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Diversity of soil bacteria in alpine coal slag mountain grassland in different vegetation restoration years

Rina Dao^{*}, Ying Zhang^{*}, XiLai Li, Linxiong Ma, Xiaolong Tie and Shengyan Lei

Abstract

Purpose This study aimed to investigate changes in the bacterial diversity of the rhizosphere soil of slag mountains in different years of revegetation restoration.

Methods Seven soil samples were selected from different years of revegetation restoration in Qinghai, China. The bacterial community of each soil was analyzed via high-throughput sequencing using the Illumina MiSeq platform.

Results Statistical analyses revealed that the diversity of the soil bacterial community was higher in the soil that was restored in 2017 than that in the soils restored from other years. 16S rRNA sequencing revealed that Proteobacteria and Actinobacteria were the dominant phyla. *Sphingomonas* was the dominant genus. Total nitrogen, available nitrogen, and total potassium influenced the horizontal community structure of the phylum, whereas total nitrogen, organic matter, and pH had a great influence on the horizontal community structure of the phylum. The richness and diversity of the bacterial community in the soils that underwent revegetation restoration were greater in the third year (2017) than in other years. In the seventh year of recovery, the richness and diversity of the bacterial community began to decline.

Conclusion The bacterial diversity of the soil in the coal mine slag mountain improved with the increase in vegetation restoration years.

Keywords Coal slag mountain, High-throughput sequencing, Microbial diversity, Proteobacteria, Vegetation restoration

Introduction

Soil is the main limiting factor that affects the restoration of degraded ecosystems in coal mining areas (Du et al. 2009; Yin et al. 2010). Soil microorganisms, such as bacteria, participate in the decomposition of organic matter, growth of plants, and other critical ecological

*Correspondence: Rina Dao daorma@163.com Ying Zhang 610354005@qq.com Key Laboratory of Alpine Grassland Ecology in Three-River Headwaters Region, Qinghai University, Xining 810016, China processes (Lu et al. 2022). The influence of the microenvironment is more significant when the soil environment changes (Chen et al. 2016); the soil microbial community can make a rapid response, and the biological community structure can change. The soil community structure is therefore a crucial indicator of soil quality change. Soil bacteria participate in the transformation of most nutrients in the soil (An et al. 2017) and occupy an absolute dominant position among soil microbes, with the proportion reaching over 95% (Li et al. 2013). Therefore, soil bacterial diversity can be used to measure soil quality levels after revegetation restoration in mining areas (Li et al. 2014; Sharma et al. 2011; Yao et al. 2013).



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The Jiangcang coal mining area is the largest coal mining area in Qinghai Province. It is located approximately 110 km northwest of Gangcha County toward the south of the upper reaches of Datong River. It is approximately 3800 m above sea level and has a cold climate. Mechanical piling up of past slag mountain has caused serious damage, including exhaustion of grassland resources, land desertification, and soil and water loss (Chang et al. 2003; Liu and Liu 2020), leading to irreversible destruction of the ecosystem; therefore, developing the structure and function of an ecosystem has a considerable effect, completely changing the growth of the vegetation survival environment. The soil microbial ecosystem has also been severely damaged by serious soil erosion, significant changes in the composition of the microbial community, and massive loss of microbial diversity, thereby affecting the material and nutrient cycling in the soil, structure and fertility of the soil, vegetation growth, and the entire ecosystem (Fan et al. 2011; Murphy et al. 2003). The alpine mining area ecosystem has suffered serious, longterm damage in the permafrost environment, which will not recover without intervention.

Most studies on ecological restoration in mining areas have focused on determining soil physical and chemical properties and vegetation reconstruction, and there are few studies on the use of soil microorganisms to analyze the soil conditions in mining areas (Zhang et al. 2014). Studies on soil bacterial diversity in China are primarily conducted in farmlands and jungles (Sun et al. 2012); however, few studies have been conducted in alpine regions. Studies on vegetation restoration in mining areas have mostly been conducted in loess hilly-gully and lowaltitude regions (Jin et al. 2019). Most studies focus on soil enzyme activity and soil biomass. However, relevant studies have shown that revegetation restoration affects soil properties (Kumar et al. 2015) and that soil bacteria can accurately reflect the restoration of degraded land (Izquierdo et al. 2005). Regarding the methods used for microbial diversity research, traditional research methods, such as the plate culture method, BIOLOG microplate method, phospholipid fatty acid analysis, gradient gel electrophoresis technology, nucleic acid molecular hybridization-based method, PCR-based method, and Sanger sequencing, have been used in previous studies (Zhao et al. 2015; Lin et al. 2010; Cai et al. 2011; Ke et al. 2011; Gao et al. 2009). These traditional methods detect relatively low bacterial community abundance and have a small range, complex operation, and high cost (Mao et al. 2015). In contrast, high-throughput sequencing technology, with the advantages of high sensitivity and quick throughput, enables simple, quick, and accurate acquisition of soil microbial information (Jones et al. 2009). Therefore, high-throughput sequencing technology was used in this study to analyze the response characteristics of soil bacterial diversity in the compound green grassland and to measure soil nutrients. This study used the grassland of the slag mountain of the Jiangcang mining area that had been planted in different years as the research objects. The relationship between the soil nutrients and soil bacterial diversity of the slag mountains of mining areas with different planting years was investigated to provide a theoretical basis for future construction and ecological reconstruction in the alpine mining area.

