Analysis of Soil and Microbial Characteristics and Microbial Response in Rare Earth Mining Areas in Jiangxi Province, China.

Zhentian Liang
  Jilin University

Wenjing Zhang (✉ zhangwenjing80@hotmail.com)
  College of Environment and Resources, Jilin University   https://orcid.org/0000-0001-6263-5384

Yuesuo Yang
  Jilin University

Jincai Ma
  Jilin University

Shuxin Li
  Jilin University

Zong Wen
  Jilin University

Research Article

Abstract

There is a certain connection between the microbial community and functional flora in rare earth mining areas, but the pollution characteristics and pollution metabolic pathways in the mining area are still unclear. The conditions of heavy metals, rare earth elements, and microorganisms after the mining of rare earth mines were studied in this paper. It was found that after mining, all the sampling areas showed low pH and low total organic carbon(TOC), accompanied by high iron and aluminum concentrations. The development of vegetation is closely related to the development level of microorganisms. In the complex environment of rare earth mining areas, the *Proteobacteria* showed an absolute competitive advantage. In the process of mine environmental recovery, the relative abundance of *Acidobacteria* will increase significantly, but with the further development of restoration, the relative abundance of *Firmicutes* in rare earth mining areas will gradually decrease. There are many genera of bacteria related to the N cycle and heavy metal metabolism in the study area, indicating important metabolic pathways of ammonia nitrogen and heavy metals in rare earth mining areas. At the same time, the study found that there are bacterial genera that promote plant nitrogen fixation in the area, further revealing the nitrogen cycle.

1. Introduction

As we know, rare earth is very important and can be used in electronic devices, the auto industry, aeronautical manufacture, energy conversion, and so on (Alonso *et al.*, 2012, Dutta *et al.*, 2016). Ion-adsorbed rare earth minerals are mainly located in the south China, especially Jiangxi Province(Zhang *et al.*, 2019). Mining activities often cause a series of hazards to the ecological environment, such as air pollution, vegetation destruction, changes in geomorphic units(Zhao *et al.*, 2012, Zhou *et al.*, 2017). More seriously, it can also reduce soil organic matter reserves and nutrient availability(Yang *et al.*, 2016)and affect soil microbial communities(Yang *et al.*, 2016, Shi *et al.*, 2017). At this stage, the main method of rare earth mining is ammonium sulfate leaching because it's efficient and cheap. To dissolve and extract the rare earth elements in the mining area, people have to inject a high concentration of ammonium sulfate solution into the ion-adsorbed mine(Moldoveanu and Papangelakis, 2012, Zhao *et al.*, 2017). In addition to the rare earth oxides, the injected ammonium ion also displaces a certain amount of soil cationic nutrients at the later stage of displacement, causing soil nutrient loss. Sulfate, as a kind of salt that is difficult to be used by plants, remains in the soil at a concentration of up to 200mg/kg.

The precipitation of heavy metal elements and rare earth elements in the soil through cation exchange will inevitably affect the survival of microorganisms and further affect the realization of ecosystem functions. It has been found that biological monitoring can be widely used in the field of atmospheric deposition by its economical and effective characteristics(Aničić Urošević *et al.*, 2020). However, the research on the interaction of microorganisms with heavy metals and rare earth elements in rare earth mining areas is still insufficient. In particular, rare-earth mining is associated with secondary hazards, such as excessive concentrations of iron and aluminum. In this study, the concentrations of iron and aluminum reached 30g/kg and 103g/kg, respectively. Microbe are very sensitive to high concentrations of salt, so they have to respond to sudden pollution(Ağdağ and Sponza, 2005, Min *et al.*, 2017). Besides, compared with soil physical and chemical properties, soil microorganisms are more sensitive to any small changes in soil or environmental stress. (Cruz-Paredes *et al.*, 2017) .Therefore, the trend of environmental change can be understood and measures can be taken in time(Valentini *et al.*, 2016). The heavy metal elements in the leaching site also have some residual effects on the community structure of microorganisms(Yang *et al.*, 2017). The complexity, richness and structural characteristics of the microbial community represent the health level of the ecological environment in a certain region and predict the natural degradation capacity of pollutants to a certain extent. The characteristics of the microbial community under the influence of the above factors should be fully understood, to judge the soil pollution situation directly in the later stage.

Considering that the structure and level of the microbial community in rare earth mining areas are seriously inadequate in the study of rare earth pollution, heavy metal pollution, and other typical pollution, this article was written. This study focused on the pollution of sulfate and heavy metals in the rare earth mining areas and determined the characteristics of iron and aluminum content in the area, the retention of rare earth elements in the area after mining, and the response of internal microorganisms in the area to these factors. At the same time, the characteristics of the microbial community in rare earth mining areas and the response of microorganisms to environmental remediation were investigated.

2. Materials And Methods

2.1 The study area

The study area is located in Longnan and Dingnan counties, Ganzhou city, Jiangxi province, China. The study area is located in a mountainous area, with hills and mountains accounting for nearly 80% of the area. The whole area is rich in vegetation and partially

exposed (caused by human factors). The area has nearly 100 large and small rare earth mines and other types of mineral deposits. Vegetation is dominated by evergreen broad-leaved forests and bamboo forests. With annual or two-year-old short plants, the ground undulates greatly, and slope runoff is easy to form during the rainfall period. Some areas with imperfect vegetation cover are easy to form typical flowing water landforms. It is introduced that the original soil in the study area is rich in organic matter, the soil quality is relatively loose, the particles are finer, and the groundwater ion content in the study area is relatively high. Based on the needs of this research, 5 typical and representative sampling points were selected from rare earth mines in the research area. They represent different recovery periods, different mining methods and recovery methods, etc.

2.2 sample collection and processing

Soil samples were collected from surface soil at a depth of 5–20 cm(All native soils in the study area). The situation in the study area is shown in Table 1. The detailed location map of the sampling points is shown in Fig. 1. Each sample was divided into two parts. The part of the sample used for testing microorganisms was sieved through a nylon sieve(aperture is 2 mm) to remove stones and plant debris(Sample processing is performed according to the recommendations provided by the testing agency). And then it was sent to Shanghai personal biotechnology Co., Ltd. for testing at low temperatures. Another part of the sample was used for testing of heavy metals and rare earth elements were also sieved through 80 mesh nylon screen and sent to Measurement Unit Northeast Institute of Geography and Agricultural Ecology, CAS for testing after being air-dried and ground(15°C,15 days).

Table 1
Geographic information and classification of samples

group	Longitude(E)	Latitude(N)	type of mining	Period of remediation
A	115.0896	24.9564	in-situ leaching	late stage
B	114.8548	24.8311	heap leaching	early stage
C	114.8950	24.8592	in-situ leaching	late stage
D	114.8244	24.8287	heap leaching	undisturbed
E	115.0894	24.9545	in-situ leaching	undisturbed

When the samples were collected, relatively scattered samples were taken at five points in each sampling point and mixed to make mixed samples, and repeated three times. Five groups of microbial samples were collected and numbered as A, B, C, D, and E, with a total of 15 samples. Five samples of physical and chemical properties were tested. In those sampling sites, group A is in the late stage of mine recovery after mining, and the restoration methods are mainly clear water leaching. The area is covered by abundant vegetation, evergreen broad-leaved forest and dense bamboo forest, plants are well developed, and decayed branches and leaves are mostly on the surface. Group B is the leaching field with high elevation. This area is the site for the early stage of restoration. Weeds are growing on the surface, but the weeds in this area grow on the guest soil, collected the soil below the guest soil layer during sample collection. Group C is a multi-stage permeable reactive barrier (PRB) restoration site, which is located in the gully and surrounded by many trees with obvious leaf cover. Group D is the slope area after heap leaching mining, with obvious erosion, severe surface exposure, basically no plants, and sandy soil mainly. The collection site of group E is similar to that of group A, but the sampling site is concentrated at the terminal of leaching restoration, which is located near the waste collection tank. The small area represents obvious exposure and the large scale is mostly covered by trees.

