Dynamic Microbial Network Structure and Assembly Process in Rhizosphere and Bulk Soils Along a Coniferous Plantation Chronosequence

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Abstract

Aims: During plantation development, microbial composition and diversity are critical for the establishment of plant diversity and multiple ecosystem functions. Here we aimed to evaluate the impacts of chronosequence and soil compartment on the bacterial and fungal community compositions, species co-occurrence, and assembly processes in forest ecosystem.

Methods: Soils were collected in rhizosphere and bulk soils along a *Pinus tabulaeformis* plantation chronosequence (15, 30 and 60 years old). The bacterial and fungal communities were determined using amplicon sequencing.

Results: The effect of stand age on the soil properties and microbial community structures was stronger than the effect of the soil compartment. In all soil samples, the dominant bacterial phyla were Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi. Basidiomycota, Ascomycota, and Mortierellomycota were the dominant fungal phyla. Higher turnover rates of soil microbial communities were observed in rhizosphere soil than in bulk soil. Dispersal limitation governed the bacterial and fungal community assembly in all soil samples, and the fungal community was more susceptible to dispersal limitation. The bacterial and fungal keystone species compositions in the rhizosphere had significant positive correlations with the soil total phosphorus and nitrite nitrogen and total nitrogen and total phosphorus, respectively, indicating their importance in soil nitrogen and phosphorus cycling. The complexity of bacterial networks increased along the chronosequence. Fungal network complexity did not show a clear age-related trend but increased from bulk soil to the rhizosphere.

Conclusions: During *Pinus tabulaeformis* plantation development, soil microbial assembly was less environmentally constrained due to an increase in resource availability.

1. Introduction

In terrestrial ecosystems, soil microorganisms are crucial regulators in biogeochemical cycling and thus play important roles in preserving soil quality and function (Dobrovol'skaya et al. 2015; Wang 2018). The temporal and spatial variations in soil microbial communities might influence their ecological function (Liu et al. 2019). The rhizosphere is considered a biological microsite in the soil tightly surrounding the roots. The physicochemical properties of rhizosphere soil are completely different from those of bulk soil. Within a microsite, diverse root exudates promote the growth of microorganisms and increase their activities, which in turn enhance mineral acquisition, pathogen protection and abiotic stress tolerance (Canarini et al. 2019; Khan et al. 2021; Pascale et al. 2020). The nutrients derived from plants play a crucial role in regulating microbial abundance, activity, and diversity and thus impact the complex interactions among plants, soil microorganisms, and soil (Pascale et al. 2020; Xiong et al. 2021). In bulk soil, most of the soil microorganisms are in a dormant state due to carbon limitation (Rüger et al. 2021). It was reported that increases in root exudates might offset carbon limitation and trigger the activity of these bulk soil microorganisms (Meier et al. 2020). However, different amounts and compositions of root

exudates only selected for and recruited the corresponding adapted plant species and genotype-specific microbes in the rhizosphere. The selection process led to significant differences in the species diversity and microbial interaction networks between the rhizosphere and bulk soil (Hartman and Tringe 2019). In recent years, most studies have focused on the microbial community structure and diversity of the rhizosphere as well as the driving factors in ecosystems, especially in forests (Goss-Souza et al. 2019; Yin and Yan 2020). The interactions among members of rhizosphere assemblages and the process of choosing niches based on functional traits in the rhizosphere remain unknown.

In an ecosystem, microorganisms live together and form complex co-occurrence networks based on various species interactions (Faust and Raes 2012). The species interactions related to potential intertaxa relationships and niches shared by community members are essential to understanding the role of soil microorganisms in maintaining ecosystem function and stability. Stochastic and deterministic processes are two fundamental ecological processes that shape communities in numerous habitats (Luan et al. 2020). The deterministic process is governed by environmental filtering, host selection (Vályi et al. 2016), and various biological interactions (e.g., predation, competition, mutualism, commensalism, and amensalism) (Zhou and Ning 2017), which result in obvious changes in community structure, especially higher β diversity under divergent environmental conditions. Stochastic processes are involved in historical contingency (e.g., priority effects), ecological drift, and/or dispersal limitation that result in community structure patterns indistinguishable from random assemblages (Chase 2010). Both processes are essential for the assembly process of most communities (Liu et al. 2021). At smaller scales, the contribution of stochastic processes may overwhelm deterministic selection due to the reduction in habitat heterogeneity from bulk soil to the rhizosphere (Chase et al. 2014). Mendes et al. (2014) reported that the microbial community in the rhizosphere was filtered by a deterministic niche; in contrast, stochastic processes seemed to shape the microbial community in bulk soil. Therefore, the contribution of deterministic or stochastic processes in different habitats remains unclear. In addition, soil properties such as soil pH (Fan et al. 2018a), organic matter (Dini-Andreote et al. 2015; Tripathi et al. 2018), and total phosphorus (Xiong et al. 2010) are important factors controlling the microorganism assembly process. Plants can also impact soil microbial communities through rhizosphere effects (Li et al. 2019), host-microbial interactions (Hassani et al. 2018), and the indirect effects of soil physicochemical properties (Pugnaire et al. 2019). Plant growth (Guo et al. 2021), composition (Zhang et al. 2021), and diversity (Dang et al. 2018) were reported to affect soil microbial communities. It was deduced that plants might be an important driving factor affecting soil microbial community assembly.

