



The succession patterns and drivers of soil bacterial and fungal communities with stand development in Chinese fir plantations

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Abstract

Background Soil microbial community composition with stand development may be changed due to the variations in canopy structure, understory vegetation, root traits, edaphic conditions, and litter inputs. However, it is still uncertain to what extent these biotic and abiotic factors shape the soil microbial community composition in Chinese fir (*Cunninghamia lanceolata*) plantations, which are widely planted in southern China.

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Methods Amplicon sequencing was used to analyze the diversity and composition of soil bacterial and fungal communities at two soil depths across four developmental stages of Chinese fir plantations.

Results Both the bacterial and fungal communities were significantly different between stand age groups. As stand aged, oligotrophic bacteria decreased in abundance while copiotrophic bacteria increased. Meanwhile, fungal functional groups connected to plants decreased in abundance while that of saprotrophic fungi significantly increased. The variance in bacterial community was mainly attributed to soil variables regarding carbon and nutrient availability, whereas the greatest fraction of fungal community dissimilarity was determined by plant-specific factors such as aboveground stand structure and tree root traits. The chemical composition of litter had a major effect on the soil fungal community, whereas the litter traits had no effect on the bacterial community.

Conclusions The potential drivers of soil fungi and bacteria differ in Chinese fir plantations. By accounting for the impacts of various environmental components, it is possible to anticipate the response of soil bacterial and fungal assemblages to forest management regime implemented in Chinese fir plantations.

Keywords Stand age · Plant community · Microbial diversity · Plant–soil interaction · Forest management

Introduction

Bacteria, the most plentiful microorganisms in soils, play a role in organic matter decomposition. Soil fungi encompass a variety of categories with varying ecological functions (Tomao et al. 2020). Saprotrophic and ectomycorrhiza (ECM) fungi can degrade complex organic material through the production of extracellular enzymes (Lindahl and Tunlid 2015; Zhang et al. 2018). Mycorrhizal fungi and endophytes, being biotrophic symbionts, facilitate the growth of plants by providing them with nutrients and conferring resistance to abiotic and biotic stresses (Rodriguez et al. 2009). Owing to their importance in controlling the processes of soil carbon (C) turnover and nutrient cycling, it is important to understand the factors that drive the assemblage of bacterial and fungal communities in forest ecosystems.

In the last decade, there has been a dramatic increase in the conversion of natural forests to commercial monoculture plantations worldwide, which are typically managed into stands of even-aged trees, ranging from clear cuts to mature forests (FAO 2020). During the development of intensively managed plantations, the plant dynamics are characterized by changes in the stand structure, tree biomass, and the biodiversity and biomass of understory plants induced by tree growth and silvicultural treatments. The changes in canopy cover and stand density at different stand developmental stages substantially modify the access of solar radiation and precipitation water beneath the crown canopy (Lucas-Borja et al. 2016). The fluctuations in the understory microclimate might have potential feedback on the activity and species composition of bacteria and fungi (Kara et al. 2008; Li et al. 2019). In addition, the forest gap created by thinning also changes the species and functional diversity of undergrowth with stand development, which represents an important component of the nutrients and C resources for microbial population (Liu et al. 2023). As a result, soil microbes have frequently been found to be related to the composition, richness and biomass of understory plants in planted forests (Qiang et al. 2023; Wan et al. 2021). In monoculture forests, trees influence soil microbiota essentially in the same way as other plants, but their effect is potentially stronger because of a greater biomass (Gömöryová et al. 2013). Trees have a direct influence on the proliferation and activity of

soil microorganisms, not only through the deposition of aboveground litter, but also through the introduction of root exudates and dead root residues to the soil (Eisenhauer et al. 2017). Root extensions also shape the soil physical environment (Angers and Caron 1998), which affects the fluid flow of nutrients and C transport to surrounding microorganisms. Tree root production, morphology, chemical traits, and vertical depth vary considerably with stand aged (Chen et al. 2018; Rosenvald et al. 2013; Zhu et al. 2022), indicating that tree adjust their resource acquisition strategy as manifested by changes in root traits, to adapt tree ecophysiological processes during stand development. Findings from studies have demonstrated that microbial properties are closely related to root functional traits (Hogan et al. 2023; Legay et al. 2014; López-Angulo et al. 2020), which indicate the amount of carbohydrates allocated and the quality of the substrate. Furthermore, some bacterial and fungal species interact with plant roots to create complex biotic associations, such as parasitism, pathogens or symbiotes (Tomao et al. 2020). Thinning or self-thinning at some specific stages of planting tree species was observed to reduce the populations of mycorrhizal species in the soil due to the removal link with host tree roots and the decreased allocation of carbohydrates from the remaining trees (Bonet et al. 2012). Thus, forest soil microorganisms might be highly sensitive to shifts in tree root traits with stand development. However, the potential role of tree fine root characteristics in explaining patterns of soil microbial communities with an age gradient of forest plantations remains largely unexplored.

As a plantation matures, changes in the stand structure, tree biomass and individual species of ground plants are closely associated with modifications in the amount, composition and decomposition rates of litter, which essentially affect the structure of belowground microbiota as a major source of available nutrients and energy (Kara et al. 2008; Liu et al. 2023). Soil physicochemical conditions are indirectly modified by forest development through changes in canopy structure, photosynthetic product releases, litter necromass, and ion uptake (Angers and Caron 1998; Le Bagousse-Pinguet et al. 2017). The physical structure (Dini-Andreote et al. 2014), pH (Lauber et al. 2009), C availability (Jiang et al. 2021), and nutrient status (Dini-Andreote et al. 2014; Lladó et al. 2018) of soils are all known to have a major impact

on both bacterial and fungal communities. Therefore, the microbial diversity and community composition of forest plantations are likely to be determined by the intricate interaction between the aboveground vegetation, litter, and soil conditions that are potentially related to the development of plantations. Although forest management practices and stand age in plantations have been studied regarding their effect on microbial species diversity and community composition (Chen et al. 2018; Dini-Andreote et al. 2014; Jiang et al. 2021), further exploration is still need to understand the influence of stand developmental stage-related factors on microbial diversity and community composition, and to identify the key factors that contribute to the response of the belowground microbial community structure. Nevertheless, there is a lack of studies with a thorough evaluation of the alterations in vegetation, litter, and soil environment on the patterns of belowground microorganisms across a range of ages in forest plantations, which prevents the development of sound guidelines and accurate management practices for sustaining site nutrient levels and stand productivity in timber plantations (Vance et al. 2010).

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is a native tree species with a cultivation history spanning over 1000 years. Because it shows rapid growth and high yields, Chinese fir has been widely planted in subtropical China and covers 24% of the plantation area nationwide (State Forestry Administration of China 2019) to satisfy the large demand for timber products. Short rotations are employed in the management of Chinese fir plantations, and the trees are usually harvested at 25 years of age (Yu 1997). Precommercial tending is applied to young coniferous stands during the first two years, and commercial thinning is conducted twice: first at 9–10 years and second at 15–16 years. During the rotation period of Chinese fir plantations, there were considerable variations in the stand structure, undergrowth vegetation composition, soil nutrient levels, fine root production and traits of trees, and litter biomass at different stand developmental stages (Ma et al. 2007; Wang et al. 2007; Zhou et al. 2016; Zhu et al. 2022). Extensive research has been conducted to examine the dynamics of bacterial and fungal communities and their correlation with abiotic soil properties in Chinese fir monocultures along a chronological sequence (Chen et al. 2021; Liao et al. 2023; Wang et al. 2022). However,

the role of above- and belowground plants as well as litter traits, such as the understory vegetation community, stand structure, and root traits, at the stand level in shaping microbial community patterns has yet to be explored. Currently, the conventional silvicultural regimes and rotation lengths of Chinese fir plantations are undergoing public scrutiny, primarily regarding the issues of soil fertility deterioration and timber yield decline during successive rotations across the area of distribution (Bi et al. 2007). A thorough understanding of the disparities in the abundance, composition and function of the soil-inhabiting microbial community across different stand stages of Chinese fir plantations, particularly the magnitude of structuring effects, driven by changes in plant, root or soil properties, which can provide a proposed reference for us to devise management strategies that regulate belowground organisms to enhance nutrient sustainability in vast areas of low-productivity Chinese fir plantations.

Here, we investigate bacterial and fungal abundance, diversity, and composition at four different developmental stages (young, middle-aged, mature and overmature) in monoculture Chinese fir plantations. Moreover, we quantify the relative contribution of plant attributes, litter traits, and edaphic properties as drivers of the dynamics of both bacterial and fungal communities during the development of Chinese fir plantations. Specifically, we tested the following three hypotheses: (1) Soil physiochemical conditions will play predominant roles in shaping both bacterial and fungal communities. The soil serves as a habitat, source of carbon and nutrients for the soil microbial community, while the plant community and litters structure most microbial groups by modifying soil habitat conditions and soil chemistry (Le Bagousse-Pinguet et al. 2017; Legay et al. 2014). (2) The fungal community of the soil has a closer relationship to the characteristics of vegetation and litter than the bacterial community. Fungal groups such as mycorrhizal fungi, endophytes, and plant pathogens live in biotrophic relationships with trees and understory plants, and their diversity and productivity are largely structured by the stand development and thinning of Chinese fir plantations (Zhou et al. 2016). Moreover, the accumulation of coniferous needles and root litters along an age gradient of Chinese fir (Ma et al. 2007; Wang et al. 2007; Zhang et al. 2017) may stimulate the growth of saprotrophic fungi that

specialize in utilizing tough, nutrient-poor carbon sources (Zhang et al. 2018). (3) Based on the above two assumptions, we consider that soil fungal communities would be more sensitive to stand development than bacteria because of the frequently reported substantial changes in soil properties, litter traits and plant communities during the development of Chinese fir plantations (Ma et al. 2007; Zhou et al. 2016). In addition, bacteria are more resilient than fungi in response to environmental disturbances due to their relatively high intrinsic growth rates and unicellular nature (Legay et al. 2014; Powell et al. 2015).

