MICROBIAL COMMUNITY STRUCTURE AND DIVERSITY IN RHIZOSPHERE AND NON-RHIZOSPHERE SOILS OF *TSUGA CHINENSIS* AT DIFFERENT TREE AGES

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Abstract. The aim of this study was to investigate the effects of tree age on the growth and development of rhizosphere and non-rhizosphere microbial community structure and diversity of *Tsuga chinensis*. The Illumina MiSeq high-throughput sequencing platform was employed to analyze the composition and diversity of bacterial and fungal communities in the rhizosphere and non-rhizosphere soils of mature and young trees in the Yuanbaoshan National Nature Reserve. The results revealed significant differences in microbial community structure under different tree ages and soil microenvironments. At the phylum level, the dominant bacterial phyla in both rhizosphere and non-rhizosphere soils of *T. chinensis* at different tree ages were Proteobacteria, Actinobacteriota, and Acidobacteriota. For fungi, the dominant phyla included Basidiomycota, Ascomycota, Mortierellomycota, unclassified_k__Fungi, and Rozellomycota. Genus-level analysis showed high similarity in the composition and abundance of bacterial communities between mature and young tree soils, regardless of rhizospheric influence. In contrast, fungal communities exhibited notable specificity. *Archaeorhizomyces* was identified as a dominant fungal genus unique to the non-rhizosphere soil of young *T. chinensis*, while *Russula* was dominant in the non-rhizosphere of mature trees and in both rhizosphere and non-rhizosphere soils of young trees. Alpha diversity analysis indicated that the diversity of bacterial and fungal communities in the rhizosphere of mature trees was generally higher than that of young trees, and the diversity in rhizosphere soils was higher than in non-rhizosphere soils. This study deepens the understanding of the interactions between *T. chinensis* and soil microorganisms and provides theoretical support for its resource conservation and scientific management.
Keywords: *rhizosphere effect, microbial ecology, bacterial and fungal diversity, alpha diversity indices, soil microbial diversity*

Introduction

Tsuga chinensis (family Pinaceae) is an evergreen tree species endemic to China and a Tertiary relict plant. It is primarily distributed in southwestern Jiangxi, southern Hunan, northern Guangdong, northeastern Guangxi (Jinxiu and Longsheng), and southeastern Guizhou, mostly growing at elevations ranging from 600 to 2,100 meters. It typically forms mixed forests with other coniferous and broad-leaved species and serves as a key constructive species in subtropical montane forest ecosystems (Zhang et al., 2011). Due to habitat fragmentation and human disturbance, the population of *T. chinensis* has drastically declined, and it is currently listed as a key protected plant species in the Guangxi Zhuang Autonomous Region (Wei et al., 2023). A deeper understanding of its

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ecological adaptation mechanisms, particularly its interactions with soil microorganisms, is essential for the effective conservation and sustainable utilization of *T. chinensis*.

Rhizosphere soil represents the interface between plants and soil microorganisms. The microbial community in this zone not only regulates plant nutrient uptake and activates secondary metabolite production but also plays crucial roles in disease resistance, root development, and responses to environmental stress (Trivedi et al., 2020). Previous studies have shown that rhizosphere microbes contribute significantly to the stability of forest ecosystems by enhancing nutrient cycling and improving plant stress tolerance (Mendes et al., 2013). Compared with non-rhizosphere soil, the microbial composition and functional complexity of rhizosphere soil are greater due to the influence of root exudates, resulting in high spatial heterogeneity (Bulgarelli et al., 2013).

Current research on *T. chinensis* mainly focuses on its population structure (Zhao et al., 2022), regeneration mechanisms (Lin et al., 2019), ecological niche characteristics (Zhao et al., 2021), and ectomycorrhizal diversity (Qian et al., 2007), with limited attention to the structure and diversity of microbial communities in its rhizosphere and non-rhizosphere soils. *T. chinensis* may exhibit varying root activity and ecological regulation capacity at different developmental stages. Therefore, in this study, tree age was used as a variable, and high-throughput sequencing technology was employed to systematically analyze the microbial community structure and diversity in rhizosphere and non-rhizosphere soils of *T. chinensis* at different ages in the Yuanbaoshan National Nature Reserve. The objective is to reveal the interaction mechanisms and community variation patterns between *T. chinensis* and soil microorganisms across developmental stages and to explore, the impact of tree age on the soil microenvironment, thereby providing theoretical support for the scientific conservation and ecological function maintenance of *T. chinensis*.

Materials and methods

Overview of the study area

The study area is located in the Yuanbaoshan National Nature Reserve in the central part of Rongshui County, Guangxi (*Fig. 1*). The terrain is complex, with elevations ranging from 1,000 to 1,500 m, the highest peak reaching 2,081 m, and the lowest elevation being 285 m. This area belongs to the mid-subtropical monsoon climate zone, characterized by abundant heat and precipitation. The annual average temperature is approximately 16.4°C, and the annual average precipitation is around 2,379 mm, making it one of the areas with the highest rainfall in Guangxi. Soil types change progressively with elevation, including mountain red soil, mountain yellow soil, and mountain yellow-brown soil. In some regions, mountain meadow soil is also present.

