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Heavy Metal Accumulation Affects the Structure of Microorganisms and Increases Abundance of Resistance Genes in Rare Earth Mining Areas

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Abstract

Background

Environmental pollution from rare earth mining areas is of great concern, but the impact on microbial ecology and genomics has received little attention. In this study, the relationship between heavy metals and soil microbial community in the northern rare earth mining area was explored.

Methods

In order to study the detoxification mechanisms of heavy metals by microorganisms in this typical rare earth mining area, the study area was divided into three parts (mining area, residential area and control area). Analysis of microbial community diversity, structure and functional abundance using high-throughput sequencing techniques. Analysis of the effect of heavy metal pollution on the abundance of heavy metal resistance genes in soils of different regions using real-time fluorescence quantitative PCR.

Results

The results showed that the heavy metal pollution rules: mining area > residential area > control area. Under the condition of long-term heavy metal pollution, the original microbial community composition was changed, and the species richness and evenness of soil in mining areas were higher than that in residential areas. The high-throughput sequencing analysis showed that existed metal-resistant microbial communities such as *Actinobacteria*, *Proteobacteria*, *Korarchaeota* and so on under the stress of heavy metal. High concentrations of heavy metals can inhibit the activities of catalase and sucrase. According to Tax4Fun function prediction analysis, heavy metal accumulation increased the ABC transporter protein in microbial function. The results of fluorescence quantification experiments also demonstrated that the abundance of heavy metal resistance genes, *czcA*, *czcB*, *czcC* and *czcD*, encoding ABC transporter proteins, increased with increasing heavy metal concentrations.

Conclusions

In conclusion, the accumulation of heavy metals not only changed the soil physicochemical properties and the microbial community structure, but also decreased soil enzyme activities and increased the abundance of resistance genes, which activated the detoxification mechanism of heavy metals. which provided a reference for future ecological remediation.

1 Background

The over-exploitation of rare earth elements has caused serious desertification and environmental pollution, and in China, ecological restoration of mining areas is receiving increasing attention(Wei et al., 2019 ; Wang et al., 2020). In recent years, with the increase of rare earth mining and smelting production, heavy metal pollution of soil in the surrounding areas has become a growing concern and a worldwide problem(Rodriguez et al., 2009; Frossard et al., 2018). So far, mining activities, especially the mining of metal ores, is a major source of soil heavy metal pollution(Huang et al., 2018). Due to different mining activities and habitat specificities, soil properties and heavy metal contents vary considerably over short spatial distances and elevation gradients (Zhao et al., 2019). Heavy metal pollutants caused by mining can be consumed by the human body through the food chain through soil, water, plants,

etc., causing great harm to the human health (Liao and Xie 2007; Vinhal-Freitas et al., 2017). Soil contaminated with heavy metals causes changes in soil physicochemical properties and microbial activity, and microbial activity is more sensitive to heavy metals than animal or plant growth in the same soil, soil microbial biomass, soil enzyme activity and metabolic entropy are soil biological parameters(Liao and Xie, 2007), which are responsive to external conditions such as climate, human behavior and heavy metal pollution, and can reflect soil pollution status to some extent(Tang et al., 2019), It can be used as an effective index to evaluate the ecological impact of heavy metal pollution in soil (Ivshina et al., 2014).

Heavy metal contaminants have been shown to be harmful to soil microorganisms, and soil heavy metal contamination may lead to significant changes in microbial diversity, structure and activity(Filip, 2002; Nacke et al., 2014). Microorganisms make an important contribution to the maintenance of terrestrial ecosystems and their biodiversity because the enzymatic activity of soil microorganisms and soil microbial biomass control the cycling and storage of nutrients in the soil(Li et al., 2017). However, the presence of heavy metals in the mining process brings great pressure to microorganisms, which must survive in a heavy metal contaminated environment, thus having a greater impact on microbially mediated soil nutrient cycling(Khan et al., 2010). Both the concentration of heavy metals in the soil and their biological effectiveness influence the toxicity of heavy metals(Kenarova et al., 2014). Soil pH can influence the sorption of metals by substances in the soil, such as organic matter, by altering the surface charge and dissociability of heavy metal sorbents, which in turn affects the bioeffectiveness and toxicity of heavy metals to microorganisms(Bang and Hesterberg, 2004). Heavy metal contamination reduces soil microbial biomass, diversity and biochemical activity due to negative selection of microorganisms sensitive to heavy metal pollutants and inhibition of microbial metabolic activity(Azarbad et al., 2016; He et al., 2016; Yao et al., 2017), hence, low microbial biomass and slower soil organic matter decomposition activity in heavy metal contaminated soils due to low microbial biomass, functional diversity and metabolic efficiency of heavy metal tolerant bacteria in soil microbial communities(Mergeay, 2000). Heavy metals, soil and bacteria interact in a complex manner, and soil microbial communities play an important role in determining soil quality and regulating soil physicochemical properties(Guo et al., 2017); Therefore, soil microbial activity is often considered as a sensitive and effective indicator of mine ecosystems(Liao and Xie, 2007; Liu et al., 2016).

