# A STUDY ON THE DIFFERENCES IN RHIZOSPHERE AND NON-RHIZOSPHERE MICROBIAL COMMUNITY STRUCTURE AND DIVERSITY OF HAINAN PLUM YEW (*CEPHALOTAXUS HAINANENSIS*) AT DIFFERENT AGES

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(Received 2nd May 2025; accepted 16th Jun 2025)

**Abstract.** To explore the characteristics of microbial communities associated with the rhizosphere and non-rhizosphere of Hainan plum yew (*Cephalotaxus hainanensis*), a rare and endemic medicinal plant in China, soils from both zones were collected from old and young trees in the Yuanbaoshan National Nature Reserve, Guangxi, China. High-throughput sequencing technology was used to study the community characteristics of soil bacteria and fungi. The study found that with increasing age, the structure and diversity of rhizosphere microbial communities changed significantly. The species composition and abundance also showed clear age specificity, indicating that rhizosphere microorganisms of *C. hainanensis* play an important role during different growth stages. In addition, the diversity indices of rhizosphere microbial communities showed clear age specificity as tree age increased, including richness, evenness, and overall diversity. At the phylum level, the dominant bacterial groups in rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages were Proteobacteria, Actinobacteriota, and Acidobacteriota. For fungi, the dominant phyla were Ascomycota, Basidiomycota, and unclassified fungi. At the genus level, the community composition and abundance of soil bacteria were similar. *Sebacina* was a dominant fungal genus specific to young trees. These results indicate that the age of *C. hainanensis* significantly affects the structure and diversity of its rhizosphere microbial community, which plays an important role in its growth and ecosystem function. This study provides an important reference for further understanding the interaction between *C. hainanensis* and its ecological environment.

**Keywords:** *Cephalotaxus hainanensis, rhizosphere soil microorganisms, microbial community diversity, bacteria, fungus*

## Introduction

Hainan plum yew (*Cephalotaxus hainanensis*) is a relict species in the genus *Cephalotaxus* (family *Cephalotaxaceae*), which is mainly distributed in Guangdong, Hainan, Guangxi, Yunnan, and Tibet (Editorial Committee of Flora Reipublicae Popularis Sinicae, 1978). It is a rare medicinal plant endemic to China. *C. hainanensis* contains several cephalotaxine-type alkaloids with anticancer activity, including cephalotaxine, homoharringtonine, isocephalotaxine, and deoxyharringtonine. Among these, homoharringtonine and cephalotaxine have been developed into clinical drugs for the treatment of leukemia (Lin et al., 2021). Wang et al. (2016) have isolated and identified 50 types of alkaloids from *C. hainanensis*, and Zhang et al. (2021) have extracted and identified 10 diterpenoid compounds from this species, including one novel compound and two compounds isolated for the first time from the genus Cephalotaxus. In addition, Xue et al. (2025) isolated and identified six phenolic compounds with anti-inflammatory activity from the n-butanol fraction of *C. hainanensis*. The cephalotaxine-type alkaloids, as the main active components of *C. hainanensis*, have shown significant therapeutic effects on leukemia and acute lymphoblastic diseases, making this species one of the most promising natural sources for anticancer drugs (Tang et al., 2023). Due to its high medicinal value, the wild resources of *C. hainanensis* have been excessively exploited. Combined with its slow growth, high ecological requirements, and low natural regeneration rate, the harvested resources are difficult to replenish and recover in a short time (Wang et al., 1992; Wang and Wang, 1994). As a result, wild populations are rapidly declining, and the species is currently listed as a national second-class protected wild plant in China.

With the advancement of science and technology, soil microbial sequencing technologies have also developed, and microorganisms play an indispensable role in soil functions. As a result, research on plant soils has gradually shifted from focusing on physicochemical properties to microbial communities (Hartmann and Six, 2023). Microbial community composition and abundance can not only be used to assess the degree of soil degradation (Yang et al., 2023) but also contribute to the remediation of soils contaminated by organic compounds (Varjani et al., 2021). In addition, soil microorganisms are the main driving force behind soil organic matter turnover and nutrient cycling, directly influencing critical ecological processes such as biogeochemical cycles (Kulhankova et al., 2006; Van Der Heijden et al., 2008). Soil microbial communities play a vital role in maintaining soil structure, nutrient cycling, and plant growth, and they are closely linked to plant development. Many plants can enhance nutrient uptake through nitrogen fixation, mycorrhizal fungi, and associated bacteria, thereby promoting their own growth (Looney et al., 2018; Padda et al., 2019). The rhizosphere soil, directly influenced by plant roots, differs from non-rhizosphere soil by exhibiting a unique microbial community structure. Variations in root exudates among different plants shape distinct microbial communities, which in turn can promote plant growth and defense mechanisms (Lu et al., 2012; Huang et al., 2014; Li et al., 2020). The rhizosphere, as the interface between plant roots and soil, is a hotspot for microbial interactions. It influences geochemical cycling, plant development, and resistance to biotic stress. Therefore, the rhizosphere is a highly complex and dynamic zone, and understanding its ecological structure is crucial for improving plant productivity (Philippot et al., 2013).

