Forest Ecology and Management 500 (2021) 119656

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Herbaceous plants influence bacterial communities, while shrubs influence fungal communities in subalpine coniferous forests

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ARTICLE INFO

Keywords:
Subalpine coniferous forests
Soil microbial communities
Shrub roots
Herbaceous roots
Litter

ABSTRACT

Forests are essential biomes for global biochemical cycles, and soil microorganisms play a key role in providing and maintaining forest ecosystem services. A comprehensive understanding of how biotic and abiotic factors drive soil microbial community composition can help predict the effects of environmental changes on forest ecosystem service functions. Therefore, this study was conducted in a subalpine coniferous forest (*Picea asperata* forests and *Sabina chinensis* forests) in the Sanjiangyuan National Natural Reserve of Qinghai Province, China, to investigate the effects of understorey vegetation on soil microbial communities by measuring shrub roots, herbaceous roots, litter and soil physicochemical properties and soil microbial communities (16S, ITS). The results showed the following: (1) In both the *P. asperata* and *S. chinensis* forests, the dominant phyla in the soil bacterial communities were Acidobacteria, Proteobacteria and Verrucomicrobia, and the dominant phyla in the fungal communities were Ascomycota and Basidiomycota. In different forest types, the soil microbial communities contained differences that were significant in beta diversity ($P < 0.05$) and nonsignificant in alpha diversity ($P > 0.05$). (2) Results from the structural equation model showed that in different forest types, bacterial community composition was significantly influenced by herbaceous roots, while fungal community composition was significantly influenced by shrub roots ($P < 0.05$), and the degree of influence was stronger than that of litter. In addition, both the shrub and herbaceous roots showed the direct influence on soil microbial communities, but not on the soil bacterial communities of *S. chinensis* forests, in which the shrub and herbaceous roots had an indirect influence on bacterial community composition through affecting soil physicochemical properties and keystone species of the bacterial communities. Therefore, in subalpine coniferous forests, the effects of understorey shrubs and herbs on soil microbial communities are more significant than those of litter. The conservation of understorey vegetation community diversity and productivity is equally important for maintaining forest ecosystem service functions and stability.

1. Introduction

Microorganisms play an important role in ecosystems, performing their ecological service functions by interacting with other components of the ecosystem (Baldrian, 2017) and are often considered to be important drivers of most ecological processes. In addition, microorganisms also influence other components, such as the effect of soil microorganisms on the diversity and productivity of plant communities (van der Heijden et al., 2008). As one of the most active components of the soil, soil microorganisms have the greatest biodiversity (van der Heijden et al., 2008). In addition, as the functions of soil microorganisms in global biochemical cycles continue to be revealed (Hesse et al., 2015; Žifčáková et al., 2016), an increasing number of studies have shown that many terrestrial ecosystem functions are maintained and stabilized by soil microorganisms (Bardgett and van der Putten, 2014; Bahnmann et al., 2018). The diversity of soil microorganisms also drives the multifunctionality of ecosystems (Delgado-Baquerizo et al., 2016). Therefore, exploring the driving factors of the diversity of microorganisms and community structure and function has been the focus of research related to microbial ecology in recent years (Lladó et al., 2018). Additionally, forest soils have become one of the main research targets for microbial ecologists in recent years due to their importance as a carbon sink and

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https://doi.org/10.1016/j.foreco.2021.119656
Received 27 June 2021; Received in revised form 23 August 2021; Accepted 25 August 2021
Available online 3 September 2021

potential carbon source, as well as their sensitivity to climate change (Llado et al., 2017).

Forest ecosystems are one of the major components of terrestrial ecosystems. With a large carbon pool function and high productivity, forest ecosystems play an irreplaceable role in regulating the global carbon balance, slowing down the rise of greenhouse gas concentrations such as CO_2 in the atmosphere and maintaining ecosystem stability (Kauppi et al., 1992; Pan et al., 2013). The different components of forest ecosystems (terrestrial vegetation and soil microorganisms) interact and affect each other, together maintaining the stability of the ecosystem (Kooch and Bayranvand, 2017). Some studies have indicated that among all kinds of factors, the types of terrestrial vegetation in forest ecosystems have a much greater influence on soil microbial communities (Xia et al., 2016). Vegetation types are able to change the physical and chemical properties of soil in a direct or indirect manner (Oh et al., 2012). Thus, Lladó et al. (2018) believed that at a small regional scale, forest soil microorganisms are driven by two main factors: litter quality on the one hand and differences in tree root traits and secretions on the other hand, both of which drive the formation of soil physicochemical properties (pH, organic matter, moisture), and thus directly or indirectly influence soil microbial communities. In natural forests, however, vegetation communities usually include understorey vegetation such as shrubs and herbs in addition to trees, and compared with coniferous trees, herbaceous and shrub roots and litter are characterized by rapid turnover and rapid decomposition (Nilsson and Wardle, 2005). Nevertheless, there is a relative lack of research on the effect of understorey vegetation on soil microbial communities.

Understorey vegetation is an important component of natural forest ecosystems. Although the tree layer plays a dominant role in the formation and construction of vegetation communities in forest ecosystems, understorey vegetation plays an important role in promoting ecosystem material cycling and maintaining the diversity and stability of community species (Gilliam, 2007). De Grandpré et al. (2003) found that in the coniferous forests of northern Canada, there are approximately 300 species of plants but only approximately 20 tree species, with understorey vegetation comprising 80% of the diversity. It has been shown that understorey plants have a greater capacity for nutrient uptake than saplings, which can inhibit sapling germination to some extent (Lyon and Sharpe, 2003). Although the biomass of understorey vegetation is usually <1% of the total biomass of a forest ecosystem, understorey vegetation can produce >10% of the total annual biomass of litter (Gilliam, 2007). As the turnover rate of understorey vegetation is much higher than that of trees, its contribution to nutrient cycling and soil carbon accumulation is likely to be greater than that of the tree layer (Chapin, 1983; Yang et al., 2017). Therefore, understorey vegetation probably plays an important role in nutrient-return processes in forest ecology, while litter and roots are important agents of plant-nutrient return (Lladó et al., 2018). As a major player in the process of ecosystem material cycling, the soil microbial community is usually influenced by the quantity, quality, biomass, and stoichiometric properties of plant litter.