Materials and methods

Study sites

The study sites were located at the Xinkou Experimental Forestry Centre (26°11'30"N, 117°26'00") of Fujian Agricultural and Forestry University, Sanming, Fujian Province, China. The region is characterized by a typical subtropical monsoon climate. The mean annual temperature is 19.6 °C, the annual rainfall is approximately 1665 mm and mainly occurs from March to August based on the data collected from 1981–2010.

The selected Chinese fir plantations were 6, 14, 23 and 36 years old; these ages were chosen to represent typical developmental stages (i.e., young, middle-aged, mature, and overmature) according to the growth properties of Chinese fir (Yu 1997). The stands were all in their first rotation and were established after clearcutting and slashing and burning a natural forest with the dominant species of *Pinus massoniana* Lamb (Table 1). The middle-aged and mature plantations were thinned (removal of alternate

rows and crown-pruning of remaining trees) to improve the growth of the remaining trees. The stand age and stand history of the Chinese fir plantations were determined using records from the local forestry department. Thus, the silviculture background, management, forest history, and origins of all four selected stands ensured a similar quantity and quality of organic matter inputs derived from vegetation to the soil compartment over the long term. The longest straight-line distance between any two forest stands was less than 10 km (Fig. 1). A geological survey map of Sanming (<http://www.ngac.org.cn>) was used to select plantation stands established on soils derived from similar pedogenetic origins, i.e., the geological parent materials were the same in the selected forest stands (granite in this study), and the soil properties were similar in the selected Chinese fir plantations. The soils in the selected stands were classified as Ultisols (Soil Survey Staff 1999), a typical soil type in southern China that is characterized by strong acidity and an abundance of aluminum and iron hydroxides. Although the selected stands were in different locations, strict conditions and factors (e.g., geographical distance, forest history, silvicultural methods, bedrock, slope aspect, and terrain slope) were imposed when choosing the experimental stands to maintain homogeneity in the initial conditions of these stands (i.e., soil, climate, and vegetation) as much as possible. Thus, the succession and driving factors for soil microbial communities among these selected Chinese fir stands were mainly determined by the developmental stages of the plantation (Table 1).

In January 2019, three south-facing quadrat subplots (20 m × 20 m) were established in each stand (young, middle-aged, mature and overmature), resulting in a total of 12 sample subplots (4 stand

Table 1 Basic characteristics of Chinese fir stands during different developmental stages

Stand developmental stage	Year of establishment	Stand Age (a)	Altitude (m)	Terrain slope (°)	Average DBH (cm)	Average tree height (m)	Tree density (stem ha ⁻¹)	Tree canopy diameter (m)
Young	2013	6	276–315	28–42°	10.37(2.38)	7.30(1.48)	2825(25.00)	2.44(0.21)
Middle-aged	2005	14	223–241	33–36°	16.92(0.72)	15.28(1.55)	1200(50.00)	3.40(0.26)
Mature	1996	23	202–239	32–37°	17.91(1.85)	16.90(0.56)	1075(139.19)	5.34(0.17)
Overmature	1983	36	192–233	33–39°	20.84(1.57)	18.70(1.01)	775 (108.97)	4.65(0.29)

Mean values with standard deviation ($n=3$) are given. DBH diameter at breast height (1.3 m above the ground)

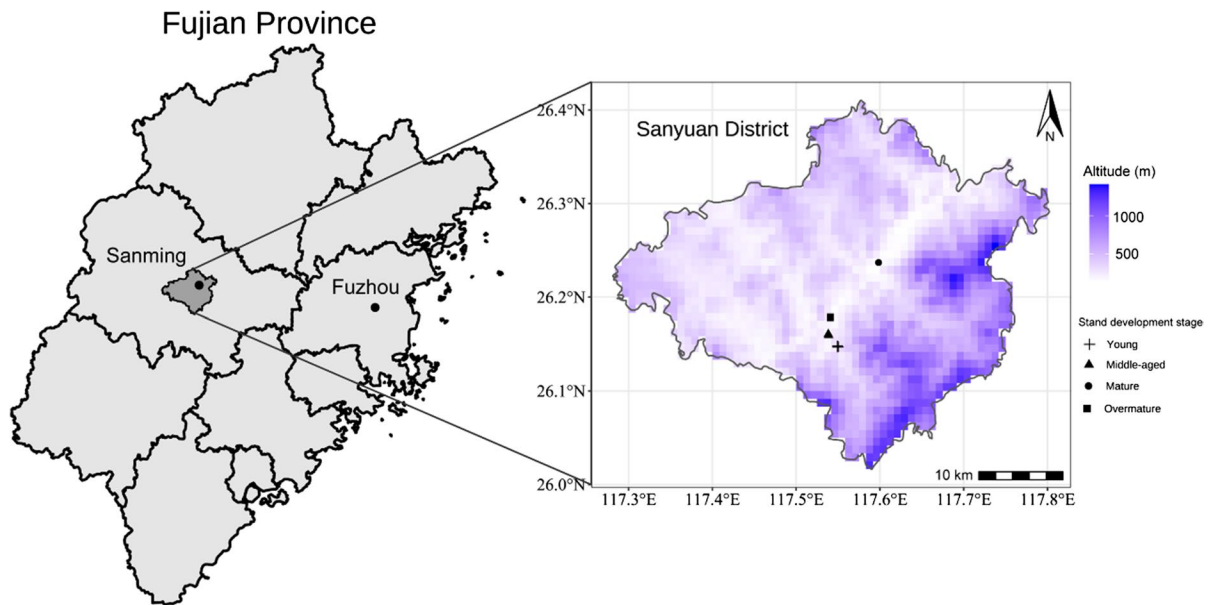


Fig. 1 Geographical locations of the sampling sites

developmental stages \times 3 subplots). Two downhill subplots and 1 upslope subplot were selected for each stand to minimize the effect of slope position on soil microorganisms.

Plant and soil sampling

Five 1.0 m \times 1.0 m quadrats that were at least 50 cm from the stem base of a Chinese fir were established in the center and four corners within each subplot using square frames. The understory vegetation within the bounds of the frames was harvested, and the litter on the forest floor was collected. In addition, fine roots (those \leq 2 mm in diameter; (Pregitzer et al. 2002)) were excavated and sampled from two trees with an average diameter at breast height (DBH) in each plot. The sampling and procedure used to determine the understory vegetation, litter and tree roots are described in the Experimental procedures of the Supplementary Information. The abbreviations of all variables measured and used for statistical analysis are provided in Table S1. The statistical results of the root traits, understory vegetation biomass, litter characteristics, and soil properties are shown in Tables S2–4.

Two soil cores were collected from 0–20 cm (A horizon) and 20–40 cm (upper portion of B horizon,

up to a depth of 20 cm) using a stainless-steel auger (7.0 cm diameter) after the litter and undergrowth vegetation were harvested from the center of each quadrat. Five soil cores within the same soil horizon from each subplot were combined to yield a composite sample, and the subsamples were chilled using ice packs and transported to the laboratory. Each soil sample was passed through a 2-mm sieve to remove stones, dead roots and litter materials and homogenized well. Each sample was separated into three parts. Subsamples for the microbial DNA analysis were stored at -80 °C. Subsamples for soil pH and available phosphorus (AP) analyses were air-dried, crushed, and passed through a 2-mm sieve. Approximately 100 g of these air-dried soils was passed through a 0.15-mm sieve for analyses of total C (TC), total N (TN), total P (P) and total K (K). In addition, the fresh soil was stored at 4 °C for the extraction of dissolved organic carbon (DOC) and inorganic N ($N-NH_4^+$ and $N-NO_3^-$). For the determination of the bulk density (BD), we obtained two soil cores from 0–20 cm and 20–40 cm using stainless steel rings (200 cm³) from the center of each subplot. The soil physical and chemical properties analyzed in the study are listed in Table S3.

DNA extraction and amplicon pyrosequencing of soil fungal and bacterial communities

Total genomic DNA was extracted from 0.5 g of fresh soil per sample using an E.Z.N.A.® stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Soil bacterial communities were characterized by amplifying fragments of the V3-V4 region of the 16S rRNA gene using the bacterial-specific primers 338F (5' ACT CCT ACG GGA GGC AGC AG 3') and 806R (5' GGA CTA CHV GGG TWT CTA AT 3') (Caporaso et al. 2012). The primers ITS1F (5' CTT GGT CAT TTA GAG GAA GTA A 3') and ITS2 (5' TGC GTT CTT CAT CGA TGC 3') were used to amplify the ITS1 region of the fungal ribosomal DNA (Gardes and Bruns 1993).

The triplicate 25- μ l reactions of each DNA sample contained 30 ng of template DNA, 1 μ l of the forward fusion primer (5 μ M), 1 μ l of the reverse fusion primer (5 μ M), 3 μ l of 2 \times Taq Plus Master Mix, 3 μ l of BSA (2 ng μ l⁻¹) and 7.5 μ l of ddH₂O. The thermal cycling conditions for the bacterial 16S rRNA gene and fungal ITS region were 94 °C for 5 min; 34 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s; and 72 °C for 7 min of extension.

The amplicons were pooled for each replicate and subjected to high-throughput sequencing by Beijing Allwegene Tech, Ltd. (Beijing, China) using an Illumina MiSeq PE300 sequencing platform (Illumina, Inc., San Diego, CA, USA).

