Postprint: Variation Characteristics of Winter Rhizosphere Soil Microorganisms of Abies yuanbaoshanensis along an Altitudinal Gradient

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Date: 2024-09-22T00:00:00+00:00

Abstract

To investigate the effects of altitude on the rhizosphere soil microbial communities of *Abies yuanbaoshanensis*, this study employed Illumina Miseq high-throughput sequencing technology to analyze variations in the composition, diversity, and influencing factors of winter rhizosphere soil microbial communities of *A. yuanbaoshanensis* along different altitudinal gradients. The results showed that: (1) Altitude had a significant effect on physicochemical properties ($P<0.05$). (2) Altitude significantly affected the composition, structure, and diversity of rhizosphere soil microbial communities ($P<0.05$). With increasing altitude, bacterial diversity exhibited a trend of first increasing and then decreasing, while fungal diversity showed a significant decreasing trend. (3) The structure and diversity of soil rhizosphere microbial communities were closely correlated with environmental factors. Soil organic matter, total soil nitrogen, total soil potassium, C:N, C:P, and N:P were important driving factors affecting changes in microbial community structure along the altitudinal gradient ($P<0.05$), while total soil phosphorus, C:P, and N:P were important driving factors influencing microbial diversity along the altitudinal gradient ($P<0.05$). This study provides a scientific basis for the conservation of the endangered plant *Abies yuanbaoshanensis* from a soil microbiological perspective, while also offering data support for improving our understanding of microbial distribution patterns in mountain forest ecosystems.

Full Text

Variation Characteristics of Winter Rhizosphere Soil Microorganisms of *Abies yuanbaoshanensis* Along an Altitudinal Gradient

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Abstract: To investigate the effects of altitude on the rhizosphere soil microbial community of *Abies yuanbaoshanensis*, we employed Illumina MiSeq high-throughput sequencing technology to analyze the composition, diversity, and influencing factors of winter rhizosphere soil microbial communities across different altitudinal gradients. The results showed that: (1) Altitude significantly affected soil physicochemical properties ($P < 0.05$). (2) Altitude significantly influenced the composition and diversity of rhizosphere soil microbial communities ($P < 0.05$). Bacterial diversity initially increased then decreased with rising altitude, while fungal diversity showed a significant declining trend. (3) Microbial community structure and diversity were closely correlated with environmental factors. Soil organic matter, total nitrogen, total potassium, C:N, C:P, and N:P were identified as important drivers of microbial community composition along the altitudinal gradient ($P < 0.05$), while total phosphorus, C:P, and N:P were key drivers affecting microbial diversity ($P < 0.05$). This study provides a scientific basis for the conservation of the endangered plant *Abies yuanbaoshanensis* from a soil microbiology perspective and offers valuable data to support research on microbial distribution patterns in mountain forest ecosystems.

Keywords: *Abies yuanbaoshanensis*; microbial diversity; altitude; high-throughput sequencing; soil physicochemical properties

In mountain forest ecosystems, numerous ecological factors change with vertical altitudinal variation, creating gradient differences in regional microclimate, vegetation distribution, and soil physicochemical properties. These factors directly or indirectly influence soil microbial communities, resulting in various patterns of microbial community change along altitudinal gradients, including monotonic decrease, increase, unimodal, bimodal, or irregular patterns (Zhang et al., 2012; Singh et al., 2013; Zhang et al., 2015; Pang et al., 2023). Microbial diversity responses to altitudinal change vary across different regions and scales, likely resulting from the combined effects of multiple environmental factors and potential factors closely related to microbial characteristics that remain insufficiently identified (Beimforde et al., 2013; Cao, 2017). Against the backdrop of global climate change, investigating the mechanisms of microbial community response to altitudinal change and how such responses affect ecological environmental factors can help us deeply understand the impacts of environmental change on Earth' s microbiome and provide a scientific basis for predicting and addressing potential effects of climate change (Meng et al., 2023). Microorganisms, as crucial components of mountain forest ecosystems, exhibit significant functions in ecosystem regulation. They not only help stabilize soil structure, enrich biodiversity, and ensure balanced forest ecosystem development, but also play important roles in enhancing plant growth adaptability (Lü, 2011; Zhang et al., 2015; Xu et al., 2021).

