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# Structural Diversity of Bacterial Community in Soft Rock and Sand Composite Soil

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## Abstract

**This study was based on the compound experimental plots of soft rock and sand (soft rock: sand (V:V), 0:1, 1:1, 1:2 and 1:5, refer to C0, C1, C2, C3, respectively) at the Yulin Field Scientific Observation and Research Station. Using high-throughput sequencing systematically study the differences in the composition of the soil bacterial community structure in the root system of crops under different compounding ratios and the influencing mechanism. The results showed that: the dominant bacterial genera were Rhodococcus and Arthrobacter in the pure sand treatment group, but the dominant genera in the community changed to lysobacteria after the addition of soft rock. The difference of the bacterial community α diversity in the different proportions was not significant; but the β diversity was significantly different, and the difference between different compounding ratios was not significant. Further research found that soil moisture and available phosphorus content were the main environmental factors affecting the changes of crop root soil bacterial community structure.**

## Keywords

**Mu Us Sandy Land; Soft Rock; Composite Soil; Bacteria; Community Structure.**

## 1. Introduction

Microorganisms are an important part of soil and one of the important factors affecting soil fertility [1]. They play various roles in nutrient cycling, fertility formation and development, ecological environment improvement, plant growth and soil-borne disease prevention and control [2-3]. It is not only an important indicator for measuring soil quality, maintaining soil fertility and crop production, but also because of its sensitivity to the living environment, it can respond rapidly to changes in soil ecological mechanisms and environmental stress, thereby changing the community structure to adapt to the environment. It is also considered as a sensitive indicator for early warning of soil ecosystem changes [4-7].

At present, the research on soil microbial germplasm resources and improvement effects mostly focus on extremely salinized land [8]. There are limited successful cases of sand land remediation and utilization at home and abroad, and there are few research literature on soil microbial succession before and after sand land consolidation and utilization [9]. Based on the urgent needs of land engineering development, this study revealed the number of microorganisms in the newly added soil and the law of community succession, so as to further explain the scientific connotation of the theory of soil organic remodeling, and to cultivate and

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excavate microbial germplasm resources in special sandy habitats, and gene bank to provide scientific basis.

## 2. Materials and Methods

### 2.1. Overview of the Study Area

The sampling point is located in the northern sandy area of Yulin, Shaanxi Province (107.2°～112.1°E, 36.1°～38.5°N). The soil is mainly aeolian sandy soil, with an altitude of about 1000 m and an average annual temperature of about 8℃. The annual rainfall is 425 mm, mainly from July to September, and the evaporation is 2000 mm.

### 2.2. Experimental Design and Sample Collection

The development of this project relies on the long-term positioning observation test field of pi sandstone and sand composite soil in the Yulin field of Shaanxi Institute of Geological Construction (constructed in 2010)), another control was set up, and no arsenic sandstone compounding was performed. There were a total of 4 treatments, each treatment was repeated three times, a total of 12 experimental plots, and the area of each plot was 15 m * 4 m. The test crop was maize, and soil samples were collected during the maize harvest period (around early October), using the five-point sampling method to collect rhizosphere soil samples at a depth of 0-20 cm. Each replicate of the same treatment was thoroughly mixed to form a mixed sample. After removing impurities such as gravel and plant residues, the soil was evenly divided into three parts. 3 samples of maize root soil samples of pure sand control group were collected as controls, totaling 12 soil samples. The fresh soil was put into a sterile sealed bag, placed in an ice box, and quickly brought back to the laboratory, and stored in a 4 ℃ refrigerator for the determination of ammonium nitrogen; the other part was put into a sterile sealed bag and placed in an ice box and brought back to the experiment quickly. The samples were stored in a refrigerator at -20 °C for high-throughput sequencing of soil bacteria (Shanghai Meiji); the remaining soil samples were air-dried and used for the determination of basic physical and chemical properties of soil.

