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Original Research

Soil Microbe and Physicochemical Characteristics in Tensile Fracture Zone Caused by Mining Subsidence

Yulian Wang, Tingyu Fan*, Jiaping Yan, Akang Lu, Wangkai Fang, Yifan Zhao, Yingxiang Chen, Shun Wang, Xingming Wang

School of Earth and Environment, Anhui University of Science and Technology,
168 Taifeng Street, Huainan, China, 232001

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Abstract

The bacterial community plays a crucial role in the soil ecosystem, and is closely linked with soil physicochemical properties. Surface tension cracks caused by underground coal mining inevitably have a significant impact on physical properties. To reveal the characteristics of the soil bacterial communities in the surface tension zones, high-throughput 16S rRNA sequencing was used to analyze the bacterial communities in surface tension fracture areas. Furthermore, the relationships between environmental factors and the diversity and abundance of the bacterial community, alpha diversity index, and variations in bacterial communities were discussed. We analyzed soil bacterial community structure and soil physicochemical properties from different dimensions. The results indicated that The abundance of bacteria in the soil surface layer was higher, and the nutrients in the middle and bottom of the slope increased with the increase of soil depth. Additionally, our data illustrated that microorganisms participated in soil carbon and nitrogen cycling in the soil samples. This study provides a reference for the study of variations in the bacterial diversity and community composition in surface tension fracture areas caused by underground mining.

Keywords: bacterial diversity, carbon and nitrogen cycle, coal mining subsidence area, surface tension fracture, soil bacteria community structure

Introduction

Soil microorganisms are closely related to soil quality, which constitute the soil ecosystem [1]. The metabolism of soil microorganisms has a certain effect on nutrient transformation and the soil structure [2]. Recently, some biogeochemical simulation studies have shown that the soil microbial community is an important part of biogeochemistry [3, 4]. In addition, there are an increasing number of studies have been directed toward the mechanisms of changes in or the generation of soil microorganisms in different types of soil, such as the effects of Quaternary volcanic activity on soil microorganisms in typical steppes [5],

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*e-mail: ayufty@163.com

the soil microbial biomass C: N response to drought in the forests of eastern China [6], and the effects of soil nutrient absorption by soil microorganisms on the growth conditions of plants [7]. Additionally, the composition of and dynamic changes in the soil bacterial community can respond to soil moisture, pH, temperature and nutrient status immediately, thus, the soil bacterial community has become an effective index to evaluate soil quality [8]. In relevant published studies, the bacterial community is also considered as a characteristic in research on mining-degraded sites [9]. Some mining activities, such as stripping, excavation, transportation and dumping, have different effects on soil chemical, physical and biological properties [10]. Therefore, the soil bacterial community is always used as an indicator to evaluate the progress of reclamation, restoration or recovery [10-13].

China is the largest coal producer and consumer worldwide. With the rapid development of its social economy, the demand for energy has accelerated, and coal mining activities have increased accordingly. In 2020, the COVID-19 pandemic and lockdowns in China caused the worldwide coal demand to drop by 11% and 7.5% year-on-year in the first and second quarters respectively, but as countries eased lockdowns, coal consumption recovered, and China's share of global coal consumption continued to increase, accounting for 56% of the global yield in 2020 [14]. In China, more than 95% of coal comes from underground mining [15]. Although coal mining in China has made outstanding contributions to the national economy, it has destroyed the local ecological balance and caused many ecological and environmental problems [16, 17], especially due to coal mining subsidence. Coal mining subsidence causes surface subsidence water [18] and surface tension fracturing. Surface tension fracturing is the most common phenomenon in mining subsidence areas [19]. It can cause soil moisture evaporation, soil nutrient erosion [20] and plant root damage [21]. Therefore, the study of subsidence soil not only is beneficial to the stability of the local economy and society, but also provides a theoretical reference for the sustainable development and ecological restoration of mining areas.

Coal mining fractures are an important type of coal mining disturbance [22], and surface tension fractures can have a negative impact on land use and the environment [23]. Research on soil quality in subsidence areas mainly focuses on the soil physicochemical properties and the process, characteristics and effects of nutrient loss. Some previous and recent studies on bacterial diversity have examined the temporal and spatial evolution of microbial populations and enzyme activities in cracks [24], the effects of postharvest sedimentation on soil bacterial diversity in sandstorm areas of western China [25] and the soil biogeochemical characteristics, bacterial diversity and metabolic adaptation heterogeneity in arid and semiarid areas of Northwest China [26]. However, there has been little research focusing on the bacterial communities of coal mining subsidence areas in surface tension fractures or in eastern China. Therefore, a better understanding of the bacterial communities in coal mining subsidence areas is of great significance for evaluating the soil quality of surface tension fractures in coal mining subsidence areas.Recently, the development of next generation sequencing (NGS) technology has made it possible to study microbial composition based on nonculture methods and pushed microbiome research into a golden age of development [27], which has allowed the study of microbiomes to move from the culture level to the DNA level. High-throughput sequencing technology has facilitated the study of microbial diversity at a previously infeasible level. This technique has been used in various studies of species diversity in medicine, aquatic environments, grasslands and farmlands, including in nucleic acid assays for the novel coronavirus.

In this study, we studied the changes in soil properties and bacterial community characteristics at different levels in Huaibei, Anhui Province, China. We assumed that the characteristics of bacterial communities are different at different levels and that there are correlations between the soil properties and bacterial community characteristics at different levels. Thus, to test our hypothesis, we chose 16S rRNA high-throughput sequencing to study the microorganisms in the top fractured zone (TA), middle fractured zone (MA) and bottom subsidence zone (SA) at different locations in the subsidence area. The correlations between soil microorganisms and soil physical, and chemical properties were detected by redundancy analysis (RDA) and correlation analysis. The main purpose of this study was to answer the following questions: (1) what are the effects of soil physicochemical properties on the bacterial community; (2) what is the spatial distribution pattern of the soil bacterial community in surface tension fracture areas in coal mining subsidence areas; and (3) The difference between soil and other types of soil in tensile fracture zone? This research is expected to provide a theoretical reference for improving the quality and efficiency of coal mining subsidence soil in surface tension fracture areas and to contribute to the sustainable development of coal mines.