At present, research on *C. hainanensis* both domestically and internationally has mainly focused on cultivation techniques (Mo et al., 2016), compound extraction (Miao et al., 2019), and the isolation of endophytic fungi that produce high levels of homoharringtonine (Yang et al., 2015; Hu et al., 2016), while studies on rhizosphere microorganisms remain largely unexplored. Investigating the structure and diversity of microbial communities in the rhizosphere and non-rhizosphere soils of *C. hainanensis* will help to better understand its ecological adaptability and growth patterns, and is of great significance for gaining deeper insights into the species' functional systems and its interactions with the surrounding environment. Therefore, this study aims to (1) characterize the microbial community structure and diversity in rhizosphere and non-rhizosphere soils of *C. hainanensis* at different tree ages using high-throughput sequencing technology, and (2) explore how microbial composition varies with tree age and soil compartment. The findings will provide important scientific evidence for the conservation and management of *C. hainanensis*, and contribute to a deeper understanding of plant–microbe interaction mechanisms in endangered and rare plant species.

## Materials and methods

### *General situation of test site*

The study area is located in the Yuanbaoshan National Nature Reserve in Guangxi Zhuang Autonomous Region, China, situated in the central part of Rongshui County, Guangxi (*Fig. 1*). The region has a mid-subtropical monsoon climate characterized by abundant heat and precipitation, with an average annual temperature of approximately 16.4 °C and an average annual rainfall of about 2,379 mm. The frost-free period lasts around 288 days. The soil type is predominantly mountain yellow soil, with a thick humus layer on the surface. The forest composition is relatively simple, with coexisting tree species mainly including *Tsuga chinensis* and *Taxus wallichiana* var. *mairei*. The understory is densely covered with *Indocalamus tessellatus* (a type of cold-resistant bamboo), and lichens and mosses are also widely distributed (Liang and Pan, 2012).

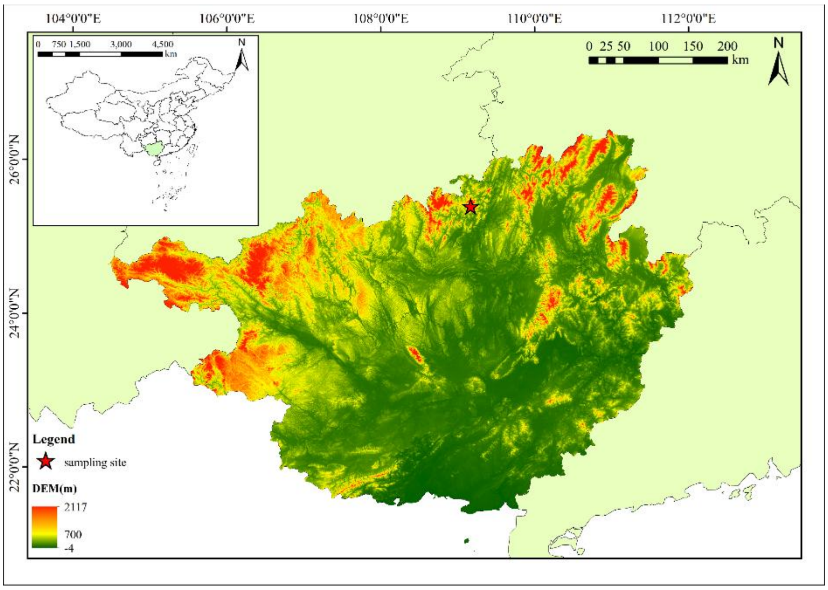

***Figure 1.*** *Map of the study area and indicate the location of the test sample*

### *Sample collection and processing*

Soil sampling was conducted following the method described by Bao (2000). In May 2022, field sampling was conducted at the summit area of the Yuanbaoshan National Nature Reserve. Three healthy mature trees (height >10 m, basal diameter >35 cm) and three saplings (height >50 cm, basal diameter >3 cm) of *C. hainanensis* were randomly selected. For each selected tree, rhizosphere and non-rhizosphere soil samples were collected. At approximately 1 meter from the tree trunk, roots were excavated using a shovel to a depth of 50–60 cm. The roots were carefully removed, and the soil particles (<1 cm in diameter) adhering closely to the root surface were collected as rhizosphere soil. Meanwhile, non-rhizosphere soil was collected from a location at least 30 cm away from the root system. All soil samples were immediately placed into a portable cooler in the field and transported to the laboratory. Samples were then stored at –80 °C until use for soil DNA extraction and subsequent high-throughput sequencing of bacterial 16S rRNA and fungal 18S rRNA genes.

### *Method*

#### *Soil microbial community diversity analysis*

Total soil microbial genomic DNA was extracted using the Fast Soil DNA Extraction Kit (Shanghai Sangon Biotech Co., Ltd., China). The extracted DNA samples were shipped on dry ice to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for sequencing. The sequencing workflow included PCR amplification, pooling and purification of PCR products, and library construction.

For bacteria, the V5–V7 region of the 16S rRNA gene was amplified using the primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'). The PCR amplification conditions were as follows: init-ial denaturation at 95 °C for 3 minutes; 28 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 45 seconds; followed by a final extension at 72 °C for 10 minutes.

For fungi, the internal transcribed spacer (ITS) region was amplified using p-rimers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). The PCR amplification conditions were: initial denaturation at 95 °C for 3 minutes; 37 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 45 seconds; with a final extension at 72 °C for 10 minutes.

All PCR products were sequenced on the Illumina platform provided by Majorbio Bio-Pharm Technology Co., Ltd.

#### *Quality control and analysis of microbiome sequencing data*

Raw sequencing data were subjected to quality control and sequence merging to obtain high-quality clean data. Operational Taxonomic Unit (OTU) clustering and taxonomic analysis were performed using the UPARSE software platform. The OTU clustering process included the following steps:

- Extracting non-redundant sequences from the optimized sequences to reduce computational redundancy during downstream analysis;
- Removing singleton sequences (those that appear only once);
- Performing OTU clustering on non-redundant sequences (excluding singletons) at 97% similarity, during which chimeric sequences were filtered out to obtain representative sequences for each OTU;
- Mapping all optimized sequences to the OTU representative sequences, and retaining those with ≥97% similarity to construct the OTU table.

