Study on the Rhizosphere Soil Microbial Community Structure Associated with Five Land Use Types in Jinchuan Mining Area

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Abstract. Five different land use types (desert, farmland, mining park, slag heap and tailing dam) were selected as variables around the Jinchuan Cu-Ni mining area in Jinchang, Gansu Province in the present study. The Atriplex canescens (Pursh) Nutt.'s rhizosphere bacterial abundance, diversity and community composition were examined taking advantage of High-throughput sequencing technology to discuss the effect of soil physicochemical properties on soil microbial community structure. The result indicated that the phylum Proteobacteria and Firmicutes was the most dominant taxon in desert, farmland and mining park, with a high abundance more than 30%. The phylum Proteobacteria was the most dominant taxon in slag heap and tailing dam, with a high abundance more than 40%. The tailing dam had the highest bacterial Chao indexes and the farmland had the highest bacterial Observed species indexes, Shannon indexes and Simpson indexes. Observed species indexes and Shannon indexes between the five sites were significantly different. The redundancy analysis and principal component analysis showed that the main environmental factors caused the different of rhizosphere bacterial community structure in five land use types were Mg, Ca, Cu, TN and moisture, followed by Ni, Cr, K, Pb, Zn content and pH. Hence, the result indicates that land use and soil environmental factors had significant impact on the diversity of soil microbial community structure.

1 Introduction

Soil microbial community is the most significant part of ecosystem and important participant of material circulation and energy flow. Soil quality and nutrient cycling were affected by human activities such as industry, forestry, and agriculture, resulting in the reconstruction of soil microbial communities. The Jinchuan mining area was one of the famous large Cu-Ni sulfide deposits with multitemal symbiosis in the world. Various land use types around the mining area might be affected by different levels of heavy metal pollution. Heavy metals not only change soil fertility, but also interfere with microbial communities, leading to the loss of biodiversity.

Research on soil microbial communities currently focused on land use types, vegetation types, heavy metal stress and nutrient elements which affected their diversity and structure. High-throughput sequencing was widely used to study the impact of environmental factors on microbial communities due to a large amount of biological information resources acquired quickly and a low cost. Previous studies used high-throughput sequencing to analyze combined effects of green manure returning and addition of sewage sludge compost on plant growth and microorganism communities in golden tailings, effects of inoculation with lignocellulose-degrading microorganisms on nitrogen conversion and denitrifying bacterial community during aerobic composting and microbial community diversity of nitrogen-contaminated groundwater[1-3]. Therefore, high-throughput sequencing technology was the most reliable method for exploring the microbial community structural characteristics changes by various types of land use types with complex soil environments and heavy metal pollution near mining areas.

Changes in the composition of contaminated soil microbial communities depended on species replacement. Including, tolerant species replaced sensitive species, resulting in a decrease in biodiversity, which was considered to be one of the main threats to soil. Studies had investigated that exposure to heavy metals could give rise to the proliferation of metal-resistant microbial community, such as Proteobacteria and Firmicutes.
Their community not only depends on the chemical properties of the soil, including pH, organic matter and soil nutrients, but also affects by the toxic heavy metals, K, Ca, Na, and Mg in the soil. Therefore, it was necessary to combine them to investigate and evaluate the sensitivity of soil microorganisms to soil ecosystem protection, but so far, there had been few such studies. Hence, five different land use types (desert, farmland, mining park, slag heap and tailing dam) were selected as variables around the Jinchuan Cu-Ni mining area in Jinchang, Gansu Province in the present study. The Atriplex canescens (Pursh) Nutt. ’s rhizosphere bacterial abundance, diversity and community composition were examined taking advantage of high-throughput sequencing technology to discuss the effect of soil physicochemical properties on soil microbial community structure.

| Site number | Distinction     | Latitude(N)       | Latitude(E)       |
|------------|----------------|-------------------|-------------------|
| A          | Desert         | 38° 20' 52.14"    | 102° 28' 52.14"   |
| B          | Farmland       | 38° 39' 16.23"    | 102° 39' 16.23"   |
| C          | Mining park    | 38° 37' 01.71"    | 102° 37' 01.71"   |
| D          | Slag heap      | 38° 29' 57.5"     | 102° 29' 57.5"    |
| E          | Tailing dam    | 38° 29' 27.6"     | 102° 29' 27.6"    |