The rhizosphere, as a special micro-ecological niche where plants, soil, and microorganisms interact, serves as a critical interface for energy exchange and material cycling and represents an important site for plant-soil ecosystem interactions. Its complexity and uniqueness are significant for understanding overall ecosystem function and stability, and its high sensitivity to environmental change can serve as a sensitive indicator of soil ecosystem changes (Yang et al., 2011; Ye, 2019). Studies have shown that rhizosphere microorganisms exert crucial influences on plant ecological adaptation, effectively decomposing organic matter to provide necessary nutrients for plants while also assisting plants in adapting to external environmental changes through secretion of soil enzymes, organic acids, and growth stimulants (Shi et al., 2018; Wang et al., 2022; Wang et al., 2023). These studies provide new perspectives for ecological restoration and plant conservation, making in-depth research on rhizosphere soil microorganisms essential for enhancing plant adaptability and stress resistance.

Abies yuanbaoshanensis is a nationally protected wild plant (first-class) that grows exclusively in Yuanbaoshan, Guangxi, with extremely high conservation and research value (Fu et al., 1980). As one of the wild plant species with extremely small populations, *A. yuanbaoshanensis* is currently critically endangered with an extremely narrow distribution range limited to the altitudinal zone of 1,750–2,050 m in Yuanbaoshan (Li & Su, 2002). The extant wild population numbers fewer than 900 individuals, with severe population decline, few adult individuals, and a predominance of old trees that show weakened growth, hollowing, and severe crown dieback (Ou et al., 2002). The cascading effects of global climate change pose a serious threat to the survival of this critically endangered species. In high-altitude regions of Yuanbaoshan, abnormal climate phenomena occur frequently, including seasonal droughts, summer rainstorms, and uncertain winter snowfall and frost (Ning et al., 2009). As existing habitats continue to be compressed and fragmented, there is an urgent need to explore the species' living environment and its adaptability to climate change to develop conservation and population expansion strategies; otherwise, the trend of population decline will be difficult to reverse, and the species will be highly vulnerable to extinction (Huang, 1998; Xiang, 2001; Wang et al., 2023). Since rhizosphere soil microorganisms are highly sensitive to environmental change while also enhancing plant adaptability to harsh environmental changes, this study conducted winter sampling of *A. yuanbaoshanensis* rhizosphere soil at different altitudes. Using the Illumina MiSeq high-throughput sequencing platform for bacterial and fungal sequencing, we analyzed the relationship between microbial community structure, diversity changes, and environmental factors across different altitudinal gradients to explore the altitudinal distribution patterns and driving factors of rhizosphere soil microbial diversity of *A. yuanbaoshanensis* in alpine habitats and understand plant adaptability under global climate change. The results of this study will not only provide a solid theoretical basis and technical support for conservation research on this endangered species but also offer valuable baseline data for studying altitudinal distribution patterns in subtropical mountain forest ecosystems.

1.1 Study Area Overview

The sampling site was located in Yuanbaoshan National Nature Reserve in Rongshui County, Guangxi (109°07 -109°13 E, 25°22 -25°32 N). The climate is mid-subtropical monsoonal, with abundant heat and precipitation, an average annual temperature of approximately 16.4 °C, average annual precipitation of approximately 2,379 mm, and a frost-free period of 288 days. The soil type is primarily mountain yellow soil with a thick surface humus layer. The forest stand is simple, with main associated tree species including *Tsuga chinensis* and *Taxus chinensis* var. *mairei*. The understory is extensively covered by cold-loving bamboo, with dense distribution of lichens and mosses (Huang, 1998; Liang & Pan, 2012).