The Loess Plateau, a semiarid and arid area located in Northwest China, has suffered serious land degradation due to perennial soil erosion. To mitigate soil erosion, extensive afforestation was carried out in the 1970s. *Pinus tabulaeformis* is widely planted in this region due to its adaptability to drought, cold, and barren soil (Bahram et al. 2013). There have been many reports about the effects of *Pinus tabulaeformis* plantations on soil nutrients, structure, and microbial communities (Dang et al. 2018; Song et al. 2021). However, these studies neglected the soil microbial assembly processes based on functional traits and the main driving factors of those processes. Therefore, in this study, we aimed to (1) investigate the changes in the microbial community structure, species co-occurrence networks, and assembly

processes in rhizosphere and bulk soils along an age gradient of *Pinus tabulaeformis* plantations and (2) elucidate the underlying assembly mechanisms, e.g., the microbial co-occurrence relationships, the microbial ecological coexistence strategies, and the ecological assembly process shaping the bacterial and fungal communities. We hypothesized that (1) the rhizospheric microbial community structure and diversity, species co-occurrence networks, and keystone taxa differ from those of bulk soil due to the differences in biotic interactions and soil properties and that (2) the relative importance of deterministic and stochastic processes might change along a chronosequence because the interaction of plant communities and soil properties among different stand ages could exert selective pressure on soil microbial communities.

2. Materials And Methods

2.1. Site description and soil sampling

The study site was located at the Guanzhuang State-owned Ecology Experimental Forest Farm (109°49'35.4"E, 35°45'1.0"N) in Huanglong County (Yan'an, Shaanxi, China). This area has a typical temperate continental semiarid to semihumid climate, with an altitude of 1,074–1,173 m, a mean annual precipitation of 550 mm and a mean annual temperature of 9.2°C. Precipitation occurs mainly from July to September, and the drought season lasts from winter to early summer of the next year. The soil is classified as a Calcaric Cambisol based on the Food and Agriculture Organization of the United Nations (FAO) classification.

The study was carried out in September 2019. Three *Pinus tabulaeformis* plantations aged 15, 30 and 60 years with similar geographical characteristics were selected as experimental sites (Table S1). Millet (*Setaria italica*) and corn (*Zea mays*) were the main crops before afforestation. To exclude spatial dependence, these experimental sites were less than 10 km apart from each other. Three 20 m × 20 m plots were randomly selected at each experimental site. Rhizosphere and bulk soils, as the two soil compartments, were collected from each plot. Rhizosphere soil was defined as soil that is tightly attached to the roots (Liu et al. 2018). Five well-growing trees in each plot were selected randomly to obtain a representative rhizosphere soil sample. For rhizosphere soil sampling, three equidistant points were located around the root system of each selected tree. At each point, the soil was dug to a depth of 20 cm to locate the roots, and then the soil that remained attached to the roots after mild mechanical disturbance was gently brushed with a sterile soft-bristled paintbrush (Philippot et al. 2013). In total, 15 points from five trees were mixed into one rhizosphere soil sample from each plot. The distance between sampling points and individual trees was approximately 1.5 m. At the same time, bulk soil samples were collected from each plot. After the litter layer was removed, nine soil cores were collected using a soil auger (5 cm in diameter) along an S-shaped pattern and then mixed into one bulk soil sample from each plot. A total of 18 samples (three plots × three stand ages × two soil compartments) were collected. All soil samples were sieved (2 mm) and divided into two portions. One portion was stored at -80°C for DNA analysis. The other portion was air dried for soil property analyses.

2.2. Soil property analyses

Soil pH was measured in 1:2.5 (soil/water, w/v) ratio suspensions using a pH electrode. The soil moisture content (SMC) was measured by oven-drying 10 g of fresh soil at 105°C for 48 h. Soil organic carbon (SOC) was measured via the potassium dichromate oxidation method. Total nitrogen (TN) was measured by the Kjeldahl method (Bremner and Mulvaney 1982). Total phosphorus (TP) and available phosphorus (AP) were measured by the molybdenum blue method after extraction with H₂SO₄-HClO₄ and sodium bicarbonate, respectively (Olsen and Sommers 1982). Nitrate nitrogen (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-N) were measured by a continuous flow autoanalyzer (AA3, Germany) after extraction with 2 M KCl (Zhang et al. 2021).

