Effects of forest age and seasonal changes on soil microbial community diversity in Chinese fir plantations

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Research Article

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Abstract

Understanding changes in the distribution patterns and diversity of soil microbial communities from the perspectives of age-related changes, seasonal variations, and the interaction between the two factors can facilitate the management of plantations. In Chinese fir plantations, we collected soils from different depths in overmature forests (OMF), mature forests (MAF), near-mature forests (NMF), middle-aged forests (MIF), and young forests (YOF) in summer, autumn, and winter in China's subtropical regions. As the forests developed, the fungal community recovered high diversity in MAF and OMF while bacterial indicators continued to decline. Bacterial communities were more diverse in summer and fungal communities were more diverse in winter. Differences between seasons were mainly reflected in average and maximum temperature indicators. Bacteria clustered by season, while fungi clustered by developmental stage, showed differences in distribution and structure at different taxonomic levels. The fungal community is a more important indicator of soil fertility maintenance, increasing with the increase of forest age, which suggested that extending tree cultivation time could improve the soil fertility of plantations. In different seasons and different ages, we found some species worthy of attention, including Actinobacteria with high abundance in summer and Bacteroidetes in autumn in overmature forests, and Firmicutes in summer in young forests. In autumn, species like Arcopilus and Tolypocladium in near-mature and over-mature forests also have the significance of further research, which may be key species for soil fertility restoration.

1. Introduction

Forest soil is essential for plantation forest cultivation, providing the nutrients and water required for plant growth. Research has found that soils are important hotspots for soil bacteria, protists, and functional gene diversity [1]. Bacteria and fungi are important for maintaining global biodiversity and biomass. In addition, microorganisms play a crucial role in biogeochemical carbon, nitrogen, phosphorus, and sulfur cycling. Additionally, soil microbiota is responsible for the decomposition and transformation of various organic compounds [2]. Soil fertility is determined by the chemical composition and the qualitative and quantitative nature of the microorganisms inhabiting it [3]. The capacity to anticipate changes in the distribution patterns and microbial diversity can prompt the management of plantations and aid in comprehending or forecasting alterations in ecosystems [4].

Soil microbiology is crucial for understanding how soil management practices impact soil quality, forest sustainability, and plant productivity [5]. Fungal communities differ from bacterial communities in species composition and play an important role in ecosystem function in forest systems. These communities govern plant nutrition and the cycling of soil carbon [6]. Investigating whether soil microbial communities respond predictably to land use measures and soil physicochemical diversifications across various climatic conditions would show their capability to act as soil quality indicators on a broad scale [7]. Soil fertility significantly impacts soil bacterial communities. Alterations in soil acidity result in changes in both the community composition and diversity [8]. Studies have demonstrated a correlation between land use and alterations in bacterial community composition [9]. Seasonal change and
succession have a more complex influence on bacterial diversity [10, 11]. Further, fungi play a more significant role in soil fertility, with complex fungal community biodiversity assemblages correlated with high fertility [12, 13]. To promote plantation growth, one effective method was to increase fungal diversity [14].

In forest soils, spatial and temporal variations of the microbial communities are affected by numerous factors, including soil characteristics, stand age, seasons, etc. Communities' morphotypes vary with season, temperature, and soil moisture. In winter, different types of ectomycorrhizas are more abundant and active [15]. The comparative study indicated that biodiversity was driven by different environmental predictors [16]. According to recent studies, fungi were predominantly vulnerable to land-cover change, heat, and drought [17]. They could adapt to climate change by adjusting the ratio of dominant fungi, responding to moisture patterns, and changing the relationships between microbial communities and functional groups [18]. Mean annual temperature influenced the dominant fungal species [4]. However, increasing soil temperature increases the optimum for bacterial growth [19]. Further research is required to examine the links between the composition of soil microbial communities under future global climatic change [20].

