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SOIL CHARACTERISTICS AND MICROBIAL RESPONSES IN POST-MINE RECLAMATION AREAS IN A TYPICAL RESOURCE-BASED CITY, CHINA

Min TAN[1, 2], Xu ZHOU[3], Gang LI[1, 2], Mengyu GE[1], Zhuang CHEN[1, 2], Junfeng QU[1, 2*]

[1]Jiangsu Key Laboratory of Coal-Based Greenhouse Gas Control and Utilization,
China University of Mining and Technology, Xuzhou, China
[2]Xuzhou Institute of Ecological Civilization Construction, Xuzhou, China
[3]Land Consolidation and Rehabilitation Center, Ministry of Natural Resource, Beijing, China

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Highlights

- Soil characteristics and microbial responses were detected in a resource-exhausted coal city.
- Considerable differences were found in microbial community structure at the class, order, family and genus levels.
- Soil physicochemical properties played vital roles in microbial community composition.

Abstract. Mining activities worldwide have resulted in soil nutrient loss, which pose risks to crop and environmental health. We investigated the effects of post-mine reclamation activities on soil physicochemical properties and microbial communities based on 16S rRNA sequencing and the further statistical analysis in the coal base in Peixian city, China. The results revealed significant differences in soil microbial relative abundance between reclamation and reference soils. *Proteobacteria* was the most abundant phyla in all seven mine sites regardless of reclamation age while considerable differences were found in microbial community structure at other levels among different sites. Notebly, *Gammaproteobacteria*, member of the phylum *Proteobacteria*, had relatively high abundance in most sites. Furthermore, Kendall's tau-b correlation heatmap revealed that potentially toxic elements and other physicochemical properties play vital roles in microbial community composition.

Keywords: reclamation, mine soil, time series, physicochemical properties, community diversity, microbial community.

Introduction

Underground mining disrupts the soil and results in the destruction of soil microbial populations and the nutrient cycles that are crucial for sustaining a healthy ecosystem. There can be multiple effects of coal mining subsidence, such as water pollution, soil degeneration, geological disasters, biodiversity losses, and ultimately a loss of local economic wealth (Jing et al., 2018; Qu et al., 2017). The topsoil in particular is damaged during mineral extraction (Xiao et al., 2011). The process of reclamation of abandoned mine land is very complex. Mining companies must ensure that post-mining subsidence lands return to a level of productivity as close as possible to its pristine condition and that they continue as a self-sustaining ecosystem. In other words, it is optimal that the productivity of the affected land is returned to its pre-mining status. Reclamation efforts to ensure the beneficial use of land resources are vital and essential. Soil remediation efforts require the management of all types of physical, chemical, and biological soil properties, including soil water content, fertility, microbial community, and various soil nutrient cycles (Cheng et al., 2018).

In general, the methods for reclaiming subsidence areas after coal mining include coal gangue filling and reclaiming technology, hydraulic dredge pump reclamation technology, and land leveling technology (Qu et al., 2018). Each method has its advantages and disadvantages. Unfortunately, sourcing local soil for coal gangue filling is often impossible, and districts are therefore incapable of supporting the complete reclamation of a collapsed area. And previous study has found that the coal gangue filling governance model can cause secondary pollution (Wang et al., 2015). Although the hydraulic dredge pump

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*Corresponding author. E-mail: *sty2016@cumt.edu.cn*

reclamation method is considered to be a high-quality option with low costs, this reclamation method is likely to cause soil salinization because of the high level of soil water content caused by the pump fillings. Given these limitations, the land leveling reclamation method is considered to be more economical and efficient in some subsidence areas. Land leveling works the soil to regularize the slopes of subsidence lands in order to return affected land into areas suitable for agriculture. Therefore, the land levelling reclamation area in Peixian city was selected as research area in this study. Research on reclaimed soil physical and chemical properties has been well represented in coal gangue filling reclamation areas and hydraulic dredge pump reclamation areas (Shrestha & Lal, 2011; Ussiri & Lai, 2008). However, there is a clear knowledge gap surrounding the development of soil nutrients in abandoned mine lands after land leveling.

Despite the small percentage of the total soil mass that microorganisms account for (0.5% w/w) (Yan et al., 2015), soil microbes have an important role in soil energy flow and nutrient cycling (Anderson & Domsch, 1980), including nitrogen fixation, oxidation, and other processes (Amato & Ladd, 1994). Moreover, microorganisms are more sensitive to environmental stresses, indicating that they can be used as an early warning index to signal disruptions in ecosystem health and function (Rosenfeld et al., 2018). Elevated concentrations of soil nutrients have also been measured in reclaimed soils, though how the soil microbial community is interacting with, or is influenced by, this effect is unclear. Microbial community dynamics may alter nutrients transformations and distributions in soils, but to date the previous studies investigating microbial communities in reclamation mine areas have focused on communities in hydraulic dredge pump reclamation areas and other reclamation soils (Li et al., 2014); the resident microbial communities in land leveling reclamation area soils remain completely uncharacterized.

Soil physicochemical and biological properties changed over time after reclamation. Mukhopadhyay et al. (2014) found that the MBC of the soil exceeded the levels of natural forest land after 17 years of reclamation in the North Karanpura area of India. Gorzelak et al. (2020) sequenced fungal and bacterial communities in the topsoil stored for one to ten years and reference soils in Western Australia. The results indicated that fungal and bacterial richness declined, but that the fungal community returned to a state similar to reference soils, whereas the bacterial community did not. These changes emphasized the importance of considering soil age during restoration.

Mining activities in the coal base overlaying Peixian city, China, have resulted in large scale soil subsidence (Liu et al., 2018). We measured community structure, diversity indices and soil physicochemical properties in Sanhejian Mine. The goal of the current study was to determine whether (i) microbial communities in land leveling soils were distinct from communities in unmined soils (ii) soils with varying nutrient concentrations and potentially toxic elements host different microbial communities and (iii) different reclamation soils host unique microbial communities, or if similar communities were present in all reclamation soils. This survey will fully reveal the ecological responses of soil microbial community to reclamation activities. And it might provide a new perspective for the ecological assessment of the post-mine reclamation areas.

1. Materials and methods

1.1. Study area

Soil samples were obtained from the Sanhejian Mine in Peixian city, Jiangsu province, eastern China. The city area (116°41′–117°09′E, 34°28′–34°59′N) has a fragile ecological environment with a semi-arid continental and temperate continental climate, with a mean annual precipitation of 776 mm, of which approximately 67.5% falls between June and September. The annual mean temperature is 14.2 °C.