2 Methods and Materials

2.1 Soil and plants

The test soil was collected from the Jinchuan mining area and its surroundings (Table 1). A site was located in the desert surrounding the Jinchuan mining area, which was not disturbed by human activities. The soils of C, D, and E site were taken from mining park, slag heap and tailing dam that had been polluted by heavy metals for a long time. The soil of B site was taken from farmland. As one of the dominant species, the Atriplex canescens (Pursh) Nutt. is widely distributed in five sites. Three plants were collected from each site for analysis. For soil sampling, the top soil was shoveled off and the soil below 5cm with the root system was collected into a sterile numbered bags. Rhizosphere soil was obtained by the method of shaking root, and the remaining soil was kept as the bulk soil. Each soil site was divided into two parts, the first part was stored at 4°C for microbial detection and analysis, while, and the others was naturally air-dried for the analysis of soil physical and chemical properties.

2.2 Determination of soil physical and chemical properties

Soil moisture was detected by infrared moisture meter (MS-100, Yuda Electronics Co., Ltd., Harbin, China). Electrical conductivity (EC) and pH were detected by conductivity meter (DDSJ-308A, Leici Electronics Co., Ltd., Shanghai, China) and pH meter (PHS-3E, Leici Electronics Co., Ltd., Shanghai, China), respectively, with a 5:1 water-soil ratio. Total nitrogen (TN) was detected by an automatic azotometer (SKD-100, Peiou Analytical Instrument Factory, Shanghai, China) with Kieldahl's method, while total phosphorus (TP) was detected by ultraviolet and visible spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd., Beijing, China) with molybdenum blue colorimetry. Calcium (Ca) and magnesium (Mg) were detected by titration and the other metallic elements were detected by a flame atomic absorption spectrophotometer (TAS-990F, Beijing Purkinje General Instrument Co., Ltd., Beijing, China).

2.3 DNA extraction, PCR amplification and sequence analysis

Genomic DNA of each soil site was extracted by PowerSoil DNA Isolation Kit (MoBio Co., Ltd.) following the provided procedure. The extracted genomic DNA was used as a template for PCR amplification of the V4 region (515F and 806R) of the microbial 16S rRNA gene. PCR amplification conditions were adjusted to: denaturation at 94 °C for 5 min, then 30 cycles (94°C/30s, 52°C/30s, 72°C/30s), and finally extended at 72 °C for 10 min. The fragment length and concentration of PCR products were detected by 1% agarose gel electrophoresis. Sites with bright main strip in between can be used for further experiments. PCR products were mixed in equidensity ratios according to the GeneTools Analysis Software (Version4.03.05.0, SynGene). Then, mixture PCR products were purified with EZNA Gel Extraction Kit (Omega, USA). Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, USA) following manufacturer’s recommendations and index codes were added. The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an IlluminaHiSeq2500 platform and 250 bp paired-end reads were generated.

2.4 Statistical analysis

Statistical analyses and visualization were performed in SPSS 23.0, Origin 2019b for Windows. Redundancy analysis (RDA) was performed with CANOCO 5.0 to reveal the relationships between bacterial community diversity and environmental properties. The data were mean ± standard error of three repeats.

3 Results and Discussions

3.1 Soil chemical properties and heavy metal concentrations
the relative abundances of these phyla in plots C and D, in which the content of Cu and Ni exceeded Soil environmental quality Risk control standard for soil contamination of agricultural land (GB15618-2018). The Cu content in B and D sites exceeded the national standard, and the Zn content was close to the national standard. The other three sites were not seriously polluted by heavy metals. Moreover, the content of TP, K, Ca, Mg, five heavy metals, pH, moisture and EC showed significant variation among these sites.