In recent years, the importance of litter has been confirmed by many experiments (Čapek et al., 2021; Fang et al., 2021; Yu et al., 2021). Additionally, the driving mechanisms of litter biomass and quality on soil microbial communities have also been reported (Bai et al., 2021; Chen et al., 2021; Liu et al., 2021). However, few studies have been conducted to investigate the interrelationships between plant roots and soil microbial communities because plant roots are mainly growing underground, and it is relatively difficult to perform experiments (Sweeney et al., 2021). Moreover, studies on forest ecosystems usually focus on trees, neglecting herbaceous roots and shrub roots in the understorey vegetation, which are sources of nutrients with relatively high turnover rates. Therefore, this study investigates the importance of understorey vegetation on soil microbial communities in forest ecosystems by comparing the effects of litter, herbaceous and shrub roots on soil microbial communities in natural forests.

This study on subalpine coniferous forests in the Sanjiangyuan National Natural Reserve is characterized by an advanced forest age and complete vertical structure, with high plant and animal diversity and irreplaceability (Qu et al., 2011; Chen et al., 2018). Based on the results of previous studies, we proposed the following three hypotheses. Among a great number of relevant studies on the effects of litter on soil microbial communities, some research results have shown that litter biomass and quality can significantly influence soil bacterial communities (Macklin et al., 2008; Leloup et al., 2018) and fungal communities (Wang et al., 2013; Otaki and Tsuyuzaki, 2019); thus, our first hypothesis is that litter has a stronger effect on soil microbial communities than understorey vegetation roots. For herbaceous roots and shrub roots, our previous study showed that in this area, the C/N ratio of herbaceous roots (C/N ratio = 74.13, Cheng et al., 2019) was significantly lower than that of shrub roots (C/N ratio = 128.89, Yang et al., 2019). A low C/N ratio is conducive to microbial decomposition and turnover rate (Maes et al., 2019); thus, our second hypothesis is that herbaceous roots have a stronger effect on the soil microbial communities than shrub roots, while shrub roots have a relatively weaker effect on the soil microbial community. In addition, since litter and plant roots are important agents in the plant nutrient-return process and soil microbial communities are often closely linked to soil-nutrient content (Fierer and Jackson, 2006; Oh, et al., 2012), our third hypothesis is that litter, herbaceous roots and shrub roots directly influence soil-nutrient contents and thus indirectly influence soil microbial communities. Finally, to verify whether the results are general or specific, we chose two typical forest types, *P. asperata* forests and *S. chinensis* forests, which are the most widely distributed forests and are the highest carbon-storage forest types in the Sanjiangyuan National Natural Reserve (Chen et al., 2018), to further verify whether differences in forest types lead to different responses of understorey vegetation to the driving effects of soil microbial communities.

2. Materials and methods

2.1. Study area description

The study area is located in the Sanjiangyuan National Natural Reserve (100.80°–102.93°E, 34.76°–35.33°N) in Qinghai Province, China, in the eastern part of the Qinghai-Tibetan Plateau. The Sanjiangyuan National Natural Reserve has a typical plateau continental climate, characterized by alternating cold and warm seasons and dry and rainy seasons (Zhang et al., 2019)), with annual precipitation of 477.2–764.4 mm and an average annual temperature of −5.6–3.8 °C. Subalpine coniferous forests are the main forest types in the Sanjiangyuan National Natural Reserve. They play an important role in climate regulation and soil and water conservation. Biodiversity conservation in the Sanjiangyuan National Natural Reserve also includes the dominant understorey shrubs–*Potentilla glabra* Lodd. and *Salix cupularis* Rehe acompanied with *Berberis vernae*, as well as the dominant herbaceous plants–*Carex* spp. and *Polygonum viviparum* L., and also some common speceis, such as *Poa*. spp, *Ranunculus*. spp, and *Potentilla*. spp (Chen et al., 2018).

2.2. Experimental design, plant investigation and soil sampling

In July and August 2015, we established twenty-four 20 m × 50 m plots (twelve *P. asperata* forest plots and twelve *S. chinensis* forest plots) that were at least 500 m distant from each other. These selected quadrats were covered with tree canopies >4 m from tree trunks. For these quadrats with shrubs and herbs, we tried to avoid disturbance of the soil microbial community by tree roots as much as possible. The sample plots were 2 m × 2 m in size, and all litter (including litter from trees, shrubs and herbs) was collected from the plots. Root samples of shrubs and herbs and soil samples were collected at a depth of 0–20 cm. The samples were sent to the laboratory immediately after collection. The shrub

and herbaceous roots were first measured for fresh weight and then dried to a constant weight for biomass determination. Plant roots were crushed and sieved through a 2 mm sieve to determine the C, N and P contents of the shrub and herbaceous roots in the same way as Cheng et al. (2019). Soil samples were sieved through a 2 mm soil sieve to filter out the rocks, roots and litter debris, and then they were divided into two parts, with one part was stored at −80 °C for DNA extraction. The second parts were air-dried and stored at air temperature for the determination of soil pH, total carbon (TC), total nitrogen (TN) and total phosphorus (TP) in the same way as Ade et al. (2018).

2.3. DNA extraction, PCR amplification, illumina sequencing and data processing

A MoBio PowerSoil DNA Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA) was used to extract the total DNA from 0.25 g soil samples according to the manufacturer's instructions. The concentration and quality of DNA were measured using a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). After the DNA samples were diluted to a concentration of 10 ng·μl^{-1}, they were stored at −20 °C as subsequent experimental samples (Liu et al., 2012).