2.3 DNA extraction and high-throughput sequencing

The DNA was quantified using Nanodrop, and the DNA was detected for DNA extraction quality by electrophoresis of 1.2% agaric gel. The corresponding primers are designed according to the conservative region of the sequence (The front primer is ACTCCTACGGGAGGCAGCA and the back primer is GGACTACHVGGGTWTCTAAT), and sample-specific Barcode sequences are added to the apcagus of rRNA gene variable regions (single or continuous multiple) or specific gene fragments. Fluorescence of PCR amplification products with Quant-iT PicoGreen dsDNA Assay Kit and fluorescence of PCR amplification products with Microplate reader (BioTek, FLx800) as a quantitative instrument and according to the quantitative results of fluorescence, the samples are mixed in proportion according to the sequence quantity requirements of each sample. The sequencing library was prepared using Illumina's TruSeq Nano DNA LT Library Prep Kit. First, the library is quality-tested on Agilent Bioanalyzer, using The Agilent High Minaty DNA Kit, Quant-iT PicoGreen dsDNA Assay Kit then quantifies the library on the Promega Quanti Fluor fluorescence quantification system and dilute the gradient of the qualified upper-

machine sequencing library (Index sequence non-repeatable) and mix in proportion to the required amount of sequencing, and be denatured by NaOH into a single chain and sequenced with high throughput, Finally.

2.4 Soil elements analysis

Pre-process the samples according to the test method and the pH was tested with the electrode method, the concentration of Fe、Al、SO_4^{2+} were tested with ICP-AES, the concentration of TOC was tested with potassium dichromate titration, ammonium ions were measured by continuous flow analysis and both heavy metals and rare earth elements are measured by ICP-MS method(Only Hg was tested by fluorescence). All samples are subject to quality control to ensure test results.

2.5 statistical analysis

The differences of microbial composition in the same group were tested with One-way ANOVA using the software IBM SPSS Statistics 25, followed by the least significant difference (LSD) at the 95% confidence level. Study microbial abundance differences in microbial groupings by statistical method to ensure test results. Analysis of microbial species diversity with alpha and beta diversity indices(Whittaker, 1960, R.H.Whittaker, 1972). The correlation between microbial community structure and the content of rare earth elements, heavy metals, and basic physical and chemical properties of the soil was analyzed using Spearman correlation. At the same time, the effect of soil physical and chemical properties on heavy metals and rare earth elements was also analyzed with the help of Spearman correlation. The R was used to analyze the relationship between microorganisms and soil elements.

3. Results And Discussion

3.1. Characteristics of conventional components and heavy metals

The study tested environmental background factors such as pH, sulfate, TOC and ammonium salts and major heavy metals, these factors represent the environmental damage caused by rare earth mining, and they also affect the survival of indigenous microorganisms and biome structure. As shown in Fig. 2(a), the pH range of soil is 4.37−4.75, all of which show a certain degree of acidity. This data indicates that microorganisms in the rare earth mining areas show a certain degree of acid resistance. From the test data, we can find that the TOC content of group A is higher than that of other groups, by an order of magnitude. Soil TOC content is between 0.08 % and 2.69 %, except for A, the TOC content of other sites is close to or lower than 0.5%, which proves that the soil properties are close to loam or sandy loam, which is not conducive to the survival of plants and microorganisms. In the Fig. 2(b), the concentration changes of sulfate and ammonia nitrogen in the sampling area are shown, The lowest value of sulfate and ammonium salt is B sample, the highest value is E sample and the concentration gap is large. As mentioned above, point E is the discharge area of in-situ ammonium sulfate leaching, where the retention effect is obvious, while point B is the soil covering planting area. The decrease in concentration indicates that human selective planting and soil improvement in the planting process also have a certain influence on the original soil layer. The corresponding relationship between the two does not meet the original injection ratio. It is speculated that in addition to its own differences in the soil, there may be more bacteria related to the nitrogen cycle that promote the nitrogen cycle. In the study of heavy metals, it was found that the content of heavy metals in the whole study area was at an acceptable level. In contrast, the heavy metals at point C were the most typical. Such stress promoted the development of functional flora(Fig. 2 (c)). The contents of iron and aluminum were high, reaching the highest levels of 33.13 g/kg and 103.1 g/kg, respectively. The concentration of lead in the tested heavy metal elements is relatively high, reaching 117.38 mg/kg, and other concentrations are low. The data is shown in Fig. 2(d).

A certain concentration of rare earth elements can be monitored in all soil samples, which is caused by incomplete leaching mining. Soil quality is an important part of ecosystem restoration, and its physical, chemical and biological characteristics are the basis for plant renewal and establishment(Zhang H 2013). Figure 2(e) shows the occurrence characteristics of rare earth elements in the study area, the total amount of rare earth elements at each sampling point and the content of each rare earth element at each point and their corresponding relationships. It is not difficult to see that the contents of rare earth elements at points A and E are higher than those at other points, which is caused by the sampling method of in-situ leaching. There are also certain differences in the proportion of rare earth elements in each sampling point, and the dominant types are quite different, which further affects the microbial community in the site.

3.2 Microbial community characteristics

16S rRNA technology was applied for analysis, and 1386422 valid sequences were obtained from all samples, which were divided into 34919 OTUs by clustering method. The number of identified bacterial OTUs in A, B, C, D E was 9028, 8613, 7784, 6595 and 7574 respectively. Among these OTUs, only 128 samples from five groups were shared, with the number of OTUs owned by A, B, C, D, E alone being 8134, 7046, 6375, 4746 and 5562, respectively. It shows that there is a very obvious separation phenomenon between the various

groups, this result is likely to be caused by environmental factors. The differences between groups of microorganisms in this study are shown in Fig. 3. When the outlier B1 was excluded, the cluster dendrogram based on the weighted pair-group method with arithmetic means (UPGMA) showed that the soil bacterial communities from the A, C were far apart from those in other places (Fig. 3II). Similar to the former two cases, ignoring outlier B1, the two-dimensional ranking diagram of samples analyzed by PCoA also shows the rationality of classification(Fig. 3I). It can be seen that certain factors affect the characteristics of the biological community in this region.