Materials and Methods

Research Area

The research area is located in the surface subsidence area corresponding to the 1047 outer working face of the Suntuan mine in Huaibei city. The working face is 560 m long. The dip width is 220 m, and the mining thickness is approximately 3.3 m. The mining time was from July 15, 2019, to the end of December, 2019. The coal-mining method was a fully mechanized mining method, which mainly recovered No. 10 coal, the mining depth was -2804~-427.3 m, and the coal

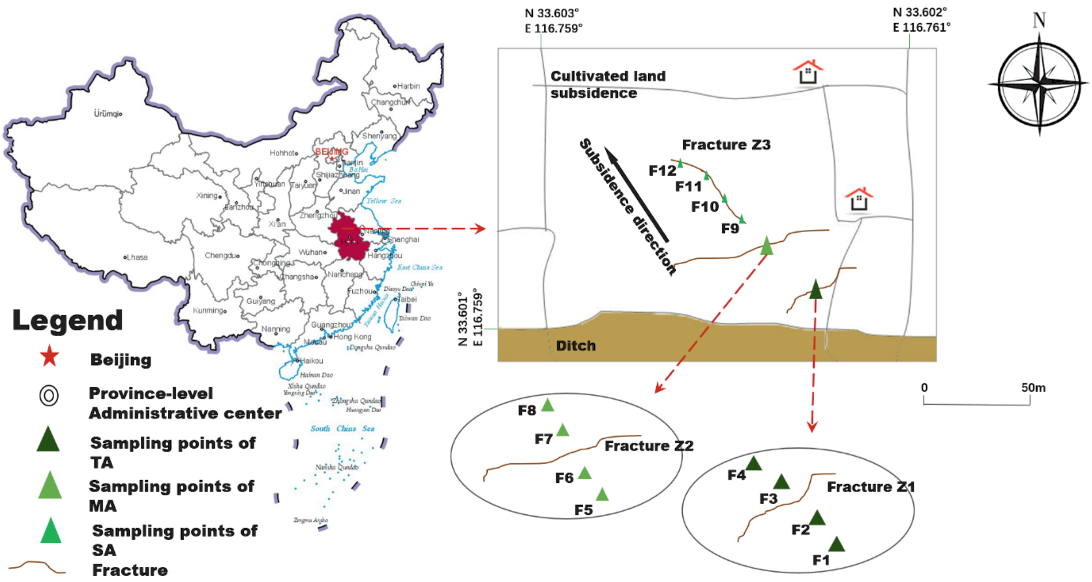

Fig. 1. Schematic diagram of sampling points.

type was 1/3 JM, with recoverable resources measuring 5,356,000 t. The face cutting direction is 150°, and the machine roadway and wind roadway directions are 60°. The region belongs to the semi-humid monsoon climate of the warm temperate zone, with an annual average temperature of 14.8ºC, extreme lowest temperature of -21.3ºC, extreme maximum temperature of 41.5ºC, annual average precipitation of 823.4 mm, and annual extreme maximum precipitation of 1441.4 mm. The dominant wind is from the southeast throughout the year, with more southwest winds in summer and more northwest winds in winter.

Sample Collection

In December 2019, soil samples were collected in the subsidence area. Four sampling points were set in the top fractured zone (TA); and middle fractured zone (MA), which were listed at 50 cm and 100 cm on the vertical sides of the fracture. Four sampling points were set in the subsidence area at the bottom subsidence zone (SA), each with an interval of approximately 15 m. At each sampling point, a soil corer with a diameter of 6 cm was used to drill and collect soil samples from the soil layer 0-60 cm away from the ground, and one sample was collected every 20 cm, for a total of 36 samples. Approximately 5 g of each sample was put into a sterile bag and immediately placed in a freezer in the field for microbial sequencing, while the remaining sample was put into a sealed bag for the determination of soil physical and chemical properties.

Experimental Method

The fresh soil samples were used to determine the soil moisture content, and the air-dried and sifted soil samples were analyzed for the following physical and chemical properties: pH, organic matter, available potassium, and available phosphorus. The moisture content was measured by the drying and weighing method. pH was measured by the potential method (water and soil ratio of 2.5:1). The tetrachloride sodium turbidimetric method was used to determine rapidly available potassium. Available phosphorus was measured by molybdenum antimony colorimetric method. Organic matter was determined by the potassium dichromate volumetric method and hydration calorimetry.

The soil samples were sent to Shanghai Partheno Biotechnology Co., Ltd. for sequencing using Illumina MiSeq high-throughput sequencing technology. The soil total DNA genome was extracted with a DNA extraction kit. Primers F: ACTCCTACGGGAGCAGCA and R: CGGACTACHVGGGTWTCTAAT were used to amplify the V3-V4 region of the 16S rRNA gene of soil microorganisms. The total DNA of the microbiome was extracted using the DunEasy PowerSoil Kit from Mobio/Qiagen, and the extracted DNA was tested. A fluorescence spectrophotometer (Quantifluor-ST Fluorometer, Promega, E6090; Quant-It Picogreen DSDNA Assay Kit, Invitrogen, P7589) was used to measure the light absorption value of DNA at 260 nm and 280 nm to detect the DNA concentration. The DNA quality was determined by 1% agarose gel electrophoresis. The concentration of the DNA solution was adjusted. The DNA working solution was stored at 4ºC and the storage solution was stored at -20ºC. The variable region of the 16S rRNA gene was first amplified by PCR and then purified by gel recovery. The purified samples were then quantitatively detected by a BIO TEK assay. The sequencing results were filtered using QIIME, and the platform was also used for data processing and mapping.

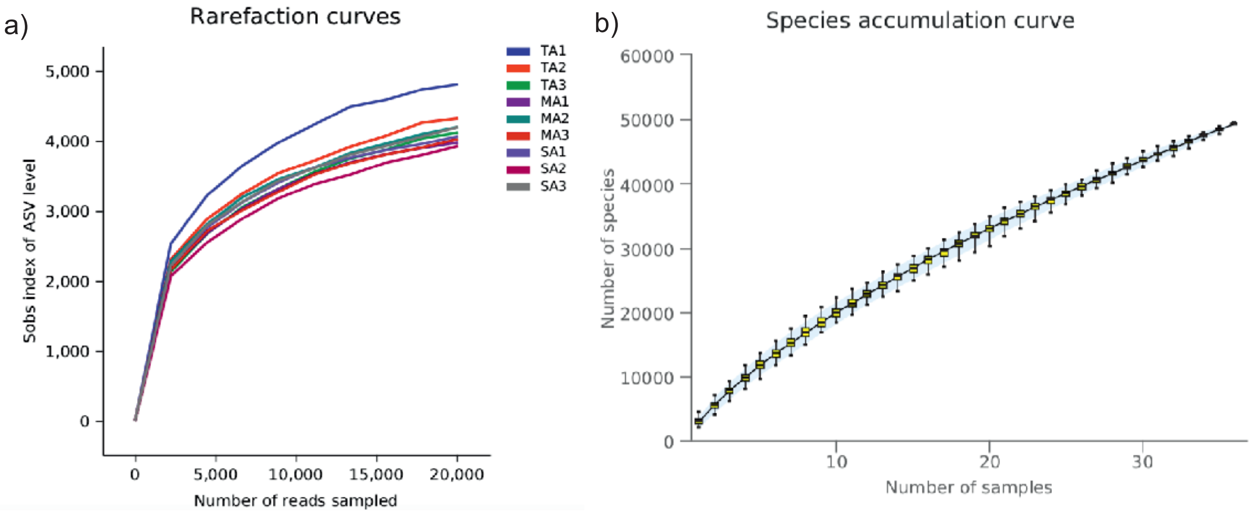

Fig. 2. Sparse curve a) and species accumulation curve b).