Taxonomic classification of the OTU representative sequences (97% similarity level) was conducted using the RDP Classifier Bayesian algorithm.

#### *Analysis of soil microbial community composition*

Based on the sequencing data, alpha diversity was analyzed using Mothur software under different random sampling depths to assess the richness and diversity of microbial communities. The indices calculated included ACE, Chao, Simpson, and Shannon. The

ACE and Chao indices reflect community richness, while the Simpson and Shannon indices indicate community diversity. Higher values of ACE, Chao, and Shannon, along with a lower Simpson index, represent greater species diversity within the samples.

Bar plots of soil microbial community composition were generated using R software. Venn diagrams were also produced using R to illustrate the number of shared and unique OTUs across multiple groups or samples.

## Results and analysis

### *Soil microbial sample sequencing results*

After sequence assembly and quality filtering, a total of 308,616 high-quality bacterial sequences were obtained, with an average sequence length of 377 bp. For fungi, 376,500 high-quality sequences were obtained, with an average sequence length of 219 bp. Rarefaction curves at 97% similarity for both bacterial and fungal communities (*Fig. 2*) showed that all sample curves tended to plateau, indicating that the sequencing depth was sufficient and the sampling was reasonable. This suggests that the data accurately represent the bacterial and fungal communities present in the samples. Additionally, all samples showed coverage rates above 99.7%, further confirming that the majority of bacterial and fungal taxa were captured, and the sequencing results can reliably reflect the microbial community structure in the soil of this region.

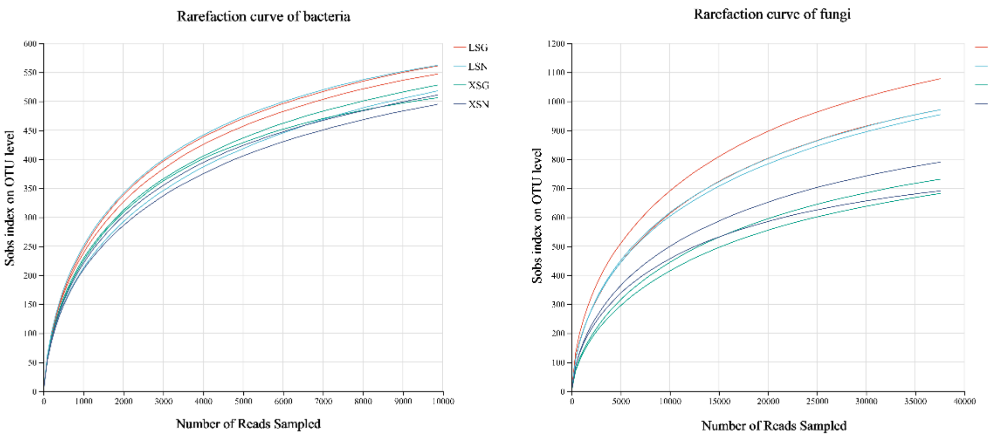

***Figure 2.*** *Dilution curves of the soil samples. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

### *Diversity and richness of soil microbial communities in rhizosphere and non-rhizosphere soils of Cephalotaxus hainanensis at different ages*

#### *Alpha diversity indices*

As shown in *Table 1*, the Shannon index for soil bacterial communities of *C. hainanensis* followed the order: old tree rhizosphere>old tree non-rhizosphere>young tree rhizosphere>young tree non-rhizosphere. In contrast, the Simpson index followed the order: young tree non-rhizosphere>young tree rhizosphere>old tree non-rhizosphere>old tree rhizosphere. These results indicate that the bacterial community diversity in soils of old trees is higher than that of young trees, and that rhizosphere soils harbor greater bacterial diversity than non-rhizosphere soils. The Chao1 and ACE indices were also higher in old tree soils than in young tree soils, while differences between rhizosphere and non-rhizosphere soils were minimal, suggesting that bacterial richness is greater in old tree soils, with little variation between rhizosphere and non-rhizosphere zones.

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

| **Microbe** | **Soil stype** | **Shannon index** | **Simpson index** | **Chao1 index** | **ACE index** | **Coverage rate/%** |
|---|---|---|---|---|---|---|
| Bacteria | LSG | 4.8732±0.0018 | 0.0203±0.0011 | 641.1598±14.8149 | 640.5955±6.8631 | 0.9878±0.0003 |
| | LSN | 4.7721±0.1639 | 0.0238±0.0049 | 640.4674±1.3497 | 644.2472±4.6115 | 0.9869±0.0014 |
| | XSG | 4.6830±0.0217 | 0.0293±0.0011 | 593.6070±32.8305 | 599.2898±31.1141 | 0.9887±0.0015 |
| | XSN | 4.5913±0.0470 | 0.0292±0.0017 | 615.0057±6.9098 | 609.5357±1.8958 | 0.9871±0.0002 |
| Fungi | LSG | 4.4259±0.1633 | 0.0447±0.0046 | 1193.2371±68.8681 | 1227.6817±64.6921 | 0.9933±0.0003 |
| | LSN | 4.1916±0.0855 | 0.0493±0.0107 | 1149.9113±19.9220 | 1186.1320±2.0416 | 0.9933±0.0002 |
| | XSG | 3.3396±0.0307 | 0.0900±0.007 | 853.8035±19.9574 | 873.6364±27.5468 | 0.9948±0.0001 |
| | XSN | 3.4324±0.0090 | 0.1158±0.0047 | 854.6336±83.9389 | 877.0799±81.8269 | 0.9954±0.0008 |