The V4-V5 region of bacterial 16S rRNA and the ITS region of fungal rRNA were targeted and amplified via PCR with the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) for bacteria (Tamaki et al., 2011) and with primers ITS3 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) for fungi (Horisawa et al., 2013). The polymerase chain reaction (PCR) system (25 μl) was composed of 10 × Ex Taq buffer (2.5 μl), 25 mM $MgCl_2$ (2 μl), 2.5 mM dNTPs (2 μl), 10 μMforward primer (1 μl) and reverse primer (1 μl), 0.5 UExTaq polymerase (TaKaRa, Dalian, China) (0.2 μl), 10 ng·μl^{-1} soil genomic DNA (5 μl) and ddH_2O (11.3 μl). PCR amplification was conducted with a Bio-read C1000 Touch Thermal Cycler system (Bio-Rad, CA, USA). The bacterial samples were amplified according to the following procedures: denaturation at 95 °C, 35 cycles of denaturation at 94 °C, 50 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min; the fungal samples were amplified according to the following procedure: 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s, and 72 °C for 10 min (Li et al., 2014). After the DNA samples were separated by gel electrophoresis, the 1% agarose gel was cut, and an SK8132 SanPrep DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China) was used for the recovery of DNA fragments according to the instructions. Subsequently, the concentration and quality of the recovered DNA fragments were tested using a NanoDrop 2000C spectrophotometer. All samples that were qualified (in terms of concentration and quality) were mixed in equal molar proportions for MiSeq sequencing.

Processing of the MiSeq sequencing data was mainly performed by the Quantitative Insights Into Microbial Ecology (QIIME) pipeline version 1.7.0 (http://qiime.org/tutorials/tutorial.html). The original sequences were sorted based on the unique sample barcodes and trimmed for sequence quality (length >300 bp, average base quality score >30) using the QIIME pipeline (Caporaso et al., 2012). Chimera sequences were removed with the UCHIME algorithm (Edgar et al., 2011). The sequences were clustered by the complete-linkage clustering method incorporated in the QIIME pipeline (Caporaso et al., 2012). Sequences were clustered into operational taxonomic units (OTUs) based on a sequence similarity threshold of 97%. Each sample was rarefied to the soil sample exhibiting the lowest number of reads (bacteria: 2446 sequences, fungi: 1364 sequences) for both alpha and beta diversity analyses, and rarefaction curves were generated from the observed species. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al., 2007). The original sequence data are available at the European Nucleotide Archive under accession number PRJEB11649 (http://www.ebi.ac.uk/ena/data/view/ PRJEB11649).

2.4. Statistical analysis

Two-tailed t-tests in SPSS 19.0 ($P = 0.05$) software were used to test the differences in the biomass of the shrub roots, herbaceous roots and litter; the contents of C, N and P; the contents of TC, TN, TP and pH in soil; and the differences in relative abundance and Shannon diversity index of each phylum of bacterial communities and fungal communities between the *P. asperata* forests and the *S. chinensis* forests. Using the OTU relative abundance tables as input data, Bray-Curtis distances were calculated using R version 3.4.0. Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances was used to assess the beta diversity of bacterial and fungal communities, and variance analysis was performed. The above steps identified great differences in litter, shrub roots, herbaceous roots, soil physicochemical properties and soil microbial communities in different forest types, yet with no differences in the same forest type. Then, we used the Molecular Ecological Network Analysis Pipeline (http://ieg4.rccc.ou.edu/mena) to conduct network analysis and calculation, and the results were visualized by using Cytoscape software. Structural equations were constructed to investigate the effects of litter and understorey plant roots on soil microbial communities in subalpine coniferous forests of different forest types, and the methods for shrub roots, herbaceous roots and soil nutrients in the study by Zhang et al. (2018) were normalized and used as indicators for application in the equations. The keystone species in the equations are the OTU numbers of keystone species of bacterial or fungal communities in the corresponding stand types obtained from the network analysis. The equations were constructed by using Amos 23.0 (Amos Development, Spring House, Armonk, PA, USA).

3. Results

3.1. Physicochemical properties of the understorey litter, shrub roots, herbaceous roots and soil

The TP content of shrub roots in the *P. asperata* forests was significantly higher than that in the *S. chinensis* forests ($P < 0.05$, Table A1). The biomass and TN content of shrub roots in the *S. chinensis* forests were significantly higher than those in the *P. asperata* forests ($P < 0.05$, Table A1). The TC content of shrub roots in different forest types had no significant differences ($P > 0.05$, Table A1). The TC content of herbaceous roots was significantly higher in the *P. asperata* forests than in that the *S. chinensis* forests ($P < 0.05$, Table A1). TN, TP and biomass of herbaceous roots were significantly higher in the *S. chinensis* forests than those in the *P. asperata* forests ($P < 0.05$, Table A1). The litter TP content was significantly higher in the *P. asperata* forests than that in the *S. chinensis* forests ($P < 0.05$, Table A1). The litter biomass and TC and TN contents were significantly higher in the *S. chinensis* forests than those in the *P. asperata* forests ($P < 0.05$, Table A1). The SOC was significantly higher in the *P. asperata* forests than that in the *S. chinensis* forests ($P < 0.05$, Table A2). Soil TN, TP and pH did not differ significantly between the two forest types ($P > 0.05$, Table A2). Soil pH ranged from 6.91 to 8.02, and the subalpine coniferous forest soils were neutral or slightly alkaline soils (Table A2).

3.2. Soil microbial community composition

From the samples, a total of 83,885 bacterial sequences and 35,238 fungal sequences were obtained. In a single sample, the number of bacterial sequences obtained ranged from 2446 to 4691, and the number of fungi ranged from 1364 to 1817. On average, in each sample, there were 3495 bacterial sequences and 1468 fungal sequences. The rarefaction analysis indicated that most bacterial and fungal communities were well represented because the rarefaction curves approached the saturation plateau (Fig. A1, Fig. A2). Some rare species, however, might not have been accessed effectively in all samples.