Peixian city has rich coal resources, with total proven resources of 2.37 billion tons (Liu et al., 2018). By the end of 2017, the total area of land subsidence in Peixian city was about 14,029 ha, occupying approximately 7.75% of the entire administrative area. The groundwater level ranged from 2.0 m to 3.0 m. Land leveling had been broadly used in the destroyed land with a subsidence depth less than 2.0 m. In recent years, Peixian city has focused on remediation of coal mining subsidence areas and the ecological environment has been improved. It has won the 2018 United Nations Human Settlements Award. The experience of coal mining subsidence in Peixian city was also being widely promoted.

The Sanhejian Mine began mineral extraction in 1988, with an annual output of 2.1 million tons of raw coal. The rapid development of the local urban and peri-urban areas has heavily relied on the mining industry. An increase in a concern for the environment has made reclamation of subsidence lands an integral feature in this landscape. In order to return the land as closely as possible to its pristine condition, developers have used different reclamation strategies in recent years. By the end of 2016, the total reclaimed land area was 426 ha in the Sanhejian Mine. Reclaimed soil was used for agricultural purposes using soy-wheat rotations.

1.2. Soil sampling

Soil samples were obtained in September 2017 from seven locations (six previously mined and land levelling reclaimed locations R07, R08, R12, R13, R16 and R17 plus one undistributed reference location CK1) at the Sanhejian Mine (Table 1). Two aspects were mainly considered when selecting the suitable sampling sites. On the one hand, the reclamation time can provide a complete time series. On the other hand, the topography, soil type and water conservancy are similar among the plots. The soil samples were used for geochemical and microbial community analysis. Three sampling points were established for each plot. And at each point, three random, sub-samples of 0–30 cm deep soil cores were collected to make a composite sample. Vegetation litter was moved prior taking the samples without altering the surface soil. The samples were sealed in polythene bags and brought to the laboratory for soil properties analysis. Composite soil samples were divided into four portions in the laboratory. The first portion was stored at –80 °C prior to molecular analysis. The second portion was oven-dried at 105 °C for 6 h to calculate soil water content. The third portion was air-dried, sieved through 2 mm mesh for a soil pH and chemical analyses. The last portion was stored at 4 °C for future analysis.

Table 1. Description of samples taken in Peixian, China

Samples	Years of Reclamation	No. of years after reclamation	Coordinates
CK1	Reference site	–	N116°46′01″, E34°53′23″
R07	Reclaimed in 2007	10	N116°46′29″, E34°53′00″
R08	Reclaimed in 2008	9	N116°49′10″, E34°54′27″
R12	Reclaimed in 2012	5	N116°47′16″, E34°54′01″
R13	Reclaimed in 2013	4	N116°46′56″, E34°53′53″
R16	Reclaimed in 2016	1	N116°49′34″, E34°53′36″
R17	Reclaimed in 2017	0	N116°46′52″, E34°54′24″

1.3. Analysis methods

1.3.1. Physicochemical analysis

Soil water content was measured by oven-drying at 105 °C for 24 h. Soil pH was measured in water with a ratio of 1:2.5 w/w using a pH meter (Sartorius PB-10, Germany). Soil organic carbon (SOC) was analyzed using the potassium dichromate oxidation–ferrous sulphate titrimetric method (Yeomans et al., 1988). Soil available nitrogen (AN) was measured by the alkali N-proliferation method. The concentration of available phosphorus (AP) was determined using the Olsen method (UV-2550, UV-Visible Spectrophotometer, Shimadzu, Japan). Soil available potassium (AK) was measured by the 1 mol L-1 neutral NH_4OAC method (iCAP 6300 ICP-OES Spectrometer, Thermo Scientific, USA). The total concentrations of potentially toxic element (cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As)) were determined by an inductively coupled plasma-mass spectrometer.

1.3.2. Molecular analysis

The soil samples were analyzed using a high-throughput sequencing analysis to compare the microbial community diversity and composition in the reclaimed regions to those in the subsided areas. We used a MOBIO Power Soil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) to extract the genomic DNA of soil samples according to the manufacturer's instructions for a further Illumina genome assay. Soils samples were added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurred by mechanical and chemical methods. Then total genomic DNA was captured on a silica membrane in a spin column format and it was washed and eluted from the membrane at last. The V4 region of microbial 16S rRNA genes was amplified using the forward primer 515F (CCGGACTACHVGGGTWTCTAAT) and reverse primer 806R (GTGCCAGCMGCCGCGGTAA). The barcode sequence of R07, R08, R12, R13, R16, R17 and CK1 were AGTACTGCAGGC, ACTGATCCTAGT, AGCGAGCTATCT, AGAGTCCTGAGC, AGTACGCTCGAG, AGCGACTGTGCA and ACGTTAGCACAC, respectively.

The PCR amplification product went into a Bio-Rad S1000 thermal cycler (Bio-Rad Laboratory, CA). Briefly, the first step was initial denaturation at 94 °C for 5 min, followed by a second step of 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 30 s. The third step was extension at 72 °C for 10 min and then stopped with a hold at 4 °C. Three repeated tests were performed for each sample and then mixed into one PCR product. The samples were evaluated using a 1.0% agarose electrophoresis and purified using the EZNA Gel Extraction Kit (Omega Kit). The resulting material was sequenced on the Illumina Hiseq 2500 platform at Magigene Company (Guangzhou, China). The DNA library was built as recommended by the manufacturer for the NEB Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs, USA).

The low-quality raw reads that were shorter than 20 bp or contained ambiguous bases were trimmed with comprehensive bioinformatics software, Trimmomatic (V0.33), before high throughput sequencing data analysis. In addition, the barcode and primer were moved in the Mothur platform (V1.35.1) to obtain the paired-end clean reads (Schloss et al., 2009). The two clean sets were then processed to include Illumina tags using Flash software. The raw tags from the Flash platform were checked with quality control procedures to get clean tags for further analysis.