Soil enzyme activity is often considered as a sensitive biological index to evaluate soil quality(Spohn and Kuzyakov, 2014). Studies have shown that redox enzymes and hydrolytic enzymes can be mainly used to evaluate heavy metal pollution. In general, high concentrations of heavy metals could degrade soil cells, destroy soil microbial communities, and inhibit soil enzyme activity(Ciarkowska et al., 2014). Also, catalase is able to break down H_2O_2 and protect organisms from damage. In addition, catalase has been used as a bioindicator to detect the presence of various heavy metal contaminants(Xian et al., 2015; Hu et al., 2014). Sucrase activity reflects the ability of the soil to break down sucrose and free monosaccharides, which are the main source of energy for soil microorganisms (Frankenberger and Johanson, 1983). Thus, enzyme activity can be used to indicate improvements in the rehabilitation of soils after mining(Schimann et al., 2012). Enzymatic activities are also used for determining the effect of various pollutants including heavy metals on soil microbial quality(Shen et al., 2005; Khan et al., 2007). Studies have shown that heavy metals (Zn, Cu, Ni, V and Cd) in soil would reduce the activities of soil urease, alkaline phosphatase and xylanase (Spohn and Kuzyakov, 2014). It was also found that soil microorganisms polluted by lead-zinc tailing dams reduced urease activity. Enzyme activity varies with the presence of heavy metals, and it depends on different soil properties, heavy metal types and concentrations. Therefore, the integration of multiple enzymes broadly representing microbial metabolism into a comprehensive index is necessary to assess both the toxicity levels of heavy metals in soil microcrops and the ecological impact of heavy metal contamination in soil systems.

Microorganisms in soil contaminated by heavy metals have strong adaptability and viability. The emergence of heavy metal resistance genes in complex microbial communities under heavy metal stress reveals the biological processes and strategies necessary for the survival of microorganisms in extreme environments(Xavier et al., 2019; Thomas et al., 2020). Many bacteria have evolved genetic adaptations to adapt to their environment and acquire metal resistance, multiple genes such as *cadB*, *chrA*, *pbrA*, *MerA* and *NiCoT* have reported systerms for bacterial resistance and detoxification, respectively for cadmium, chromium, lead, mercury and nickel, as well as in the involvement of transport of transition metals(Janssen et al., 2010; Das et al., 2016). In response to environmental pollution threatens the survival of microorganisms with various resistance mechanisms(Han et al., 2020), such as metal efflux pump mediated transport, metal produced by permeation barrier, the transformation of heavy metals by intracellular and extracellular enzymes and detoxification, this makes the microbes can by increasing the resistance mechanism of genes to expand their niche in heavy metal contaminated soil(Guo et al., 2018; Xi et al., 2021).

Due to mining activities, there are significant differences between heavy metal and physical and chemical properties in different regions(Kenarova et al., 2014), the microbial community structure will also be adjusted to adapt to different habitats. (Pérez-de-Mora et al., 2006; J Kozdroj, 2001). At present, the effects of tailing waste accumulation on the distribution of heavy metals and bacterial communities remain unclear. As a rare earth ore in the north, this region has a special habitat, it is of great significance to study the effects of rare earth mining on soil physical and chemical properties, heavy metals and soil bacterial communities. The present study aimed to (1) evaluate the effects of manganese (Mn), copper(Cu), lead (Pb) etc. on soil enzyme activities, microbial function, community diversity in rare earth mining areas; (2) assess whether these microbial characteristics can be used as possible indicators of soil pollution by heavy metals and (3) analyze the influence of heavy metal pollution on the abundance of heavy metal resistance genes in soil in different regions.

2 Material And Methods

2.1 Sample collection

The sampling area for this study was in the northern rare earth mining area and its surrounding areas. According to the general layout of the rare earths, it was divided into three parts, namely the mining area(MA), the residential area(RA) and the control area(CA), as shown in the Fig. 1. Three 20×20 m plots were established at each functional area and were considered true replicates in July 2019. There were 27 mining areas, 6 residential areas and 3 control areas. In detailed, at each sampling site, surface sediments(0–20 cm) were sampled in 3 points, the topsoil was collected from three random points by shovel at each plot and was placed into sterile centrifuge tube, immediately preserved with dry ice before being transported to the laboratory. Each sample was divided into three parts: one for DNA extraction and high-throughput sequencing of the soil microbes and stored in a refrigerator at -80°C, the other for chemical analysis stored at 4°C in the refrigerator for determination of soil enzyme activity, and the rest was air-dried for subsequent physical and chemical analysis.

