Variations of Soil Cyanobacteria Communities Accompanied by Different Habitat Types in Abandoned Ion-Absorbed Rare Earth Tailings

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Abstract: Soil fertility declined, companied with the poor capacity of water holding, after rare earth mining for a long time, mine tailings remediation produced in conventional rare earth elements REEs exploitation are currently urgent issues. Cyanobacteria are prokaryotic oxygenic phototrophs, played a vital role in the biogeochemical cycling of carbon (C) and nitrogen (N), reducing soil loss, are the pioneer organisms of biological soil crusts BSCs. However, studies on cyanobacteria inoculation in abandoned ionic rare earth mines were still insufficient. The abundance and composition of microorganisms in the topsoil of abandoned ionic rare earth mines were analyzed using high-throughput sequencing of the 16S rRNA V4 gene in this study. Fifteen samples were selected in and around rare earth mine tailings based on five different habitat types to assess the difference of main bacteria component and the dominant cyanobacteria caused by environmental factors. A total of 713,057 effective 16S rRNA genes were classified into 30 bacteria phyla, and 7 cyanobacterial genera were determined in phyla of Cyanobacteria. Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria, Planctomycetes, and Cyanobacteria were dominant groups in all samples (>5% of total effective sequences). Only *Microcoleus*, *Leptolyngbya*, *Microcystis*, *Stigonema*, *Chroococcidiopsis*, *Phormidium,* and *Lyngbya* were determined in rare earth mine tailings. *Leptolyngbya* was wildly distributed in rare earth tailings except for natural woodland, while *Microcoleus* was found in all of the studying areas in this study. Mining activities could cause the amount of unknown cyanobacteria specie pnr_Chloroplast abnormal. Additionally, results showed that cyanobacteria community composition was not correlated with soil organic matter SOM, but cyanobacteria richness and diversity were limited by the high content of ammonia nitrogen, and *Leptolyngbya* and *Microcoleus* could apply to abandoned ion-absorbed rare earth mining tailings repairment.

Keywords: Cyanobacteria, High-throughput sequencing, Ion-absorbed rare earth mines, Eco-restoration.

1. INTRODUCTION

Mineral resources were rich in Ganzhou, located to the south of Jiangxi Province, China, belong to ion-absorbed rare earth mines (Zheng *et al*., 2017). Rare earth elements (REEs) have wildly applied across various high-tech industries, such as non-ferrous metal industries and many other fields, and the demand for REEs is sharply increasing in the decades (Zhang *et al*., 2020b). REEs are absorbed on the clay minerals in the form of ions, which enter the solution by ion-exchange desorption reactions, offering convenience for rare earth elements extract (Feng *et al*., 2012). Simple leach process, low cost, and lack of management, excess Ammonium sulfate $(NH_4)_2SO_4$ solutions were injected into the soil and stably remained during the mining process, generating a large area of rare earth tailings, abandoned mine tailings have also become a pollution source, associated pollutant could cause health risks without manage, topsoil structure balance and ecological function were also damaged in the past (Li *et al*., 2020; Wei *et al*., 2019). Ammonia contamination mainly exists in the form of water-soluble ammonium, existing in deeper soil and the surrounding environment under the effect of surface runoff and groundwater (Feng *et al*., 2012; Liu *et al*., 2019; Zhang *et al*., 2020b). A series of environmental issues including soil acidification, highly concentrated ammonia, poor soil organic matter, high bulk density, declined soil fertility, low biological diversity level *et al*., come to attention as increased environmental awareness (Shi *et al*., 2019; Wei *et al*., 2019). Economical and sustainable processing techniques coupled with mine tailings remediation produced in conventional REEs exploitation are currently urgent issues (Dushyantha *et al*., 2020).

The topsoil of mining tailings has still been barren in the long-time exposed environment and cannot hold water and nutrients, resulting in the difficulty of native vegetable growth and artificial remediation. Plants' growth was limited by absent soil organic matter and nutrient-deficient not heavy metal pollution recorded at a low level, vegetable remediation and soil modifier were applied to control water and soil erosion (Yang *et al*., 2020). However, the ecological structure was still simple in rare earth mining tailings, with a weak