Methods

Site description and soil sampling

The research site is located in the Jiangcang coal mine area, Gangcha County, Haibei Prefecture, Qinghai Province, China. It is located in the alpine zone at an altitude of 3800-4200 m (Jin et al. 2019). Its geographical coordinates are 99°27′-99°35′E and 38°02′-38°03′N. The annual average temperature is -2.8 °C, ranging from – 35.6 °C to 19.8 °C. A large number of alpine meadows and marshes are distributed in the Jiangcang mining area, which are classified as alpine marsh wetland. The matrix of the slope surface is composed of coal gangue and accumulated residue. On the sunny slope, cofferdams have been built along the bottom edge of the slag mountain to increase the stability of the slope surface. The planting methods for all plots were the same, where Elymus nutans, Poa crymophila, and Puccinellia tenuiflora were planted artificially at a ratio of 2:1:1. The proportion of 1 was planted in May 2015, May 2017, and late May 2019, respectively, at a sowing rate of 8.0943 kg/hm², and plants were covered with a nonwoven fabric after sowing. During this period, no fertilization, watering, or grazing activities were performed. The elevation of the undisturbed original vegetation in the mine area is 3788 m, and the geographical coordinates are 99°38'E and 38°03'N. The dominant species in this area are Kobresia pygmaea, Kobresia humilis, and Carex rigescens.

In the experiment area (Fig. 1), the vegetation restoration duration from 2013 to 2019 was selected, and the grasslands had vegetation restoration durations of 1 year (2019), 2 years (2018), 3 years (2017), 4 years (2016), 5 years (2015), 6 years (2014), and 7 years (2013). We also collected samples of native vegetation soil (YS) from the undisturbed original vegetation area. Three soil samples of 0-20 cm were collected from each area, stones, and residual plant roots were removed, and samples were placed into ziplock bags before being brought to the laboratory for determination of their physical and chemical properties. Then, ground vegetation and surface coverings were removed



Fig. 1 The geographical location of the layout of the sample plot

from the sample site, and sterile soil samples were collected using a sterilized shovel. The sterile samples were returned to the laboratory at low temperature (-5 °C) and placed in the refrigerator for correlation analysis of soil chemical properties and soil microorganisms. The sterile soil of native vegetation was soil that had not been applied to guest soil and had not undergone revegetation restoration in the coal mine slag mountain.

Soil chemical property measurements

Soil pH was measured using a pH meter in a suspension of soil and water with a soil:water ratio of 1:2.5. Total nitrogen (TN) content was measured using the potassium dichromate sulfuric acid boiling method. Total phosphorus (TP) content was determined using the sulfuric acid and perchloric acid boiling and molybdenum antimony resistance colorimetric methods. Total potassium (TK) content was determined using the molten NaOH and flame photometry method. Available nitrogen (AN), available phosphorus (AP), and available potassium (AK) contents were measured using the alkaline hydrolysis diffusion, molybdenum blue, and flame photometry methods, respectively. Soil organic matter (SOM) was determined using the potassium dichromate volumetric method. All methods were followed according to a previous report (Chao and Bunge 2002).

DNA extraction, sequencing, and data analysis

Nucleic acid extraction was performed using the TGuide S96 magnetic bead method soil genomic DNA Extraction Kit (DP812). The concentration of extracted nucleic acid was detected using a microplate reader (Synergy HTX). The integrity of the amplified PCR products was detected via electrophoresis in a 1.8% agarose gel.