Bioinformatics analysis

The paired-end bacterial and fungal reads were demultiplexed and quality-filtered using the QIIME Version 1.8.0 pipeline (Caporaso et al. 2010). The primers were removed, and sequences with a quality score < 20 bp or with any truncated reads shorter than 50 bp were discarded. Chimeric sequences were identified and removed using UCHIME. To minimize the effect of sequencing depth on the intersample variation, the number of sequences per sample was rarefied to the smallest sequence size (19570 sequences in the study) of all 24 soil samples by random sampling using MOTHUR (Version 1.33.3). The rarefaction curve of all soil samples was shown in Fig. S2. Operational taxonomic units (OTUs) were constructed by clustering consensus sequences at the 97% sequence similarity level (Lundberg et al. 2012) using

USEARCH (Version 2.7.1). Taxonomic annotations were assigned to the representative sequence of each OTU by searching the SILVA 16S rRNA database (<http://www.arb-silva.de>) for bacteria (Quast et al. 2013) and the UNITE fungal ITS database (<http://unite.ut.ee/index.php>) for fungi (Koljalg et al. 2013). Estimates of OTU richness and the Shannon index were calculated using the QIIME pipeline to estimate bacterial and fungal alpha diversity and evenness. The percentages of shared and unique OTUs were calculated for the soil horizons or for stand developmental stages and visualized in Venn diagrams (Fig. S3).

The ecological functions of fungal OTUs at the genus level were aligned with the FUNGuild database (<http://www.funguild.org/>) (Nguyen et al. 2016) using the *FUNGuildR* package. Only retained taxa were assigned to a single guild along with the confidence rankings of 'probable,' 'possible' or 'highly probable' from the ectomycorrhizal, all pathogenic, and all saprotrophic fungal guilds.

Quantitative real-time PCR (qPCR) analysis of bacterial and fungal abundance

The gene copy numbers of the bacterial 16S rRNA and fungal ITS rRNA were determined using qPCR with an ABI7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The same primers employed for the sequencing approach were used to analyze the 16S and ITS sequences. The reaction mixture contained 2 μ l of the DNA template, 10 μ l of 2 \times Taq Master Mix, and 0.5 μ l each of forward primers and reverse primers at 10 μ M, and ddH₂O was added to reach a final 20 μ l volume. The qPCR conditions were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s for denaturation, 55 °C for 30 s for annealing and an extension step at 72 °C for 30 s. Plasmids generated from cloned targets (101 to 105 copies) were diluted tenfold to establish standard curves for each target region (Frey et al. 2011). An average of the triplicate results was calculated. The quantities of DNA obtained were converted to copy numbers per dry soil weight.

Statistical analysis

The effects of the stand developmental stage and soil depth on the abundance of 16S rRNA and fungal ITS gene copies and diversity indexes (OTU richness

and Shannon evenness) were analyzed using two-way analysis of variance. Significant differences in microbial gene abundance and relative abundances of bacterial and fungal phyla among stand developmental stages in each soil layer were examined using one-way analysis of variance, and mean values were compared with Tukey's honest significant differences test at the 5% level.

Due to challenges associated with the technical and statistical reproducibility of OTU abundance for low abundance OTUs, only OTUs with abundances greater than 0.5% in more than 5 samples were used to determine differences in abundance. The normality and homogeneity of variances were assessed using the Shapiro–Wilk test and the Bartlett test with the *car* package before conducting the ANOVA. When the assumption of a constant variance was violated, data were log transformed with or calculated as square roots to satisfy the assumptions of ANOVA.

The distance matrices of the bacterial and fungal communities were calculated with the Bray–Curtis method with the Hellinger-transformed OTU relative abundance. Subsequently, nonmetric multidimensional scaling (NMDS) ordination was performed based on Bray–Curtis distances to explore the dissimilarity between bacterial and fungal OTUs at two soil depths among different developmental stages of Chinese fir (*metaMDS* function). Permutational multivariate ANOVA (PerMANOVA) was conducted to test the significant effects of stand developmental stage and soil depth on microbial communities with the *adonis* function using 999 permutations. Pairwise comparisons with the Holm correction for multiple testing were used to compare microbial communities among different forest stands with the custom function *pairwise.adonis* (<https://github.com/pmartinezarbizu/pairwiseAdonis/blob/master/pairwiseAdonis/R/pairwise.adonis.R>).

Variance partitioning analysis (VPA) was performed (*varpart* function; *vegan* package) using Hellinger transformed OTU abundance data as the response variable to quantify the relative contribution of the three environmental matrices of soil, litter and plant traits (Table S1) to the variance in the structure of the bacterial and fungal communities. Soil, litter and plant explanatory sets were independently fitted with bacterial and fungal abundance matrices (*rda* function). The importance of variables in explaining models was determined by step-forward selection

to remove nonsignificant variables from each of the explanatory sets using the generalized Akaike information criterion (AIC, *ordistep* function). Variance partitioning for the bacterial and fungal communities by each environmental matrix (soil properties, plant attributes and litter traits) was computed with *RsquareAdj* and the *anova.cca* function. Additionally, the selected variables for VPA, including plant, soil and litter variables (listed in Table S13), were used to carry out distance-based redundancy analysis (db-RDA) (*rda* function). Variables with variance inflation factors (VIFs) higher than 10 were excluded from the VPA to avoid collinearity among variables that arose from many zeros (*vif.cca* function). The *anova.cca* function was used to evaluate the significance of the global RDA model. A Monte Carlo permutation (999 permutations) test was used to test the predicted significance of the selected variables to the fitting model (*envfit* function). We further evaluated the significance of correlations between the NMDS scores and soil, plant and litter traits by conducting post-hoc permutational correlations (*envfit* function), the results of which are depicted in Fig. S4. The PerMANOVA, NMDS, VPA and db-RDA were conducted with the *vegan* package.

The relative abundance of each bacterial and fungal phylum was correlated with all soil, plant and litter variables by calculating Spearman's correlation coefficient (*corr.test* function; *psych* package). All analyses were performed using R (R 4.0.4; R Core Team), and statistical significance was set to the level of $P < 0.05$. All NMDS, RDA, VPA, and linear correlation results were visualized with the *ggplot2* package.

Results

Abundance and diversity of the soil bacterial and fungal communities

The amount of bacterial 16S gene significantly decreased ($P = 0.010$) while the amount of fungal ITS gene as well as the ratio of bacteria/fungi did not significantly change with stand age (Table 2). Soils at the depth of 0–20 cm had higher abundances of bacteria and fungi than the depth of 20–40 cm ($P < 0.001$).

After quality filtering and chimera assessments, 993,750 (ranging from 20,241 to 82,709 per sample)

Table 2 The abundance of soil bacteria and fungi as well as the bacteria/fungi ratio in the soils at depths of 0–20 cm and 20–40 across the four stand development stages of Chinese fir plantations

Stand developmental stage	Soil depth (cm)	Bacteria (10^8 copies g^{-1} soil)	Fungi (10^6 copies g^{-1} soil)	Bacteria/Fungi
Young	0–20	1.99 (0.49) a	1.61 (0.56)	140.63 (25.64)
	20–40	0.59 (0.11) A	0.19 (0.03)	298.66 (35.16)
Middle-aged	0–20	0.78 (0.45) b	2.21 (1.11)	421.06 (393.38)
	20–40	0.22 (0.04) B	0.11 (0.02)	213.68 (50.73)
Mature	0–20	0.82 (0.31) ab	0.99 (0.45)	108.34 (38.84)
	20–40	0.11 (0.04) B	0.08 (0.03)	187.08 (111.37)
Overmature	0–20	0.61 (0.13) b	0.93 (0.42)	104.57 (44.17)
	20–40	0.09 (0.06) B	0.10 (0.06)	74.29 (11.62)
Two-way ANOVA (<i>P</i> value)				
SDS		0.010*	0.600	0.487
D		<0.001***	<0.001***	0.294
SDS×D		0.343	0.982	0.796

Mean values with standard error ($n=3$) are given. The effects of stand developmental stages (SDS), soil depths (D) or their interaction (SDS×D) on soil bacterial and fungal abundance were assessed using two-way ANOVA (** $P<0.01$, *** $P<0.001$, * $P<0.05$). Lower-case letters and capital letters within the column indicate significant differences among stand developmental stages at 0–20 cm and 20–40 cm, respectively, using Tukey's honest significant difference ($P<0.05$). A lack of letters indicates that there are no significant differences among stand developmental stages ($P<0.05$)

bacterial reads and 658,425 (ranging from 8,232 to 65,594 per sample) fungal reads were recorded from 24 soil samples (Table S5). These sequences were clustered into 3381 bacterial OTUs and 2713 fungal OTUs at the 97% similarity threshold of sequences; among these OTUs, 1568 core bacterial OTUs (46.4%) and 732 core fungal OTUs (27.0%) were detected in all 24 samples (Figs. S2–3). A total of 824 fungal OTUs accounting for 30.7% of the total reads were exclusively detected in the 4 age groups (Fig. S3). The number of these specific bacterial OTUs decreased with stand development, and 200, 141, 74 and 89 bacterial OTUs were exclusive to the young-aged, middle-aged, mature, and overmature stages, respectively (Fig. S2).

Bacterial OTU species richness in the soils at a depth of 0–20 cm decreased with stand age ($P=0.018$, Fig. 2a), but no significant changes were found for bacterial species richness in soils at the depth of 20–40 cm (Fig. 2a) and fungal OTU richness in soils at two depths across forest stands ($P=0.929$, Fig. 2b). The Shannon diversity index for both the bacterial and fungal communities did not change with stand age ($P=0.119$ for bacteria and $P=0.063$ for fungi; Fig. 2). Moreover, the OTU richness and Shannon indexes of bacteria and fungi followed a

significant declining trend from 0–20 cm to 20–40 cm ($P<0.001$).

Composition and structure of the soil bacterial and fungal communities

The taxa *Acidobacteria* (32.2%), *Chloroflexi* (29.7%), and *Proteobacteria* (18.5%) were the most abundant members in all soil samples (Fig. 3a). The top five abundant bacterial phyla did not differ among stand developmental stages. The phyla with a mean relative abundance less than 5.0%, namely, *Firmicutes* (1.9%), *Gemmatimonadetes* (0.7%), and *GAL15* (2.3%), were significantly affected by the stand developmental stage. The *Gemmatimonadetes* groups displayed a higher abundance in the mature stand while the relative abundance of *Firmicutes* was significantly lower in the mature stand. The mean abundance of *GAL15* across the two soil depths decreased from 5.46% to 2.51% with stand age (Table S6).