2.3. DNA extraction and Illumina sequencing

Soil total DNA was extracted by a Fast DNA spin kit for soil (MP Biomedicals, Cleveland, USA). A NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, North Carolina, USA) and 1.2% agarose gel electrophoresis were used to assess the quantity and quality of DNA. The primer pairs 338F/806R (Muyzer et al. 1993) and ITS 5F/ITS 1R (Ji et al. 2021) were used to amplify the bacterial 16S rRNA gene and fungal ITS gene, respectively. PCR was performed in a volume of 25 µL containing 5 µL GC buffer (5×), 5 µL reaction buffer (5×), 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 2 µL dNTPs (2.5 mM), 2 µL DNA template, 0.25 µL Q5 High-Fidelity DNA Polymerase (5 U µL⁻¹), and 8.75 µL ddH₂O. The reaction conditions were as follows: 2 min of initial denaturation at 98°C, 25 cycles of 15 s at 98°C, 30 s at 55°C, and 30 s at 72°C, and a final elongation for 5 min at 72°C. The purified PCR amplicons were sequenced on a paired 250-bp Illumina MiSeq PE 250 platform (Illumina Corporation, USA).

Microbial raw sequences were analyzed using QIIME 2 2019.4 (Bolyen et al. 2019). With the use of the DADA2 pipeline, sequences were quality filtered, denoised, and merged, and the chimeras were removed (Callahan et al. 2016). Nonsingleton amplicon sequence variants (ASVs) were used to construct the phylogenetic tree. Taxonomic classification of ASVs was assigned using the SILVA (release 132, <http://www.arb-silva.de>) and UNITE (release 8.0, <https://unite.ut.ee>) databases for bacteria and fungi, respectively. A total of 91 906 and 79 700 sequences per sample of bacteria and fungi were generated after rarefaction.

2.4. Co-occurrence network analysis

To reveal species coexistence across the chronosequence and soil compartment, co-occurrence networks of bacteria and fungi were constructed based on Sparse Correlations for Compositional data (SparCC). According to a previous method of combining samples in data analysis (Qiu et al. 2021; Zheng et al. 2021; Wang et al. 2022), six samples (combining bulk and rhizosphere samples) were used in co-occurrence networks of each chronosequence, and nine samples (combining samples of three stand ages) were contained in co-occurrence networks of different soil compartments. ASVs with average relative abundances greater than 0.05% and that occurred in more than 50% of samples in each corresponding subdataset were selected (Jiao et al. 2016). The correlations of SparCC < 0.7 and > -0.7

and $P > 0.01$ were removed. To further describe the topological properties of networks, a series of indexes (e.g., average degree, average path length, network diameter, graph density, clustering coefficient, and modularity) were calculated using the 'igraph' package in R (<https://www.R-project.org/>). The networks were visualized using the Gephi platform (<https://gephi.org/>).

In addition, according to the within-module connectivity (Z_i) and among-module connectivity (P_i), the nodes were divided into four categories: (1) network hubs (highly connected within the entire network, $Z_i > 2.5$; $P_i > 0.62$); (2) module hubs (high connectivity within a module, $Z_i > 2.5$; $P_i < 0.62$); (3) connectors (link modules, $Z_i < 2.5$; $P_i > 0.62$); and (4) peripherals (less connectivity with other species, $Z_i < 2.5$; $P_i < 0.62$) (Deng et al. 2012; Poudel et al. 2016). Nodes belonging to network hubs, module hubs and connectors were defined as the keystone species owing to their crucial roles in networks (Guimera and Amaral 2005; Toju et al. 2018).

2.5. Community assembly analysis

The weighted β nearest taxon index (β NTI) and Bray-Curtis-based Raup-Crick (RC_{bray}) were used to evaluate the assembly processes of microbial communities by a null model approach (Stegen et al. 2013). The β NTI was calculated by determining the standard deviation between observed data and the null distribution of phylogenetic β -diversity metrics using the "picant" package in R. The RC_{bray} value was quantified by estimating the standard deviation between the empirical data and the null distribution of taxonomic β -diversity metrics. The combination of β NTI and RC_{bray} was used to quantify the relative importance of deterministic (e.g., heterogeneous selection and homogeneous selection) and stochastic processes (e.g., dispersal limitation, homogenizing dispersal and drift). β NTI > 2 or < -2 represent heterogeneous selection or homogeneous selection, respectively. $|\beta$ NTI| < 2 with $RC_{\text{bray}} > 0.95$ or $RC_{\text{bray}} < -0.95$ represent the contribution of dispersal limitation or homogenizing dispersal, respectively, and $|\beta$ NTI| < 2 with $|RC_{\text{bray}}| < 0.95$ represents the contribution of drift (Stegen et al. 2013). The same method of combining soil samples with co-occurrence network analysis was used in community assembly analysis.