Note: In the table, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree

Fungal community diversity and richness exhibited a similar trend to that of bacteria. The Shannon index of fungal communities followed the order: old tree rhizosphere>old tree non-rhizosphere>young tree non-rhizosphere>young tree rhizosphere, while the Simpson index followed the order: young tree non-rhizosphere>young tree rhizosphere>old tree non-rhizosphere>old tree rhizosphere. Again, the Chao1 and ACE indices for fungi were higher in old tree soils than in young tree soils, with only slight differences between rhizosphere and non-rhizosphere soils. These findings suggest that fungal community diversity is higher in old tree soils than in young tree soils, and that old tree rhizosphere soils harbor higher fungal diversity communities than their non-rhizosphere counterparts, whereas for young trees, the non-rhizosphere soil shows slightly higher diversity than the rhizosphere. Overall, the fungal richness in old tree soils exceeds that in young tree soils, with limited differences between rhizosphere and non-rhizosphere samples.

All sample libraries exhibited coverage rates above 99.7%, indicating that the microbial community information in each sampling site was well-represented.

### *OTU abundance*

As shown in *Fig. 3*, a total of 447 bacterial OTUs were detected in the soils of *C. hainanensis*, primarily composed of members from the phyla Proteobacteria, Actinobacteriota, and Acidobacteriota. For fungi, a total of 321 OTUs were identified, mainly consisting of Ascomycota, Basidiomycota, and unclassified fungal taxa. The unique OTUs of bacterial and fungal communities in rhizosphere and non-rhizosphere soils showed certain similarities across different tree ages: old tree rhizosphere soils contained more unique OTUs than old tree non-rhizosphere soils, and young tree non-rhizosphere soils had more unique OTUs than young tree rhizosphere soils. Additionally, the number of unique OTUs was higher in the rhizosphere of old trees than that of young trees, while the young tree non-rhizosphere soils had more unique OTUs than those of old trees. These patterns suggest a high similarity in microbial community diversity between rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages.

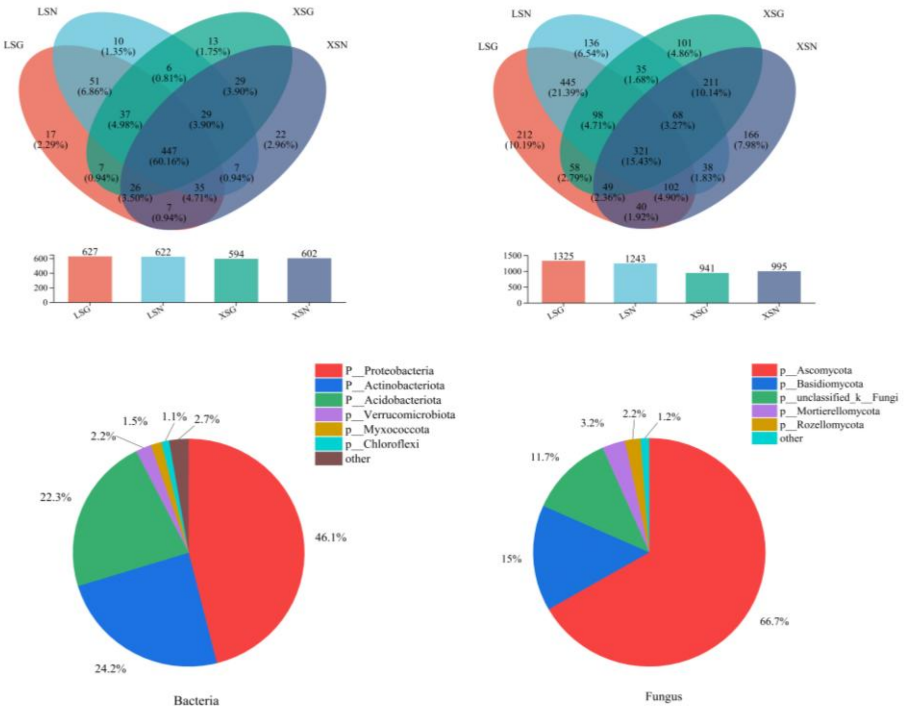

***Figure 3.*** *OTUs of bacteria and fungal in rhizosphere and non-rhizosphere soil of Cephalotaxus hainanensis at different ages. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

## ***Soil microbial community structure of Cephalotaxus hainanensis at different ages***

### *Microbial community composition at the phylum level*

As shown in *Fig. 4*, at the bacterial phylum level, the dominant phyla in the rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages are Proteobacteria, Actinobacteriota, and Acidobacteriota. These three bacterial phyla accounted for 92.50%, 92.50%, 92.42%, and 92.68% of the total bacterial abundance in the four samples, respectively. Among them, Proteobacteria had the highest relative abundance, with proportions of 46.07%, 48.39%, 40.34%, and 41.58% in the four samples. At the fungal phylum level, the dominant phyla in the rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages were Ascomycota, Basidiomycota, and unclassified fungi. These three fungal phyla accounted for 93.41%, 89.79%, 97.10%, and 92.51% of the total fungal abundance in the four samples, respectively. Among them, Ascomycota had the highest relative abundance, with proportions of 66.68%, 50.19%, 58.97%, and 45.04%, and its abundance was higher in rhizosphere soils than in non-rhizosphere soils. In contrast, the relative abundance of Basidiomycota was higher in non-rhizosphere soils than in rhizosphere soils. Overall, the dominant microbial taxa in the rhizosphere soils of *C. hainanensis* exhibited higher relative abundance compared to those in the non-rhizosphere soils.