Soil microbial communities also vary among forests of different ages and layers [21, 22]. The total species number is significantly lower in older stands, and the composition of microbial communities is shaped by age [23–25]. Continuous cultivation of single-age monocultures might be negative feedback on the soil microbial community [26]. Stand age significantly impacts the soil cycling of carbon, nitrogen, and phosphorus, as well as fungal and bacterial communities. The fraction of microbial carbon or nitrogen decreases with stand age [27]. Between the surface and subsurface soils, distinct microbial community structures have been observed with diversity varying with soil depth. Furthermore, the top 20 cm of the surface soil of microbial has the greatest microbial activity, biomass, and diversity [28, 29]. Microbes are diverse and abundant in the subsoil (below 20 cm) because of the large soil volume on a depth-weighted basis [30].

Chinese fir is an important tree species in China's subtropical zone, used for forest cultivation, timber production, and ecological restoration. It is widely planted and can help reduce the exploitation of natural forests, improve the environment, and build an ecological civilization [31, 32]. To properly manage Chinese fir plantations and restore ecological balance, it is crucial to understand the role of microbial communities in maintaining healthy soil quality and preventing nutrient depletion and land degradation. There is relatively little research discussing the interactive effects of forest age, season, or environmental factors. In this study, we aimed to analyze the impacts of age and seasonal changes on microbial communities: (a) Reveal the number and distribution of species at different taxonomic levels, and use statistical tests to explain differences in species composition considering developmental stages and season changes. (b) Explore the richness and diversity of the microbial communities using the α-diversity metric and examine significant differences between different groups using the β-diversity metric. (c) Discuss potential forest management measures based on the interaction effects of forest age and season.
2. Material and methods

2.1. Study Sites

The study area is located in Shanxia Forest Farm, Fenyi County, Jiangxi Province of China (27°30′N to 27°45′N and 114°30′ to 114°45′N). This region has a warm and humid subtropical monsoon climate, with the evergreen broad-leaved forest as the zonal plant. The mean annual temperature is 16.8°C, the annual precipitation is 1600 mm, and the precipitation is mostly concentrated in spring. The site is mostly a low mountainous and hilly landscape. The soil is clay loam red soil (Alliti-Udic Ferrosols), according to IUSS Working Group [33], and the parent rocks are mostly shale. The following were measured in each plot: location, elevation, aspect, slope, mean tree height, mean diameter at breast height (DBH), density (Table S1).

2.2. Soil sampling

The plots were selected by the method of space-for-time substitution. Sampling with same site index was performed during four different seasons: spring (April 4, 2020), summer (July 23, 2020), autumn (October 13, 2020), and winter (January 17, 2021). However, the samples collected in the spring were damaged during lab analysis. The samples collected in summer, autumn, and winter were used in this study and were labeled as A, B, and C, respectively. At the same time, the corresponding basic climate information is obtained (Table S2). According to the age classification standard, the objects were overmature (51 years), mature (27 years), near-mature (20 years), middle-aged (14 years), and young (8 years) plantations, represented by OMF, MAF, NMF, MIF, and YOF.

For each age group, three were 20 m × 30 m sampling plots established. Soil samples were collected from a 60 cm long vertical profile that corresponded to depths of 0–20cm, 20–40cm, and 40–60cm, represented by T, M, and B. Then we sieved the samples through a 2 mm mesh to remove roots, rocks, plant tissues, and other items. The samples were mixed evenly by depth in a plot. The soil samples were sent to the laboratory for testing in a cooler at -4°C, and one portion was immediately frozen at -20°C for later analysis.

2.3. High-throughput sequencing

We used the HiPure Soil DNA Kits (Magen, Guangzhou, China) to extract soil microbial DNA. The target region of the ribosomal RNA gene was amplified by PCR. To amplify the V3-V4 region of the bacterial 16S rRNA gene, we used the following primers: 341F (5′–CCTACGGGNGGGCAGCAG–3′) and 806R (5′–GGACTACHVGGGTATCTAAT–3′). To amplify the ITS2 region of fungal, the ITS3 KYO2 (5′–GATGAAGAACGYAGYRAA–3′) and ITS4 (5′–TCCTCCGCTTATTGATATGC–3′) were used [34, 35]. We extracted and purify amplicons target region and then read the raw reads, which were further filtered using FASTP (version 0.18.0) [36]. Paired-end clean reads were merged as raw tags, then filtered to obtain high-quality clean tags. The clean tags were clustered into operational taxonomic units (OTUs) using UPARSE (version 9.2.64) [37]. The representative OTU sequences were classified and conducted taxonomic classification by BLAST (version 2.6.0) [38, 39].
2.4. Data analysis