After PCR amplification and Illumina high-throughput sequencing to extract total sample DNA, bacterial V4+V5 (F: 5′-GTGCCAGCMGCCGCGG-3′, R: 5′-CCGTCAATTCMTTTRAGTTT-3′) and fungal ITS1+ITS2 (F: 5′-GTGCCAGCMGCCGCGG-3′) and fungal ITS1+ITS2 (F : 5'-CTTGGTCATTTAGAGGAAGTAA-3', R: 5'-GCTGCGTTCTTCATCGATGC-3') synthesized primers, combined primer linkers, and carried out PCR amplification. PCR reaction system: 10.0 μl of 10× Buffer containing 15 mmol/L MgCl2, 2.0 μl of 2.5 mmol/L dNTPs, 5.0 μl of each 10 μmol/L primer, 1.0 μl of 5 U/μl Taq enzyme, 4.0 μl of DNA template, sterilized Deionized water 73.0 μl, total volume 100 μl. PCR reaction conditions: 94°C for 5 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; 72°C for 10 min. After PCR amplification, the products were purified, quantified, and homogenized to form a sequencing library. The constructed library was first subjected to library quality inspection. The library that passed the quality inspection was sequenced with IlluminaHiSeq PE250. The sequencing was performed by Nanjing Jisihuiyuan Biotechnology Co., Ltd.

Remove long reads with an average quality value below 20 and long reads with more than 3 N-containing bases (the length of reads ranges from 220 to 500 nt), and use Mothur software to optimize the sequence for OTU clustering analysis, using Usearch Clustering was performed at a similarity of 0.97, and after chimera filtering was performed on the clustered sequences, OTUs for species classification were obtained, and each OTU was considered to represent a species. Data analysis OTUs with a similarity of 97% were randomly selected to generate dilution curves, and the software Mothur was used to calculate the richness index Chao and Observed species index, and the diversity index Simpson and Shannon. Species annotation of OTU was performed

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based on RDP and UNITE taxonomic database, and R language was used for data processing and mapping.

### 2.3. Data Analysis

The experimental data were summarized by Excel 2007. The differences of bacterial community in different compounding ratios in roots of different species were analyzed by SPSS and plotted by R.

## 3. Results and Discussion

It can be seen from Figure 1 that the dilution curve tends to be flat, indicating that the detection ratio of microbial communities in environmental samples is close to saturation, and the current sequencing volume can cover most of the species in the samples. It can also be seen from Figure 1 that after adding a certain proportion of arsenic sandstone, the OTU of maize root bacteria in the compound soil has an obvious trend of increasing, but there is little difference between different proportions.