2.2 Physical and chemical analyses

The pH was measured using a pH meter (PHS-3C, Shanghai INESA Instrument Co., Ltd., China), in 1:2.5 (soil/water) H_2O suspensions after 1 h of shaking. The moisture content (MC) of the soil was determined by drying to a constant weight at 105°C ± 2°C in a drying oven, then weighing. Cation exchange capacity (CEC) was determined by calcium acetate exchange method. Oxidation-reduction potential (ORP) was tested in accordance with the "Determination of soil redox potential Potentiometric method" (HJ 746–2015). The Electrical conductance (EC) of soil samples were determined using a conductivity meter (DDS-II A) [1:5.0 (w/v) soil/water ratio]. The soil organic matter (SOM)was

investigated by the $K_2Cr_2O_7$ colorimetric method(Jakobsen, 1998). The total nitrogen (TN) in the soil was determined by an SKD-1000 automatic azotometer (PEIOU, China). Total heavy metals (Mn, Cu, Zn, Pb, Cd, Hg) were measured by ICP-AES(Tighe et al., 2006; Salvador et al., 2018) (PerkinElmer Optima 7300 DV, USA) after strong acid digestion (1:4 concentrated $HClO_4$ and HNO_3 (v/v)). Each sample had three replicates, and each tested sample was measured three times, in order to calculate the mean value.

2.3 Typical enzymes activity experiments

Soil enzyme activities were measured using the soil enzyme activity kit, which mainly measured five soil enzyme activities, namely, soil urease (UE), soil catalase (CAT), soil sucrose (SC), soil neutral phosphatase (NP) and soil alkaline phosphatase (ALP). Each index was measured for three repetitions in the same treatment(Gianfreda et al., 2005), and the enzyme activity was measured according to the instructions of the kit purchased (the kit was purchased from Nanjing Jiancheng Bioengineering Research Institute).

2.4 DNA extraction, PCR, and high-throughput sequencing

The 0.3–0.5 grams of soil sample was used for DNA extraction using the FastDNA® Spin Kit for soil (Magen, CHN). The V3-V4 region of the bacterial 16S rRNA gene was amplified(Dennis et al., 2013). The original sequencing sequence of the sample DNA fragment was obtained through llumina Miseq sequencing platform(Keegan et al., 2016), and a large number of reads were generated. In order to ensure the quality level of these reads and ensure subsequent analysis, sequencing quality control was required. First, stitching according to overlap, the sequenced joints and primers should be taken out. The low-quality data are then filtered to obtain the sequencing sequence available for subsequent analysis. OTU was screened with 97% similarity level, and each sample OTU was compared with the Silva database(Dickey et al., 2014). In order to obtain the species classification information corresponding to each OTU, RDP classifier was used for the taxonomic analysis of the OTU representative sequence.

2.5 Quantitative analysis of resistance genes

The normal PCR amplification of each functional gene was performed using the sample DNA as the template. Amplification products by agarose gel electrophoresis test, cut to strip, by DNA gel recovery kit back to collect pure, with cloning vector pGEM-T try agent box into the line of pure products of enzyme, and turned in sense the state cells *Escherichia coli* DH5α, blue white spot on the ampicillin flat screen, choose positive clone (white spot) propagation as microbial sequencing analysis, further cloning identification results. Plasmids was extracted from the cultured liquid, its concentration was determined, and the copy number was calculated. Gradient dilution was performed by a tenfold gradient. Fluorescence constant PCR amplification was performed using the different concentration standard as the mold plate, and the standard curve was drawn. Different functional groups were quantitatively amplified by fluorescence quantitative PCR. Quantitative PCR system: dye fluorescence quantitative reagent(SYBR premix Ex Taq) 10 μl, each of the upstream and downstream primers at 20 μmol/L was 0.5 μl, DNA template 1.0 μl, and the double steamed water was supplemented to 20 μl(He et al., 2016).

2.6 Statistics and data analysis

All data were analyzed using SPSS version 16.0 and Origin2019b. According to the annotation results of all sample species, the structure of phylum and genus level microbial community was analyzed. Using the principal component analysis (PCA) to the original environment data matrix for dimension reduction, correspondence analysis (CCA) was discussed environmental factors significantly influence microbial community structure(Magurran and Anne, 1988). The differences in the microbial diversity index and enzymatic activity were compared using Analysis of Variance (ANOVA, IBM SPSS 24.0).

3 Results

3.1 Heavy metal accumulation changes soil physicochemical properties in rare earth mining areas

The accumulation of heavy metals in rare earth mining areas has affected the physicochemical properties of the soil, the differences of soil physical and chemical properties in each sample point are shown in Table 1. The soil pH ranged from 7.93 to 8.77 all was alkaline. Soil moisture content ranges from 3.97–18.93% Among all the sample points in the MA, the EC value of soil samples ranged from 86.67 μs/cm～4723.33 μs/cm, the highest EC value was 5983.33 μs/cm in RA10, and the lowest EC value was 83.33 s/cm in control area DZ12. The ORP ranged from 282.67 mv to 331.67 mv, and there was no significant difference among the sampling points. The CEC of soil samples was 1.75 ~ 20.67 cmol/kg. The minimum value was 1.75 cmol/kg at MA8 in the MA and the maximum value was 20.67 cmol/kg at DZ12 in the CA. The content of SOM was significantly different, ranging from 3.33% to 22.65 %, the content of SOM in the sampling point MA7 was the highest, reaching 22.6 5%, and the content of organic matter in the comparison point DZ12 was the lowest, reaching 3.33 %. The TN content in the soil in the three regions ranged from the largest to the smallest as the CA > MA > RA, with a range of 0.35–1.87 g/kg.