Data Analysis

We used Excel software to process and analyze the experimental data, and SPSS. 19.0 software (SPSS Inc., Chicago, IL, USA) was used to calculate Spearman's rank correlation coefficient to determine the relationship between environmental factors and bacterial community diversity or abundance. QIIME2 software to process the raw microbe data. The DADA2 method mainly includes primer removal, mass filtration, denoising, splicing and chimerism removal. Each reduplication sequence generated after quality control with DADA2 is called an ASVS (amplicon sequence variants) or feature sequence, and the abundance table of these sequences in the sample is called a feature table. Alpha diversity indices were calculated using the software package GGPLOt2 and included the Chao1 (Chao, 1984) and Shannon (Shannon, 1948a, b) indices representing diversity, and Good's Coverage (Good, 1953) index representing coverage. For β-diversity, NMDS analysis was performed using the software package Vegan. For redundant discriminant analysis (RDA), the R basic software package was used to perform Monte Carlo permutation tests to reveal the relationship between bacterial assemblages and environmental factors.

Results

Alpha and Beta Diversity of Bacterial Communities

The abundance and diversity of bacterial communities in the soil subsidence area are shown in Table 1. A total of 2,923,937 valid sequences and 111,738 ASVs were generated from 36 soil samples from 12 sampling sites. The coverage rate was above 94%, and the species rarefaction curve of all samples tended to be close to saturation (Fig. 2a). The species accumulation curve indicated that the sample size was sufficient for subsequent data analysis (Fig. 2b). The abundance of bacteria in TA1 and TA2 soil was significantly higher than that in other sampling sites, and the average Chao1 index values were 4816.05 and 4305.03, respectively (Table 1). The abundance in the MA1 and SA2 samples was relatively low ($P<0.05$), and the average Chao1 index values were 3966.29 and 3922.08, respectively. For soil bacterial diversity, the Shannon index in these samples ranged from 9.87 to 10.53, which was higher than the Shannon index values in TA1, TA2, and MA2 ($P<0.05$), and the lowest value was 9.87 in SA2.

A nonmetric multidimensional scale (NMDS) based on the Bray-Curtis distance matrix was used to explore the β-diversity of all samples in the subsidence area (Fig. 3). The NMDS plot showed the differences in the bacterial community structure. ANOSIM further validated the NMDS analysis (Fig. 3). According to the NMDS analysis, there was little difference in bacterial communities on the soil surface (0-20 cm), and there were significant differences between the samples of 20-60 cm soil at the basin floor and the samples of 0-20 cm soil at the surface of the subsidence area.

Taxonomic Composition of the Bacterial Communities

The main bacterial community composition of 36 soil samples were analyzed at the phylum and genus levels (Fig. 4). The abundances and relative abundances of the dominant phylum were different among the 36 soil samples. The results showed that *Actinomycetes* were the most important phylum in all soils, accounting for 37.03% on average. *Proteus* was the second most dominant phylum, accounting for 24.44% on average. *Acidobacteria* and *Chloroflexi* were the third and fourth dominant phyla, accounting for 13.5% and 10.74% on average, respectively.

Table 1. Abundance and diversity index of bacterial communities in different study areas of coal mining subsidence area

Group[a]	Vseqs[b]	ASV	Chao1	Shannon	Coverage
TA1	82720	3537	4816.05	10.53	0.94
TA2	81566	3172	4305.03	10.29	0.95
TA3	85391	3021	4121.98	10.10	0.95
MA1	73239	2995	3966.29	10.16	0.95
MA2	82630	3143	4193.78	10.30	0.95
MA3	76757	3010	4037.05	10.14	0.95
SA1	84171	3082	4078.42	9.98	0.95
SA2	81931	2865	3922.08	9.87	0.95
SA3	82580	3094	4188.36	10.21	0.95

[a] TA represents the fractured area at the top of the slope, MA represents the fractured area in the middle slope, and SA represents the subsidence area at the bottom of the slope. Numbers 1, 2 and 3 represent the soil depth of 0-20 cm, 20-40 cm and 40-60 cm respectively.

[b] Vseqs = valid sequences, ASVs = amplicon sequence variants

At the genus level, the dominant species and quantity of bacteria differed among different samples. In some cases, a species was highly represented and was the dominant bacterium in some samples, while in others, its proportion was small or nonexistent. A total of 515 different genera were identified in 36 soil samples. The dominant genera were *MB-A2-108* (4.42%), *Subgroup_6* (6.54%), *Rokubacteriales* (3.50%), *KD4_96* (2.34%) and *Rubrobacter* (2.32%). *MB-A2-108* was the dominant genus on average, except in TA1, MA1 and SA1. The *MB-A2-108*, *Subgroup_6, Rokubacteriales* and *Rubrobacter* amounts in SA were all high er than those in TA and MA.

Soil Physiochemical Properties and Redundancy Analysis

The differences in the physiochemical property parameters of soils in different regions and layers in the tensile fracture zone are shown in Fig. 5. From the vertical direction, the general trend of the soil moisture content showed an increase with soil depth. The content of available potassium in TA decreased with soil depth. The content of available phosphorus increased with soil depth in MA and SA. The pH of the surface layer was less than 7 and showed a trend of increasing with soil depth. In the horizontal direction, the content of soil

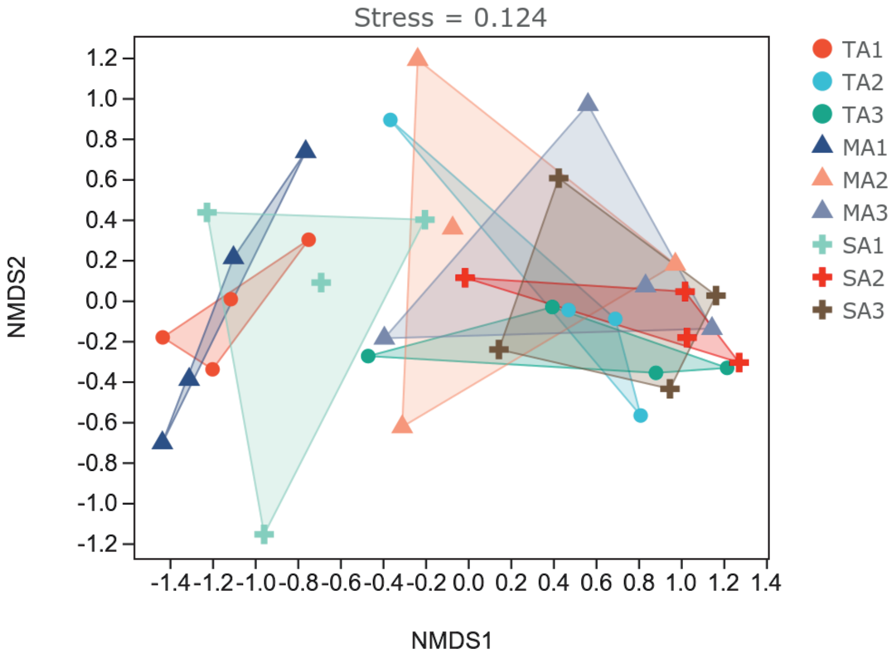

Fig. 3. NMDS analysis.