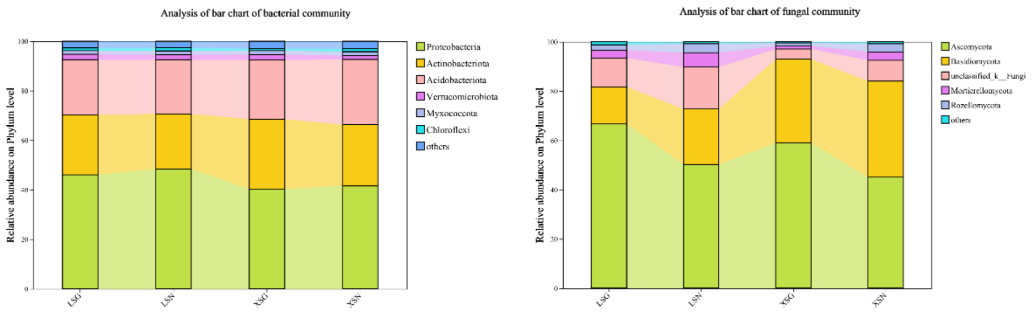

***Figure 4.*** *Composition of soil microbial communities in rhizosphere and non-rhizosphere of Cephalotaxus hainanensis at phylum level. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

### *Microbial community composition at the genus level*

As shown in *Fig. 5*, the dominant bacterial genera in the rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages were norank_f__Xanthobacteraceae, *Acidothermus*, and norank_f__norank_o__Subgroup_2. The bacterial community composition and abundance were similar across all four soil samples. For older *C. hainanensis* trees, the dominant fungal genera in both rhizosphere and non-rhizosphere soils included unclassified_p__Ascomycota, unclassified_c__Archaeorhizomycetes, unclassified_k__Fungi, and unclassified_c__Agaricomycetes. For younger trees, the dominant fungal genera in both rhizosphere and non-rhizosphere soils included unclassified_p__Ascomycota, unclassified_c__Archaeorhizomycetes, *Sebacina*, and *Archaeorhizomyces*. Among them, *Sebacina* was identified as a dominant genus unique to the rhizosphere of younger trees. These results indicate that the composition and abundance of fungal communities in the rhizosphere and non-rhizosphere soils of *C. hainanensis* differ at the genus level depending on tree age.

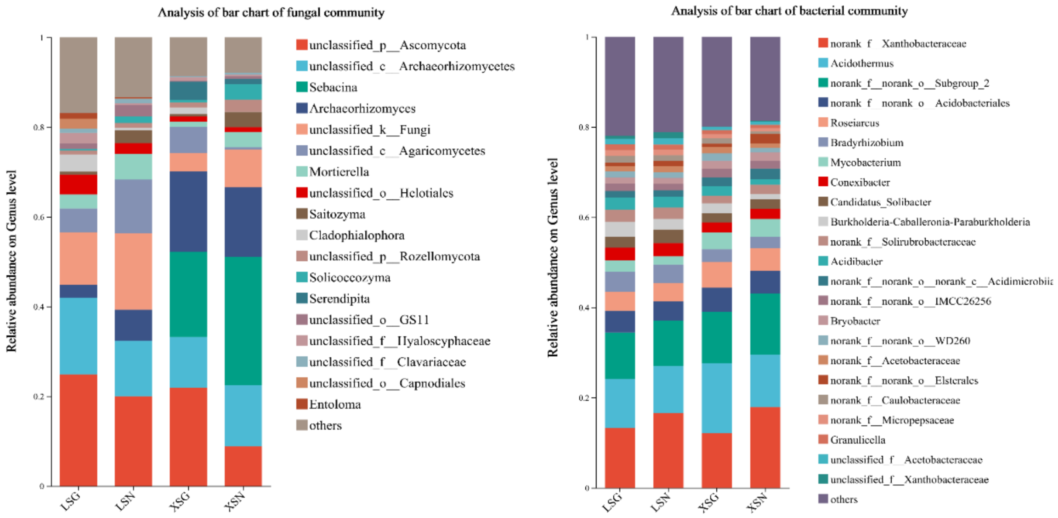

***Figure 5.*** *Composition of soil microbial communities in rhizosphere and non-rhizosphere of Cephalotaxus hainanensis at genus level. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

### *Cluster heatmap of microbial communities*

According to the cluster analysis (*Fig. 6*), the bacterial community composition and abundance in rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages were generally similar. The dominant bacterial genera across all samples were norank_f__Xanthobacteraceae, *Acidothermus*, and norank_f__norank_o__Subgroup_2. In contrast, fungal communities exhibited more pronounced differences in both composition and abundance between rhizosphere and non-rhizosphere soils. For older trees, the fungal communities in rhizosphere and non-rhizosphere soils were relatively similar, likely due to their well-developed root systems and long-term interactions with surrounding soil microbes, which help establish stable microbial assemblages. However, in younger trees, the rhizosphere fungal community showed reduced diversity compared to that of older trees. This may be attributed to the less developed root systems and lower quantity and complexity of root exudates in younger trees, which limit microbial colonization and the formation of complex symbiotic relationships. Despite these differences, several fungal genera were consistently dominant across all samples, including *Archaeorhizomyces*, unclassified_c__Agaricomycetes, unclassified_p__Ascomycota, unclassified_c__Archaeorhizomycetes, and unclassified_k__Fungi. Notably, *Sebacina* was identified as a dominant genus specifically enriched in the rhizosphere of younger trees. As members of *Sebacina* are known to be involved in early-stage root colonization and mutualistic interactions, their presence may indicate an initial establishment of root–fungal associations in young *C. hainanensis*.