Sample collection and processing

Soil sampling was conducted in May 2022 at the mountaintop of the Yuanbaoshan National Nature Reserve, following the method described by Bao et al. (2000). One sampling plot was established, within which three healthy mature *T. chinensis* trees (height > 10 m, diameter at breast height > 35 cm) and three healthy young trees (height > 50 cm, diameter at breast height > 3 cm) were randomly selected. For each tree, soil was excavated to a depth of 50–60 cm approximately 1 m from the trunk using a spade to expose the roots. Soil particles (<1 cm in diameter) adhering to the roots were

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collected as rhizosphere soil samples, while soil located approximately 30 cm away from the root zone was collected as non-rhizosphere soil samples. Two samples were collected from each tree, corresponding to rhizosphere and non-rhizosphere zones, resulting in a total of 12 soil samples. Sampling information was recorded using labeled tags. All samples were immediately placed in a portable cooler in the field and transported to the laboratory, where they were stored at –80 °C for subsequent soil DNA extraction and high-throughput sequencing of bacterial 16S rRNA and fungal 18S rRNA genes.

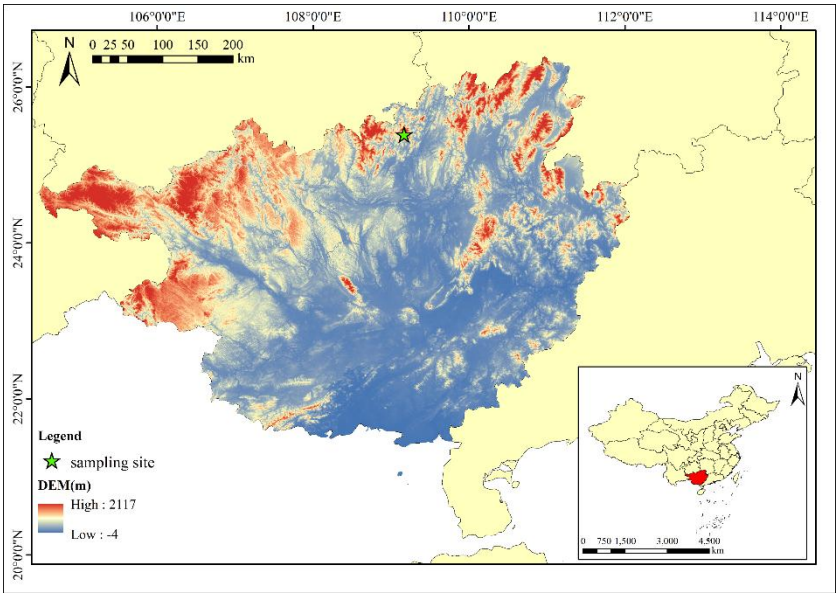

Figure 1. *Map of the study area and the location of the test sample*

Determination of soil microbial community diversity

Total DNA of soil microorganisms was extracted using the Fast Soil Genomic DNA Extraction Kit provided by Shanghai Sangon Biotech Co., Ltd. The extracted DNA samples were shipped on dry ice to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for sequencing. Sequencing procedures included PCR amplification, pooling and purification of PCR products, and library construction. The bacterial V5–V7 region was amplified in two rounds. The first-round PCR used primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1392R (5'-ACGGGCGGTGTGTRC-3'), with the following program: initial denaturation at 95°C for 3 min; 30 s denaturation at 95°C; 30 s annealing at 55°C; 45 s extension at 72°C; 27 cycles; final extension at 72°C for 10 min. The second-round PCR used primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'), with the following program: initial denaturation at 95°C for 3 min; 30 s denaturation at 95°C; 30 s annealing at 55°C; 45 s extension at 72°C; 13 cycles; final extension at 72°C for 10 min. The fungal ITS region was amplified in a single round using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'), with the program: initial denaturation at 95°C for 3 min; 30 s denaturation at 95°C; 30 s annealing at 55°C; 45 s extension at 72°C; 35 cycles; final extension at 72°C for 10 min. The amplification products were sequenced using the Illumina platform provided by Majorbio.

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Quality control and analysis of microbiome sequencing data

Raw sequencing data were subjected to quality control and merging to obtain high-quality valid data. OTU (Operational Taxonomic Unit) clustering and taxonomic analysis were performed using the Uparse software platform. The OTU clustering procedure was as follows: (1) extract non-redundant sequences from optimized sequences to reduce redundancy in intermediate analysis; (2) remove singleton sequences without duplicates; (3) cluster non-redundant sequences (excluding singletons) at 97% similarity, and remove chimeras during clustering to obtain representative OTU sequences; (4) map all optimized sequences to the representative OTU sequences and select those with ≥97% similarity to construct the OTU table. Taxonomic analysis of OTU representative sequences at the 97% similarity level was performed using the RDP Classifier Bayesian algorithm.