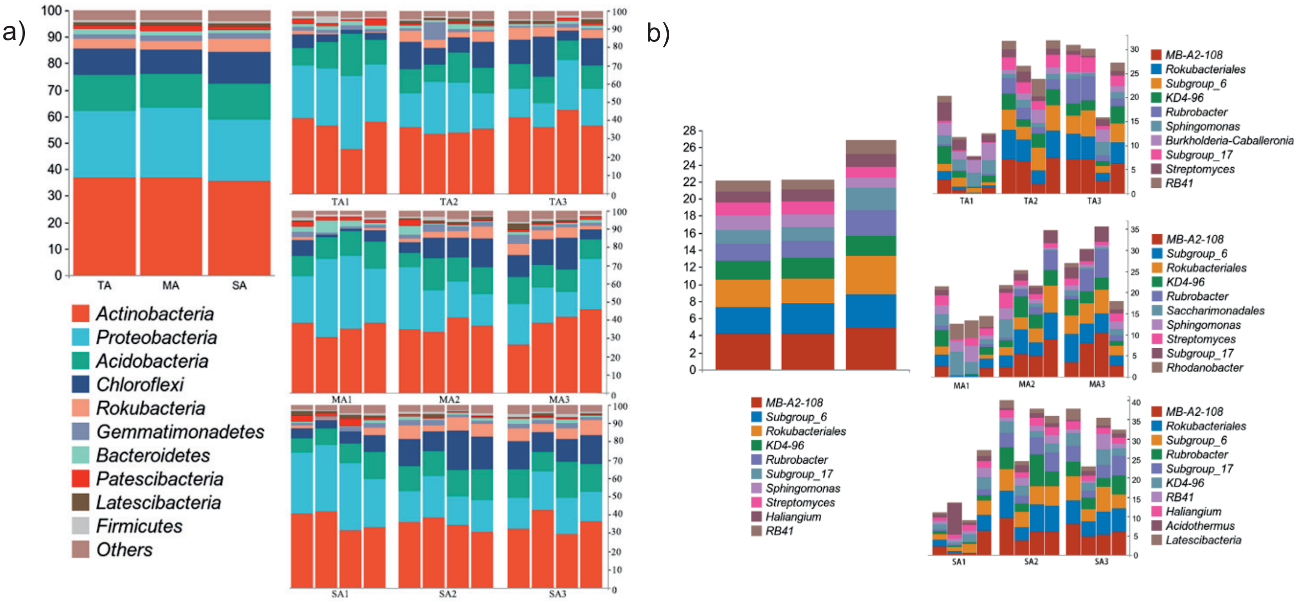

Fig. 4. Relative abundance of bacterial communities at phylum(a) and genus(b) level in the coal mining subsidence area.

organic matter in the surface layer was significantly higher than those in the middle layer and bottom layer. The content of soil organic matter in the surface layer of MA was the highest, followed by that in TA, and the lowest was found in SA. In addition, the content decreased with soil depth.

The correlation between physiochemical indices and bacterial communities in different areas and soil layers of coal mining sloping farmland were determined by redundancy analysis (RDA). The first RDA axis explained 44.47% of the bacterial community variation, while the second RDA axis explained 1.92% (Fig. 6). The RDA model revealed that soil organic matter and pH play key roles in the distribution of bacterial communities in different areas and layers of the coal mining subsidence area (Fig. 6).

Abundance of Functional Genes Related to the Carbon and Nitrogen Cycle

In this study, PICRUST was used to predict the functions of bacterial communities of each sample,

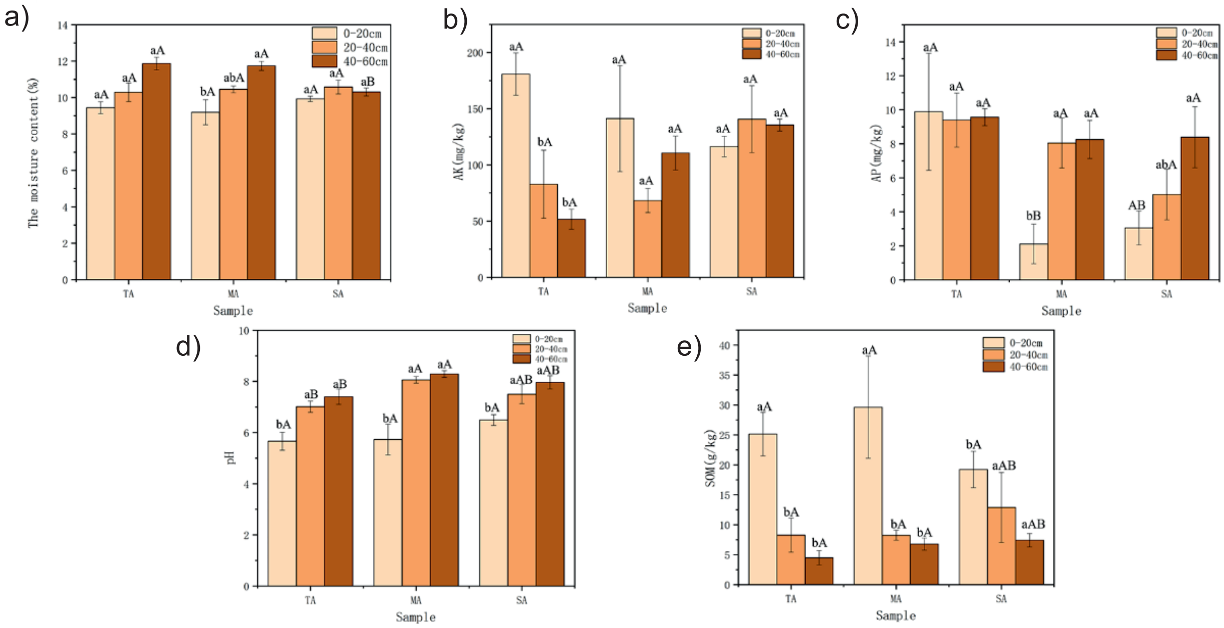

Fig. 5. Physicochemical parameters of bacterial communities in different study areas of coal mining subsidence area (Lowercase letters indicate the importance of differences in soil depths in the same area; capital letters indicate the importance of differences between different areas of the same soil layer. If the letters are different, there is a significant difference(P<0.05)).

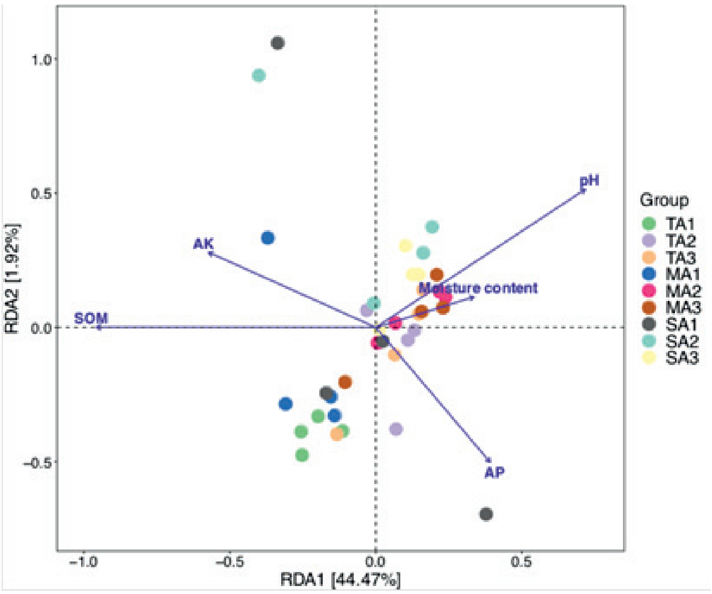

Fig. 6. Redundancy analysis (RDA) and environmental factors of soil bacterial community composition in coal mining subsidence area.

