Postprint: Bacterial Community Diversity in Different Types of Saline-Alkali Soils in Arid Regions

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Abstract

Taking four types of saline-alkali soils in the Manas River Basin—sulfate-chloride type, chloride-sulfate type, carbonate type, and sulfate type—as research subjects, bacterial communities were sequenced using high-throughput sequencing technology. The study revealed that a total of 137,822 bacterial gene sequences were obtained from the four saline-alkali soil samples. The Shannon index and Chao1 index exhibited the following pattern: carbonate type > sulfate type > sulfate-chloride type > chloride-sulfate type, indicating that the diversity and abundance of bacterial communities in carbonate-type soil were significantly higher than those in the other three samples. The bacterial communities in the four saline-alkali soils mainly comprised 10 phyla, among which Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant phyla in sulfate-chloride type, chloride-sulfate type, and sulfate type soils, whereas carbonate-type soil was dominated by Firmicutes, Proteobacteria, and Actinobacteria. At the genus level, the community composition varied considerably among the samples. Cluster analysis divided the four bacterial communities into three groups, with sulfate-chloride type and chloride-sulfate type grouped together, while sulfate type and carbonate type each formed separate groups. Among environmental factors, total salt, chloride ions, sulfate ions, and nitrate ions had significant effects on species distribution ($P<0.05$). Bacterial communities in different types of saline-alkali soils in the Manas River Basin exhibited significant differences, with numerous types of bacterial communities present, particularly in carbonate-type soil. These findings reveal the composition of bacterial communities in different types of saline-alkali soils in arid regions and the key environmental factors influencing community structure, providing a scientific basis for exploring microbial resources in saline-alkali soils and for saline-alkali land improvement.

Full Text

Abstract

High-throughput sequencing was used to analyze bacterial communities in four types of saline-alkaline soils, including sulfate-chloride, chloride-sulfate, carbonate, and sulfate types. A total of 137,822 gene sequences were obtained from the soil samples. The Shannon and Chao1 indices followed the order: carbonate > sulfate > sulfate-chloride > chloride-sulfate, indicating that the diversity and abundance of soil bacterial communities in carbonate soil samples were significantly higher than those in other soil samples. The saline-alkaline soils were mainly comprised of ten phyla. The dominant bacterial phyla were Proteobacteria, Bacteroidetes, and Actinobacteria in sulfate-chloride, chloride-sulfate, and sulfate soils, respectively. Firmicutes, Proteobacteria, and Actinobacteria were the dominant populations in carbonate soil. At the genus level, the composition of soil bacterial communities differed among soil types. Cluster analysis divided the bacterial communities into three groups: one comprising sulfate-chloride and chloride-sulfate soils, and the others composed of sulfate and carbonate soils. The effects of total salt, chlorine, sulfate, and nitrate contents on bacterial communities were significant ($P < 0.05$). The results revealed significant differences in bacterial communities among different saline-alkaline soil types in the Manas River Basin, with particularly diverse communities in carbonate-type soils. These findings provide a scientific basis for developing microbial resources in saline-alkaline soils and their improvement.

Keywords: saline-alkaline soil; soil microorganism; high-throughput sequencing; bacterial community; diversity; Xinjiang

1. Introduction

Saline-alkaline soils are widely distributed in arid and semi-arid regions worldwide, particularly in Xinjiang, China, where they cover approximately 3.09×10 km^2. These soils are characterized by high pH, high salt content, and poor soil structure, which severely limit agricultural productivity. Previous studies have shown that soil microorganisms play crucial roles in nutrient cycling and soil improvement. However, the composition and diversity of bacterial communities in different saline-alkaline soil types remain poorly understood. This study investigated bacterial community structure in four typical saline-alkaline soil types in the Manas River Basin using high-throughput sequencing technology.

2. Materials and Methods

2.1 Sample Collection and Site Description

Soil samples were collected in July 2017 from the Manas River Basin. Four typical saline-alkaline soil types were selected: sulfate-chloride (?@–BCDAE), chloride-sulfate (BC–?@AE), sulfate (F@AE), and carbonate (?@AE) soils. Three replicate plots (10 m × 10 m) were established for each soil type, with

each plot spaced more than 10 m apart. Within each plot, five sampling points were randomly selected and mixed to obtain a composite sample. Soil samples were collected from 0–20 cm depth using a soil auger. Visible plant residues and stones were removed, and samples were passed through a 2 mm sieve. Each composite sample was divided into two portions: one for physicochemical analysis and one for DNA extraction. The DNA samples were stored at -80°C until analysis.

2.2 Soil Physicochemical Analysis

Soil pH was measured using a pH meter (soil:water ratio 1:2.5). Soil organic matter content was determined by the potassium dichromate oxidation method. Total nitrogen was measured using the Kjeldahl method. Available phosphorus was extracted with sodium bicarbonate and analyzed by molybdenum-blue colorimetry. Available potassium was extracted with ammonium acetate and measured by flame photometry. Total salt content was determined by gravimetric analysis. Cl and SO^{2} were measured by ion chromatography (Thermo Fisher ICS-1100). NO was analyzed by UV spectrophotometry. All analyses were performed in triplicate.

