influence soil biogeochemical processes.

Methods We used fungi ITS and bacteria 16S rDNA to investigate soil microbial communities and functional genes in three monoculture plantations consisting of a non-native evergreen conifer (*Pinus sibirica*), a native deciduous conifer (*Larix gmelinii*), and a native deciduous angiosperm (*Betula platyphylla*) to compare with two 1:1 mixed-species plantations (*P. sibirica* and *L. gmelinii*, *P. sibirica* and *B. platyphylla*).

Results The fungal community structure of the conifer-angiosperm mixed plantation was similar to that of the non-native evergreen conifer, and the bacterial community structure was similar to that of the angiosperm monoculture plantation. Fungal communities were strongly related to tree species, but bacteria communities were strongly related to soil nitrogen. Microbial co-occurrence patterns varied according to plantation types and altered soil nutrient cycling. Microbial communities in forest plantations of conifer-angiosperm mixed plantation contribute to soil nitrogen fixation and coniferous mixed plantation contribute to soil carbon fixation.

Conclusions Our results provide a comparative study of the soil microbial ecology in afforestation of different functional traits species. This knowledge enhances the understanding of the relative control of soil microbial community structure.

Key Message

Functional traits species afforestation influences microbial community and functional genes.

Introduction

Soil microorganisms play important roles in global biogeochemical cycles, but their communities and functions can be negatively influenced by environmental changes, such as climate warming, nitrogen deposition, and the loss of biodiversity (IPCC 2007; Vitousek et al. 1997). Understanding the factors controlling microbial community structure and their functions can potentially mitigate consequences of ecological disturbances (Fierer 2017) or inform strategies for forest management to influence ecosystem process (Maron et al. 2011). Recent methodological developments have facilitated determination of soil microbial diversity, which has provided a more comprehensive understanding of factors controlling microbial community structure (Tyson et al. 2004). While it is clear that tree species and a variety of abiotic factors can have a large influence on soil microbial community structure (Prescott and Grayston 2013), general patterns of how tree functional characteristics (e.g., phyla, leaf habit) influence forest soil microbial community structure and C and N cycling remain relatively unexplored (Dawud et al. 2017).

Indeed, general principles of microbial community assembly may be more difficult to establish than previously realized because fungal and bacterial components may respond differently to biotic and abiotic factors, including critical interactions between the components themselves.

Fungal community structure appears to be largely determined by the dominant tree species. For example, fungal species abundance in conifer and broad-leaved forest in Estonia increased with tree species abundance (Tedersoo et al. 2016). Leff et al. (2018) and Chen et al. (2017) suggested that certain fungal populations were strongly associated with individual plant species. However, the primary control on fungal community structure is not always clear and can be confounded by other factors. For example, both tree and fungal diversities were low in sandy soil and relatively high in rich clay soil in the western Amazon (Peay et al. 2013). Overall, our understanding of factors influencing soil fungal community structure remains insufficient (Whittaker 2006), especially compared to our understanding of factors influencing bacterial community structure.

Bacterial community structure appears to be determined largely by abiotic factors. Recent studies have documented soil pH (Lauber et al. 2009), aridity (Wang et al. 2015), and geographical factors controlling bacterial community structure (Dunbar et al. 2002; Hanson et al. 2012; Staley and Anna-Louise 2003; Tringe et al. 2005). Across large spatial scales, bacterial communities are strongly influenced by soil pH (Bahram et al. 2018), soil nitrogen and carbon (Trivedi et al. 2016) but they do not appear to be strongly influenced by plant species (Walther et al. 2002). The bacterial community structures influenced by the environment, may be determined by the preference of the bacterial community for niches with higher nutrient contents and organic matter quality (Baldrian et al, 2012). Acidobacteria represented 20% of all bacteria and their relative abundance in acidic forest soil rich in organic matter exceeded 60% (Lauber et al 2009). An increase in studies examining spatial microbial ecology has indicated that ecological processes affecting bacterial communities are largely controlled by abiotic factors (Martiny et al. 2006). For example, pH has been proved to be the most important driver of bacterial community composition in several studies on forest soils. since bacteria inhabit small environments, transient changes in some physicochemical factors (e.g., soil moisture, temperature) affect bacterial dispersal and thus bacterial community structure (Llado et al. 2017; Prescott and Vesterdal 2013).

Despite substantial evidence that fungal community structure is largely influenced by biotic factors and that bacterial community structure is largely influenced by abiotic factors, the interaction between fungi and bacteria can also influence the overall microbial community structure (Landi et al. 2018). For example, fungi release acidic substances in the process of decomposition, and only those bacteria that can grow and utilize C in fungal filaments persist in the microbial community (Folman et al. 2008; Rinta-Kanto et al. 2016; Valaskova et al. 2009). Changes in functional traits of vegetation accompany by changes in microclimate (shading, protection and transpiration of soil water), litter (both aboveground and roots) and production of root exudate may also influence interactions between fungi and bacteria and result in different community structures (Prescott and Grayston 2013).

Network analysis can be a powerful tool for understanding interactions among the components of microbial communities (Barberan et al. 2012). Briefly, network analysis provides insight into positive or negative correlations among community components (Faust and Raes 2012), network connectedness (degree) and complexity (clustering coefficient), and can also identify potential 'keystone' taxa in communities (Banerjee et al. 2016). Network analysis may also reveal potential implications of the microbial community on ecosystem processes. For example, changes in network structure could lead to an alteration in soil nutrient cycling (Tylianakis et al. 2010). The microbial network structure can indicate decomposition, denitrification and is more sensitive. Therefore, microbial community structure in co-occurrence network can be used as an indicator of ecosystem functional response (Karimi et al. 2017). Studies have shown that a less complex microbial network releases less CO₂ (Blouin et al. 2015). Quantifying the changes in soil ecosystem function due to network can be achieved through microbial functional genes.

Here we used a combination of monoculture and mixed-species stands, representing a range of functional types (i.e., evergreen conifer, deciduous conifer, and deciduous angiosperm), to explore how tree species, soil characteristics, and the interaction of microbial community components influence the microbiome. This approach allows us to separate the influence of different tree species and soil characteristics to explore potential assembly mechanisms that determine soil fungal and bacterial community structure. We hypothesized that i) tree species (i.e., a biotic factor), exerts the strongest control on fungal community assemblage, whereas soil characteristics (i.e., abiotic factors), exert the strongest controls on bacterial community assemblage. ii) Microbial community under mixed-species plantations would form novel networks compared to that under monocultures. iii) Mixed-species plantations would alter the microbial community functional genes in abundance.

Materials And Methods

Study sites and experimental design

This study site was located in northern Greater Khingan Range, Inner Mongolia, China (51° 44' 46"N, 121° 44' 46"E), the region in the cold temperate zone, mean annual air temperature and precipitation were -5.31°C and 437.4mm. Due to the cold climate, the ecosystem exhibits low tree diversity with only a few tree species (e. g. *Larix gmelinii*, *Pinus sylvestris*, *Picea koraiensis* Nakai, *Betula platyphylla*). *Pinus sibirica* has been used for afforestation in the area since 2000 for its high economic value.

Our study site contains several 20-yr-old monoculture and mixed-species plantations with no anthropogenic disturbance (i.e., harvesting, fire, etc) in the last 5 year. Monoculture plantations comprised of an evergreen conifer (*Pinus sibirica*, P), a deciduous conifer (*Larix gmelinii*, L), and a deciduous angiosperm (*Betula platyphylla*, B). The mixed-species plantations included *P. sibirica* mixed with *L. gmelinii* (PL), and *P. sibirica* mixed with *B. platyphylla* (PB). Within each of the five plantation types described above, we established three 120 m² (30 m×40 m) plots and 200m buffer zone between them, for a total of 15 plots.

Although the plantations were established on similar soil in 2000, we expected that two decades of forest growth and different above- and belowground inputs from different species and their mixtures would have changed soil characteristics (Laganière et al. 2010, Halina et al. 2019) and these characteristics may influence the soil microbial community.