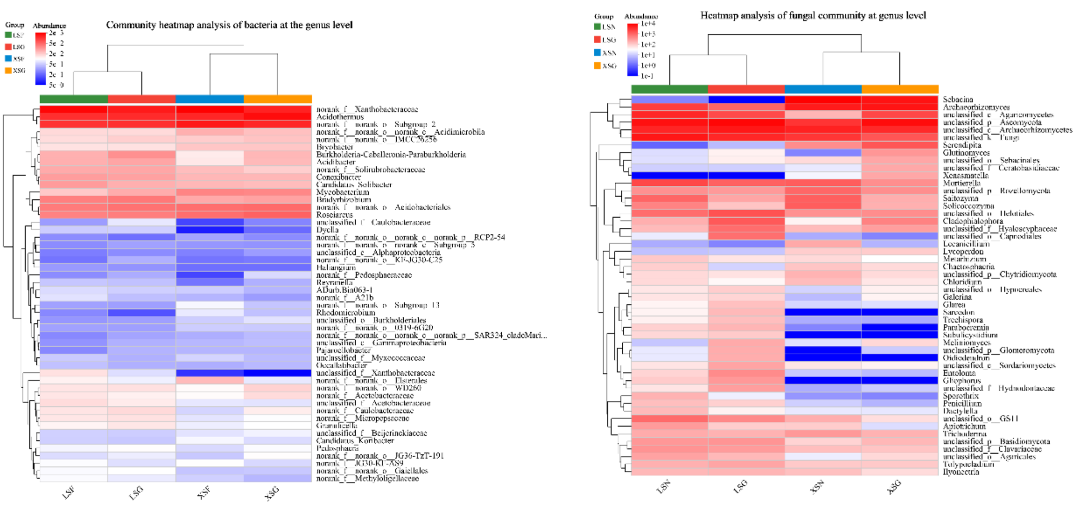

***Figure 6.*** *Cluster heat map of microbial communities of rhizosphere and non-rhizosphere soil of Cephalotaxus hainanensis. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

### *Principal Component Analysis*

Principal Component Analysis (PCA) is a widely used dimensionality reduction technique that simplifies complex datasets by capturing the main sources of variation, thereby revealing the underlying structure of microbial communities across different samples. As illustrated in *Fig. 7*, PCA was applied to compare the microbial community compositions in rhizosphere and non-rhizosphere soils of *C. hainanensis* at different ages. For bacterial communities, the first two principal components (PC1 and PC2) explained 27.85% and 16.71% of the total variation, respectively. The bacterial communities showed relatively dispersed distributions within each group but pronounced differences between rhizosphere and non-rhizosphere soils. This pattern suggests a distinct separation in bacterial community composition influenced by proximity to roots, reflecting the strong selective effect of root exudates and rhizosphere conditions that shape bacterial assemblages differently from bulk soil. The low similarity between rhizosphere and non-rhizosphere bacterial communities highlights the rhizosphere as a dynamic hotspot for bacterial diversity and functional specialization, likely contributing to nutrient cycling, root health, and plant growth promotion. In contrast, fungal communities exhibited a slightly higher variation explained by PC1 (29.46%) and PC2 (16.45%). Non-rhizosphere soil samples from different age groups clustered tightly, indicating relatively stable and conserved fungal communities in bulk soil. However, rhizosphere fungal communities were more dispersed, implying greater heterogeneity and plasticity influenced by plant age and root-associated factors. This variation in rhizosphere fungi may correspond to shifts in symbiotic relationships, such as mycorrhizal associations, which can vary with tree developmental stage, root architecture, and nutrient demands. The observed clustering patterns thus suggest that fungal communities in the rhizosphere adapt dynamically to the host plant’s growth conditions, potentially modulating stress responses, nutrient acquisition, and disease resistance. Overall, these PCA results reflect distinct microbial niche differentiation between rhizosphere and non-rhizosphere soils, with bacterial communities showing more pronounced compartmentalization and fungal communities displaying greater variability linked to plant age. This spatial and developmental differentiation of microbial assemblages likely underpins critical ecological functions, including organic matter decomposition, nutrient transformation, and plant-microbe interactions that collectively influence the health and growth of *C. hainanensis*. Further functional assays and metagenomic analyses would be valuable to elucidate the specific microbial processes and interactions driving these patterns.