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capacity of resisting disturbance (Zhou *et al*., 2015). The topsoil of mine tailings was still in early succession, although it was accelerated by human activities (Liu *et al*., 2017). Mine tailings with widely long-time phytoremediation, formed a favorable microsite, microbial populations were significantly affected by afforestation, promoting microbial community development, showed that natural succession could be accelerated by the artificial approaches for initial stage rehabilitation (He *et al*., 2019a; Liu *et al*., 2017; Xu *et al*., 2020). Biological soil crusts BSCs are micro forest ecosystems, including soil particles and cyanobacteria, algae, fungi, licenses, and bryophytes which live within or on the top of the uppermost millimeters of soil (Belnap, 1995). BSCs can enhance soil nutrient accumulation and accumulate fine particles by extracellular polymeric substances (EPS), promoting edaphic conditions and reducing the possibility of soil loss by water erosion. Cyanobacteria are prokaryotic oxygenic phototrophs, the pioneer organisms of BSCs (Lan *et al*., 2014; Zhou *et al*., 2020). Soil cyanobacteria played a vital role in the biogeochemical cycling of carbon (C) and nitrogen (N), reducing soil loss (Kheirfam *et al*., 2020). Meanwhile, cyanobacteria could also increase the capacity of water holding by the exocellular excretion, one of the secretions was cyanobacteria exopolysaccharides, as the main soil organic component, could adhere to soil particles (Tiwari *et al*., 2019). The released organic matter during cyanobacteria proliferation had a highly hydrophilic character in different growth stages, and the dissolved organic matter from BSCs was the carbon source of heterotrophic organisms, complex BSCs systems formed by inoculating cyanobacteria were favorable to modify properties of impaired soil substrates (Leloup *et al*., 2013; Munoz-Rojas *et al*., 2018). Therefore, they are likely to contribute to ecosystem resilience to disturbance (He *et al*., 2019b; Souza-Egipsy *et al*., 2004). Studies suggested microbial community structure was correlated with pH, Soil organic matter SOM, and ammonia nitrogen, and the absence of plants on rare earth mine tailings was positively associated with the lack of SOM (Liu *et al*., 2021). However, studies on the microbial distribution, diversity, and ecological function under the condition of high ammonia nitrogen content mainly performed to the effects of ammonia-oxidizing archaea and nitrification in N circulation (Isobe *et al*., 2018). Few studies on soil cyanobacteria diversity and application for the waste rare earth mines remediation of highly concentrated ammonia nitrogen in soil were reported.

A hypothesis that cyanobacteria in mining areas was supplied mainly from surrounding unexploited areas transported mainly by precipitation and atmosphere but was limited by the high content soil ammonia nitrogen and low-level SOM was formulated. Misqe high-throughput sequencing was applied to identify the distribution characterizes of cyanobacteria species and other microbial communities. Those results were contributing to realizing the bacteria distribution and cyanobacteria component, to accelerate the formation and development of BSCs and modifying microsite on the topsoil of mining tailings (Zhou *et al*., 2020). It’s suggested that a combination of cyanobacteria inoculation and rational structure of vegetables could facilitate the development of BSCs, (N) circulation, soil particle fixation, and the formation of a more stable microecology structure (He *et al*., 2019b; Warren *et al*., 2018). Realizing the distribution coupled with abundance and forming artificial BSCs, by dominant cyanobacteria species are crucial for modifying soil microhabitat and decreasing water erosion.

2. MATERIALS AND METHODS

2.1. Study Site

The Shangjia Rare-earth Mine is situated in the south of Jiangxi Province, China (115°21′22″～115°54′25″E, 24°30′40″～25°12′10″N), where mining was discontinued more than 20 years ago. The region has a typical subtropical monsoon climate, with mean annual temperatures of 18.9 ℃ and mean annual precipitation falls of 1643 mm, the average elevation is between 500 and 1000 m. The soil types are mostly lateritic red earth with an acidic pH ranging from 4.5 to 5.5. Both of the top and deep soil across mining areas were mostly composed of rare earth tailings, which were produced by pool leaching and heap leaching process. Traditional processing operations caused substantial vegetation damage, resulting in serious soil erosion. Furthermore, the topsoil of the study area, modified by soil modifier and planting tolerant vegetables to improve water and soil holding capacity, was covered by plants and mainly comprised of Dense *Paspalum wettsteinii* Hack with a short growing period, *Miscanthus sinensis*, and sparse and undersized *Pinus massoniana,* where were also covered by biocrusts from incipient to well-developed biocrusts but less original plants. Characteristics of the sample plots were summarized in Table **1**.

2.2. Sampling and Processing

Samples were collected in September 2019, sampling areas of 1 m×1 m in each sampling site including five habitat types were randomly selected,

Table 1: Basic Information on Sample plots. BG_Woodland was Native Woodland, the Rest were Mining Areas and Restored by Artificial Planting