1.2 Sample Collection and Processing

Sampling was conducted in winter (December 2022). Based on the growth and distribution of *A. yuanbaoshanensis*, altitudinal gradients were set at 100 m intervals: 1,750 m, 1,850 m, 1,950 m, and 2,050 m. As extremely small populations often have insufficient numbers of individuals in the wild, sampling was limited by natural conditions and species characteristics, making it difficult to meet conventional sampling standards. Consequently, individual trees sampled exhibited differences in growth traits (Hu et al., 2023). Within each altitudinal plot, three vigorous individuals with relatively similar traits were randomly selected for rhizosphere soil collection, with sampling details shown in Table 1 . At 0.5 m from the trunk, surface litter and humus layers were removed, soil was excavated, and fresh fine roots were selected. Soil attached to fine roots was shaken off, and soil tightly adhering to roots was gently brushed off with a sterile soft brush as rhizosphere soil. Fresh soil samples were cleared of visible roots and residues, then divided into three subsamples: one placed in an aluminum box for soil water content determination, one immediately stored in a sealed incubator (with dry ice) for microbial community diversity analysis, and one air-dried and passed through a 2 mm sieve for physicochemical property measurement.

1.3 Soil Physicochemical Property Determination

Soil water content (SWC) was determined using the drying method; soil pH was measured using the potentiometric method (National Standard HJ 962-2018); soil organic carbon (SOC) was determined by potassium dichromate hydration heating method; total nitrogen (TN) by concentrated sulfuric acid digestion-sodium salicylate spectrophotometry; total phosphorus (TP) by sulfuric acid digestion-molybdenum antimony anti-colorimetry; and total potassium (TK) by flame photometry.

1.4 Soil Microbial Total DNA Extraction, PCR Amplification, and High-Throughput Sequencing

Total DNA was extracted from 0.25 g frozen soil samples following the ALFASoil DNA Extraction Kit (FINDROP, Guangzhou) protocol. The PCR amplification program was: 94 °C pre-denaturation for 5 min; 31 cycles of 94 °C denaturation for 30 s, 53 °C annealing for 30 s, and 72 °C extension for 30 s; final extension at 72 °C for 8 min; and hold at 12 °C. Primer regions: fungal ITS region amplification primers were ITS3-F (5 -GCATCGATGAAGAACGCAGC-3) and ITS4-R (5 -TCCTCCGCTTATTGATATGC-3); bacterial 16S rRNA gene amplification primers were 338F (5 -ACTCCTACGGGAGGCAGCA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3). Quantification, normalization, library construction, and sequencing of PCR amplification products were completed by Guangdong Meige Technology Co., Ltd.

1.5 Statistical Analysis

Data calculations were performed using Excel 2013, one-way ANOVA using SPSS 20.0, and plotting using Origin 8.0. Microbial analysis was conducted using the Ilumina NovaSeq6000 (PE250) platform from Guangdong Meige Technology Co., Ltd. Alpha diversity was characterized using ACE, Chao1, Shannon, and Simpson indices. Beta diversity was analyzed using Bray-Curtis distance and principal coordinates analysis (PCoA) to explore differences in rhizosphere soil bacterial and fungal communities across altitudes. Redundancy analysis (RDA) was used to reveal correlations between rhizosphere soil environmental factors and microbial composition, and Mantel test was used to explore correlations between environmental factors and bacterial and fungal community diversity.

2.1 Effects of Altitude on Rhizosphere Soil Physicochemical Properties

The measured results of rhizosphere soil physicochemical properties and stoichiometric ratios of *A. yuanbaoshanensis* along the altitudinal gradient are shown in

Table 2 and Table 3 . The data indicate that rhizosphere soil nutrient content is relatively high, with significant differences in soil physicochemical properties and stoichiometry across altitudes. Specifically, organic carbon, C:P, and N:P showed significant differences among the four altitudinal gradients, demonstrating that altitude significantly affects soil physicochemical properties and stoichiometry ($P < 0.05$).