Rarefaction curves about the OTUs reached the plateau with the increase of the number of sequences per sample, stressing that the 38, 000 sequences for each sample were sufficient to characterize soil bacterial communities in the studied soils. The corresponding alpha diversity index is shown in Table 2. Through the study on the microbial diversity of rare earth mining areas, the species richness in this area is relatively high. Five groups of samples Chao1 index are distributed between 3200 and 4800, and the groups with the highest index and the lowest index are group A and group D, respectively. The Observed species index has A similar distribution pattern, with the highest value still appearing in group A and the lowest values appearing in group D, 4707 and 3,226, respectively. When it comes to the study of species diversity, it can be found that group A still showed certain advantages, while group D showed less obvious disadvantages. This study found that there was no significant difference between Simpson index and Shannon index, with both the Simpson index above 0.99 and Shannon index around 10. Then, the diversity, evenness and coverage of sample evolution were analyzed in depth, which was characterized by Faith's PD index, Pielou's evenness index and Good's coverage index respectively. The study found that group A still maintained the highest evenness and group E the lowest evenness. The difference between the two groups was about 0.07, indicating that relative richness level and richness followed the same change rule. The coverage level study found that although the sample diversity index and richness index of group D had no advantages, the coverage level was the highest, but the differences between the groups were minimal, reaching above 0.99, and the sequencing coverage level was high.

Table 2
Soil bacterial microbe alpha diversity index

group	Chao1	Simpson	Shannon	Pielou_e	Observed _soecies	Faith_pd	Good_ coverage
A	4832.83	0.998	10.628	0.878	4707.24	221.10	0.995
B	4466.52	0.996	10.080	0.836	4358.30	327.86	0.997
C	4122.14	0.996	9.806	0.821	3971.20	266.36	0.994
D	3280.95	0.994	9.381	0.820	3226.04	290.16	0.999
E	3650.08	0.995	9.484	0.805	3532.73	292.82	0.998

The OTUs could be divided into different categories, and 11 dominant bacterial phyla with the relative abundances > 1% were obtained, including *Proteobacteria, Firmicutes, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Patescibacteria, Verrucomicrobia, Spirochaetes, Gemmatimonadetes*. *Proteobacteria* dominated, accounting for more than a third of the genome, and *Firmicutes*, the second-largest, accounted for nearly 50 percent of the total. (Fig. 4T). When it came to groups, the dominant phylum of all groups was *Proteobacteria*(This is because *proteobacteria* are the largest species of bacteria), and OTUs contained in all groups were ranked in the first place, while those in the second place were divided. Among them, *Actinomycetes* were ranked in the second place in group A and group C, with a similar proportion, while *Firmicutes* were ranked in the second place in group B, group D and group E. *Firmicutes* also ranked second in the overall level of the community, mainly due to the data volume advantage. However, group A and group C together again also coincides with the previous clustering results, indicating that the factors affecting clustering also affect the development of the microbial community.

At the genus level, the relative abundance is greater than 1.5% were *Bacterium, Burkholderiaceae, Gammaproteobacteria, Acidothermus, Paraburkholderia, Pseudomonas, Acidobacteriaceae, Rhodanobacteraceae, Clostridiales*. Based on Euclidean distance, the composition of taxa at the genus level was used as the analysis object, and the abundance data of the genus with the average abundance of the top 50 digits were used to draw the heatmap. It can be seen from the heat map data that the bacterial community is divided into two parts, in which group A and C are similar, while group B, D and E are similar, but some differences between group A and group C can be found, which is consistent with the previous PCoA and other analysis.

The development level and abundance level of regional microorganisms are closely related to the geochemical process of the site. The study found that *Burkholderiaceae* is a typical ammonia nitrogen degrading bacteria(Feng *et al.*, 2017). In this study, it was found that the

relative abundance of *Burkholderiaceae* was very high, indicating that there is obvious ammonia oxidation in the study area. The secondary damage caused by human mine restoration, the natural environment can be restored spontaneously. Research on ammonium conversion in the desert of Oman found that *Gammaproteobacteria* play a huge role in ammonia oxidation. Sequencing revealed that *Gammaproteobacteria*'s ammonia oxidation gene has the largest copy number(Abed *et al.*, 2015). In Brazil's iron mine studies, it was found that Fe(III) reduction was related to *Gammaproteobacteria*, *Acidobacteriaceae*, and *Chloroflexus*(Parker *et al.*, 2017), which further explained the relatively high abundance of these bacteria in the site and also revealed the existence of the study area Fe(III) metabolic pathway. The existence of these genera will also strengthen the survival of plants under the threat of aluminum pollution. It shows that these genera have certain promotion significance to the vegetation restoration in the study area(Shi *et al.*, 2020). This finding further illustrates the self-repair ability of the study area.

The *Chujaibacter* was discovered and named by Korean scientists in 2015. Due to the short time of discovery, there are few related studies, but it is known that the strain is closely related to *Metallobacterium Schaeffleri*(Kim *et al.*, 2015). It can be inferred that the bacteria participated in the recovery of heavy metal pollution in the study area, especially the E area where the eluent was discharged. According to the heat map analysis, the *Pandoraea* genus is very typical in the E study area, and this genus has a strong effect on ammonia nitrogen removal(Hao *et al.*, 2012), indicating that high concentrations of ammonia nitrogen are likely to be removed by this microbial action. It can be easily found in the heat map that *Occallatibacter*, *Gemmatimonas*, and *Cellulomonas*, and others have close clustering relationships and are quite different from other strains(Chee-Sanford *et al.*, 2019, Friedrich *et al.*, 2020, Добровольская *et al.*, 2020). The abundance of these genera at the C research site is relatively large, indicating that these strains are more typical in this area. Most of these bacterial genera are closely related to the nitrogen cycle or plant nitrogen fixation. It can be seen that the PRB function in the treatment of ammonia nitrogen secondary pollution strengthens the ammonia nitrogen cycle of the site, and enhances the ammonia nitrogen microbial removal of the site based on enhanced chemical remediation. At research point A, because the in-situ ammonium salt leaching ore deposits the amount of ammonia nitrogen in the deep soil layer is still large, and the types and numbers of surface vegetation are at a high level, the *rhizobia* resistant to ammonia nitrogen has become a more typical genus(Sen and Appunu, 2008, Crovadore *et al.*, 2016, de Andrade Santos *et al.*, 2018). The ecological restoration of the site provides strong support.

3.3. Correlations between soil elements and soil microbial communities

To better reveal the characteristics of the influence of geochemical parameters on the microbial environment, we used R to do a CCA analysis of the study area(Fig. 6), Through screening to remove unreasonable factors, pH, TOC, NH4-N, Al, Cd, Dy, Ho, Er, etc. were determined as influencing factors for analysis. It was found that the key factors related to the composition of the microbial community can be roughly divided into three parts. Among them, the group controlled by NH4-N is the most obvious. This group is mainly composed of three groups of samples B, D, and E. There is an obvious positive correlation with NH4-N, which further illustrates that there is a certain ammonia nitrogen removal potential and trend in the unrepaired area or the area before the restoration. The TOC and Al in area A have more obvious effects on microorganisms. Further research found that the more abundant *Bradyrhizobium* in study area A is a very typical acid-tolerant Al-resistant genus(Ozawa *et al.*, 1999), revealing the possible metal metabolism that may occur on the surface of rare earth mining areas. At the same time, the appearance of rare earth elements and high TOC also confirmed that in-situ leaching has less damage to the ecology.

The correlation between soil element characteristics and microbial community diversity index is listed in Table 3. Surprisingly, there is no significant correlation between the sulfate and ammonium ions introduced in the rare earth mining process and the species diversity index. Although not significant, it can still be found that there is a negative correlation between the above two factors and the α diversity index, indicating that the introduction of ammonium sulfate is not conducive to the survival of microorganisms. According to the correlation coefficient judgment, heavy metal elements and bacteria community diversity did not show a significant correlation. But through comparing data can still be found, heavy metal elements in addition to arsenic and almost all negative correlation between the diversity of the biotic community. It shows that the presence of heavy metals is still detrimental to the microbial community. As an essential element for the survival of almost all organisms, C has a great impact on the diversity of the bacterial community and shows a significant positive correlation. The correlation coefficient between TOC content and the diversity index of this study is large, with the maximum value of 0.91, which is the impact on the evenness of the microbial community.