The dominant phyla in the soil bacterial communities (relative

abundance > 10%) in both the *P. asperata* and *S. chinensis* forests were Acidobacteria (*P. asperata*: 17.58%, *S. chinensis*: 19.20%), Proteobacteria (*P. asperata*: 16.36%, *S. chinensis*: 17.00%), Verrucomicrobia (*P. asperata*: 11.19%, *S. chinensis*: 16.71%) and Planctomycetes (*P. asperata*: 12.38%, *S. chinensis*: 13.40%). The relative abundance of Acidobacteria, Proteobacteria, and Planctomycetes varied insignificantly between the *P. asperata* and *S. chinensis* forests. However, the relative abundance of Verrucomicrobia in the *P. asperata* forests was significantly higher than that in the *S. chinensis* forests ($P < 0.05$, Fig. 1a).

The dominant phyla in the soil fungal communities in the two forest types (relative abundance > 10%) were Ascomycota (*P. asperata*: 36.58%, *S. chinensis*: 46.03%) and Basidiomycota (*P. asperata*: 33.88%, *S. chinensis*: 13.71%). The relative abundance of Ascomycota varied insignificantly between the *P. asperata* and *S. chinensis* forests. However, the relative abundance of Basidiomycota in the *P. asperata* forests was significantly higher than that in the *S. chinensis* forests ($P < 0.05$, Fig. 1b).

3.3. Soil microbial diversity in the two forests

We regarded the observed OTU number, phylogenetic diversity, Shannon diversity index, Chao1 estimator of richness and Simpson diversity as the alpha diversity of the soil microbial communities. Through two-tailed T-tests, it was found that there was no significant difference in the alpha diversity between bacterial and fungal communities in the different forest types (Table 1).

We analysed the structure and differences in soil bacterial and fungal communities between the *P. asperata* and *S. chinensis* forests through NMDS. The results showed that the NMDS of bacterial stress = 0.12 and fungal stress = 0.16, which indicated the respective unique structures in the soil bacterial and fungal communities in the different forest types ($P < 0.01$, Fig. 2).

3.4. Co-occurrence network structure of microbial communities

To elucidate the potential microbial-microbial interactions in soils in different forest types and to identify keystone species in the bacterial and fungal communities in the different forest types, we constructed two bacterial symbiosis networks (Fig. 3a) and two fungal symbiosis networks (Fig. 3b). All networks obtained exhibited scale-free features with power law R^2 values ranging from 0.84 to 0.94. The results of the network analysis showed that the soil bacterial and fungal communities in the subalpine coniferous forest were dominated by positive interrelationships (*P. asperata* forest bacteria: 83.27%, *P. asperata* forest fungi: 72.35%, *S. chinensis* forest bacteria: 82.67%, *S. chinensis* forest fungi: 60.13%) and that the soil microbial communities in the different forest types were mainly mutually beneficial, with relatively weak competition. For the bacterial communities, the keystone species in the *P. asperata* forests were the phyla Proteobacteria with 137 OTUs, mainly comprising members of the Family Hyphomicrobiaceae (23 OTUs), Bradyrhizobiaceae (8 OTUs), Sphingomonadaceae (7 OTUs) and Comamonadaceae (6 OTUs). In the remaining 93 OTUs, 17 OTUs belongs to the Order Rhizobiales, which also contained the Hyphomicrobiaceae, Bradyrhizobiaceae, and the left 76 OTUs belongs to other 21 Orders, such as Burkholderiales, Myxococcales and Xanthomonadales, et al. While the keystone species in the *S. chinensis* forests were Acidobacteria with 128 OTUs, mainly comprising members of Ellin6075 (15 OTUs), mb2424 (9 OTUs), Solibacteraceae (4 OTUs) and RB40 (3 OTUs). In the remaining 97 OTUs, 35 OTUs belongs to the Order RB41, and 27 OTUs belongs to iii1-15, 7 OTUs belongs to Solibacterales, et al. For the fungal communitites, the most keystone species of the soil fungal community in both the forest types were unidentified species. However, there were 8 and 6 subordinated OTUs belonging to Ascomycota in the *P. asperata* forests and *S. chinensis* forests, respectively. The difference is that in the *P. asperata* forests 3 OTUs belong to Sordariomycetes containg a dentified genus Nectriaceae, and 3 OTUs belong to Eurotiomycetes, the other two OTUs belong to Leotiomycetes and Lecanoromycetes containing a dentified genus Umbilicaria. While in the *S. chinensis* forests, only 1 OUT was dentified to the Family Herpotrichiellaceae, and the others were still undentified at the low level of resolution.

3.5. Influence of litter, shrub and herbaceous roots on soil microbial communities

Based on the data (RMSEA < 0.08, chi-square test P > 0.05), a structural equation model of the relationship between bacterial (Fig. 4a) and fungal (Fig. 4b) community composition and environmental factors was developed. The structural equation model results showed that bacterial community composition in the *P. asperata* forests was significantly affected by herbaceous roots, litter and bacterial community keystone species (standardized direct effects: −0.25, −0.17, and −0.59; $P < 0.05$, Fig. 4a). Bacterial community composition in the *S. chinensis* forests was significantly affected by herbaceous roots, soil-nutrient conditions and bacterial community keystone species (standardized direct effects: −0.66, 0.47, and −0.46; $P < 0.05$, Fig. 4a). Fungal community composition in the *P. asperata* forests was significantly affected by shrub roots (standardized direct effects: 0.31; $P < 0.05$, Fig. 4b). Fungal community composition in the *S. chinensis* forests was significantly affected by shrub roots and keystone species of the fungal community (standardized direct effects: 0.31; P < 0.52, −0.66; $P < 0.05$, Fig. 4b).

