Postprint: Diversity Analysis of Rhizosphere Soil Bacterial Communities of Populus euphratica in the Lower Reaches of the Tarim River

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Abstract

High-throughput sequencing technology was employed to sequence the rhizosphere soil bacteria of Populus euphratica at different growth stages (young stage, middle-aged stage, over-mature stage, and declining stage) in the lower reaches of the Tarim River. Canonical correspondence analysis (CCA) and Spearman correlation analysis were combined to explore the correlation between bacterial community composition and environmental factors. The results showed that: (1) A total of 7287 operational taxonomic units (OTUs) were obtained from the soil samples, and after comparative identification, 73 phyla, 165 classes, 339 orders, 454 families, 651 genera, and 205 species were identified. (2) The richness and diversity of the rhizosphere soil bacterial communities of Populus euphratica showed a trend of first increasing and then decreasing with growth stage, but there was no significant difference among different growth stages. (3) The dominant bacterial phyla in the rhizosphere bacterial communities of Populus euphratica were Proteobacteria, unidentified_{Bacteria}, and Halobacterota, while the dominant bacterial genera were Marinobacter, Halomonas, and Woeseia. Compared with the phylum taxonomic level, the bacterial community composition showed greater differences at the genus level, with different dominant genera in the rhizosphere bacterial communities of Populus euphratica at different growth stages. (4) The rhizosphere soil bacterial community composition of Populus euphratica at different growth stages could be divided into two major groups: soil samples from the middle-aged stage and declining stage clustered into one group, while those from the young stage and over-mature stage clustered into another group. (5) CCA analysis indicated that soil water content, total potassium, total salt, and pH were environmental factors that significantly influenced the rhizosphere soil bacterial community composition of Populus euphratica ($P<0.05$). The research results provide a scientific basis for enriching studies on rhizosphere microorganisms in arid regions and exploring plant-microbe interactions in arid areas.

Full Text

Analysis of Bacterial Community Diversity in the Rhizosphere Soil of *Populus euphratica* in the Lower Reaches of the Tarim River

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Abstract

This study employed high-throughput sequencing technology to analyze rhizosphere soil bacteria associated with *Populus euphratica* at different developmental stages (young, medium, overripe, and decline periods) in the lower reaches of the Tarim River. Canonical correspondence analysis (CCA) and Spearman correlation analysis were used to investigate the relationships between bacterial community composition and environmental factors. The results demonstrated that: (1) A total of 98,028 effective sequences and 7,287 operational taxonomic units (OTUs) were obtained from the soil samples, with 3,701, 4,543, 4,297, and 3,710 OTUs identified in the young, medium, overripe, and decline periods, respectively. Comparative annotation revealed 73 phyla, 165 classes, 339 orders, 454 families, 651 genera, and 205 species. (2) Alpha diversity analysis indicated that bacterial community richness and diversity in the rhizosphere soil of *P. euphratica* exhibited a trend of initial increase followed by decrease across developmental periods, though no significant differences were observed between periods. (3) *Proteobacteria*, *unidentified_{Bacteria}*, and *Halobacterota* were the dominant bacterial phyla, while *Marinobacter*, *Halomonas*, and *Woeseia* were the dominant genera. Compared with the phylum level, bacterial community composition showed greater variation at the genus level, with different dominant genera characterizing each developmental period. (4) Cluster analysis revealed two distinct groups: the medium and decline periods clustered together, while the young and overripe periods formed another cluster. (5) CCA identified soil water content, total potassium, total salt, and pH as the primary environmental factors significantly influencing rhizosphere bacterial community composition ($P<0.05$). These findings provide a scientific basis for advancing research on rhizosphere microorganisms and plant-microbe interactions in arid regions.

Keywords: rhizosphere; soil microorganism; high throughput sequencing; bacterial community; Tarim River

1. Introduction

The Tarim River, China' s longest inland river, supports extensive cultivated land and the world's largest natural *Populus euphratica* forest. In recent decades, human water development activities and altered surface hydrology have caused flow interruption and severe ecological degradation in the lower reaches. Since 2000, ecological water conveyance projects have been implemented 19 times, leading to preliminary vegetation recovery and ecological improvement. However, recent studies indicate that *P. euphratica* populations in the lower Tarim River suffer from weak regeneration and an inverted pyramid age structure, with declining proportions of young individuals and a predominance of overmature trees.