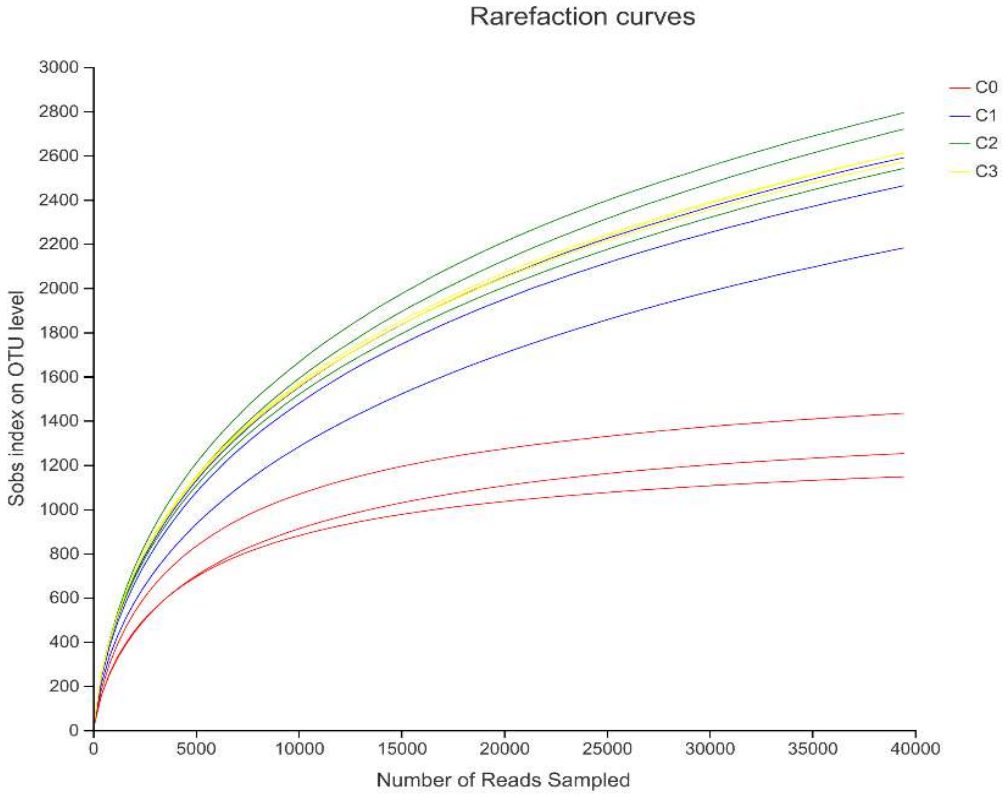


**Figure 1.** Bacterial dilution curve of corn root soil

The Shannon index was selected to characterize the α-diversity of soil microorganisms in root soil (Figure 2), and the difference between different treatments was tested by the index between-group difference test. The results showed that the alpha diversity of maize root bacteria did not reach a statistically significant level between different compounding ratios and the control group, although the bacterial alpha diversity of the compound soil was relatively higher than that of the control group.

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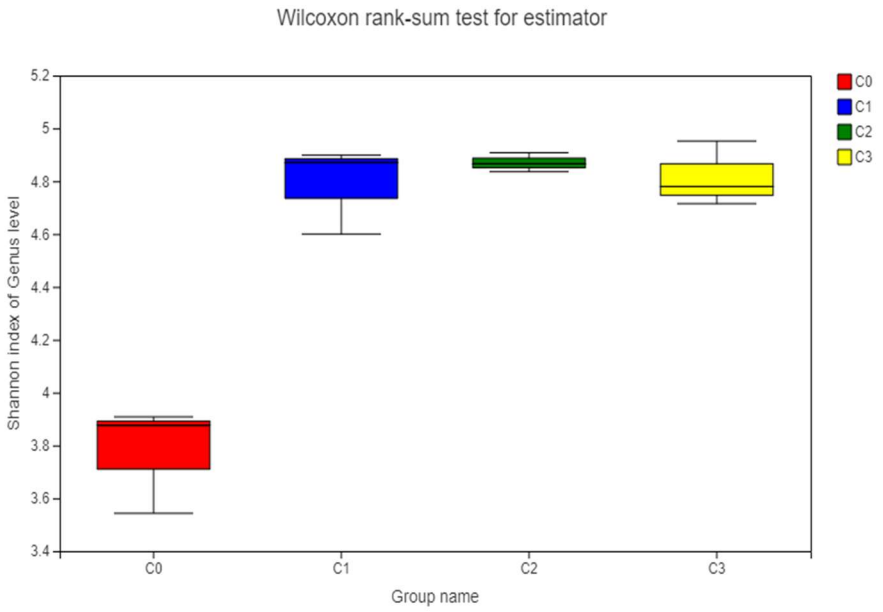


**Figure 2.** Analysis of alpha diversity differences of maize root soil bacteria

The results of this study showed that the number of OTUs in C1, C2 and C3 treatments were 3605, 3822 and 3814, respectively, which were much higher than those in pure sand (2087, C0).