The neutral model was further used to determine the effects of stochastic processes on community assembly (Sloan et al. 2006). This model predicts the relationship between the frequency of observed species occurring in given localities and their abundance distribution in the metacommunity. The migration rate (m) represents the probability that an individual locally lost by chance would be replaced by dispersal from the metacommunity. A higher m value indicates that the microbial community is less restricted by dispersal. Levin's niche breadth index was calculated to help illustrate the patterns of deterministic processes and stochastic processes and their contribution to the microbial community under specialized habitats. A wider niche breadth indicates a microbial group with higher metabolic flexibility at the community level (Pandit et al. 2009). The "stats4" and "hmisc" packages in R were employed to carry out neutral model analysis, and the "spaa" package in R was used to calculate the niche breadth.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) and *t*-tests were used to estimate the differences in the soil physicochemical properties, microbial alpha diversity, and niche breadth along the chronosequence and between the soil compartments, respectively. Two-way ANOVA was used to estimate the effects of the chronosequence and soil compartment on the soil physicochemical properties and microbial alpha diversity. Spearman's rank analysis was used to estimate the correlations of the soil physicochemical properties with dominant microbial taxa and alpha diversity. One- and two-way ANOVAs, *t*-tests and Spearman's rank analysis were conducted using SPSS 22.0 software. Principal coordinate analysis (PCoA) and two-way permutation multivariate ANOVA (PERMANOVA) with 999 permutations were used to determine the difference in the microbial community structure across the chronosequence and between the soil compartments using the "cmdscale" and "adonis" functions of the "vegan" package in R. Time-decay relationships (TDRs) of community similarity versus time in the bulk soil and rhizosphere were performed by fitting a linear model based on the community similarity distance (1 – Bray-Curtis distance) and pairwise temporal interval. Mantel tests were used to unravel the relationship between soil physicochemical properties and microbial community composition, keystone species composition and β NTI. Furthermore, multiple regression on distance matrices (MRM) analysis was performed to understand which soil variables were associated with the keystone species composition of bacteria and fungi in the rhizosphere soil using the 'MRM' function of the "ecodist" package in R.

3. Results

3.1. Soil properties

Most of the soil physiochemical properties showed significant differences between the rhizosphere and bulk soil along the chronosequence (Table S2). The contents of SOC, SMC and TN increased gradually along the chronosequence and peaked in the 60-year-old plantation. However, soil pH showed an opposite trend. The SOC, AP, and C:N ratios were higher in the rhizosphere soil than in the bulk soil. Additionally, compared to the bulk soil, the pH in the rhizosphere soil was lower and more stable. Two-way ANOVAs indicated that the interaction between the chronosequence and soil compartment was significant for soil pH, TN, and NO_3^- -N.

3.2. Microbial community composition and diversity

In total, 62 771 bacterial ASVs and 6 906 fungal ASVs were obtained. Across all samples, eight bacterial phyla/classes, namely, Acidobacteria (21.66%), Actinobacteria (19.21%), Gammaproteobacteria (11.81%), Chloroflexi (10.60%), Alphaproteobacteria (9.32%), Gemmatimonadetes (7.96%), Deltaproteobacteria (5.37%) and Rokubacteria (4.62%), dominated the bacterial communities (Fig. S1). The most abundant fungal class was Agaricomycetes (65.11%), followed by Sordariomycetes (3.60%), Pezizomycetes (3.44%), Archaeorhizomycetes (1.84%), Mortierellomycetes (1.52%), and Leotiomyces (1.29%) (Fig. S1). The relative abundance of Alphaproteobacteria and Gammaproteobacteria in the rhizosphere soil increased gradually along the chronosequence and was significantly higher than that in the bulk soil. The abundance of all of the detected fungi except Agaricomycetes and Sordariomycetes in the bulk soil

decreased along the chronosequence. Pezizomycetes, Mortierellomycetes, and Eurotiomycetes were more abundant in the rhizosphere soil than in bulk soil from 30 to 60 years.

For bacteria, the alpha diversity (Shannon and Chao1) showed no significant differences along the chronosequence but significantly ($P < 0.05$) increased from the bulk soil to the rhizosphere. The chronosequence and soil compartment had no effects on the Shannon diversity and richness of the fungal communities (Table S3).

PCoA showed that the bacterial and fungal compositions were clearly separated among the three forest ages (Fig. 1). The first two axes explained 41.21% and 41.62% of the total variation in the soil bacterial and fungal communities, respectively. Two-way permutational multivariate analysis of variance (PERMANOVA) revealed that the chronosequence had a stronger influence than the soil compartment on the bacterial and fungal community structures (Fig. 1). The Mantel tests indicated that the bacterial community structure had significant correlations with SMC, SOC and AP. SMC was the key factor correlated with the fungal community structure (Table S4). The dominant bacterial taxa and diversity appeared to have a stronger interrelationship than fungi with the soil properties (Table S5).