The rhizosphere represents the zone where root activity most directly and intensely influences soil. Bacteria, fungi, and other microorganisms attracted by root exudates accumulate in this region, playing crucial roles in plant growth and development. With the advent of metagenomics and rapid sequencing technology, high-throughput sequencing has become an essential tool for investigating soil microbial diversity and community structure. Previous research has demonstrated that rhizosphere microbial abundance and composition vary substantially with habitat, vegetation type, soil physicochemical properties, and even plant genotype or growth stage. Studies show that rhizosphere bacterial community abundance initially increases then decreases with growth stage, while diversity gradually declines. Investigations of different tree ages have revealed decreasing bacterial and actinomycete contents with increasing age, with diversity following a hump-shaped pattern.

This study examines rhizosphere soil from *P. euphratica* at different developmental stages in the lower Tarim River using high-throughput sequencing. By analyzing bacterial communities and their relationships with environmental factors, we aim to identify key drivers of community composition and provide a theoretical foundation for understanding rhizosphere microorganisms and plant-microbe interactions in arid regions.

1.1 Study Area Overview

The study site is located in a natural *P. euphratica* forest near the Yingsu section of the lower Tarim River (40°28 -40°55 N, 87°51 -87°75 E). The region experiences a typical continental extreme arid climate with scarce precipitation (annual average <50 mm), intense evaporation (annual total 2,960 mm), and high annual temperatures (10.5–11.4°C). Annual solar radiation ranges from 5,692 to 6,360 $kJ \cdot m^{-2}$, creating extremely harsh ecological conditions. Dominant vegetation includes *Populus euphratica*, *Tamarix ramosissima*, *Glycyrrhiza uralensis*, *Alhagi sparsifolia*, and *Phragmites australis*.

1.2 Experimental Design and Sample Collection

Soil sampling was conducted in July 2020 within 100 m × 100 m plots. Following the classification standards from Wang Shiji et al.' s *Populus euphratica Forest*, we selected trees from four developmental periods: young (4–10 cm diameter), medium (30–70 cm), overripe (59–67 cm), and decline (8–12 cm). For each period, three healthy, pest-free trees with similar growth characteristics were chosen for morphological measurements (Table 1) and rhizosphere soil collection. Additionally, bare land without vegetation cover was sampled at three points as a control, with three replicate composite samples collected.

Rhizosphere soil was collected by excavating a soil profile beginning 0.5 m from the main root. Fine roots ($ $2 mm) were identified at 60 cm depth, and soil adhering to these roots after gentle shaking was defined as rhizosphere soil. Each tree provided three replicate samples. Collected soil was divided into three portions: one stored in liquid nitrogen for bacterial community analysis, one air-dried and sieved for physicochemical property determination, and one placed in aluminum boxes for moisture content measurement via oven drying at 105°C.

1.3 Soil Physicochemical Property Determination

Soil physicochemical properties were measured following standard methods from Bao Shidan' s *Soil and Agricultural Chemistry Analysis*. Parameters included pH, organic matter, total nitrogen (TN), total phosphorus (TP), total potassium (TK), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), available phosphorus (AP), available potassium (AK), electrical conductivity (EC), and total salt content. Specific methods included: potassium dichromate oxidation for organic matter; $HClO_4$-H_2SO_4 digestion with FOSS Kjeltec automatic analyzer for TN; acid dissolution with Agilent UV spectrophotometry for TP; atomic absorption spectroscopy for TK; $CaCl_2$ extraction with BRAN+LUEBBE AA3 flow analyzer for nitrate and ammonium nitrogen; $NaHCO_3$ extraction with molybdenum-antimony colorimetry for AP; ammonium acetate extraction with atomic absorption for AK; pH meter (soil:water = 1:2.5) for pH; conductivity meter for EC; and residue drying (soil:water = 1:5) for total salt.