Quality tags were clustered into Operational Taxonomic Units (OTUs) in the Usearch platform (V8.0.1517) at the 97% similarity level, based on the uparse cluster method (Edgar, 2010). The highest occurrence frequency sequences of OTUs were selected as the representative sequence. The taxonomy was then assigned based on the Ribosomal Database Project (RDP) classifier and the python script of Qiime with 0.5 similarity (Caporaso et al., 2010). Nonparametric indicator indices of microbial diversity (Chao1, Simpson, Shannon) were analyzed across the soil samples using Qiime. Chao 1 is a richness estimator, which corrects the observed richness by adding a term based on the abundances of OTUs represented by singletons and doubletons (Chao & Bunge, 2002). The Simpson index and the Shannon index indicate the species richness and diversity. All sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database under BioProject number PRJNA515548, BioSample numbers SAMN10761821 to SAMN10761830.

1.4. Statistical analysis

The correlations between soil physical and chemical properties and microbiological characteristics were performed using the Kendall's tau-b correlation in SPSS (version 19.0). And the cluster analysis method was used to emphasize the heterogeneity and homogeneity among the elements. The statistical comparisons and plotting of all three community -diversity metrics were carried out in MATLAB.

2. Results and discussion

2.1. Basic physicochemical properties

The descriptive statistic for the physicochemical properties in different soil samples were shown in Table 2. Similar to other studies on the soil pH in the mining areas with high phreatic water levels in eastern China (Li et al., 2015a; Sheoran et al., 2010), the soil pH was alkaline in our study. This can be attributed to many factors, including the climatic feature, soil's parent material, soil base cations, organic matter accumulation, and the organic acids released from decomposition processes (Pataki et al., 2006). Specifically, Peixian City is located north of the Yangzi River, where rainfall is low, eluviation is weak, and the basic ion content in soil is high. In addition, the soil parent material in the area is yellow moist soil, which has a low organism content and high free calcium carbonate content. Therefore, soils in this district tend to be alkaline. However, we failed to find any obvious regularity between the soil pH and the reclamation age in this study, which was inconsistent with other studies (Clough & Skjemstad, 2000; Deng et al., 2016). The lack of correlation is probably because the soil in the mining area has a site-specific pH level inherited directly from parent soil material, as parent material is the most important factor for determining soil pH level (Yarwood et al., 2015). However, the effect of the reclamation age on soil pH cannot be overlooked. In other words, soil pH level may remain stable under natural farming conditions over a short period of time, but obvious patterns with the reclamation age might be found if the time series is long enough (Xie et al., 2020).

We found that soil water content was at a lower level under the same cultivation and management conditions in the R13, R16 and R17 sites. This may be attributed to the destruction of reclaimed soil structure caused by soil compaction, which affects the capacity of soil water transmission and retention. This is consistent with the fact that the exploitation of mineral resources often results in the variation of bulk soil moisture content (Sheoran et al., 2010).

Many factors jointly determine the change in SOC with the reclamation age, such as soil management practices, parent soil material, and the influence of plants and microorganisms (Clough & Skjemstad, 2000; Li et al., 2015a; Pataki et al., 2006). In this study, the concentration of SOC in CK1 was higher than that of the reclaimed plots, which can be explained by the loss to the atmosphere of a large amount of SOC in the reclaimed land (Deng et al., 2016). Moreover, SOC content increased constantly with the reclamation age. And SOC concentrations reached 88.92% of that of the non-mined site over the 10 years following reclamation (Table 2).

The AN content in the CK1 was found significant higher than the other reclamation ages. And the AN content did not significant differ with the reclamation ages. Similar to the AN, no obvious regularity between the AP and AK content and the reclamation age was found in our study.

2.2. Potentially toxic element contamination level

All of the potentially toxic element concentrations do not exceed the China Soil Environmental Quality Risk Control Standard for Soil Contamination of Agricultural Land (GB15618-2018) (China Ministry of Ecology and Environment [CMEE], 2018). However, all the potentially toxic element concentrations exceeded the mean elemental concentrations throughout Jiangsu Province except AS. With the analysis of the potential toxic element concentrations and the reclamation ages, the method of quadratic polynomial fitting was used. The results were shown in Figure 1. It indicated that the elements concentration decreased with the reclamation ages except Zn. This phenomenon might be due to the absorption and transfer of heavy metals by vegetation. The highest Zn contamination was found in R12. It was largely because of the application of more commercial zinc fertilizer in R12 than other mined soils.

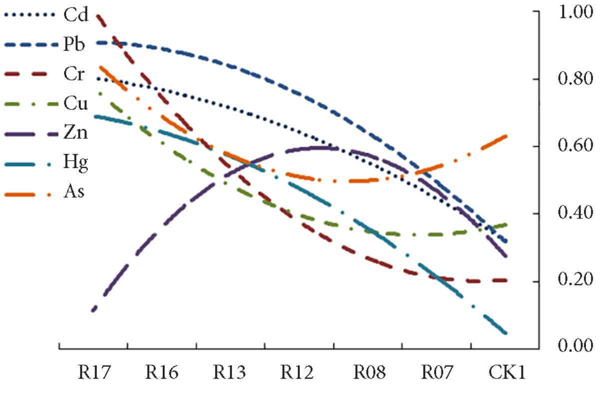


Figure 1. Changes of the potentially toxic element concentrations with the reclamation ages

Table 2. Physical and chemical properties in soils collected from the Sanhejian Mine

Sample ID	Ph	Water Content (%)	SOC (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Hg (mg/kg)	As (mg/kg)
CK1	8.09±0.04	25.73±2.57	10.43±0.99	192.44±6.07	19.4±1.87	119.75±4.88	0.11±0.02	15.81±0.82	52.01±8.12	17.48±0.96	48.03±1.64	0.02±0	7.46±0.3
R07	8.15±0.04	21.67±1.9	9.47±0.8	59.27±3.95	12.57±2.98	70.58±12.44	0.09±0.02	13.5±0.99	49.16±0.9	19.59±1.37	69.03±4.36	0.02±0.01	8.3±0.1
R08	8.04±0.08	30.35±0.85	9.22±0.65	90.07±2.35	24.02±7.41	101.32±2.82	0.12±0.01	17.33±0.57	55.9±2.67	17.3±1.71	55.31±2.92	0.03±0	8.43±0.5
R12	8.14±0.1	32.63±2.71	8.67±0.15	62.01±6.98	40.32±7.61	50.38±3.98	0.12±0.01	17.37±0.76	48.83±1.06	18±0.57	62.62±2.74	0.02±0.01	6.87±0.71
R13	8.12±0.11	19.72±1.16	7.82±0.45	73.68±0.95	17.7±6.73	151.33±12.5	0.13±0.01	17.63±1.03	58.75±7.73	19.3±0.71	56.43±3.58	0.03±0.01	7.88±0.42
R16	7.95±0.07	22.18±2.38	4.93±0.58	64.42±6.78	9.21±0.89	96.77±13.38	0.1±0.01	16.28±1.2	57±2.93	17.57±0.61	52.26±2.11	0.02±0.01	7.82±0.44
R17	8±0.07	25.76±2.91	3.37±1.07	60.6±3.94	8.68±0.93	47.98±4.48	0.13±0.01	17.9±1.99	61.03±2.25	19.64±0.83	53.57±3.36	0.03±0	8.55±0.4

Note: Abbreviations: soil organic carbon (SOC); available nitrogen (AN); available phosphorus (AP); available potassium (AK).