Previous studies have found that rare earth ore mining will cause quite obvious in the process of iron and aluminum content increases, iron and aluminum is analyzed emphatically in the survey, the study found that the concentration of aluminum species diversity of microbial communities showed a significant positive correlation, the correlation coefficient reached 0.965, so aluminum is one of the important influencing factors of the microbial community structure of rare earth mining area. The iron content had little effect on the microbial community structure and the whole was not significant. Rare earth elements have a certain positive influence on the diversity

and abundance of the community structure, and the correlation coefficient is almost above 0.5, especially Dy, Ho, Er and Y. The contents of these four elements all show a significant positive correlation with the diversity of the microbial community, and the correlation coefficient is about 0.9. At the same time, there was a positive correlation between the contents of rare earth elements and the evenness of the microbial community. However, the diversity and coverage of microbial community evolution were negatively correlated with the content of rare earth elements, and the influence of various elements was significant.

The nutrients and vegetation added in the early stage of plant restoration may not necessarily achieve long-term restoration, so the development of microbial community becomes particularly important(Orozco-Aceves *et al.*, 2017). The microbial community can not only indicate the current status of the soil to a certain extent but also respond to environmental changes and participate in participatory remediation. From Table 2, it can be seen that there is a large difference between the alpha diversity index of the three groups of microbial communities of A, B and C and the two groups of D and E. First of all, we found that Chao1 and Observed_species indexes have obvious laws. These two indexes show the species richness of the microbial community. The values of group A are in the first place. The two index values of group B exceed group C. It is speculated that it is the input of fertilizers and other substances that stimulated the microorganisms of group B during the artificial repair process, so that they developed rapidly. Given that the area is in the same sedimentary layer and close to each other, we assume that the original soil was the same or similar before the soil was mined. Therefore, we can predict that the effects of artificial root clefts will have a great impact on the restoration of microbial community richness, and purpose-oriented planting of vegetation can improve the microbial environment of soil. It can be found from the table that the Chao1 index of Groups D and E drops rapidly. Among them, the high concentration of sulfate stimulated some microorganisms, making it difficult to survive. The species richness decreased greatly. In the sampling points of group D, the strong erosion made the TOC in this area only 0.08%, which greatly restricted the development of microbial communities. Shannon index and Simpson index indicated the diversity of the community. There was little difference between the two indexes, but the rule was still obvious. Group A and group C with lush vegetation and group B with artificial plant restoration had higher indexes, moreover, the value of group A is still the highest. Previous studies have found that the development of plant communities can promote the development of microbial communities(Pepper *et al.*, 2012, Li and Huang, 2014). Similarly, some studies find that the presence of vegetation in tailings can improve the activity of microorganisms, which is related to the ability of the environment to repair itself(Sun *et al.*, 2018, Shrestha *et al.*, 2019).

Furthermore, the results of the beta diversity of microbial sequencing were analyzed. It can be found through the PCoA chart that the research objects of this study can be divided into two groups according to the PCoA of beta diversity, namely A and C as a group, B, D and E As a group. Such results are consistent with the previous analysis, indicating that there are roughly two different ecosystems. The important influencing factor of these two ecosystems is the presence or absence of plants. Although there is a plantation in group B, the addition of vegetation-related microorganisms is insufficient considering the time of action, so there is no similarity. There have been similar reports before(Bi *et al.*, 2018, Kang *et al.*, 2018). The long-term existence of plants has a significant effect on the microbial community structure, and then it can be speculated and verified that microbes, in turn, affect plants, and explore the mutually beneficial relationship between the two(For example, the *rhizobia* found in the previous Sect. (3.2) are of great significance to the maintenance of nutrients in plants).

This study found that at the phylum level, the predominant flora was *Proteobacteria, Firmicutes, Actinobacteria, Acidobacteria, and Bacteroidetes.* From the relative abundance, it can be seen that the microorganisms of these phyla have strong adaptability to the acid environment and the tailings environment of rare earth mines, and play an important role in such an ecosystem. No matter whether it is overall or grouped, *Proteobacteria* is an absolute dominant phylum, indicating that it has the highest adaptability to harsh environments. This is consistent with the results of previous studies on zinc smelting slag (Youfa *et al.*, 2018)and tailings in Tongling, Shaoguan and Jiangxi(Chen *et al.*, 2013, Liu *et al.*, 2014). It can be seen that *Proteobacteria* often occupy an advantageous position in the harsh environment of mine tailings. From the phylum level relative abundance graph, we can see that the dominant flora of the two groups A and C are *Proteobacteria*. The proportion of phylum is far more than that of the other three groups. There is very little development of *Firmicutes* bacteria in these two groups. This is a clear difference from the other three groups. It can be seen that the *Firmicutes* in the two groups will be significantly reduced after ecological restoration. When studying the difference between Group B and D and E, it is not difficult to find that the relative abundance of *Acidobacteria* is significantly increased. It can be seen that during the process of ecological restoration, *Acidobacteria* is the primary active phylum, and is the representative phylum of ecological restoration. Besides, the results also confirmed that plants can cause changes in *Acidobacteria*, especially in rare earth mining areas. However, it is clear that research on rare earth minerals is still lacking at this stage, and further studies on vegetation types and element types need further research. Further research found that there are corresponding laws not only at the phylum level but also at the genus level. These results indicate that the existence and restoration of plants are of great significance to the ecological environment. But to be specific to the micro-process of ecological environment restoration, further study of gene metabolism is needed.

Table 3
Correlation analysis of soil bacterial alpha diversity and environmental factors