1.4 Soil Bacterial DNA Extraction, PCR Amplification, and Sequencing

Microbial genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method. DNA purity and concentration were assessed via 1% agarose gel electrophoresis. Samples were diluted to 1 ng· l^{-1} with sterile water. The 16S V4 region was amplified using specific primers with New England Biolabs' Phusion® High-Fidelity PCR Master Mix with GC Buffer. PCR products were detected via electrophoresis, purified using a gel extraction kit, and used for library construction with the TruSeq® DNA PCR-Free Sample Preparation Kit. After Qubit quantification and quality control, libraries were sequenced

on the Illumina NovaSeq6000 platform at Beijing Compass Biotechnology Co., Ltd.

1.5 Sequencing Data Processing

Raw reads from Illumina NovaSeq6000 were assembled using FLASH software and quality-filtered to obtain clean tags. UCHIME was used for chimera filtering to generate effective tags. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using QIIME1. Representative sequences were annotated against the Silva132 database, and α-diversity indices were calculated.

1.6 Data Analysis

α-Diversity was assessed using the Shannon, Simpson, Chao1, and ACE indices. β-Diversity was analyzed via Unifrac distance and UPGMA clustering. Canonical correspondence analysis (CCA) and Spearman correlation analysis were performed to examine relationships between bacterial communities and environmental factors. Statistical significance was determined using one-way ANOVA in SPSS 22.0.

2. Results

2.1 Soil Physicochemical Properties

Rhizosphere soil physicochemical properties varied across developmental periods (Table 2). Soil pH ranged from 7.78 to 9.49. Total N, P, and K remained relatively stable throughout *P. euphratica* development, with highest values in the decline period. Available K and P showed significant variation, with the decline period having 3–7 times higher available K and 2–3 times higher available P than other periods. Nitrate nitrogen was lower in all rhizosphere soils compared to bare land, while ammonium nitrogen showed no significant differences ($P>0.05$). Electrical conductivity and total salt content showed no significant differences among young, medium, and overripe periods ($P>0.05$), but were significantly higher in the decline period (EC = 7.96 $ms \cdot cm^{-1}$, total salt = 30.01 $g \cdot kg^{-1}$).

2.2 Sequencing Results and Depth Assessment

High-throughput sequencing of rhizosphere and bare land soils yielded 98,028 effective sequences, which were clustered into 7,287 OTUs. Rarefaction curves plateaued for all samples (Figure 1), indicating adequate sequencing depth. The Venn diagram revealed 1,370 shared OTUs across all samples (Figure 2). Rhizosphere soils contained more OTUs than bare land (3,701, 4,543, 4,297, and 3,710 for young, medium, overripe, and decline periods, respectively, vs. 2,425 for bare land). Unique OTUs accounted for 9.92%, 20.49%, 13.89%, and 11.13% of total OTUs in young, medium, overripe, and decline periods, respectively (Figure 3).

2.3 Alpha Diversity of Rhizosphere Bacterial Communities

α-Diversity indices are presented in Table 3. Coverage indices exceeded 0.98 for all samples, confirming comprehensive sequence detection. The overripe period exhibited the highest bacterial diversity (Shannon and Simpson indices), while the medium period showed the highest richness (Chao1 and ACE indices). Overall, bacterial richness and diversity displayed a hump-shaped pattern across developmental periods, though no statistically significant differences were observed between periods.

2.4 Bacterial Community Composition

At the phylum level (Figure 4), *Proteobacteria*, *unidentified_{Bacteria}*, and *Halobacterota* were dominant across all samples, with relative abundances varying by developmental period. *Proteobacteria* reached its highest abundance (29.05%) in the young period. At the genus level (Figure 5), *Marinobacter*, *Halomonas*, and *Woeseia* were the dominant genera, with *Marinobacter* peaking in the young period (5.43%), *Halomonas* in the young period (4.35%) and declining thereafter, and *Woeseia* reaching maximum abundance in the decline period (3.51%). UPGMA clustering based on Unifrac distances revealed two distinct groups: medium and decline periods clustered together, while young and overripe periods formed a separate cluster (Figure 6).