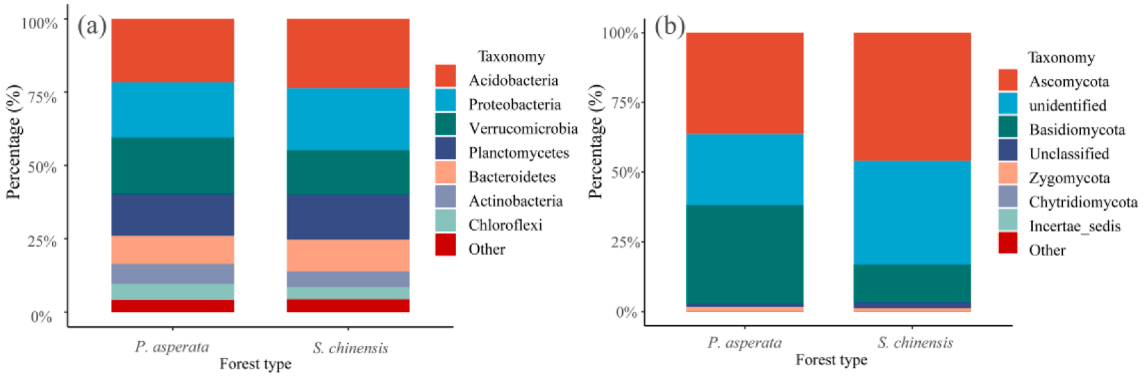

Fig. 1. Bacterial and fungal phylum relationships of *P. asperata* and *S. chinensis*. (a) Soil bacterial communities; and (b) soil fungal communities.

Table 1
Bacterial and fungal community alpha diversity of *P. asperata* and *S. chinensis* forest soil. The same letters represent nonsignificant differences ($P > 0.05$).

		Observed OTU number	Chao1 estimator of richness	Phylogenetic diversity	Shannon diversity index	Simpson diversity index
Bacteria	*P. asperata*	2190.27 ± 220.24 a	3834.85 ± 335.54 a	139.04 ± 11.74 a	8.54 ± 0.39 a	0.98 ± 0.01 a
	S. chinensis	2217.98 ± 196.23 a	3982.84 ± 289.11 a	146.5 ± 10.89 a	8.42 ± 0.57 a	0.98 ± 0.01 a
Fungi	*P. asperata*	821.23 ± 92.80 a	1347.48 ± 142.51 a	175.79 ± 20.58 a	6.16 ± 0.51 a	0.95 ± 0.03 a
	S. chinensis	826.06 ± 82.41 a	1353.64 ± 100.96 a	185.42 ± 17.26 a	5.80 ± 0.56 a	0.92 ± 0.03 a

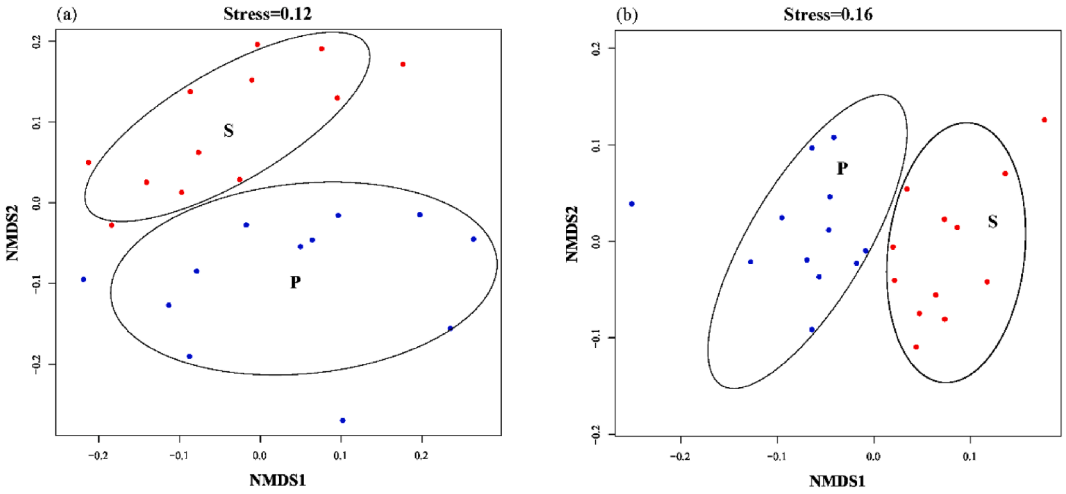

Fig. 2. Samples plotted in the plane of NMDS1 and NMDS2 from a principal coordinate analysis of the bacterial and fungal communities in the *P. asperata* and *S. chinensis* forest soil samples based on Bray-Curtis distances for all samples. (a) Soil bacterial communities; and (b) soil fungal communities.

4. Discussion

4.1. Composition and diversity of soil microbial communities in subalpine coniferous forests

Forest soils are one of the most diverse microbial habitats on earth, and bacteria are among the most abundant microbial groups and play an important role in soil organic matter mineralization, nitrogen fixation, cellulose and lignin decomposition. In this study, the dominant bacterial community phyla in both the *P. asperata* and *S. chinensis* forests were Acidobacteria, Proteobacteria and Verrucomicrobia (Fig. 1a). The dominance of Acidobacteria and Proteobacteria is more common in coniferous forest soils (Baldrian et al., 2012; Uroz et al., 2013), as both phyla are able to decompose cellulose (Kim et al., 2014; Lopez-Mondejar et al., 2016) and obtain the nutrient resources needed in an environment where nutrient resources are relatively difficult to obtain through hard-to-decompose organic matter or weathering of minerals (Štursová et al., 2012; Lladó et al., 2016; Llado et al., 2017). This was also demonstrated in the keyston species analyses. The keystone speceis in the two forest soils were different, which affected other community members and perform key ecosystem functions. Overall, these major candidate keystone species were significantly correlated with the carbohydrate metabolism in the two coniferous forest soils. For instance the major candidate keystone species of the *P. asperata* forests were from phyla Proteobacteria, such as families Hyphomicrobiaceae, Sphingomonadaceae and Comamonadaceae. Each of them performed different ecosystem functions adapting to the environments. Most of the Hyphomicrobiaceae were oligotrophic species, and the speceis of Comamonadaceae is hydrogen-oxidizing. The Sphingomonadaceae species are able to utilize a wide diversity of organic compounds and to grow and survive under a low-nutrient conditions (Gregorio et al., 2017). The keystone speceis Solibacteraceae of the *S. chinensis* forests were also demonstrated to be active in carbohydrate mineralization (Nelkner et al. 2019). Bacteria in Verrucomicrobia are free-living, facultative anaerobic and saccharolytic (Dawkins and Esiobu, 2018), and poor soil aeration due to the high soil bulk density (Table A2) of subalpine coniferous forest soils may be the main reason for their high relative abundance.