Plots	Plot Label	Vegetable Type	Vegetable Coverage, %	Renew Time (year)	Restoration effects	Slope	Orientation	Notes
BG_woodland	A	*Dicranopteris dichotoma* Bernh etc.	56	0	None	24°	Northeast	Control (CK)
Impact_buffer_zone	B	*Imperata cylindr*	5	2	bad	44°	Southeast	
Bare_land	C	None	0	2	bad	< 5°	None	
Sparse_grassland	D	*Paspalum wettsteinii* Hack	78	2	slightly effective	< 5°	None	
Artificial_woodland	E	*Pinus massoniana*	15	5	effective	28°	East	

were started to recover the damaged surface soil by planting in 2017. A total of 5 mixed plots in areas of mining tailings were sampled to evaluate the effect of polluted mining tailings on soil cyanobacteria communities in different habitat types divided according to vegetable coverage and restoring methods. The upper tailings and underlying soil (totally 0~5 cm depth) were collected. Parallel experiments were done marked as 1, 2, and 3 (n=3). The background soil samples in an unexploited natural woodland, water erosion region, bare areas, sparse grassland and artificial woodland were gathered, marked as BG_woodland (A). Impact_buffer_zone (B), Bare_land (C), Sparse_grassland (D) and Artificial_woodland (E), respectively (Figure **1**). BSCs samples were placed in sterile Petri dishes (90 mm diameter, 10 mm deep), and stored at -80°C until DNA extraction. Removing plant debris and pebbles, air-dried and sieved (2 mm mesh) in all the soil samples for the analysis of environmental factors, including pH, SOM, ammonia nitrogen.

2.3. Soil Physicochemical Analysis

The soil samples were air-dried and pass through a 2 mm sieve. Soil physicochemical properties in this study, including pH, SOM, and ammonia nitrogen were

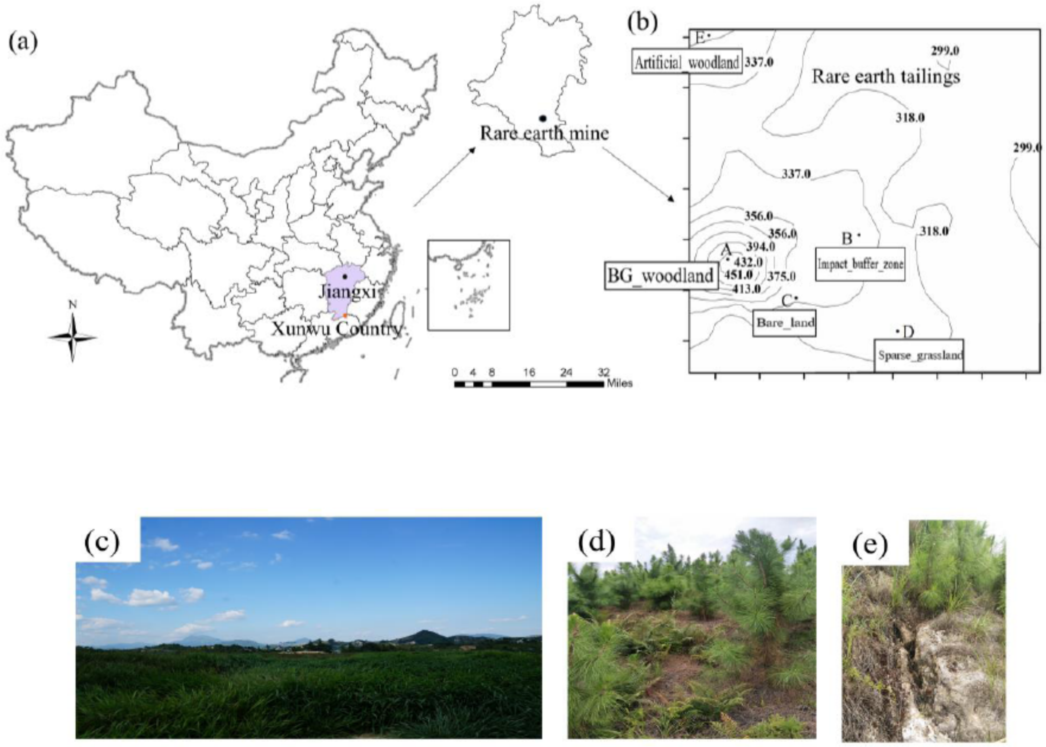

Figure 1: Study and Sampling sites of Shangjia rare earth tailings, Xunwu Country (Jiangxi, China) (**a**), an elevation model (**b**) indicating the sampling sites and vegetable characteristics. Mining areas covered mainly by dense *P. wettsteinii* coverage (**c**), sparse and undersized *P. massoniana* (**d**), but severe water erosion still happened (**e**). **A:** native woodland; **B:** water erosion region; **C:** bare areas; **D:** sparse grassland; **E:** artificial woodland.

measured. The pH was measured in a 1:2.5 ratio (*w/v*) of soil in a water suspension for 30 min by a glass electrode pH meter. SOM was determined by the potassium dichromate-oxidation external heating method. The soil samples were extracted with 1M KCl, and the content of ammonia nitrogen in the extracts was measured using a UV spectrophotometer at a wavelength of 690 nm.