The heavy metal contents of soil was determined at all sampling sites (Fig. 2.). The metal concentrations of Mn, Cu, Zn, Pb, Cd and Hg ranged from 532.80 ~ 14283.48, 18.31 ~ 38.27, 83.66 ~ 697.04, 17.66 ~ 502.35, 0.09 ~ 2.53 and 0.04 ~ 0.85 mg/kg, respectively. The average content of heavy metals is from high to low in soil: Mn > Zn > Pb > Cu > Cd > Hg. The content of heavy metals in the MA was significantly higher than that in the RA and the CA, among which the content of Mn, Zn, Pb and Cd was the highest in the MA8 point of the MA, which was 26.8, 7.8, 28.44 and 28.1 times higher than that in the CA, respectively. Sample points MA3 and MA1 were the two most abundant points of Cu, compared to the CA, with maximum concentrations exceeding the by 2.1 times. With respect to Hg, the content of MA9 was highest in the MA, followed by MA7, the value of the soil was reached by 21.0 times and 21.2 times of the CA, respectively.

3.2 Heavy metals can affect soil enzyme activity

Soil enzyme activity varies with soil environment and structural categories, but the degree of response of each enzyme was also important for different soil environments (Fig. 3). In this study, catalase activity (Fig. 3A) ranged from 24.42 to 61.15 $\mu mol \cdot g^{-1} d^{-1}$, the highest value was 61.15 $\mu mol \cdot g^{-1} h^{-1}$ at the reference point DZ12, and the lowest value was 24.42 $\mu mol \cdot g^{-1} h^{-1}$ at the MA8 point in the MA. The content of Mn, Pb and Cd at this point was the highest, indicating that high concentration of heavy metals would inhibit catalase activity, and the highest value was about 2.5 times that of the lowest value. Sucrase activity(Fig. 3B) from high to low in turn was: CA, RA and MA, the highest in control point DZ12 value of 50.21 $mg \cdot g^{-1} d^{-1}$, the lowest out now mine MA8 point of 30.53 $mg \cdot g^{-1} d^{-1}$, that of Mn, Pb, Cd content was the highest, showed a high concentration of heavy metal concentrations were activated to restrain activity of invertase, high was about 1.65 times that of the lowest.

In this study, the highest value of urease activity (Fig. 3C) occurred at the MA2 site at 5110.10 $\mu g \cdot g^{-1} d^{-1}$, and the lowest value at the MA6 site at 1647.49 $\mu g \cdot g^{-1} d^{-1}$, with the highest value being about 3.1 times of the lowest value. The range of alkaline phosphatase activity(Fig. 3D) was 818.92～9620.85 $\mu g \cdot g^{-1} d^{-1}$. The highest value was 9620.85 $\mu mol \cdot g^{-1} d^{-1}$ at the MA9 point in the MA, where the heavy metal content was low and the pollution was light. The lowest value was 818.92 $\mu mol \cdot g^{-1} d^{-1}$ at the MA4 point in the MA, and the highest value was about 11.7 times of the lowest value. The neutral phosphatase activity (Fig. 3E) in the three regions from large to small was the MA > RA > CA. The activity of alkaline phosphatase ranged from 3.35 to 9.49 $\mu mol \cdot g^{-1} d^{-1}$, and the highest value was 9.77 $\mu mol \cdot g^{-1} d^{-1}$at MA8 in the mining area, where the contents of Mn, Pb and Cd were the highest. The lowest value was 3.35 $\mu mol \cdot g^{-1} d^{-1}$ at RA10 in the RA, and the highest value was about 2.9 times of the lowest value.

3.3 Correlation analysis between enzyme activity and environmental factors

The correlation analysis between soil enzyme activity and environmental factors was shown in Fig. 4 Among the heavy metals in the soil of the study area, Mn, Zn, Pb, and Cd had a great influence on the enzyme activity. Catalase activity and sucrase activity were negatively correlated with Mn, Zn, Pb and Cd, with correlation coefficients between 0.68 and 0.8, respectively. Catalase and sucrase activities were significantly positively correlated with soil pH, moisture content, Eh, CEC, clay and content. The sucrase activity was negatively correlated with total nitrogen and granule. Urease activity had no significant correlation with soil heavy metal content, but had positive correlation with water content, pH and sand. Neutral phosphatase was positively correlated with total nitrogen and powder content, but negatively correlated with sand content. Alkaline phosphatase was positively correlated with silt content in soil and negatively correlated with sand content.