Determining Soil Physicochemical Parameters

In each plot, soil was sampled in the upper 0–10 cm soil layers in 30 randomly selected locations in June 2018. After visible root and plant residues were removed, the 30 samples were combined to form a single composite sample from each plot, and then divided into two subsamples. One sub-sample was air-dried and sieved through a 2-mm mesh for chemical analysis and the other sub-sample was stored in an ice-box, transferred to the laboratory, and stored at -80 °C for DNA extraction. Soil pH was measured using a pH meter (1:2.5w/v). Soil organic carbon (SOC) and total nitrogen (TN) were determined using an Elemental Analyzer (Multi N/C 2100s, Analytik Jena). Total phosphorus was determined by colorimetry, using the ammonium molybdate method after treatment with H₂SO₄ -H₂O₂ (Murphy and Riley 1958).

DNA extraction and sequencing

DNA was extracted from 5 g freeze-dried soil using the MoBIO PowerSoil DNA Isolation Kit (Carlsbad, CA). The V3V4 region of bacterial 16S rRNA was amplified using primers 515F and 806R (Caporaso et al. 2012); for fungi, the fungal internal transcribed spacer (ITS) region was amplified using primer ITS3-F GCATCGATGAAGAACGCAGC and ITS4-R TCCTCCGCTTATTGATATGC (Prober et al. 2015; Qiao et al. 2016). PCR was conducted on 20 ng/ul of

template DNA employing an initial denaturation of 5 min at 94°C, followed by (30

for 16S and 30 cycles for ITS) 30 s at 94°C, 30 s at 52°C and 30 s at 72°C; followed by 10 min final elongations at 72°C.

Amplicon sizes were determined using an Agilent Bioanalyzer 2100 system (~550bp: 16S and ~325-425bp: ITS) and libraries were sequenced on an IlluminaHiSeq2500 platform and 250 bp paired-end reads were generated.

Bioinformatic and statistical analyses of sequencing data

Quality control was performed on paired-end raw reads using Trimmomatic (V 0.33). Clean reads were joined using FLASH (V 1.2.11) and quality control was performed using Mothur (V 1.35.1). Chimeras were removed with Usearch (V10). Resulting sequences were clustered into Operational Taxonomic Units (OTU) at 97% identity. Species annotation information was obtained by comparing representative sequences of each OTU with the Silva and UNITE databases. Fungi functional genes were identified based on FunGuild and the C and N cycling genes were identified using the KEGG database.

Data Analysis and Statistics

We performed analysis of similarities (ANOSIM) to test the effect of plantation type on fungal and bacterial communities. The structure of the microbial community was analyzed by non-metric multidimensional scaling (NMDS) based on Bray with the relative abundance of the dominant species at genes level. We analyzed relative abundance >1% phylum in fungal and bacterial communities and variation in species abundance using one-way ANOVA with $\alpha = 0.05$ and Fisher's LSD to perform mean comparisons. Redundancy analysis (RDA) identified relationships between environmental variables and taxon. All statistical analysis were conducted in "vegan" (Oksanen et al. 2018) and "ggplot2" (Wickham 2016) packages of R software (Core Team 2019).

Microbial co-occurrence networks of microbial communities in mixed-species plantations were analyzed following the method of Wu et al. (Wu et al. 2019). Briefly, we combined P, L, and B represent the monoculture plantation (P-L-B); P, L, and PL to represent coniferous mixed forest (P-L-PL); P, B, and PB to represent mixed stands of coniferous and broad-leaved forest (P-B-PB) (S. Table.1). To reduce the complexity of the network and facilitate the identification of core taxon, we selected OTU relative abundance for analysis (Barberan et al. 2012). All possible OTU pairs used to calculate spearman's rank correlations and the p-values were based on the false discovery rate (FDR). Only Spearman's rank strong positive ($\rho > 0.8$), strong negative ($\rho < -0.8$) and FDR q-value < 0.001 relationship (Barberan et al. 2012; Chao et al. 2016) were identified as having significant co-occurrence relationships. Networks were visualized using Cytoscape (V 3.7.2) and network topology parameters obtained by Network Analyzer. Species with high mean degree were considered keystone taxa (Berry and Widder 2014).

Results

Change in soil microbial community structure

We observed a significant difference in the soil fungal community among monoculture plantations (ANOSIM $R = 0.613$, $p = 0.024$) and between monoculture and mixed species plantations (ANOSIM $R = 0.508$, $p = 0.002$) (S. Table. 2). The fungal community structure in mixed-species plantations resembled a mixture of fungal communities from monoculture plantations, but this depended on the plantation mixture. For example, P and L monoculture plantations exhibited some overlap in fungal communities and the PL mixed-species plantation exhibited a fungal community that clustered between that of P and L. Although P and B monoculture plantations did not exhibit overlap in fungal communities and the PB mixed-species plantation did not overlap with either monoculture, it was much more closely clustered toward P compared to B monoculture plantations (Fig. 1a).

We observed a significant difference in the bacterial community structure among monoculture plantations (ANOSIM $R = 0.473$, $p = 0.003$) and between monoculture and mixed species plantations (ANOSIM $R = 0.508$, $p = 0.002$). Bacterial communities were unique among monoculture plantations and did not exhibit overlap. For instance, the bacterial community of the PL mixed-species plantation overlapped with that of P, but not L monoculture plantation. The PB bacterial communities were unique,

and the bacterial community of the PB mixed-species plantation did not overlap with the bacterial community of either monoculture plantation, but it was more closely clustered toward that of B (Fig. 1b).

Change In Soil Microbial Community Species

The primary difference of soil microbial community structure was not the change of dominant species, but the change of relative abundance of species (Fig. 2). In fungal communities, the relative abundance of Basidiomycota decreased from monoculture B to PB mixed-species plantations, whereas Ascomycota abundance increased (Fig. 3a). In bacterial communities, several key taxa such as actinobacteria, proteobacteria, and WPS-2 relative abundance increased from monoculture L to mixed-species plantation PL, and decreased from monoculture to P mixed-species plantation PB. Chloroflexi and Gemmatimonadetes relative abundance increased from monoculture P to mixed-species plantation PB and decreased from monoculture L to mixed-species plantation PL. Verrucomicrobia abundance did not significantly change from monoculture to mixed-species plantation (Fig. 3b).

The Relationship Between Physicochemical Parameters And Soil Microbial Community

Edaphic variables accounted for 41.28% of the total variation in the fungal community, with axis 1 of the redundancy analysis (RDA) accounting for 24.23% and axis 2 accounting for 22.12% (Fig. 4a). The most important factor regulating soil fungal community composition was tree species ($R^2 = 0.36$, $p = 0.001$). Ascomycota was positively correlated with P; however, Basidiomycota was not. Edaphic variables in bacterial communities accounted for 33.17% of variance, with axis 1 accounting for 59.76% and axis 2 accounting for 4.83% (Fig. 4b). The most important factors were TN ($R^2 = 0.71$, $p = 0.004$). Proteobacteria, Actinobacteria, and WPS-2 were positively correlated with TN, while Chloroflexi and Gemmatimonadetes were negatively correlated with TN.

Fungal And Bacterial Co-occurrence Network Analysis

We used significant correlations to construct three co-occurrence networks (i.e., P-L-B, P-B-PB and P-L-PL) and observed the difference between monoculture and mixed species plantations on fungal and bacterial community co-occurrence. The number of nodes in the mixed-species plantation network was higher than in any monoculture plantation, but this was primarily due to bacterial and not fungal dynamics. For example, bacterial nodes increased in the mixed species plantations relative to monocultures, whereas fungal nodes exhibited little difference and the number of edges in the mixed-species plantation was higher than in any monoculture plantation. Bacteria were more connected than fungi and contained more edges. Fungi-fungi had the least number of edges. The number of fungi-bacteria and bacteria-bacteria edges increased in the mixed-species plantation compared to that of monoculture plantations (Table 1). We analyzed the connectedness and complexity of three networks and found that P-B-PB (Fig. 5c) had

the highest average network connectedness (degree) (Fig. 6a) and complexity (clustering coefficient) (Fig. 6b), followed by P-L-PL (Fig. 5b) and P-L-B (Fig. 5a). We observed that several taxa played a central role (degree of connectivity) in the networks. Our analysis indicated that the keystone of P-L-B, P-B-PB and P-L-PL were FuKun57 (Proreobacteria), Coxiella (Proreobacteria), and Occallatibacter (Acidobacteria) respectively.