2.4. DNA Extraction and Quantitative PCR

DNA was extracted from a duplicate 0.5 g of BSCs sample using MIO-BIO PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The obtained DNA was checked by running the samples on 1.2% agarose gels. The following universal primers for the 16S rRNA were used: F: 5'-GTGCCAGCMGCCGCGGTAA-3' and R: 5'-GGACTACHVGGGTWTCTAAT-3' to amplify the V4 regions of the cyanobacterial 16S rRNA gene. Amplicons were purified using DNA gel extraction kit (Axygen, USA) and quantified using the FTC-3000 TM real-time PCR (Funglyn Shanghai, China). Purified Amplicons were pooled in equimolar ratios and paired-end sequencing on the Novaseq platform using Novaseq 6000 SP 500 Cycle Reagent Kit (Illumina USA).

2.5. Statistical Analysis

Data were initially processed with Microsoft Excel 2016. The α-diversity indices were calculated by the Mothur software. One-way ANOVA was applied to test the differences of environmental parameters and cyanobacterial α-diversity using the SPSS 22. Significant differences among treatments were determined using Duncan's test ($p < 0.05$ was considered significant). The spearman correlation analysis was used to analyze the relationship between microbial diversity indices and environmental factors (pH, SOM, and ammonia nitrogen).

3. RESULTS

3.1. Analysis of Soil Physicochemical Properties

Results of different sampling plots are showed in Table **2**, in the tested soil, the soil pH was weakly acidic and in the range of 4.5-5.5, and had no significant differences among five different habitat types ($p > 0.05$). The content of SOM in unexploited woodland was high, ranging from 29.6 to 50.95 g. kg^{-1}, which was significantly higher than that of the rare earth tailings ($p < 0.01$). Moreover, the average SOM values were in order of BG_woodland > Sparse_grassland > Impact_buffer_zone > Artificial_woodland > Bare_land. There was no apparent difference between bare land and artificial woodland, with an average of 2.74 and 2.92 g. kg^{-1}, respectively. No significant differences in ammonia nitrogen content were found among five habitat types, and the values in mining areas mainly ranged from 6.6 to 14.1 mg. kg^{-1}. However, apparent differences happened in two of all habitat types were Bare_land and Artificial_woodland, with the abnormal values of 54.95 and 45.93 mg. kg^{-1}, respectively. And the SOM values of BG_woodland were highest, with an average of 24.2 mg. kg^{-1}.

3.2. Microbial Community Diversity and Composition

Rarefaction analysis was used to detect the sample effort and compare microbial richness between different samples. The curve tended to be flat, suggesting results believable and reasonable (Figure **2a**). At the 97% similarity level, all the effective sequences were distributed into operational taxonomic units (OTUs). The OTUs could be divided into different categories, dominant microflora mainly was as follows: Proteobacteria, Chloroflexi, Acidobacteria,

Table 2: Means of pH, SOM, Ammonia Nitrogen at Five Sampling sites. SOM, Soil Organic Matter. Means ± SDs are Indicated by the Error Bars

Habitat Types	pH	SOM (g/kg)	Ammonia Nitrogen (mg/kg)
BG_woodland	4.89±0.13a	36.07±12.93a	24.20±10.48a
Impact_buffer_zone	4.95±0.16a	4.78±1.19b	17.46±18.58a
Bare_land	4.81±0.20a	2.74±0.44b	23.77±19.22a
Sparse_grassland	5.03±0.15a	5.37±1.87b	9.80±2.66a
Artificial_woodland	4.81±0.20a	2.92±1.14b	23.77±19.23a

Note: N =15. ^{a} Different letters in a row indicate differences among different soil samples (P < 0.05).

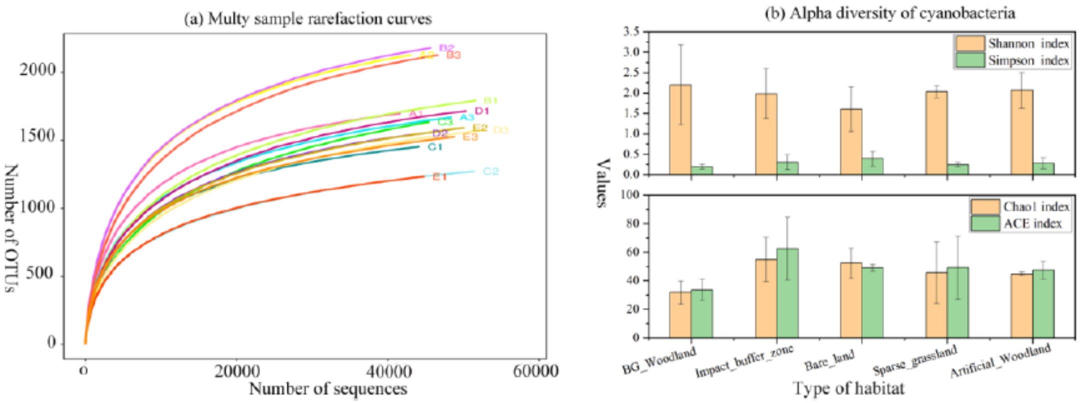

Figure 2: Analysis of cyanobacterial communities in different habit types. (**a**) Rarefaction curve of soil microbial; **(b)** analysis of cyanobacteria α-diversity.