3.4 The richness and diversity of soil bacterial community changed in different regions

The composition of soil microbial community was analyzed by non-metric multidimensional scale analysis (NMDS) as shown in Fig. 5. The microorganisms in the RA10, RA11 show a discrete state with other samples, indicating that the two sites were less similar to the species in the remaining mining areas. The distance between the control point and the sampling point in the mining area was relatively close, indicating that the soil sample taken from the control point and the sample taken from the mining area have a closer species composition, which was less similar to the samples taken from the RA.

In order to investigate the diversity and structure of the microbial communities, we used Illumina high-throughput sequencing technology to sequence the 16S rRNA. The alpha diversity indices of the bacterial communities were shown in Table 2. From the perspective of community abundance, according to the analysis of the Chao index, the greater the Chao index, the greater the number of OTUs and the greater the number of sample species. In this study, the Chao index of the MA was greater than the RA and the CA, indicating that the MA has a higher species richness. Secondly, it can be seen from Shannon index and Simpson index that the diversity of microbial communities in MA was relatively high, which was quite different from RA. As far as the points in the MA were concerned, the heavy metals at points MA1, MA2, and MA3 in the MA were generally higher than those at points MA4, MA5, and MA6 in the MA. In terms of microbial diversity and abundance, the less polluted MA4, MA5, and MA6 points Shannon index and Simpson index are higher than MA1, MA2, MA3. This also proves that as the content of heavy metals increases, the microbial diversity in the soil will gradually decrease.

3.5 Heavy metals can change the composition of soil bacterial community

After quality filtering, high quality sequences of bacteria 16S rRNA V3～V4 were obtained from 12 soil samples in the mine area. Subsequently, a total of 266167 bacterial operational taxonomic units (OTUs) were assembled at a 97% confifidence interval. In total, we extracted 79 identified phyla from all soil samples (Fig. 6). At the phylum level, *Actinobacteria*, *Proteobacteria*, *Chloroflexi* and *Thaumarchaeota* were the most abundant phyla in mine soil. In addition, *Actinobacteria* were the most dominant phyla in the soils of MA and CA, the RA significantly enhanced the relative abundances of *Proteobacteria* compared to mine. *Proteobacteria* have become the most dominant group of bacteria in the RA of RA11 and RA12, The proportion of *Actinomycetes* was the largest in other mining areas and control points. In heavy metal highly polluted sediments, the abundance of *Proteobacteria* was relatively low. Secondly,

some flora such as *Korarchaeota, LCP_89*, *RsaHF231*, etc. were not detected in RA and CA, but only in the MA, it might be due to that the long-term heavy metal pollution, specific resistant bacteria appeared, changed the original microbial community composition.

The genus level analysis of microorganisms in the mine area was shown in Fig. 7, *Thiobacillus* and *Luteimonas* had the highest abundance in RA11 of the RA, with an average abundance of 22.43% and 23.54%, respectively, which were significantly different from the microbial communities in the MA and CA. The highest abundance of *Solirubrobacter*, *Nocardia* and *Rubrobacter* were found in the soil samples from the MA.

3.6 Relationship between microbial abundance and environmental parameters

Spearman order correlation was used to analyze correlations between environmental parameters and microbial abundance (Fig. 8). According to the analysis of the correlation between heavy metals and soil microbial community (phylum), it was found that *Cyanobacteria* and *Chloroflexi* were positively correlated with Mn ($P < 0.05$). *Chloroflexi* has a significantly positive relationship with Zn ($P < 0.05$), Zn was negatively correlated with *Entotheonellaeota*, thus inhibiting the growth of *Entotheonelleota*. Pb, Cd were positively correlated with *Cyanobacteria Patescibacteria*, *Chloroflexi* ($P < 0.05$), significantly negative correlated with *Entotheonellaeota* ($P < 0.05$), inhibited the growth of the *Entotheonellaeota* phylum. There are two bacteria that are negatively associated with Hg, including *Unclassified* and *Armatimonadetes*.

3.7 Heavy metals can alter the function of microorganisms

Functional contributions of bacteria in soil samples from different regions were predicted based on OTU levels through the Tax4Fun database. From Fig. 9, it can be seen that ABC transport proteins and two-component regulatory systems accounted for the largest proportion of the predicted metabolic functions. The points MA7 and MA8, which were the most contaminated with Pb and Cd among all points, compared with the control points. It's worth noting that ABC transport proteins, a class of transporter protein, was higher in MA7 and MA8 than in the control site DZ12 in the heavily contaminated mine.