Table 1
Number of nodes and edges in bacterial-fungal co-occurrence network. +, represents positive correlation; -, represents negative correlation.

	P-L-B	P-L-PL	P-B-PB
Nodes	129	147	149
Bacterial nodes	76	94	95
Fungal nodes	53	53	54
Edges	156	180	274
Fungal-Fungal	13 ⁺ ; 16 ⁻	12 ⁺ ; 6 ⁻	21 ⁺ ; 12 ⁻
Fungal-Bacterial	20 ⁺ ; 24 ⁻	32 ⁺ ; 29 ⁻	56 ⁺ ; 48 ⁻
Bacterial- Bacterial	44 ⁺ ; 39 ⁻	56 ⁺ ; 45 ⁻	75 ⁺ ; 62 ⁻
Network density	0.019	0.017	0.025
Clustering coefficient	0.244	0.185	0.30

Change in soil physicochemical and soil microbial community functional genes

The soil physicochemical features were significantly changed by both mixed after 20 years. Compared with results in L, TOC (P = 0.001) was increased by 11.33% in PL mixed-species plantation, compared with results in B, it decreased by 16.8% in PB mixed-species plantation. TN (P = 0.001) of PB and PL plantations were 17.2% and 12.7% higher than that in B and L. The pH values in both mixed-species plantations soil were significantly decreased compared to those in monoculture plantations soil (Fig. 7).

Fungi functional genes were identified based on FunGuild (S. Table. 3) and the C and N cycling genes were identified based on the KEGG database (S. Table. 4). A total of 55,318,299 genes related to the C cycle and 442,863 genes related to the N cycle were identified in all metagenomes. Ectomycorrhizal fungi relative abundance in PL and PB mixed-species plantations was smaller than in monocultural plantations

(Fig. 8a) whereas the relative abundance of saprotroph fungi higher in the PL and PB mixed-species plantations than in monoculture plantations (Fig. 8b). Compared with monoculture plantations, the relative abundance of C cycle genes (Fig. 9a) and N cycle genes (Fig. 9b) in PB and PL mixed-species plantations were lower than that in B and L monoculture plantations, respectively, and higher than in P monoculture plantation.

Discussion

Influence of mixed-tree species on microbial community structure

The fungal community structure of the mixed-species plantation comprised of trees from different phyla (PB) more closely resembled that of the coniferous monoculture plantation (P), and bacterial community structure more closely resembled that of the angiosperm monoculture plantation (B). Tree species was the most important factor affecting fungal community structure, and soil nitrogen was the most important factor affecting bacterial community structure, which supported the first hypothesis. Our results indicate that microbial community structure of mixed-species plantations from the same phylum was comprised mainly of fungal and bacterial components that exhibited high abundance in the monoculture plantations.

That the fungal community structure was most strongly influenced by tree species is likely due to the greater dependence of fungi on plant product (Millard and Singh 2010), being a major mediator of decomposition and nutrient cycling, and possibly also affecting species coexistence by altering nutrient utilization. This may explain our finding that the relative abundance of ectomycorrhiza fungi was reduced in both mixed-species forests relative to monocultures, and the mixed species plantation comprised of two conifers (PL) exhibited lower ectomycorrhizal fungal abundance than the mixed species plantation comprised of a conifer and an angiosperm (PB). The opposite was true for saprotrophic fungi, with both types of mixed-species plantations exhibiting higher abundance than monocultures and mixed forests comprised of two conifers (PL) exhibiting higher abundance than mixed forests comprised of a conifers and angiosperm (PB; Fig. 8a). Thus, tree species is an important factor affecting the beta-diversity of fungal community structure (Peay et al. 2013). Other studies have found that the tree species effect is stronger than abiotic factors in shaping fungal diversity (Sasse et al. 2018). Even though soil characteristics differed among plantations in our study, differences in fungal community structure were not influenced by soil characteristics. Similarly, a previous study found that fungal community structure in forests with ectomycorrhizal tree was not influenced by soil characteristics (Henkel et al. 2002). This on account of the ectomycorrhizal mycelium, which symbiotic with most temperate tree species, accounts for 80% of the fungal community in forest soils (Högberg and Högberg, 2002, Urbanova et al., 2015).

Compared to fungi, bacteria are universal, we found that bacterial community structure similar to one of the conifer plantation in coniferous mixed plantation, and similar to the angiosperm plantation in the conifer-angiosperm mixed plantation. Also we found that the main factor affecting bacterial community

structure was soil nitrogen content. Dukunde et al. (2019) found in angiosperm mixed forests, the bacterial community structure was more similar to one of them. As bacterial Nitrogen fixation is responsible for more than 90% of the nitrogen input process (Berthrong et al. 2014), the variation in bacterial community may not be strictly due to tree species, but rather because this species is the dominant species in the soil nitrogen cycle in mixed forest, so although the community structure is similar to a particular species, the main factor controlling the community structure is soil nitrogen. Since soil bacterial communities are more influenced by stochastic processes than deterministic changes (Zhang et al. 2016), and soil pH and MTP (Bahram et al. 2018) are the main factors influencing bacterial community structure in large-scale research studies, but these factors have little variation at local scales, so we believe that although tree species may contribute to differences in bacterial communities structure, it may be related to stochastic processes (such as soil fertility) are more relevant (Siciliano et al. 2014).

Variation in fungal and bacterial community structure in our study was reflected in species relative abundance rather than taxonomic changes. For fungi, the main reason for the difference in community structure between monoculture plantations and mixed-species plantations was species abundance. In addition to ectomycorrhizal interactions, the relationship between fungal community structure and tree species may relate indirectly to litter chemical properties (Thoms and Gleixner 2013) or root exudate composition (Paterson et al. 2007; Sasse et al. 2018). Although we did not investigate root exudates in this study, we did observe differences in soil characteristics. Tree species influenced the fungal community structure in the two mixed-species plantations. Specifically, the presence of *P. sibirica* increased the relative abundance of Ascomycota, and suppressed Basidiomycota. Ascomycota has been associated with cellulose decomposition (Fabian et al. 2017) and was particularly abundant in soil of PL, due to lignocellulose degradation rate in mixed litter was significantly higher than in monoculture (Wang et al. 2020).

Actinobacteria, Proteobacteria, and WPS-2 relative abundance were positively correlated with total soil nitrogen, whereas Gemmatimonadetes and Chloroflexi were negatively correlated with total soil nitrogen. The addition of inorganic nitrogen to forest soil showed that Actinobacteria abundance increased, whereas Acidobacteria and Verrucomicrobia abundance decreased (Ramirez et al. 2012). Molecular understanding of N cycling also provided evidence that nitrogen decreased the abundance of key protein-coding genes in bacterial responsible for N fixation and denitrification (Freedman et al. 2013). The relative abundance of Acidobacteria, Actinobacteria, and Proteobacteria across forest soils is very high (Nacke et al. 2011; Uroz et al. 2013), and responsible for most bacterial transcription (Baldrian et al. 2011; Zifcakova et al. 2016). Taxa that grow fast and rely on labile carbon sources, such as Proteobacteria (Fierer et al. 2007), while other groups, such as Gemmatimonadetes (DeBruyn et al. 2011), and Chloroflexi (Maestre et al. 2015) that enriched in lower nutritional conditions may decline. Our observations suggest bacterial community structure variation induced by total nitrogen could alter the function and metabolic potential of the communities, as well as the decomposition rate.

