Postprint: Bacterial Diversity and Community Structure in the Rhizosphere Soil of Two Halophytes

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Abstract

High-throughput sequencing technology was employed to investigate the diversity and community structure of rhizosphere soil bacteria in two halophytes, *Lycium ruthenicum* and *Kalidium caspicum*, in the arid regions of northwestern China, aiming to reveal the differences between the rhizosphere soil bacterial communities of the two salt-tolerant plants and between rhizosphere and bulk soil bacterial communities, thereby providing a theoretical foundation for in-depth studies on the relationship between rhizosphere soil microorganisms and salt tolerance in halophytes. The results demonstrated that the abundance of bacterial diversity in the rhizosphere of *L. ruthenicum* and *K. caspicum* was higher than that in bulk soil, with the rhizosphere soil of *L. ruthenicum* exhibiting greater bacterial diversity than that of *K. caspicum*. Significant differences were observed in the composition and abundance of bacterial communities between rhizosphere and bulk soils: 21 phyla and 289 genera were detected in the rhizosphere soil of *L. ruthenicum*, and 22 phyla and 304 genera in that of *K. caspicum*, whereas 28 phyla and 285 genera were identified in the bulk soil associated with *L. ruthenicum*, and 24 phyla and 336 genera with *K. caspicum*. In the rhizosphere soils of both halophytes, Proteobacteria and Firmicutes were the dominant phyla; Bacteroidetes, Actinobacteria, Cyanobacteria, and Planctomycetes showed significantly higher abundance in rhizosphere soils compared to bulk soils, while Firmicutes exhibited lower abundance in rhizosphere soils. The numbers of dominant bacterial phyla and genera in the rhizosphere soils of both plants were higher than those in bulk soils, with 10 and 9 dominant bacterial genera identified in the rhizosphere soils of *L. ruthenicum* and *K. caspicum*, respectively, compared to 4 dominant genera in each of the corresponding bulk soils, among which *Pseudomonas* was a shared dominant genus in both rhizosphere and bulk soils. The composition and abundance of bacterial communities differed between the rhizosphere soils of *L. ruthenicum* and *K. caspicum*, with

only *Pseudomonas* and *Halomonas* being shared dominant genera in the rhizosphere soils of both plants. UniFrac analysis and cluster analysis revealed that the similarity between rhizosphere soil bacterial communities of the two halophytes was greater than that between rhizosphere and bulk soil communities. Bacterial diversity was positively correlated with soil organic carbon, organic matter, and total nitrogen, and negatively correlated with pH and electrical conductivity; electrical conductivity and pH were the primary factors influencing species composition of bulk soil bacterial communities, while organic carbon and total nitrogen were the main determinants for rhizosphere soil bacterial communities.

Full Text

Preamble

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Bacterial Diversity and Community Structures in Rhizosphere Soil of Two Halophytes, *Lycium ruthenicum* and *Kalidium caspicum*

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Abstract

High-throughput sequencing was employed to investigate the diversity and community structure of rhizosphere soil bacteria associated with two halophytes—*Lycium ruthenicum* and *Kalidium caspicum*—in the arid region of northwestern China. The study aimed to reveal differences in rhizosphere bacterial communities between these two salt-tolerant plants and between rhizosphere and bulk

soils, providing a theoretical foundation for further investigation into the relationship between rhizosphere microbes and halophyte salt tolerance.

Results demonstrated that rhizosphere soil bacterial diversity was higher than that of bulk soil, with *L. ruthenicum* rhizosphere exhibiting greater diversity than *K. caspicum*. Bacterial community composition and abundance differed significantly between rhizosphere and bulk soils. A total of 21 phyla and 289 genera were detected in *L. ruthenicum* rhizosphere, while 22 phyla and 304 genera were found in *K. caspicum* rhizosphere. Bulk soils contained 28 phyla and 285 genera for *L. ruthenicum*, and 24 phyla and 336 genera for *K. caspicum*. Proteobacteria and Firmicutes were dominant in all samples. The abundance of Bacteroidetes, Actinobacteria, Cyanobacteria, and Planctomycetes was significantly higher in rhizosphere soil, whereas Firmicutes abundance was lower compared to bulk soil. The number of dominant genera was higher in rhizosphere soils, with 10 and 9 dominant genera identified in *L. ruthenicum* and *K. caspicum* rhizospheres, respectively, compared to only 4 genera in their corresponding bulk soils. *Pseudomonas* was the only genus dominant in both rhizosphere and bulk soils, while *Pseudomonas* and *Halomonas* were shared dominant genera between the two plant rhizospheres.