Soil depth-related differences in the relative abundances of bacterial phyla were substantial: 9 of 10 phyla were significantly influenced by soil depth (Table S6). The relative abundance of *Chloroflexi* sequences increased to 41.44% and replaced *Acidobacteria* as the dominant phylum at 20–40 cm.

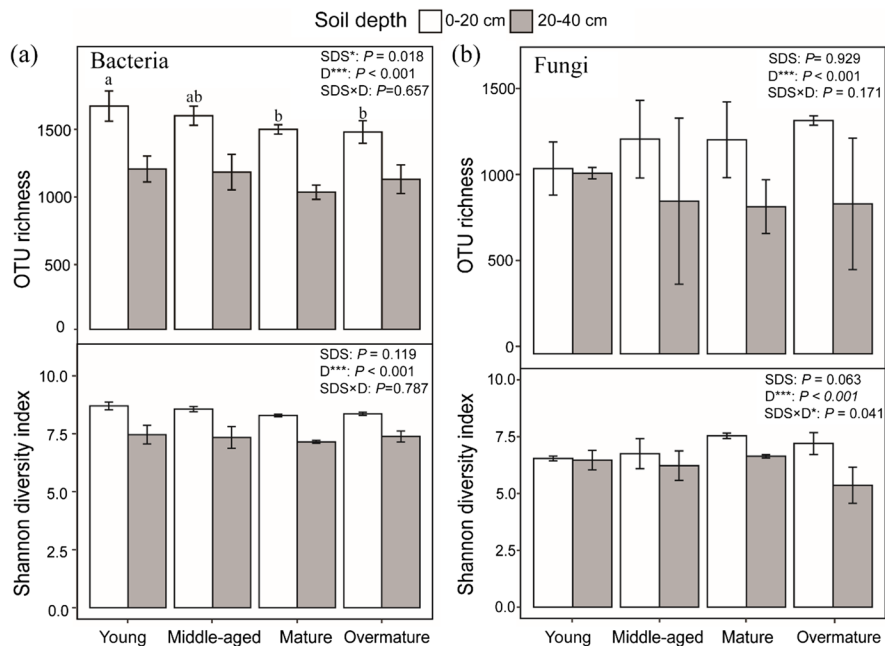


Fig. 2 Richness and diversity index of soil bacterial (a) and fungal (b) communities at soil depths of 0–20 cm and 20–40 cm across four stand developmental stages of Chinese fir plantations. The effects of stand developmental stages (SDS), soil depths (D) or their interaction (SDS×D) on the diversity and richness of soil bacterial and fungal communities were assessed using two-way ANOVA (** $P < 0.001$,

** $P < 0.01$, * $P < 0.05$). Lowercase letters on the top of bars indicate there are significant differences among stand developmental stages within the same soil depth ($P < 0.05$) using Tukey's honest significant difference. A lack of letters indicates that there are no significant differences among stand developmental stages ($P < 0.05$)

Proteobacteria represented 25.0% of the total sequence at 0–20 cm but decreased to 11.9% at 20–40 cm. Similarly, a significant decrease was also recorded for the low-abundance phyla (Fig. 3a and Table S6).

PerMANOVA based on Bray–Curtis dissimilarities indicated that the overall composition of the active bacterial community differed significantly among the different stand stages ($P < 0.001$; Fig. 3b). Following a post hoc pairwise comparison, it was determined that the bacterial communities in soils of the young and overmature stands differed from one another ($P = 0.049$), and those in the middle-aged stands were distinct from those observed in the mature stands ($P = 0.035$; Table S7). The NMDS results showed a clear separation between samples from two different soil depths, which was further corroborated by the PerMANOVA, which revealed that soil depth had a significant influence on bacterial community composition (Fig. 3b).

The soil fungal communities were predominantly composed of members of the phyla *Ascomycota* (73.7%) and *Basidiomycota* (17.0%), which accounted for $90.74 \pm 3.70\%$ and $90.68 \pm 3.88\%$ of the relative abundance at 0–20 and 20–40 cm, respectively (Fig. 4a and Table S8). The stand developmental stage had little effect on the two dominant phyla but significantly affected the phyla present at low relative abundances, e.g., *Glomeromycota*, *Mortierellomycota*, and *Chytridiomycota* (Fig. 4a and Table S8). The relative abundance of *Mortierellomycota*, *Glomeromycota*, and *Chytridiomycota* in the 0–20 cm soil layer increased with stand age, reaching its highest concentration at the mature stage. (Table S8). The abundance of *Rozellomycota* grew over the course of stand development, culminating in the overmature stage with a concentration of $1.70 \pm 0.58\%$. Unlike bacterial species which were sensitive to soil depth, *Rozellomycota* and *Chytridiomycota* were the only two phyla

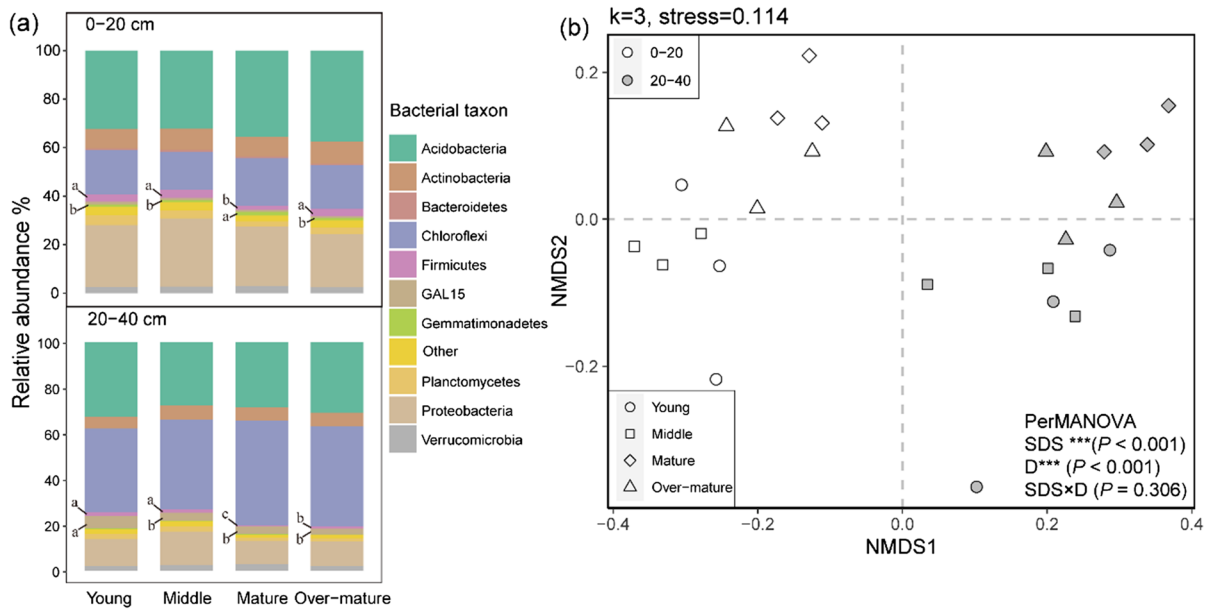


Fig. 3 Average relative abundances of bacterial phyla (a) and NMDS ordination of bacterial OTU matrices based on Hellinger-transformed Bray–Curtis distances (b) at depths of 0–20 cm and 20–40 cm across four stand developmental stages of Chinese fir plantations. The average relative abundances from three replicates were calculated at the phylum level. Taxa with significant differences in average relative abundances among stand developmental stages are marked with different

lowercase letters ($P < 0.05$). A lack of letters indicates there are no significant differences among Chinese fir plantations of different stand developmental stages ($P < 0.05$).

The results from NMDS revealed a significant effect of stand age, with samples from the young and middle-aged stages being distinct from the mature and overmature stages along the first axis (PerMANOVA, $P < 0.001$). According to the pairwise comparison test, a significant disparity was observed in the fungal communities among all age groups ($P < 0.05$, Table S7). Additionally, the fungal community also exhibited significant differences in phylogenetic composition between the 0–20 and 20–40 cm soil layers (PerMANOVA, $P < 0.001$).

For fungal functional guilds, the proportions of ectomycorrhizal, endophyte, litter saprotroph, undefined saprotroph, wood saprotroph, and plant pathogen fungi varied significantly between the different stand developmental stages (Fig. 5; Table S9). Putative saprotrophs were less abundant at the young and middle-aged stages, but ECM fungi had the highest abundance during the middle-aged stage. Endophytes

had a significantly high relative abundance in the mature and overmature stages, while plant pathogens displayed the opposite pattern across the age gradient. All the fungal guilds, apart from plant pathogenic fungi, showed no significant difference in relative abundance between the topsoil and subsoil (Table S9).