Significant TDRs were found for the soil bacterial community in the different soil compartments (Fig. 2). The slopes of the bacterial communities in the rhizosphere soil were significantly steeper than those in the bulk soil, revealing rapid community turnover in the rhizosphere soil. However, a significant TDR was observed for fungal communities only in the rhizosphere soil, which suggested that the turnover rates of fungal communities changed dramatically along the chronosequence.

3.3 Microbial community networks

Multiple network topological properties revealed that the co-occurrence patterns of the bacterial and fungal networks changed constantly along the chronosequence (Fig. 3 and Table S6). All networks showed strong power-law distributions of degrees, indicating typical scale-free degree characteristics (Fig. S2). For bacteria, the network diameter and average path length decreased along the chronosequence, while the number of edges, graph density, average degree, and clustering coefficient tended to increase, suggesting a more connected network structure with stand age (Table S6). In addition, the positive correlations in the bacterial network in 60-year-old plantations increased by 9.98% and 12.55% compared with those in 30-year-old and 15-year-old plantations, respectively. This result indicated that the conflicting interactions (the competition among the bacterial species) decreased gradually along the chronosequence (Table S6). For fungi, the clustering coefficient, graph density, and average degree increased from 15 years to 30 years and later decreased at 60 years. The trends of the positive correlations were the opposite of those found in bacterial networks, indicating that the increase in stand age might lead to more conflicting interactions (Table S6). In addition, the bacterial networks were similar between the rhizosphere and bulk soil. However, fungal networks were different between the different soil compartments. The rhizosphere soil had higher numbers of nodes (86) and edges (188) and a higher average degree (4.37) than bulk soil, suggesting more complex relationships among the fungal species.

There were a total of 321 connectors, 13 module hubs (network hub ASVs), and 2 network hubs identified in the bacterial networks (Fig. S3 and Table S7). However, the numbers and their taxonomic affiliations differed among the three stand ages. The greatest number of connectors was observed in the 15-year-old plantation, which mainly belonged to Acidobacteria, Actinobacteria, Chloroflexi, and Gammaproteobacteria. There were three module hubs belonging to Alphaproteobacteria (1 ASV) and Chloroflexi (2 ASVs). In the 30-year-old plantation, 83 connectors and 5 module hubs were detected among various taxonomic groups. The connectors were mainly related to Acidobacteria and Gammaproteobacteria. Module hubs were related to Acidobacteria (1 ASV), Actinobacteria (2 ASVs) and Alphaproteobacteria (2 ASVs). In the 60-year-old plantation, the 103 connectors were mainly assigned to Acidobacteria, Gemmatimonadetes, and Rokubacteria. The 5 module hubs were assigned to Actinobacteria (1 ASV), Chloroflexi (1 ASV), Deltaproteobacteria (1 ASV) and Gammaproteobacteria (2 ASVs). The only 2 network hubs detected at this site belonged to Actinobacteria and Alphaproteobacteria. In addition, the number of connectors increased from the rhizosphere (63) to the bulk soil (77). No module hubs or network hubs were identified in the rhizosphere soil. In the fungal networks, the number of connectors decreased gradually with stand age (Fig. S3). A higher number of connectors was observed in the rhizosphere (39) than in the bulk soil (32). These connectors originated from a variety of taxonomic groups and were mainly related to Saccharomycetes, Sordariomycetes, Agaricomycetes, and Mortierellomycota (Table S7). A module hub (1 ASV) was identified only at the 30-year-old site, which was related to Tremellomycetes. No network hub was detected in the fungal networks.

The correlation between soil properties and keystone species suggested that bacterial keystone species in the rhizosphere had significantly positive correlations with TP and NO_3^- -N, and fungal keystone species in the rhizosphere were positively and significantly correlated with SOC, TN, and TP (Table S8). The MRM models revealed that TP and NO_3^- -N had strong effects on bacterial keystone species in the rhizosphere, and fungal keystone species in the rhizosphere were strongly affected by SMC, TN, TP, and NO_3^- -N (Table 1).

Table 1
Multiple regression with distance matrices (MRM) to estimate the contribution of soil properties on the rhizospheric keystone species composition.

Soil properties	Bacterial keystone taxa	Fungal keystone taxa
	$R^2 = 0.506$	$R^2 = 0.642$
	$P < 0.05$	$P < 0.01$
pH	-0.240	0.484
SMC	ND	-0.064*
SOC	0.003	0.009
TN	-0.013	0.220*
TP	0.210*	0.590*
NO ₃ ⁻ -N	0.032*	0.150*
NH ₄ ⁺ -N	-0.031	ND
AP	ND	ND
C/N	ND	-0.003
<i>Note.</i> ND, not determined. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.		