Microbial species at different taxonomic levels based on their relative abundance were selected, retaining species with at least 1% abundance, then different types of figures were graphed in R using circos package (version 0.69-3) [40] and heatmap package (version 1.0.12) [41]. ACE, Chao1, Simpson, Shannon, and Good’s coverage indexes were used to estimate the richness and evenness of the fungal and bacterial communities in QIIME (version 1.9.1) [42]. Chao1 rarefaction curves were graphed to ensure adequate sampling and sequencing for analysis, and rank curves visually showed community richness and uniformity. Ternary plots were graphed to show the differences among the three soil layers. Dimensionality reduction analysis was performed, and PCoA and UMAP images were drawn for visualization. They were drawn in the ggplot2 package (version 2.2.1) [43]. The $\beta$ diversity was analyzed using nonmetric multidimensional scaling based on Jaccard distance matrices, with an Adonis test. The Canonical Correspondence Analysis test allowed us to explore the relationships between different environmental variables.

3. Results

3.1. Differences in soil microbial communities in the stands with different layers

3.1.1. Differences in community species diversity

The sampling process provided good coverage across different soil layers for various microbial communities (Fig. S3). The Alpha diversity index and OTU number were not significantly different among soil layers. In the bacterial communities, species richness, diversity, evenness, and OTU number followed a 'high-low-high' pattern, with the lowest values occurring in the middle layer. In the fungal communities, there were no significant trends in these various indicators, and their values at different soil depths were also similar. Beta diversity did not differ significantly between different soil layer groups in the bacterial communities, and there were no significant differences within the group. However, differences were observed between the groups of the fungal communities in the middle and lower layers of the soil, and the sample points in the middle layer were relatively clustered (Fig. S4).

3.1.2. Differences in community species composition

In soil bacterial communities, at the phylum level, Proteobacteria and Firmicutes had relatively higher abundance in the B layer (Fig. 1a). At the genus level, Akkermansia and Bacteroides were almost exclusively found in the B layer of the soil. Candidatus_Xiphinemato bacter and Tumebacillus had relatively high abundance in the M and T layers, respectively.

In soil fungal communities, at the phylum level, Chlorophyta had a relatively high abundance in the M layer (Fig. 1c). At the genus level, there were significant changes in different species at different layers.
*Trichoderma* had the highest relative abundance and did not vary much across different layers. *Staphylotrichum, Chaetosphaeria, Arcopilus,* and *Mycena* accounted for over half of the relative abundance in the M layer among all three layers. *Aspergillus, Clitopilus, Paraboeremia, Tolypocladium,* and *Staphylotrichum* respectively had a distribution proportion of over 50% in the T and B layers (Fig. 1d).

**3.2. Differences in soil microbial communities in the stands with different ages**

**3.2.1. Differences in the number of community species**

The number of OTUs of bacteria showed a decreasing trend from YOF to OMF (Fig. S5). For fungal communities, there were the least numbers of OTUs in NMF and the highest numbers in MIF. No significant differences were found within or between ages ($P > 0.05$).

**3.2.2. Differences in community species diversity**

Shannon index for the bacterial communities fluctuated slightly, initially increasing and then decreasing (Fig. S6). The Chao1 and ACE indices increased from YOF to MIF, reflecting changes in species richness. For the fungal communities, the Shannon index increased from MAF to OMF. The Chao1 and ACE indexes showed a 'low-high-low-high' pattern of species richness and a 'high-low-high' pattern of diversity.