Factors driving the taxonomic composition and community structure of soil bacteria

Correlation analysis showed that *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes*, and *Bacteroidetes* had a positive correlation with TC, TN, DOC, and AP, as well as the C:N:P stoichiometry ($P < 0.001$; Fig. 6a). On the other hand, the abundances of *Chloroflexi* and *GAL15* were negatively correlated with these soil parameters ($P < 0.001$). A positive correlation was observed between the $N-NH_4^+$ content and the abundance of some low-abundance phyla, such as *Actinobacteria*,

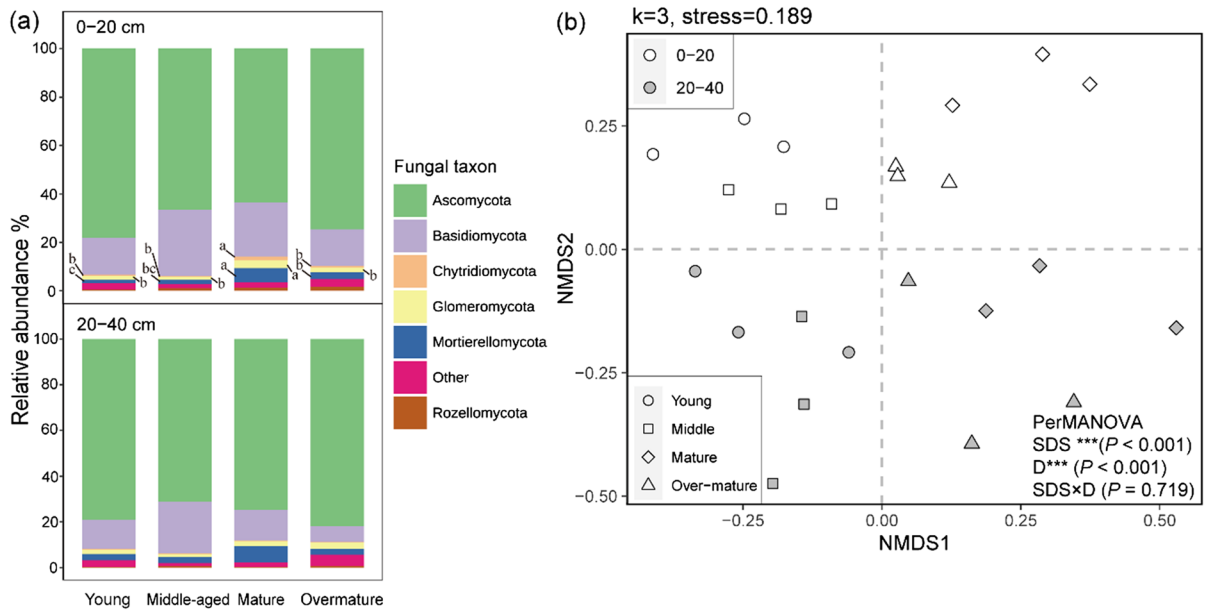


Fig. 4 Average relative abundances of fungal phyla (a) and NMDS ordination of fungal OTU matrices based on Hellinger-transformed Bray–Curtis distances (b) at depths of 0–20 cm and 20–40 cm during different stand developmental stages of Chinese fir plantations. The average relative abundances from three replicates were calculated at the phylum level. Taxa with significant differences in average relative abundances among

stand developmental stages are marked with different lower-case letters ($P < 0.05$). A lack of letters indicates that there are no significant differences among stand developmental stages ($P < 0.05$). The effects of stand development stage (SDS), soil depth (D) and their interaction (SDS × D) on the composition structure of the fungal community were assessed using PerMANOVA (** $P < 0.001$, * $P < 0.01$, $P < 0.05$)

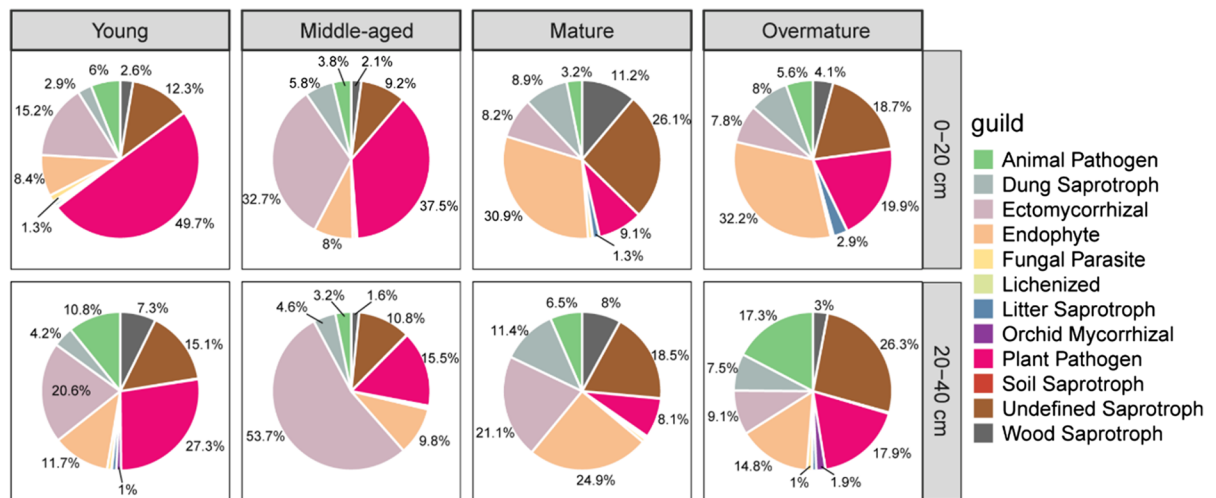


Fig. 5 Relative abundance of soil fungal functional guild assignments at depths of 0–20 cm and 20–40 cm across four stand developmental stages of Chinese fir plantations identified using FUNGuild. Relative abundances less than 1% are not labeled in the figure

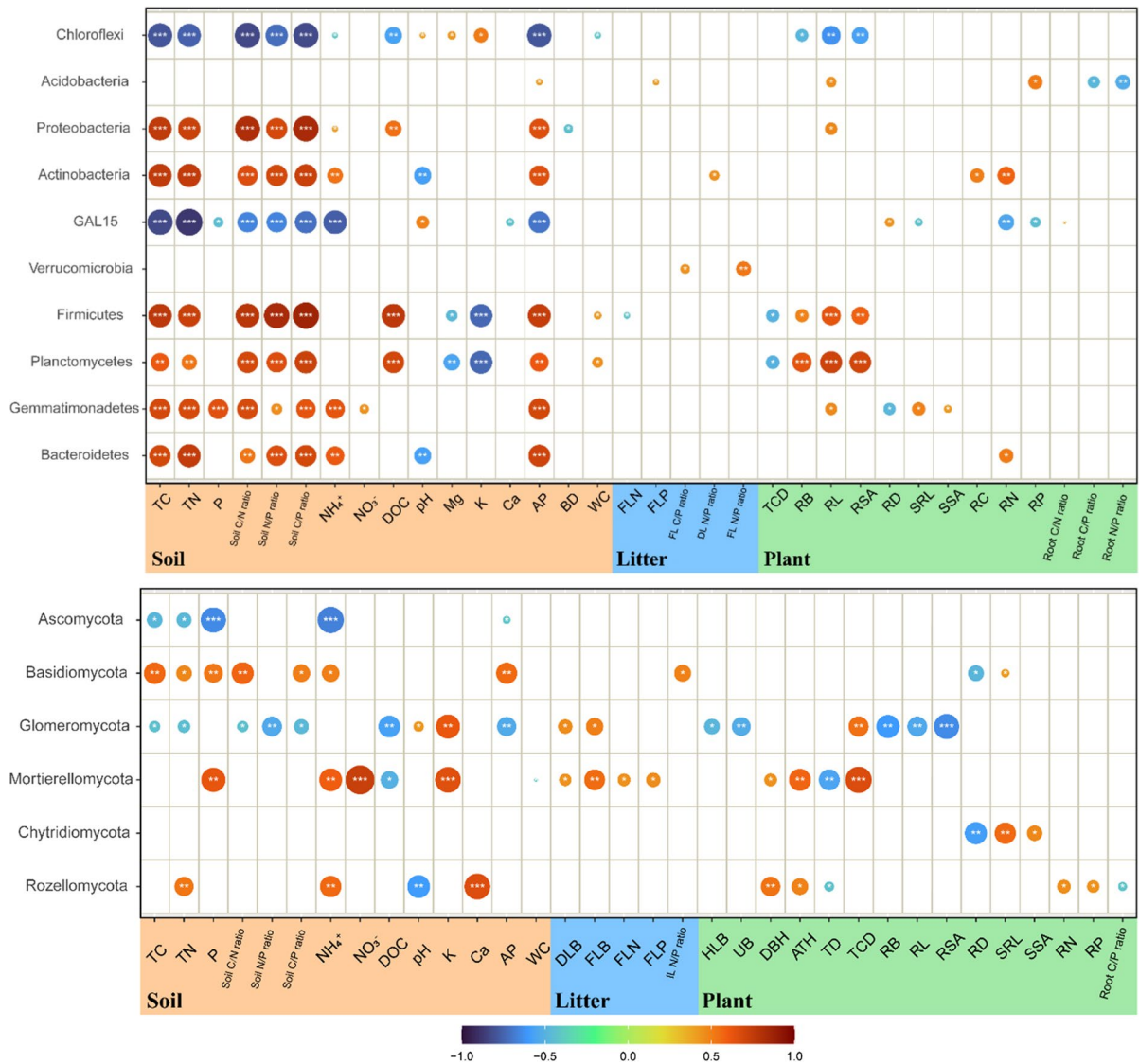


Fig. 6 Heatmap of Spearman's correlation coefficients (ρ values) between the relative abundance of bacterial (a) and fungal (b) phyla and individual variables in the set of soil, plant and litter. The size of the circle indicates the absolute value of the correlation coefficient. Variables with significant correlations with the abundance of any bacterial or fungal phylum are shown as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. TC, total carbon; TN, total nitrogen; P, total phosphorus; N–NH₄⁺, ammonium, N–NO₃⁻, nitrate; DOC, dissolved organic carbon; K, potassium; Mg, manganese; Ca, avail-

able phosphorus; WC, water content; BD, bulk density; DLB, decomposed litter biomass; FLB, fragmented litter biomass; FLN, fragmented litter N; FLP, fragmented litter P; SLB, shrub layer biomass; HLB, herb layer biomass; UB, understory biomass; DBH, diameter at breast height; ATH, average tree height; TCD, tree crown diameter; RB, root biomass; RL, root length; RSA, root surface area; RD, root diameter; SRL, specific root length; SSA, specific surface area; RN, root nitrogen; RP, root phosphorus

Proteobacteria, *Gemmatimonadetes*, and *Bacteroidetes*; however, a negative correlation was seen between the N–NH₄⁺ content and the abundance of *Chloroflexi* and GAL15. Only 5 of 21 litter traits were

significantly associated with 4 bacterial phyla (*Firmicutes*, *Actinobacteria*, *Acidobacteria* and *Verrucomicrobia*) with lower correlation coefficients. Root morphological and chemical traits showed a stronger

correlation with bacterial phyla than other plant variables ($P < 0.05$).