index	PH	SO42-	TOC	NH4-N	Hg	Cr	As	Cd	Pb
Chao1	-0.13707	-0.40692	0.758426	-0.2507	-0.2643	0.146363	0.078783	-0.38235	-0.02402
Simpson	0.084847	-0.42619	0.810333	-0.3647	-0.40886	-0.10803	0.016402	-0.13511	0.00368
Shannon	-0.0669	-0.43428	0.877169	-0.26231	-0.35558	-0.02648	0.220841	-0.278	-0.19244
Pielou_e	0.102858	-0.50686	.910*	-0.3382	-0.52002	-0.27358	0.427245	-0.10078	-0.33052
Observed_species	-0.13021	-0.43717	0.763415	-0.26805	-0.28909	0.142158	0.124582	-0.39318	-0.0349
Faith_pd	-0.20311	-0.17353	-0.83756	-0.05639	0.164131	0.678092	0.189544	-0.52321	0.500789
Goods_coverage	-0.15561	0.054214	-0.55633	0.217314	0.162157	0.164179	0.547106	-0.14838	-0.2289
index	Al	Fe	Ce	Pr	Nd	Sm	Eu	Gd	Tb
Chao1	.949*	0.32957	0.545371	0.54763	0.508736	0.640058	0.571587	0.725942	0.762656
Simpson	.890*	0.253634	0.535191	0.56167	0.511663	0.684555	0.59301	0.804212	0.869076
Shannon	.965**	0.409399	0.670132	0.686201	0.639658	0.763477	0.716798	0.827313	0.838578
Pielou_e	0.860108	0.390544	0.703662	0.741147	0.683073	0.79593	0.782811	0.835522	0.824338
Observed_species	.953*	0.326639	0.549101	0.552279	0.511301	0.640435	0.57831	0.722407	0.755463
Faith_pd	-0.47648	-0.51787	-0.7372	-0.79094	-0.76868	-0.86724	-0.79929	− .907*	− .900*
Goods_coverage	-0.58769	-0.10826	-0.29625	-0.32131	-0.29645	-0.49163	-0.3291	-0.66325	-0.78719
index	Dy	Ho	Er	Tm	Yb	Lu	Y	La	Sc
Chao1	0.775931	0.787254	0.767344	0.672457	0.555519	0.524148	0.812198	0.544523	0.769556
Simpson	.894*	.909*	.899*	0.816983	0.705679	0.672018	.919*	0.555616	0.790461
Shannon	0.832141	0.830753	0.784791	0.64643	0.50112	0.45925	0.86583	0.682329	.892*
Pielou_e	0.80119	0.789477	0.723732	0.558231	0.402873	0.353613	0.829674	0.736102	.931*
Observed_species	0.766789	0.777643	0.755248	0.656372	0.537638	0.505381	0.804686	0.549214	0.780076
Faith_pd	-0.86565	-0.82965	-0.75986	-0.60512	-0.45912	-0.41321	-0.84157	-0.78395	-0.74912
Goods_coverage	-0.84709	-0.87355	− .913*	− .923*	-0.87574	-0.86209	-0.84329	-0.31409	-0.46085

3.4 Research deficiencies and future research

The types of rare earth minerals are extremely complex, but the overall scale is small, and the types of rare earth minerals involved in this study are still insufficient. In this study, the characteristics of soil elements and the response of microorganisms to the environment in the late restoration, early restoration and undisturbed areas were included. However, the number of sampling mines limited the number of sites in the early restoration period, so only one mine was found in the early restoration period. In this study, although it was confirmed that vegetation development had a certain impact on biology, due to the small scale of rare earth mines, vegetation coverage was not quantitatively studied, and the impact of vegetation types and growth conditions on microorganisms was not further analyzed.

After studying the characteristics of soil elements and the corresponding characteristics of microorganisms in the environment in the rare earth mining area, further research will be performed. First of all, in the mining field, by testing the regional characteristics of microorganisms, the occurrence of rare earth elements in the region can be analyzed to achieve rapid screening of mines, but specific indicator microorganisms still need in-depth research. Secondly, *Proteobacteria* microorganisms can be used as an option for rapid repair of the site, and a better solution of mine repair through the combination of multiple technologies. To make up for the shortcomings of this research, the later research will find more research sites to enrich the types of mines, and at the same time try to quantify the vegetation

coverage level, and further refine the vegetation type and vegetation root development level. Study the mechanism of vegetation's impact on microorganisms and the environment and verify the current results. Finally, vegetation and microorganisms will be restored together.

4. Conclusion

The interaction between the indigenous microbial communities of rare earth mines and the geochemical characteristics and processes of the mine itself is the key to reveal the biogeochemical evolution of rare earth mines. This study selected a typical research area of a typical mining area and found that the microbial community will change as the mine recovers.

In general, the soil environment of the rare earth tailings site is significantly affected by the mining effect. Among them, the soil of the research site shows acidic characteristics, and the pH is less than 4.8. At the same time, the TOC content in the soil is seriously insufficient. There are a lot of iron and aluminum elements in the soil after mining, which is caused by the residue of iron-rich and aluminum-rich clay minerals after leaching, and the aluminum element content reaches 103 g/kg. The content of rare earth elements in the collected soil samples is quite different, and the analysis found that the content of rare earth elements is positively correlated with TOC and negatively correlated with Pb content. This internal relationship needs further exploration. Because the mineral leaching agent is an ammonium sulfate solution, the content of sulfate in the soil is generally high, The drainage area repair in the in-situ leaching area has a lag effect, and long-term management should be realized. The characteristics of the microbial community in different recovery periods are different. NH4-N has the most obvious impact on microorganisms in the unrecovered area or at the initial stage of recovery.

The microbial community can make different responses to different environments, and the development of vegetation directly affects the structural characteristics of the microbial community. The regions with relatively complete vegetation coverage (group A) have the highest alpha diversity index, and Several indicators are positively correlated with the development of vegetation. The dominant bacteria in the study area are *Proteobacteria* and the relative abundance of *Proteobacteria* in each sampling area is the highest, indicating that *Proteobacteria* have the strongest adaptability to the rare earth mine environment. In the process of mine restoration, the bacterial community will also change accordingly, which is mainly reflected in the gradual decrease of the relative abundance of *Firmicutes* and the gradual increase of the relative abundance of *Acidobacteria*. Among the research points, the typical bacterial genera are mostly related to the nitrogen cycle or plant nitrogen fixation(*Burkholderiaceae, Gammaproteobacteria, Gemmatimonas*) and heavy metal metabolism(*Chujaibacter)*. Among them, point C is the most typical, indicating that multi-stage PRB restoration has a good role in promoting ecological restoration, especially the restoration of ammonia nitrogen.

Declarations

Data availability

All the data obtained in this study are presented in this paper, and the specific sequencing results and element determination data can be obtained from the author.