Influence of mixed-tree species on co-occurrence network

Microbial community networks in mixed-species plantations were more robust than monoculture stands, which supported the second hypothesis. In particular, conifer-angiosperm mixed plantation (PB) contained more nodes and edges.

Co-occurrence network results provide insight into vegetation change in belowground networks and highlight the role of plant species in this response, as well as the role of interactions between microbial community components. Our results showed that network topological properties changed association with different treatments. Mixed-species plantation networks contained more nodes, edges, and exhibited higher average network connectedness, complexity, especially the conifer-angiosperm plantation. Nodes and edges increased indicating that mixed-species plantations were more complicated than monoculture plantations. Previous studies have reported that microbial co-occurrence networks in a natural broad-leaved forest were more complex and robust than monoculture plantations (Nakayama et al. 2019). Our results suggest that stability was higher in mixed-species than in monoculture plantations. Positive and negative correlations between fungal and bacterial components increased in mixed-species plantations compared to monoculture plantations. This may indicate vegetation not only provides resources for microbes, but also intensifies their competition (Mau et al. 2015); however, as available resources increased, members may respond synergistically to habitat change, resulting in positive feedback and co-oscillations (Coyte et al. 2015), and higher clustering coefficient of network complexity. Our results indicated that network complexity is mainly caused by the fungi-bacterial and bacterial-bacterial interactions, although the soil bacterial community exhibited weak stress resistance, but strong resilience (de Vries et al. 2018). In the mixed-species plantations, especially coniferous-angiosperm mixed plantation, the overall microbial network is more robust than the network of the monoculture plantations. The bacterial community structure was mainly affected by soil nitrogen content, suggesting that nitrogen availability will affect the complexity and stability of the microbial co-occurrence network.

In microbial networks, key taxa that affect community structure and integrity are known as keystone taxa (Bahram et al. 2018) because their removal results in drastic shifts in microbial community composition and function (Barabasi et al. 2011; van der Heijden and Hartmann 2016). Fungal and bacterial communities connect as a network through keystone taxa in mixed-species plantations. Relative abundance of keystone taxa is of lesser importance to their impact on community structure than is their presence or absence (Lynch and Neufeld 2015). We observed that FukuN57 (Rhizobiales), *Occallatibacter* (Acidobacteria), and *Coxiella* (Gammaproteobacteria) were the keystone in P-L-B, P-L-PL, and P-B-PB respectively, all the keystones in co-occurrence network are bacterial, and were the member of Proteobacteria and Acidobacteria, which are two of the most abundant phyla in forest soils (Kuffner et al. 2012; Kurth et al. 2013; Lipson 2007), and have important functions. We know relatively little about these keystone taxa; however studies have shown that bacteria accumulate more cellulosic-C in coniferous forest litter than fungi, and most of these bacteria belong to Acidobacteria, Proteobacteria (Rhizobiales) (Brown and Chang 2014; Eichorst and Kuske 2012). Most of the isolates in Gammaproteobacteria (Esson et al. 2016) have proved to be the methanotrophic (heterotrophic) bacteria, which may represent sinks of atmospheric methane especially in boreal forests soil. That fungus needs these bacteria to cooperate

with other bacteria may be due to the energy released by the decomposition of fungi needs to be transformed by specific types of bacteria to be used by other bacteria.

Influence of mixed-tree species on potential microbial functional genes

We observed that the abundance of functional genes in fungal and bacterial communities were significantly different between the two mixed-species plantations, which supported the third hypothesis. We found that the relative abundance of mycorrhizal fungi decreased in mixed-species plantations compared to monocultures, whereas the relative abundance of saprophytic fungi increased. The C and N cycling genes decreased dramatically in mixed-species plantations.

Fungi have important ecological functions and their abundance can be highly variable (Izzo et al. 2005) in forest ecosystem. One key ecological function is that mycorrhizal fungi promote plant nutrient absorption (Smith and Read 2008). In our study, the relative abundance of ectomycorrhizal fungi was higher in conifer-angiosperm mixed plantation (PB) than in coniferous and coniferous mixed plantation (PL), and the pattern of saprotrophic fungi was reversed. A recent study by Ge et al. (2017) indicated, ectomycorrhizal abundance was positively correlated with soil pH. Our results confirm this conclusion, the soil pH of conifer-angiosperm mixed plantation was indeed higher than coniferous mixed plantation. Saprophytic fungi regulate nutrient cycling, via decomposition of organic matter (Boddy and Frankland 2008), so it is possible that their abundance in the mixed plantation comprised of two conifers (PL) was higher than that of the mixed plantation comprised of a conifer- angiosperm (PB) due to litter chemistry and recalcitrance. However, the slow metabolism rate of fungi slows nutrient cycling in the ecosystem, so that the rate of nutrient release matches the rate of nutrient uptake by plants (Hattenschwiler and Vitousek 2000). This coordinated nutrient cycling not only preserves the resources in the ecosystem, but also increases the retention time of the assimilated carbon (Makkonen et al. 2012). This may result in higher soil carbon content in coniferous mixed plantation (PL) than conifer-angiosperm (PB) mixed plantation, but slower nutrient cycling.

Based on the results of the KEGG database, it can be seen that the introduction of non-native species *P. sibirica* in mixed-species plantations reduced soil carbon and nitrogen cycling genes compared to monocultures. A number of studies have shown that invasive species support more microbial nutrient release through litter or root secretions (Zhang et al. 2018). For example, readily degradable plant metabolites will destabilize soil C stocks by promoting microbial co-metabolism of recalcitrant compounds, leading to soil C loss (Fontaine and Barot 2005). Therefore, soil C stocks in the mixed plantation comprised of a conifer and angiosperm were significantly lower and soil N stocks were significantly higher than in the mixed plantation comprised of two conifers. At the same time, we also observed that their soil N stocks were reversed. Although there was no significant difference between the two mixed-species plantations in the total amount of C and N cycle genes, suggesting that there were differences in the processes of fixation and release. Therefore, the selection of different tree species for afforestation will influence rates of C and N cycling (Hobbie 1996; Mitchell 2010), which could initiate profound changes in resilience and function of the ecosystem (Gessner et al. 2010; Valentin et al. 2014).

Conclusions

Our results showed that fungal community structure was mainly affected by tree species. Whereas bacterial community structure was mainly affected by soil nitrogen. The soil microbial co-occurrence relationship varied with different types of mixed-species plantation. Conifer-angiosperm mixed plantation contribute to soil nitrogen fixation and coniferous mixed plantation contribute to soil carbon fixation. Our results are robust in that all of our plantations are the same age and from the same site, thereby eliminating variation due to stand development, climate, and soil parent material. An improved understanding of the factors influencing microbial community structure can be applied to forest management ecosystem processes and ecosystem services. Future work should aim to assess the relative stability of the microbial community structure following natural (e.g., windthrow or wildfire) and artificial disturbances (e.g., forest thinning or harvesting).

Declarations

Author contributions

Conceptualization, Y. Gao, X.W. Wang, and D.P. Aubrey; Investigation, Y. Gao, L. Yang, and Z.Y. Jiang; Methodology, Y. Gao, Z.J. Mao, and X.W. Chen; Data creation and Formal analysis, Y. Gao and X.W. Wang; Software and Visualization, Y. Gao and X.W. Wang; Writing - original draft Y. Gao and X.W. Wang; Writing - review & editing, Y. Gao, X.W. Wang, and D.P. Aubrey; Funding acquisition, X.W. Wang and X.W. Chen.

