Postprint: Rhizosphere Soil Microbial Diversity of Paris polyphylla var. chinensis at Different Growth Years

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Abstract

To investigate the relationship between the growth and development of *Paris polyphylla* and changes in soil microbial community structure, this study employed Illumina high-throughput sequencing technology to sequence and analyze bacterial 16S rRNA and fungal 18S sequences in the rhizosphere soil of *Paris polyphylla* at four different growth stages (3-year-old, 5-year-old, 7-year-old, and 9-year-old). The results demonstrated that: (1) The dominant bacterial phyla in rhizosphere soils across different growth years were consistently Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi; the dominant fungal phyla were Ascomycota, Basidiomycota, and Mucoromycota. (2) Bacterial species were more abundant and exhibited higher diversity than fungi in the rhizosphere soil of *Paris polyphylla* across different growth years. Bacterial diversity displayed an " "-shaped pattern of decreasing, increasing, and then decreasing again with increasing growth years of *Paris polyphylla*, reaching its nadir at year 5 and its peak at year 7; fungal diversity exhibited a "Λ" -shaped pattern of initially increasing and subsequently decreasing with increasing growth years, with the peak at year 7. Bacterial community richness followed a "Λ" -shaped pattern of initially increasing and subsequently decreasing with increasing growth years, attaining maximum richness at year 7; whereas fungal richness showed minimal variation with increasing growth years. (3) UPGMA cluster analysis revealed that the rhizosphere soil microbial community structure evolved significantly with advancing growth years, with the period of more pronounced bacterial community evolution occurring at year 7 post-planting, while for fungal communities it was at year 5 post-planting. (4) Spearman correlation analysis identified available potassium and total nitrogen as the primary factors influencing bacterial composition in the rhizosphere soil of *Paris polyphylla*, while total potassium was the principal factor affecting fungal composition. In summary, these results indicate that the composition and structure of rhizosphere soil

microbial communities vary across different growth and development stages of *Paris polyphylla*, with years 5–7 representing the critical period during which major changes in rhizosphere soil microbial community diversity occur.

Full Text

Microbial Diversity in Rhizosphere Soil of *Paris polyphylla* var. *chinensis* Across Different Growth Years

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Abstract: To investigate the relationship between the growth and development of *Paris polyphylla* var. *chinensis* and changes in rhizosphere soil microbial community structure, this study employed Illumina high-throughput sequencing technology to analyze bacterial 16S rRNA genes and fungal 18S sequences in rhizosphere soils from four different growth years (3-year, 5-year, 7-year, and 9-year-old plants). The results revealed: (1) The dominant bacterial phyla across all growth years were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi*, while the dominant fungal phyla were *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. (2) Bacterial species were more abundant and diverse than fungi in the rhizosphere soil across different growth years. Bacterial diversity exhibited an " " -shaped pattern of decreasing, increasing, and then decreasing again with growth year, reaching its lowest point at year 5 and highest at year 7. Fungal diversity showed a "Λ"-shaped pattern of first increasing then decreasing, peaking at year 7. Bacterial community richness followed a "Λ" -shaped pattern, also peaking at year 7, whereas fungal richness showed minimal variation across growth years. (3) UPGMA cluster analysis demonstrated clear evolution of rhizosphere microbial community structure with increasing growth year, with bacterial communities undergoing more dramatic changes at year 7 after planting, while fungal communities changed most significantly at year 5. (4) Spearman correlation analysis identified available potassium and total nitrogen as the primary factors influencing bacterial composition, while total potassium was the main factor affecting fungal composition. These findings indicate that rhizosphere soil microbial community composition and structure vary across different growth and development stages, with years 5–7 representing a critical period for major changes in microbial community diversity.