The decomposition of organic matter in forest ecosystems is mainly accomplished by fungi, which are important for forest soil carbon input processes (Baldrian and Lindahl, 2011). In this study, the dominant phyla in the soil fungal communities in the *P. asperata* and *S. chinensis* forests were both Basidiomycota and Ascomycota (Fig. 1b). Both Basidiomycota and Ascomycota are the common dominant phyla in the soil fungal communities of the subalpine forests on the Tibetan Plateau (Cao et al., 2020), and some of the groups can form ectotrophic mycorrhizae with the roots of higher plants and have high cold tolerance to adapt to the low-temperature environments in the subalpine region of the Qinghai-Tibetan Plateau (Gu et al., 2017). Additionally, enzymes secreted by Basidiomycota fungi can efficiently hydrolyse lignin, which is more readily available in forest ecosystems (Kirk and Farrell, 1987). The enzymatic decomposition system of Ascomycota fungi is not as efficient as that of Basidiomycota and usually uses relatively easy-to-decompose organic matter as a reaction substrate, which has an important role in the early stages of litter decomposition (Lindahl et al., 2010).

The alpha diversity in the soil bacterial and fungal communities in the *P. asperata* and *S. chinensis* forests was not significantly different ($P > 0.05$, Table 1), but the beta diversity was significantly different ($P < 0.05$, Fig. 2). Forest ecosystems provide diverse carbon sources for soil microorganisms, and the high redundancy of ecological niches causes the alpha diversity of microbial communities (bacteria and fungi) to be less correlated with vegetation and usually not significantly different between forest types in the same area (Barberán et al., 2015; Prober et al., 2015; Rivest et al., 2019). Beta diversity could represent varied species alternations by varied ecological niches (Heděnec et al., 2018), which are relatively more driven by environmental factors and are usually controlled by a variety of factors, such as soil pH, soil organic matter content, and soil water content (Lladó et al., 2018). Therefore, the differences in plant communities and soil physicochemical properties between the *P. asperata* and *S. chinensis* forests may be the main

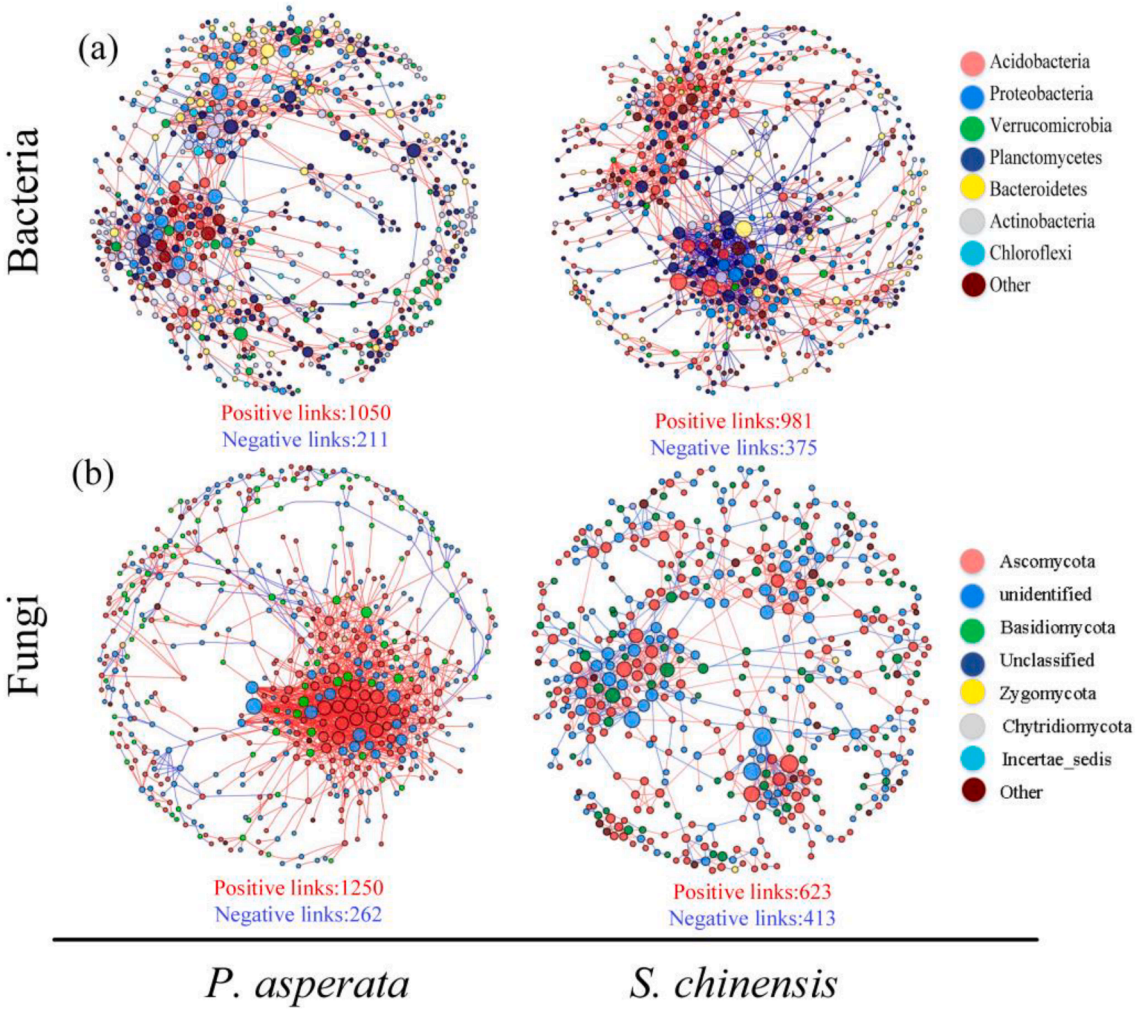

Fig. 3. Classification of nodes to identify putative keystone species within the bacterial (a) and fungal (b) networks.

reason for the significant differences in soil microbial communities.