Excel 2007 was used for data collation, and SPSS Statistics 26 was used for one-way analysis of variance of soil nutrients. Duncan's complex range method was used to test significant differences (P < 0.05). Using a 70% confidence threshold, the phylogenetic relationship of each 16S rRNA gene sequence was analyzed against the SILVA (SSU123) 16S rRNA database using a ribosomal database project (RDP) classifier for taxonomic analysis. Operational taxonomic units (OTUs) with similarities of at least 97% were selected for OTU cluster analysis and species taxonomy analysis to generate a dilution curve, and Mothur was used to calculate the Chao1, ACE richness, and Shannon and Simpson diversity indices (Schloss et al. 2011). The RDP classifier Bayesian algorithm was used for the taxonomic analysis of representative OTU sequences, and each sample's community composition was determined at the classification levels of phylum and class (Wang et al. 2007). The relationships between the fungal community structure and soil factors were analyzed using the redundancy analysis of the Baimaike Cloud platform.

Results

Soil chemical properties

The analysis was based on the data of the physical and chemical properties of the soil from the study plots with different vegetation restoration years, as shown in Table 1. The pH values of all soil samples were within the range of 7.6-8.6, indicating that all soils were slightly alkaline. The SOM initially increased and then decreased with the increase in the duration of vegetation restoration and reached a maximum value at the fourth vegetation restoration year (2016). There were no significant differences in TN, AK, and AN contents among the greenfield plots. The AP index reached the highest value in the soil from restoration year 2019. The maximum value of TP was 1.67 ± 0.16 in the soil from restoration year 2015. The TN, AN, AK, and SOM were higher in YS (native vegetation soil) than in the other soil samples, whereas the TK content in YS was lower than that in the other soil samples. The nutrient status of YS was significantly different from that of other soils. YS was neither applied to the guest soil nor was it greened, resulting in a significant difference from the greened soils. Therefore, there was a correlation between soil nutrients and vegetation, and changes in vegetation also change soil nutrients.

Alpha diversity of soil bacterial communities

As shown in Table 2, bacteria isolated from the 2017 soil had the highest Chao1 and ACE indices among the eight samples in this study, indicating the highest bacterial community richness, while YS had the lowest. The soil planted in 2017 had the highest Shannon value (8.594). The Shannon indices of the YS and 2019 soils were lower than those of the other samples, indicating that the bacterial community diversity was relatively low.

Composition of the soil bacterial community

As shown from the analysis in Fig. 2, 146 OTUs were obtained from sample bacteria after OTU clustering, and 63 OTUs were common. Each sample had an OTU count ranging from 4 to 67, with YS having the most (64). The lowest OTU counts were found in the soils planted in 2014 and 2016, both of which had OTU values of 4. The number of OTUs in YS accounted for approximately 33% of the total number of OTUs, indicating that the bacteria in YS did not have a high similarity with the bacteria in the other soils.

At the phylum level, 11 bacterial phyla were detected among the eight samples, as depicted in Fig. 3. The distribution of the soil bacterial community was uniform in various plots. The planted samples were mainly dominated by Proteobacteria, Actinobacteria, Bacteroidetes,

Table	1	Ph	/sical	and	chemical	material	s of so	bil
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	TN(g⋅kg ⁻¹⁾	TP ₍ g⋅kg ⁻¹)	TK(g⋅kg ⁻¹)	AN(m g⋅kg ⁻¹)	AP(m g⋅kg ⁻¹)	AK(m g⋅kg ⁻¹)	OM(g⋅kg ⁻¹)	pН
Y13	0.86 ± 0.02^{b}	1.04 ± 0.24^{abc}	23.32 ± 1.87 ^{abc}	28.00 ± 0.00^{b}	$10.77 \pm 2.36^{\circ}$	124.00 ± 3.47^{ab}	24.02 ± 2.06^{d}	8.21
Y14	1.99 ± 0.84^{b}	$0.84 \pm 0.12^{\circ}$	24.22 ± 2.71^{ab}	68.00 ± 38.74^{b}	16.40±9.55 ^{bc}	148.33 ± 47.65^{ab}	$66.02 \pm 12.71^{\circ}$	8.54
Y15	0.94 ± 0.05^{b}	1.67 ± 0.16^{a}	27.27 ± 1.10^{ab}	38.67 ± 12.90^{b}	21.57±11.05 ^{abc}	145.00 ± 20.08^{ab}	$75.10 \pm 1.80^{\circ}$	7.99
Y16	1.62 ± 0.25^{b}	$0.80 \pm 0.14^{\circ}$	24.59 ± 1.34^{ab}	27.00 ± 10.39^{b}	13.90 ± 4.00^{bc}	127.67 ± 53.72^{ab}	158.93±49.57 ^b	8.42
Y17	1.32 ± 0.11^{b}	1.12 ± 0.20^{abc}	23.90 ± 2.63^{ab}	25.67 ± 4.04^{b}	29.93 ± 7.82^{ab}	129.33±18.56 ^{ab}	$81.98 \pm 10.03^{\circ}$	7.62
Y18	1.41 ± 0.18^{b}	$0.89 \pm 0.22^{\circ}$	23.32 ± 0.90^{abc}	35.33 ± 9.45^{b}	21.23±11.02 ^{abc}	153.7 ± 7.64^{ab}	$80.68 \pm 5.79^{\circ}$	8.14
Y19	1.02 ± 0.10^{b}	0.99 ± 0.23^{bc}	21.22 ± 1.38^{bcd}	26.00 ± 1.73^{b}	34.77 ± 17.72^{a}	118.67 ± 15.01^{ab}	56.79 ± 0.89^{cd}	8.48
YS	9.62 ± 5.73^{a}	1.63 ± 0.92^{ab}	18.76 ± 3.95^{d}	425.6 ± 220.26^{a}	$13.17 \pm 4.30^{\circ}$	164.33 ± 25.81^{a}	328.68 ± 27.28^{a}	8.46