VPA revealed that a combination of soil, plant and litter variables accounted for 50% of the variation in the bacterial community. Soil properties had the highest contribution to the variance, accounting for 15%, while plant variables and litter traits had a lesser impact, explaining 4% and 1%, respectively. A combined 27% of the variance in bacterial community composition was accounted for by the shared

variation between the two groups of soil characteristics and plant variables (Fig. 7a). The variance in the bacterial community was significantly explained by soil properties and plant characteristics (Adj. $R^2 = 0.56$ and 0.51 respectively), whereas the influence of litter traits was not significant (Adj. $R^2 = 0.03$; $P > 0.05$; Table 3).

According to RDA, the bacterial community was clearly split into two clusters, one from 0–20 cm and the other from 20–40 cm, along the first axis

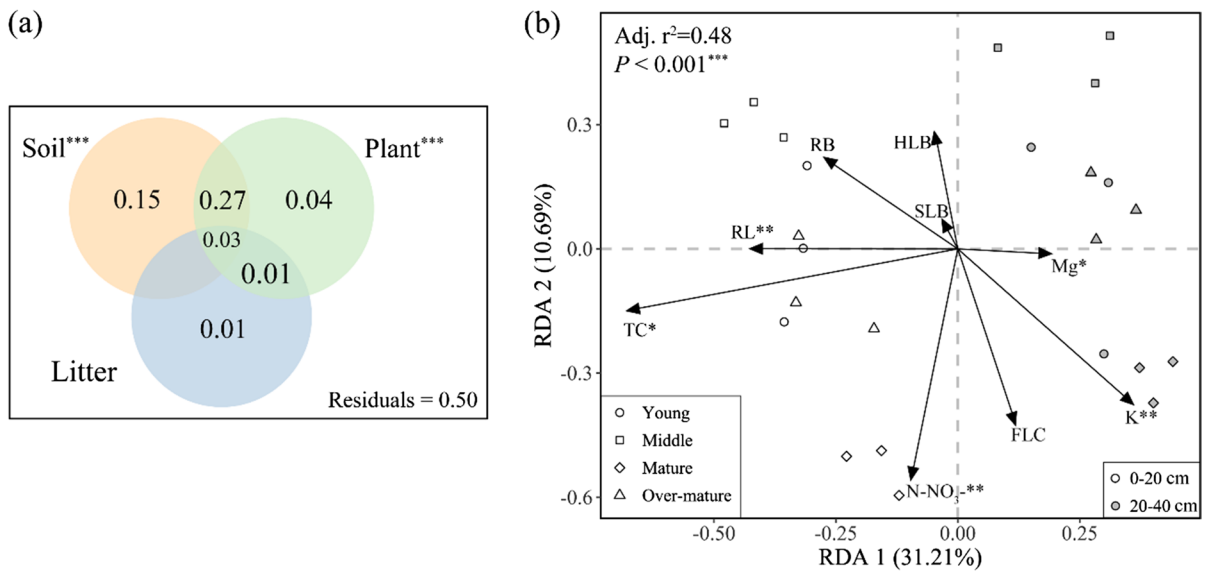


Fig. 7 (a) VPA depicting the proportions of the variance in the bacterial community explained by three sets of predictors: soil properties, plant traits and litter characteristics. The selected variables are listed in Table S12. Numbers in circles are the proportion of explained variables (adjusted R^2 values). The intersection area without values represents 0% explained by shared variance. The significance of the fitting model explained by each variable set is shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (b) dt-RDA of the relationship between the

Bray–Curtis dissimilarity of bacterial communities and 9 individual variables selected based on the VPA result. The significance of each variable explained to the variance in bacterial community is shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. TC, total carbon; N–NO₃⁻, nitrate; K, potassium; Mg, manganese; SLB, shrub layer biomass; HLB, herb layer biomass; FLC, fragmented litter carbon; RB, root biomass; RL, root length

Table 3 The VPA results for the significance level and contribution of the bacterial and fungal communities by three environmental matrices (soil properties, plant traits and litter characteristics)

	Soil properties			***	Plant attributes			***	Litter traits			
	df	R^2	Adj. R^2		df	R^2	Adj. R^2		df	R^2	Adj. R^2	
Bacteria	5	0.56	0.44	***	6	0.51	0.34	***	1	0.07	0.03	ns
Fungi	3	0.31	0.21	***	8	0.55	0.30	***	5	0.34	0.16	***

The df value indicated the degrees of freedom of the selected variables. The values of R^2 and Adj. R^2 indicated the proportion and adjust proportion of variance explained by the fitting models for the response variables. The significance of the fitting models is shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns $P > 0.05$

(Fig. 7b). The profiles of the bacterial communities from 0–20 cm tended to align with higher TC content and longer RL, and lower Mg content. The second RDA axis distinguished the bacterial species composition between the middle and mature stages, and this dissimilarity was strongly linked to the changes in the contents of K, $N-NO_3^-$, FLC, and understory biomass (SLB and HLB). Monte Carlo permutation tests indicated that among these predictors, soil TC (25%, $P=0.022$), K (33%, $P=0.007$), Mg (23%, $P=0.050$), $N-NO_3^-$ (34%, $P=0.005$), and RL (37%, $P=0.009$) were the significant contributors to the total variance (Table 4).

Factors driving the taxonomic composition and community structure of soil fungi

The correlation between major fungal groups and environmental factors indicated that *Ascomycota* was negatively correlated with TC, TN, P, $N-NH_4^+$, and AP, whereas *Basidiomycota* was significantly and positively correlated with the ratios of C/N and C/P. The two most abundant fungal phyla, *Basidiomycota* and *Ascomycota*, rarely showed correlations with plant and litter factors (Fig. 6b). However, *Basidiomycota* had a marginally significant correlation with the N/P ratio of litter, SRL and RD ($P<0.05$). Numbers of biotic and abiotic factors had a significant relationship to the abundance of *Glomeromycota* and *Mortierellomycota*, with 17 and 14 environmental factors respectively.

The soil, plant, and litter variable sets collectively accounted for 41% of the fungal communities (Fig. 8a). Plant factors were the most influential in explaining the variation in fungal community composition, contributing 30% of the variation with a pure effect of 19% (Fig. 8a). Edaphic and litter variables together accounted for 14% and 19% of the variation in fungi, with individual contributions of 4% and 8%, respectively. The dissimilarity of fungal assemblages across stand development stages was significantly predicted by UB, SLB, TCD, FLC, DLN, and IL N/P ratio ($P<0.001$, Table 3). However, the only factor that had a significant impact was the C content in the fragmented litter that directly contacted the soil interface ($P=0.007$, Table 4). RDA also identified that

Table 4 Results from a Monte Carlo permutation test showing the significance and the percentage of variation in bacterial and fungal composition explained by individual variables

Explanatory sets	Variables	Adj. R ²	P value
<i>Bacterial community</i>			
<i>Soil</i>			
	TC	0.25	0.022*
	$N-NO_3^-$	0.34	0.005**
	K	0.33	0.007**
	Mg	0.23	0.050*
<i>Plant</i>			
	RL	0.37	0.009**
	RB	0.18	0.133
	SLB	0.02	0.865
	HLB	0.12	0.251
<i>Litter</i>			
	FLC	0.04	0.738
<i>Fungal community</i>			
<i>Soil</i>			
	TC	0.30	0.029*
	Mg	0.03	0.758
<i>Plant</i>			
	TCD	0.12	0.046*
	ATH	0.02	0.811
	SLB	0.08	0.440
	UB	0.02	0.854
	RD	0.28	0.042*
	RL	0.10	0.308
	RC	0.06	0.535
	RP	0.34	0.010**
<i>Litter</i>			
	DLB	0.04	0.672
	FLC	0.36	0.007**
	DLN	0.08	0.417
	FLB	0.16	0.148
	IL N/P ratio	0.04	0.664

TC total carbon; $N-NO_3^-$, nitrate; K potassium, Mg manganese, RB root biomass, RL root length, SLB shrub layer biomass, HLB herb layer biomass, FLC fragmented litter carbon, DLB decomposed litter biomass, DLN decomposed litter nitrogen, FLC fragmented litter carbon, FLB fragmented litter biomass

root traits (RD and RP), and TC were the major factors that significantly impacted the fungal community