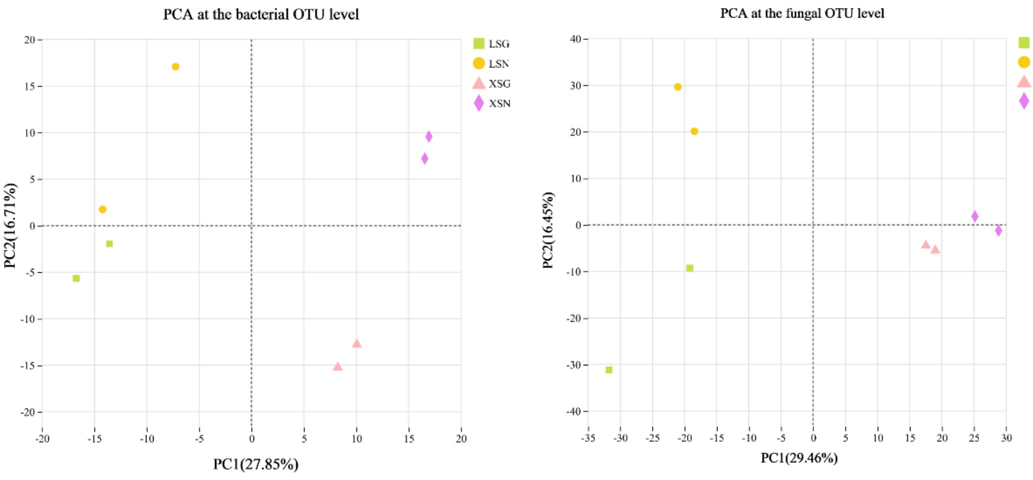

***Figure 7.*** *Principal component analysis of microbial community structure in rhizosphere soil and non-rhizosphere soil of Cephalotaxus hainanensis. Note: In the figure, LSG represents rhizosphere soil of old tree, LSN represents non-rhizosphere soil of old tree, XSG represents rhizosphere soil of new tree, and XSN represents non-rhizosphere soil of new tree*

## Discussions

Generally, rhizosphere soil microbial diversity is expected to be higher than that of non-rhizosphere soil. However, the results of this study show that not all rhizosphere soil microbial diversities are higher than their corresponding non-rhizosphere counterparts. For *C. hainanensis* saplings, both the Chao1 index for bacteria and fungi, as well as the Shannon index for fungi in rhizosphere soil (XSG), were slightly lower than those in non-rhizosphere soil (XSN). Similar findings have been reported in *Lycium ruthenicum* (Li et al., 2018), *Acacia* (Liu et al., 2022), and *Paris polyphylla* (Zeng et al., 2020). This may be related to a high litterfall rate of branches and leaves in the surrounding habitat, which enriches the humus layer and thereby increases microbial diversity in non-rhizosphere soils. In contrast, the root systems of saplings are still underdeveloped, secreting fewer exudates, which could lead to the observed results (Liu et al., 2020).

Within the same soil type, both Shannon and Chao1 indices for bacteria and fungi in rhizosphere and non-rhizosphere soils of mature *C. hainanensis* trees were higher than those of saplings, suggesting that the microbial community structure becomes richer with increasing tree age. This may be attributed to the developmental stage of sapling roots, where the rhizosphere ecosystem is not fully established and the root exudates are insufficient to effectively recruit diverse microbial taxa. For example, the fungal diversity in the soil of *Ormosia hosiei* has been shown to significantly increase with plantation age, with 20-year-old stands having much higher fungal diversity than 7-year-old ones (Wan et al., 2021). Similarly, the structure of bacterial and fungal communities in the rhizosphere soil of *Carya cathayensis* is significantly affected by tree age. This is because rhizosphere microbial community structure and diversity tend to change with plant growth years and respond differentially to various soil environmental factors (Fu and Huang, 2014; Meng et al., 2023).

The dominant bacterial phyla in rhizosphere and non-rhizosphere soils of *C. hainanensis* of different ages were Proteobacteria, Actinobacteriota, and Acidobacteriota, while the dominant fungal phyla were *Ascomycota* and *Basidiomycota*—common microbial groups in forest soils (Liu et al., 2018; Wei et al., 2018; Qiu et al., 2022). In this study, microbial community structures varied among soil samples. In the four sample types (LSG, LSN, XSG, XSN), Proteobacteria and Ascomycota represented the dominant bacterial and fungal groups, respectively. *Proteobacteria* are known to be abundant in sediment recovery environments (Rojas et al., 2016), and Ascomycota are active in lignin degradation and decomposition of various plant residues (Manici et al., 2024).

At the genus level, the dominant bacterial genera in soils from different *C. hainanensis* age groups were identified as norank_f__Xanthobacteraceae, *Acidothermus*, and norank_f__norank_o__Subgroup_2. The dominant fungal genera included *Archaeorhizomyces* and several unclassified groups. *Acidothermus* is known as a bacterial genus capable of decomposing organic matter and utilizing various carbon sources, representing approximately 20% of the total bacterial abundance in this study (Wang et al., 2023). *Archaeorhizomyces* are recognized as important and widespread members of rhizosphere soil microbial communities (Menkis et al., 2014).

Notably, the genus *Sebacina* was uniquely observed in the rhizosphere and non-rhizosphere soils of saplings (XSG and XSN samples), comprising about 20–30% of the fungal community. Given that *Sebacina* has been reported to promote germination and early development of orchid seeds (Durán et al., 2019), it may play a role in facilitating early root development and microbe-root interactions in *C. hainanensis* saplings. This observation suggests a potential ecological function of *Sebacina* in creating a favorable microecological environment conducive to sapling growth, though further experimental studies are needed to confirm this relationship.

In conclusion, the microbial community structure in the rhizosphere and non-rhizosphere soils of *C. hainanensis* exhibits notable differences across tree ages. While bacterial communities remain relatively stable, fungal communities are more significantly influenced by tree age, particularly during the sapling stage, where distinct microbial characteristics were observed. These findings not only provide a theoretical basis for understanding the dynamic relationship between microbial community structure and tree development but also have important practical implications for the conservation and management of *C. hainanensis*. Enhancing and maintaining microbial diversity, especially in the rhizosphere, may improve soil health, nutrient cycling, and tree resilience, thereby supporting the growth and ecological adaptation of this nationally protected species. Therefore, integrating microbial community considerations into conservation strategies can contribute to more effective protection and sustainable restoration efforts of *C. hainanensis* populations in their natural habitats.re soils of *C. hainanensis* exhibits notable differences across tree ages. Bacterial communities appear relatively stable, while fungal communities are more significantly influenced by tree age, especially in the sapling stage, where distinct microbial characteristics were observed. These findings offer a theoretical basis for further exploration of the relationship between microbial community structure and tree age in *C. hainanensis*, as well as its role in ecological adaptation.