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Figures

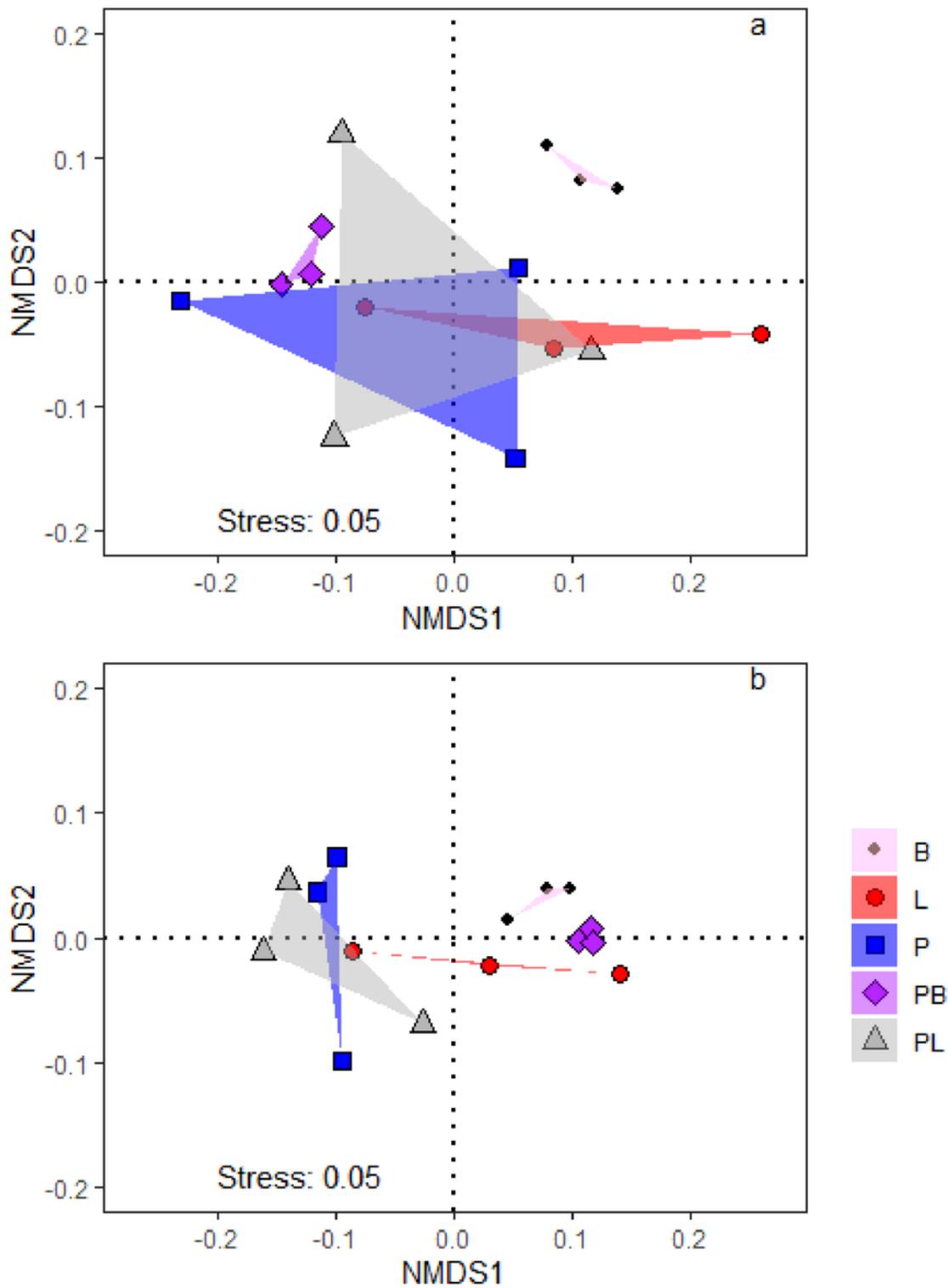


Figure 1

Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis analysis of community structure of fungi (a) and bacterial (b) in monoculture and mixed forests soil. Colors of symbols represent forest types. B, *Betula platyphylla*; L, *Larix gmelinii*; P, *Pinus sibirica*; PB, *P. sibirica* and *B. platyphylla*; PL, *P. sibirica* and *L. gmelinii*.

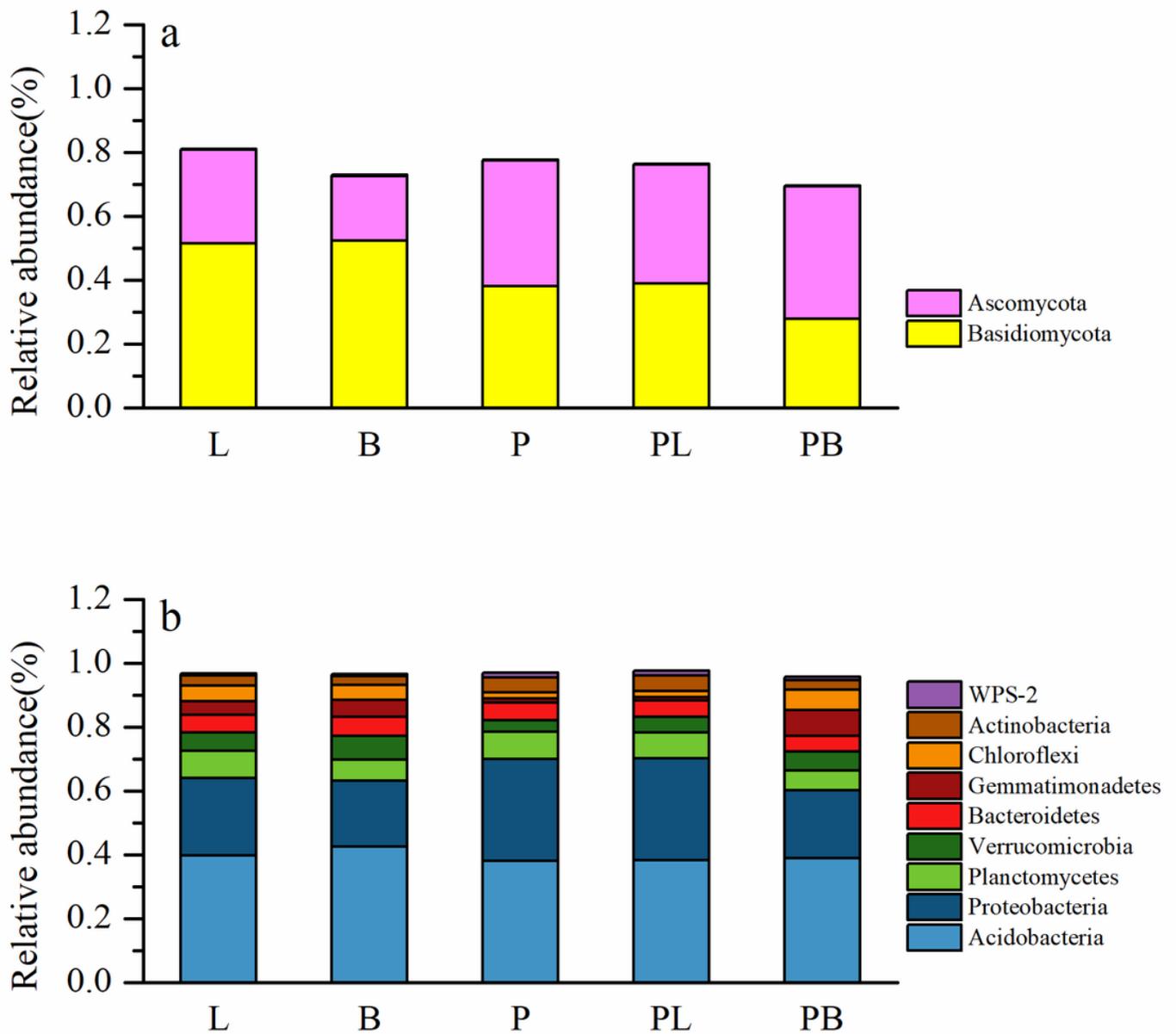


Figure 2

Relative abundance of fungal (a) and bacterial species (b). The transverse coordinate is the sample name and the longitudinal coordinate is the relative abundance. The figure shows species with a relative abundance of more than 1% in fungal and bacterial communities. Details are described in Fig.1.