2.3. Differences in microbial diversity

The average ratio of assigned sequences to clean sequences was 80.55%, indicating that there was a high coverage of sequencing. The ratio range of assigning to the genus of the soil samples was from 18.82% to 46.30%. A higher ratio also indicated the OTU annotation of the sample was better; the lower ratio showed species complexity of the samples was higher.

All three indices (Shannon diversity index, Chao 1, Simpson diversity index) based on OTU data were made of the species present at a 3% dissimilarity level. Refraction curves of the Chao 1 and Shannon indices showed distinct patterns in microbial richness and diversity (Figure 2). Both the sets of curves approached plateaus, reflecting that further sequencing would cause only a small amount of new species.

As shown in Figure 2, both the Shannon index and the Simpson index revealed similar trends. The value of the R12 sample was higher than that of other samples, indicating that microbial diversity and richness were higher. Chao 1 diversity ranged from 2769 to 8141, with an average value of 6310. In the Sanhejian Mine, all three indices in most mined samples were lower compared to the unmined soil, though there was an exception in R12.

The microbial communities in land leveling soils had distinctly lower abundances and diversity when compared to the unmined soils except R12. There might be two reasons. First of all, R12 had higher levels of soil water content, TP concentration, and Zn element concentration than other samples, which have a positive impact on the activity of microbiota. Second, the As concentration was the lowest. It had a relatively weak inhibitory effect on microorganisms. Quadros (Quadros et al., 2016) observed significant losses in bacterial community diversity after coal mine reclamation. Microbial communities are known to be highly dependent on local environmental conditions, including soil properties and vegetation types, which depend on the length of post-reclamation time and agricultural land management (Yarwood et al., 2015). Some studies (Bier et al., 2015; Lewis et al., 2012; Mummey et al., 2002) have identified that soil might have experienced a persistent and perhaps irreversible shift as a result of mining.

2.4. Changes of bacterial community in different sampling sites

In total, sequences were allocated to 55 different phyla, 164 classes, 240 orders, 261 families, and 346 genera. The major phyla with relative abundances greater than 4% were *Proteobacteria* (38.25%), *Firmicutes* (13.91%), *Crenarchaeota* (9.12%), *Acidobacteria* (7.49%), *Bacteroidetes* (6.35%), and *Planctomycetes* (5.51%), accounting for 80.62% of the total defined phyla across all sites. The relative abundance of dominant phyla for each sample is shown in Figure 3a. Regardless of reclamation age, *Proteobacteria* was the most abundant phyla in all seven mine sites, with a relative abundance of 33.10%, 39.66%, 33.77%, 33.27%, 50.39%, 44.18% and 33.37% for the R07, R08, R12, R13, R16, R17 and CK1 samples, respectively, which was in agreement with other studies (Li et al., 2015b; Rastogi et al., 2010). *Proteobacteria* is a ubiquitous and prevalent phylum in soil, which has the largest proportion in terms of both quality and variety (Zhang et al., 2016).

Different patterns were observed at the class level (Figure 3b). The most dominant classes in land leveling soils were altered compared to unmined soil in the mine. To be specific, the most abundant class was *Gammaproteobacteria* in R07, R08, R12, R16 and R17, while *Thaumarchaeota* was the most abundant class in CK1 and R13. At the order level, *Nitrososphaerales* made up 12.32%, 10.82% and 16.88% of the total bacterial communities in CK1, R07, and R13 while *Bacillales* was more prevalent in younger sites, accounting for 39.98% in R16 and 25.31% in R17 (Figure 3c). The relative abundance of the dominant family was consistent among the older reclamation samples and unmined sample while there was a different relative abundance in the younger reclamation samples. *Nitrososphaeraceae* was the most abundant family in older reclamation samples (CK1, R07, R08 and R13) while the dominant family in younger samples (R16 and R17) was *Exiguobacteraceae* (Figure 3d). The difference among these plots may be partially due to a difference in the microbial communities caused by the different reclamation years.

At the genus level, seven predominant genera were obtained in reclaimed and subsided soils (Figure 3e).