UniFrac and cluster analyses revealed greater similarity between the rhizosphere bacterial communities of the two halophytes than between rhizosphere and bulk soil communities. Bacterial diversity correlated positively with soil organic carbon and total nitrogen, but negatively with electrical conductivity. Electrical conductivity, pH, total organic carbon, and total nitrogen were the primary factors influencing both bulk and rhizosphere soil bacterial communities.

Keywords: halophytes; rhizosphere; bacteria; diversity; community structure

Introduction

Soil microorganisms constitute one of the most important and active components of terrestrial soil systems, playing irreplaceable roles in soil formation and development, organic matter transformation, soil environmental purification, and bioremediation. The rhizosphere, defined as the soil region directly influenced by plant roots and their exudates, represents a critical zone for interactions between soil microbes and plants. Plant growth-promoting microorganisms, particularly certain bacteria and fungi, significantly contribute to plant growth and stress resistance. Rhizosphere microbial diversity and community structure are shaped by plant influences, exhibiting species-specific characteristics and developmental stage specificity. Plant activities alter the rhizosphere microenvironment, creating distinct differences in community structure and composition between rhizosphere and bulk soils. Soil microbial communities are also influenced by soil physicochemical properties, moisture, and other factors.

In saline-alkaline environments, soil salt content significantly affects microbial activity, diversity, and community structure. Halophytes have evolved unique salt tolerance mechanisms through long-term adaptation and evolution to cope with saline-alkaline stress. In addition to intrinsic plant mechanisms, rhizosphere microbes and some endosymbionts also contribute to plant salt tolerance. Investigating halophyte rhizosphere microbial diversity and community structure enhances understanding of halophyte salt tolerance and plant-microbe interactions. While numerous studies have examined rhizosphere microbial diversity in non-halophytes under normal or salt-stress conditions, research on rhizosphere microbial community composition in halophytes remains limited.

Lycium ruthenicum and *Kalidium caspicum* are two halophytes distributed in the arid regions of northwestern China, with *K. caspicum* occurring exclusively in Xinjiang. Both species inhabit saline soils in desert areas and exhibit strong salt tolerance. Previous research has investigated their salt-alkaline tolerance mechanisms from physiological and ecological perspectives, but in-depth studies on rhizosphere microbial diversity and its relationship with salt tolerance are lacking. This study employed high-throughput sequencing technology to analyze the community structure of rhizosphere and bulk soil bacteria associated with these two halophytes, examine similarities and differences between their rhizosphere bacterial communities, and provide a theoretical basis for elucidating the relationship between rhizosphere bacteria and halophyte salt tolerance.

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1. Materials and Methods

1.1 Sample Collection

The study area was located in the Ebinur Lake Wetland National Nature Reserve in Jinghe County, Xinjiang, in the southwestern Junggar Basin. The region features an arid climate with intense surface evaporation and high soil salinity. The average soil electrical conductivity (0–10 cm depth) was 5.41 ms/cm, pH was 8.77, and average water content was 7.19%. Soil bulk density was approximately 1.38 g/cm^3. The area supports vegetation including *Populus euphratica*, *Haloxylon ammodendron*, *Halostachys caspica*, *Halocnemum strobilaceum*, *Kalidium foliatum*, *Kalidium caspicum*, *Lycium ruthenicum*, and *Tamarix ramosissima*.

Soil samples were collected in October from saline-alkaline soils in the reserve. The *K. caspicum* and *L. ruthenicum* communities were approximately 2 km apart. Healthy individuals with similar growth status and genetic background were selected from each population. Plant root systems were excavated, and loosely attached soil was shaken off, leaving only soil tightly bound to roots. This soil was collected in sterile centrifuge tubes as rhizosphere soil. For *K. caspicum*, samples were collected from individuals spaced 30–50 m apart. Samples were transported to the laboratory on ice.

Bulk soil samples were collected simultaneously from the same locations at 0–

30 cm depth after removing surface soil. These were placed in sterile bags and transported on ice. Due to limited rhizosphere soil quantity, replicate samples were pooled for analysis.

1.2 Soil Physicochemical Analysis

Soil electrical conductivity (EC) and pH were measured using a pH meter and conductivity meter. Soil organic carbon (TOC) and soil organic matter (SOM) were determined by potassium dichromate digestion. Total nitrogen (TON) was measured using the Kjeldahl method. Bulk density was determined by the ring knife method.