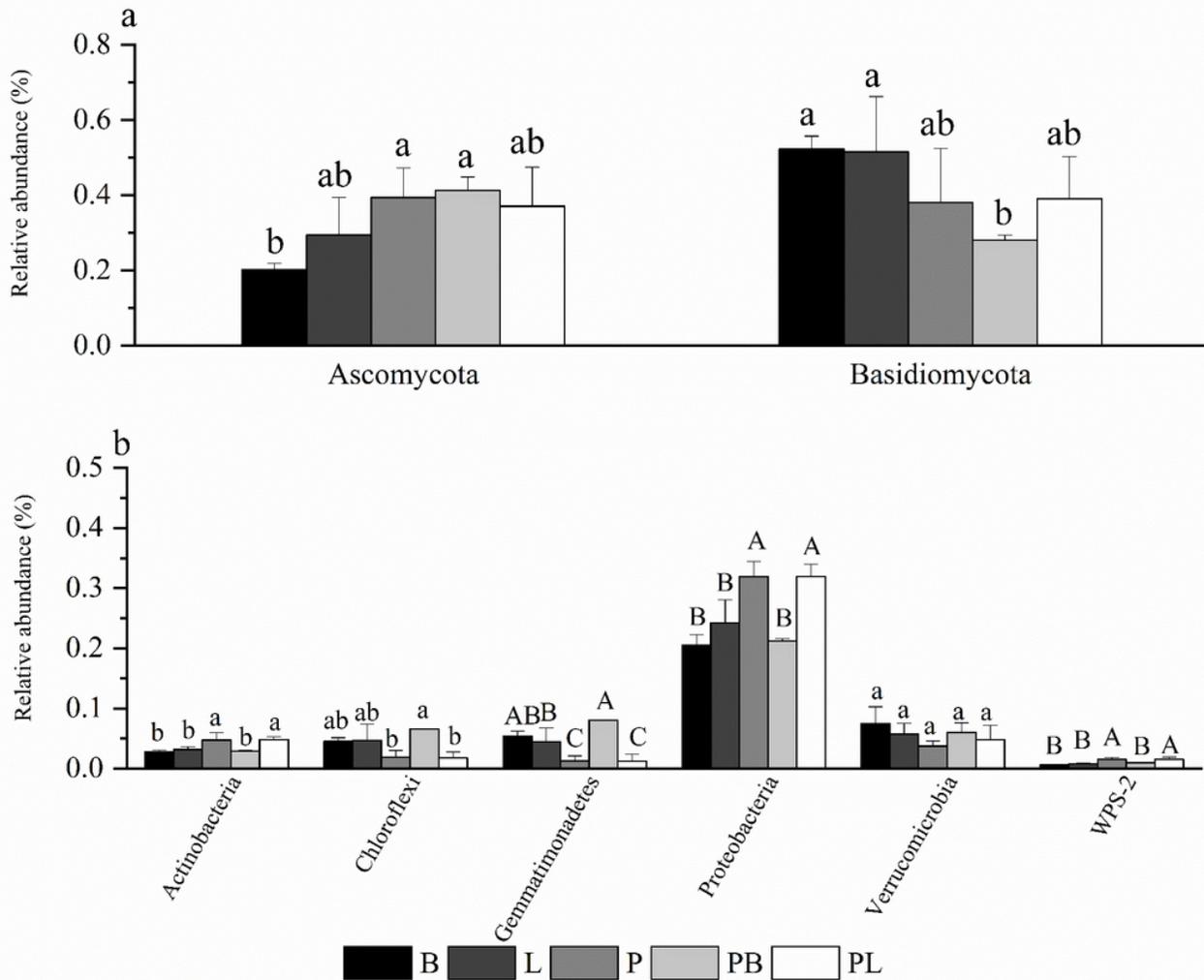


Figure 3

Relative abundance changes of fungi (a) and bacterial (b) sensitive species in five plantation types. Different lowercase letters represent significant differences $p < 0.05$. Capital letters indicate extremely significant $p < 0.001$. Details are described in Fig.1.

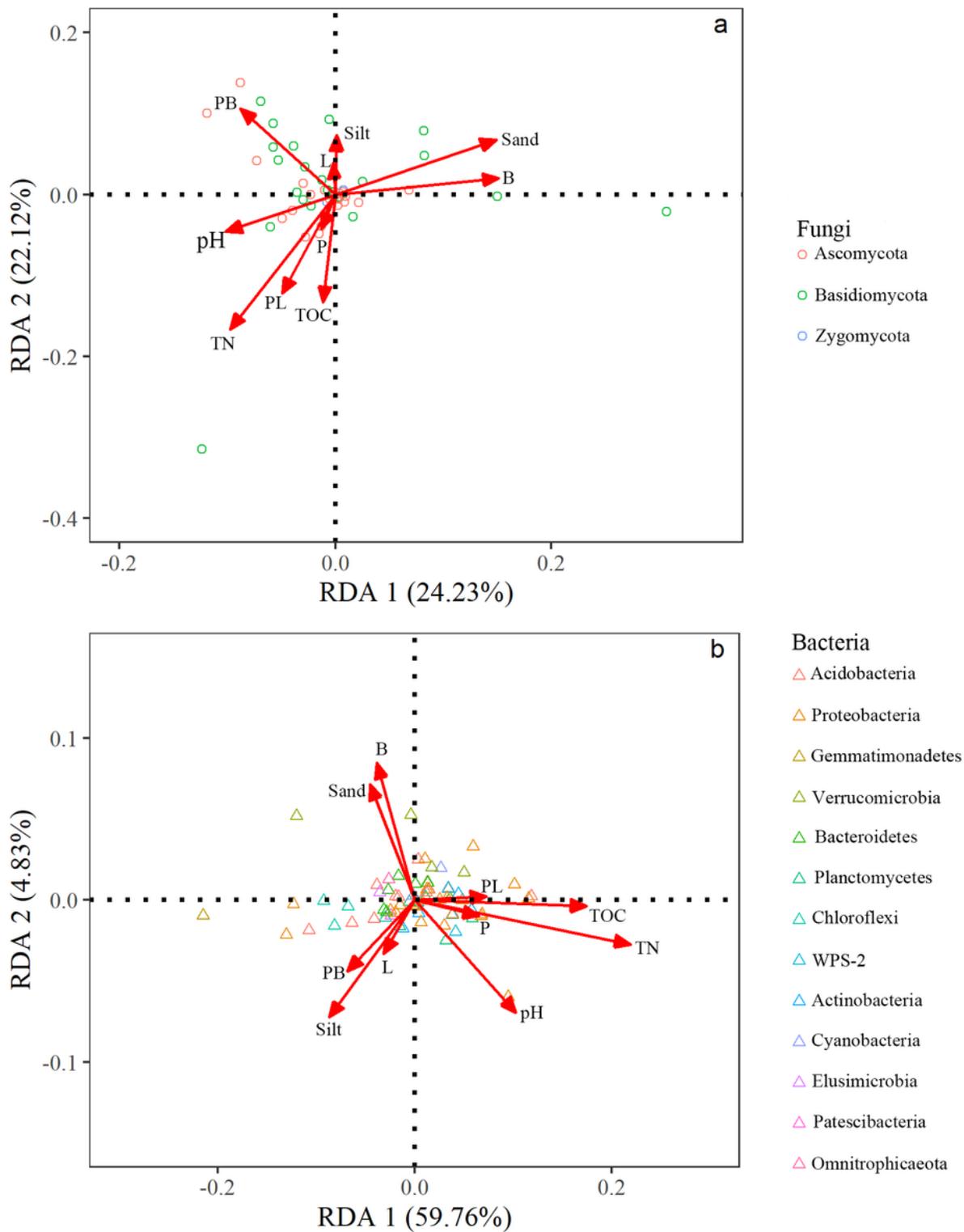


Figure 4

Redundancy analysis of fungi (ITS) (a) and bacterial 16S rRNA(b). Data were calculated by mean relative abundance > 0.1% families and colored according to phyla.