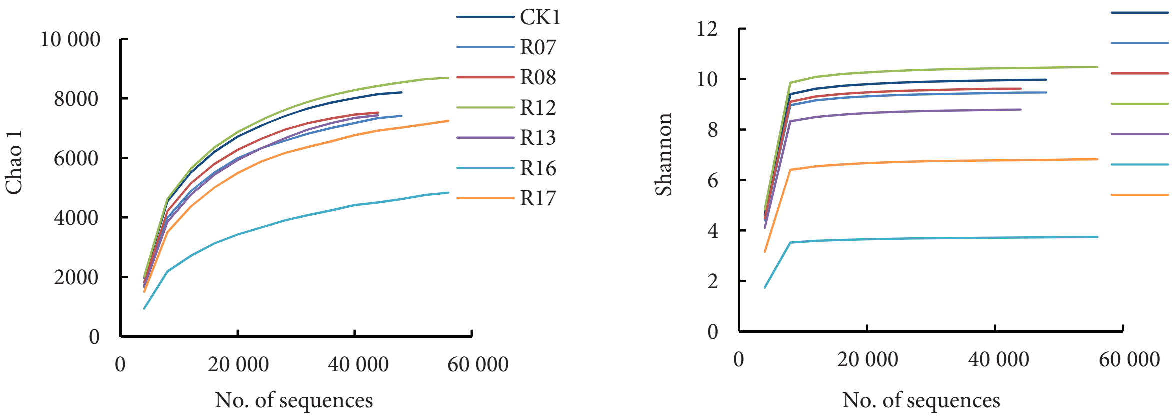
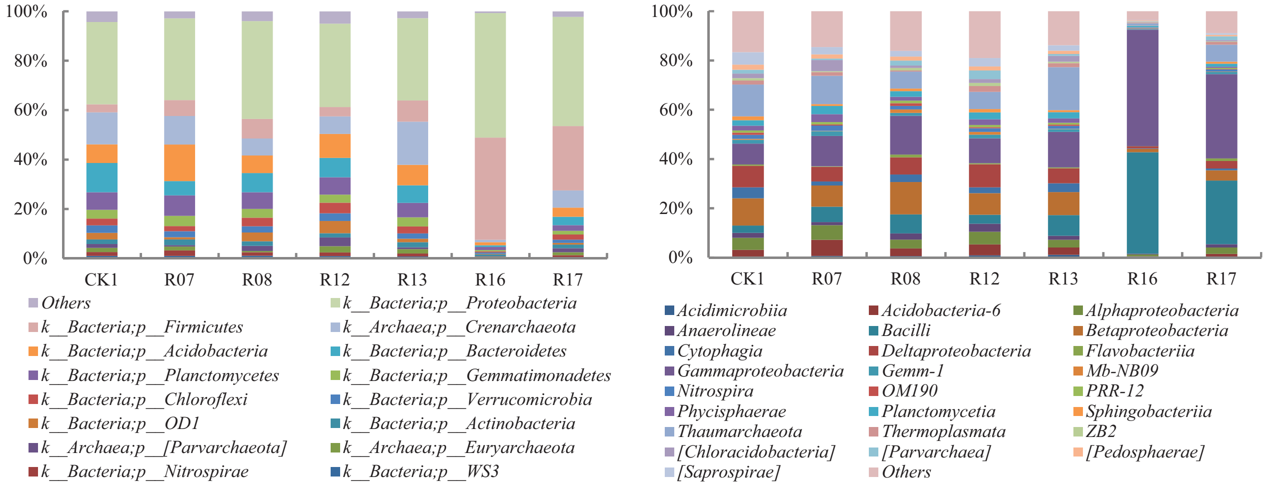
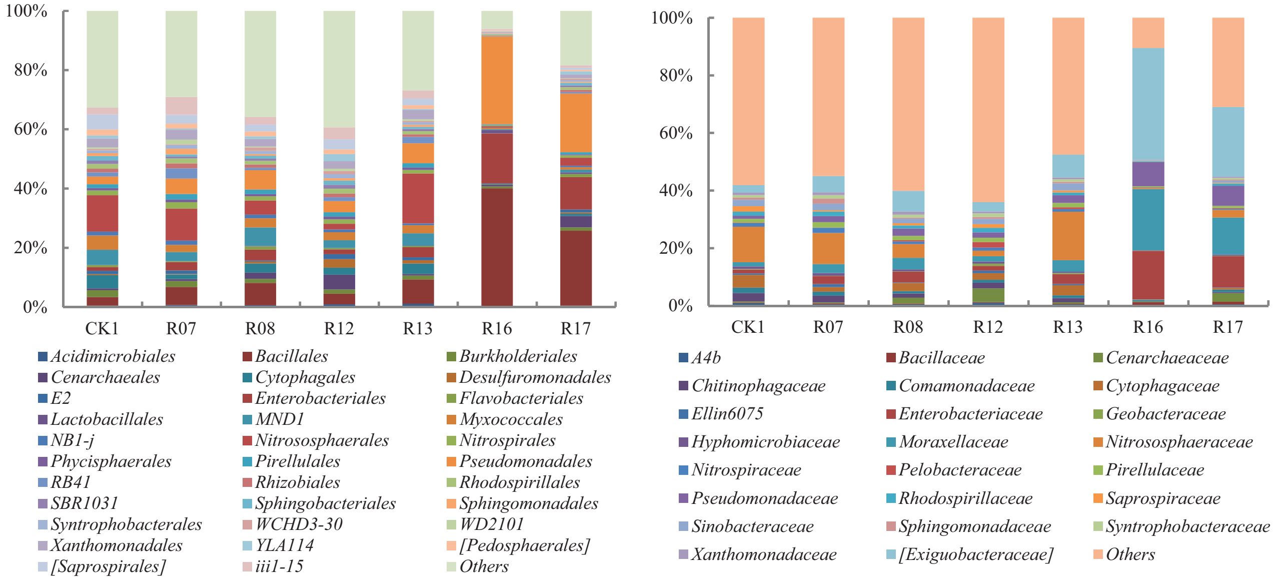


Figure 2. Rarefaction curves of the Chao 1 and Shannon indices at 97% sequence identity for samples



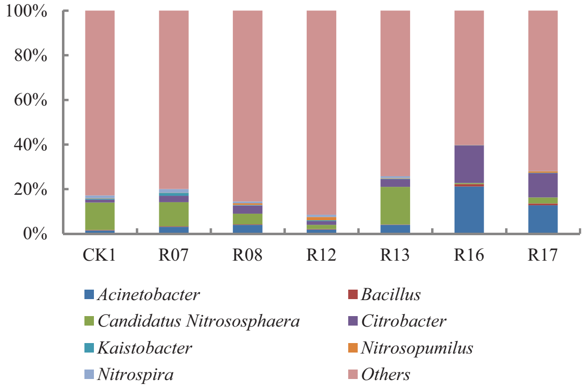
a) percent of community abundance on phyla level

b) percent of community abundance on class level



c) percent of community abundance on order level

d) percent of community abundance on family level



e) percent of community abundance on genus level

Figure 3. Relative abundance of staple microbial taxa on phyla/ class/ order/ family and genus across all samples. Phylogenetic groups with <1% relative abundance are classified as others

The most common genus differed distinctly among collected samples. The relative abundances of the dominant genus were quite consistent among its older reclamation sites (R07, R08, R12, R13) and unmined soil, but there was a different relative abundance in the younger reclamation sites (R16, R17) compared to the unmined soil. To be specific, the *Candidatus Nitrososphaera* was the most abundant genus in older reclamation sites and unmined soils while the dominant bacterial genera in younger reclamation sites were *Acinetobacter* and *Citrobacter*. These findings indicate that the composition and diversity of the soil microbial community is very different among plots of different reclamation periods, and further show that the soil microbial community could be used as an index of the effects of reclamation period on collapsed land.