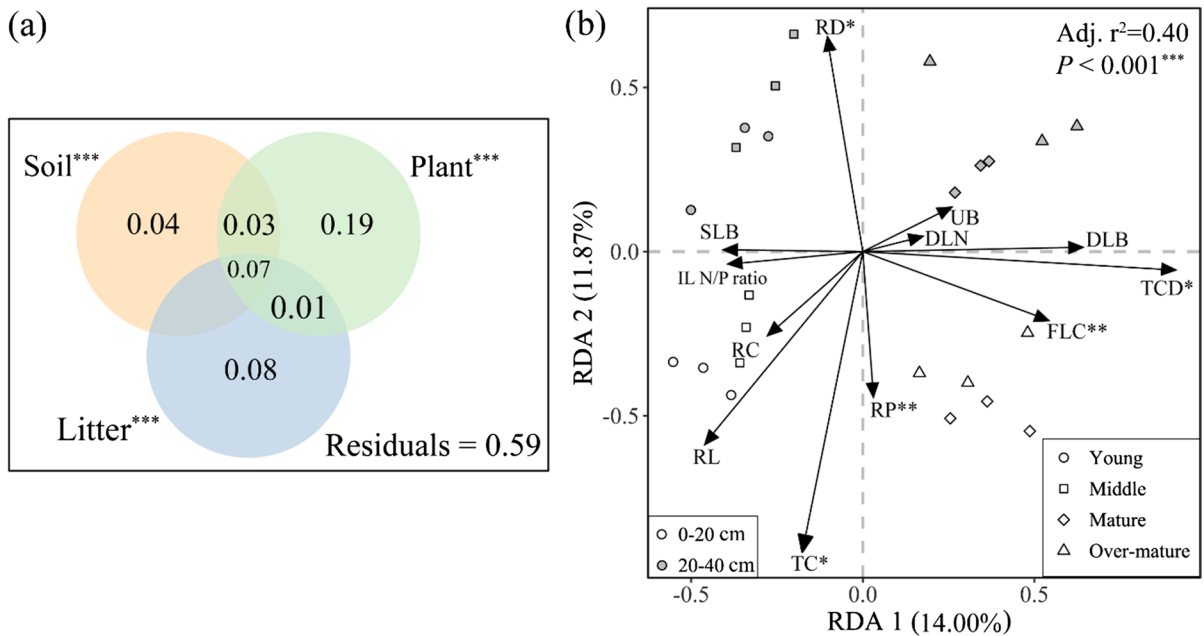


Fig. 8 (a) VPA depicting the proportions of the variance in the fungal community explained by soil properties, plant traits and litter characteristics. The selected variables are listed in Table S12. Numbers in circles are the proportion of explained variables (adjusted R^2 values). The intersection area without values represents 0% explained by shared variance. The significance of the fitting model explained by each variable set is shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. (b) dt-RDA of the relationship between the Bray–Curtis dissimilarity of fun-

gal communities and 15 individuals. The significance of each variable explained to the variance in the fungal community is shown as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. TC, total carbon; DLB, decomposed litter biomass; DLN, decomposed litter nitrogen; FLC, fragmented litter carbon; SLB, shrub layer biomass; UB, understory biomass; DBH, diameter at breast height; ATH, average tree height; TCD, tree crown diameter; SLB, shrub layer biomass; UB, understory biomass; RL, root length; RD, root diameter; RP, root phosphorus

across horizontal soil layers ($P < 0.05$, Table 4 and Fig. 8b).

Discussion

Plant characteristics as drivers of soil bacterial and fungal communities

Although plant attributes significantly explained the variance in the bacterial community, they only accounted for a unique proportion of 4.0% (Fig. 7a). Of all plant variables, RL was the only significant plant parameter able to predict the bacterial community in the RDA model, and it was mainly associated with the bacterial variation across soil depth (Fig. 7b). Plant variables independently accounted for the largest proportion (19%) of the total variance in fungal community composition (Fig. 8a), pointing to their significance as drivers of the soil fungal

community. Our hypothesis that plants have a more profound impact on the fungal community, as some fungal groups can establish either obligatory or facultative symbiotic relationships with plants, was validated by the results.

Among all plant factors, the canopy structure and root traits of canopy-dominant trees were the main factors that explained the variation in the species composition of fungi. TCD, acting as a proxy of forest structure, was significantly related to the transition of fungal community structures from younger stages to older stages (Fig. 8a and Table 4). With the developmental progression of Chinese fir plantations, tree growth and commercial thinning substantially shape stand structure (Zhou et al. 2016), which in turn affects the amount of precipitation and light that reaches the forest floor, thus altering the microclimatic conditions beneath the crown canopy (Lucas-Borja et al. 2016; Tomao et al. 2020). The assembly and physiological activities of soil fungi have been

reported to be strongly controlled by environmental heterogeneity, microclimate and the soil biochemical cycle within canopy gaps (Li et al. 2019; Qiang et al. 2023).

Our findings also highlight the potential role played by roots as a driver of both bacterial and fungal communities. Bacterial biomass, diversity, and some bacterial taxa were correlated with root morphological traits, such as RL, RB, and RSA (Tables S10–11 and Figs. 6a, 7b). Root morphological traits, including root surface area and root tips, were used to successfully predict root exudation rates and amounts of conifer species (Akatsuki and Makita 2020), thereby suggesting a tight link between root abundance and the amount of rhizodeposition. It was frequently reported that higher RL, RB, and root tips would support higher bacterial activity and biomass by enlarging the distribution area and promoting partitioning production and flow rates of photosynthates delivered to soils (Eisenhauer et al. 2017; Legay et al. 2014; López-Angulo et al. 2020; Maherali 2014). Together, the significant decline in RL and RSA along the soil profile and stand age (Table S1) may lead to a decline in rhizodeposition production that hinders bacterial biomass and leads to a shift in the bacterial community with stand development.

In contrast to bacteria, RD, SRL and RP were the significant explanatory variables for the separation of fungal assemblages across different soil depths. An increase in RD but decreases in SRL and SSA with soil depth (Table S2) indicated that shallow fine roots were significantly thinner, and roots distributed in deep soils tended to be thicker. Thinner roots in resource-rich surface soils are often related to better exploitation capacities and more acquisitive strategies (i.e., high SRL and thin RD) (Prieto et al. 2015). Conversely, thicker roots in deeper soils are associated with resource conservation strategies (Gu et al. 2014) and display greater root cortical areas and C investment for symbiotic partnerships, especially arbuscular mycorrhizal fungi (AMF), in return for limiting resources (Bergmann et al. 2020). The root symbiosis of Chinese fir with AMF is an important approach to increase phosphorus (P) uptake in P-limited subtropical forests (Tian et al. 2023). Large differences in soil P availability across soil layers (Table S4) likely drive disparities in root resource acquisition strategies that successively shape fungal community assembly via root selection and environmental filtering. Thus,

suites of root traits that relate to resource acquisition strategy and root nutrient status were frequently observed as the drivers shaping fungal composition (Hogan et al. 2023; Prieto et al. 2015). Moreover, greater RSA increases the probability of contact between propagules and roots (Maherali 2014), leading to higher incidences of root infections by fungal pathogens. In line with these studies, the relative abundance of *Chytridiomycota*, of which some members are plant pathogens, was positively correlated with SSA and SRL (Fig. 6b), which decreased with soil depth. The relative percentage of plant pathogens also showed a decreasing pattern with soil depth (Table S9), indicating that higher RSA in surface soils was more vulnerable to those fungal groups.

A previous study demonstrated that the long-term removal of understory vegetation in Chinese fir plantations resulted in a decrease in soil fungal biomass (Wan et al. 2021), indicating that understory vegetation plays a significant role in sustaining soil biological processes. However, we found that understory vegetation, including herbs and shrubs, did not exert a significant effect on the succession of either bacterial or fungal composition (Fig. 7b, Fig. 8b and Table 4). The biomass of the understory was observed to account for a small fraction of the total stand productivity, amounting to less than 0.02%, and this proportion decreased as the stand aged (Table S3). According to the estimate of Ma et al. (2007), the understory litter constituted a minor fraction, ranging from 1–9% of the nutrient restitution in a young Chinese fir stand. The small contribution of the understory layer to the soil C and nutrients in Chinese fir plantations could explain why the understory vegetation had a minimal effect on soil microbial community. Furthermore, species composition, coverage, and root traits were not accounted for when assessing the contribution of understory vegetation to the variation in the microbial community, potentially masking the effect of the understory vegetation.

Soil properties as drivers of soil bacterial and fungal communities

The VPA results revealed that individual soil abiotic factors had a considerable impact on both bacterial and fungal communities, with a more pronounced effect on the soil bacterial composition, thus verifying our hypothesis (Fig. 7 and Fig. 8). Edaphic

parameters constituted the major drivers of bacterial community structure and diversity, as demonstrated in previous studies (Dini-Andreote et al. 2014; Jiang et al. 2021; Lauber et al. 2009; Lladó et al. 2018). The db-RDA and correlation analyses between variables and the NMDS ordination showed that the differences in soil bacterial communities across the age gradient of Chinese fir plantations were linked to higher concentrations of inorganic N, TP, and K (Fig. S4a and Fig. 7b). It is widely recognized that high soil nutrient availability supports bacterial growth and activity, which consequently shape the structure and functions of the bacterial community (Fierer et al. 2009; Legay et al. 2014). It was therefore not surprising that *GAL15*, which has been demonstrated to be oligotrophic (Brewer et al. 2019), was found to decrease in abundance with increasing stand age, while the *Gemmatimonadetes* groups, possessing copiotrophic attributes (Rime et al. 2015), displayed a higher abundance at the later stages. The changes in the relative abundance of *GAL15* and *Gemmatimonadetes* were significantly associated with an enhanced availability of N and P in soils throughout the succession of the Chinese fir plantation (Fig. 6a), as indicated by the decreasing ratios of C/N and C/P and TP in parallel with an increase in the $N-NH_4^+$ content (Table S4). Copiotrophic bacteria may be capable of outcompeting oligotrophic bacteria due to their ability to flourish when nutrients are abundant and reproduce quickly (Fierer et al. 2009).

Apart from bacterial community structure, bacterial biomass and α -diversity were also significantly correlated with most soil properties (Tables S10–11). Among the soil factors that responded significantly to forest age, the concentrations of DOC and WC decreased with stand development and were positively correlated with bacterial abundance and richness. DOC is the main source of available C substrates for soil microorganisms (Marschner and Kalbitz 2003) and plays vital roles in maintaining the growth of microbial populations. Bacterial groups were reported to preferentially use labile organic materials and be sensitive to the availability of soil resources (Sayer et al. 2017). As surrogates for root exudate production (López-Angulo et al. 2020), root growth traits (i.e., RL and RB) showed a decreasing trend following the establishment of the Chinese fir plantation and were also positively correlated with the bacterial biomass and the alpha diversity index. Thus,

these results provide evidence that the depletion of carbohydrates and water in older stands (Table S3) may suppress the catabolic activity of bacteria and subsequently reduce the abundance and diversity of bacterial species via competitive exclusion.