2.2 Changes in Rhizosphere Soil Microbial Community Composition of *A. yuanbaoshanensis* Across Altitudinal Gradients

Processing of raw sequencing data from soil samples at four altitudes using the Illumina MiSeq platform yielded 402,729 bacterial sequences and 314,371 fungal sequences, which were annotated into 13,489 bacterial OTUs and 8,103 fungal OTUs, with bacterial numbers exceeding fungal numbers. Figure 1 [Figure 1: see original paper] shows that rhizosphere soil microbial community composition structures were similar across altitudes, with certain differences in relative abundance but no obvious altitudinal distribution pattern. The relative abundance of unclassified or unidentified microorganisms was relatively high. Bacterial community composition was relatively stable across altitudes, while fungal community composition showed greater variation with altitude.

2.2.1 Analysis of Bacterial Community Composition Differences

Based on taxonomic analysis of soil genomic DNA sequences, bacterial OTUs from *A. yuanbaoshanensis* rhizosphere were classified into 13 phyla, 36 classes, 97 orders, 182 families, 269 genera, and 360 species. Results (Figure 1, Table 4) showed that at the phylum level, Acidobacteria (46.15%), Proteobacteria (28.69%), and Actinobacteria (10.52%) were dominant bacterial phyla, but none showed significant changes with increasing altitude. At the genus level, *Bryobacter* (5.42%), *Acidothermus* (5.34%), *Candidatus_{Solibacter}* (4.88%), *Roseiarcus* (3.53%), and *Acidibacter* (2.16%) were dominant bacterial genera. Among these, *Acidothermus* and *Acidibacter* showed significant differences across altitudinal gradients ($P < 0.05$), while other genera showed no significant changes. Figure 2 [Figure 2: see original paper] indicates that TK, C:N, and N:P showed significant correlations with *Bradyrhizobium* ($P < 0.05$); TP showed significant negative correlation with *Acidothermus* ($P < 0.05$); C:P and N:P showed significant positive correlations with *Acidothermus* ($P < 0.05$); SWC showed extremely significant positive correlation with *Acidothermus* ($P < 0.01$) and significant negative correlations with *Bryobacter* and *Candidatus_{Solibacter}* ($P < 0.05$); pH showed significant positive correlation with *Roseiarcus* ($P < 0.05$). Both *Acidibacter* and *Bradyrhizobium* showed significant correlations with altitude ($P < 0.05$), indicating that altitude has certain effects on bacterial community composition.

2.2.2 Analysis of Fungal Community Composition Differences

Based on taxonomic analysis of soil genomic DNA sequences, fungal OTUs from *A. yuanbaoshanensis* rhizosphere were classified into 27 phyla, 66 classes, 122 orders, 168 families, 231 genera, and 76 species. Results (Figure 1, Table 4) showed that at the phylum level, Ascomycota (44.39%) and Basidiomycota (40.00%) were dominant fungal phyla, with Basidiomycota showing significant differences across altitudes ($P < 0.05$). At the genus level, *Elaphomyces* (11.59%), *Sebacina* (5.47%), *Russula* (4.97%), and *Cortinarius* (4.97%) were dominant fungal genera. Among these, *Russula* showed a significant decreasing trend with increasing altitude ($P < 0.05$), while other fungal genera showed no significant changes. Additionally, *Pseudohydnum* and *Leuconeurospora* were found to be unique fungal genera at 1,750 m altitude. Figure 2 shows that TK and TP showed significant negative correlations with *Phaeocollybia* ($P < 0.05$), while TK and TP showed significant positive correlations with *Cenococcum* ($P < 0.05$); N:P and C:P showed significant positive correlations with *Phaeocollybia* ($P < 0.05$); SWC showed significant positive correlations with *Russula* and *Amanita* ($P < 0.05$); pH showed significant positive correlation with *Cortinarius* ($P < 0.05$). *Amanita*, *Russula*, and *Phaeocollybia* showed significant correlations with altitude ($P < 0.05$), indicating that altitude has certain effects on fungal community composition.