and carbon and nitrogen metabolism-related functional gene KEGG orthology was obtained from the KEGG database (Table 1). The genes related to carbon and nitrogen metabolism in the surface tension and fracture soil in the coal mining subsidence area were involved in three reactions (carbon fixation, carbon reduction, nitrotoluene degradation and nitrogen metabolism). The abundances of carbon and nitrogen metabolism-related functional genes in the coal mining subsidence area are shown in Fig. 7. In Fig. 7a), *CynT* is a carbon fixation gene, and its abundance was the highest. *CoxS, coxM* and *coxL* are carbon reduction genes, and their abundances were higher in all samples. The abundances of *nifU, ncd2* and *glnG* were significantly higher in all samples, as shown in Fig. 7b). Additionally, the abundances of *pnbA, ntrY* and *ncd2* were higher in the top layer of the surface tension soil. *GlnK, glnL,glnG* and *glnB* are nitrogen regulatory genes, and along with *nifU*, their abundances were higher in the middle and bottom layers of the surface tension soil.

Discussion

Variations in the Bacterial Community Structure of the Coal Mining Subsidence Area

Extremely diverse bacterial populations existed in the soil and affected the biogeochemical cycle of soil. The 4 dominant phyla were *Actinomycetes*, *Proteus*, *Acidobacteria* and *Chloroflexi*, which have also been the main phyla found in other different types of coal mining subsidence soil research [28-30]. The abundances of *Actinomycetes* and *Acidobacteria* showed little

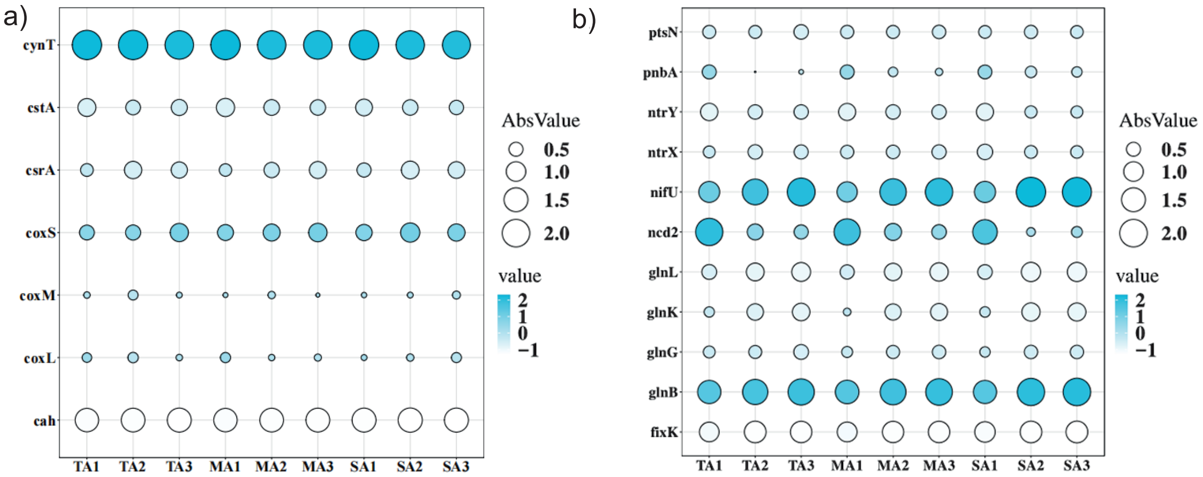

Fig. 7. Relative abundances of carbon and nitrogen metabolism genes in coal mining subsidence area.

difference among samples. However, the abundance of *Proteobacteria* was higher in the surface soil than in the middle layer of the surface tension soil, and higher in the middle of the slope than at the top and bottom of the slope. The existence of surface fractures can inhibit the flow of surface runoff or nutrients along the slope and increase vertical flow. Thus, both the abundance and diversity of bacterial communities at the top of the slope were higher than those in other locations. In the horizontal direction, the bacterial community structure in the surface soil samples (0-20 cm) and deep soil samples (40-60 cm) showed a trend of aggregation. In the middle soil samples (20-40 cm), TA and MA showed significant differences, but the bacterial community structure was similar to that of SA. The soil compaction caused by mining subsidence has a strong effect on soil microorganisms [31]. The surface tension fracures caused by underground mining can cause soil compaction, which increases the soil bulk density and changes the size distribution, curvature and connectivity of soil pores, affecting the soil physical properties [32-35] and causing the aggregation of the bacterial community structure at different depths in the fracture.

Effects of Soil Physicochemical Properties on Microbial Diversity and Abundance

Environmental factors such as the climate, soil type, soil properties and geographic location, can all affect soil microbial communities [25]. In an ecosystem, the soil nutrient status is closely related to microorganisms. From Table 1 and Fig. 2, we can see that both the abundance and diversity of the TA1 and TA2 bacterial communities were relatively high, while the abundance and diversity of the SA2 bacterial communities were the lowest. These results indicated that the soil nutrient contents in TA1 and TA2 were at least higher than those in SA2. Large-area collapse in coal mining areas will cause the soil to sink and change the soil texture structure, resulting in the concentration of soil nutrients and water content as well as other physical and chemical properties with different crack depths. Through the correlation analysis of the abundance of the first 4 dominant phyla and environmental parameters, it can be seen (Table 2) that the abundances of the first dominant *Actinomycetes* and the third dominant *Actinomycetes* were not affected by environmental factors. PH, soil organic matter and available potassium were significantly correlated with the abundances of *Proteobacteria* and *Chloroflexi* bacteria. The contents of organic matter and available potassium were significantly positively correlated with the abundance of *Proteobacteria*, but negatively correlated with the abundance of *Chloroflexi* bacteria. *Proteobacteria* and *Chloroflexi* bacteria were in a competitive relationship. Fig. 5 shows that the soil moisture content and available P decreased significantly near the crack, while the soil pH, available K and organic matter contents increased near the crack. The change in the water content at the basin floor was small. The organic matter, available P and available K tend to increase. The soil pH followed no obvious rule.

Recently, many studies have been conducted on soil microorganisms after reclamation in coal mining subsidence areas [10, 36-39]. By studying the physical and chemical properties of soil and the diversity and abundance of the bacterial community before and after reclamation, the recovery status of coal mining subsidence soil can be determined. Li et al. [39] studied changes in the composition and diversity of bacterial communities before and after reclamation in abandoned mine land in eastern China. They found that *Actinomycetes, Acidobacteria, Proteobacteria* and *Prongella* were the dominant phyla in the coal mining subsidence soil before reclamation. After reclamation, the dominant groups were *Blomonas, Greenrot, Actinomycetes* and nitrifying bacteria. In 36 soil samples, the top 4 dominant phyla were *Actinomycetes, Proteus, Acetobacter* and *Chlorobacteria*, accounting for almost 85.71%, which was consistent with the above research results.