2.3 DNA Extraction and PCR Amplification

Total soil DNA was extracted from 0.5 g of fresh soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer' s instructions. The V3-V4 region of the bacterial 16S rRNA gene was amplified using primers 338F (5' -ACTCCTACGGGAGGCAGCAG-3') and 806R (5' -GGACTACHVGGGTWTCTAAT-3'). PCR reactions were performed in a 20 L mixture containing 4 L of 5× FastPfu Buffer, 2 L of 2.5 mM dNTPs, 0.8 L of each primer (5 M), 0.4 L of FastPfu Polymerase, and 10 ng of template DNA. Thermal cycling conditions were: 95°C for 3 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA).

2.4 High-Throughput Sequencing

Purified amplicons were pooled in equimolar concentrations and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using the 2 × 300 bp paired-end protocol at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) database.

2.5 Data Processing and Analysis

Raw sequences were quality-filtered using QIIME (version 7.1). Operational taxonomic units (OTUs) were clustered at 97% similarity using UPARSE. Chimeric

sequences were identified and removed using UCHIME. Taxonomic classification was performed using the RDP Classifier (version 2.2) against the Silva database (Release 123) with a confidence threshold of 0.7. Alpha diversity indices (Chao1, ACE, Shannon, and Simpson) were calculated using Mothur (version v.1.30.1). Beta diversity analysis was performed using vegan package in R. Pearson and Spearman correlations were used to analyze relationships between bacterial communities and soil properties. Mantel tests were conducted to assess correlations between environmental factors and community composition. Cluster analysis was performed based on Bray-Curtis distances. Redundancy analysis (RDA) was used to identify key environmental factors shaping bacterial communities. Significance was determined at $P < 0.05$ unless otherwise stated.

3. Results

3.1 Soil Physicochemical Properties

The physicochemical properties of the four soil types are shown in Table 2. Soil pH ranged from 9.05 to 10.89. The carbonate soil (?@AE) had the highest pH (10.89) and highest total salt content (34.45 g/kg). The sulfate-chloride soil (?@–BCDAE) showed the lowest organic matter (1.33 g/kg) and available phosphorus (0.28 mg/kg). Significant differences ($P < 0.05$) were observed among soil types for all measured parameters.

Table 2. Physicochemical properties of soil sample plots (0–20 cm depth)

Soil Type	pH	Organic Matter (g/kg)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Total Salt (g/kg)	Cl (g/kg)	SO_4^{2-} (g/kg)	NO (mg/kg)
?@–BCDAE	9.05d	1.33b	3.56c	0.28c	0.36b	10.25b	4.32d	340.58c	21.06a
BC–?@AE	9.20c	3.38a	9.62a	0.67a	0.62a	34.45a	14.56a	505.64b	10.96b
F@AE	10.89a	0.36c	7.41b	0.41b	0.59a	33.1a	11.68b	585.41a	0.03d
?@AE	9.53b	0.68bc	8.32a	0.72a	0.58a	32.18a	8.07c	593.63a	0.34c

Note: Different lowercase letters indicate significant differences among soil types ($P < 0.05$, Duncan' s test).

3.2 Sequencing Results and Alpha Diversity

A total of 137,822 high-quality sequences were obtained from 12 soil samples, with 32,339–37,413 sequences per sample (Table 3). The number of OTUs

ranged from 22,379 to 23,864, with no significant differences among soil types. However, alpha diversity indices showed significant variation. The Shannon index was highest in carbonate soil (?@AE) and lowest in sulfate-chloride soil (?@–BCDAE). The Chao1 and ACE indices followed the order: carbonate > sulfate > sulfate-chloride > chloride-sulfate, indicating that carbonate soil harbored the most diverse bacterial communities.

Table 3. MiSeq sequencing results and diversity indices for each sample

Soil Type	Sequences	OTUs	Chao1	ACE	Shannon	Simpson
?@–BCDAE	32,339	22,379	290.62	384.89	337.04	289.62
BC–?@AE	37,413	23,864	377.81	344.3	2.90	0.89
F@AE	35,621	23,156	289.62	377.81	3.44	0.93
?@AE	34,508	22,789	337.04	344.3	3.78	0.95

Rarefaction curves (Fig. 1) showed that all samples approached saturation, indicating adequate sequencing depth. The carbonate soil curves were highest, confirming its greater bacterial diversity.

3.3 Bacterial Community Composition

At the phylum level, ten bacterial phyla were identified across all samples. Proteobacteria, Bacteroidetes, and Actinobacteria dominated in sulfate-chloride, chloride-sulfate, and sulfate soils. In carbonate soil, Firmicutes (42.47%) was the most abundant phylum, followed by Proteobacteria (35.29%) and Actinobacteria (6.7%). The relative abundance of unclassified bacteria ranged from 1.25% to 10.04%.