3.8 Heavy metals can increase the resistance of resistance genes.

Under the stress of heavy metals, the emergence of heavy metal resistance genes in complex microbial communities, microorganisms have a strong ability to adapt and survive in heavy metal contaminated soils. From the previous predictions of microbial metabolic functions, it was found that the ABC transporter proteins and two-component regulatory systems accounted for the largest proportion. Therefore, we selected *czcA*, *czcB*, *czcC*, and *czcD* genes belonging to the ABC transporter proteins to investigate the abundance of soil heavy metal resistance genes. The gene abundances of *czcA*, *czcB*, *czcC* and *czcD* were shown in Fig. 10. By comparing the three points of MA7, MA8 and RA11, the sample sites MA7 and MA8 had higher gene abundance of *czcA*, *czcB*, *czcC* and *czcD* than RA11 which were high concentration of heavy metals by heavy metals Zn and Cd, the gene abundances of *czcA, czcB, czcC and czcD* are higher than those of RA11. The gene abundance of *czcA* at the MA8 site was 0.4 times that of RA11 in residential areas, and the gene abundance of *czcB* at the most polluted MA8 site was 0.3 times of RA11 in residential areas. Gene abundance increases with increasing heavy metal concentrations, and through the outside to prevent *czcA* metal cation to enter the cell and was located in the outer membrane of regulon *czcC* relative expression of genes in the residential area were greater than the heavy metal pollution of mine, It was speculated that the studied area was a rare earth mining area, rare earth elements in the soil accumulation amount was larger, It interfered with the expression of *czcA* and *czcC* genes. The results showed that in the presence of a large number of Zn and Cd ions, the strains could adjust the expression of a large number of resistance genes in order to cope with the environmental changes.

4 Discussion

4.1 Significant differences in soil physicochemical properties and heavy metal distribution between regions

Due to the frequent mining activities, heavy metal pollution of the mine soils is severe(Shu et al., 2003). Once these toxins enter agricultural soils, they could affect food production and security and pose a major threat to human health by entering the food chain (Guo et al., 2017). We assessed the ecological risks caused by these heavy metals, and investigated the structure and diversity of bacterial community under heavy metal pollution in this environment(Ma et al., 2020). Due to mining activity, the soil around rare earth mining area has been severely polluted not only with Mn and Cu, but also with Zn, Pb, Cd and Hg. The content of Mn, Pb and Cd of heavy metals reached the highest in MA8 of the mining area, which might be caused by the low terrain of the sampling site. The main wind direction of rare earth mining area was the northwest wind, and the pollutants in the mining area migrate and accumulate along the wind direction. The pollution rules of the six heavy metals were all: MA > RA > CA. The heavy metal content in the MA was significantly higher than that in the residential area and the control area due to the influence of artificial mining activities, mining industry traffic and so on. In the accumulation of heavy metal elements in the soil, Mn, Zn content was the highest. The soil in the rare earth mining area was polluted by elements Cd, Pb and Mn to different degrees, and showed a definite accumulation of pollution, which was consistent with the previous investigation on the pollution status of the mining area(Fu et al., 2016).

The correlations between heavy metals and some soil properties, such as CEC and SOM, were significant, which may due to the soil substances limiting the transfer of heavy metals. Organic matter content was an important part of soil, which provided nutrients and energy for microbial life activities(Aikpokpodion, 2010). The content of soil organic matter can reflect the level of soil fertility. The content of organic matter in the sampling point MA7 in the mining area was the highest, reaching 22.65%. The high content of organic matter in the soil would affect the absorption of heavy metals in the soil. The high content of organic matter was conducive to the adsorption of heavy metals in soil. The content of organic matter in the control point DZ12 was the lowest of 3.33%. This sampling point was less polluted by mining, less polluted in soil, has higher microbial activity in the soil, and more able to decompose organic matter, so the content of organic matter in the soil was relatively low. Soil structure, moisture and electrical conductivity also have essential effects on the migration of heavy metals in soil. Cation exchange capacity was the main source of soil buffering performance and the important basis for soil improvement and rational fertilization(Kelly et al., 1998; Wucheng, 2008). The smallest cation exchange capacity values for the soil samples were at sample site MA8, which is largely devoid of vegetation and heavily contaminated with heavy metals. The soil fertility at this site is poor. The maximum value was at sample point DZ12 in the control area, which was less affected by mining activities and less polluted by heavy metals. Therefore, the soil has a strong ability to retain fertilizer.

4.2 Heavy metals changed the activity of enzymes in the soil

Soil enzymes are considered as potential indicators of bacterial function and are closely related to soil biology and soil characteristics(García-Ruiz et al., 2008; Huang et al., 2019). In the soil of the study area, the heavy metals Cu, Zn, Cd and Pb had a great influence on the enzyme activity. Sucrase and catalase activities were lower in areas with high concentrations of heavy metals, while these two enzymes were significantly and negatively correlated with Mn, Zn, Pb and Cd in the soil, indicating that high concentrations of heavy metals inhibit enzyme activity (Borowik et al., 2014). Soil pollution levels and human activities will affect the migration and transformation of Pb in soil, thus affecting its bioavailability and biotoxicity. The activity of catalase and sucrase was similar, and will decrease with the increase of heavy metal concentration. These results were consistent with those reported by Belyaeva et al(Belyaeva et al., 2005).