According to the data analysis, we can also find that some soil physico-chemical properties, such as the contents of organic matter, pH and available potassium, were major drivers of soil bacterial communities [40]. However, this finding did not definitely indicate whether the degree of bacterial community aggregation was directly related to a certain nutrient element, and more research is needed.

Table 2. Correlation analysis between environmental parameters and the abundance of the first 4 dominant phyla.

	Actinomycetes	Mycetozoan	Acid bacillus	Chloroflexi
Moisture content	0.143	-0.277	0.065	0.274
pH	0.188	-0.637**	0.017	0.586**
SOM	-0.259	0.84**	0.053	-0.769**
AP	0.076	-0.372*	0.152	0.263
AK	-0.271	0.495**	0.133	-0.434**

*The correlation was significant at a confidence level of 0.05 (double test).

**The correlation was significance at a confidence level of 0.01 (double test).

Predicted Functions of Bacterial Communities Associated with Carbon and Nitrogen Metabolism

Our data illustrated that microorganisms participated in soil carbon and nitrogen cycling in the soil samples (Fig. 7a-b). The genes related to carbon and nitrogen metabolism in the surface tension and fracture soil in the coal mining subsidence area included carbon fixation, carbon reduction, nitrotoluene degradation and nitrogen metabolism. Microbial communities are mainly involved in the biogeochemical cycle of carbon and nitrogen [41]. Microorganisms can accumulate energy and nutrients in nutrient-poor environments through the nitrogen cycle [42]. Overall, 18 carbon and nitrogen cycling-related functional genes were detected, including 3 carbon reduction genes, 4 carbon fixation genes, 4 nitrogen regulatory genes and 7 nitrogen metabolism genes (Fig. 7). The intensity of the main carbon and nitrogen cycling-related functional genes found in the surface tension and fracture soil in the coal mining subsidence area are shown in Table 3. The presence of genes encoding proteins involved in the phosphonate recycling mechanism (such as *pnbA* and *ptsN* in the soil samples) suggested that these proteins form a carbon phosphorus lyase complex that participates in methane production from methyl phosphonate [42, 43]. The abundance and distribution of carbon and nitrogen genes may be related to the organic matter and organic carbon contained in the tensile fracture soil in coal mining subsidence areas, leading to the existence of some nitrogen fixing bacteria. *Actinomycetes* are considered to be involved in organic matter decomposition as they can secrete extracellular enzymes to help breakdown lignin in lignocellulose [44] and degrade polysaccharides or phenolics in dead plants. *Acidobacteria* are also important soil microorganisms and play an important role in soil material circulation and ecological environment construction. At the same time, they also degrade plant residue polymers, participate in the iron cycle, have photosynthic ability, participate in single-carbon compound metabolism, etc. The abundance of *Proteobacteria* were significantly lower in soils with a high abundance of *Chlorobacteria*, and both *Chlorobacteria* and *P. proteobacteria* belong to nitrogen metabolism-related fungi, suggesting a possible competitive relationship between *Chlorobacteria* and *Proteobacteria*. This assumption agreed with Zhang [45] and Huang [46], who suggested that the level of organic carbon/organic matter is the main factor affecting the abundance and diversity of nitrogen-fixing bacteria [47]. Change in seasons and arable land can also explain this finding.

Comparison of Soil Properties between Mining Subsidence Soil and Other Soil Types

Soil nutrients and organic matter have high spatial variability, especially in coal mining subsidence areas [48]. Thus, soil nutrients and organic matter are always used as indicators in soil property or reclamation studies. The results of this study on environmental parameters showed that there was no significant difference in soil pH between different regions. The surface layer content of soil organic matter was significantly higher than those in the middle layer and deep layer. Additionally, there was no significant difference in soil available phosphorus among different soil layers in the TA region, and MA and SA in the surface layer were significantly lower than those in the middle and deep layers, which was the opposite of the trend of organic matter. The content of soil available potassium was the highest in TA1 and decreased with soil depth. In contrast, the content of available potassium increased with soil depth in SA. The available potassium content in MA was the lowest in MA2. The results indicate that fractures in the research area lead to changes in the distribution of soil nutrients. Also, it may be due to the planting of plants on the surface, the nutrient content of the topsoil is relatively higher. Zhang et al. [48] used a stochastic simulation method to study the 3D spatial distribution of SOM in the Zhaogu mining area in Henan Province, and the results revealed the maximum spatial variation in SOM in the surface layer (0-20 cm). Bao 's [49] research conducted as early as 2008 showed that surface subsidence changes the soil chemical microenvironment in coal mining areas; and has a more significant impact on surface soil. This is consistent with the research results obtained in this study. [26] found that the contents of many nutrient elements decreased significantly with soil depth when they studied soil in subsidence areas of arid and semiarid underground coal mining, again indicating that soil nutrients infiltrated vertically due to mining subsidence. In recent years, many scholars have studied soil organic matter and nutrient elements in coal mining subsidence areas for land restoration and the planting of crops or vegetation [38, 39, 48, 50, 51]. Consistently, the nutrient content and biological structures of all mining soils were much better after reclamation than during mining subsidence. Plants, soil microorganisms and soil physical and chemical properties are closely related. Plant roots continuously secrete large amounts of organic materials into the soil, forming rhizosphere deposits, which provide abundant nutrients and energy for microorganisms. The species and quantity of plant root exudates determine the species and quantity of rhizosphere microorganisms and affect their growth, reproduction and metabolism [52]. The dynamic changes in the rhizosphere microbial community in turn affect the material cycle and energy flow of the plant rhizosphere ecosystem, and thus affect the growth and development of plants and the changes in vegetation diversity [53]. It has been further indicated that the physical and chemical properties of plants and soil, material exchange between microorganisms and nutrient cycling are complex open systems [54].

Table 3. Distribution of carbon and nitrogen metabolism genes in each group of samples.

	TA1	TA2	TA3	MA1	MA2	MA3	SA1	SA2	SA3
cynT	3574.496	3567.734	3456.711	3591.738	3440.615	3450.321	3584.304	3435.499	3370.238
cah	175.724	146.448	111.983	176.752	144.136	131.110	116.300	84.588	106.518
cosS	2009.792	2029.492	2280.213	2092.911	2176.852	2298.773	2113.231	2370.994	2191.241
coxM	1398.740	1265.924	1418.435	1439.669	1370.104	1456.995	1429.380	1418.600	1347.727
coxL	1697.201	1241.840	1398.277	1729.196	1402.308	1378.540	1566.301	1370.376	1260.238
csrA	1111.702	777.657	856.634	1127.921	935.498	806.292	1020.949	739.832	832.812
cstA	728.060	982.365	879.842	702.799	907.065	927.379	826.488	926.888	1008.646
ncd2	2938.866	1742.646	1619.221	2708.184	1813.945	1646.339	2520.695	1345.603	1440.998
ptsN	821.273	830.536	734.992	814.336	823.632	799.020	842.875	811.824	846.839
pnbA	1610.788	1184.429	1145.560	1614.736	997.67	1076.308	1598.417	907.600	962.375
nifU	2149.102	2661.53	2908.276	2087.344	2735.093	2850.054	2168.803	3108.206	3068.212
glnB	2386.333	2600.042	2718.498	2418.411	2667.596	2762.623	2374.377	2846.567	2877.102
glnK	966.946	600.793	519.063	1069.544	618.761	599.3136	958.114	465.424	478.915
glnL	711.817	496.0591	412.043	753.937	532.323	499.604	728.349	379.360	389.087
glnG	890.933	832.214	718.155	937.352	822.034	799.977	963.399	803.586	806.427
ntrY	531.782	743.054	743.875	554.635	738.586	746.690	566.253	873.692	884.409
ntrX	887.292	740.711	764.129	812.318	797.882	754.076	676.478	839.851	855.997
fixK	341.464	157.242	177.101	326.869	186.466	175.137	268.353	155.131	144.660