Analysis of soil microbial composition

Based on sequencing data, Alpha diversity indices reflecting microbial community richness and diversity under different random subsampling depths were calculated using the mothur software, including Ace, Chao, Simpson, and Shannon indices (Schloss et al., 2009). Among them, Ace and Chao indices reflect the richness of communities in the samples, while Simpson and Shannon indices reflect community diversity. Higher Ace, Chao, and Shannon values and lower Simpson values indicate greater species diversity in the samples. Bar plots of microbial community composition were generated using R (R Core Team, 2023) and the ggplot2 package (Wickham, 2016). Venn diagrams were employed to visualize the number of shared and unique OTUs among multiple groups or samples and were generated using the VennDiagram package (Chen, 2022). Alpha diversity analysis was also supported by the vegan package (Oksanen et al., 2022).

Results and analysis

Sequencing results of soil microbial samples

After splicing and quality control, 415,085 valid bacterial sequences with an average length of 377 bp and 376,103 valid fungal sequences with an average length of 257 bp were obtained. At 97% sequence similarity, the dilution curves (*Fig. 2*) of bacterial and fungal communities in each sample plateaued, indicating adequate sequencing depth and reasonable sampling. This suggests that the microbial communities were reliably represented. The coverage rate for all samples exceeded 99.7%, showing that most bacterial and fungal taxa were captured and that the results largely reflect the structure of microbial communities in the soils.

Microbial diversity and richness in rhizosphere and non-rhizosphere soils of Tsuga chinensis at different ages

Alpha diversity indices

According to *Table 1*, the Shannon index of bacterial communities followed the order: old tree non-rhizosphere > young tree non-rhizosphere > young tree rhizosphere > old tree rhizosphere. The Simpson index followed the order: old tree rhizosphere > young tree rhizosphere > old tree non-rhizosphere = young tree non-rhizosphere. These results indicate that bacterial community diversity was generally higher in old tree soils compared to young tree soils, and slightly higher in non-rhizosphere soils than in

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rhizosphere soils. Both the Chao1 and ACE indices showed greater richness in old tree soils than in young tree soils, with non-rhizosphere soils having slightly higher richness than rhizosphere soils in both age groups.

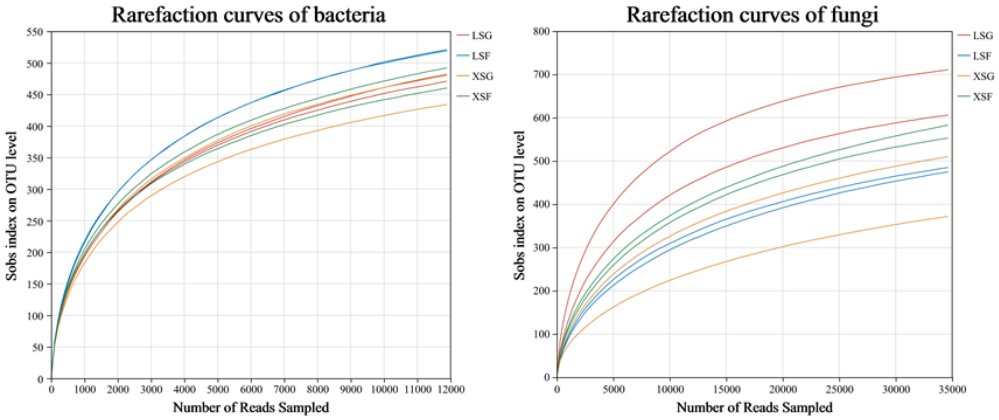

Figure 2. *Dilution curves of the soil samples. Note: LSG: Rhizosphere of old trees; LSF: Non-rhizosphere of old trees; XSG: Rhizosphere of young trees; XSF: Non-rhizosphere of young trees. The same abbreviations apply hereafter*

Table 1. *Alpha diversity of bacterial and fungal communities in rhizosphere and non-rhizosphere soils of Tsuga chinensis at different ages*

Microbe	Soil stype	Shannon index	Simpson index	Chao1 index	ACE index	Coverage rate/%
Bacteria	LSG	4.4022± 0.0384C	0.0348± 0.002A	577.7856± 9.4318a	575.1708± 14.8666ab	0.9902± 0.0003b
	LSN	4.6965± 0.0363A	0.0229± 0.002B	597.4534± 10.5873a	598.8061± 5.1733a	0.9907± 0.0003ab
	XSG	4.4379± 0.0837C	0.0311± 0.004A	535.0551± 25.9584b	537.9676± 27.0517b	0.9913± 0.0005a
	XSN	4.5957± 0.0202B	0.0229± 0.0005B	574.8041± 23.5746a	568.1888± 22.0286ab	0.9905± 0.0005ab
Fungi	LSG	3.4647± 0.3759A	0.1359± 0.0341C	732.9367± 45.382a	729.8238± 42.9566a	0.9966± 0.0001A
	LSN	2.9804± 0.0281B	0.1906± 0.0046B	688.9622± 40.6194ab	706.2363± 35.5976ab	0.9955± 0.0004B
	XSG	2.5106± 0.1115C	0.2295± 0.0006B	571.2429± 70.2277c	588.2047± 64.6853c	0.9959± 0.0003B
	XSN	2.2181± 0.0215C	0.3269± 0.0428A	608.864± 11.328bc	625.5835± 11.6161bc	0.9958± 0.0002B