TN Total nitrogen, TP Total phosphorus, TK Total K, AN Available nitrogen, AP Available phosphorus, AK Available K, OM Organic matter. Different lowercase letters for the same index indicate significant difference (P < 0.05)

Table 2	Soil bacterial	data of g	rassland w	ith different v	egetation	restoration	years in slag	mountain c	of coal	mine

Sample name	ACE indices	Chao1 indices	Shannon indices	Simpson indices	Coverage indices
Y13	1383.8049	1415.75	8.1788	0.9904	0.9981
Y14	1019.2238	1060.0769	7.0091	0.9782	0.9975
Y15	1342.3753	1340.6783	8.3371	0.9909	0.9983
Y16	1183.5284	1193.3942	7.3217	0.9748	0.9975
Y17	1438.892	1459.2844	8.594	0.9942	0.9974
Y18	1322.5432	1374.0964	8.3281	0.9925	0.9975
Y19	1149.3683	1198.8571	6.3991	0.9455	0.9969
YS	257.3057	231.8571	6.7737	0.9857	0.9997



Fig. 2 Venn diagram of bacterial operational taxonomic unit distribution in the sample



Fig. 3 Distribution map of bacterial communities at the phylum level

Chloroflexi, and Acidobacteria, while Proteobacteria, Firmicutes, and Acidobacteria were the dominant phyla in YS. Proteobacteria accounted for 39% of the soil bacteria in YS and 33%–54% of the soil bacteria in the other samples. Proteobacteria species can degrade complex pollutants and repair soil. The proportion of Acidobacteria was less than 10% in the 2019 and YS samples and more than 15% in the other samples. Actinomycetes accounted for the highest proportion of bacteria in the 2019 samples. Actinobacteria play a role in nitrogen cycling, and the proportion of Actinobacteria in native vegetation soils was approximately 6%. Firmicutes accounted for 23.7% of soil bacteria in YS and approximately 1% in the other samples.

As shown in Fig. 4, the dominant genera in the 2013–2018 soil samples were *Sphingomonas* and *Ellin6067*. In the 2019 and YS samples, *Sphingomonas* was the dominant genus. *Sphingomonas* strains have a high metabolic capacity and strong adaptability to the environment (Tai et al. 2011) and accounted for approximately 5% of the bacteria in YS and more than 6% of the bacteria in the 2013–2019 samples. The genus *Ellin6067* accounted for less than 0.01% of the bacteria in YS and between 0.1% and 7% of the bacteria in the other samples. The group of *Gemmatimonas (Blomonas)* in the 2011–2019

samples was more than 0.7%, while it was not found in YS.

The link between diversity and composition of bacterial communities and soil environmental variables

In this study, the physical and chemical soil properties were analyzed at the phylum and genus levels, respectively, and the analysis results are shown in Fig. 5. At the bacterial phylum level (Fig. 5a), TN, AN, and TK had longer rays, indicating that they significantly influenced the community structure at the phylum level. TP showed a significant positive correlation with the dominant Proteobacteria and a negative correlation with Actinobacteria, Bacteroidetes, and Acidobacteria. Total potassium was positively correlated with Proteobacteria, Bacteroidetes, and Acidobacteria, while Actinobacteria was positively correlated with AP and pH. At the genus level (Fig. 5b), TN, SOM, and pH had longer rays, indicating that they significantly influenced the bacterial community structure at the genus level. SOM, TP, TN, pH, and AK were positively correlated with Ochrobactrum, while Sphingomonas was positively correlated with AP and pH. Total potassium was associated with *Ferruginibacter*, Hymenobacter, Terrimonas, Ellion6067, and Haliangium but was negatively correlated with Ochrobactrum and