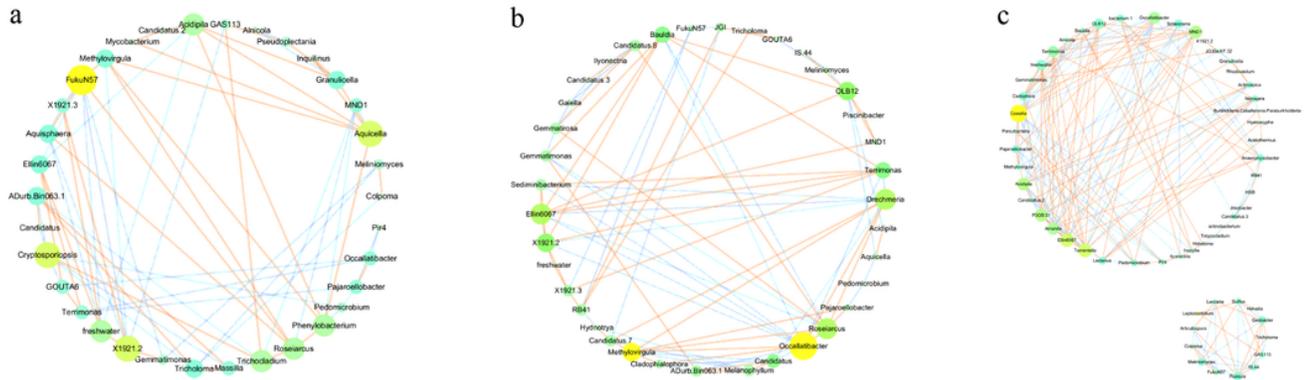


Figure 5

Co-occurring network of microorganism community in Monoculture, PL, and PB based on correlation analysis. The connections stand for a strong (spearman's $p > 0.8$ and $p < -0.8$) and significant ($p < 0.001$) correlations. a, P-L-B; b, P-L-PL; c, P-B-PB. The avg. neighbor of node is more than 4, nodes colored from green to yellow and size ranked according to degree index. Edges colored by correlations, positive (orange) and negatively (blue).

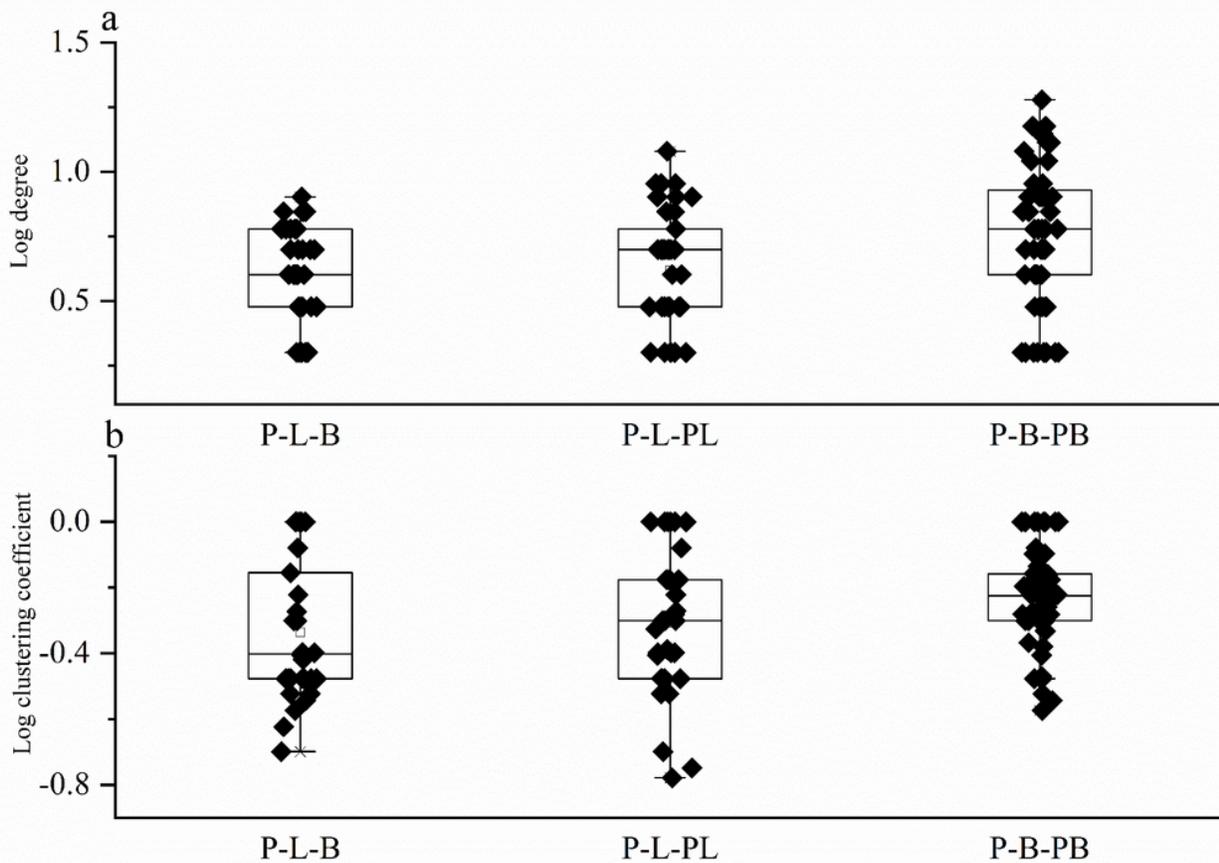


Figure 6

Node connectedness and complexity of microbial networks. a, degree; b, log clustering coefficient. Each box represents the interquartile range, the line in each box represents the median, top and bottom of the boxes represent first and third quartiles, and whiskers represent the range of 1.5 interquartile range, dots represent single observations.

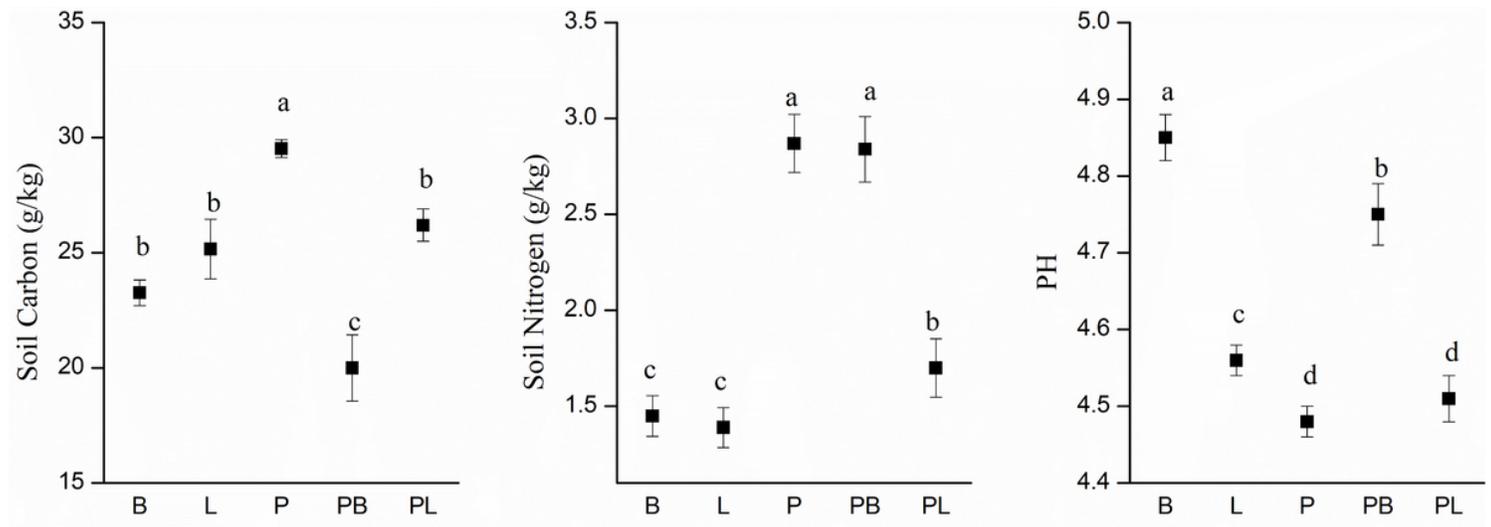


Figure 7

Variations of soil properties among monocultures and mixed-species plantations.