1.3 Soil Microbial Genome Extraction, Amplification, and Sequencing

Rhizosphere soil solution (0.2 g) was transferred to sterile centrifuge tubes for microbial genomic DNA extraction. Bulk soil DNA was extracted from approximately 0.2 g of soil using a sterile spoon. The OMEGA E.Z.N.A.TM Mag-Bind Soil DNA Kit was used for extraction. DNA quality was assessed by agarose gel electrophoresis, and concentration and purity were determined using NanoDrop.

A two-round PCR approach was employed to amplify the bacterial 16S rDNA V3–V4 region. The first round used universal primers fused with barcode sequences: 341F (5 -ccctacacgacgctcttccgatctg(barcode)cctacgggnggcwgcag-3) and 805R (5 -gactggagttccttggcacccgagaattccagactachvgggtatctaatcc-3). Each 30 L reaction contained 15 L 2×Taq master mix, 1 L each primer (10 mol/L), and 20 ng template DNA. Cycling conditions were: 94°C for 3 min; 30 cycles of 94°C for 30 s, 45°C for 20 s, 65°C for 30 s; final extension at 72°C for 5 min.

The second round added Illumina sequencing adapters. Cycling conditions were: 95°C for 30 s; 5 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 30 s; final extension at 72°C for 5 min. PCR products were purified using magnetic beads, and replicate samples were pooled in equal amounts before sequencing by a commercial company using the Illumina platform.

Raw sequences were processed by removing primer adapters, low-quality bases (Phred Quality Score = 20), non-specific amplification products, and chimeras. Sequences were assembled with a 200 bp threshold. Operational taxonomic units (OTUs) were defined at 97% similarity. Representative sequences were taxonomically classified using the RDP classifier. Species composition was analyzed at phylum and genus levels, and community bar charts and heatmaps were generated.

1.4 Data Analysis

Alpha diversity analysis was performed using Mothur 1.30 and QIIME 1.8. Diversity indices (ACE, Chao1, Shannon, Simpson) were calculated, and rarefaction curves were constructed. Weighted UniFrac analysis was used to assess community similarity and construct clustering trees. Canonical correspondence

analysis (CCA) was employed to identify major environmental factors influencing bacterial community structure.

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2. Results

2.1 Soil Physicochemical Properties

Soil physicochemical properties are presented in . Total organic carbon (TOC), soil organic matter (SOM), and total nitrogen (TON) contents were higher in rhizosphere soils than in bulk soils for both halophytes. Electrical conductivity (EC) and pH values were lower in rhizosphere soils compared to bulk soils. The rhizosphere soil of *K. caspicum* exhibited higher EC than *L. ruthenicum* rhizosphere soil, while *L. ruthenicum* bulk soil showed higher EC than its rhizosphere soil. For *K. caspicum*, rhizosphere EC was slightly higher than bulk soil EC.

** Chemical factors of rhizosphere and bulk soils of *Lycium ruthenicum* and *Kalidium caspicum***

Sample	Species	Soil type	TOC (g/kg)	SOM (g/kg)	TON (g/kg)	EC (ms/cm)	pH
L.r_R	*L. ruthenicum*	Rhizosphere	10.25	17.68	0.757	1.96	8.09
L.r_B	*L. ruthenicum*	Bulk	6.71 (0.80)	11.55 (1.37)	0.523 (0.04)	8.90 (0.07)	6.26 (1.26)
K.c_R	*K. caspicum*	Rhizosphere	23.40	40.34	0.77	7.35	4.94
K.c_B	*K. caspicum*	Bulk	2.67 (0.39)	4.60 (0.68)	0.112 (0.03)	8.98 (0.18)	4.02 (1.26)

Note: Values are mean (standard deviation). Due to limited rhizosphere soil, replicates were pooled for analysis, yielding single values.

2.2 Sequencing Data Analysis

Effective sequence lengths ranged from 400–440 bp, with most approximately 420 bp. The numbers of analyzed sequences from rhizosphere and bulk soils were 10,881, 15,911, 17,881, and 12,941 for *L. ruthenicum* rhizosphere, *K. caspicum* rhizosphere, *L. ruthenicum* bulk soil, and *K. caspicum* bulk soil, respectively. Rarefaction curves approached saturation, and sequencing coverage exceeded 99%, indicating adequate sampling depth and representation of bacterial communities.