Compliance with ethical standards

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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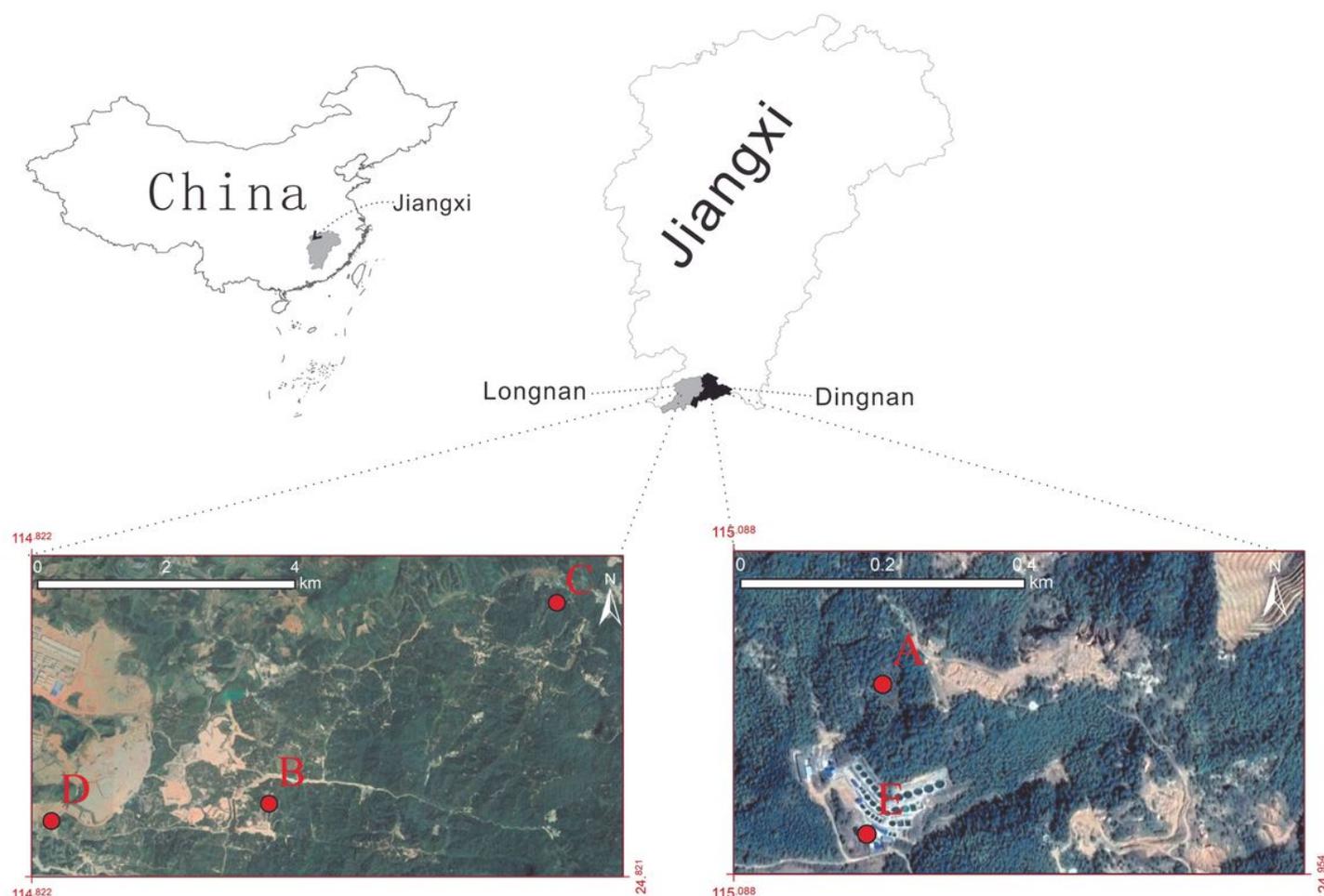
Figures

Figure 1

Location of the study area and the sampling point(The figure shows the microtopography and vegetation cover of the sample area) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
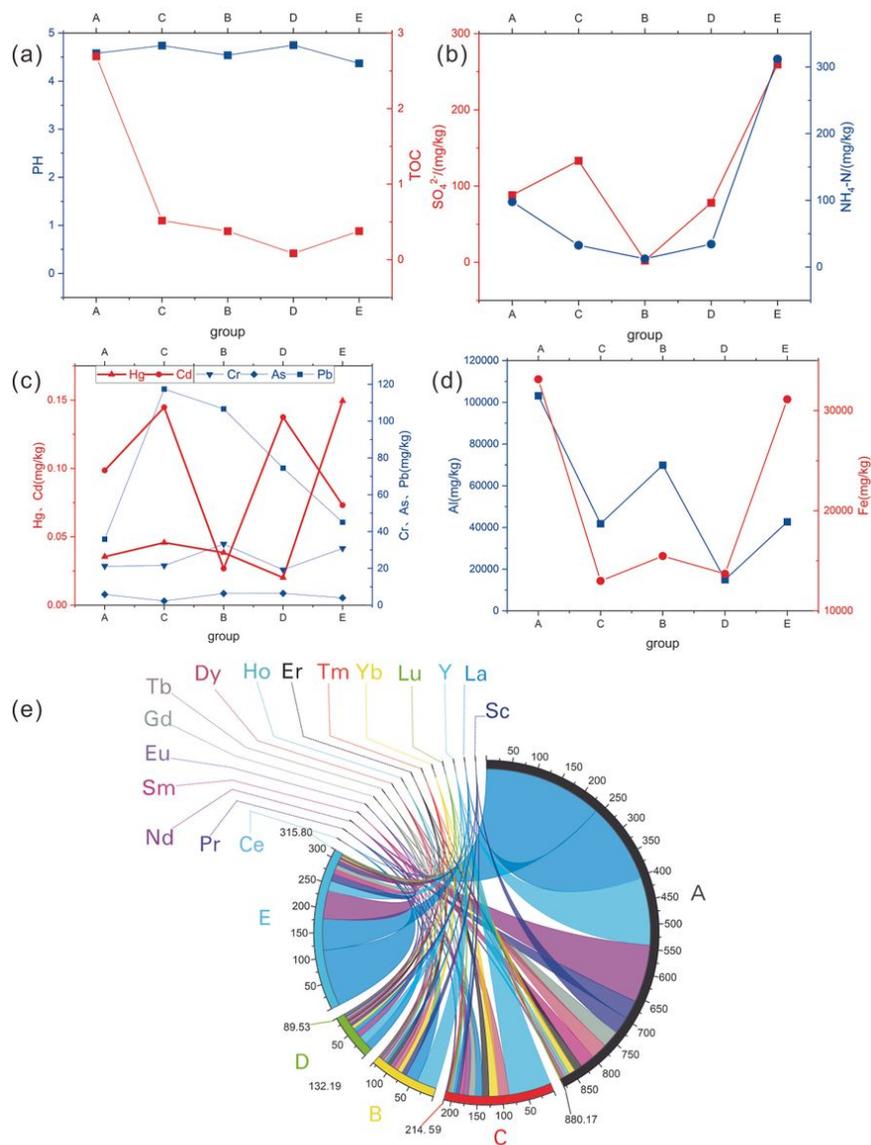
Figure 2