Keywords: *Paris polyphylla* var. *chinensis*, rhizosphere soil, microbial community, diversity

Paris polyphylla var. *chinensis* is a perennial herb of the Liliaceae family and a traditional Chinese medicinal plant renowned for its heat-clearing, detoxifying, anti-inflammatory, and analgesic properties. It serves as the primary raw material for numerous famous Chinese patent medicines including Yunnan Baiyao, Gongxuening, Baibaodan, Reduqing, Jidesheng Snake Medicine, Antiviral Granules, and total saponin tablets (Yuan et al., 2004; Tang et al., 1998), holding significant value for development and application. The rhizosphere represents a zone where soil, root systems, and microorganisms interact intimately and influence one another (Butler et al., 2003). Appropriate growth of rhizosphere microorganisms can stimulate root development, increase absorption area, and promote nutrient uptake, while also serving as important indicators of soil fertility and nutrient status (Wang et al., 2015; Li et al., 2016; Quan et al., 2016). Medicinal plants produce abundant secondary metabolites such as flavonoids, alkaloids, and terpenoids that readily leach into soil, altering rhizosphere physicochemical properties and consequently modifying microbial communities (Nihorimbere et al., 2011). Research has shown that different plant species, different developmental stages of the same plant, or different genotypes exhibit varying nutrient preferences and absorption capacities, resulting in differences in root exudate composition, secretion quantity, and accumulation levels, which in turn drive changes in rhizosphere microbial structure (Garbeva et al., 2008; Han et al., 2010). Wei et al. (2010) studied rhizosphere microorganisms and soil enzyme activities in 2-year and 10-year-old *Forsythia suspensa*, finding that total rhizosphere microbial biomass increased with plant age, with bacterial and fungal populations rising while actinomycetes declined. Most studies on continuous cropping obstacles in medicinal plants indicate that prolonged monoculture shifts rhizosphere microbes from high-fertility "bacterial-type soil" to low-fertility "fungal-type soil" or disrupts microbial population balance (Li, 2006; Qiao et al., 2009; He et al., 2008). Imbalanced rhizosphere microbial community structure and increased pathogen populations constitute primary causes of continuous cropping obstacles in medicinal plants (Li et al., 2010).

Therefore, investigating how different growth years of medicinal plants affect rhizosphere microbial community diversity is crucial for understanding soil status, promoting plant growth, controlling underground pests and diseases, and improving medicinal material quality. *P. polyphylla* var. *chinensis* has a long growth cycle, typically requiring 8–10 years from seed to harvest, and extended monoculture easily triggers continuous cropping obstacles that compromise quality and yield. Recent research on *P. polyphylla* rhizosphere microbes has focused on different cultivation environments and varieties (Zhou et al., 2015; Zhang et al., 2016; Zheng et al., 2020), while studies examining the relationship between different developmental stages and rhizosphere microbial diversity remain scarce, leaving unclear how soil microbial community composition changes with growth year. To address this knowledge gap, this study collected rhizosphere soils from wild *P. polyphylla* var. *chinensis* at different growth years, employed Illumina high-throughput sequencing of bacterial 16S rRNA and fungal 18S sequences, and investigated microbial community composition and diversity to elucidate

how *P. polyphylla* growth influences microbial community structure, providing scientific basis for artificial cultivation, understanding physiological-ecological mechanisms of rhizome disease occurrence, and developing biological control methods.

1.1 Soil Sample Collection and Processing

P. polyphylla stems wither above ground each winter, leaving scars on the rhizome that can be counted to determine plant age (Su et al., 2020). Following Bao' s method (Bao, 2000), we collected samples in September 2018 from the wild natural distribution area of *P. polyphylla* in Jinxiu County, Guangxi. Using random multi-point mixing principles, we selected 10 healthy, uniformly growing plants with identical scar counts (one scar representing one year of growth) at each sampling point. Rhizosphere soil attached to roots (0–0.5 cm) was collected, mixed, placed in sterile plastic bags, labeled, and immediately transported in a cooler to the laboratory, with three replicates per sampling point. Each soil sample was divided into two portions: one stored at -80°C for DNA extraction and subsequent bacterial 16S rRNA and fungal 18S rRNA sequencing, and one air-dried, ground, and passed through a 2 mm sieve for physicochemical property analysis. Due to the uncontrollable nature of wild plant ages and considering the accumulation pattern of major medicinal components (polyphyllin saponins) (Zhang et al., 2011; Wang et al., 2018), we selected rhizosphere soils from 3-year, 5-year, 7-year, and 9-year-old plants for microbial diversity analysis. Soil information is presented in Table 1.