Note: Data are expressed as mean ± SD (n = 3). Different lowercase letters in the same row indicate significant differences ($P < 0.05$); different uppercase letters indicate extremely significant differences ($P < 0.01$)

For fungi, the Shannon index followed the order: old tree rhizosphere > old tree non-rhizosphere > young tree rhizosphere > young tree non-rhizosphere, while the Simpson index followed the order: young tree non-rhizosphere > young tree rhizosphere > old tree non-rhizosphere > old tree rhizosphere. The highest fungal diversity was observed in the rhizosphere soil of old trees. Chao1 and ACE indices for fungi followed a consistent

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trend: old tree rhizosphere > old tree non-rhizosphere > young tree non-rhizosphere > young tree rhizosphere, suggesting that old tree soils had higher fungal richness than young tree soils, and that rhizosphere and non-rhizosphere soils in old trees showed comparable richness. All samples exhibited coverage rates above 99.5%, indicating that the sequencing depth was sufficient to comprehensively capture the microbial community composition in each soil type.

OTU abundance

As shown in *Fig. 3*, a total of 371 bacterial OTUs were detected, mainly belonging to Proteobacteria, Actinobacteriota, and Acidobacteriota. A total of 169 fungal OTUs were detected, primarily from Basidiomycota, Ascomycota, Mortierellomycota, unclassified_k__Fungi, and Rozellomycota. Unique bacterial OTU numbers followed: old tree non-rhizosphere > old tree rhizosphere > young tree non-rhizosphere > young tree rhizosphere, with more unique OTUs in older trees. Unique fungal OTU numbers followed: old tree rhizosphere > old tree non-rhizosphere > young tree non-rhizosphere > young tree rhizosphere, again with more OTUs in older trees. This suggests high similarity in microbial diversity between rhizosphere and non-rhizosphere soils across tree ages.

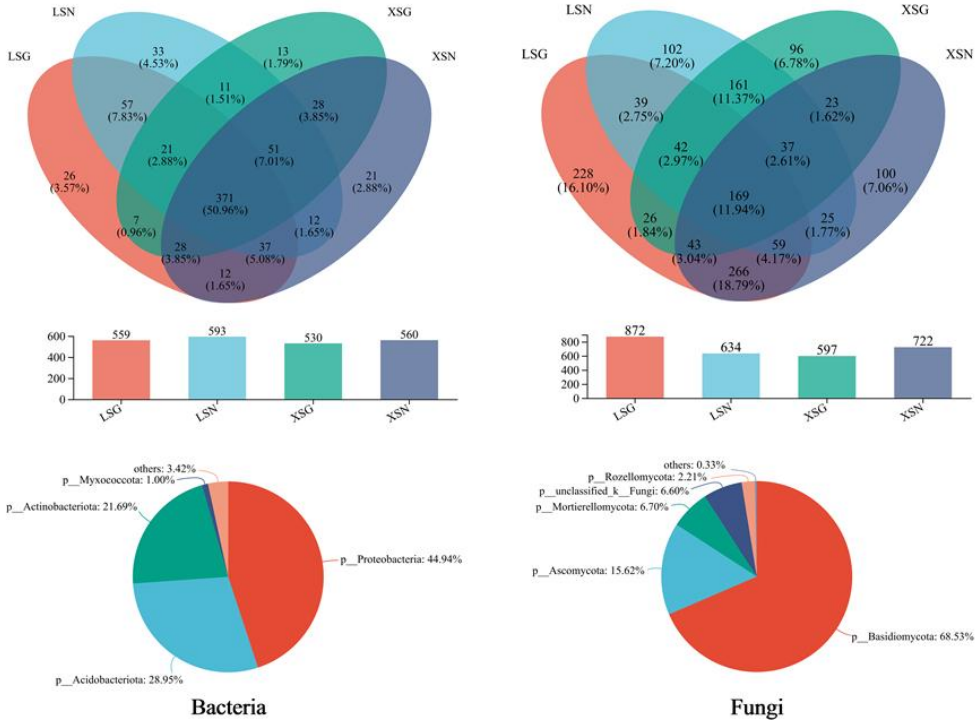

Figure 3. *OTUs of bacteria and fungi in rhizosphere and non-rhizosphere soil of Tsuga chinensis at different ages*