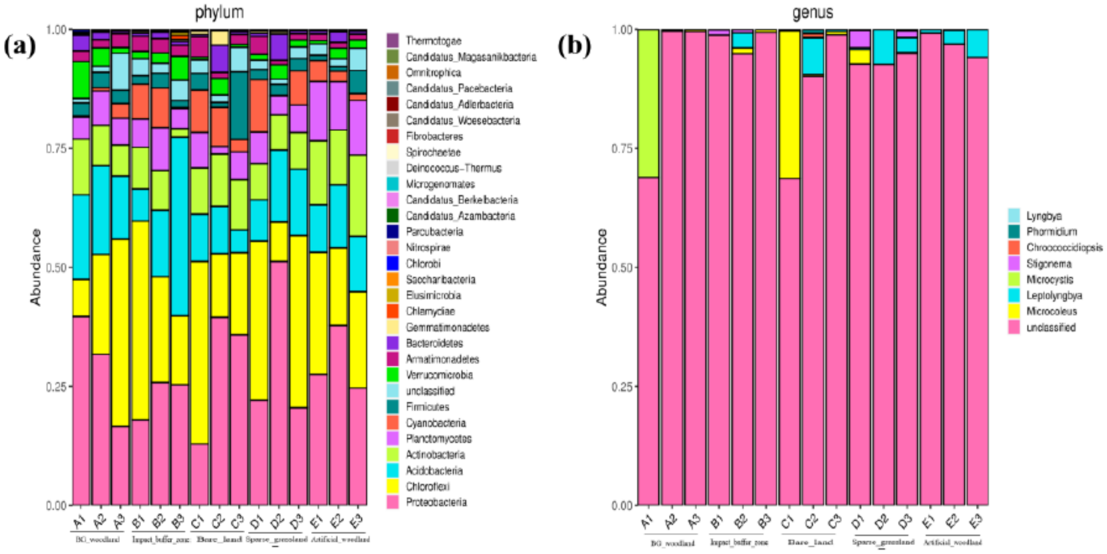

Figure 3: Relative abundance of microbial in different sampling sites. (**a**) The abundance of bacteria on phylum level; (**b**) the abundance of cyanobacterial on genus level.

Planctomycetes, Cyanobacteria, Firmicutes, Verrucomicrobia, Armatimonadetes, Bacteroidetes (accounting for more than 1%) (Figure **4a**). Based on the sequencing data, the OTUs for cyanobacteria were drawn out from the original microbial communities. All samples contained 141 OTUs for cyanobacteria, assigned to 7 genera, including *Microcoleus*, *Leptolyngbya*, *Microcystis*, *Stigonema*, *Chroococcidiopsis*, *Phormidium*, *Lyngbya* (Figure **4b**), contributed 2.74% of the total OTUs for bacteria. Chao1 and ACE indices were applied to analyze cyanobacteria diversity. As shown in Figure **2b**, the lowest average values of chao1 and ACE indices occurred at BG_woodland, with the average of 31.63 and 33.20, respectively. Communities showed a higher alpha diversity in BG_woodland than Bare_land calculated by Shannon and Simpson indices showed that the species diversity of BG_woodland was abundant, with a maximum of 2.20 and minimum of 0.98, respectively.

Venn diagram was performed to test the common and unique quantity of OTUs for analyzing the difference between the sampling plots. The overlap of cyanobacteria had 22 OTUs, accounting for 15.60% of total OTUs in all habitat types, and the common bacteria was 17.81% (Figure **5**). *Pnr_Chloroplast* was the common cyanobacteria that existed in all the habitat types but less relative taxonomic studies, which was abundant in mining areas, and approximately 9 times than native woodland, causing the unclassified taxa rate of relative abundance was higher (Figure **4b**).

3.3. Effect of Environmental Factors on Soil Cyanobacteria

Abundance analysis of bacteria at the phylum level.