2.2.3 Relationship Between Microbial Community Structure and Environmental Factors

Altitudinal gradient changes often alter forest stand climate and soil properties, thereby affecting soil microbial community composition. This study used RDA to analyze the effects of rhizosphere soil environmental factors on community composition. Figure 3 [Figure 3: see original paper] shows that altitude is the most important factor limiting rhizosphere soil microbial community composition of *A. yuanbaoshanensis*. In bacterial communities, the first axis explained 19.41% of all environmental factors, and the second axis explained 13.08% of all variables. In fungal communities, RDA1 and RDA2 explained 15.11% and 12.87% of structural variation, respectively. Results showed significant correlations between environmental factors and variation in rhizosphere soil microbial communities, with altitude having significant effects on both bacterial and fungal communities ($P < 0.05$). Among environmental factors, SOC and TN had significant effects on bacterial communities ($P < 0.05$), while TK, C:N, C:P, and N:P had extremely significant effects ($P < 0.01$). SOC, TN, and N:P had significant effects on fungal communities ($P < 0.05$), while TK, C:N, and C:P had extremely significant effects ($P < 0.01$), indicating that soil nutrient limitation and carbon-nitrogen-phosphorus ratios are key factors driving structural variation in rhizosphere soil bacterial and fungal communities of *A. yuanbaoshanensis* across altitudes.

2.3.1 Alpha Diversity

ACE and Chao1 indices reflect community richness (higher values indicate greater richness), while Shannon and Simpson indices reflect community diversity (higher Shannon values indicate greater diversity, lower Simpson values indicate greater diversity). Results (Figure 4 [Figure 4: see original paper]) showed that alpha diversity of *A. yuanbaoshanensis* rhizosphere soil varied significantly with altitude ($P < 0.05$). Bacterial diversity was highest at 1,950 m altitude, while fungal diversity was highest at 1,750 m altitude. With increasing altitudinal gradient, bacterial and fungal richness both showed a trend of initial decrease followed by increase; bacterial diversity showed an overall trend of initial increase then decrease, while fungal diversity showed a significant decreasing trend.

2.3.2 Beta Diversity

PCoA results based on Bray-Curtis distance are shown in Figure 5 [Figure 5: see original paper]. In bacterial communities, samples from four altitudes were relatively concentrated, indicating similar bacterial community structure across altitudes. Samples within the 1,850 m altitude group were relatively concentrated, indicating good sample repeatability. In fungal communities, samples from four altitudes were relatively dispersed. Samples within the 1,850 m altitude group were relatively concentrated and distant from other altitude groups, indicating good within-group repeatability but relatively large between-group differences. Samples within the other three altitude groups were relatively dispersed, indicating large within-group community structure differences. Beta diversity of rhizosphere soil bacteria and fungi of *A. yuanbaoshanensis* did not show regular patterns with altitude.

To further explore the significance of differences in bacterial and fungal community structures across altitudinal groups, multiple statistical methods were comprehensively applied, including MRPP, Adonis, Anosim, and Amova. Table 5 data show that in Anosim analysis, R values greater than 0 indicated significant differences in bacterial and fungal community composition and structure across altitudinal groups of *A. yuanbaoshanensis* ($P < 0.05$).

2.3.3 Relationship Between Microbial Community Diversity and Environmental Factors

Mantel test analysis was used to reveal relationships between α-diversity and β-diversity of rhizosphere soil microbial communities and environmental factors (Figure 6 [Figure 6: see original paper]). Results showed that altitude was significantly correlated with bacterial α-diversity ($P < 0.05$). In bacterial communities, soil TP, TK, C:N, N:P, and C:P were the main environmental factors affecting bacterial α-diversity ($P < 0.05$), while soil TP, N:P, and C:P were the main factors affecting bacterial β-diversity ($P < 0.05$). In fungal communities, soil TN, TP, C:P, and N:P were key environmental factors affecting fungal α-diversity ($P < 0.05$), while fungal β-diversity showed no significant correlation with environmental factors ($P > 0.05$).