Table 2. Soil chemical properties and heavy metal concentrations

| Land use types | A              | B              | C              | D              | E              |
|---------------|----------------|----------------|----------------|----------------|----------------|
| Moisture(%)   | 0.79±0.03c     | 7.19±0.05a     | 2.64±0.09c     | 1.18±0.11d     | 2.96±0.04b     |
| pH            | 8.65±0.10b     | 8.34±0.02e     | 8.52±0.3c      | 8.74±0.12a     | 8.37±0.08d     |
| TN(mg/kg)     | 0.03±0.01c     | 0.08±0.00a     | 0.06±0.01b     | 0.04±0.01bc    | 0.05±0.00b     |
| TP(mg/kg)     | 22.72±0.21a    | 16.95±0.12c    | 8.81±0.07e     | 18.88±0.15b    | 10.5±0.15d     |
| EC(µS/cm)     | 85.09±2.56e    | 539.47±17.21a  | 370.51±11.21b  | 146.14±7.28c   | 121.98±5.36d   |
| K(mg/kg)      | 33.24±1.25c    | 60.55±1.56d    | 89.66±2.01b    | 63.01±1.99c    | 109.53±2.38a   |
| Ca(mg/kg)     | 0.65±0.01c     | 4.07±0.08a     | 1.32±0.03c     | 2.72±0.05b     | 0.75±0.01d     |
| N(mg/kg)      | 0.05±0.00d     | 0.49±0.01a     | 0.05±0.00cd    | 0.06±0.00cd    | 0.12±0.00b     |
| Mg(mg/kg)     | 0.08±0.01e     | 0.95±0.02a     | 0.10±0.01d     | 0.13±0.01c     | 0.28±0.01b     |
| Cu(mg/kg)     | 10.63±0.12d    | 17.72±0.17d    | 113.12±9.53c   | 211.66±7.37b   | 222.1±7.62a    |
| Zn(mg/kg)     | 6.52±0.35e     | 9.85±0.41d     | 156.32±8.65b   | 52.43±2.46c    | 591.2±15.37a   |
| Pb(mg/kg)     | 7.58±0.08e     | 11.62±0.16d    | 31.66±0.31b    | 15.53±0.15c    | 130.42±3.58a   |
| Cr(mg/kg)     | 30.22±0.74c    | 33.43±0.96d    | 42.35±1.27b    | 70.47±1.37a    | 35.50±1.88c    |
| Ni(mg/kg)     | 47.93±2.39e    | 85.12±1.47c    | 104.56±2.18b   | 295.69±7.15a   | 91.17±3.24d    |
| TP(mg/kg)     | 22.72±0.21a    | 16.95±0.12c    | 8.81±0.07e     | 18.88±0.15b    | 10.5±0.15d     |
| PN            | 0.14           | 0.23           | 0.89           | 1.59           | 2.38           |^aDifferent small letters indicate significant differences between sites for that parameter.
^bThe grading standards: PN<1, warning limit; 1≤PN≤2, slight pollution; 2≤PN≤3, moderate pollution; PN≥3, heavy pollution.

3.2 Analysis of microbial diversity and composition of rhizosphere soil

Soil microbial biomass and diversity were influenced by various soil environmental variables[^8]. The soil microbial community compositions at the phylum level in the five sites were shown in Fig. 1, and a total of 15 bacterial phyla were detected. Proteobacteria were dominant bacteria in many types of soils, such as mining area soil, wetland soil, karst soil and farmland soil. The predominant bacterial phyla were Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes in A, B, E sites, which accounted for 77%, 80%, and 87% of the total soil bacteria. The predominant bacterial phyla were Proteobacteria and Actinobacteria in C, D sites, which accounted for 99% and 97% of the total soil bacteria. The dominant microorganisms were relatively abundant in the soil and played an important role in the regulation of ecological functions. Because the envelope of Proteobacteria was mainly composed of lipopoly saccharide, it could protect internal genetic material from external interference, so that it could survive and reproduce in most environments as the dominant bacteria phyla[^1-6]. Additionally, some species were highlighted even though their relative abundances were low, such as Planctomycetes, Verrucomicrobia and Chloroflexi. However, the relative abundances of these phyla varied greatly at the five sites. Interestingly, the relative abundance of Actinobacteria, Bacteroidetes, Acidobacteria, Chloroflexi in the C, D sites were significantly lower than in the other three sites. When heavy metals entered the soil in large quantities, tolerant species increased, microbial diversity decreased and community structure substantially changed[^7]. In present study, compared with the other three land types, the relative abundance of Proteobacteria, the dominant phyla in plots C and D, increased significantly. The relative abundances of Firmicutes, Actinomycetes and Acidobacteria were significantly reduced.