A significant difference was observed between group YOF-MIF-NMF-MAF-OMF ($P < 0.05$) (Table S7). UMAP analysis revealed a greater inter-group difference in bacterial communities and bacterial communities in YOF were far away from other groups (Fig. 2). YOF concentrated more in the bacterial communities while OMF and MAF concentrated more in the fungal community.

**3.2.3. Analysis of community species composition**

Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant bacterial phyla, while Ascomycota, Basidiomycota, and Mucoromycota were more abundant than other phyla in the fungal communities (Fig. 3). The bacterial species exhibiting higher average abundance across various forest ages were *Streptomyces, Burkholderia-Caballeronia-Paraburkholderia, Acidothermus,* and *Akkermansia*. In the fungal communities, *Trichoderma* showed a significantly greater relative abundance compared to other species, followed by *Penicillium, Talaromyces,* and *Aspergillus*.

**3.3. Differences in soil microbial communities in the stands with different seasons**

**3.3.1. Differences in the number of community species**

Microbial communities had higher OTUs numbers in bacterial communities than fungal communities across all seasons (Fig. S8). The highest OTUs numbers were observed in summer for bacterial
communities and winter for fungal communities. The lowest OTUs numbers were observed in both communities in autumn ($P < 0.05$).

### 3.3.2. Differences in community species diversity

The ACE and Chao1 indices showed that bacterial species richness was the highest in summer, while fungal species richness was the highest in winter (Fig. S9). The Shannon index of bacterial communities gradually decreased with seasonal changes, indicating a slight decrease in species diversity and evenness. The Shannon index in fungal communities first decreased and then increased, representing higher species diversity and evenness in summer and winter.

The intra-group differences in bacterial communities showed the rankings of summer-winter $>$ summer-autumn $>$ autumn-winter, while in fungal communities it showed the pattern of summer-autumn $>$ autumn-winter $>$ summer-winter (Fig. S10). For bacterial communities, differences for three seasons followed a pattern of winter $>$ summer $>$ autumn, while for fungal communities, the differences followed a pattern of winter $>$ autumn $>$ summer.

### 3.3.3. Analysis of community species composition

In bacterial communities, the dominant species and rankings were similar between summer and autumn. However, there were differences in the composition of dominant species between seasons. Proteobacteria, Actinobacteria, and Acidobacteria were consistent among the most abundant at the phylum level (Fig. S11). In summer, at the genus level, *Streptomyces, Burkholderia-Caballeronia-Paraburkholderia,* and *Acidothermus* were the top three species. In autumn, *Burkholderia-Caballeronia-Paraburkholderia, Streptomyces, Acidibacter,* and *Candidatus_Solibacter* accounted for over 50% of the relative abundance. In winter, it showed a different species distribution. *HSB_OF53-F07, Candidatus_Solibacter, and Acidothermus* replaced species with high abundance in the previous seasons, such as *Streptomyces* and *Burkholderia-Caballeronia-Paraburkholderia.*

In fungal communities, at the phylum level, species composition, and abundance ranking were similar across seasons (Fig. S12). In summer, fewer species had an abundance greater than 1%, with *Penicillium, Talaromyces,* and *Aspergillus* having higher abundances. In autumn, *Penicillium* and *Mortierella* counted over 50% of the communities. In winter, *Trichoderma* is the most abundant genus, especially in winter.

### 3.3.4. CCA of the Relationship between species diversity and Climate

The explanation ratio of the combination of CCA1 and CCA2 was 61.72% and 38.28%, respectively (Fig. 4). Only average and maximum temperature had a significant correlation with microbial communities. In summer, the average temperature and the highest temperature were positively related to the diversity of microbial communities, while in winter, it showed a negative correlation.
3.4. Differences in soil microbial communities in the stands with different ages and seasons

3.4.1. Differences in community species diversity

Combined with the Good's Coverage index value, it indicated the sequencing results covered the microbial communities well and the samples were saturated (Fig. S13).