3.1.1 Correlation Between Soil Environmental Factors and Microbial Community Structure

This study found that at phylum and genus levels, dominant microbial groups in *A. yuanbaoshanensis* rhizosphere soil did not change with altitude, though their relative abundances differed across altitudes without showing obvious altitudinal distribution patterns. Acidobacteria, Proteobacteria, and Actinobacteria were dominant bacterial phyla, while Ascomycota and Basidiomycota were dominant fungal phyla, consistent with results from small-scale altitudinal gradient studies by Wang et al. (2023) and large-scale studies by Cao et al. (2022), suggesting that altitudinal scale does not significantly affect the composition structure of dominant microorganisms. As decomposers in food chains, microorganisms can effectively decompose and transform humus to improve soil nutrients. Meanwhile, soil nutrient factors affect microbial metabolism and soil enzyme activity, representing important factors causing microbial community structure changes. In this study, all five dominant phyla were beneficial for organic matter decomposition and nutrient cycling, likely because Yuanbaoshan National Nature Reserve is located in a subtropical monsoon climate zone with favorable hydrothermal conditions and concurrent rainfall and heat periods. These climatic conditions facilitate soil organic matter accumulation and litter decomposition, providing abundant substrate sources for various microorganisms. This allows nutrient-loving bacterial and fungal groups to proliferate and grow extensively, making the relative abundances of microorganisms related to metabolism and decomposition significantly dominant across all altitudinal gradients. Such microorganisms not only benefit local soil nutrient cycling but also provide important support for ecological balance and biodiversity conservation.

Acidobacteria can effectively promote lignin decomposition in soil, while Proteobacteria play key roles in cycling of organic matter, nitrogen, phosphorus, and sulfur in soil, with their activity directly reflecting soil nutrient status—consistent with our findings of higher abundances at nutrient-rich altitudes. Many groups in Actinobacteria can secrete various enzymes including cellulase, chitinase, and peroxidase, which are crucial for soil organic matter mineralization, facilitating organic matter decomposition and soil fertility improvement (De Vries et al., 2013). As the largest group in the fungal kingdom, Ascomycota are renowned for their excellent decomposition and degradation capabilities. Although no significant differences were found across altitudes in this study, their abundance at 1,950 m altitude was 8.85%–23.60% higher than at other altitudes. This phylum can form symbiotic relationships with hosts, thereby enhancing ecosystem nutrient cycling efficiency and resistance to environmental stress, making Ascomycota a likely reason for the stronger environmental adaptability at 1,950 m altitude. Basidiomycota have stronger capabilities in decomposing recalcitrant compounds, particularly complex lignified plant fibers (Wang & Tu, 2014; Xiao et al., 2023). Basidiomycota gradually decreased with increasing altitudinal gradient ($P < 0.05$), with significantly higher abundance at 1,750 m altitude than at higher altitudes, possibly because low-altitude areas of *A. yuanbaoshanensis* have more associated tree species, richer understory litter, and higher lignin content, which favor Basidiomycota growth and make them dominant soil fungal groups that can accelerate local soil nutrient cycling —potentially one reason why richness was highest at 1,750 m altitude.

This study found that among dominant genera, only the bacterial genera *Acidothermus* and *Acidibacter* and the fungal genus *Russula* showed significant differences across altitudinal gradients ($P < 0.05$), while others showed no significant changes. Soil water content showed significant positive correlation with *Acidothermus* ($P < 0.05$), while *Acidibacter* and *Russula* showed significant negative correlations with altitudinal gradient ($P < 0.05$). Most dominant fungal genera were ectomycorrhizal fungi, such as *Russula*, *Sebacina*, and *Cortinarius*. Ectomycorrhizal fungi form mycorrhizal networks connecting plant hosts with fungi, facilitating water and nutrient absorption by plant hosts, enhancing stress resistance in harsh environments, and helping maintain stability of tree population ecosystems (Li & Gao, 2021; Xiong et al., 2021; Gao et al., 2022). Therefore, these dominant microbial genera play important roles in improving soil nutrients and promoting plant growth. *Russula* is speculated to potentially enhance the adaptability of *A. yuanbaoshanensis* at low altitudes and strengthen stress resistance to harsh environments, though this speculation requires further research. Additionally, *Pseudohydnum* and *Leuconeurospora* were found to be unique fungal genera at 1,750 m altitude.