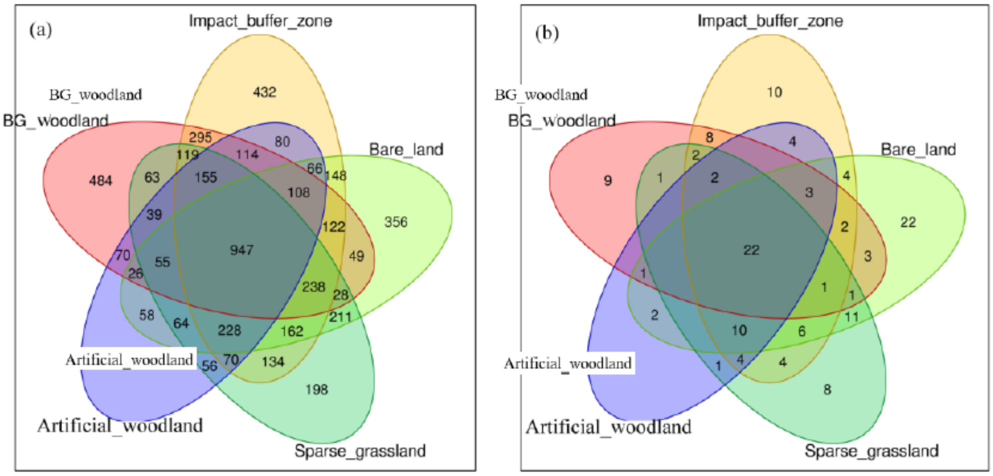

Figure 4: Venn diagram of microbials population types in different sampling sites. (**a**) Bacteria structure; (**b**) cyanobacteria structure.

Table 3: Descriptive Statistics of Cyanobacteria α-Diversity in Surface Soils

Indicators	Groups	Chao1 index	ACE index	Shannon index	Simpson index
SOM (g/kg)	0-5	31.79±7.94a	33.62±7.60a	2.20±0.30	0.19±0.06
	5-10	59.37±4.72a	62.55±3.07a	1.93±0.14	0.29±0.06
	20-55	46.0±3.59b	48.56±15.94b	1.92±0.51	0.31±0.16
Ammonia nitrogen (mg/kg)	6-10	59.86±6.56c	66.02±13.36a	1.71±0.31	0.39±0.13
	10-20	49.61±11.60b	47.57±8.45b	2.03±0.67	0.26±0.14
	20-55	36.32±10.36c	40.25±12.90a	2.13±0.37	0.23±0.14
Habitat types	BG_woodland	31.79±7.94	33.62±7.60	2.20±0.30	0.19±0.06
	Impact_buffer_zone	54.92±15.48	62.53±22.10	1.98±0.61	0.30±0.18
	Bare_land	52.20±10.50	48.96±2.40	1.60±0.54	0.39±0.18
	Sparse_grassland	45.67±21.35	49.19±22.16	2.03±0.15	0.24±0.06
	Artificial_woodland	44.80±1.32	47.54±6.31	2.06±0.44	0.28±0.14

5317 OTUs of bacteria were detected, the main bacteria species were detected in our study including Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria, Planctomycetes, and Cyanobacteria (Figure **3a**). Based on Figure **4a**, the overlap of bacteria had 947 OTUs, accounting for 17.81% of total OTUs in all habitat types. In terms of cyanobacteria abundance, no significant differences were found among five different habitat types. Shrub canopies were not the limiting factor in this studying area for cyanobacteria by comparing artificial remediation area, Impact_buffer_zone, and bare land, the apparent differences among different treatments at the same habitat types could be caused by vegetable coverage and water erosion (Figure **2b**). Results showed that apparent differences between cyanobacteria abundance were observed with low-level SOM content and high ammonia nitrogen content. No significant difference in cyanobacteria α-diversity indices was found between native woodland and downslope mining areas of water erosion but the common portion was 42% (Table **3**, Figure **4b**).

3.4. Relationship between Environmental Factors and Cyanobacteria Diversity

Simpson index was significantly negatively correlated with the Shannon index ($p < 0.01$, Table **4**). No apparent relationship among cyanobacteria α-diversity, pH, and SOM was found ($p > 0.05$). Cyanobacteria richness was limited but the diversity of cyanobacteria was increased with the content of ammonia nitrogen increasing, showing that the balance for native cyanobacteria community structure was destroyed but tolerant and unknown species were

Table 4: The Correlation and Significance between Relative Abundances of Cyanobacteria Diversity and Soil pH, SOM, and Ammonia Nitrogen Contents. Significance are Demonstrated as: $^{*}p < 0.05$; $^{**}p < 0.01$ (Two Tailed)

	pH	SOM	Ammonia Nitrogen	Chao1 index	ACE index	Shannon Index
SOM	0.31					
Ammonia	-0.41	0.06				
Chao1 index	0.34	-0.26	-0.60^{*}			
ACE index	0.21	-0.21	-0.53^{*}	0.93^{**}		
Shannon index	-0.13	0.41	0.61^{*}	-0.72^{**}	-0.64^{*}	
Simpson index	0.01	-0.43	-0.95^{**}	0.81^{**}	0.74^{**}	-0.943^{**}