Table 1 Sources of rhizosphere soils of *P. polyphylla* var. *chinensis* in different growth years

Sample	Years of growth	Collection location	Latitude and longitude	Altitude (m)
3 years	Vanilla Ridge, Jinxiu County	110°12 23 E, 24°8 54 N		
5 years	Vanilla Ridge, Jinxiu County	110°12 10 E, 24°8 55 N		
7 years	Vanilla Ridge, Jinxiu County	110°12 10 E, 24°8 57 N		
9 years	Vanilla Ridge, Jinxiu County	110°12 12 E, 23°54 60 N		

1.2.1 Soil Microbial DNA Extraction, PCR Amplification, and Sequencing

Total soil microbial DNA was extracted using a soil genomic DNA rapid extraction kit from Shanghai Sangon Biotech Co., Ltd. Extracted DNA samples were shipped on dry ice to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. for sequencing. The entire sequencing workflow included PCR amplification, pooling and purification of PCR products, and library construction. Primers 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) were used to amplify the bacterial V3–V4 region. The amplification program consisted of: initial denaturation at 95°C for 3 min; 28 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s; and final extension at 72°C for 10 min. Primers SSU0817F (5 -TTAGCATGGAATAATRRAATAGGA-3) and 1196R (5 -TCTGGACCTGGTGAGTTTCC-3) were used to amplify the fungal V5-V7 region. The amplification program consisted of: initial denaturation at 95°C for 3 min; 37 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s; and final extension at 72°C for 10 min. Amplicons were sequenced on the Illumina platform at Majorbio.

1.2.2 Microbial Sequencing Data Quality Control and Analysis

Raw sequencing data were assembled and quality-controlled to obtain valid data. OTU (operational taxonomic units) clustering and taxonomic analysis were performed using the Uparse software platform. The OTU clustering procedure was as follows: (1) non-repetitive sequences were extracted from optimized sequences to reduce redundant computational load; (2) single sequences without repeats were removed; (3) non-repetitive sequences (excluding single sequences) were clustered into OTUs at 97% similarity, with chimeras removed during clustering to obtain representative sequences; (4) all optimized sequences were mapped to OTU representative sequences, and sequences with $ $97% similarity to representative sequences were selected to generate OTU tables. The RDP classifier Bayesian algorithm was used for taxonomic analysis of OTU representative sequences at the 97% similarity level.

1.2.3 Analysis of Rhizosphere Soil Microbial Composition in *P. polyphylla* var. *chinensis*

Based on sequencing data, mothur software was used to analyze Alpha diversity indices (Ace, Chao, Simpson, and Shannon) under different random samplings to reflect microbial community richness and diversity. Ace and Chao indices reflect community richness, while Simpson and Shannon indices reflect community diversity. Higher Ace, Chao, and Shannon values and lower Simpson values indicate greater species diversity. R language tools were used to generate bar charts of soil microbial communities, and Venn diagrams were created using R to statistically analyze the number of shared and unique OTUs among multiple groups or samples.

1.2.4 Analysis of Differences in Rhizosphere Soil Microbial Community Structure

Beta diversity analysis was performed among microbial communities from different samples to explore similarity or dissimilarity in community composition. Qiime data analysis pipeline was used to calculate Beta diversity distance matrices, followed by UPGMA cluster analysis and dendrogram generation using R language.

1.3 Determination of Rhizosphere Soil Physicochemical Properties

Soil physicochemical properties were determined according to *Soil Agrochemical Analysis* (Bao, 2000). Measured parameters and methods included: total nitrogen (Kjeldahl method), total phosphorus (molybdenum-antimony colorimetry), total potassium (flame photometry), soil organic carbon (potassium dichromate heating method), ammonium nitrogen and nitrate nitrogen (Kjeldahl analyzer method), available phosphorus (hydrochloric acid-ammonium fluoride extraction), available potassium (extraction + atomic absorption spectroscopy), and pH.

1.4 Correlation Analysis Between Microbial Species and Soil Physicochemical Factors

Based on soil DNA sequencing and physicochemical analysis data, R language tools were used to calculate correlation coefficients (Spearman rank correlation coefficient, Pearson correlation coefficient, etc.) between selected microbial species and environmental factors. The resulting numerical matrices were visualized through heatmaps, where color intensity reflects data values in the two-dimensional matrix or table.