Fig. 4 Distribution of community structure of all samples at the genus level



Fig. 5 RDA of soil microbial community structure and soil physicochemical properties. **a** Horizontal community structure of bacterial phylum. **b** Horizontal community structure of bacterial generic level

Sphingomonas. It can be seen that there is a specific interaction between soil bacterial community structure and the soil's physical and chemical properties in different restoration degrees of coal mine slag mountain grassland.

Linear discriminant analysis effect size

Linear discriminant analysis (LDA) effect size (LEfSe) can find important species with significant differences between groups, and the statistical results include three parts. The LDA value distribution histogram shows the significantly enriched species and their importance in each group, while the species branching evolution diagram shows the different species and their evolutionary relationships. It can be seen from Fig. 6 that there were 20 bacterial populations with significant differences in the treatment model. There were significant differences in species effect size (importance) in the top four taxa (Sphingomonadales, Sphingomonadaceae, Sphingomonas, and Bacteroidetes, respectively). There were 16 populations with significant differences to the control (YS). The bacteria with significant differences ranked in order of impact size (importance) were Lachnospiraceae, uncultured-bacterium-f-SC-I-84, f-SC-I-84, unculturedbacterium-f-SC-I-84, and Rhizobiales. The number of microbial species significantly enriched in the treatment model was significantly higher than that in the control soil (YS).

In the treatment model, the bacterial taxa enriched with significant differences were *Sphingomonas*, *Micrococcus*, Chitinobacteriaceae, Blastocatellales, and Blastocatellaceae. In the control soil, Rokubacteriales, Firmicutes, *Clostridium*, Clostridiales, and Lachnospiraceae were the main bacterial taxa with significant differences and evolutionary relationships.

Discussion

The primary method for restoring a fragile ecosystem is to conduct revegetation restoration, which promotes the recovery of the overground and underground soil quality, so that the ecosystem of the coal mine slag mountain can reach a stable state. Soil microorganisms can reflect the soil restoration status of the ecosystem and provide a scientific basis for future restoration. The methods for studying microbial diversity include biochemical techniques, which include traditional methods and molecular phylogenetic analysis techniques, such as high-throughput sequencing. This study aimed to investigate the bacterial communities in the grassland soils of coal mine slag mountains after various periods of vegetation restoration.

Jin (2019) found that after 4 years of restoration, nutrients in the soil increased to varying degrees compared with that in the bare land in the slag mountain before restoration, which is inconsistent with the results of this experiment. We found that the levels of nutrients in the soil 1–7 years after revegetation restoration were lower than those of native soil. In addition, although the pH of the soil decreased to varying degrees after 2 years of revegetation restoration, it remained weakly alkaline. This was because that the revegetation restoration period was short, the microbial species were few, and that nutrients were not replenished in this time, resulting in poor soil nutrient content and a continuous alkaline state.

Chen Laihong et al. (Chen et al. 2012) observed that the bacterial diversity changed irregularly after various reclamation periods. In the present study, differences in



Fig. 6 Indicator microbes in groups with linear discriminant analysis (LDA) scores higher than 4. **a** LDA score of LDA effect size (LefSe). Evolutionary branch diagram of differential bacterial communities or species. **b** Cladogram of LEfSe. The size of the circle from inside to outside of the evolutionary branching graphs in a group indicates the importance of abundance from the phylum to genus or species levels, and groups with the same color indicate the importance of the phylum in this group. The LDA value (influence value of linear discriminant analysis) distribution histogram shows the bacterial community or species (biomarker) with LDA scores > 4. All the displayed bacterial communities or species exhibited significant differences among all groups. The letters k, p, c, o, f, g, and s indicate kingdom, phylum, class, order, family, genus, and species, respectively. Bacterial communities or species with significant abundance differences in different groups are shown, and the length of the histogram represents the influence of the biomarkers