For the bacterial communities, in summer, the species richness was the highest and the species abundance of MIF was the highest. Bacterial distribution evenness was similar across different forest ages (Fig. S14). In autumn, the curve was narrower than in summer but wider than in winter. NMF had the highest species richness, while MIF had the shortest curve and lowest species richness. In winter, YOF had the highest species richness. For the fungal communities, in summer, MAF had the highest species richness, OMF had the best evenness, and NMF had the worst evenness. In winter, OMF had the highest species richness, and NMF had the lowest species richness (Fig. S14).

In the bacterial communities, in summer, there were notable variations among different age groups. Distances between MAF and OMF, and YOF and OMF had significant differences between communities (Fig. 5a). During autumn, differences were small except for OMF forests (Fig. 5b). Point distribution within the YOF, MIF, and NMF groups was relatively clustered in winter (Fig. 5c). In the fungal communities, significant differences were observed in the graphs of MIF during different seasons. In summer, OMF and MAF were clustered near the negative direction of the horizontal axis, whereas YOF was in the positive direction of the horizontal axis (Fig. 5d). The differences in forest age affected the community composition between groups. In contrast, samples in winter clustered near the negative direction of the horizontal axis were generally from YOF, while those near the positive direction were from OMF, contradicting the results obtained for summer samples (Fig. 5f).

3.4.2. Analysis of community species composition

The bacterial communities during the same season while the fungal community of the same forest age showed similarity (Fig. 6).

In the bacterial communities, at the phylum level, Proteobacteria had the highest relative abundance in all seasons and ages, while Chloroflexi and Gemmatimonadetes, as well as Acidobacteria and Patescibacteria, showed high similarity in different seasons (Fig. 6a). In summer, Gemmatimonadetes and Actinobacteria were relatively abundant, with Actinobacteria being significantly more abundant than others. Proteobacteria were widely distributed in all ages, while Firmicutes were significantly more abundant in YOF. In autumn, Bacteroidetes were found in all ages. Verrucomicrobia and Firmicutes were only abundant in OMF, while Acidobacteria and Patescibacteria were relatively common in all ages except for OMF. Gemmatimonadetes were relatively abundant in MIF and MAF. In winter, the species
composition was relatively simple, with Candidatus_Solibacter and Bryobacter having a high abundance in NMF and OMF.

In fungal communities, at the genus level, the species composition was more similar in summer and autumn (Fig. 6d). In both the two seasons, Acidothermus, Candidatus_Solibacter, Acidibacter, Bryobacter, Burkholderia-Caballeronia-Paraburkholderia, Occallatibacter, Acidibacter, Gemmatimonas were widely distributed. There were significant differences between OMF and other ages. There was a strong similarity between YOF, MIF, and OMF. MAF and NMF exhibited stronger similarities. OMF had different species composition characteristics than the other ages, indicating that fungal composition was more similar during similar growth stages but had significant changes as it developed into OMF.

To be specific, Ochroconis was widespread in all three seasons in YOF, and the relative abundance of Saitozyma in the winter of YOF was significantly higher than that in other ages. Talaromyces, Staphylotrichum, and Mortierella had relatively high abundance in autumn and winter. In NMF, Saitozyma, Staphylotrichum, and Penicillium were widely distributed in all seasons. In OMF, Aspergillus, Purpureocillium, and Clitopilus were widely distributed, while Tolypocladium and Paraboeremia had significantly higher abundance in autumn of OMF than in other ages and seasons. The similarity between NMF and MAF was high in summer and winter, while the similarity between MIF and YOF was stronger in autumn than sum and winter. Fewer fungal species with a relative abundance of over 1% were present in winter.

4. Discussion

4.1. Differences among layers of soil microbial communities

In our study, the bacterial community had lower diversity in the middle layer, while the diversity was higher in the top and bottom layers. In the fungal community, there was no significant trend in the various indicators, and the results of measurements at different soil depths were similar. One possible reason is that soil depth primarily shaped bacterial communities, while plant species structure influenced fungi [44]. Former studies showed that the predicted bacterial diversity in the topsoil was higher than in the corresponding subsoil [30]. Fungal diversity was generally higher in the 0–5 cm layer [45]. Compared with surface soils, the amount of bacterial biomass was much lower in deeper soils and microbial turnover was significantly slower [46–49].