** Genomic DNA sequence data statistics and alpha diversity analysis for soil samples**

Sample	Valid sequences	OTUs (97%)	ACE index	Chao1 index	Shannon index	Simpson index	Coverage
L.r_R	10,881	1,880	2,794.76	2,656.83	6.23	0.0039	0.996
K.c_R	15,911	2,015	3,012.45	2,892.31	5.98	0.0045	0.995
L.r_B	17,881	1,294	1,891.22	1,802.15	5.67	0.0056	0.998
K.c_B	12,941	1,788	2,456.78	2,301.45	5.71	0.0052	0.996

2.3 Bacterial Diversity

Alpha diversity analysis revealed that rhizosphere soil bacterial diversity was significantly higher than that of bulk soil ($p < 0.01$). *Lycium ruthenicum* rhizosphere exhibited higher bacterial diversity than *K. caspicum* rhizosphere, though the difference was not statistically significant ($p > 0.05$). The Shannon index was higher for *L. ruthenicum* rhizosphere, while the Simpson index was lower, indicating lower bacterial abundance compared to *K. caspicum* rhizosphere. No significant differences were observed in bulk soil bacterial diversity between the two plant species.

2.4 Bacterial Community Composition and Structure

Taxonomic analysis identified 37 bacterial phyla across all soil samples. Proteobacteria and Firmicutes were dominant in all samples, with relative abundances ranging from 37.26%–81.65% and 1.73%–45.67%, respectively. A total of 21 phyla and 289 genera were detected in *L. ruthenicum* rhizosphere, and 22 phyla and 304 genera in *K. caspicum* rhizosphere. Bulk soils contained 28 phyla and 285 genera for *L. ruthenicum*, and 24 phyla and 336 genera for *K. caspicum*.

Dominant phyla in *L. ruthenicum* rhizosphere, in descending order of abundance, were Proteobacteria (37.26%), Firmicutes (33.07%), Bacteroidetes (16.08%), Actinobacteria (5.27%), Cyanobacteria (4.32%), and Planctomycetes (1.07%). In *K. caspicum* rhizosphere, the dominant phyla were Proteobacteria (81.65%), Firmicutes (8.55%), Bacteroidetes (4.33%), and Actinobacteria (2.28%). Bulk soils showed different abundance patterns, with Firmicutes reaching 45.67% and 55.4% in *L. ruthenicum* and *K. caspicum* bulk soils, respectively.

At the genus level, 289 and 304 genera were detected in *L. ruthenicum* and *K. caspicum* rhizospheres, respectively, compared to 285 and 336 genera in corresponding bulk soils. The number and composition of dominant genera differed between rhizosphere and bulk soils. *Pseudomonas* was the only genus dominant in both rhizosphere and bulk soils, while *Pseudomonas* and *Halomonas* were shared dominant genera between the two plant rhizospheres.

In *L. ruthenicum* rhizosphere, the 10 dominant genera were *Planococcus* (16.7%), *Salinimicrobium* (6.58%), *Planomicrobium* (5.34%), *Pseudomonas* (4.1%), *Bacillus* (1.77%), and *Gillisia* (1.37%). In *K. caspicum* rhizosphere,

the 9 dominant genera included *Planococcus* (14.47%), *Halomonas* (12.24%), *Acinetobacter* (4.19%), *Exiguobacterium* (3.56%), *Aliifodinibius* (2.45%), and *Cobetia* (1.51%). Bulk soils were dominated by *Exiguobacterium*, *Acinetobacter*, and *Citrobacter*, with abundances of 44.01% and 41.45% in *L. ruthenicum* and *K. caspicum* bulk soils, respectively. Many low-abundance genera in bulk soil, such as *Halomonas*, *Salinimicrobium*, *Pontibacter*, and *Thioalkalispira*, became dominant in rhizosphere soils.

[**Figure 1: see original paper**] shows rarefaction curves of soil bacterial communities at 97% identity constructed with the Shannon index. [**Figure 2: see original paper**] illustrates bacterial community structures at phylum and genus levels.

2.5 Bacterial Community Similarity and Difference Analysis

Significant differences in bacterial community composition and abundance were observed between rhizosphere and bulk soils of both plants and between rhizosphere communities of different plants. UniFrac analysis revealed that the distance between rhizosphere communities of the two plants was 0.44, while the distance between their bulk soil communities was 0.04. Distances between rhizosphere and bulk soils were 0.65 for *L. ruthenicum* and 0.61 for *K. caspicum*.

Cluster analysis showed that rhizosphere bacterial communities of both plants grouped together, while their bulk soil communities formed a separate cluster, indicating greater similarity between rhizosphere communities than between rhizosphere and bulk soils. [**Figure 3: see original paper**] presents a heatmap and clustering dendrogram of bacterial communities at the genus level.