The phylogenetic relationships of various organisms were analysed (Figure 4). The phylogenetic circle tree represented different classification levels (kingdom-genus) from the inside to the outside. The size of the circle was proportional to the abundance of the species. Different colours represented different phyla. The top 40 abundance species were represented by solid circles. The species names in the circles were marked with alphanumerics, etc., and were shown in the legend on the left. The resulting phylogenetic tree consisted of several clusters. In our study, *Proteobacteria* was the most abundant phyla in all seven mine sites, which is consistent with the findings reported in previous studies (Li et al., 2014; Thavamani et al., 2017). *Proteobacteria* encompassed huge morphological, physiological and metabolic diversity. Members of the phylum *Proteobacteria* including *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and other classes played* a key role in global nutrient cycling.

2.5. Influence of soil geochemistry on microbial diversity

The correlation coefficients were shown in Table 3. The results indicated that the SOC and AP were positively correlated with the Shannon, Chao 1 and Simpson at 0.01 level, which is similar to the research reported by Li et al. (2015b). The research summarized that SOC, soil water content and TP were identified as more important indicators to structure the microbial community than Cu and

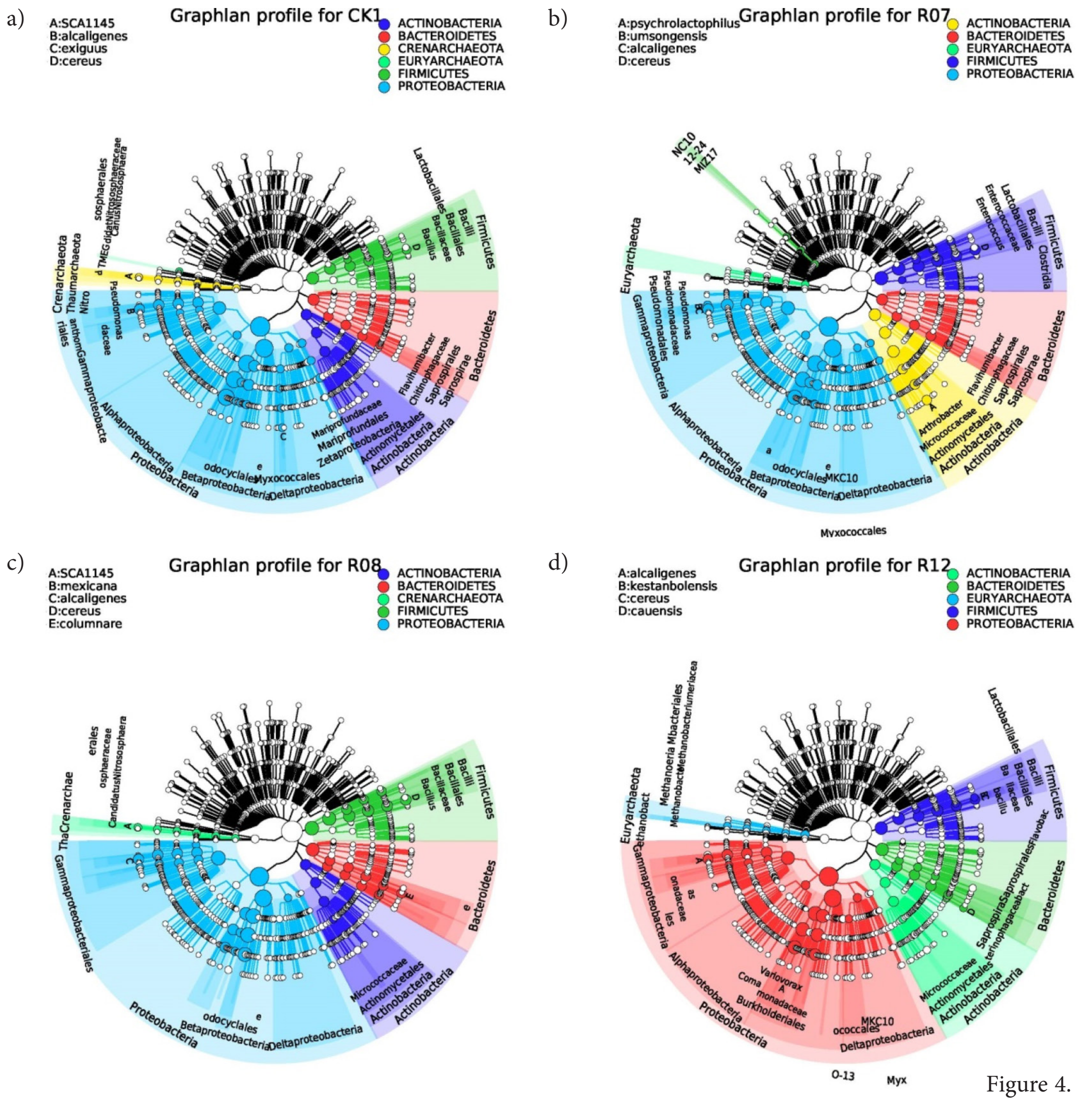


Figure 4. *to be continue*

Pb. Application of organic fertilizers could improve soil physical and chemical properties, which are important factors responsible for the change in microbial community structure, and therefore affect their function. Concentration of SOC and AP in the soil could act as an important indicator of the health of the ecological system in the reclaimed area.

Soil pH has a strong influence on the activity of microbiota. Results showed that soil pH level was positively correlated with Shannon and Chao 1 ($p < 0.05$), and positively correlated with Simpson ($p < 0.01$) in our study. The research by Lauber et al. (2009)ţ suggested that soil pH played an important role in microbial community dynamics, among the identifiable deterministic factors. And Shi et al. (2018) found that the effect of soil pH on the microbial community was evident across the North China Plain.

According to our statistical analyses, soil water content had a strong influence on microbial community, which was different from previous studies. Soil water content affected microbes via two primary mechanisms: as a transport medium for substrates, and as a participant in the hydrolysis process (Yan et al., 2015). Microbial activity and growth were influenced by the soil water content. Lack of water might contribute to C and N mineralization in the surrounding environment and the accumulation of osmolytes in microbial cells (Pulleman & Tietema, 1999).

A positive correlation was found between AN and Chao 1 ($p < 0.05$). And AN content is likely to be affected by soil moisture, temperature variation, and soil organism activities. Furthermore, AN content plays an important role in the evolution of reclaimed soil by affecting the nutrient cycling and microbial communities (Kong et al., 2019; Shrestha et al., 2009; Yuan et al., 2017).