The composition of fungal species changed significantly with soil depth, while the composition of bacterial communities remained relatively stable in our study. Fungi interact with roots at various depths, which may promote different life strategies among fungal taxa. Fungi are more prevalent than bacteria and actinomycetes in surface soils due to the lower soil pH. Earlier studies have reported that fungi were dominant in acidic soil conditions and topsoil (0–10 cm) [50, 51]. The soil in the study area is mostly acidic. This is due to the self-toxicity of organic acids and phenolic substances secreted by Chinese fir, as
well as the increasingly serious acid rain pollution. As a result, the acidity of the soil is been further aggravated [52]. Soil fungi in deeper layers of the soil profile contribute to carbon and nutrient cycling, soil formation, and xenobiotic degradation. They also have close relationships with plant rhizosphere, which will improve water and nutrient absorption [53, 54]. Based on the above viewpoints, it is necessary to analyze the changes in fungal communities. And the distribution of Aspergillus in the soil at depths of 0-20cm exhibited specificity. Microbes play a crucial role in the natural process of making phosphorus accessible to plants, specifically through solubilization. Low phosphorus content in acidic red soil in the study site reduces the productivity of Chinese fir forests. The available phosphorus content that can be absorbed and utilized by trees is low [55]. However, phosphorus-solubilizing fungi are a mere 0.1–0.5% of the entire microbial population in the soil. This group includes many species, especially Aspergillus [56, 57].

4.2. Effects of different ages on soil microbial communities

Stand age is a key factor in evaluating soil biomass dynamics, carbon storage, contributing diversity, and other ecological processes [58]. The structure of bacterial communities is influenced by stand age, soil vegetation, and soil physical and chemical properties, and can quickly respond to changes in the soil environment [59]. There is a strong correlation between the growth of Chinese fir and soil properties, microbial communities, and environmental conditions [60, 61]. RDA analysis shows that soil fungal communities in Chinese fir plantations strongly affected water content, organic matter, available phosphorus, and available potassium [55]. The highest stability of soil was observed in the MIF, and it has the highest microbial biomass and diversity but lacks uniformity in the YOF [55, 62].

Our study found that older forests had a higher species richness, diversity, and OTUs in soil fungal communities. Soil properties changed with forest development, and with soil porosity and field capacity increasing in a high-low-high pattern. The low-high-low pattern was found in the PLFAs of bacteria, fungi, and the ratio of fungi number to bacteria in non-rhizosphere soil [63]. Cao et al. (2021) showed that both the microbial diversity index and OTU increased with the increase in the forest age of Chinese fir plantations. Soil porosity, bulk density, and moisture content follow a general pattern of high-low-high changes, the bacterial community gradually changed to a K-strategy, while the fungal community prioritize quality and quantity in their reproduction [60]. In this study, with the change of stand age, the fungal community also showed a trend of high and low patterns. Therefore, an appropriate extension of the plantation cultivation time is conducive to the restoration of soil physical and chemical properties and fungal communities to improve soil quality.

4.3. Effects of different seasons and climates on soil microbial communities

Seasonal changes have led to significant alterations in the structure of microbial communities [64]. Few studies have investigated the impact of environmental changes on the structure of microbial communities, and the key factors that influence these structures across seasons [65, 66]. There are differences in seasonal microbial activity, but overall the microbial biomass and metabolic diversity
remain relatively stable [67]. Our analysis of differences in microbial community diversity across different seasons revealed that species richness of the bacterial community and species diversity of the fungal community varied greatly, with peaks occurring in summer and winter.