2.1.1 Bacterial Community Diversity Analysis

The abundance indices (Ace, Chao) and diversity indices (Shannon, Simpson) of bacterial and fungal communities in the tested soil samples are shown in Table 2. Bacterial Ace and Chao indices exhibited a trend of first increasing then decreasing with growth year, with significant differences among years and peaks occurring at year 7. This indicates that bacterial richness in *P. polyphylla* rhizosphere soil increased initially then declined with plant age, reaching maximum richness at year 7 and minimum at year 3. Shannon and Simpson indices also differed significantly among years, showing clear fluctuations with growth year. At year 5, Shannon index reached its minimum while Simpson index peaked, suggesting that the soil environment at this stage favored growth of certain specific bacterial taxa. Consequently, bacterial richness did not change substantially, but competition suppressed growth of other bacterial taxa, causing an exceptionally large decline in bacterial diversity. By year 7, Shannon index peaked and Simpson index reached its minimum, indicating maximum bacterial diversity. These results demonstrate that *P. polyphylla* growth significantly

affects both the quantity and types of bacteria in rhizosphere soil.

2.1.2 Fungal Community Diversity Analysis

As shown in Table 2, fungal Ace and Chao indices did not differ significantly across years, indicating that *P. polyphylla* growth had minimal impact on fungal richness. However, Shannon and Simpson index analysis revealed that fungal Shannon index increased then decreased with growth year, while Simpson index showed the opposite trend, with peaks and troughs both occurring at year 7. Significance analysis confirmed that *P. polyphylla* growth significantly affected fungal diversity, with fungal taxa first increasing then decreasing, reaching maximum abundance at year 7.

Table 2 Abundance and diversity indices of soil microbial communities

Years of growth	Bacterial				Fungal			
	Ace index	Chao index	Shannon index	Simpson index	Ace index	Chao index	Shannon index	Simpson index
3 years	1,062.69$±32.53*d*|1,088.86±37.67*d*|5.73±0.10*c*|0.0061±0.0001*b*|160.62±11.55*a*|159.10±8.43*a*|1.89±0							

Note: Different lowercase letters within the same column indicate significant differences (P<0.05). The same below.

2.2.1 Bacterial Community Composition Analysis

A total of 25 phyla, 56 classes, 134 orders, 195 families, 280 genera, and 484 species of bacteria were detected across the four rhizosphere soil samples. At the phylum level (Fig. 1A), the relative abundance of different bacterial phyla changed markedly with plant growth. The dominant phyla across all growth years were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi*, with *Proteobacteria* and *Acidobacteria* comprising over 60% of total bacterial abundance. *Proteobacteria* abundance increased then decreased with growth year, showing minimal change from year 3 to 7 but declining substantially (22.36%) from year 7 to 9. *Acidobacteria* showed the opposite pattern, decreasing then increasing, with significant declines of 41.35–43.64% from year 3 to 5–7, followed by a rapid 51.88% recovery at year 9, indicating that *P. polyphylla* exerts greater influence on *Acidobacteria* than on *Proteobacteria*. *Chloroflexi* and *Bacteroidetes* both increased then decreased, showing no substantial rise from year 3 to 5 but significant increases from year 5 to 7 (164.90% and 456.21%, respectively). Both subsequently declined, with *Chloroflexi* showing a small decrease while *Bacteroidetes* dropped dramatically by 72.27%. *Actinobacteria* reached minimum abundance at year 7, with no significant differences among other

years. Additionally, *Firmicutes* peaked at 18.16% at year 5 but remained below 5% in other years, while *Nitrospirae* showed significantly increased abundance in 7-year-old rhizosphere soil compared to other years.

At the family level (Fig. 1B), communities were dominated by Solibacteraceae-subgroup-3, norank-o-Subgroup-2, *Xanthobacteraceae*, and norank-o-norank-c-Subgroup-6. Solibacteraceae-subgroup-3 reached maximum abundance (12.9%) in 5-year soil and minimum (1.2%) in 7-year soil, while norank-o-norank-c-Subgroup-6 showed the opposite pattern (minimum 1.1% at year 5, maximum 10.9% at year 7). norank-o-Subgroup-2, *Xanthobacteraceae*, norank-o-*Acidobacteriales*, and unclassified-o-*Gammaproteobacteria*-Incertae-sedies all decreased then increased, reaching minimum abundance at year 7. Furthermore, norank-o-norank-c-norank-p-WPS-2, *Acidothermaceae*, norank-o-norank-c-AD3, *Caulobacteraceae*, and *Koribacteraceae* were detected in 3-year, 5-year, and 9-year soils but were nearly absent in 7-year soil. *Nitrosomonadaceae* existed only in 7-year soil at 6.7% abundance, while Clostridiaceae-1 was present exclusively in 5-year rhizosphere soil at 17.2% abundance.

