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 multimetal 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 changed 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*.

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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.

Table 1. Site code and latitude and longitude information

Site number	Distinction	Latitude(N)	Latitude(N)
A	Desert	38° 28' 52.14"	38° 28' 52.14"
B	Farmland	38° 39' 16.23"	38° 39' 16.23"
C	Mining park	38° 37' 01.71"	38° 37' 01.71"
D	Slag heap	38° 30' 57.5"	38° 30' 57.5"
E	Tailing dam	38° 29' 27.6"	38° 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 shovel 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℃ 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 ℃ for 5 min, then 30 cycles (94℃/30s, 52℃/30s, 72℃/30s), and finally extended at 72 ℃ 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 soil moisture content of five sites was all at a relatively low level, and belong to alkaline soil (8.34-8.74, Table 2). The overall pollution of the five metals was evaluated using the Nemerow Pollution Index (PN). The content of TN, EC, TK, Ca, Mg, and Na was the highest in B site. E site had the highest PN, achieving moderate pollution, 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±0.03e	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±0.03c	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	10.50±0.13d
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±1.25e	60.55±1.56d	89.66±2.01b	63.01±1.99c	109.53±2.38a
Ca(mg/kg)	0.65±0.01e	4.07±0.08a	1.32±0.03c	2.72±0.05b	0.74±0.01d
Na(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±0.12e	17.72±0.17d	113.12±9.51c	211.66±8.37b	222.31±7.62a
Ni(mg/kg)	6.52±0.35e	9.85±0.41d	156.32±8.65b	52.43±2.46c	591.20±15.37a
Cr(mg/kg)	7.58±0.08e	11.62±0.16d	31.66±0.31b	15.54±0.0.15c	130.42±3.58a
Pb(mg/kg)	30.22±0.74e	33.43±0.96d	42.35±1.27b	70.47±1.37a	35.50±1.88c
Zn(mg/kg)	47.93±2.39e	85.12±1.47c	104.56±2.18b	295.69±7.15a	91.17±3.24d
PN	0.14	0.23	0.89	1.59	2.38

[a]Different small letters indicate significant differences between sites for that parameter.
[b]The 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[4].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 (relative abundance>9%) 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[5-6]. 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.

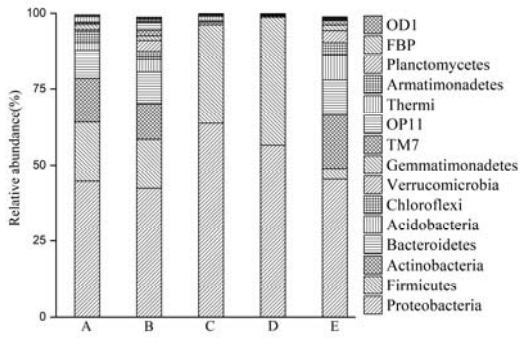

Fig. 1. Soil bacterial relative abundance at phylum level

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. Observed species and Shannon indexes were 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	527.79±188.79c	242.56±102.04d	3.06±0.29d	0.77±0.01d
B	1859.93±58.10a	1068.67±13.50a	7.86±0.08a	0.98±0.00a
C	721.82±170.02bc	347.56±39.64c	3.56±0.18c	0.81±0.03c
D	1686.95±58.15b	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

[a]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	Ca	Na	Mg	Cu	Ni	Cr	Pb	Zn	
1	.90	-.58	.87	.06	.76	.55	.91	.86	.92	.87	.51	.533	.68	.66	52.81
2	-.41	.69	-.46	-.85	-.36	.79	-.30	-.49	-.37	.43	.82	.80	.63	.65	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, 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

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, this is consistent with previous studies[12]. The relative abundance of *Planctomycetes* was significantly 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.

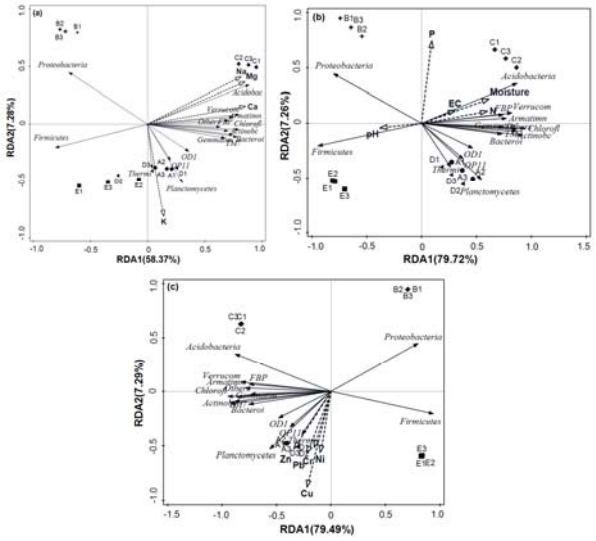

Fig. 2. Redundancy analysis for bacterial and environmental factors at phylum level

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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