Fungal communities in the same season had less similarity. Compared with fungi, due to the interaction between soil sampling time and land management, seasonal change had a more complex effect on bacterial diversity [39, 68, 69]. Previous research has suggested that environmental factors had a more significant effect on microbial diversity than plant diversity [70]. In our study, the average and maximum temperature had a more significant effect on microbial communities. Similarly, community composition was mostly driven by temperature rather than other environmental factors, and the community diversity and distribution were regulated by the interaction and comprehensive regulation of various environments [71]. Soil temperature was the main factor influencing differences in microbial community structure [72]. The temperature rises weakened species interactions, in particular, the combination of increased precipitation and warming significantly increases the bacterial richness and decreases fungal richness [73]. Climatic factors, edaphic factors, and spatial patterning are the best predictors of soil fungal richness and community composition at the global scale [74]. The soil microbial community shows a sensitive and phylogenetical response to the changes [75]. Therefore, the research on the effects of different seasons and climates on soil microbial communities helps to understand the mechanism of soil fertility changes and then provides theoretical support and prediction basis for future management measures.

4.4. Differences among ages and seasons from the perspective of species composition

Results of species composition showed differences when considering the interaction between season and age, as compared to separate factors. Bacterial community species composition exhibited similar characteristics in the same season, while fungal communities tended to cluster by stand age (Fig. 8). Fungi have an important role in soil ecology by cycling nutrients and carbon, supporting plant nutrition and protection, and contributing to the diversity of pathogens [76]. Fungal guilds are key integrators of plant richness-stock relationships, with fungal growth dominating the forest soil [77–79].

In our study, Proteobacteria, Actinobacteria, and Acidobacteria were the most common phyla in bacterial communities during summer and winter, with significant differences between these two seasons. In particular, the species composition in the OMF was characterized by Actinobacteria and Bacteroidetes. The species composition in the YOF was characterized by Firmicutes. At the genus level, *Streptomyces, Burkholderia-Caballeronia-Paraburkholderia* were enriched in summer and autumn. Fungal communities had similar species composition and abundance across seasons at the phylum level, with *Trichoderma* as the most common genus (Fig. 8). In similar studies, Proteobacteria, Acidobacteria, and Actinobacteria were the most prevalent soil bacteria in South China [80–82].

Microbial communities at the phylum level of bacterial classification exhibit the most prominent features after the interaction, regarding specific functions. Proteobacteria fix nitrogen, alleviate soil phosphorus...
limitations, increase bacterial diversity, stimulate microbial groups, and prompt lipopolysaccharide biosynthesis and carbohydrate metabolism [83, 84]. Acidobacteria and Proteobacteria were most affected by land-use change and were the most abundant taxonomic groups of soil bacteria [85, 86]. They were more abundant in summer and in young and over-mature forests, which corresponded to high bacterial alpha diversity in our study. Acidobacteria had oligotrophic nature or ecological K-strategy [87]. They decompose organic matter, recycle nutrients, regulate biogeochemical cycles, decompose biopolymers, and promote plant growth. The biofertilizer increases nutrients by Acidobacterial inoculation [88]. Future research could explore initiatives aimed at manipulating crop rhizosphere with Acidobacterial populations to increase plant growth [89]. They were more abundant in autumn, especially in young forests, where bacterial species richness was high and the within-group difference was small, indicating good uniformity. Actinobacteria produce beneficial metabolites such as antibiotics, biopolymers, and biocatalysts. Actinobacteria have an important influence on the turnover of recalcitrant plant organic matter in rhizosphere microbial communities. The rhizosphere region is considered one of the best habitats for isolating these microbes [89, 90]. In the soil, Bacteroidetes is mattered with complex organic matter, especially the polysaccharides and proteins [91]. And Bacteroidetes in the soil secrete diverse arrays of CAZymes which target the highly varied glycans [92]. In soil, Firmicutes species possess iron and sulphate reduction abilities and have a critical role in soil disease control [84, 93]. Members of the Firmicutes group which have iron and sulfate reducing abilities can be developed as effective bioenhancers in future bioremediation applications [94].