Note: JY1, JY2, JY3, and JY4 correspond to soil samples from 3-year, 5-year, 7-year, and 9-year-old plants, respectively. The same below.

Figure 1 Relative abundance of bacteria in rhizosphere soil at phylum (A) and family (B) levels

2.2.2 Fungal Community Composition Analysis

A total of 8 phyla, 26 classes, 56 orders, 76 families, 78 genera, and 96 species of fungi were detected. At the phylum level (Fig. 2A), fungi were dominated by *Ascomycota*, *Basidiomycota*, and *Mucoromycota*, with *Ascomycota* and *Basidiomycota* comprising over 80% of total abundance and showing the greatest variation among growth years. *Ascomycota* abundance increased annually, reaching 88.11% in 9-year soil after minimal levels in 3-year soil. *Basidiomycota* showed the opposite trend, declining as *Ascomycota* increased, with maximum abundance in 3-year soil. *Mucoromycota* increased then decreased, peaking in 7-year soil. *Chytridiomycota* abundance declined progressively with growth year. At the family level (Fig. 2B), dominant taxa included *Archaeorhizomycetaceae*, *Russulaceae*, norank-p-*Mucoromycota*, and unclassified-c-*Agaricomycetes*, with varying abundances across years. *Archaeorhizomycetaceae* increased annually (except being undetectable in 7-year soil), reaching 66.5% abundance in 9-year soil. *Russulaceae* existed only in 3-year rhizosphere soil at 64.8% abundance. norank-p-*Mucoromycota*, unclassified-c-*Agaricomycetes*, unclassified-c-*Sordariomycetes*, unclassified-p-*Ascomycota*, *Aspergillaceae*, unclassified-o-*Onygenales*, *Ophiocordycipitaceae*, and *Herpotrichiellaceae* all increased then decreased, with maximum abundances occurring in 7-year soil. Notably, 7-year rhizosphere soil showed richer fungal diversity (Fig. 2B).

Figure 2 Relative abundance of fungi in rhizosphere soil at phylum (A) and family (B) levels

2.3 Correlation Analysis of Rhizosphere Soil Microbial Communities Across Different Growth Years

Venn diagrams of OTUs (Fig. 3) revealed 322 shared bacterial OTUs across different growth years, representing 33.68%, 28.59%, 19.60%, and 22.90% of total bacterial OTUs in each growth year, respectively. The number of unique bacterial OTUs increased then decreased with growth year, reaching maximum abundance (869 OTUs) in 7-year rhizosphere soil—approximately 5.9–37.8 times higher than other years. For fungi, 50 shared OTUs represented 35.21%, 30.86%, 48.54%, and 33.11% of total fungal OTUs across growth years, with unique fungal OTUs also peaking (26 OTUs) in 7-year soil. These results indicate that both bacterial and fungal OTU types changed with growth year, but bacterial communities exhibited greater variation, suggesting that *P. polyphylla* growth influences bacterial communities more strongly than fungal communities.

Note: A. Bacteria; B. Fungi. The same below.

Figure 3 Venn diagram of bacteria and fungi in rhizosphere soil of *P. polyphylla* var. *chinensis* across different growth years

2.4 Analysis of Differences in Rhizosphere Microbial Community Structure Across Different Growth Years

UPGMA cluster analysis of bacterial and fungal community composition similarity among samples is shown in Figure 4. Bacterial communities (Fig. 4A) clustered into three groups: 3-year and 5-year soils grouped together, while 7-year and 9-year soils formed separate groups, indicating highest similarity between 3-year and 5-year soils and lowest similarity between 7-year soil and other years. Fungal communities (Fig. 4B) also formed three clusters: 5-year and 9-year soils grouped together, while 7-year and 3-year soils were separate, showing highest similarity between 5-year and 9-year soils and lowest between 3-year soil and others. These results demonstrate that both bacterial and fungal communities evolved with plant age, but the timing and pattern differed: bacterial communities changed most dramatically at year 7, then converged with year 3 and 5 patterns by year 9, whereas fungal communities changed most significantly at year 5, showed some changes at year 7, and converged with the year 5 pattern by year 9.