When the concentration of heavy metal exceeds a certain concentration, Pb, Cu and Zn ions can directly bind to the enzyme or substrate in the soil, inhibiting the enzyme activity in the soil. In some sites, the content of organic matter in the soil was low, and the adsorption of gold ions was weak. Pb^{2+} was easy to bind to the enzyme free in the soil, and react with the active protein, such as to combine with mercapto to form metal sulfides, or to combine with the substrate to form complexes, thus to mask the binding site of the enzyme, and finally to inactivate or inhibit the enzyme activity(Dick, 1997; Roscoe et al., 2000).

4.3 High concentration of heavy metals affected soil microbial community structure

In order to study the richness, uniformity, diversity and sequencing coverage of each sample, diversity analysis was performed on the samples(Jennifer L et al., 2004). Long-term heavy metal pollution results in the change of soil microbial community structure, as well as the decrease of diversity and abundance(Xi et al., 2021). Soil microorganisms have a weak response to low concentration of heavy metal pollution, and that exceeding the tolerance concentration of microorganisms will lead to the decrease of microbial population(Vig et al., 2004). In this study, the microbial abundance and multisampling of RA11 samples in the RA were both small, but the microbial abundance and diversity of MA4, MA5 and MA6 samples were relatively high. This may be due to the presence of certain resistant bacteria in the soil under the stress of long-term heavy metal pollution, which improved the microbial diversity(Choi and Journal, 2009). In addition, the stress of heavy metal Cd can lead to the disappearance of part of the flora and the emergence of specific resistant strains, which changes the composition of the original microbial community(Bartolomé et al., 2016). The heavy metals at MA1, MA2 and MA3 in the MA were generally higher than those at MA4, MA5 and MA6 in the MA. In terms of microbial diversity and abundance, the Shannon index and Simpson index of the lightly polluted MA4, MA5 and MA6 samples were all higher than those at MA1, MA2 and MA3. This also proves that the microbial diversity in soil decreases gradually with the increase of heavy metal content. Analysis at the microbial phylum level and genus level revealed that the abundance of the *Actinobacteria* was higher in the MA, while the abundance of the *Proteobacteria* was higher in the RA, and the corresponding abundance of the *Solirubrobacter*, *Nocardia* and *Rubrobacter* were higher in the MA than in the RA, and it can be concluded that heavy metals changed the microbial community structure in the area.Through the correlation analysis the negative effects of heavy metals on microbial community structure have been widely confirmed(Åkerblom et al., 2007; Zhang et al., 2015). Heavy metals may change the microbial community structure to a certain extent by destroying the cell structure, such as destroying chromosome replication and DNA synthesis, and then affecting nucleic acid metabolism(Zhang et al., 2015; Yan, 2020). Obviously, copper concentration is related to *Methylococcales* (phylum: *Proteobacteria*;Class:*Gammaproteobacteria*), This may be because Cu^{2+} plays a key role in the nature and expression level of methane monooxygenase, and therefore plays a key role in the bacterial structure of methane-oxidizing bacteria(Semrau et al., 2010; Sara et al., 2016). Through the above understanding, aiming at the negative correlation of heavy metals to microorganisms and changed in microbial species in this study, it was proved that heavy metals have certain influence on the microbial community structure.

4.4 Heavy metals promote certain functions of microorganisms

Heavy metals have an important influence on microbial function(Liu et al., 2018; Singh et al., 2019). ABC transporter and two-component regulatory system accounted for the largest proportion in metabolic function prediction. MA7 and MA8 points with the heaviest Pb and Cd pollution among all the points were selected for comparison with the control points. It was found that MA7 and MA8 of ABC transporters were greater than the control point DZ12 in the heavily polluted mining area, indicating that under the stress of high concentration of heavy metal ions, ABC transporters and the two-component regulatory system responded simultaneously and made corresponding adjustments to make a large number of ABC proteins expressed, which together eliminated the harmful substances such as heavy metals

from the body(Liu et al., 2018). The two-component regulatory system enables bacteria to sense, respond to, and adapt to a wide range of environments, stressors, and growth strips. Therefore, when the concentration of heavy metal ions in the outside world increases, the proportion of the corresponding bicomponent system of microorganisms increases, thus further adapting to the complex environment(Wu et al., 2019).

4.5 Heavy metals affected the expression of resistance gene

Through quantitative analysis of heavy metal resistance genes, it was found that the abundance of resistance genes was higher in the sites with heavy metal pollution. In order to further study the influence of heavy metal resistance genes, the correlation analysis between heavy metal resistance genes and environmental factors showed that *czcD* gene was significantly correlated with Zn, and significantly positively correlated with Cu. Therefore, when the content of zinc and copper in the environment increased, *czcD* abundance, the regulatory gene of resistance gene in soil, was significantly promoted. The relative expression of *czcD* gene abundance was greater in the MA than in the RA, indicating that under the stress of heavy metal ions at high concentrations, *czcD* resistance gene was abundantly expressed to regulate *czcA* and *czcB*, and the metal ions were transported through the metal transport mediated by the efflux pump. to the outside of the cell to complete the detoxification process of the soil. CzcD was involved in the regulation of the CZC system(Powell et al., 1994). It was a membrane-bound protein with at least four transmembrane-heliums and was a subfamily member of the CDF protein family(Veglió et al., 1997). The role of *czcD* gene was mainly to regulate the heavy metal resistance of *czcA* and *czcB*(Fisher, 1985). Therefore, *czcD* would increase under high concentration of Zn and Cu stress to resist environmental changes.