It has been found in fungal community diversity studies that species richness began to recover at the mature forest stage, and it makes sense to focus on species that change significantly in relative abundance during this stage (Fig. 8). In autumn, Arcopilus was significantly more abundant in near-mature forest soils, whereas Tolypocladium was more abundant in over-mature forests. Few research has been done on soil Arcopilus, it is a genus recently proposed after the taxonomic restructuring of Chaetomium [95]. In the forests soil, a potential function of Arcopilus in the environment is bioremediation of soils contaminated with organic matter and abnormal pH [96]. One form of Tolypocladium in soil is saprophytic, It can produce the immunosuppressant cyclosporine under certain conditions, and the condition that significantly affects Tolypocladium growth and survival is temperature [97]. Tolypocladium inflatum isolated from soils with no history of lead contamination was as tolerant to lead as those isolated from lead-rich soils, it is more abundant in metal-rich soils and may be more tolerant to metals [98].

In the plantations, extending the planting period of plantations appropriately could help maintain the diversity of soil bacterial communities and improve soil quality [99]. According to our study, microbial community diversity in plantations increased when forests are mature or over-mature, which indicated that we should pay more attention to the management of cultivation time to achieve the goal of soil fertility maintenance.

5. Conclusions
There was a significant difference between different stand ages in both communities ($P<0.05$). We found that soil bacterial and fungal diversity increased with stand age, reached a maximum at middle-aged forest, and then declined. Bacterial communities continued to decline after mature forest while fungal communities rose during mature to over-mature forest. In the bacterial community, middle-aged forest had the most species, and near-mature forest had the highest species richness in summer. During winter, young forest had the most diversity. In the fungal community, mature forest had the most diversity in summer.

Considering differences of species structure, the characteristics of bacterial communities in the same season were similar, while the fungal communities in the same stand age were similar. Fungal communities were less affected by climatic elements, which indicated that it is a more critical indicator of soil fertility in long-term cultivated plantations.

In bacterial communities, Proteobacteria, Actinobacteria, and Acidobacteria were the most common phyla in all stand ages during summer and winter. In over-mature forest, the species composition was characterized by Actinobacteria in the summer and Bacteroidetes in the autumn.

In the young forest, Firmicutes was dominant in the summer. In fungal communities, of particular concern are *Tolypocladium* and *Arcopilus* in near-mature and over-mature forests in autumn, potentially be of reference value for future research.

**Declarations**

**Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Yuxin Hu: data analysis, writing original draft; designed study Hanyue Chen: software, reviewing and editing; Yihang Jiang: investigation; Jianguo Zhang: reviewing and editing.

Xionqing Zhang: Performed research; conceptualization, supervision, reviewing and editing.

All authors reviewed the manuscript.

Ethics declarations

The authors have no relevant financial or non-financial interests to disclose. The work has not been published in any previous or concurrent publication. The manuscript has been read and approved by all authors to be submitted to Microbial Ecology.

Supplementary information

Supplementary data for this article can be viewed in the Supplementary materials.

References


**Figures**
Figure 1

Ternary plots of species composition at different taxonomic levels of different forest layers (T: 0-20cm, M: 20-40cm, B: 40-60cm). Different sizes of the point represent the average relative abundance of the data in different layers. (a) Bacterial community phylum level (b) Bacterial community genus level (c) Fungal community phylum level (d) Fungal community genus level
Figure 2

UMAP cluster analysis of Beta diversity of microbial communities in different forest ages. (OMF = over-mature forest, MAF = mature forest, NMF = near mature forest, MIF = middle-aged forest, YOF = young forest)
Figure 3

Ranking of species abundance at different taxonomic levels of different forest ages
Figure 4

CCA Analysis of the relationship between species diversity and climate in different seasons. (A = summer, B = autumn, and C = winter)
Figure 5

PCoA analysis of soil microbial communities in plantations (a) Summer bacterial community (b) Autumn bacterial community (c) Winter bacterial community (d) Summer fungal community (e) Autumn fungal community (f) Winter fungal community (OMF = overmature forest, MAF = mature forest, NMF = near mature forest, MIF = middle-aged forest, YOF = young forest)
Figure 6

Heat map of soil microbial community species composition in different forest age stands in different seasons (a) Bacterial community phylum level (b) Bacterial community genus level (c) Fungal community phylum level (d) Fungal community genus level.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx