Postprint: Study on Rhizosphere Soil Microbial Diversity of *Anemone altaica*

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Abstract

To understand the differences in rhizosphere soil microbial diversity between wild and cultivated Anemone altaica, this study investigated the community composition and diversity of rhizosphere soil microorganisms in wild and cultivated A. altaica using Illumina MiSeq high-throughput sequencing technology. The results showed that: (1) The fungal diversity in the rhizosphere soil of wild A. altaica was significantly higher than that of cultivated A. altaica ($P < 0.05$), whereas the difference in bacterial diversity was not significant ($P > 0.05$). NMDS analysis results demonstrated that the differences in fungal community structure between wild and cultivated A. altaica rhizosphere soils were more pronounced. (2) Bacterial 9,566 operational taxonomic units (OTUs) involved 39 phyla, 127 classes, 315 orders, 500 families, and 886 genera, while fungal 2,670 OTUs involved 15 phyla, 57 classes, 138 orders, 293 families, and 597 genera. At the phylum level, Proteobacteria, Acidobacteria, and Actinobacteria in bacterial communities, as well as Basidiomycota, Ascomycota, and Mortierellomycota in fungal communities, were all dominant phyla in the rhizosphere soils of wild and cultivated A. altaica, but their relative abundances differed under different growth conditions. (3) Redundancy Analysis (RDA) results indicated that organic matter was the primary factor influencing soil bacterial communities ($P < 0.05$), while soil pH, alkali-hydrolyzable nitrogen, and available phosphorus were the main factors affecting fungal communities ($P < 0.05$). In summary, this study demonstrates that significant differences exist in the composition and diversity of rhizosphere soil microbial communities between wild and cultivated A. altaica, and these differences may be closely related to soil physicochemical properties under different growth conditions. The findings hold certain significance for the scientific cultivation of A. altaica and soil improvement.

Full Text

Microbial Diversity Study in Rhizosphere Soil of *Anemone altaica*

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Abstract

To understand the differences in rhizosphere soil microbial diversity between wild and cultivated *Anemone altaica*, this study employed Illumina MiSeq high-throughput sequencing technology to investigate the community composition and diversity of rhizosphere soil microorganisms. The results revealed: (1) The fungal diversity in wild *A. altaica* rhizosphere soil was significantly higher than that in cultivated *A. altaica* ($P < 0.05$), whereas bacterial diversity showed no significant difference ($P > 0.05$). Non-metric multidimensional scaling (NMDS) analysis further demonstrated more pronounced differences in fungal community structure between wild and cultivated samples. (2) A total of 9,566 bacterial operational taxonomic units (OTUs) were identified across 39 phyla, 127 classes, 315 orders, 500 families, and 886 genera, while 2,670 fungal OTUs were classified into 15 phyla, 57 classes, 138 orders, 293 families, and 597 genera. At the phylum level, Proteobacteria, Acidobacteria, and Actinobacteria dominated bacterial communities, while Basidiomycota, Ascomycota, and Mortierellomycota dominated fungal communities in both wild and cultivated rhizosphere soils, though their relative abundances varied between growth conditions. (3) Redundancy analysis (RDA) indicated that organic matter was the primary factor influencing bacterial communities ($P < 0.05$), whereas soil pH, alkali-hydrolyzable nitrogen, and available phosphorus were the main factors affecting fungal communities ($P < 0.05$). In summary, this study demonstrates significant differences in rhizosphere microbial community composition and diversity between wild and cultivated *A. altaica*, likely associated with variations in soil

physicochemical properties under different growth conditions. These findings provide valuable insights for the scientific cultivation and soil improvement of *A. altaica*.

Keywords: *Anemone altaica*, soil microorganism, high-throughput sequencing, community structure, species diversity

Introduction

Anemone altaica, a perennial herb belonging to the family Ranunculaceae and genus *Anemone*, is a valuable traditional Chinese medicinal plant known by various local names including Chuanguqi, Xuan Shen, and Jiujie Changpu [**?**, **?**]. This species thrives in cool, moist, and shaded environments, typically distributed across mountainous regions, understories, and stream banks at elevations ranging from 800 to 2,000 meters [**?**]. However, due to its specific habitat requirements and excessive harvesting, wild populations of *A. altaica* have experienced declining yields and quality in recent years. While advances in cultivation technology have enabled commercial-scale production and partially alleviated ecological pressure from over-collection [**?**], understanding the optimal soil conditions for *A. altaica* cultivation remains critical for achieving high yield, quality, and sustainable development. Consequently, comprehensive research on the developmental biology and growth mechanisms of this species is urgently needed.

The rhizosphere represents a unique zone where plant roots interact intimately with soil microbial communities [**?**]. Rhizosphere microorganisms constitute essential components of soil ecosystems, playing pivotal roles in nutrient and energy cycling, soil structure maintenance, and microecological balance [**?**, **?**]. Moreover, these microbes decompose organic matter into inorganic nutrients available for plant uptake, while secreting soil enzymes, organic acids, and growth-promoting substances that enhance plant growth and environmental adaptation [**?**]. The diversity and stability of soil microbial communities are fundamental to maintaining healthy soil systems and plant productivity [**?**]. Previous studies have demonstrated that the quantity and composition of rhizosphere microorganisms under different habitats are key factors influencing plant ecological adaptability [**?**]. Therefore, investigating rhizosphere soil microbial community structure can provide novel insights for the cultivation management of *A. altaica*.

Given the crucial role of rhizosphere microorganisms in soil biochemical processes and plant development, researchers have hypothesized that imbalances in rhizosphere microbial community structure may be the primary cause of soil quality degradation, yield reduction, and disease occurrence in medicinal plants [**?**]. Similarly, differences in rhizosphere microbial community structure likely represent a key factor underlying the yield and quality disparities between wild and cultivated *A. altaica*. Unfortunately, current research on *A. altaica* has

primarily focused on its pharmacological effects, chemical composition, clinical applications, and habitat adaptation, while studies on its rhizosphere microbial communities remain scarce [**?**]. This research gap underscores the significant value of investigating the rhizosphere soil microbial community structure of *A. altaica*.

This study was conducted in the Baiyunshan National Nature Reserve, utilizing both wild and cultivated *A. altaica* as research subjects. Through high-throughput sequencing technology, we investigated the rhizosphere soil microbial communities to address three key questions: (1) How do microbial diversities differ between wild and cultivated *A. altaica* rhizosphere soils? (2) What are the compositional differences in rhizosphere soil microbial communities between wild and cultivated *A. altaica*? (3) What are the primary environmental drivers influencing these rhizosphere soil microbial communities? The findings provide theoretical guidance for the scientific cultivation and soil improvement of *A. altaica*.

1.1 Study Site and Sample Collection

The Baiyunshan National Nature Reserve (111°48'–112°16' E, 33°33'–33°56' N) is located in the hinterland of the Funiu Mountains in southern Song County, Luoyang City, Henan Province, covering a total area of 16,800 km^2 with an average elevation of 1,800 meters. The study area features a typical continental monsoon climate with hot, rainy summers and cold, dry winters. Annual temperatures range from 13.1 to 13.9 °C, with annual precipitation of 1,200 mm. Situated in the transitional zone from warm temperate to north subtropical zones, the reserve hosts a convergence of northern and southern flora with lush vegetation and highly diverse geographic landscapes, creating favorable conditions for *A. altaica* growth and reproduction.

Sampling points were established within the reserve. Cultivated *A. altaica* was propagated by cutting rhizomes into 3–5 cm segments, which were planted in furrows at 30 cm spacing, resulting in relatively sparse planting density. The cultivation site was an artificially managed germplasm resource nursery under forest canopy, characterized by shaded, humid conditions without direct sunlight exposure and generally requiring no irrigation. The artificial cultivation habitat closely mimicked the wild environment, with both wild and cultivated populations growing under the dominant tree species *Quercus aliena* var. *acuteserrata*, and both soil nutrient conditions were essentially consistent. The cultivated plot was located approximately 2 km from the wild population, with plants aged 2–3 years and a cultivation area of about 1 hm^2.

In May 2021, rhizosphere soils were collected from both wild and cultivated *A. altaica*. Three replicate 20 m × 20 m plots were randomly established for each growth type, with intervals exceeding 30 m between plots. Within each plot, five-point sampling was employed, and rhizosphere soils from at least five *A. altaica*

plants near each point were collected and thoroughly mixed into a composite sample, yielding three samples per growth type. Rhizosphere soil was defined as soil tightly adhering to plant roots (including lateral roots) after gently shaking off loosely attached soil and brushing with a sterile brush. Soils from the five sampling points within each plot were combined into a single sample, after which residual roots and gravel were removed. Each soil sample was divided into two portions: one was placed in sterile 5 mL centrifuge tubes, transported to the laboratory, and stored at –80 °C for subsequent microbial DNA extraction; the other was sieved through a 2 mm mesh to remove impurities and air-dried for physicochemical property analysis.

1.2 Soil Physicochemical Property Determination

Soil physicochemical properties including organic matter, pH, available phosphorus, and alkali-hydrolyzable nitrogen were determined using conventional methods from *Soil Agrochemical Analysis* [**?**]. Soil pH was measured using a glass composite electrode pH meter. Organic matter content was determined by the potassium dichromate volumetric method. Alkali-hydrolyzable nitrogen was measured using the petri dish diffusion method. Available phosphorus was extracted with $NaHCO_3$ and analyzed by molybdenum blue colorimetry [**?**].

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

Total DNA was extracted from 0.5 g of frozen soil samples using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Norcross, GA, U.S.) following the manufacturer's instructions. The primer sets targeted the following regions: bacterial 16S rRNA genes were amplified using primers 515F (5 -GTGCCAGCMGCCGCGGTAA-3) and 806R (5 -GGACTACHVGGGGTWTCTAAT-3); fungal ITS regions were amplified using primers ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3) and ITS2R (5 -GCTGCGTTCTTCATCGATGC-3) [**?**]. The PCR amplification program consisted of initial denaturation at 95 °C for 5 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min [**?**, **?**]. Purified PCR products were used for DNA library construction, and high-throughput sequencing was performed on the Illumina MiSeq platform at Shanghai Majorbio Bio-pharm Technology Co., Ltd.

1.4 Data Processing and Analysis

Raw data were processed and classified using the Qiime2 platform (https://qiime2.org). Chimeric sequences were removed, and operational taxonomic units (OTUs) were constructed based on 97% sequence similarity [**?**]. To analyze differences between wild and cultivated *A. altaica* rhizosphere microbial communities, rarefaction curves were generated to assess sampling adequacy. Venn diagrams, NMDS analysis, and alpha diversity indices were employed to evaluate community structure differences.

Microbial community composition was visualized using Circos diagrams and species abundance stacked bar charts. Differential species between groups were identified using t-tests in STAMP software. To examine relationships between rhizosphere soil microbial communities and environmental factors, redundancy analysis (RDA) was performed. The significance of each environmental factor's influence on microbial community distribution was tested using ANOVA functions in R software.

2.1 Soil Physicochemical Properties

As shown in Table 1 , the pH and alkali-hydrolyzable nitrogen content in wild *A. altaica* rhizosphere soil were significantly higher than those in cultivated rhizosphere soil.

2.2 Rhizosphere Soil Microbial Diversity of *A. altaica*

Sequencing of 16S rRNA and ITS genes yielded 9,566 bacterial OTUs and 2,670 fungal OTUs. Taxonomic annotation revealed that bacterial OTUs belonged to 39 phyla, 127 classes, 315 orders, 500 families, and 886 genera, while fungal OTUs were classified into 15 phyla, 57 classes, 138 orders, 293 families, and 597 genera. Rarefaction curves (Figure 1 [Figure 1: see original paper]) showed that species richness increased gradually with sequencing depth and eventually plateaued, indicating that the sequencing data approached saturation and the sampling was adequate to reflect true community characteristics.

OTU analysis revealed that wild and cultivated rhizosphere soils shared 5,332 bacterial OTUs (55.74% of total) and 740 fungal OTUs (27.72% of total). The numbers of unique bacterial OTUs were 2,296 and 1,938 for wild and cultivated samples, respectively, while unique fungal OTUs numbered 1,207 and 723, respectively.

NMDS analysis (Figure 3 [Figure 3: see original paper]) further demonstrated significant differences in both bacterial and fungal community composition between wild and cultivated rhizosphere soils. Alpha diversity indices (Table 2) showed no significant difference in bacterial community diversity ($P > 0.05$) but

revealed significantly higher fungal diversity in wild *A. altaica* rhizosphere soil compared to cultivated soil ($P < 0.05$). Both bacterial and fungal community diversities were higher in wild conditions.

2.3 Analysis of Soil Microbial Community Structure Under Different Cultivation Modes

Circos diagrams were constructed using OTUs representing the top 1% contribution rates (Figure 4 [Figure 4: see original paper]), including 97 bacterial OTUs and 27 fungal OTUs. Most representative sequences were detected in both wild and cultivated rhizosphere soils, such as the bacterial genera *Bradyrhizobium* (OTU106), *Candidatus_{udaeobacter}* (OTU84), and unclassified Xanthobacteraceae (OTU7178), and the fungal genus *Mortierella* (OTU5055, OTU1740, OTU7098). However, fungal taxa including unclassified Hyaloscyphaceae (OTU6763) and *Sebacina* (OTU32) were predominantly present only in cultivated *A. altaica*.

At the phylum level, all six soil samples contained 39 bacterial and 15 fungal phyla (Figure 5 [Figure 5: see original paper]). Dominant bacterial phyla in both wild and cultivated rhizosphere soils were Proteobacteria (21.50%–28.18%), Acidobacteria (14.76%–18.42%), Actinobacteria (11.68%–14.69%), Verrucomicrobia (10.61%–15.40%), Chloroflexi (6.44%–7.43%), Planctomycetes (5.09%–6.83%), and Methylomirabilota (4.20%–5.12%), collectively accounting for approximately 85% of total communities. Proteobacteria and Actinobacteria showed higher relative abundances in wild soil, whereas Acidobacteria and Verrucomicrobia were more abundant in cultivated soil. Dominant fungal phyla were Basidiomycota (27.94%–44.94%), Ascomycota (27.51%–28.70%), Mortierellomycota (16.50%–34.83%), and Rozellomycota (5.20%–9.48%), representing approximately 98% of total fungal communities. Notably, Mortierellomycota abundance was significantly higher in wild rhizosphere soil, while Basidiomycota relative abundance was significantly lower in wild compared to cultivated soil.

At the genus level (Table 3), dominant bacterial genera in wild rhizosphere soil were *Candidatus_{udaeobacter}*, unclassified Xanthobacteraceae, and *Rokubacteriales*, whereas cultivated soil was dominated by *Candidatus_{udaeobacter}*, *Vicinamibacter*, and *Rokubacteriales*. Dominant fungal genera in wild rhizosphere soil included *Mortierella*, *Sebacina*, and *Russula*, while cultivated soil was dominated by *Sebacina*, *Mortierella*, and unclassified Hyaloscyphaceae.

Significance testing across taxonomic levels identified microbial groups with significantly different abundances between growth modes (top 10 differentially abundant groups). Bacterial taxa including *Elusimicrobia*, unclassified lineage_{IV}, unclassified norank_o_{{{lineage}}*{{IV}}}, lineage*{IV}, and *Elusimicrobiota* showed significantly higher abundances in wild rhizosphere soil

($P < 0.05$). Fungal taxa such as Mortierellales, *Mortierella*, Mortierellomycota, Mortierellaceae, and Mortierellomycetes exhibited significantly different abundances between groups.

2.4 RDA Analysis of Soil Environmental Factors

RDA analysis revealed that the four environmental factors collectively explained 82.45% of the variation in bacterial communities and 77.59% of the variation in fungal communities (Figure 7 [Figure 7: see original paper]). Alkali-hydrolyzable nitrogen, pH, and available phosphorus showed acute angles between their vectors, indicating positive correlations. Organic matter significantly influenced bacterial community structure ($P < 0.05$), while soil pH, alkali-hydrolyzable nitrogen, and available phosphorus significantly affected fungal community structure ($P < 0.05$) (Table 4).

3 Discussion and Conclusion

Cultivation of *A. altaica* appears to alter soil nutrient status and physicochemical properties. Although initial cultivation conditions mimicked the wild habitat with consistent soil nutrient status, subsequent analysis revealed significantly lower pH and alkali-hydrolyzable nitrogen in cultivated rhizosphere soil, suggesting that cultivated plants absorb and utilize soil nutrients, potentially causing declines in organic carbon and imbalances in essential nutrients like nitrogen and phosphorus, ultimately leading to soil acidification. The root systems of cultivated *A. altaica* may release substantial organic acids, and microbial metabolic activities could further contribute to soil acidification [**?**].

This study employed high-throughput sequencing to investigate rhizosphere soil microbial community diversity in wild and cultivated *A. altaica*. The results indicated that growth mode did not significantly affect bacterial diversity ($P > 0.05$), likely because both sample sets were collected within the Baiyunshan Reserve, sharing similar geographic location, macroclimate, and ecological conditions. However, fungal diversity was significantly higher in wild rhizosphere soil, possibly because soil pH < 5.0 adversely affects the growth and reproduction of acid-sensitive fungi [**?**, **?**]. Additionally, cultivation practices may disrupt soil physical and chemical structure, reduce nutrient content, and compromise soil health, potentially inhibiting certain rhizosphere microorganisms and resulting in lower fungal diversity in cultivated soil [**?**]. Furthermore, nitrogen content changes significantly influence fungal community structure in cultivated *A. altaica* rhizosphere soil [**?**]. Previous studies have shown that fungal communities may be more susceptible to soil nutrient effects than bacterial communities [**?**], and that fungal communities are more strongly affected by cultivation practices than bacterial communities in ginseng [**?**]. These findings align with our

results, suggesting that fungal communities are more sensitive to environmental changes, which may explain the significant differences observed in wild *A. altaica* rhizosphere soil.

This study represents the first investigation of rhizosphere soil microbial community composition in wild and cultivated *A. altaica*. Bacterial community composition was dominated by Proteobacteria, Acidobacteria, and Actinobacteria, consistent with previous research [**?**, **?**]. Proteobacteria serve as an important indicator of soil nutrient status and were more abundant in nutrient-rich wild rhizosphere soil, functioning as primary decomposers of litter [**?**]. Acidobacteria are typically oligotrophic and acidophilic, thriving in low-nutrient, acidic soils [**?**], which aligns with their higher abundance in cultivated soil in this study. Among fungal communities, Basidiomycota, Ascomycota, and Mortierellomycota were dominant in both wild and cultivated soils, though their relative abundances differed, consistent with previous findings [**?**, **?**]. Basidiomycota play crucial roles in degrading complex lignocellulose components in litter and nutrient cycling [**?**]. Ascomycota, primarily saprotrophic fungi, typically inhabit soils with higher pH and exhibit strong resistance to environmental stress [**?**, **?**], supporting our results. Additionally, bacterial taxa such as *Elusimicrobia*, lineage_{IV}, and unclassified Xanthobacteraceae, and fungal taxa including *Mortierella* and *Solicoccozyma*, were dominant in wild *A. altaica* rhizosphere soil, potentially playing important roles in promoting nutrient cycling and plant growth [**?**]. The observed differences in microbial composition and abundance between wild and cultivated soils suggest distinct ecological strategies among dominant microbial groups under different growth conditions.

Soil properties are critical factors influencing rhizosphere microbial community structure. This study identified soil organic matter as the most significant factor affecting bacterial communities, consistent with previous research [**?**]. Soil pH, alkali-hydrolyzable nitrogen, and available phosphorus were the primary environmental factors influencing fungal communities, aligning with findings from other studies [**?**]. Soil pH affects microbial diversity by influencing enzyme formation, activity, and cell membrane permeability. Previous research has demonstrated that nitrogen and phosphorus contents significantly affect rhizosphere fungal communities, as nitrogen transformation and cycling are closely related to soil fungi, and phosphorus plays an important role in fungal reproduction and growth [**?**]. Therefore, the primary drivers influencing bacterial and fungal communities in *A. altaica* rhizosphere soil differ substantially.

This study systematically analyzed differences in rhizosphere soil microbial community structure between wild and cultivated *A. altaica*, revealing the impacts of different cultivation practices on microbial diversity. Compared to wild populations, cultivated *A. altaica* exhibited lower bacterial and fungal diversity in rhizosphere soil. The relative abundances of Acidobacteria, Actinobacteria, and Mortierellomycota were significantly higher in wild *A. altaica*. Environmental factor analysis further demonstrated that organic matter was the primary factor affecting bacterial communities, while soil pH, alkali-hydrolyzable nitrogen,

and available phosphorus were the main factors influencing fungal communities. These results provide theoretical guidance for the scientific cultivation and soil improvement of *A. altaica*.

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