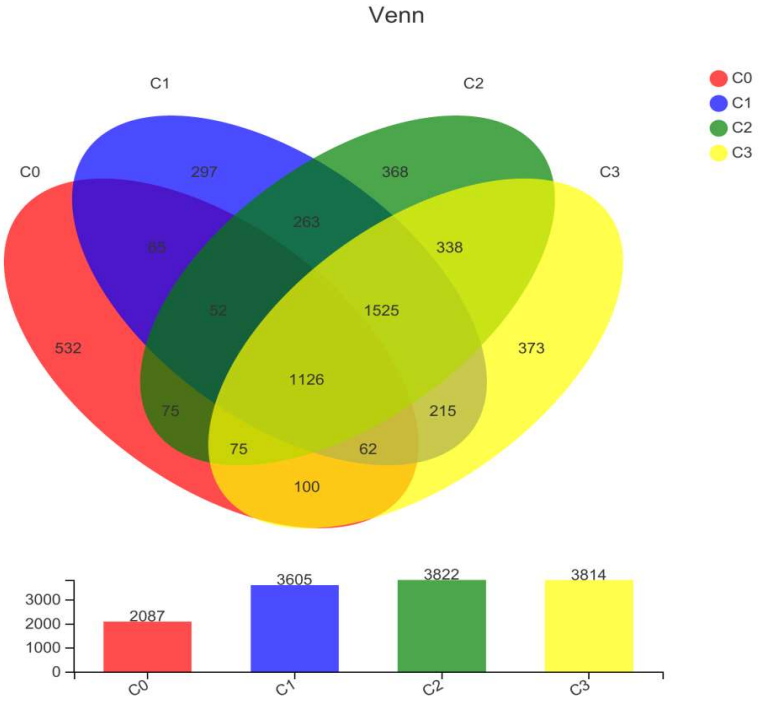


**Figure 3.** Venn diagram of the number of bacterial OTUs in maize root soil with different compounding ratios

Without adding arsenic sandstone (pure sand, A0), the dominant genus was Rhodococcus. After adding arsenic sandstone, the dominant genus in the community changed significantly, and the abundance of Rhodococcus and Arthrobacter decreased, and Rhodococcus disappeared even after the addition of arsenic sandstone. In this study, the Kruskal-Wallis rank sum test method was used to test the hypothesis of species between different groups (or samples) of microbial communities based on the genus level, and to evaluate the significance level of species

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abundance differences. Figure 28 shows that 15 species such as Nocardioides have significant differences among the 4 treatments (all $P < 0.05$).