Geochemical parameters, the content of heavy metals and rare earth elements at different research sites and their changes with site types ((a) pH and total organic carbon(TOC), (b) SO42- and NH4-N, (c) Hg, Cd, Cr, As and Pb, (d) Al and Fe, (e) The content of each rare earth element at each sampling point, the total content of rare earth elements at the sampling point and their proportional relationship. The length of the ring on the right represents the content of rare earth elements, and the ring on the left represents the type of rare earth elements. Unit: mg/kg.)
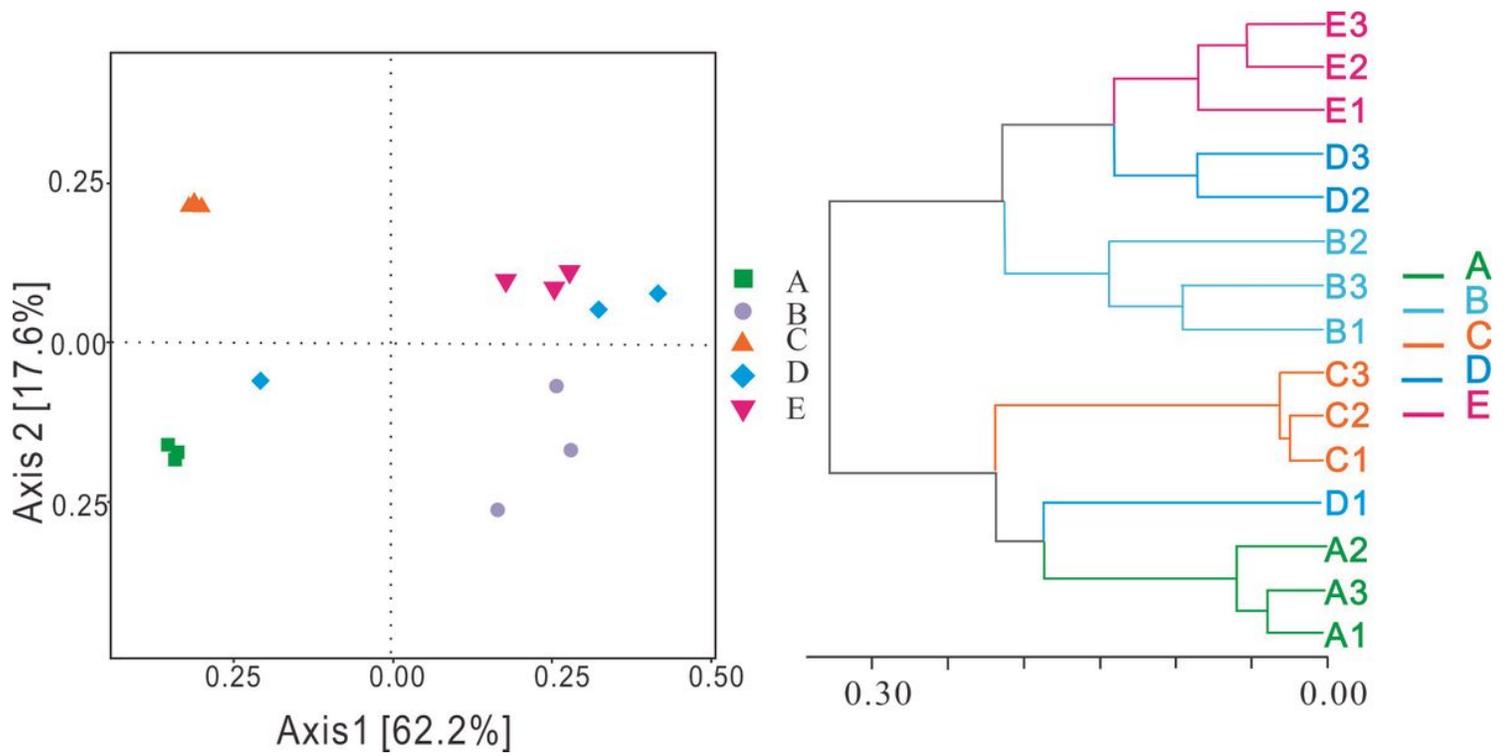
Figure 3

I, II: PCoA and UPGMA plots of the soil bacterial OTUs based on weighted UniFrac metric among all samples(In the figure, A, B, C, D and E are the groups of sample collection, respectively. A and C are the late stage of restoration, B is the early stage of restoration, and D and E are the undisturbed stage).
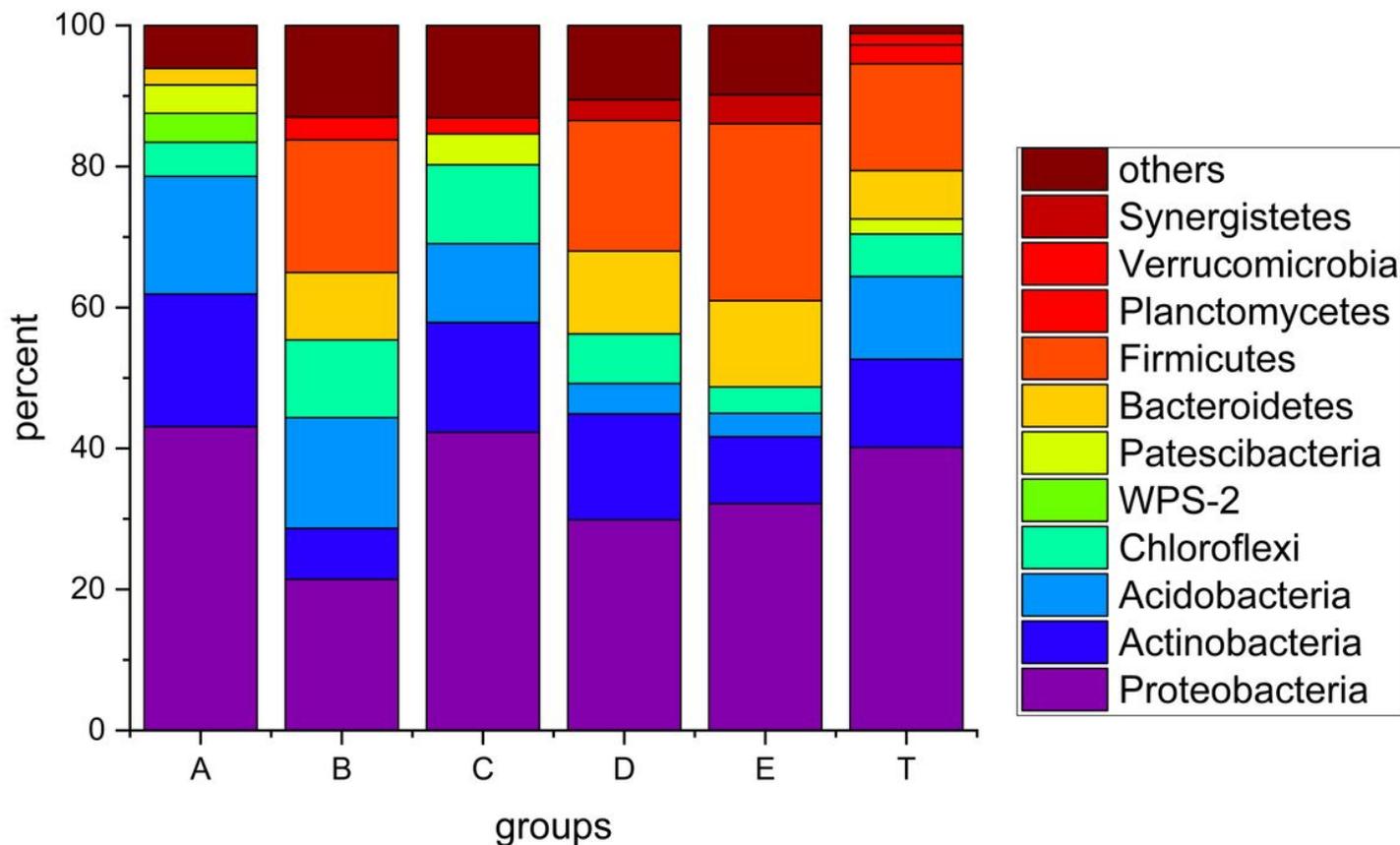
Figure 4