4.2. *Driving factors of soil microbial community composition in subalpine coniferous forests*

Microbial diversity is closely related to the multifunctionality of terrestrial ecosystems, and exploring the driving factors of forest soil microbial community diversity has been a popular topic of research in recent years (Delgado-Baquerizo et al. 2016; Delgado-Baquerizo et al., 2017; Yu et al., 2021). Previous studies have shown that the main driving factors of forest soil microbial community composition include soil pH, litter, soil nutrients, and tree species (Landesman et al., 2014; Nie et al., 2021). Nevertheless, our experimental design focused more on the driving effects of litter and understorey vegetation on microbial communities. The results of our study do not support our first hypothesis. The driving effect of litter on the compositions in the soil bacterial and fungal communities was weaker in both the *P. asperata* and *S. chinensis* forests than that of shrub roots or herbaceous roots (Fig. 4), and the effect of litter on soil microbial communities was not as strong as we predicted. The reasons for this may be as follows: to begin with, according to the physical distance mechanism proposed by Sokol and Bradford (2019), the carbon input from the underground part of vegetation may be stronger than that of aboveground parts, and compared with litter, the vegetation roots have a closer physical distance with the soil microbial communities, making it easier to affect the microbial communities. In addition, a higher soil bulk density further restricts the litter from entering the soil, thus making it more difficult for it to be decomposed and absorbed by microorganisms. This will reduce the effects of litter on microbial communities. Second, previous studies have shown that litter has a strong controlling effect on soil microorganisms. However, studies have usually been conducted in broad-leaved forests (Čapek et al., 2021) and broad-leaved litter is more easily decomposed by soil microorganisms than coniferous litter, having a faster decomposition rate (Niu et al., 2020) and possibly a stronger driving effect on forest soil microbial communities. In contrast, compared with broad-leaved litter, coniferous litter may have a weaker driving effect on soil microbial communities due to its relatively difficult decomposition. In addition, we collected all aboveground litter during soil sampling in this study and tried to minimize the mix of litter in the collected soil samples, which relatively reduced the number of litter saprophytes in the samples. This may be another major reason why the driving effect of litter on the soil microbial communities was not as strong as we expected.

Roots are an important mediator of the plant-soil interface and can significantly influence the composition of soil microbial communities (Sweeney et al., 2021). These results do not support our second hypothesis, as the composition of the soil bacterial communities is significantly influenced by herbaceous roots in both the *P. asperata* and *S. chinensis* forests, while the composition of the soil fungal communities is significantly influenced mainly by shrub roots (Fig. 4). The main reasons for the differences in the factors controlling bacterial and fungal communities are probably as follows. (1) Bacteria and fungi differ in their ability to decompose substrates. In forest soil, bacteria are more

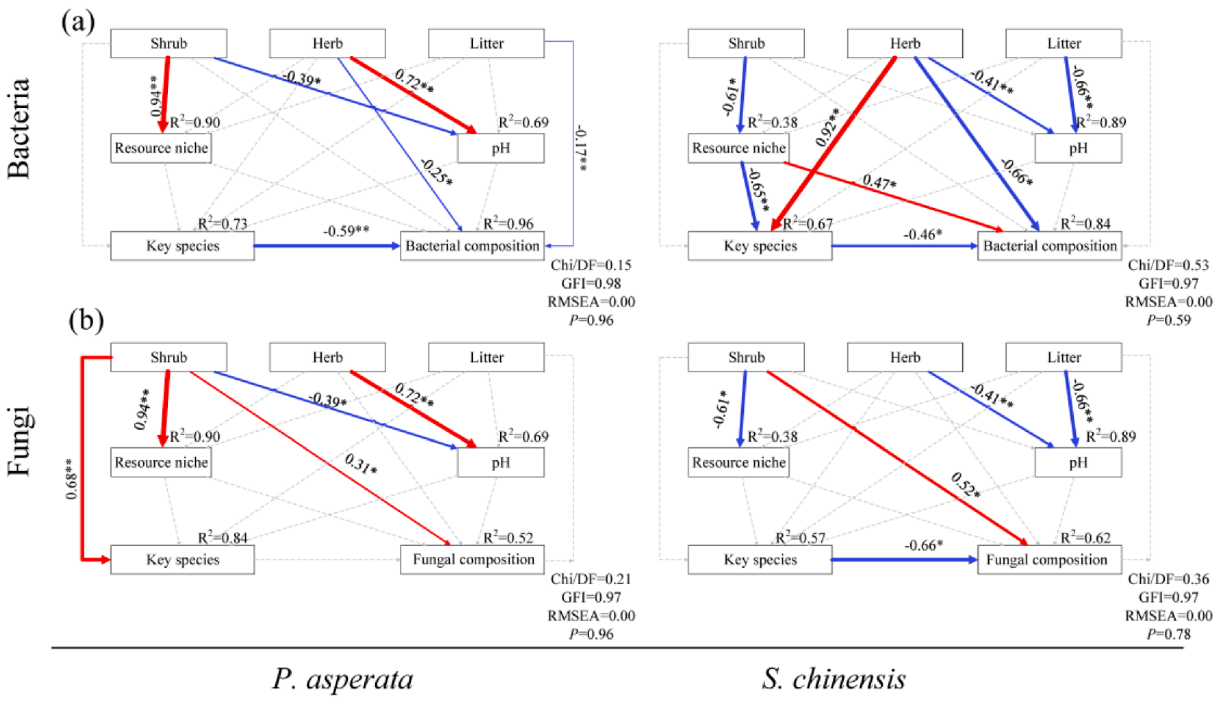

Fig. 4. The final structural equation for bacterial (a) and fungal (b) community composition affected by environmental variables. Red lines represent significant positive affect paths; blue lines represent a significant negative affect path ($P < 0.05$); and grey dash lines represent a nonsignificant correlation ($P > 0.05$).