microorganisms in the soil of the revegetated grassland were not evident after 4–7 years of recovery, which may be due to the slow improvement of microorganisms in the coal slag mountain due to the cold climate, shallow surface soil, and poor nutrient supply of vegetation. Dangi et al. (2012) discovered that the soil microbial structure could recover to the normal soil structure after 5-14 years of revegetation restoration. In the current study, the soil bacterial community was found to return to the normal soil structure level after 5-8 years of revegetation restoration, which is consistent with Dangi's study but differed from the findings of Kumar's study (Kumar et al. 2015) on the revegetation restoration of coal gangue mountains in India, mainly because soil microorganisms are associated with changes in various related factors in the soil and the geographical structure and climatic conditions of the study area. In the current study, we discovered that regardless of the vegetation restoration year, bacteria dominated. This finding is consistent with the research conclusions of Tan (Tan et al. 2014) and Wang (Wang et al. 2016). Overall, soil disturbances, such as cultivation and revegetation restoration, cause significant harm to fungal populations, and it is common for bacterial dominance to increase after physical disturbances. The research of Li et al. (Li et al. 2015) showed that bacteria of the green bent phylum were dominant in different soil types in the mining area, which differed from the findings of the current study. This difference may be due to the dominant species of bacteria being different due to the different types of polygreen plants, soil structure, geographical structure, and climate between the two studies. In two other previous studies (Tai et al. 2011; Jin et al. 2019), it was discovered that Proteobacteria and Actinobacteria were the dominant bacteria in samples with different years of revegetation restoration

in the slag mountain of the permafrost mining area, which was consistent with the findings of the current study, where the main microbial dominant groups in the samples with different vegetation restoration years were Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, and Acidobacteria at the phylum level, and Sphingomonas, uncultured-bacterium-c-Subgroup-6, and Ellin6067 were the dominant bacteria at the genus level. Among these bacteria, Sphingomonas gradually became the dominant microbial group as the number of revegetation restoration years increased. Sphingomonas has a high metabolic capacity, multi-functional physiological characteristics and a special degradation mechanism, strong adaptability to the environment, and has great application potential in environmental protection (Yang et al. 2015). More attention should be paid to Sphingomonas in future mine vegetation restoration projects.

The results of this study indicated that TN, AN, and TK had a significant influence on the bacterial phylum community structure. TP was significantly positively correlated with the dominant Proteobacteria and negatively correlated with Actinobacteria, Bacteroidetes, and Acidobacteria. TK was positively correlated with Proteobacteria, Bacteroidetes, and Acidobacteria, while Actinobacteria was positively correlated with AK and pH. These results contradict those of Li et al. (2018), who found that Chloroflexi was significantly positively correlated with AK and SOM, while Actinobacteria was negatively correlated with AP, SOM, and AK, and nitrate nitrogen had little effect. Soil nutrients and structure may be altered due to the various restoration mechanisms, and soil microbes may change accordingly.

In the Jiangcang coal mine area of Qinghai Province, the bacterial species composition of grassland soil in different vegetation restoration years (from 2013 to 2019) was rich, and the microbial community composition of grassland soil was significantly different in the soil from different vegetation restoration years. Land reclamation and ecological reconstruction in mining areas should not only restore the surface vegetation and organisms but should also restore the underground ecosystem. Soil microorganisms are extremely important for the stability of soil ecosystems and their related functions. The importance of ecological indicators, such as soil microorganisms, should be noted, and a comprehensive and scientific index of land reclamation and soil quality dynamic monitoring should be established. It is necessary to grasp the changing trend of reclaimed soil quality to realize the reconstruction and coordinated development of the above- and below-ground ecosystems and realize the highly coordinated and unified ecological economy and social benefits of land reclamation in mining areas.

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Authors' contributions

Rina Dao did the field sampling and physiochemical data analysis, and wrote the draft manuscript. Ying Zhang and XiLai Li did the field sampling. Xiaolong Tie, Linxiong Ma, and Shengyan Lei supervised the project and finalized the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria. That the article is original, has not already been published in a journal, and is not currently under consideration by another journal. That you agree to the terms of the BioMed Central Copyright and License Agreement.

Competing interests

The authors declare that they have no competing interests.