The relative abundances of dominant soil bacterial communities at the phylum level (In the figure, A, B, C, D and E respectively represent the relative abundance of each phylum after grouping, while T represents the relative abundance of each phylum when all samples are not grouped, that is, the relative abundance of microbial phylum level in all samples. A and C are the late stage of restoration, B is the early stage of restoration, and D and E are the undisturbed stage.)
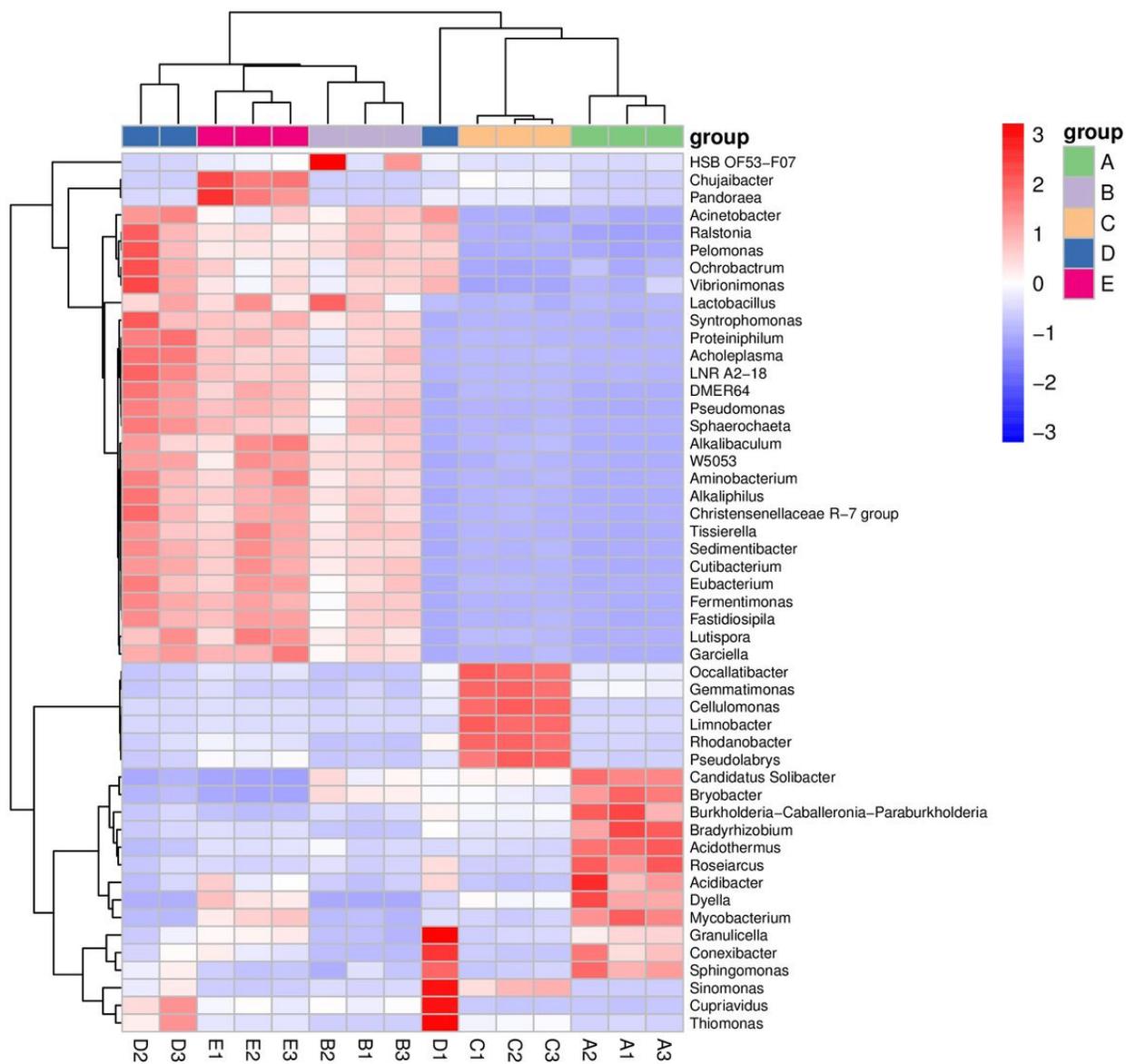
Figure 5

Heat map of genus horizontal species composition of soil bacterial bi-clustering (In the figure, A, B, C, D and E are the groups of sample collection, respectively. A and C are the late stage of restoration, B is the early stage of restoration, and D and E are the undisturbed stage.)
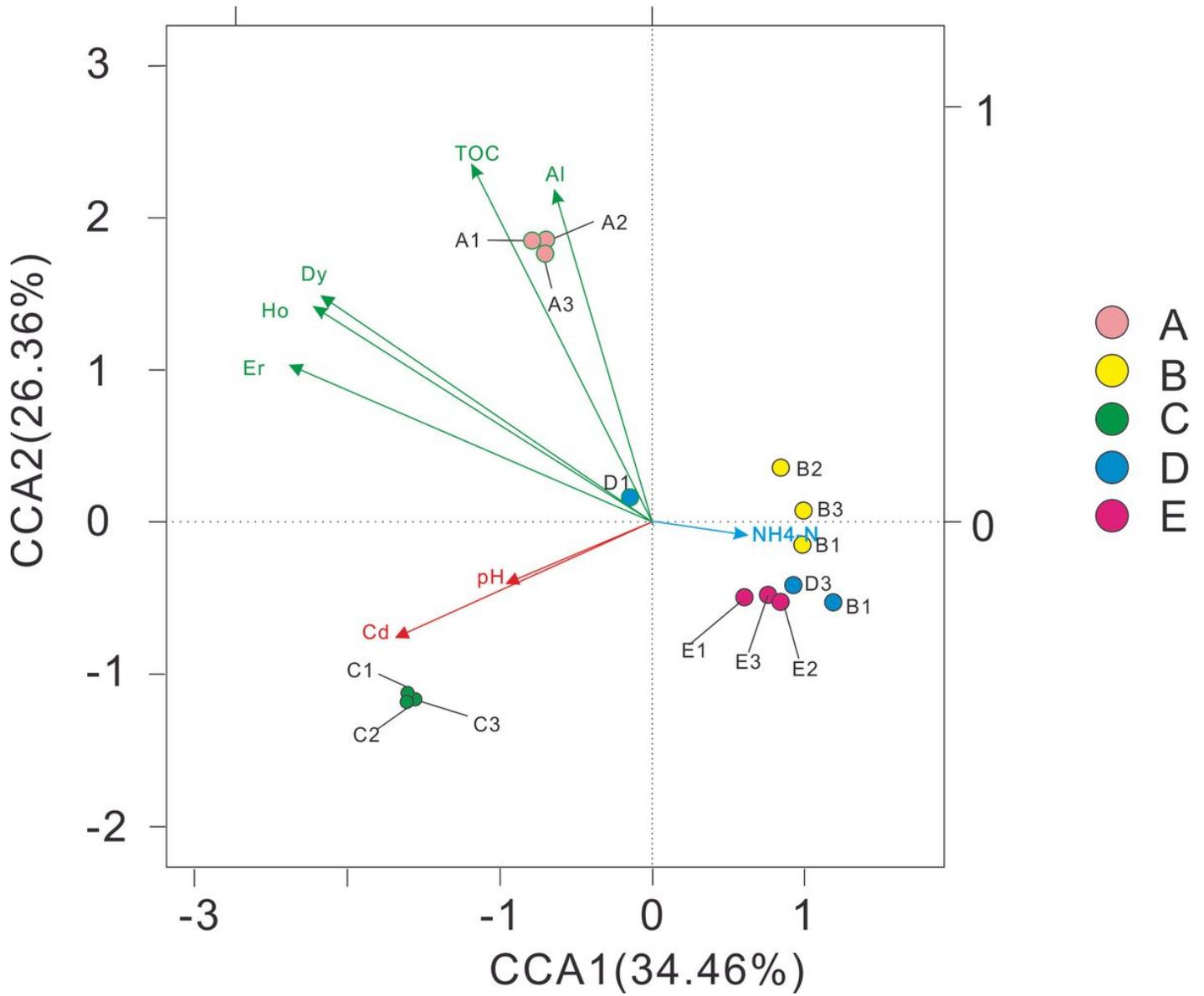
Figure 6

CCA diagram of hydrogeochemical elements and indigenous microorganisms in the study area