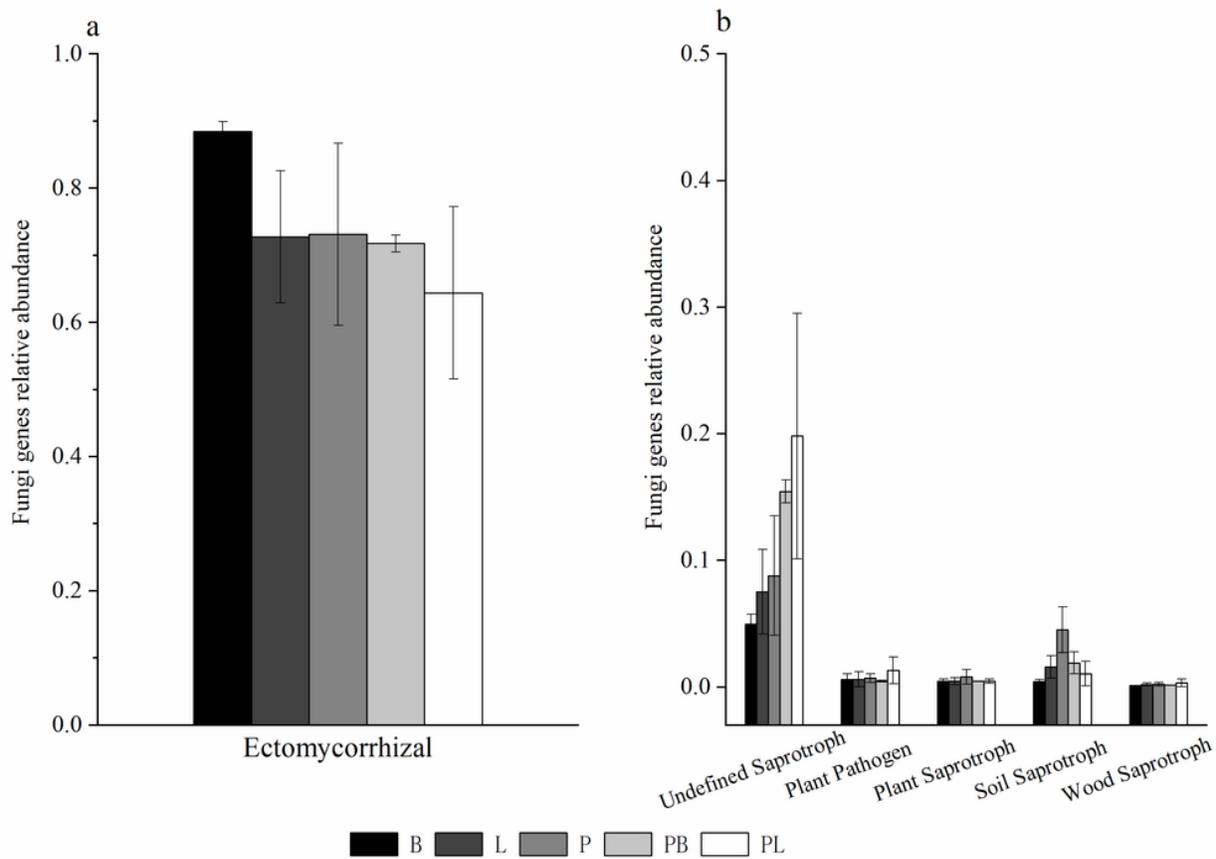


Figure 8

The abundance of fungi functional genes identified based on FunGuild database.

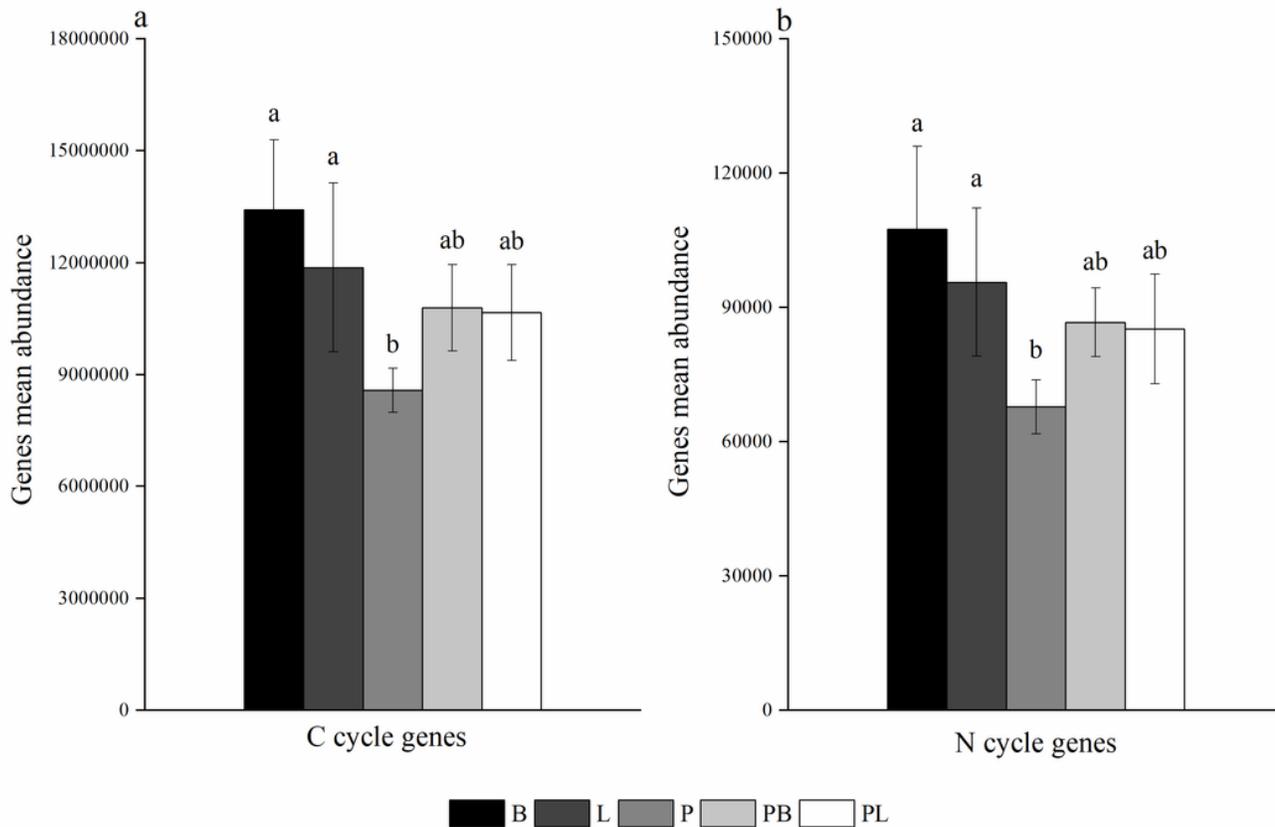


Figure 9

The abundance bacterial functional genes identified based on the KEGG database C cycle genes and N cycle genes. Different lowercase letters represent significant differences $p < 0.05$.

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