Influencing factors on biological distribution show diverse differences across altitudinal gradients at different scales. At large scales, climate is the key factor dominating microbial community distribution, while at small scales, differences in soil factors among different locations dominate changes in soil microbial composition and abundance because populations adapted to a certain climate zone over the long term usually occupy similar climatic niches (Yelle et al., 2008; Miao et al., 2016; Hanaka et al., 2019). This study was conducted across a small-scale altitudinal gradient (1,750–2,050 m) and found that altitude is the most important factor limiting rhizosphere soil microbial community composition of *A. yuanbaoshanensis*. Among all environmental factors, SOC, soil TN, TK, C:N, C:P, and N:P all had significant effects, with soil physicochemical factors closely related to microbial composition changes. Specifically, soil nutrients and carbon-nitrogen-phosphorus ratios at different altitudes were important causes of differences in rhizosphere soil microbial community composition of *A. yuanbaoshanensis*.

3.1.2 Correlation Between Soil Environmental Factors and Microbial Community Diversity

Mountain forest ecosystems are natural venues for studying soil microbial communities and climate change. Altitudinal gradient changes alter climatic environments, affect vegetation types, change soil properties, and thereby profoundly influence microbial diversity (Guo & Tang, 2020). Although rhizosphere soil accounts for only 1%-3% of total soil volume, it is the most active and unique ecological niche for plant-soil-microorganism interactions with environmental conditions. Its microorganisms enhance plant adaptability to adverse environments by improving soil nutrients (Wang et al., 2022). Compared with animals and plants, research on altitudinal distribution patterns of soil microorganisms is still in its early stages, with most studies focusing on surface soils. Research on altitudinal distribution patterns of rhizosphere soil microorganisms of single woody plant species is even rarer. Previous studies have indicated that soil microbial diversity often lacks universal altitudinal gradient patterns (Zhang et al., 2012; Singh et al., 2013; Zhang et al., 2015; Pang et al., 2023).

This study showed that microbial diversity and richness of *A. yuanbaoshanensis* rhizosphere soil differed significantly across altitudinal habitats ($P < 0.05$), with relatively obvious distribution patterns along altitudinal gradients. Bacterial diversity was highest at 1,950 m altitude, while fungal diversity was highest at 1,750 m altitude. With increasing altitudinal gradient, bacterial and fungal richness both showed a trend of initial decrease followed by increase, with significant differences in microbial community richness indices across altitudinal gradients ($P < 0.05$), consistent with results from small-scale altitudinal gradient studies by Wang et al. (2023). Bacterial diversity showed an initial increase then decrease trend with rising altitude, peaking at 1,950 m, similar to trends observed in *Abies fabri* and *Picea purpurea* on Gongga Mountain (Xiong et al., 2021; Gao et al., 2022). Fungal community diversity showed a significant decreasing trend with rising altitude ($P < 0.05$), highest at 1,750 m and lowest at 2,050 m, similar to trends in *Rhododendron delavayi*, *Ginkgo biloba*, and *Picea crassifolia* (Xiong, 2021; Wu et al., 2022; Meng et al., 2023).