At the genus level, 517 genera were identified. Eight dominant genera (relative abundance > 5%) were found: *Halomonas*, *Gillisia*, *Pseudarthrobacter*, *Bacillus*, *Rubellimicrobium*, *Adhaeribacter*, *Salegentibacter*, and *Nitriliruptoraceae*. The distribution of these genera varied significantly among soil types. For example, *Gillisia*, *Salegentibacter*, and *Nitriliruptoraceae* were significantly more abundant in sulfate-chloride soil, accounting for 57.2%, 29.94%, and 12.86% of the community, respectively.

3.4 Beta Diversity and Cluster Analysis

Cluster analysis based on Bray-Curtis distances revealed three distinct groups (Fig. 5). Sulfate-chloride and chloride-sulfate soils clustered together, while sulfate and carbonate soils formed separate clusters. This grouping pattern was supported by the Venn diagram analysis (Fig. 2), which showed that sulfate-chloride and chloride-sulfate soils shared more OTUs (46) than other pairs.

3.5 Relationships Between Bacterial Communities and Soil Properties

Redundancy analysis (RDA) revealed that soil pH, total salt content, and Cl concentration were the primary factors influencing bacterial community composition (Fig. 6). The first and second RDA axes explained 31.6% and 18.4% of the total variation, respectively. Spearman correlation analysis (Fig. 7) showed that Firmicutes abundance was positively correlated with pH ($r = 0.85$, $P < 0.01$) and total salt content ($r = 0.79$, $P < 0.01$). Proteobacteria abundance was negatively correlated with Cl concentration ($r = -0.68$, $P < 0.01$).

Mantel test results (Table 4) confirmed significant correlations between bacterial community composition and soil properties ($r = 0.429$, $P = 0.039$). Individual factors showing significant correlations included total salt ($r = 0.443$, $P = 0.046$), Cl ($r = 0.499$, $P = 0.047$), and SO^{2} ($r = 0.555$, $P = 0.046$).

Table 4. Mantel test for environmental factors at phylum level

Factor	Mantel statistic	P-value	Permutations	Tail Type
Overall	0.42915	0.039	93841	two-side
pH	0.394	0.042	913	two-side
Total Salt	0.44333	0.046	46601	two-side
Cl	0.49944	0.047	338	two-side
SO^{2}	0.5553	0.046	46	two-side

4. Discussion

This study demonstrated significant differences in bacterial community composition among four saline-alkaline soil types in the Manas River Basin. Carbonate soil exhibited the highest bacterial diversity, likely due to its unique chemical environment. The dominance of Firmicutes in carbonate soil (42.47%) contrasts with previous studies in neutral soils, where Proteobacteria typically dominate. This suggests that Firmicutes species possess specific adaptations to high pH and carbonate stress.

The clustering of sulfate-chloride and chloride-sulfate soils indicates that anion composition significantly influences bacterial community structure. High Cl concentrations may select for halotolerant bacteria such as *Halomonas* and *Bacillus*, which were more abundant in these soils. The significant correlation between bacterial communities and soil salinity parameters (Table 4) supports this hypothesis.

Our findings align with previous research showing that pH is a primary driver of bacterial community composition in saline-alkaline soils. However, the relative importance of different salts (Cl vs. SO^{2}) varies among soil types. The identification of specific indicator genera for each soil type provides potential targets for developing microbial inoculants tailored to specific saline-alkaline conditions.

4.1 Adaptation Mechanisms of Dominant Bacteria

The prevalence of *Halomonas* and *Bacillus* in high-salt soils suggests these genera possess effective salt tolerance mechanisms, including compatible solute accumulation and efficient ion transport systems. *Nitriliruptoraceae*, enriched in sulfate-chloride soil, may participate in sulfur cycling, contributing to soil nutrient transformation.

4.2 Implications for Soil Improvement

Understanding the relationship between bacterial communities and soil properties provides a basis for microbial resource development. For carbonate soils, Firmicutes-based inoculants may be effective, while Proteobacteria-based approaches might suit sulfate-dominated soils. Future work should isolate and characterize functional strains from these communities to develop targeted soil amendments.

5. Conclusions

(1) Bacterial diversity and abundance in carbonate soil were significantly higher than in sulfate-chloride, chloride-sulfate, and sulfate soils.

(2) Ten bacterial phyla were identified, with Proteobacteria, Bacteroidetes, and Actinobacteria dominating in sulfate-chloride, chloride-sulfate, and sulfate soils, while Firmicutes, Proteobacteria, and Actinobacteria dominated in carbonate soil.

(3) Bacterial communities clustered into three groups based on soil type, with significant differences in community composition among the four saline-alkaline soil types.

(4) Total salt, Cl^-, SO_4^{2-}, and NO_3^- contents significantly affected bacterial community structure ($P < 0.05$).

These results provide a scientific foundation for exploiting microbial resources and developing improvement strategies for saline-alkaline soils in arid regions.

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