3.4. Microbial assembly processes

Figure 4 shows that stochastic processes, especially dispersal limitation, dominated both bacterial and fungal community assembly. However, the relative contribution of stochastic and deterministic processes in explaining the variation in microbial community assembly changed with stand age. The relative importance of dispersal limitation decreased by 33.33% from the 15-year-old to the 30-year-old plantation and then increased by 26.67% for the bacterial community assembly, while the reverse trend was observed for the fungal community assembly. Regarding the soil compartment, dispersal limitation explained 55.56% of the variation in the bacterial community assembly in the rhizosphere soil, which was the same as that in the bulk soil. The contribution of dispersal limitation to the bacterial community assembly in the rhizosphere soil was the same as that in the bulk soil. However, dispersal limitation explained more of the variation in the fungal assembly patterns, increasing from 77.78% in the bulk soil to 88.89% in the rhizosphere soil. Homogeneous selection, homogenizing dispersal, drift, and heterogeneous selection slightly affected the assembly of the microbial community. In addition, β NTI and soil properties had no significant correlation, indicating less environmental filtering in controlling microbial community assembly (Table S9).

The migration rates of bacteria were lowest in the 30-year-old plantation, and fungi showed the opposite trend, indicating that the bacterial community was more limited and that the fungal community was less limited by dispersal in the 30-year-old plantation than in the 15- and 60-year-old plantations. In addition, the bacterial migration rates tended to be higher in the rhizosphere than in the bulk soil. The fungal migration rates were contrary to the bacterial migration rates, which suggested that the fungal community was more limited by dispersal in the rhizosphere than in the bulk soil (Fig. 5). Similarly, the habitat niche breadth of bacteria and fungi exhibited the same trends as the migration rates in the different plantations and soil compartments.

4. Discussion

4.1. Microbial community diversity and structure

Our data showed that there was no significant difference in the alpha diversity of the soil microbial community during the development of the *Pinus tabulaeformis* plantations. Generally, soil microbial diversity and richness increase with vegetation succession due to the increase in understory vegetation composition and diversity (Xu et al. 2020; Zhong et al. 2020). However, in the vegetation succession process, the changes in the microbial community lag behind those in the vegetation and soil (Niu et al. 2007). The microbial community has high resilience and resistance to environmental disturbance (Griffiths and Philippot 2012). Furthermore, the dominant trees determine the soil microbial community composition (Urbanová et al. 2015). The microbial community structure remained relatively stable throughout the three stand ages. Therefore, the alpha diversity of the soil microbial communities did not change.

Soil properties regulated the dominant groups in the soil bacterial and fungal communities and thus led to differences in the soil microbial community composition (Liu et al. 2018). In the present study, Proteobacteria, Acidobacteria, Actinobacteria and Chloroflexi were the dominant bacterial phyla at all experimental sites. The relative abundance of Proteobacteria increased gradually with stand age and peaked in the 60-year-old plantation. Proteobacteria are defined as copiotrophic bacteria that have high nutritional requirements. Actinobacteria belong to the oligotrophic group and are adapted to growth in stressful environments (Zhang et al. 2021). The development of *Pinus tabulaeformis* plantations was favorable for labile substrate input and soil nutrient accumulation and thus promoted the growth of copiotrophic bacteria. The decrease in the relative abundance of Actinobacteria might also be demonstrated from the opposite perspective. Basidiomycota and Ascomycota dominated fungal communities in the *Pinus tabulaeformis* plantation. The relative abundance of Basidiomycota in the 60-year-old plantation increased by 27.80% compared to that in the 15-year-old plantation. This might be because Basidiomycota are responsible for the decomposition of lignin and aromatic compounds in a forest ecological system (Dang et al. 2018). The plant litter source and amount increase with stand age, and the needles of *Pinus tabulaeformis* are rich in lignin and cellulose, which might be degraded by Basidiomycota. In addition, *Pinus tabulaeformis* is an ectomycorrhizal tree species. Most ectomycorrhizal fungi belong to Basidiomycota, which increase the availability of water and nutrients (Varenus et al.

2016). The relative abundance of Ascomycota decreased with stand age. Recent studies have revealed that Ascomycota thrive under oligotrophic conditions, especially on low carbon substrates (Dang et al. 2018). Therefore, the higher abundance of Ascomycota revealed that the fungal communities adapted to poor soil nutrient conditions. The variation in Basidiomycota and Ascomycota with plantation development indicated that the growth of vegetation changed the soil nutrient conditions. In addition, plant roots select the specific microorganisms that colonize the rhizosphere to enhance nutrient acquisition and protect against pathogenic bacteria (Dennis et al. 2010). In the present study, the decrease in soil available phosphorus with stand age indicated that the requirement for phosphorus continues to increase in plants as plantation development. It was reported that Alphaproteobacteria might produce and secrete phosphomonoesterase by regulating the expression of the *phoD* gene and thus improve phosphorus availability (Mise et al. 2018). Actinobacteria are also crucial for phosphorus solubilization (Sahu et al. 2007). The higher relative abundance of Alphaproteobacteria and Actinobacteria in the rhizosphere soil may reflect the plant demand, and these bacteria may facilitate plant phosphorus absorption.