However, inconsistent with other work (Zhang et al., 2018), our results showed that soil AK was negatively correlated with the richness and diversity of the microbial community. The supplementation of AK relies on applying fertilizer (Zhang & Kong, 2014). In this paper, the negative influence may be related to the excessive content of AK in some plots. The content of AK in agricultural areas is not "the more, the better". For example, there is a strong interaction among soil elements in the natural environment

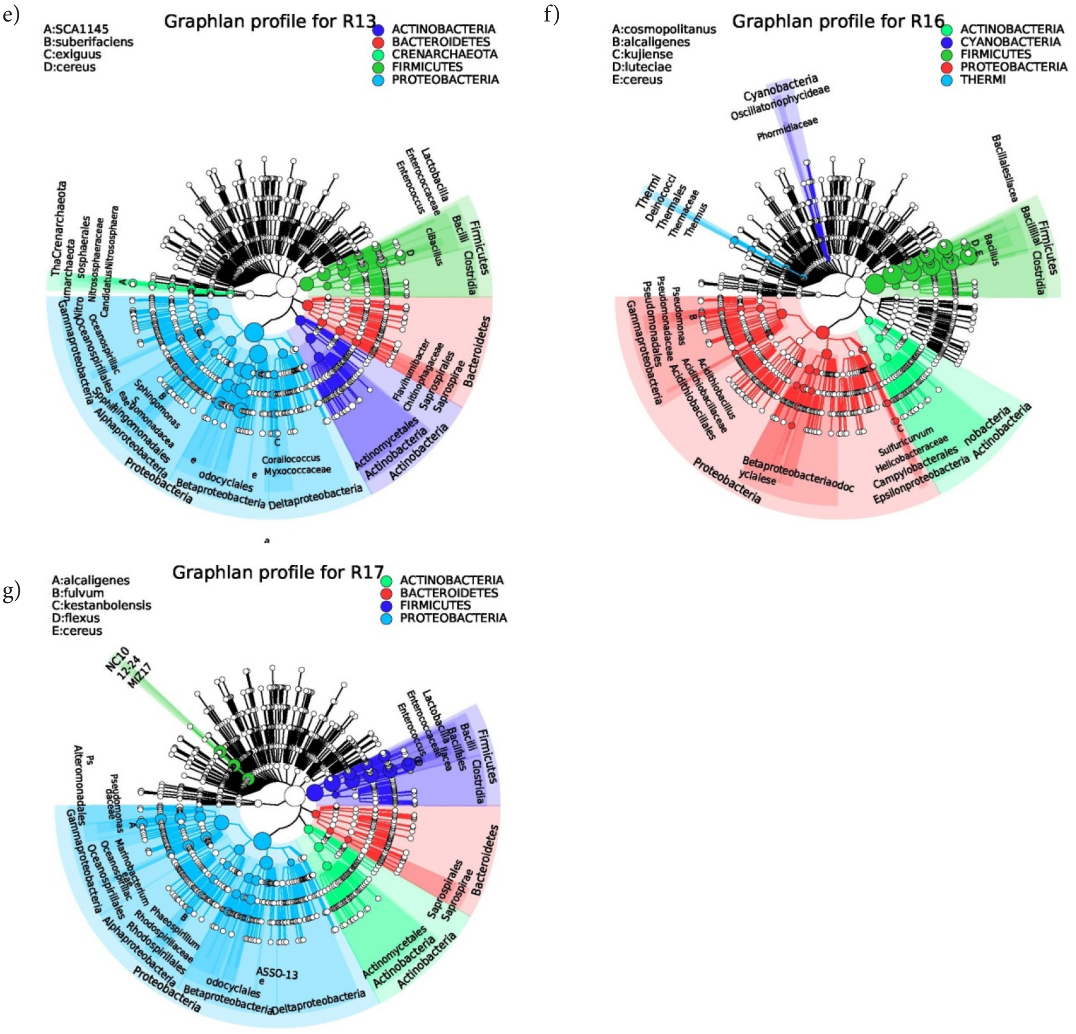


Figure 4. Phylogenetic relationship analysis of various organisms that are associated in the mining area with the organism obtained by 16Sr RNA sequencing

Table 3. Correlations among physical and chemical properties and microbiological diversity indices in soils collected from the Sanhejian Mine

	Shannon	Chao 1	Simpson	pH	Water Content	SOC	AN	AP	AK	Cd	Pb	Cr	Cu	Zn	Hg	As
Shannon	1.000															
Chao 1	0.905**	1.000														
Simpson	0.926**	0.823**	1.000													
Ph	0.394*	0.374*	0.486**	1.000												
Water Content	0.447**	0.376*	0.434*	−0.010	1.000											
SOC	0.553**	0.463**	0.538**	0.375*	0.129	1.000										
AN	0.256	0.346*	0.130	0.068	−0.010	0.368*	1.000									
AP	0.678**	0.708**	0.651**	0.437**	0.324*	0.444**	0.210	1.000								
AK	−0.015	0.075	−0.163	−0.019	−0.238	0.234	0.524**	0.133	1.000							
Cd	0.022	0.120	−0.029	−0.026	0.181	−0.227	0.067	0.139	−0.026	1.000						
Pb	0.105	0.196	0.065	−0.155	0.371*	−0.081	0.181	0.190	0.105	0.500**	1.000					
Cr	−0.362*	−0.292	−0.451**	−0.229	−0.091	−0.316*	0.100	−0.196	0.205	0.331*	0.215	1.000				
Cu	−0.196	−0.206	−0.152	0.068	−0.219	−0.205	−0.200	−0.229	−0.067	0.098	−0.086	0.167	1.000			
Zn	0.166	0.075	0.325	0.311	0.076	0.167	−0.400*	0.257	−0.267	0.005	−0.038	−0.167	0.190	1.000		
Hg	−0.104	−0.069	−0.087	−0.339	0.011	−0.153	−0.055	0.033	0.120	0.136	0.251	0.005	−0.175	−0.033	1.000	
As	−0.287	−0.357*	−0.228	−0.180	−0.100	−0.124	−0.062	−0.253	−0.081	0.000	−0.024	0.287	0.358*	0.119	0.213	1.000

Note: ** Significant at $P < 0.01$;* Significant at $P < 0.05$.

(Kumar et al., 2020). When AK content is excessively high, the transformation and absorption of other soil nutrient elements will be affected. For example, a high level of soil AK might lead to a decrease in the ability of the soil to absorb calcium and other cations. In addition, the high content of soil AK could be responsible for the ecosystem trophic structure and dynamic balance. Therefore, the negative correlation between soil AK and the richness and diversity of microorganisms in this study can be fully explained.