Conclusions

This study investigated the variations in the bacterial community structure of the surface tension fracture area. The results indicated that soil nutrient content was relatively high in the surface layer, and the similarity of the bacterial community structures in the surface layer (0-20 cm) and deep layer (40-60 cm) soil samples showed a tendency of aggregation in the horizontal direction. The soil moisture content and effective phosphorus near the crack decreased significantly, and the pH, available potassium and organic matter content near the crack increased, while the abundance and diversity of bacterial communities in TA1 and TA2 were relatively high. The abundance and diversity of the SA2 bacterial community were the lowest, showing that soil nutrients have a certain influence on the abundance and similarity of the bacterial community structure. We also observed that the genes related to the carbon and nitrogen cycles in the surface tension fractures in the coal mining subsidence area were involved carbon fixation, carbon reduction, nitrotoluene degradation and nitrogen metabolism. This study provides a reference for the study of variations in bacterial diversity and variations in bacterial community composition in surface tension fracture areas caused by underground mining.

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Conflict of Interests

The authors declare no conflicts of interest.

Reference

1. WU B., LUO H., WANG X., LIU H., and XU H. Effects of environmental factors on soil bacterial community structure and diversity in different contaminated districts of Southwest China mine tailings, Sci Total Environ, **802** (16), **2021**.
2. FAN L., TARIN MWK, ZHANG Y., HAN Y., ZHENG Y. Patterns of soil microorganisms and enzymatic activities of various forest types in coastal sandy land, Glob Eclo Conserv, **28 2021**.
3. HAGERTY S.B., GROENIGEN KJ VAN, ALLISON S.D., HUNGATE B.A., SCHWARTZ E., KOCH G.W., KOLKA R.K., DIJKSTRA P. Accelerated microbial turnover but

constant growth efficiency with warming in soil, Nat Clim Chang, **4** (10), **2014**.

4. WIEDER W.R., BONAN G.B., ALLISON S.D. Global soil carbon projections are improved by modelling microbial processes, Nat Clim Chang, **3** (10), **2013**.
5. JIN CABC, DXB C., HLB C., LCB C., YZB C., HQB C., FLB C., LI MBC, BWB C., BC A. Quaternary volcanic activities influence core soil microorganisms in a typical steppe, Catena, **207**
6. SUN Y., CHEN HYH, JIN L., WANG C., YANG J. Drought stress induced increase of fungi:bacteria ratio in a poplar plantation, Catena, **193,** 104607, **2020**.
7. QMA B., WP A., SHENG T.A., XS B., YX A., DRC B., PWH B., LS C., LWA D., DLJB E. Maize and soybean experience fierce competition from soil microorganisms for the uptake of organic and inorganic nitrogen and sulphur: A pot test using 13C, 15N, 14C, and 35S labelling, Soil Biol Biochem, **2021**.
8. ZHALNINA K., DIAS R., QUADROS PATRICIA DRR D.E., DAVIS-RICHARDSON A., TRIPLETT E.W. Soil pH Determines Microbial Diversity and Composition in the Park Grass Experiment, Microb Ecol, **69** (2), **2015**.
9. BANDYOPADHYAY S., MAITI S.K. Evaluation of ecological restoration success in mining-degraded lands, Environ Qual Manag, **29** (1), **2019**.
10. A YU FENG, B JINMAN WANG A, B ZHONGKE BAI A, and C LUCY. Effects of surface coal mining and land reclamation on soil properties: A review, Earth Sci Rev, **191**, 12, **2019**.
11. CHENG Z., ZHANG F., GALE W.J., WANG W., YANG H. Effects of reclamation years on composition and diversity of soil bacterial communities in Northwest China, Can J Microbiol, **64** (1), **2018**.
12. DANGI S.R., STAHL P.D., WICK A.F., INGRAM L.J., and BUYER J.S. Soil Microbial Community Recovery in Reclaimed Soils on a Surface Coal Mine Site, Soil Sci Soc Am J, **76** (3), **2012**.
13. MUMMEY DANIEL L., STAHL PETER D., BUYER JEFFREY S. %J APPLIED SOIL ECOLOGY, Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation, Appl Soil Ecol, **21** (3), **2002**.
14. IEA, Available online: https://www.iea.org/coal2017/ (accessed on 1 June **2019)**
15. HUANG, JIU, TIAN, CHUYUAN, XING, LONGFEI, BIAN, ZHENGFU, MIAO, XIEXING. Green and Sustainable Mining: Underground Coal Mine Fully Mechanized Solid Dense Stowing-Mining Method, Sustainability-basel, **9**, 1418, **2017**.
16. ZHEN-QI H. U., WANG XIN JING, AN-MIN H.E. Distribution characteristic and development rules of ground fissures due to coal mining in windy and sandy region, Coa Soc Chi J, **39** (1), **2014**.
17. ZHOU D.W., WU K., CHENG G.L., LI L. %J ARABIAN JOURNAL OF GEOSCIENCES, Mechanism of mining subsidence in coal mining area with thick alluvium soil in China, Arab J Geosci, **2015**.
18. FAN T.Y. ZHANG J.P., WANG S., YU L., WANG X.M., SU J.F Comparison of Eutrophication Evaluation Methods in Closed Coal Mining Subsidence Watering Area, J AUST, **40**, **2020**.
19. YAN J.P., CHEN X.Y., CAI Y., CHENG F.K., FAN T.Y. A review of genetic classification and characteristics of soil cracks, Open Geosci, **13** (1), **2021**.
20. ZHU Y., WANG J., BAI Z., QIN Q., WANG H. Recent Research Progresses of Influences of Coal Mining Subsidence on Soil Physical and Chemical Properties, Soils, **2016**.
21. WANG S.M., HUA-DONG D.U., WANG S.Q. Analysis of damage process and mechanism for plant community and soil properties at northern Shenmu subsidence mining area, Coa Soc Chi J, **2017**.
22. ZHANBIN, LUO, JING, CHEN, XIAOXIAO, HUPING, HOU, SHAOLIANG, Cracks Reinforce the Interactions among Soil Bacterial Communities in the Coal Mining Area of Loess Plateau, China, Int J Env Res Pub He, **16** (24), **2019**.
23. ZHENGFU, BIAN, AND, HILARY, INYANG, AND, JOHN, Environmental issues from coal mining and their solutions, Sci Technol, **2010**.
24. TAO D.U., YIN-LI B.I., HUI J.U., ZHENG JIAO LONG, and LIU SHENG. Effects of surface cracks caused by coal mining on microorganisms and enzyme activities in rhizosphere of Salix psammophila, Coa Soc Chi J, **38** (12), **2013**.
25. SHI P., ZHANG Y., HU Z., MA K., WANG H., CHAI T. The response of soil bacterial communities to mining subsidence in the west China aeolian sand area, Appl Soil Ecol, **2017**.
26. GUO J., ZHANG Y., HUANG H., YANG F. Deciphering soil bacterial community structure in subsidence area caused by underground coal mining in arid and semiarid area, Appl Soil Ecol, **163** (2), **2021**.
27. WHITE R.A., CALLISTER S.J., MOORE R.J., BAKER E.S., JANSSON J.K. The past, present and future of microbiome analyses, Nat Protoc, **11** (11), **2016**.
28. CHEN J., NAN J., XU D., MO L., BAO Y. Response differences between soil fungal and bacterial communities under opencast coal mining disturbance conditions, CATENA, **194**, 104779, **2020**.
29. JIN C.A., LI M.A., ZZ B., JI N.A., DX A., LC A., XZ A., YB A. Evaluation of the ecological restoration of a coal mine dump by exploring the characteristics of microbial communities - ScienceDirect, Appl Soil Ecol, **147**
30. WU Z., SUN L., LI Y., SUN Q. Shifts in Vegetation-Associated Microbial Community in the Reclamation of Coal Mining Subsidence Land, Environ Eng Sci, **37** (12), **2020**.
31. BEYLICH ANNEKE, OBERHOLZER HANS RUDOLF, SCHRADER STEFAN, H?PER HEINRICH, WILKE BERNDT MICHAEL. Evaluation of soil compaction effects on soil biota and soil biological processes in soils, Soil Till Res, **109** (2), **2010**.
32. ZHANG S., GRIP H., L?VDAHL L. Effect of soil compaction on hydraulic properties of two loess soils in China, Soil Till Res, **90** (1-2), **2006**.
33. PAGLIAI A.M., MARSILI B.A., SERVADIO B.P., VIGNOZZI A.N., PELLEGRINI A.S. Changes in some physical properties of a clay soil in Central Italy following the passage of rubber tracked and wheeled tractors of medium power - ScienceDirect, Soil Till Res, **73** (1-2), **2003**.
34. PAGLIAI M., VIGNOZZI N., PELLEGRINI S. Soil structure and the effect of management practices, Soil Till Res, **79** (2), **2004**.
35. B., SCHFFER, AND, M., STAUBER, AND, T. L., Soil and macro-pores under uniaxial compression. I. Mechanical stability of repacked soil and deformation of different types of macro-pores - ScienceDirect, Geoderma, **146** (1-2), **2008**.
36. WANG X., LI Y., WEI Y., MENG H., HONG J. Effects of fertilization and reclamation time on soil bacterial