Microbial community structure of soils under Tsuga chinensis at different ages

Microbial community composition in rhizosphere and non-rhizosphere soils

As shown in *Fig. 4*, at the phylum level, the dominant bacterial phyla in rhizosphere and non-rhizosphere soils of *T. chinensis* with different tree ages were Proteobacteria,

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Actinobacteriota, and Acidobacteriota, with a cumulative relative abundance of 95.86%, 95.10%, 96.03%, and 95.08% in old-tree rhizosphere, old-tree non-rhizosphere, young-tree rhizosphere, and young-tree non-rhizosphere soils, respectively. Among them, Proteobacteria had the highest relative abundance, accounting for 41.45%, 45.50%, 47.48%, and 45.22%, respectively. At the fungal phylum level, the dominant phyla were Basidiomycota, Ascomycota, Mortierellomycota, unclassified fungi, and Rozellomycota, which accounted for 96.32%, 99.48%, 99.81%, and 99.77% of the total fungal abundance in the respective soils mentioned above. Among these, Ascomycota had the highest relative abundance (56.62%, 79.34%, 73.67%, and 64.21%, respectively), and its abundance was higher in rhizosphere than in non-rhizosphere soils. In contrast, Basidiomycota was more abundant in non-rhizosphere soils than in rhizosphere soils. Overall, the dominant microbial populations in rhizosphere soils of *T. chinensis* showed higher relative abundance than those in non-rhizosphere soils.

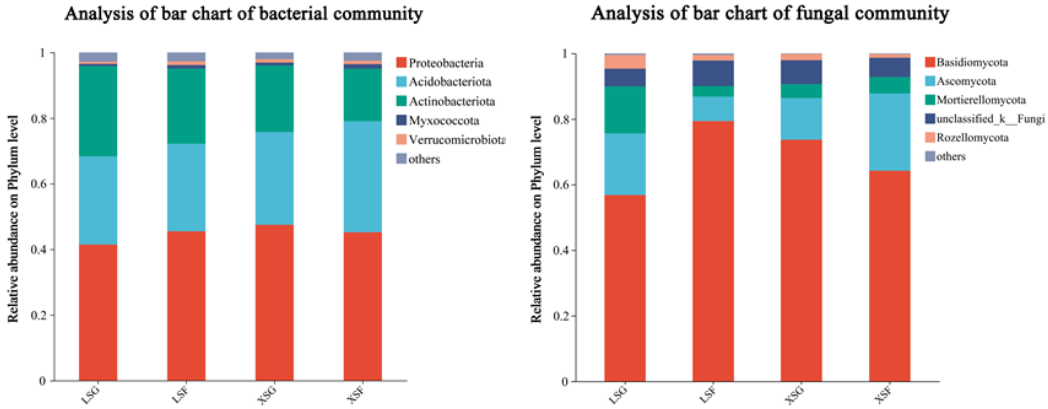

Figure 4. *Composition of soil microbial communities in rhizosphere and non-rhizosphere soils of Tsuga chinensis at the phylum level*

As shown in *Fig. 5*, the dominant bacterial genera in rhizosphere and non-rhizosphere soils of *T. chinensis* with different tree ages were *norank_f__Xanthobacteraceae*, *norank_f__norank_o__Subgroup_2*, *Acidothermus*, *Roseiarcus*, and *norank_f__norank_o__Acidobacteriotales*. The community composition and abundance of these bacterial genera were similar across the four soil types. In contrast, the dominant fungal genera exhibited certain differences among tree ages and soil types. In the old-tree rhizosphere soil, the dominant fungal genera included *Sebacina*, *Mortierella*, *Cortinarius*, *unclassified_k__Fungi*, and *Clavulina*. In the old-tree non-rhizosphere soil, dominant genera were *Russula*, *Sebacina*, *Mortierella*, *Cortinarius*, and *unclassified_k__Fungi*. In the young-tree rhizosphere soil, the dominant genera were *Russula*, *Mortierella*, *Cortinarius*, *unclassified_k__Fungi*, *unclassified_p__Ascomycota*, and *Clavulina*. In the young-tree non-rhizosphere soil, the dominant genera were *Russula*, *Sebacina*, *Mortierella*, *unclassified_k__Fungi*, *unclassified_p__Ascomycota*, *Archaeorhizomyces*, and *Clavulina*. Notably, *Archaeorhizomyces* was a unique dominant fungal genus found only in the young-tree non-rhizosphere soil. *Russula* was a dominant genus in the old-tree non-rhizosphere, young-tree rhizosphere, and young-tree non-rhizosphere soils. These results suggest that fungal community composition and abundance at the genus level differ between rhizosphere and non-rhizosphere soils of *T. chinensis* of different ages.