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References

- An LY, Li JJ, Yan JX, Li HJ (2017) Effects of microbial diversity on soil carbon mineralization. Environ Sci. 38:4420–4426. https://doi.org/10.13227/j.hjkx. 201702058
- Cai CQ, Li T, Long CL (2011) Soil microbial diversity and its research methods. J Anhui Agric Sci. 39:17274–17276. https://doi.org/10.13989/j.cnki.0517-6611.2011.28.159
- Chang YN, Da T, Hao FH (2003) Estimating the economic losses of farmland degradation in China at the end of the 20th century. China Popul Resour Environ 13:20–24. https://doi.org/10.3969/j.issn.1002-2104.2003.03.005
- Chao A, Bunge J (2002) Estimating the number of species in a stochastic abundance model. Biometrics 58:531–539. https://doi.org/10.1111/j. 0006-341X.2002.00531
- Chen LH, Qiao GH, Dong HL, Ma WL (2012) Effects of reclamation on soil bacterial diversity in Zhumger open-pit mining area. J Arid Land Resour Environ 2:120–122. https://doi.org/10.13448/j.cnki.jalre.2012.02.016
- Chen XY, Fang W, Yan JP, Liu Y, Tan HY, Zhou YZ (2016) Effects of soil cover thickness on diurnal variation of soil respiration during reclamation in mining area. J China Univ Mining Technol 45:164–169. https://doi.org/10. 13247/j.cnki.jcumt.000462
- Dangi SR, Stahl PD, Wick AF, Ingram LJ, Buyer JS (2012) Soil microbial community recovery in reclaimed soils on a surface coal mine site. Soil Sci Soc Am J 76:915–924. https://doi.org/10.2136/sssaj2011.0288
- Du YJ, Zhang CL, Li MS, Wang SH, Hu XB (2009) Study on moisture characteristics of spontaneous combustion gangue mountain based on DIVINER2000. Soil Water Conserv China 37:54–56. https://doi.org/10. 14123/j.cnki.swcc.2009.05.001
- Fan WH, Bai ZK, Li HF, Qiao JY, Xu JW (2011) Effects of different reclamation modes and reclamation years on soil microorganisms. Trans Chin Soc

Agric Eng 27:330–336 (https://www.cnki.com.cn/Article/CJFDTOTAL-NYGU201102058.htm)

- Gao QY, Xu GC, Li XY (2009) Study on denaturing gradient gel electrophoresis (DGGE) in microbial diversity. J Biol 26:80–81 (https://www.cnki.com.cn/ Article/CJFDTOTAL-STXB200308014.htm)
- Izquierdo I, Caravaca F, Alguacil MM, Hernández G, Roldán A (2005) Use of microbiological indicators for evaluating success in soil restoration after revegetation of a mining area under subtropical conditions. Appl Soil Ecol 30:3–10. https://doi.org/10.1016/j.apsoil.2005.02.004
- Jin LQ, Li XL, Sun HF, Yang XG, Zhang J (2019) Effects of different restoration years on vegetation and soil characteristics of slag mountain in alpine open-pit coal mine area. Chin J Ecol 38:121–128. https://doi.org/10. 13292/j.1000-4890.201901.014
- Jin LQ (2019) Effects of artificial vegetation on microbial community restoration in permafrost mining areas [D]. Qinghai University.https://doi.org/10. 27740/d.cnki.gqhdx.2019.000074
- Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N (2009) A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J 3:442–453. https://doi.org/10.1038/ ismej.2008.127
- Ke H, Wang LB, Du HL (2011) Effects of combined application of microbial agent and slow-release fertilizer on microbial ecology in reclaimed soil. J Soil Water Conserv 25:86–93. https://doi.org/10.13870/j.cnki.stbcxb.2011. 05.033
- Kumar S, Maiti SK, Chaudhuri S (2015) Soil development in 2–21 years old coalmine reclaimed spoil with trees: a case study from Sonepur-Bazari opencast project, Raniganj Coalfield, India. Ecol Eng 84:311–324. https:// doi.org/10.1016/j.ecoleng.2015.09.043
- Li FL, Liu M, Li ZP, Jiang CY, Han FX, Che YP (2013) Changes in soil microbial biomass and functional diversity with a nitrogen gradient in soil columns. Appl Soil Ecol 64:1–6. https://doi.org/10.1016/j.apsoil.2012.10.006
- Li YY, Chen LQ, Wen HY, Zhou TJ, Zhang T (2014) Pyrosequencing-based assessment of bacterial community structure in mine soils affected by mining subsidence. Int J Min Sci Technol 24:701–706. https://doi.org/10.1016/j. ijmst.2014.07.002
- Li JR, Hou HP, Wang C, Zhang SL, Ma J, Ding ZY, Huang L, Dong J, Yang YJ (2018) Bacterial diversity in reclaimed soil based on high-throughput sequencing. Environ Sci Technol 41:148–157. https://doi.org/10.19672/j. cnki.1003-6504.2018.12.022
- Li YY (2015) Study on microbial diversity and enzyme activity of soil reclaimed by mud pump in coal mining subsidence [D]. Xuzhou: China University of Mining and Technology (1–131). https://cdmd.cnki.com.cn/Article/ CDMD-10290-1015972417.htm
- Lin H, Zhang YX, Song XQ (2010) Study on soil microbial activity and fauna in coal mine dump contaminated area of Xiejiaji. Coal Mine Environ Prot 16:84–91
- Liu T, Liu S (2020) The impacts of coal dust on miners' health: A review. Environ Res. 190:109849. https://doi.org/10.1016/j.envres.2020.109849
- Lu ZX, Wang P, Ou HB, Shou XW, Li CW, Yi J, Ren JW, Xiong SL, Zhi HW, Li JC, Zhi ML (2022) Effects of different vegetation restoration on soil nutrients, enzyme activities, and microbial communities in degraded karst landscapes in southwest China. Forest Ecol Manage. 508(15):120002. https:// doi.org/10.1016/j.foreco.2021.120002
- Mao WH, Wu SL, Zhang X (2015) Construction and application of highthroughput detection method of 16S rDNA ion Torrent PGM for soil microorganisms. J Zhejiang Agric Sci 27:2165–2170. https://doi.org/10. 3969/j.issn.1004-1524.2015.12.19
- Murphy DV, Stockdale EA, Brookes PC, Goulding KWT (2003) Impact of microorganisms on chemical transformations in soil. In: Soil Biological Fertility (eds D. V. Murphy and L. K. Abbott) pp. 37–59. Kluwer Academic Publishers, Netherlands. https://doi.org/10.1007/978-1-4020-6619-1-3
- Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA based studies. PLoS One 6:27–31. https://doi.org/10.1371/journal.pone.0027310
- Sharma SK, Ramesh A, Sharma MP, Joshi OP, Govarts B, Steenwerth KL, Karlen DL (2011) Microbial community structure and diversity as indicators for evaluating soil quality. Biodivers Biofuels Agroforestry Conserv Agric 5:317–358. https://doi.org/10.1007/978-90-481-9513-8-11
- Sun LJ, Qi YC, Dong YS, Peng Q, He YT, Liu XC, Jia JQ, Cao CC (2012) Review on the effects of global change on soil microbial community diversity