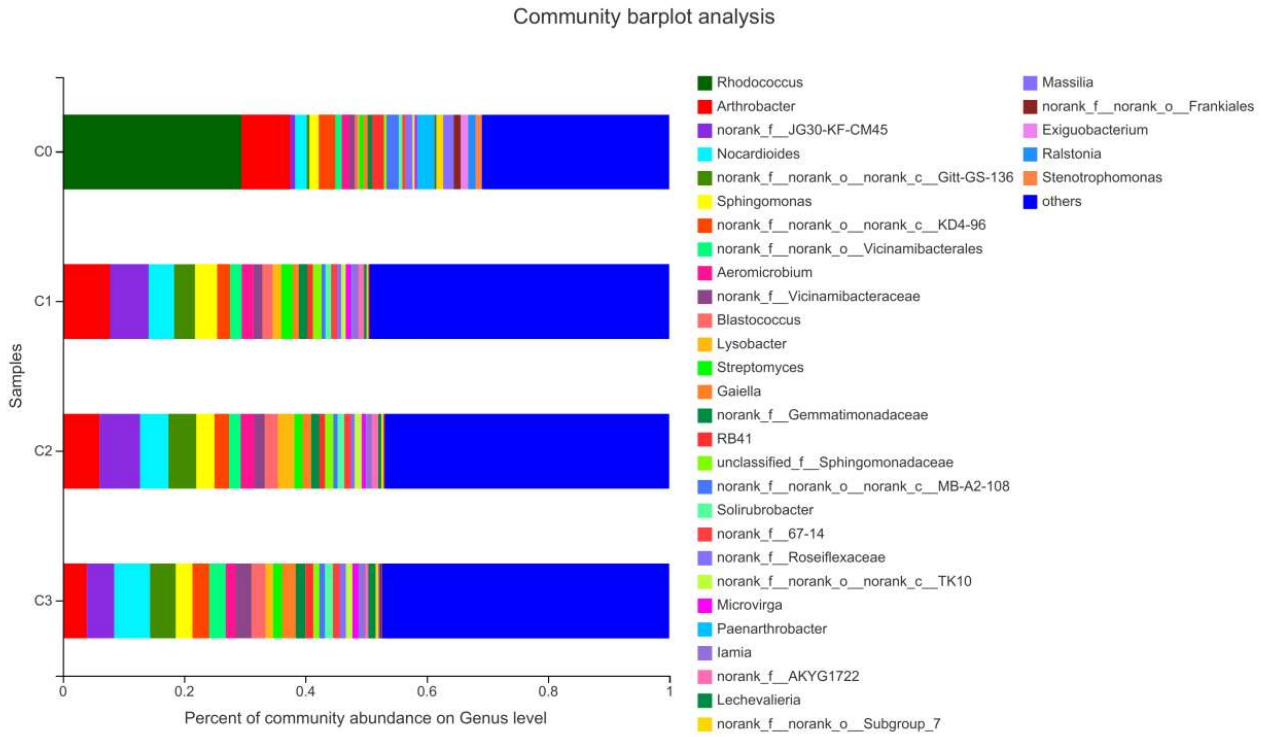


**Figure 4.** Community composition of maize root soil bacteria at different genus levels with different compounding ratios

Figure 5 shows that soil moisture and available phosphorus content were significantly correlated with changes in bacterial communities in maize root soil samples.

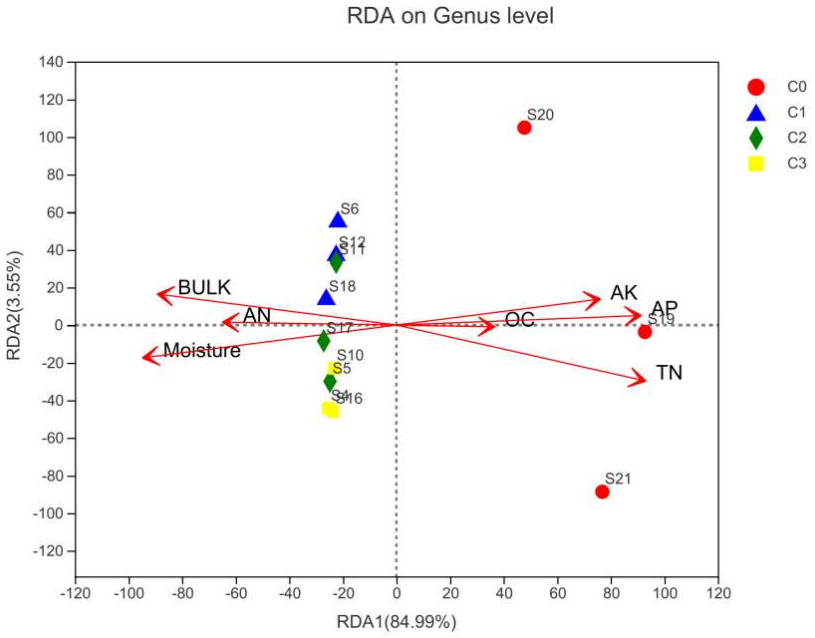


**Figure 5.** RDA analysis of maize root soil bacteria at different genus levels with different compounding ratios

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## 4. Conclusion

1) The dominant bacterial genera were Rhodococcus and Arthrobacter in the pure sand treatment group, but the dominant genera in the community changed to lysobacteria after the addition of soft rock.

2) The difference of the bacterial community α diversity in the different proportions was not significant; but the β diversity was significantly different, and the difference between different compounding ratios was not significant.

3) Soil moisture and available phosphorus content were the main environmental factors affecting the changes of crop root soil bacterial community structure.

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