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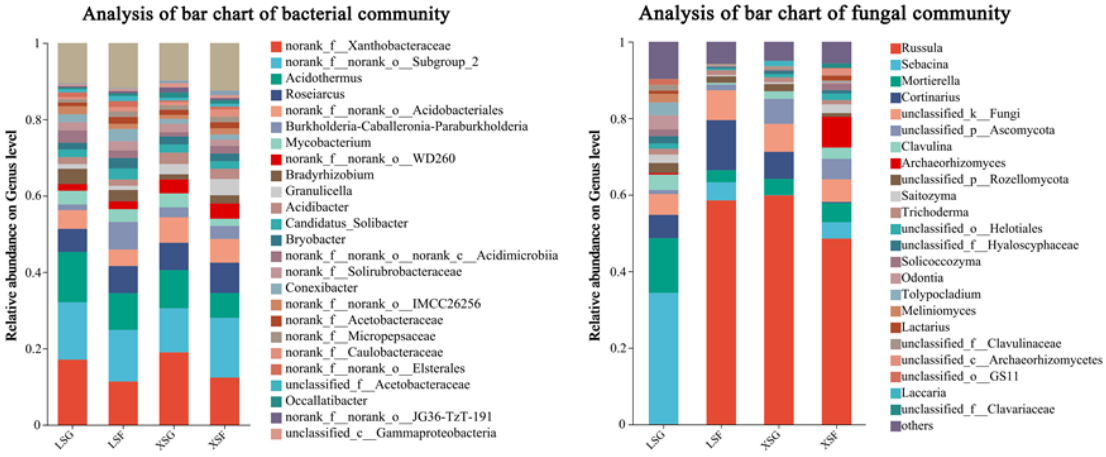

Figure 5. *Composition of soil microbial communities in rhizosphere and non-rhizosphere soils of Tsuga chinensis at the genus level*

As shown in the clustering analysis (*Fig. 6*), the bacterial communities in rhizosphere and non-rhizosphere soils of *T. chinensis* with different tree ages were compositionally and quantitatively similar, with dominant bacterial genera being *norank_f__Xanthobacteraceae*, *norank_f__norank_o__Subgroup_2*, *Acidothermus*, *Roseiarcus*, and *norank_f__norank_o__Acidobacteriotales*. However, fungal communities showed greater differences. The fungal community composition and abundance were similar between the rhizosphere and non-rhizosphere soils of old trees, and likewise between those of young trees. The shared dominant fungal genera across all soil types were *Mortierella* and *unclassified_k__Fungi*. *Archaeorhizomyces* was exclusive to young-tree non-rhizosphere soil, while *Russula* was a dominant genus in the old-tree non-rhizosphere, young-tree rhizosphere, and young-tree non-rhizosphere soils.

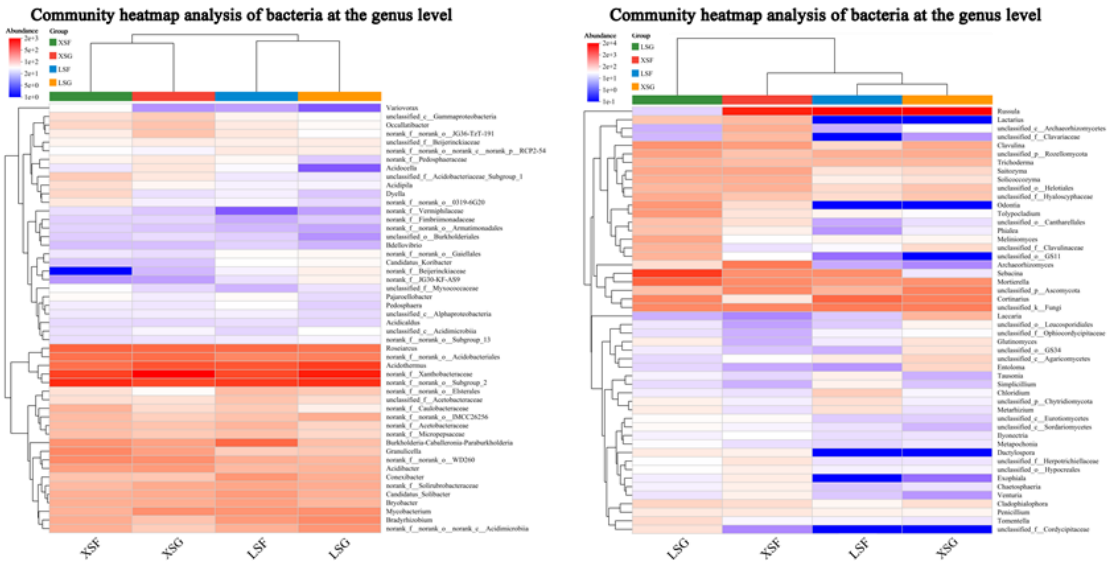

Figure 6. *Cluster heat map of microbial communities of rhizosphere and non-rhizosphere soil of Tsuga chinensis*

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Principal component analysis

Principal component analysis (*Fig. 7*) was used to reduce the dimensionality of the complex data and reveal underlying structures. Analysis of microbial community compositions among samples helps to assess similarities and differences between groups. For bacterial communities, principal component 1 (PC1) and principal component 2 (PC2) explained 28.61% and 16.95% of the variation, respectively. The distribution of bacterial communities was relatively dispersed within groups and showed large inter-group differences, indicating low similarity between rhizosphere and non-rhizosphere bacterial communities. For fungal communities, PC1 and PC2 explained 31.67% and 18.69% of the variation, respectively. The community compositions among old and young trees, as well as between rhizosphere and non-rhizosphere soils, were highly dispersed, suggesting significant differences in fungal community structures. These results indicate that microbial community structures in rhizosphere and non-rhizosphere soils of *T. chinensis* vary significantly with tree age.