**** Correlations between rhizosphere soil bacterial diversity indices and soil physicochemical factors

Diversity index	EC	pH	TOC	SOM	TON
Shannon index	-0.75	-0.73	0.81	0.89	0.85
Simpson index	-0.04	-0.59	0.58	0.41	0.39
ACE index	-0.81	-0.58	0.95	0.93	0.91
Chao1 index	-0.89	-0.41	0.92	0.90	0.88

2.6 Relationship Between Bacterial Diversity and Soil Physicochemical Properties

Correlation analysis revealed positive relationships between rhizosphere bacterial diversity (Shannon, ACE, Chao1 indices) and soil organic carbon, organic matter, and total nitrogen, though correlations were not statistically significant ($p > 0.05$). Negative correlations were observed with electrical conductivity and pH. CCA analysis identified EC, pH, TOC, and TON as the primary factors influencing both bulk and rhizosphere soil bacterial communities. [**Figure 4: see**

original paper] shows the CCA ordination of rhizosphere bacterial community composition and soil factors.

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3. Discussion

Plants actively select rhizosphere bacterial communities through root activities that modify the rhizosphere environment, selectively enriching beneficial bacteria while reducing detrimental ones. This creates plant-specific rhizosphere bacterial community structures. Our findings align with previous studies showing significant differences in diversity and composition between rhizosphere and bulk soils of *L. ruthenicum* and *K. caspicum*.

Alpha diversity analysis confirmed higher bacterial diversity in rhizosphere versus bulk soil, consistent with literature reports. Proteobacteria and Firmicutes dominated both rhizosphere and bulk soils, which aligns with studies identifying these phyla as predominant in saline soils and various plant rhizospheres. Proteobacteria includes diverse pathogens and nitrogen-fixing bacteria, while Firmicutes bacteria are often associated with stress resistance and can tolerate extreme conditions. The higher abundance of Proteobacteria in rhizosphere soils may reflect their adaptation to the nutrient-rich root environment.

The enrichment of Bacteroidetes, Actinobacteria, Cyanobacteria, and Planctomycetes in rhizosphere soils, coupled with reduced Firmicutes abundance, suggests plant-mediated selection. Actinobacteria and Bacteroidetes are known plant-associated groups, while Cyanobacteria and Planctomycetes may contribute to nutrient cycling in the rhizosphere.

Cluster analysis revealed greater similarity between the rhizosphere communities of the two halophytes than between rhizosphere and bulk soils, suggesting convergent selection of rhizosphere bacteria under saline stress. This may represent an adaptive mechanism where halophytes develop similar rhizosphere microbiomes to cope with salt stress.

The number of dominant bacterial genera was higher in rhizosphere soils, but their abundance patterns differed between plant species. *Pseudomonas* and *Halomonas* were shared dominant genera, reflecting their adaptation to saline conditions. *Pseudomonas* is a well-known salt-tolerant genus that can degrade various organic compounds, while *Halomonas* comprises halophilic bacteria that thrive in saline environments. Other genera such as *Salinimicrobium*, *Planococcus*, *Pontibacter*, and *Thioalkalispira* were enriched in rhizosphere soils, many of which exhibit halotolerance.

Plant-specific differences were evident in community composition and abundance. *Planomicrobium*, *Bacillus*, and *Gillisia* were dominant in *L. ruthenicum* rhizosphere, while *Aliifodinibius*, *Gracilibacillus*, and *Fodinicurvata* were characteristic of *K. caspicum* rhizosphere. These differences reflect distinct plant

genetic backgrounds and root exudate profiles, which shape unique rhizosphere microenvironments.

Soil salinity is a major limiting factor for microbial communities in saline regions. High salt content inhibits microbial growth, but salt-tolerant and halophilic bacteria proliferate under these conditions. The positive correlation between bacterial diversity and soil organic carbon/total nitrogen suggests that rhizosphere enrichment of these nutrients promotes microbial diversity. Salt stress may increase rhizosphere bacterial diversity and biomass in salt-tolerant plants, potentially alleviating salt stress effects. Our findings indicate that electrical conductivity and pH are primary determinants of bulk soil bacterial communities, while organic matter and nitrogen are key drivers of rhizosphere community composition, consistent with previous research.

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Supplementary Figures

[FIGURE:S1] Differential analysis of bacterial community composition between rhizosphere and bulk soil of *L. ruthenicum* and *K. caspicum* at the phylum level.

[FIGURE:S2] Differential analysis of bacterial community composition between rhizosphere and bulk soil of *L. ruthenicum* and *K. caspicum* at the genus level.

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