Figure 4 UPGMA cluster analysis of microorganisms in rhizosphere soil

2.5.1 Analysis of Rhizosphere Soil Physicochemical Properties

Total nitrogen (TN), total phosphorus (TP), total potassium (TK), total organic carbon (TOC), ammonium nitrogen (A), nitrate nitrogen (N), available phosphorus (AP), available potassium (AK), and pH were measured in rhizosphere soils from 3-year, 5-year, 7-year, and 9-year-old plants (Table 3), with evaluation based on the 1980 National Second Soil Survey nutrient classification standards. Results showed variation in physicochemical properties across growth

years. Total N was abundant or higher, peaking at year 5 at 2.2–2.4 times other years' levels. Total P approached or exceeded abundant levels, being lowest at year 3 (0.78 $g \cdot kg^{-1}$), peaking at year 5 (1.62 $g \cdot kg^{-1}$), declining at year 7 (1.16 $g \cdot kg^{-1}$), and recovering near peak levels at year 9 (1.57 $g \cdot kg^{-1}$). Total K increased with growth year but remained below adequate levels. Available N (ammonium + nitrate N) exceeded 150 $mg \cdot kg^{-1}$ (abundant level) at years 3, 5, and 7, peaking at year 5, but declined substantially at year 9 to scarce levels (<90 $mg \cdot kg^{-1}$). Available P varied considerably among years, increasing with growth year and exceeding abundant levels. Available K increased then decreased, peaking at year 7 (407.92 $mg \cdot kg^{-1}$, abundant level), while being scarce at year 3 and moderate at years 5 and 9. Soil organic matter (organic carbon) declined with growth year, being abundant or higher except at year 9 (moderate). Soil pH increased progressively from 4.10 to 6.96, showing gradual alkalinization but remaining within the suitable range for *P. polyphylla* growth. These changes in soil nutrients and pH may relate to nutrient consumption by plant growth, alteration of nutrient forms through root exudation, and shifts in microbial activity and community structure.

Table 3 Physicochemical properties of rhizosphere soil of *P. polyphylla* var. *chinensis* across different growth years

Years of growth	Total nitrogen ($g \cdot kg^{-1}$)	Total phosphorus ($g \cdot kg^{-1}$)	Total potassium ($g \cdot kg^{-1}$)	Total organic carbon ($g \cdot kg^{-1}$)	Ammonium nitrogen ($mg \cdot kg^{-1}$)	Nitrate nitrogen ($mg \cdot kg^{-1}$)	Available phosphorus ($mg \cdot kg^{-1}$)	Available potassium ($mg \cdot kg^{-1}$)	pH
3 years	2.69$±0.06A|1.96±0.06B|0.78±0.06C|3.18±0.08F|77.57±4.90A|32.73±1.90a|152.7±11.01b|50.39±7.34D|99.98±2.60								

Note: Letters A, B, C, D, E, and F in the same column respectively indicate soil nutrient classification standards of rich, above average, medium, scarce, scarcer, and scarcest.

2.5.2 Correlation Analysis Between Microbial Species and Soil Physicochemical Factors

Soil physicochemical properties are crucial factors affecting both *P. polyphylla* yield and quality and soil microbial community composition and abundance. Spearman correlation analysis between rhizosphere microbial taxa and soil physicochemical factors is presented in Figures 5 and 6, showing varying degrees of correlation.

Figure 5 displays Spearman correlations between the top 15 bacterial phyla and soil physicochemical factors. *Proteobacteria*, *Firmicutes*, and *Gemmati-*

monadetes abundances showed significant positive correlations with total nitrogen (TN), total phosphorus (TP), and available potassium (AK), respectively. *Acidobacteria*, WPS-2, and *Verrucomicrobia* showed significant negative correlations with available potassium (AK), available potassium (AK), and total nitrogen (TN), respectively. *Planctomycetes* showed significant negative correlation with nitrate nitrogen (N) and positive correlation with available phosphorus (AP). The remaining eight phyla showed no significant correlations. Among the 15 bacterial phyla, available potassium (AK) significantly influenced three phyla, total nitrogen (TN) influenced two, and total phosphorus (TP), available phosphorus (AP), and nitrate nitrogen (N) each influenced one phylum. Although organic carbon (TOC) and ammonium nitrogen (A) showed no significant correlations with the 15 bacterial phyla, both exhibited similar correlation patterns, showing strong negative correlations with *Chloroflexi*, *Bacteroidetes*, *Planctomycetes*, *Rokubacteria*, *Nitrospirae*, and *Latescibacteria*. Considering the proportional abundance of each phylum, available potassium (AK) and total nitrogen (TN) emerged as the primary physicochemical factors shaping bacterial community composition.

The correlation coefficient R ranges from -1 to 1, with values displayed in different colors in the right panel. R>0 indicates positive correlation, R<0 negative correlation; *** indicates P$ $0.05. The same below.

Figure 5 Spearman correlation heatmap of different soil bacterial species and environmental factors at phylum level

Figure 6 shows Spearman correlations between the top eight fungal phyla and soil physicochemical factors. *Ascomycota*, some unclassified fungal taxa (unclassified_k__{fungi}), and *Zoopagomycota* showed significant positive correlations with total potassium (TK), total nitrogen (TN), and available potassium (AK), respectively. *Basidiomycota* showed significant negative correlation with total potassium (TK), while *Chytridiomycota* showed significant negative correlation with pH and positive correlations with soil organic carbon (TOC) and ammonium nitrogen (A). The remaining three phyla showed no significant correlations. Among the eight fungal phyla, total potassium (TK) significantly influenced two phyla, while pH, organic carbon (TOC), ammonium nitrogen (A), total nitrogen (TN), and available potassium (AK) each influenced one phylum. Organic carbon (TOC) and ammonium nitrogen (A) showed nearly identical correlation patterns with the eight fungal phyla and strong correlations with *Ascomycota* and *Basidiomycota*. Although total phosphorus (TP) showed no significant correlations, it exhibited strong relationships with *Ascomycota* and *Basidiomycota*. Considering fungal community composition, total potassium (TK) was the primary factor influencing fungal community structure, followed by pH, organic carbon (TOC), ammonium nitrogen (A), total phosphorus (TP), total nitrogen (TN), and available potassium (AK).

Figure 6 Spearman correlation heatmap of different soil fungal species and environmental factors at phylum level

3 Discussion and Conclusion

Soil microorganisms constitute essential components of soil ecosystems, with microbial community diversity sensitively reflecting changes in plant growth, reproduction, and metabolic activity. Soil organisms exhibit mutual constraints and dependencies while interacting with environmental factors in a cyclical regulatory process (Hirsch et al., 2010). Therefore, studying plant rhizosphere microbial diversity changes helps reveal plant growth and development patterns and is highly significant for cultivation of medicinal plants. This study investigated changes in rhizosphere microbial diversity of wild *P. polyphylla* var. *chinensis* across different growth years, finding that bacterial and fungal community composition and structure differed among growth years, with years 5–7 representing a critical period for major changes in microbial community diversity and composition. The two most abundant bacterial phyla in *P. polyphylla* rhizosphere soil were consistently *Proteobacteria* and *Acidobacteria*, whose relative abundances changed substantially in opposite directions during plant development —a pattern also observed in studies of soil bacterial communities in Chinese fir plantations at different developmental stages (Wei et al., 2017). Zheng et al. (2020) similarly reported that *Proteobacteria* was most abundant in *P. polyphylla* rhizosphere soil, followed by *Acidobacteria* and *Actinobacteria*. Kang et al. (2018) found that pepper cultivation soils were dominated by *Proteobacteria* and *Acidobacteria* as bacterial phyla and *Ascomycota*, *Basidiomycota*, and *Zygomycota* as fungal phyla. Huang et al. (2010) identified *Proteobacteria*, *Firmicutes*, and *Acidobacteria* as dominant bacterial groups in Hainan banana orchard soils. These studies indicate that microbial community composition varies among plant rhizospheres, while *P. polyphylla* rhizosphere soil shares general characteristics with other soil microbial communities.