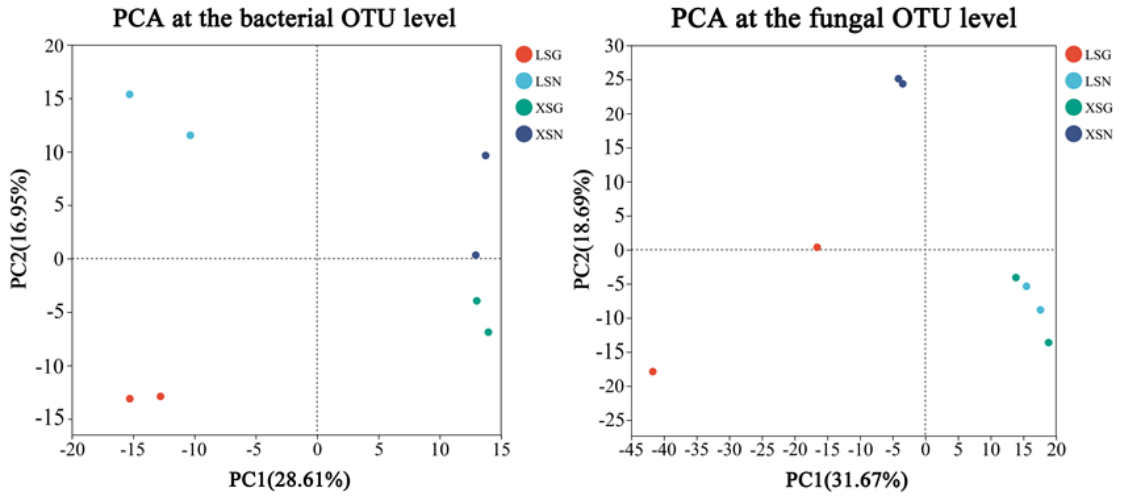

Figure 7. *Principal component analysis of microbial community structure in rhizosphere and non-rhizosphere soils of Tsuga chinensis*

Discussion

Soil microbial diversity is a crucial indicator for evaluating ecosystem functionality and stability, and its dynamic variation is closely related to plant developmental stages, root exudates, and the soil microenvironment (Berendsen et al., 2012). *T. chinensis*, as an endemic tree species in China, plays a vital role in forest ecosystems. With the growing focus on forest ecology, the interaction between trees and microorganisms has attracted increasing attention. This study investigated the composition and diversity of rhizosphere and non-rhizosphere microbial communities associated with *T. chinensis* at different tree ages, providing scientific insight into the potential effects of tree age on microbial communities and contributing to a deeper understanding of ecosystem functioning in forest environments.

The results showed that, at both bacterial and fungal levels, the microbial diversity in the rhizosphere soil of older trees was generally higher than that of younger trees, but different microbial groups exhibited distinct patterns between rhizosphere and non-

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rhizosphere soils. For bacterial communities, the Shannon index was highest in the non-rhizosphere soil of old trees, indicating the greatest diversity. The Chao1 index was also higher in both the rhizosphere and non-rhizosphere soils of old trees compared to those of young trees, suggesting that older tree soils harbor more abundant bacterial communities. This may be attributed to the mature root system and enriched exudates providing more favorable ecological niches and nutrient sources for microbial colonization. However, fungal communities responded more complexly: The Shannon indices of rhizosphere and non-rhizosphere soils in older trees were similar and both higher than those in younger trees; fungal diversity in the non-rhizosphere soil of young trees exceeded that in the rhizosphere, suggesting that fungal communities are less responsive to root influence and may even be more active in non-rhizosphere environments. This highlights the high sensitivity of fungi to environmental stress and nutrient patterns. Similar trends have been observed in other plants—for instance, the rhizosphere soil of *Lycium ruthenicum* supports higher bacterial but lower fungal diversity compared to the non-rhizosphere soil (Li et al., 2018), whereas *Paris polyphylla* exhibits higher bacterial diversity in non-rhizosphere soils (Zheng et al., 2020). These findings indicate that the influence of roots on soil microbial communities varies among plant species and is affected by plant developmental stages and environmental factors.