in grassland. Prog Geogr. 31:1715–1723. https://doi.org/10.11820/dlkxjz. 2012.12.018

- Tai XS, Feng JL, Mei L, Li SW (2011) Research progress on biodegradation of Sphingomonas. Hunan Agric Sci 7:21–25. https://doi.org/10.16498/j.cnki. hnnykx.2011.07.001
- Tan HW, Yang SD, Wu J, Liu YX, Xiong LM, Zhou LQ, Xie RL, Huang GQ, Zhao QG (2014) Comparison of soil microbial activity and bacterial diversity between eucalyptus plantation and different stand in red soil region. Acta Pedologica Sinica 51:575–584. https://doi.org/10.11766/trxb201309 170421
- Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of 16S rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM. 00062-07
- Wang LD, Yao T, Wang FL, Wei LY, Guo CX, Wu CR, Li FM (2016) Effects of soil microbial diversity on soil enzyme activity in the lower reaches of Shiyang River. Acta Ecologica Sinica 36:4769–4779 (https://www.cnki.com. cn/Article/CJFDTOTAL-STXB201615022.htm)
- Yang N, Yang MY, Lei YL, Zhao LF, Chen SH, Fu MY, Lin ZJ (2015) Seasonal variations of soil microbial communities on sloping - land with purple soils. Ecol Environ Sci 24:34–40. https://doi.org/10.16258/j.cnki.1674-5906.2015. 01.006
- Yao HM, Zhang SY, Lei Z, Yu JX, Li JY (2013) Diversity of soil microbes in vineyard under two different cropping patterns. J Jiangsu Agric Sci 41:372–374. https://doi.org/10.15889/j.issn.1002-1302.2013.08.122
- Yin W, Hu YK, Liu YY, Gong YM, Zhang W, Liu W (2010) Study on soil biological characteristics of artificial grassland at different planting stages in Bayanbulak. Acta Prataculturae Sinica 19:218–226. https://doi.org/10. 11686/cyxb20100529
- Zhang ZJ, Cao YG, Wang SF, Guo CY, Wang X, Lu N, Zhou W, Bai ZK (2014) Analysis of soil microorganism and enzyme activity in the reclaimed land of Pingshuo Loess open-pit mining area. Acta Ecologica Sinica 1:110–123. https://doi.org/10.5846/stxb202002240326
- Zhao AH, Du XJ, Zang J, Zhang S, Jiao ZH (2015) Soil bacterial diversity in the Baotianman deciduous broad-leaved forest. Biodiversity Science 1(23):649–657. https://doi.org/10.17520/biods.2015032

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