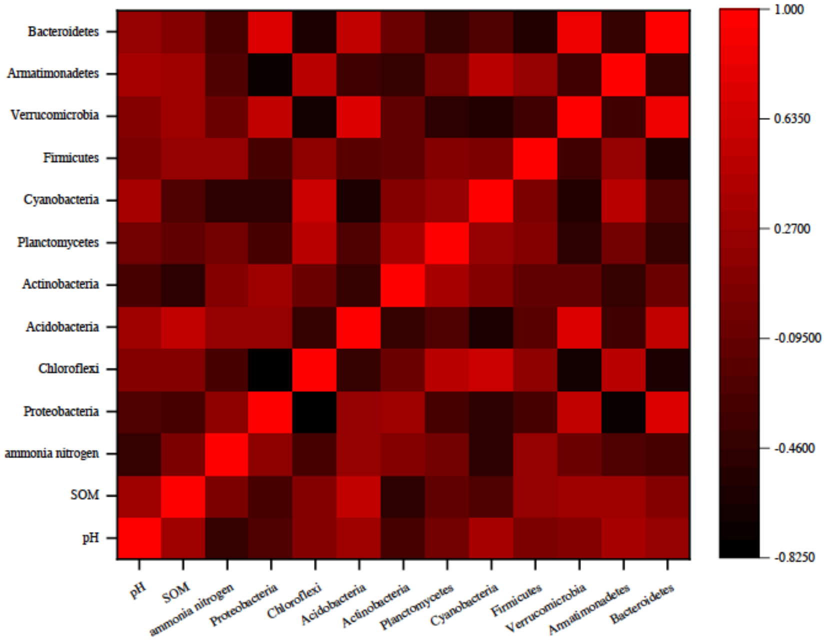

Figure 5: Heatmap of the correlation values between relative abundances of the top 10 bacterial phyla and soil pH, SOM, and ammonia nitrogen.

sharply growing (Figure **3b**). As shown in Figure **5**, results showed that the relative abundance of cyanobacteria was correlated with Chloroflexi, Acidobacteria, and Verrucomicrobia. No correlations between abundant microbes and soil pH, ammonia nitrogen, were observed, which could be the reasons for long-term high ammonia nitrogen content, the quantity of microbes participating in substances circulation decreased, such as N circulation.

4. DISCUSSION

Ecological remediation, including stabilizing damaged topsoil and reshaping microhabitats of waste residue generated by mineral resources mining was crucial. Compared with similar rare earth mines, there were still the common characters of low SOM and soil acidification (Zhou *et al*., 2015). Precipitation was associated with supplement of soil moisture and substrate diffusion, was important for microbial development, could accelerate the shift of soil bacterial community structure mostly contributing to the changes in soil water and internal environmental factors (Hu *et al*., 2020). As shown in Table **3**, the distribution character of topsoil ammonia nitrogen from the in-situ leaching process was similar to those in this study ranging from 6.16 to 54.95 mg. kg^{-1} (Zhang *et al*., 2020b). The cations (NH_4^+) in the leaching agents are absorbed on the soil particles, fixed ammonium binds more strongly to the mineral soil phase and cannot be easily removed, water-resolution ammonium could be the reason for the distribution differences in same habitat types (Xu *et al*., 2020; Zhang *et al*., 2020b), causing the content of soil ammonia nitrogen in rare earth mine tailings were still high and uneven (Isobe *et al*., 2018; Liu *et al*., 2021; Zuo *et al*., 2019), ecological functions were damaged.

Soil microbes play an important role in the circle of substances, it's important to increase the quality of the microsites. Cyanobacteria could have a favorable application due to extracellular polysaccharide and morphological characteristics (Munoz-Rojas *et al*., 2018). However, the spatial differences of soil cyanobacteria were determined by biotic and abiotic factors, complex environmental factors affect microbial community structure (Wang *et al*., 2020), such as soil properties, artificial plantations, and soil microbes and precipitation, etc. (Xu *et al*., 2020). The predominant bacterial communities in mining tailings including Proteobacteria, Acidobacteria, and Firmicutes were formed, the relative abundance of Cyanophyta less than 1% (Liu *et al*., 2021; Wei *et al*., 2019; Zhang *et al*., 2020a). The dominant microbial community structure composition slightly changed compared with earlier studies for the microbial community structure in rare earth tailings, and the relative abundance of cyanobacteria increased (Wei *et al*., 2019), results in our study showed that Cyanophyta was widely distributed in abandoned rare earth mines, and the relative abundance had increased to 2%. Most cyanobacteria richness was limited with the degradation degree of the topsoil ecosystem increased (Roncero-Ramos *et al*., 2020).