likely to be driven by substrates with high nitrogen contents due to their weaker decomposition capacity (Brabcová et al., 2018), whereas fungi are able to use lignin and cellulose as substrates, which are relatively difficult to decompose and have low nitrogen contents (Hodge et al., 2010; Štursová et al., 2012). In this study, the C/N ratios of herbaceous roots in the coniferous forests were significantly lower than those of shrub roots (Table A1), which may lead to a situation in which resource competition between bacteria and fungi affects their use of shrubs versus herbaceous roots as their substrate: herbaceous roots were the main carbon source for bacteria, while shrub roots were the main carbon source for fungi. (2) Bacterial and fungal communities are influenced by the volume of their substrates. Through experiments with different volumes of litter, Angst et al. (2018) found that the number of bacteria increases significantly on smaller substrates, while fungi are mainly found on larger substrates. The volume of herbaceous roots is significantly smaller than that of shrub roots, which may also be one of the reasons for the different driving factors between bacterial and fungal communities. (3) Shrub roots can form symbiotic relationships with ectomycorrhizal fungi and can influence soil fungal communities by nutrient exchange or their mycelium (Nara and Hogetsu, 2004; Hewitt et al., 2020), resulting in a stronger driving effect on soil fungal communities utilizing shrub roots.

4.3. *Driving pathways for soil microbial communities utilizing shrub roots, herbaceous roots or litter*

For the driving pathways of the soil microorganisms utilizing litter, herbaceous roots or shrub roots, the results of the structural equation model (Fig. 4) show that shrubs, herbaceous roots and litter mainly drive soil microbial community compositions through direct effects in the *P. asperata* and *S. chinensis* forests. Among them, only shrub roots had significant effects on soil nutrients ($P < 0.05$). In addition, only the shrub roots in the *S. chinensis* forests had a significant indirect effect ($P < 0.05$) on bacterial community composition by controlling the soil-nutrient conditions and keystone species of the bacterial communities, while herbaceous roots and litter did not have a significant effect ($P > 0.05$) on soil nutrients. In contrast to our third hypothesis, as our studied sites were located in the natural reserves without artificial disturbance, the heterogeneity is relatively weak in the same forest type, which would led to an inconspicuous driving effect on microbial communities (Lladó et al., 2018). In addition, vegetation root exudates may also have been an unknown factor contributing in these results. A study by Wang et al. (2021) simulating the dynamic effects of root secretions on soil by adding glucose, glycine and oxalic acid into forest soils showed that even a small amount of root secretions could effectively stimulate microorganism activity and could inhibit its degradation, thus exerting a strong control on soil microbial community structure. However, from the perspective of nutrient return, for contributions to the carbon, nitrogen and phosphorus contents of forest soil more than one hundred years old, these relatively small amounts of bioactive substances may be negligible. Therefore, the effects of understorey vegetation roots on soil microbial communities are mostly direct rather than indirect through the alteration of soil physicochemical properties. In view of the above reasons, in future studies, we hope to further investigate the driving mechanisms of subalpine coniferous forest understorey vegetation on soil microbial communities by using controlled experiments or observations on longer time scales, combined with isotope labelling and metagenomic sequencing to elucidate the importance of understorey vegetation on soil microbial communities and forest ecosystem functions.

Furthermore, the relative abundance of the keystone species in microbial communities generally had a significant effect on community composition in this study, but only the keystone species in bacterial communities in the *S. chinensis* forests were significantly influenced indirectly or directly by shrub roots and herbaceous roots (Fig. 4). The keystone species in the bacterial community in the *S. chinensis* forests are Acidobacteria species, which are usually characterized by a wide range of ecological amplitudes and available substrates (Ward et al., 2009; Angst et al., 2018) and are dominant in relatively barren soil environments (Llado et al., 2017). Therefore, the diversified substrate sources for shrub and herbaceous roots can directly or indirectly contribute to their relative abundance and can influence microbial community composition through their dominance in the communities.

5. Conclusion

Shrub and herbaceous roots in natural subalpine coniferous forests have strong driving effects on soil microbial community compositions, and bacterial communities are significantly influenced mainly by herbaceous roots, while fungal communities are significantly influenced mainly by shrub roots, and litter has a relatively weak driving effect on microbial communities. Therefore, in natural forest ecosystem management, understorey vegetation, as an important regulatory factor on soil microbial communities, should also be included as an object of protection and management to maintain the stability of forest soil microbial community functions. For planted forests, based on the results of this study, we believe that shrubs and herbs should also be planted appropriately during the process of planting new forests to enhance the complexity of the understorey vegetation structure and to diversify the sources of microbial substrates, thus providing a diverse soil microenvironment for stabilizing soil microbial community diversity, better sustaining the feedback mechanisms in the plant-soil system, and improving the quality of planted trees and the sustainability of forest ecosystem service functions.

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.

Acknowledgments

The authors appreciate our colleagues who provided assistance with the fieldwork. This study was supported by the National Natural Science Foundation of China (U20A2008; 31870407), The Second Tibetan Plateau Scientific Expedition and Research (STEP) program (Grant No. 2019QZKK0302), and the Fundamental Research Funds for the Central Universities (No. 2020NZD03).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.119656.

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