The temporal turnover of soil microbial communities is a vital indicator of the successional dynamics of biological communities owing to its tight association with ecological functions (Zhang et al. 2019). Our results showed that the bacterial and fungal succession rates in the rhizosphere were higher than those in the bulk soil. This finding supported the first hypothesis that temporal turnover decreased from the rhizosphere soil to the bulk soil. A possible reason might be that plentiful carbon substrates are derived from carbon fixed by photosynthesis, root exudates, and plant residue decomposition in the rhizosphere (Song et al. 2019). Consequently, the microbial activity, turnover, and copiotrophic bacteria (e.g., Proteobacteria) increased significantly in this zone. Furthermore, a series of specific bacterial and fungal that are responsible for improving nutrient absorption and protecting against pathogens. The extensive changes in the temporary turnover of the soil microbial community depended on variations in plant and soil nutrient status during plant growth and development.

4.2. Microbial species co-occurrence networks and keystone species

Co-occurrence network analysis reflects the assembly principles and interspecies relationships of soil microbial communities and thereby provides a greater understanding of the relationships between ecosystem complexity and stability (Zhu et al. 2020). In the present study, the complexity of the bacterial networks tended to increase markedly with increasing stand age (Fig. 3 and Table S6), which led to higher community stability. Furthermore, the bacterial network in the 60-year-old plantation had more positive correlations than those in the 15- and 30-year-old plantations (Table S6), revealing that the competition among bacterial species decreased gradually during plantation development. This pattern might be attributed to the increase in resource availability and niche availability across the plantation chronosequence (Fan et al. 2018b). However, the fungal network structure showed no clear age-related trends from 15 years to 60 years (Fig. 3 and Table S6), indicating that soil environmental heterogeneity had minor effects on the fungal community dynamics in our study. In contrast, fungal networks in the

rhizosphere soil had more nodes and edges than those in the bulk soil, suggesting an increase in network complexity. Abundant ectomycorrhizal (ECM) fungi form mutualistic symbioses with *Pinus tabulaeformis* and are regarded as key organisms involved in plant nutrient and carbon cycling (Wang et al. 2021), driving fungal communities to enhance their interspecific communication in the form of complex co-occurrence networks during plantation development.

Generally, keystone species are connectors, module hubs, and network hubs that play critical roles in network structure and regulate important ecosystem functions (e.g., soil organic matter metabolism, nitrogen metabolic pathway) within the whole community (Xun et al. 2021). Variations in keystone species might reflect variations in the interspecies relationship to some extent, thus impacting soil nutrient cycling. In the present study, the keystone species changed with stand age and differed in the rhizosphere and bulk soils due to the interactions between vegetation development and soil properties (Table S9). The specific functions of the keystone species also changed. The bacterial network hub Actinobacteria has the ability to decompose a range of recalcitrant organic substances (e.g., cellulose, organic acids, polysaccharides) (de Menezes et al. 2015) and to produce antibiotics (e.g., streptomycin, aureomycin, and terramycin) (Dhanasekaran and Jiang 2016). Alphaproteobacteria mainly include Rhizobiales, which supply phytohormones and a variety of nutrients to plants and perform nitrogen fixation through symbiosis with their host (Erlacher et al. 2015). The bacterial module hub Chloroflexi, composed of phototrophic bacteria, has anoxygenic photosynthetic activity (Hanada 2014). This phylum can also ferment carbohydrates and degrade some complex organic polymeric compounds (Speirs et al. 2015). Deltaproteobacteria and Gammaproteobacteria belong to Proteobacteria, which grow quickly by utilizing carbon substrates derived from roots (Fan et al. 2018b). The bacterial connector Gemmatimonadetes has been reported to have chlorophyll-based reaction centers that might be responsible for the synthesis of carbon (Zeng et al. 2014). Rokubacteria participate in the TCA cycle, glycolysis, and complex transport and thus play important roles in carbon cycling (Becraft et al. 2017). The fungal module hub Tremellomycetes can effectively resist drought by cryptobiosis (Esser 2014). The fungal connectors belong to Dothideomycetes (e.g., Saccharomycetes and Sordariomycetes) (Luo et al. 2019). Agaricomycetes (Sterkenburg et al. 2018) and Mortierellomycota (Loit et al. 2020) have the capacity to enhance nutrient availability by degrading cellulose, promoting nutrient release from litter, resisting disease, and cooperating with other species. In summary, during the development of plantations, these keystone species had a variety of metabolic functions and thus affected the interspecies relationships in the whole community.