The content of ammonia nitrogen ranging from 1 to 3 $mg{\cdot}kg^{-1}$ and SOM was significantly positively with fungi diversity and soil bacteria community composition (Xu *et al*., 2020). Shannon and Simpson indices were negatively correlated with evenness and divergence, respectively, which could be used to access the response to environmental disturbance (Zhong *et al*., 2020). Shannon and Simpson indices for cyanobacteria in our study had an average value of 1.98 and 0.28, respectively, which showed that cyanobacteria among the BSCs had a low richness and uniform distribution, cyanobacteria communities were more dispersed, and the resource utilization was high. Compared to the similar study on the effect of artificial plantation for damaged mine ecosystems, Shannon and Simpson indices were similar to those in this study (Deng *et al*., 2020). The cyanobacteria community structure of BG_woodland and Artificial_woodland showed a great similarity due to the unique OTUs quantity of cyanobacteria in Artificial_woodland equal to zero (Figure **4b**). Nevertheless, the similar elevation of BG_woodland and Artificial_woodland but in a different sloping field, showing that cyanobacteria transformed by natural force, such as atmosphere (Figure **1**, **4b**; Warren *et al*., 2018).

Shrub canopies can accelerate the development of BSCs, including DNA content and moss abundance (Zhou *et al*., 2019; Serpe *et al*. 2013). Meanwhile, with the development of BSCs, the inter-community competition across bacteria increase, microorganisms could facilitate the formation of more stable BSCs, but the relative abundance of cyanobacteria decreased with the succession of BSCs. Cyanobacteria-cover soil can increase the abundance of the nitrification rate due to increased nitrification-related genes (Hu *et al*., 2020; Ye *et al*., 2020; Zhou *et al*., 2020). Additionally, the bacterial composition in this study was similar to the in-situ mining area. Bacteria, such as Chloroflexi , Nitrospirae, and Actinobacteria, were associated with the nitrogen removal. The augment for Chloroflexi and EPS could facilitate the ammonia nitrogen transform and bind phosphorus, respectively.

It needed to identify whether the key limiting factors for the development of BSCs was the absence of *Nostoc*, *Tolypothrix*, and *Scytonema* in rare earth tailings, which could offer favorable support for the inoculation of cyanobacteria in rare earth mines. According to the present study, cyanobacteria richness was limited but diversity was increased with the content of ammonia nitrogen increased, the cyanobacteria diversity of BG_woodland was lower than other barren areas, the condition with low content of SOM could contribute to cyanobacteria inoculation. The reorganization and construction of soil microbial by cyanobacteria inoculation accelerating the formation of BSCs was significant for the eco-remediation of rare earth mine tailings topsoil. Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi, and Acidobacteria occurring at rare earth mines were widely distributed, these common microbial communities could facilitate the formation of a stable soil biosphere (He *et al*., 2019a; Zhang *et al*., 2020a).

5. CONCLUSIONS

According to the present study, the soil pH ranging 4.5 to 5.5 was weakly acidic, and mining tailings with low-level SOM has still been barren and high ammonia-nitrogen content with a average value of 19.4mg/kg. The species diversity of BG_woodland was abundant along with high content of SOM, and no significant differences in ammonia-nitrogen content were found among five habitat types. 7 cyanobacterial genera were found by high-throughput sequencing, and the lowest average values of chao1 and ACE indices occurred at BG_woodland, no significant differences of cyanobacteria abundance were found among five different habitat types. Meanwhile, an unknown cyanobacteria species pnr_Chloroplast was largely and widely existing in the topsoil of mining areas based on OTUs quantity, which was 10 times, even 100 times compared with others, showing cyanobacteria species were uneven. Cyanobacteria richness was limited but diversity was increased with the content of ammonia nitrogen increased. And no apparent differences were observed among cyanobacteria α-diversity, pH, and SOM in different habitat types. Cyanobacteria had a positive correlation with Acidobacteria and Verrucomicrobia and negatively correlated with the content of ammonia. *Microcoleus*, *Leptolyngbya*, *Microcystis*, *Stigonema*, *Chrooccidiopsis*, *Phormidium*, *and Lyngbya* were determined in this study, which were uneven and mainly existed in sparse_grassland and artificial_woodland.

Combined with morphological characteristics, extracting cyanobacteria species from BSCs and inoculation to facilitate the formation of BSCs and the balance of micro-ecosystem were contributing to fixing the incompact soil particles in abandoned rare earth mines. Eventually, this study on the diversity of cyanobacteria has a favorable effect on the selectivity and determination of cyanobacteria, providing a good initiation for inoculation.

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ABBREVIATIONS AS FOLLOWS

BSCs	Biological soil crusts
REEs	Rare earth elements
EPS	Extracellular polymeric substances
SOM	Soil organic matter
OTUs	Operational taxonomic units

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