The results suggested that microbial diversity had a correlation with potentially toxic elements. Some research reported that most mircoorganisms could tolerate varying level of toxicity through extracellular precipitation, enzymatic oxidation, cell wall adsorption and intracellular complexation (Liu et al., 2020; Yang et al., 2017).

2.6. Influences of soil physicochemical properties on bacterial community

The Kendall's tau-b correlation heatmap analysis was used to investigate the relationship among the soil physicochemical parameters and relatively microbial abundance of dominated bacterial phyla (Figure 5). The analysis indicated that the variation in the composition of bacterial community was mainly driven by soil pH, water content, SOC and AP. The abundance of the *Nitrospirae* and *Planctomycetes* were positively correlated with pH ($p < 0.05$) while *Proteobacteria* was negatively correlated with pH ($p < 0.05$). Soil pH has been widely recognized as a key factor affecting the distribution of soil bacteria (Fierer & Jackson, 2006; Lauber et al., 2008). pH can affect the community composition and abundance of nitrifying microorganisms by affecting the chemical form, concentration and effectiveness of the substrate in the nitrification reaction. And soil water content had a positive correlation with the abundance of *Parvarchaeota* ($p < 0.05$). Soil water status resulted in changes of enzyme activities and soil respiration, affecting microbial growth and metabolic activity. Furthermore, the abundance of *Nitrospirae*, *Planctomycetes* were positively correlated with SOC ($p < 0.05$). It was consistent with the study of Cong et al. (Cong et al., 2020). The reason is that the bacteria had a close relationship with carbon conversion and accumulation (Zhao et al., 2018). The Firmicutes had a negative relationship with SOC. This is because the Firmicutes are oligotrophs and tend to be associated with low carbon environments (Chen et al., 2017). Interestingly, the results showed that AP was positively correlated with *Verrucomicrobia*, *Chloroflexi* and *Bacteroidetes* ($p < 0.05$), and positively correlated with OD1($p < 0.01$).

Several studies have reported heavy metals significantly determined microbial community composition in soil (Åkerblom et al., 2007; Congyan et al., 2018). In our study, elements Cd, Pb, and Cr showed uniformly negative

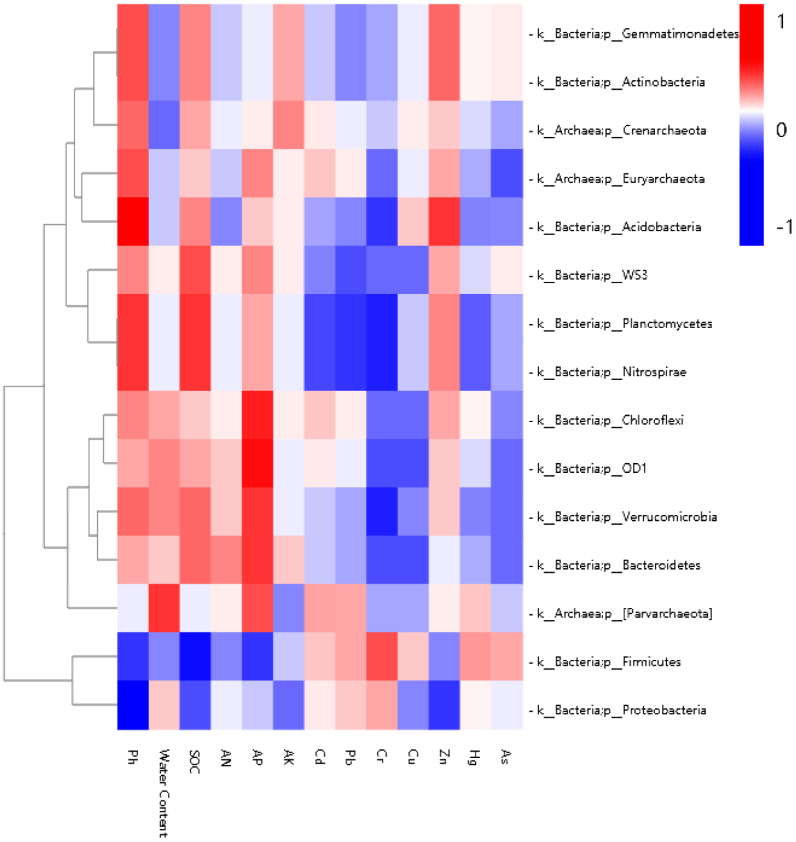


Figure 5. Heatmap of Kendall's tau-b correlation between the relative abundance of series of bacteria phyla and physicochemical properties

associations with the relative abundance of bacteria Nitrospirae, Planctomycetes, and Acidobacteria, which is similar to the study of Li et al. (Li et al., 2020). This contributes to heavy metals could destroy the process of chromosome replication and DNA synthesis, modify bases in organisms, then cause structural changes. Conspicuously, there was a positive correlation between element Zn and the relative abundance of Nitrospirae, Actinobacteria, Gemmatimonadetes, Planctomycetes, and Acidobacteria, which might be due to the synergistic effect of Zn in the soil N cycle, since it serves as a cofactor for some enzymes involved in N metabolism.

Conclusions

The study provided an original insight into the abundance and composition of bacterial communities based on 16 rRNA sequences in the post-mine reclamation areas in a typical resource-based city. We found that *Proteobacteria* was the ubiquitous and prevalent phylum in both reclamation and reference soils. However, a considerable difference in the relative abundances was found when comparing reclamation to reference soils at the class, order, family and genus levels. These findings indicate that the composition and diversity of the soil microbial community is very different among plots of different reclamation periods, and further show that the soil microbial community could be used as an index of the effects of reclamation period on collapsed land. Subsequent further analysis indicated pH, water content, SOC, AP and potentially toxic elements were positively correlated with the abundance and communities.

These findings might provide a new perspective for the ecological assessment of the post-mine reclamation areas. The ecological environmental factors including soil physical and chemical indicators, vegetation coverage, biodiversity index and other related indicators should be considered to establish comprehensive evaluation model or other related models in the evaluation of the coal mining area. The factors reflect the main characteristics of the ecological environment quality.

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Author contributions

Min Tan, Xu Zhou, Jun-Feng Qu, and Gang Li conceived and designed the experiments; Min Tan, Meng-Yu Ge, and Zhuang Chen performed the experiments; Min Tan analyzed the data; Min Tan and Jun-Feng Qu wrote the paper; all authors read and approved the final manuscript. Min Tan and Xu Zhou contributed equally to this work.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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