Many studies have shown that changes in soil carbon-nitrogen-phosphorus content are often indirect pathways through which plants affect soil microbial community diversity. Plants regulate C, N, and P content through litter and root exudates, improve soil physicochemical properties, and thereby influence soil microbial communities (Zheng et al., 2010; Cao et al., 2022; Wang et al., 2023; Xiao et al., 2023). Our results confirm this viewpoint, finding that soil TP, C:P, and N:P jointly and significantly affected bacterial and fungal α-diversity ($P < 0.05$). Additionally, altitude, SWC, TK, and C:N were key factors significantly affecting bacterial α-diversity ($P < 0.05$), while TN was the key factor significantly affecting fungal α-diversity ($P < 0.05$). Bacterial β-diversity of *A. yuanbaoshanensis* rhizosphere soil was mainly affected by soil TP, N:P, and C:P, while fungal β-diversity was unrelated to environmental factors, differing from results by Wang et al. (2023). Studies have shown that microbial diversity across different regions and scales often responds differently to altitudinal change, with highly complex and diverse influencing factors. The difference from our study may be because Wang et al. (2023) focused on the low-altitude evergreen broadleaf species *Castanopsis fargesii*, while this study targeted the high-altitude coniferous species *A. yuanbaoshanensis*. Microbial community structure is regulated by different types of litter and root exudates, while environmental factors such as soil organic matter content, pH, and microclimate also indirectly affect microbial communities, leading to significant differences in how soil microbial diversity responds to soil properties. Soil physicochemical factors are closely related to microbial diversity changes, with soil nutrients and carbon-nitrogen-phosphorus ratios at different altitudes being important causes of differences in rhizosphere soil microbial community diversity of *A. yuanbaoshanensis*.

3.1.3 Limitations

The study species is an extremely small population—*A. yuanbaoshanensis*. Due to limitations from natural conditions and species characteristics, the small number of individuals in the wild made it difficult to meet standard sampling methods, meaning our data were affected not only by altitude but also by individual tree traits. Future studies will investigate microbial community characteristics associated with individual trait differences to supplement these data. Although many studies have indicated that seasonal changes affect soil temperature, moisture, and plant growth status, thereby indirectly influencing soil microbial communities, the specific mechanisms of community composition and diversity changes remain unclear (Zheng et al., 2018). Seeds of *A. yuanbaoshanensis* typically mature and naturally fall in mid-to-late October as temperatures gradually decline, and begin to germinate as temperatures rise and rainfall increases (Wang et al., 2023). During this process, safety during the overwintering period is crucial for seed germination. Although low-temperature environments weaken plant root effects on rhizosphere microorganisms, lower soil temperatures can create relatively stable habitats in mountain ecosystems, reducing nutrient fluctuations and microbial metabolic activities. Meanwhile, rhizosphere soil nutrients are relatively high in winter (Xiong et al., 2021), providing favorable conditions for seed overwintering. Therefore, this study selected winter sampling to explore the response mechanisms of soil characteristics and microbial community features of *A. yuanbaoshanensis* rhizosphere at different altitudes in alpine habitats to environmental change, and to identify microorganisms that promote *A. yuanbaoshanensis* growth and development. However, this study lacks seasonal variation research, and winter sampling alone cannot thoroughly investigate temporal and spatial distribution patterns of *A. yuanbaoshanensis* microbial communities. In future research, we plan to conduct multi-seasonal sampling and analysis to comprehensively explore microbial community variation patterns across different seasons.

3.2 Conclusion

This study used high-throughput sequencing technology to analyze differences in rhizosphere soil microbial communities of *A. yuanbaoshanensis* across altitudinal gradients, revealing the altitudinal distribution patterns and driving factors of rhizosphere microbial communities of a single plant species in mountain forest ecosystems. Results showed that microbial diversity had relatively obvious patterns along altitudinal gradients, with bacteria showing an initial increase then decrease trend and fungi showing a continuous decreasing trend ($P < 0.05$). Altitude significantly affected rhizosphere soil microbial structural composition ($P < 0.05$) but did not show obvious altitudinal distribution patterns, with fungal communities more strongly affected by altitude than bacterial communities. Environmental factor correlation analysis further revealed that soil nutrients and carbon-nitrogen-phosphorus ratios at different altitudes were key factors causing differences in rhizosphere soil microbial community diversity and composition of *A. yuanbaoshanensis* ($P < 0.05$). This study can provide more precise and scientific basis for subsequent ecological conservation and restoration of *A. yuanbaoshanensis*.

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