Bacterial Ace, Chao, and Shannon indices were significantly higher than fungal indices, while bacterial Simpson index was significantly lower, indicating that bacterial species were more abundant and diverse than fungi, representing a healthy "bacterial-type" soil (Kang et al., 2018). At year 7, both microbial richness and diversity reached maximum levels, but declined significantly after year 7, with rhizosphere soil showing a tendency to shift from "bacterial-type" to "fungal-type." Zhou et al. (2015) also found that bacterial quantities exceeded fungal quantities in *P. polyphylla* var. *yunnanensis* rhizosphere soil, with fungal and potassium-solubilizing bacteria increasing while bacteria and actinomycetes decreased with growth year, suggesting similar microbial change patterns between *P. polyphylla* varieties, possibly due to similar root exudate compositions. Root exudate types and quantities directly affect rhizosphere microbial metabolism and growth, thereby influencing microbial taxa, abundance, and distribution (Nihorimbere et al., 2011). Studies on *Ophiopogon japonicus*, *Panax quinquefolius*, and *Fritillaria thunbergii* (Li, 2006; Li et al., 2010; Sun et al., 2011; Liao et al., 2011) found that shifts from high-fertility "bacterial-type" to low-fertility "fungal-type" soils increased pests and diseases, reducing crop yield and quality. Therefore, timely harvesting is recommended during artificial

cultivation of *P. polyphylla*, and microbial agents can be used to regulate soil microbial environments for pest control and quality improvement.

Microbial community characteristics are influenced by climate, soil properties, and other factors (Zhao et al., 2016). Liu et al. (2015) reported that *Proteobacteria* prefer nutrient-rich soils and correlate significantly with soil organic matter and total nitrogen, consistent with its abundance changes in *P. polyphylla* rhizosphere soil. Spearman analysis also revealed significant positive correlation between *Proteobacteria* and total nitrogen. Available potassium (AK) showed significant negative correlation with *Acidobacteria*, while Wang et al. (2012) reported significant positive correlation between total rhizome saponin content and both total and available potassium, suggesting that *Acidobacteria* may not favor saponin accumulation. Soil pH is an important factor affecting *Acidobacteria* abundance and diversity, typically showing significant negative correlation (Griffiths et al., 2011). However, this study found no significant negative correlation between *Acidobacteria* abundance and pH, but instead significant negative correlation with available potassium, which increased then decreased with growth year, indicating that available potassium and plant growth exert stronger effects on *Acidobacteria* than pH in *P. polyphylla* rhizosphere soil. Total phosphorus and available phosphorus showed significant positive correlations with *Firmicutes* and *Planctomycetes*, respectively, but not with dominant phyla. Since *Firmicutes* and *Planctomycetes* represent small proportions of the bacterial community while *Proteobacteria* and *Acidobacteria* are most abundant, changes in these two phyla cause substantial shifts in overall community structure. Therefore, available potassium and total nitrogen are the primary physicochemical factors shaping bacterial community composition, with phosphorus having less influence.

Ju et al. (2008) considered soil pH the most important factor affecting soil fungal quantity and diversity in *Taxus chinensis* forests, followed by organic matter and moisture content. Paul et al. (2012) found that total C/N ratio was most important for soil fungal community changes, with soil pH as a secondary factor. This study identified total potassium (TK) as the primary factor influencing fungal composition in *P. polyphylla* rhizosphere soil, followed by pH, organic carbon (TOC), ammonium nitrogen (A), total phosphorus (TP), total nitrogen (TN), and available potassium (AK). These results differ from but also share similarities with previous studies, indicating that primary influencing factors vary among plant species and habitats. Organic carbon (TOC) and ammonium nitrogen (A) showed nearly identical correlation patterns with both bacterial and fungal phyla, suggesting similar effects on overall microbial community composition. Furthermore, organic carbon (TOC), ammonium nitrogen (A), and total phosphorus (TP) showed stronger correlations with dominant fungal phyla than bacterial phyla, indicating greater influence on fungal community composition. The unique microbial community structure in *P. polyphylla* rhizosphere soil may relate to specific root exudates that affect microbial distribution (Liu et al., 2019), though the types of exudates and their interaction mechanisms with soil and microorganisms remain unclear and require further investigation.

This study preliminarily revealed the changing patterns of rhizosphere soil microbial diversity in wild *P. polyphylla* var. *chinensis* across different growth years, identifying years 5–7 as a critical period for major changes in microbial community diversity and composition. These findings establish a foundation for further exploration of the relationship between *P. polyphylla* growth and microorganisms, provide scientific basis for investigating relationships between effective component accumulation and rhizosphere microbial environments, and offer insights into disease occurrence mechanisms and control measures, thereby promoting sustainable utilization and industrial development of *P. polyphylla*.

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Note: Figure translations are in progress. See original paper for figures.