Fig. 1. Soil bacterial relative abundance at phylum level

Four alpha diversity indexes (Chao1, Observed species Shannon and Simpson) were used to evaluate the soil microbial community richness, diversity, coverage, evenness and dominant species status, respectively (Table 3). Chao index ranged from 527.79 to 1987.65. Observed species index ranged from 242.56 to 1068.67. Shannon index ranged from 3.06 to 7.86. Shannon index ranged from 0.77 to 0.98. The soil at E site had the highest Chao indexes, while that at B site had the highest
Observed species, Shannon and Shannon indexes. The contribution rate was significantly different among the five sites.

Table 3. Community diversity analysis of soil rhizosphere microorganisms at phylum level

| Sites | Chao1       | Observed species | Shannon | Simpson |
|-------|-------------|------------------|---------|---------|
| A     | 527.70±188.79c | 242.56±102.04d  | 3.06±0.20d | 0.77±0.01d |
| B     | 1859.93±58.10a  | 1068.67±15.50a  | 7.86±0.08a | 0.98±0.00a |
| C     | 721.82±176.52bc | 347.56±39.64dc | 3.56±0.18c  | 0.81±0.03c |
| D     | 1563.65±58.13bc | 919.60±17.46b  | 6.95±0.09b  | 0.96±0.01ab |
| E     | 1987.65±47.55a  | 1047.11±14.38a  | 7.05±0.12b  | 0.96±0.00b |

Different small letters indicate significant differences between sites for that parameter.

3.3 Redundancy analysis of bacterial community structure and soil properties

The composition of the microbial community in the long-term contaminated soil had changed. However, this change both depended on the soil chemical properties and heavy metals. For this study, the main contributions of heavy metal fractions and soil chemical properties to bacterial communities were analyzed using redundancy analysis (RDA) and principal component analysis (PCA) (Figure 2, Table 4). The first two comprehensive indicators were retained (52.80%, 36.60%), in accordance with the principle that the contribution rate was greater than 80%. The cumulative contribution rate was 89.40%, indicating that fourteen environmental factors could explain the differences in the composition of microbial communities among the sites. The first main component mainly includes the content of Mg, Ca, Cu, TN and moisture. They were the main environmental factors which caused differences in the rhizosphere bacterial community structure of five land use types. Next, the second main component mainly includes the content of Ni, Cr, K, Pb, Zn content and pH. According to the contribution rate, the relative importance of each comprehensive index could be known.

Table 4. Coefficient and contribution of comprehensive indexes

| Principal component | Each comprehensive indexes | Contribution% |
|---------------------|-----------------|---------------|
| moisture, pH, TN, TP, EC, K, Cu, Na, Mg, Ni, Cr, Pb, Zn |  | |
| 1                   | .90 - .58 - .06  | 52.81         |
| 2                   | .41 - .69 - .85  | 36.60         |