The abundance, α -diversity, and most phyla of both bacteria and fungi were significantly correlated with the concentrations of TC and TN, and the ratios of C:N:P (Table S10–11; Fig. 6a) and TC were the primary controls on the depth-integrated separation of microbial communities (Figs. 7, 8 and Table 4). Variations in TC, as well as the ratios of C/N and C/P across the soil profile (Table S3), suggest a sharp decrease in the availability of carbon and mineralizable carbon with depth. These findings provide evidence of the major influence of C availability on microbial diversity, composition, and distribution along the soil profile in our study area, which agrees with previous research (Fierer et al. 2009; Hansel et al. 2008). For example, *Chloroflexi* and the candidate phylum *GAL15*, both of which are chemolithotrophic or phototrophic (Bennett et al. 2020; Brewer et al. 2019), were more abundant in deeper soil layers with limited resources. Therefore, it was observed that there was a negative correlation between the concentrations of TC and TN and the ratios of C:N:P and the relative abundance of these two phyla.

Litter traits as drivers of soil bacterial and fungal communities

Our results revealed that the structure of soil fungal communities among younger and older stands was associated with the C content of the decaying fragmented litter, while none of litter traits had no significant influence on bacterial communities (Figs. 7, 8 and Table 4). The results are in line with our assumptions that litter parameters would have a greater contribution to the structure of soil fungal communities than bacterial communities. A special phenomenon that occurs in Chinese fir plantations is that more than 90% of the dead branches and senescent leaves remain on tree stems as the stand canopy closes (Zhang and Sheng 2001). The labile components present in these senescent twigs are rapidly released, driven by strong leaching processes, and strongly decompose over several years because of the heavy rainfall and high temperatures in the study area (Mao et al. 2021). Higher standing biomass, TC content and C/N ratio of litters at the

later stages were observed in this study (Table S3), suggesting gradual accumulation of these recalcitrant litters on the forest floor after senescent twigs fell off stems during the development of the Chinese fir plantations. It is well known that some fungal groups are highly specific to decompose lignin and other recalcitrant components in soils by producing lignin-degrading enzymes (Zhang et al. 2018). The lack of correlation between the bacterial community and litter properties in this study was probably related to the substantial loss of easily available matter in the litter materials because bacteria have been shown to generally prefer to utilize easily decomposable compounds (Legay et al. 2014), and most bacteria lack lytic enzymes for recalcitrant substrate decomposition (Lindahl and Tunlid 2015; Zhang et al. 2018). We thus suggest that shifts in soil fungal communities during the stand development of Chinese fir plantations are associated with the accumulation of recalcitrant litters on the forest floor.

Moreover, we also found that a higher abundance of *Mortierellomycota* at later stages, of which the members are dominantly saprotrophic (Tedersoo et al. 2014), was associated with biomass and N and P contents of fragmented litter (Fig. 6a and Table S5), where nutrient cycles are intensified and thus provide available niches for a variety of saprotrophic fungi (Uroz et al. 2016). The existing biomass of the partly decomposed and fragmented litter materials was significantly correlated with the relative abundance of *Glomeromycota*. AMF, a phylogenetically distinct group of fungi from the phylum *Glomeromycota*, that colonize and proliferate in leaf litter have been commonly found across a variety of ecosystems (Bunn et al. 2019). The hyphae of AMF within litter access litter-bound nutrients and release nutrients to their associated host plants as well as nearby soil microbiota (Bunn et al. 2019). Increases in these fungal taxa and their association with the complexity and recalcitrance of litter along the age gradient suggest that the overall pattern and functions of the soil fungal community would shift toward intensifying litter decomposition and nutrient cycling with the development of Chinese fir plantations.

Soil bacterial and fungal community responses to stand development of Chinese fir plantation

In this study, we observed that the unique bacterial OTU number decreased with succession in

the Chinese fir plantations (Fig. S3a); in contrast, a decrease in fungal OTU number did not occur during stand development (Fig. S4a). Bacterial abundance and OTU diversity significantly decreased with increasing stand age whereas the abundance and diversity of soil fungi remained relatively stable with respect to different stand ages of Chinese fir plantations (Fig. 2). These results were inconsistent with our prediction that the soil fungal community is more sensitive than the bacterial community to the stand development stages of Chinese fir. Yang and Wu (2020) found that bacterial communities are more vulnerable than fungal communities to environmental change caused by vertical climate zones corresponding to different forest types. Lan et al. (2022) reported that fungal networks shared more links and became more complex than bacterial communities when rainforests were replaced with rubber plantations on Hainan Island, China, suggesting that fungal communities have a higher resistance to unfavorable conditions. Fungal species are well known to efficiently exploit resources through spores and hyphae or through mutualistic symbiosis with plants (Tomao et al. 2020). Saprotrophs and ECM fungi show higher decomposition capabilities of producing various types of enzymes than bacteria, allowing them to first obtain resources from many of the relevant available substances (Lindahl and Tunlid 2015; Zhang et al. 2018). Thus, the constant dynamics of fungal abundance and alpha diversity with stand age might be related to stable connections among fungal species and higher adaptability to environmental changes.

The relative abundance of the dominant bacterial and fungal taxa remained largely unchanged with increasing stand age, and the shifts in both fungal and bacterial communities among the different stand developmental stages were mainly contributed by the change in subordinate taxa (Fig. 3a and 4a; Table S6 and Table S8). *Acidobacteria*, *Chloroflexi*, and *Proteobacteria* dominated the soil bacterial community in the four forest stands, while *Ascomycota* and *Basidiomycota* were the dominant groups of soil fungal communities, accounting for more than 90% of all taxonomic fungal sequences. The dominant bacterial and fungal phyla are consistent with previous observations in different soil types across the tropics to cold temperate regions (Jiang et al. 2021; López-Mondéjar et al. 2015; Vieira et al. 2020). The relative abundances of dominant species of both the bacterial

community and fungal community among four stand stages were similar, probably because these dominant organisms are generalists with a broad range of tolerances and resource use (Fierer et al. 2009). The changes in vegetation and climate during successional stand development in such local monoculture plantation forests are not enough to change the relative composition of these generalist taxa that experience high natural variation. In contrast, taxa with high resource specificity or specialist functions are likely to be more susceptible to natural or human-induced disturbances (Fierer et al. 2009; Sayer et al. 2017).

For fungal guilds, we found that ECM fungi showed a higher abundance in the middle-aged stand than in the other stands (Fig. 5 and Table S9). Mycorrhizal communities have been continually observed to positively correlate with the diversity of existing plant communities (López-Angulo et al. 2020; Sayer et al. 2017; Zhang et al. 2018). Since Chinese fir is a tree species colonized by AMF, ECM fungi should be found on plants beneath the canopy layer. Small openings and gaps created by thinning at the middle-aged stage drive vigorous species (Zhou et al. 2016) and biomass proliferation (Table S3) in the layer of understory vegetation, which may increase opportunities for mycorrhizal symbiosis. In addition, various saprotrophic fungi (i.e., wood, dung, litter and undefined saprotrophs) showed higher abundances at the mature and overmature stages, which are consistent with those reported by Chen et al. (2021), who found that trees in Chinese fir plantations rely more on saprotrophic fungi to recycle nutrients from complex organic matter originating from litter deposition at older stages, accompanied by a decrease in the abundance of ECM fungi. Saprotrophic fungi were able to exploit the void left by ECM fungi, as the two shared a similar fundamental niche (Kyaschenko et al. 2017a). Our results also revealed that the shifts in the fungal community were closely related to the biomass and quality of the fragmental litter layer (Fig. 6 and Fig. 8a). Saprotrophic fungi were favored by the accumulation of recalcitrant litter; thus, ECM activities may be suppressed in the later stages. The increasing availability of N and P and cation concentrations (K, Mg, and Ca) in older stands (Table S4) may have resulted from the mobilization of nutrients bonded within organic matter, which was contributed by a high abundance of free-living saprotrophs via their soil N and P cycling capacities (Kyaschenko

et al. 2017b). The greater availability of these elements allows host plants to reduce their dependency on mycorrhizal fungi while inducing a shift in the dominance of oligotrophic and copiotrophic bacteria from younger to older stages (Fig. 3a).

The fungal abundance of plant pathogens and endophytes showed opposite trends with increasing stand age (Fig. 5), implying that an antagonistic relationship seems to exist between endophytes and plant pathogens in the soils of Chinese fir plantations. Root-associated endophytes in some plants were suggested to confer inhibitory effects on soil-borne plant pathogenic fungi and contribute to healthy soils and the nutrition and stress resistance of host plants (Hallmann and Sikora 1996; Rodriguez et al. 2009). Based on the observations described above, an increase in endophytes and a decline in plant pathogens along the age gradient may be related to enhanced tree disease defense and habitat adaptation with stand development.

Conclusions

The biomass and α -diversity of bacteria declined, whereas the fungal biomass and diversity remained relatively constant along an age sequence of Chinese fir plantations. The functional groups of both bacteria and fungi undergo a shift as stand development progresses. There was a decrease in the abundance of bacterial phyla with oligotrophic attributes, while an increase was observed in copiotrophic bacteria. The fungal groups associated with plants were gradually overshadowed by saprotrophic fungi. Soil abiotic properties related to the availability of carbon and nutrients were the main factors influencing the soil bacterial populations. The variation in the soil fungal community is largely determined by the characteristics of living vegetation. The root traits of trees were the most significant determinants among the plant explanatory variables in accounting for the variance in both bacterial and fungal communities; however, distinct root traits were responsible for explaining the variations in bacterial and fungal communities. A correlation was found between the structure of soil fungal communities and the chemical composition of fragmented litter across various stands, while no significant impact of litter parameters on bacterial communities was identified. The results highlight the

importance of considering the unique responses of bacterial and fungal communities to alterations in forest structure, litter layer, roots, and soil matrix when assessing the impacts of improved management practices and potential climate variations on soil biochemical processes in Chinese fir plantations.

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Data availability The data that support the findings of this study are available on request from the corresponding authors, [Y. Zhang and R. Mao], upon reasonable request.

Declarations

Ethical approval The authors have no relevant financial or nonfinancial interest to disclose.

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