The bacterial communities in both rhizosphere and non-rhizosphere soils of *T. chinensis* at different ages were compositionally similar, with dominant phyla being Proteobacteria, Actinobacteriota, and Acidobacteriota. This pattern is consistent with findings from studies on *Rhododendron delavayi* (Fang et al., 2019) and *R. liliiflorum* (Li et al., 2022) , which also reported Acidobacteriota as abundant in rhizosphere soils, possibly due to the acidic microenvironment created by Rhododendron roots. Moreover, Acidobacteriota abundance has been positively correlated with soil nitrogen content (Zhang et al., 2023). Proteobacteria include many facultative or obligate anaerobic heterotrophs, playing a key role in organic matter decomposition and nutrient cycling, and thus serving as indicators of soil fertility (Li et al., 2016). Actinobacteriota are involved in organic matter degradation and antibiotic metabolism (Cui et al., 2023). Among fungi, Basidiomycota and Ascomycota were dominant. Basidiomycota are known for their strong spore dispersal ability, enabling long-distance colonization and optimization of microbial habitats (Egidi et al., 2019). Ascomycota are primary decomposers with high environmental adaptability and competitive advantage (Yan et al., 2021; Li et al., 2022). At the genus level, dominant bacterial genera in both rhizosphere and non-rhizosphere soils included *norank_f__Xanthobacteraceae*, *norank_f__norank_o__Subgroup_2*, *Acidothermus*, *Roseiarcus*, and *norank_f__norank_o__Acidobacteriotales*. *Acidothermus* species are known for decomposing organic matter and utilizing various carbon sources, and their abundance is positively associated with soil organic matter (Du et al., 2017). In contrast, fungal communities differed significantly by tree age and soil compartment. Common dominant fungal genera included *Mortierella* and *unclassified_k__Fungi*. *Archaeorhizomyces* was uniquely dominant in the non-rhizosphere soil of young trees, and *Russula* was a dominant genus in the non-rhizosphere soil of old trees as well as both rhizosphere and non-rhizosphere soils of young trees. *Mortierella* not only enhances plant stress tolerance and productivity (Xia et al., 2022), but also improves acidic soil pH through organic acid secretion (Ning et al., 2022), mobilizes phosphorus (Osorio and Habte, 2013), and chelates toxic aluminum ions (Kochian et al., 2015), thereby improving nutrient availability. *Archaeorhizomyces*, though slow-growing, are widely distributed in root

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environments and are closely linked to soil nutrient turnover (Carrino-Kyker et al., 2016); their relative abundance increases with organic fertilization (Guan Hongzhi et al., 2023). *Russula* forms ectomycorrhizal associations with *Larix*, *Pinus*, and *Fabaceae*, enhancing root nutrient and water uptake and improving stress tolerance (Li et al., 2015). In this study, fungi from the genus *Russula* were dominant in the rhizosphere and non-rhizosphere soils of young *T. chinensis* trees, as well as in the non-rhizosphere soil of old trees. This suggests that *Russula* fungi may play an important ecological role in the germination and growth of *T. chinensis*.

In summary, the age of *T. chinensis* significantly influences the structure and diversity of its rhizosphere microbial communities, with particularly notable differences in fungal communities across tree ages and soil compartments. This study provides a new perspective on the relationship between tree growth and microbial ecology. However, there are some limitations to this research: first, it focuses solely on *T. chinensis*, and future studies could expand to include other tree species and environmental conditions to improve generalizability; second, although metagenomic sequencing was used to analyze microbial communities, some potential microbial taxa may not have been detected. Future research could increase sequencing depth and breadth to further enhance our understanding of rhizosphere microbial diversity.

Conclusion

There were clear differences in the microbial community structure between rhizosphere and non-rhizosphere soils of *T. chinensis*, and these differences were significantly affected by tree age. This indicates that the development of the rhizosphere and non-rhizosphere soil environments during tree growth has a marked influence on the composition and structure of microbial communities. Specifically, the bacterial diversity in the non-rhizosphere soil of old trees was the highest, and the bacterial richness in both rhizosphere and non-rhizosphere soils of old trees was higher than that of young trees; bacterial diversity in the non-rhizosphere soils of both old and young trees was higher than that in their respective rhizosphere soils. However, fungal diversity showed contrasting trends: the rhizosphere fungal diversity in older trees was higher than in their non-rhizosphere soils, whereas younger trees exhibited the opposite pattern, with lower rhizosphere fungal diversity.

In terms of community composition, bacterial communities in rhizosphere and non-rhizosphere soils were relatively similar across tree ages, while fungal communities varied more noticeably. Specifically, the fungal community composition and abundance in both rhizosphere and non-rhizosphere soils of older trees were relatively consistent, and a similar pattern of structural stability was also observed in younger trees.

In conclusion, tree age is a key factor influencing the structure and diversity of rhizosphere and non-rhizosphere soil microbial communities in *T. chinensis*. Future studies could further explore how changes in tree age, soil physicochemical properties, and other ecological factors collectively drive the dynamic succession of rhizosphere microbial communities. This would provide valuable insights into the plant–microbe interactions within forest ecosystems. Moreover, the findings of this study offer important theoretical support for forest ecosystem conservation and sustainable management strategies.

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