The content of K, Ca, Na, Mg and so on, would affect the toxicity of heavy metals in the different land use types[8-9]. Figure 2(a) showed that the relationship between soil bacterial community structure and the content of K, Ca, Na, Mg. The characteristic values of axis 1 and 2 were 0.5837 and 0.0728, respectively. The cumulative interpretation of changes in all microbial community data is 65.65%. The cumulative interpretation of soil environmental factors by change was 100.00%. It contained most information of the microbial community and environmental, which not only explained the factors’ relationship, but also expressed the relationship between the soil bacterial community structure and K, Ca, Na, Mg content. We could easily find that Ca, Na Mg content had significant effects on rhizosphere bacterial community structure. K content had a negative effect on soil bacterial community structure. Among the dominant bacteria phyla, the relative abundance of Proteobacteria and Firmicutes was significantly negatively correlated with the content of Ca, Na, Mg in the soil (P<0.05). The relative abundances of Actinobacteria and Bacteroidetes were significantly positively correlated with the Na content in the soil (P<0.01). Combined Table 2 and Figure 1, as the first principal components, it could be seen that the Ca and Mg content was positively correlated with Actinobacteria, Bacteroidetes, Acidobacteria and Chloroflexi relative abundance. Interestingly, the relative abundance was the higher in B and E sites with higher Ca content. The relative abundance of Chloroflexi and Actinobacteria was the highest in the E site with the highest Ca content. The relative abundance of Actinobacteria and Bacteroidetes was the higher in A, B and E sites with higher Mg content. It could be seen that the Ca and Mg content was positively correlated with Planctomycetes relative abundance. Moreover, the relative abundance of Planctomycetes was the lowest in the C site with the lowest Ca content. This was similar to the results of previous studies[10-11].

Figure 2(b) showed that the relationship between soil bacterial community structure and the content of moisture, pH, TN, EC, TP and TN. The characteristic values of axis 1 and 2 were 0.7992 and 0.0726, respectively. The cumulative interpretation of changes in all microbial community data is 87.18%. The cumulative interpretation of soil environmental factors by change was 99.99%. We could easily find that pH had significantly positive effects on the relative abundance of Proteobacteria and Firmicutes, and had significantly negative effects on the relative abundance of Actinobacteria, Bacteroidetes, Acidobacteria and Chloroflexi. It was the same as TP, without significantly correlation with the phylum bacteria. Moisture was negatively related to the relative abundance of Firmicutes (P<0.05). TN was negatively related to the relative abundance of Firmicutes, and was positively correlated with the proportion of Bacteroidetes and Actinobacteria.
related to the relative abundance of Chloroflexi (P<0.05). Combined Table 2 and Figure 1, as the first principal components, it could be seen that the relative abundance of Proteobacteria and Firmicutes was the highest in C sites with lowest moisture, meeting the negative correlation between them. Moisture was significantly positively related to the relative abundance of Actinobacteria and Chloroflexi (P<0.05). Although it had no significant correlation with Acidobacteria, Bacteroides, Chloroflexi and Verrucomicrobia, they were positively correlated. In addition, their relative abundance was also the highest in the E example with the highest moisture.

Figure 2(c) showed that the relationship between soil bacterial community structure and the content of Cu, Cr, Ni, Zn, Pb. The characteristic values of axis 1 and 2 were 0.7949 and 0.0729, respectively. The cumulative interpretation of changes in all microbial community data is 86.78%. The cumulative interpretation of soil environmental factors by change was 100.00%. We could easily find that among the dominant bacterial, the relative abundance of Proteobacteria was significantly negatively related to the content of Cu (P<0.05), and the relative abundance of Proteobacteria was negatively related to the content of Ni, Cr, Pb, Zn. The relative abundance of Firmicutes was negatively related to the content of Cu (P<0.05), and the relative abundance of Firmicutes was positively related to the content of Ni, Cr, Pb, Zn. This was the opposite of the condition of Bacteroidetes. The relative abundance of Actinobacteria was positively related to the content of Cu (P<0.01). In addition, the relative abundance of Planctomycetes was also the highest in the C site with the highest Cu content, indicating that it had a better adaptability to Cu pollution.

4 Conclusions

(1) Proteobacteria and Firmicutes were dominant bacteria in the five land use types, accounting for more than 40% and 30%, respectively.

(2) The tailing dam had the highest Atriplex canescens (Pursh.) Nutt.'s rhizosphere bacterial richness, and the farmland had the highest bacterial diversity, coverage, evenness and dominant species status. Observed species indexes and Shannon indexes between the five sites were significantly different.

(3) The main environmental factors which caused differences in the rhizosphere bacterial community structure of five land use types, were the content of Mg, Ca, Cu, TN and moisture, followed by the content of Ni, Cr, K, Pb, Zn and pH.

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