communities in coal mining subsidence areas, Sci Total Environ, **739,** 139882, **2020**.

37. BANDYOPADHYAY S., MAITI S.K. Evaluation of ecological restoration success in mining-degraded lands, Environ Qual Manag, **29** (1), **2019**.
38. ZHANG, GENGJIE, ZHOU, WEI, LIU, XIAOYANG, BAI, ZHONGKE, CAO, Changes in soil properties in the soil profile after mining and reclamation in an opencast coal mine on the Loess Plateau, China, Envrion Qual Manag, **98**, 228, **2017**.
39. LI Y.Y., CHEN L.Q., WEN H.Y., Changes in the composition and diversity of bacterial communities 13 years after soil reclamation of abandoned mine land in eastern China, Ecol Res, **30** (2), **2015**.
40. ALBERTO O., ERICA L., HENRIK N.R., MARIANGELA G., ALFREDO V., PAOLA B., VALERIA B., and MARI M. Unravelling Soil Fungal Communities from Different Mediterranean Land-Use Backgrounds, PLOS ONE, **7** (4), **2012**.
41. SHREYAS V., KUMBHARE, DHIRAJ, P., DHOTRE, SUNIL, KUMAR, DHAR, Insights into Diversity and Imputed Metabolic Potential of Bacterial Communities in the Continental Shelf of Agatti Island, PLOS ONE, **10** (6), **2015**.
42. MANDAL S.D., CHATTERJEE R., KUMAR N.S. Dominant bacterial phyla in caves and their predicted functional roles in C and N cycle, BMC Microbio, **17** (1), **2017**.
43. WU L., BODEN R., HILLEBRAND A., KUMARESAN D., MOUSSARD H., BACIU M., CHEN Y., LU Y. Life without light: microbial diversity and evidence of sulfur- and ammonium-based chemolithotrophy in Movile Cave, ISME J, **3** (9), **2009**.
44. XU L., HAN Y., YI M., YI H., GUO E., ZHANG A. Shift of millet rhizosphere bacterial community during the maturation of parent soil revealed by 16S rDNA high-throughput sequencing, Appl Soil Ecol, **2018**.
45. JING, ZHAN, QINGYE, Diversity of free-living nitrogen-fixing microorganisms in wastelands of copper mine tailings during the process of natural ecological restoration, J Environ Sci, **3**, 476, **2011**.
46. HUANG L.N., TANG F.Z., SONG Y.S., WAN C.Y., WANG S.L., LIU W.Q., SHU W.S. Biodiversity, abundance, and activity of nitrogen-fixing bacteria during primary succession on a copper mine tailings, Chi J Inorg Chem, **3**, 439, **2011**.
47. LI Y., WU Z., DONG X., JIA Z., SUN Q. Dependency of biological nitrogen fixation on organic carbon in acidic mine tailings under light and dark conditions, Appl Soil Ecol, **2019**.
48. ZHANG HUIJUAN, LIU WENKAI, ZHANG HEBING, FAN LIANGXIN, Spatial distribution of soil organic matter in a coal mining subsidence area, Mater Sci Forum
49. HCA B., MS B., YL A. Soil desiccation in the Loess Plateau of China, GEODERMA, **143** (1–2), **2008**.
50. XU Z., ZHANG Y., YANG J., LIU F. Effect of Underground Coal Mining on the Regional Soil Organic Carbon Pool in Farmland in a Mining Subsidence Area, Sustainability-basel, **11** (18), 4961, **2019V**
51. HE YIMING, HE XIAO, LIU ZHERONG, ZHAO SHUWEN, BAO LIYING, LI QI, and YAN LING. Coal mine subsidence has limited impact on plant assemblages in an arid and semi-arid region of northwestern China, Ecosci, **1**, **2017**.
52. SINGH G., MUKERJI K. Root exudates as determinant of rhizospheric microbial diversity. Soil Bil, **2006**.
53. BARDGETT, RICHARD, The diversity of life in soil. **24**, **2005**.
54. WU L.K., LIN X.M., LIN W.X. Advances and perspective in research on plant-soil-microbe interactions mediated by root exudates, Chi J Pla Eco, **38** (3), **2014**.