2.5 Correlations Between Rhizosphere Bacterial Communities and Environmental Factors

CCA was performed to identify environmental factors influencing bacterial community composition across developmental periods (Figure 7). After excluding autocorrelated variables (available K, EC), the analysis retained water content, organic matter, TN, TP, TK, nitrate N, ammonium N, available P, pH, and total salt. Soil water content, total salt, pH, and TK significantly affected bacterial community composition ($P<0.05$). The first and second ordination axes explained 14.73% and 14.94% of the variation, respectively. Spearman correlation analysis between the top 10 phyla and environmental factors (Figure 8) revealed significant positive correlations between organic matter, TN and species distribution ($P<0.05$), and negative correlations between TP, EC, total salt and community composition ($P<0.05$). Water content was significantly negatively correlated with *Desulfobacterota* ($P<0.05$), while TK showed significant positive correlation with *Thermoplasmatota* ($P<0.05$). Nitrate N was positively correlated with *Bdellovibrionota* ($P<0.05$), and available P and K were negatively correlated with *Planctomycetota* and *Nanoarchaeota* ($P<0.05$).

3. Discussion

Water and nutrients are essential for vegetation survival, particularly in arid regions where water availability and soil nutrient content are critical limiting factors. Our analysis revealed that rhizosphere soil water content was consistently

higher than in bare land, peaking in the young period. Young *P. euphratica* trees were typically found in areas prone to water accumulation. Soil nutrients and total salt were significantly higher in the decline period, consistent with previous research showing nutrient accumulation and reduced salt uptake in aging trees.

Rhizosphere microbial community structure is influenced by multiple biotic and abiotic factors. Soil physicochemical properties broadly shape microbial assemblages, while plant species and developmental stage determine which microorganisms thrive in the rhizosphere. *Proteobacteria*, the dominant phylum across all growth stages, is characteristic of arid saline soils and plays a crucial role in maintaining ecosystem stability. This copiotrophic group, along with *Bacteroidetes* and *Actinobacteria*, facilitates nutrient cycling by releasing and helping roots absorb available potassium, phosphorus, and micronutrients.

At the genus level, dominant taxa varied significantly across developmental stages. *Marinobacter* dominated the young period, *Halomonas* was optimal in medium and overripe periods, and *Woeseia* peaked in the decline period. This variation likely reflects changing root exudate profiles and soil conditions throughout tree development. The UPGMA clustering supported this pattern, grouping medium with decline periods and young with overripe periods based on community similarity.

Our correlation analyses identified soil water content, total potassium, total salt, and pH as the primary environmental drivers of bacterial community composition. These factors directly influence microbial survival and indirectly affect plant health by modulating nutrient availability and salt stress. The significant negative correlation between water content and *Desulfobacterota*, and positive correlation between total potassium and *Thermoplasmatota*, suggest specific physiological adaptations among bacterial taxa to arid soil conditions.

4. Conclusion

This study investigated rhizosphere bacterial communities of *Populus euphratica* at different developmental stages in the lower Tarim River using high-throughput sequencing. The main conclusions are:

1. Rhizosphere bacterial community richness and diversity exhibited a hump-shaped pattern across developmental periods, though no significant differences were detected between periods.
2. *Proteobacteria* was the dominant phylum, while *Marinobacter*, *Halomonas*, and *Woeseia* were the dominant genera. Community composition varied more substantially at the genus level than at the phylum level.
3. Bacterial communities clustered into two groups: medium and decline periods formed one cluster, while young and overripe periods formed another.
4. Soil water content, total potassium, total salt, and pH were the key envi-

ronmental factors significantly shaping rhizosphere bacterial community composition ($P<0.05$).

These results provide valuable insights into rhizosphere microbial ecology in arid regions and establish a foundation for future studies on plant-microbe interactions. Future research should focus on functional diversity to provide more comprehensive scientific references for rhizosphere microbiology in arid ecosystems.

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