4.3. Microbial assembly process

Revealing the mechanisms mediating microbial community assembly is a crucial topic in microbial ecology (Zhou and Ning 2017). It is important to clarify how assembly processes respond to environmental factors, at both temporal and spatial scales. In our study, stochastic processes were vitally important for microbial assembly during *Pinus tabulaeformis* plantation development in both the rhizosphere soil and bulk soil, indicating weak selection from environmental variation. One possible explanation is that the increase in soil nutrients (e.g., SOC and TN) (Table S2) provided diverse and

alternative nutrient resources for taxa, which widened the habitat niche breadth and thus weakened environmental filtering (Shu et al. 2021). Additionally, Chen et al. (2020) found that body size might be used as an important indicator to evaluate the dispersal capacity of microorganisms. According to the “size plasticity” (Farjalla et al. 2012) and “size dispersal” hypotheses (Shurin et al. 2009), bacteria might be less niche limited and thus dispersal limited than fungi. In the present study, the fungal community in the rhizosphere soil was more affected by dispersal limitation than the bacterial community, supporting these hypotheses. However, the bacterial community had lower dispersal limitation in the 30-year-old plantation, which was attributed to the selectivity of the habitat niche (Chen et al. 2020). When bacteria disperse to a new niche, the occupancy of the niche is restricted, and the niche is not colonized by all individuals, revealing limited dispersion.

5. Conclusions

Our results showed that the soil microbial communities in the rhizosphere differed significantly from those in the bulk soil during *Pinus tabulaeformis* plantation development. The influences of afforestation age on the soil microbial communities were greater than those of the soil compartments. The complexity and connection of the bacterial network structure increased with stand age but showed no clear variation between the soil compartments. The complexity of the fungal network structure did not show a clear age-related trend but increased from the bulk soil to the rhizosphere. The stochastic process and dispersal limitation played an essential role in the microbial community assembly along the chronosequence regardless of the soil compartment. Bacterial communities with a wider niche breadth were less dispersal limited than fungal communities. These results indicated that plantation development might enhance forest ecosystem stability, and thus reduce the influence of environmental filtering on soil microbial assembly. Our results highlighted the dynamics of the microbial assembly process during afforestation. Further studies should focus on the linkage between the assembly of the soil microbial community and its functional properties, which will be critical for understanding the mechanism by which soil microbial communities mediate ecosystem function.

Declarations

CRedit authorship contribution statement

Conceptualization: Ying Wang, Liguang Dong, Xuan Yu. Data curation: Ying Wang, Min Zhang, Xuan Yu. Formal analysis: Xiaoxiong Bai, Jiawen Zhang, Xuan Yu. Funding acquisition: Xuan Yu. Investigation: Ying Wang, Min Zhang, Jiawen Zhang, Xiaoxiong Bai, Xuan Yu. Methodology: Ying Wang, Xuan Yu, Liguang Dong. Project administration: Xuan Yu. Software: Ying Wang, Min Zhang. Supervision: Jiawen Zhang, Liguang Dong. Visualization: Ying Wang, Liguang Dong, Min Zhang. Writing – original draft: Ying Wang, Xuan Yu. Writing – review & editing: Ying Wang, Liguang Dong, Min Zhang, Xuan Yu.

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.

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Figures

Figure 1

Principal co-ordinates analysis (PCoA) of soil bacterial (a) and fungal (b) community composition based on Bray-Curtis distance. 15Y, 15 years-old; 30Y, 30 year-old; 60Y, 60 year-old; BS, bulk soil; RS, rhizosphere soil. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2

Time-decay relationships for bacterial (a) and fungal (b) community across the chronosequence in different soil compartments. BS, bulk soil; RS, rhizosphere soil. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3

Bacterial (a-e) and fungal (f-j) co-occurrence networks across the chronosequence and soil compartment. Nodes represent ASVs. Edges indicate significant correlations (SparCC greater than 0.7 or less than -0.7 with P value < 0.01). The colors of nodes indicate different taxa they belonging to. The size of each node represents its relative abundance. 15Y, 15 years-old; 30Y, 30 year-old; 60Y, 60 year-old; BS, bulk soil; RS, rhizosphere soil.

Figure 4

The relative importance of ecological processes bacterial (a, b) and fungal (c, d) community assembly across the chronosequence and soil compartment. 15Y, 15 years-old; 30Y, 30 year-old; 60Y, 60 year-old; BS, bulk soil; RS, rhizosphere soil; HeS, heterogeneous selection; HoS, homogeneous selection; DL, dispersal limitation; HD, homogenizing dispersal; DR, drift.

Figure 5

Multiple biological models showing microbial community assembly across the chronosequence and soil compartment. Migration rates (m) value based on neutral model of bacteria and fungi across the chronosequence (a) and soil compartment (b). Habitat niche breadth of bacterial (c, d) and fungal (e, f) communities across the chronosequence and soil compartment. 15Y, 15 years-old; 30Y, 30 year-old; 60Y,

60 year-old; BS, bulk soil; RS, rhizosphere soil. Different letters indicate significant difference with ($P < 0.05$). ns, not significantly different; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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