## Conclusion

The observed significant differences in microbial community structure between rhizosphere and non-rhizosphere soils of *C. hainanensis*, as well as across different tree ages, highlight the dynamic interactions between trees and their soil microbiomes during development. The higher bacterial and fungal diversity found in older trees suggests a more complex and potentially stable microbial ecosystem associated with mature plants, which could contribute to enhanced nutrient cycling, disease resistance, and overall tree health. The contrasting patterns of fungal diversity between rhizosphere and non-rhizosphere soils in young versus old trees imply that microbial assemblages shift in response to changing root exudates and soil conditions as the tree grows. Practically, understanding these microbial dynamics can inform conservation and restoration strategies for *C. hainanensis*, particularly given its status as a protected species. Promoting soil microbial diversity, especially beneficial fungi like *Sebacina* which appear uniquely in saplings, may improve seedling establishment and early growth success. This could be applied through microbiome management, such as inoculation with beneficial microbes during nursery cultivation or in reforestation efforts. Future research should aim to experimentally verify the ecological roles of key microbial taxa identified in this study, especially *Sebacina*, to elucidate their functional contributions to sapling development and soil health. Longitudinal studies tracking microbial community succession throughout tree ontogeny, combined with assessments of soil physicochemical properties and tree physiological status, would provide deeper insight into microbe-host interactions. Such integrative approaches would strengthen the foundation for microbiome-based conservation practices and improve the management of *C. hainanensis* populations in natural and managed ecosystems.

**Acknowledgements.** This work was supported by the Guangxi Natural Science Foundation (2024GXNSFAA010452 and 2023GXNSFAA026253); the Independent Projects of the Key Laboratory of Functional Phytochemicals Research and Sustainable Utilization of Guangxi Plants (ZRJJ2024-3 and ZRJJ2024-11); the Hechi Science and Technology Base and Talent Project (Hechi AC231113); and the Operational Fund of Guangxi Institute of Botany (No. GUIZHIYE25009).

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# APPENDIX

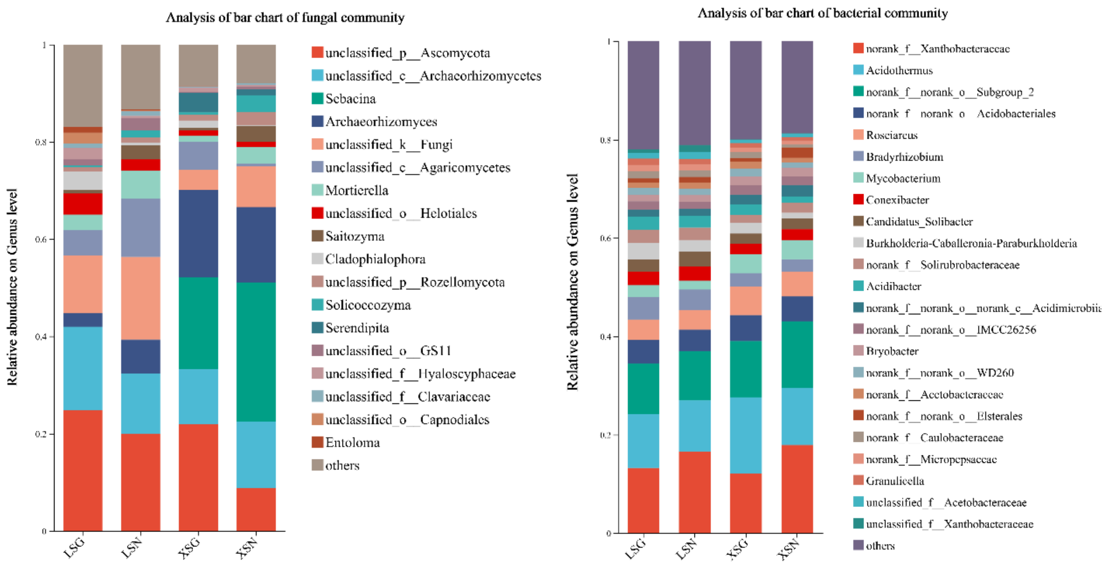

***Figure 1.*** *Composition of soil microbial communities in rhizosphere and non-rhizosphere of Cephalotaxus hainanensis at genus level*

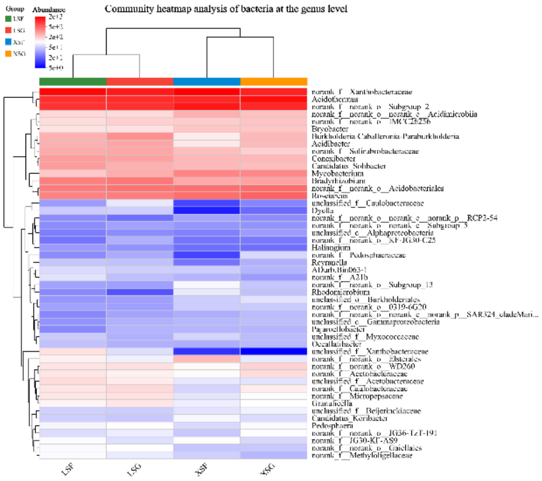

***Figure 2.*** *Cluster heat map of microbial communities of rhizosphere and non-rhizosphere soil of Cephalotaxus hainanensis*

DOI: http://dx.doi.org/10.15666/aeer/2304_83358350