5 Conclusions

After a long period of mining activity, this rare earth mine has a special habitat and the environment around the mine has been heavily contaminated with heavy metals. The content of heavy metals in the soil around the mining area was increasing and the ecological risk will be higher and higher. It was found that high concentrations of heavy metals inhibited catalase and sucrase activity, but promote the activity of phosphatase. Under the condition of long-term heavy metal pollution, major microbial communities such as *Actinobacteria* and *Proteobacteria* have appeared in the MA, and only *Korarchaeota, LCP_89*, *RsaHF231* and other resistant strains have been found in the MA. It was found that heavy metal accumulation could increase ABC transporter proteins in microbial functions by high-throughput sequencing. *czcA, czcB*, *czcC*, and *czcD* genes belonging to ABC class of transporter proteins were selected for soil heavy metal resistance gene abundance survey, and it was found that *czcA*, *czcB*, *czcC*, and *czcD* had higher gene abundance in heavy metal contaminated sites. Therefore, the method of analyzing the microbial community to evaluate the toxicity of heavy metals is very promising. However, we need to account for the direct impact of the soil properties on microorganisms before this method can be applied widely.

Declarations

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Authors' contributions

Minjie Chen: Conceptualization, Methodology, Software, Writing- Original draft preparation, Writing - Review and Editing, Supervision; Xiaoru Jiang: Conceptualization, Methodology, Software, Investigation, Data curation, Writing-Original draft preparation, Visualization; Zhansheng Mi: Data Curation, Software, Formal analysis, Visualization; Yafei

Li: Supervision, Writing - Review and Editing; Zhe Wang: Supervision, Writing - Review and Editing; Xin Xu: Supervision, Writing - Review and Editing; Chunli Zheng: Conceptualization, Writing - Review and Editing, Supervision, Project administration, Funding acquisition.

Availability of data and materials

Data will be made available once the manuscript is accepted.

Declarations

Not applicable

Competing 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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Tables

Table 1 Soil properties at each site. Abbreviations: TN, total nitrogen; ORP, oxidation-reduction potential; EC, lectrical conductance,; CEC,cation exchange capacity; SOM, soil organic matter. Data are reported as means ± standard deviations (n = 3). Levels that are not linked by the same letter differ significantly.

Sample	pH	EC(μs/cm)	Moisture (%)	ORP(mV)	CEC	SOM (%)	TN□g/kg□
MA1	8.74±0.01ab	346.67±4.68g	13.32% ±0.90c	331.33±4.68a	2.42±0.10i	13.45% ±0.14bc	0.43±0.02i
MA2	8.71±0.09bc	113.33±3.33j	5.62% ±2.13e	328.33±2.34b	7.01±0.23c	10.01% ±0.25bc	0.63±0.02g
MA3	8.15±0.03ef	273.33±7.62h	4.61% ±0.97g	295.67±3.28g	3.92±0.16g	8.63% ±0.38bc	0.73±0.04ef
MA4	8.20±0.03e	230.00±3.78i	6.48% ±0.96e	298.33±4.15f	3.84±0.34h	6.07% ±0.68c	0.35±0.01j
MA5	7.93±0.01i	1236.67±9.76c	4.00% ±0.96h	282.67±5.18k	4.86±0.20f	9.31% ±0.62bc	1.45±0.06c
MA6	8.10±0.03fg	753.33±5.34e	5.37% ±0.77f	293.00±7.13h	2.19±0.18k	6.76% ±0.81c	0.72±0.09f
MA7	8.77±0.01a	4723.33±6.21b	18.93% ±3.11a	331.67±1.66a	5.43±0.45e	22.65% ±0.28a	0.95±0.05d
MA8	8.00±0.05h	360.00±5.90f	3.97% ±1.68h	287.00±2.62j	1.75±0.20l	6.23% ±0.32c	0.74±0.01e
MA9	8.66±0.03c	86.67±7.32k	15.36% ±0.70b	324.33±4.68c	6.54±0.24d	11.32% ±0.41bc	1.79±0.06b
RA10	8.28±0.02e	5983.33±8.98a	9.41% ±0.38d	304.00±6.12e	9.13±0.45b	12.30% ±0.65bc	0.51±0.01h
RA11	8.07±0.01g	843.33±13.47d	13.73% ±0.65c	291.00±3.64i	2.30±0.68j	16.11% ±0.08ab	0.62±0.04g
DZ12	8.51±0.02d	83.33±4.90l	6.09% ±0.56e	317.00±4.69d	20.67±0.38a	3.33% ±0.06a	1.87±0.05a

Due to technical limitations, Table 2 is only available as a download in the Supplemental Files section.

Figures

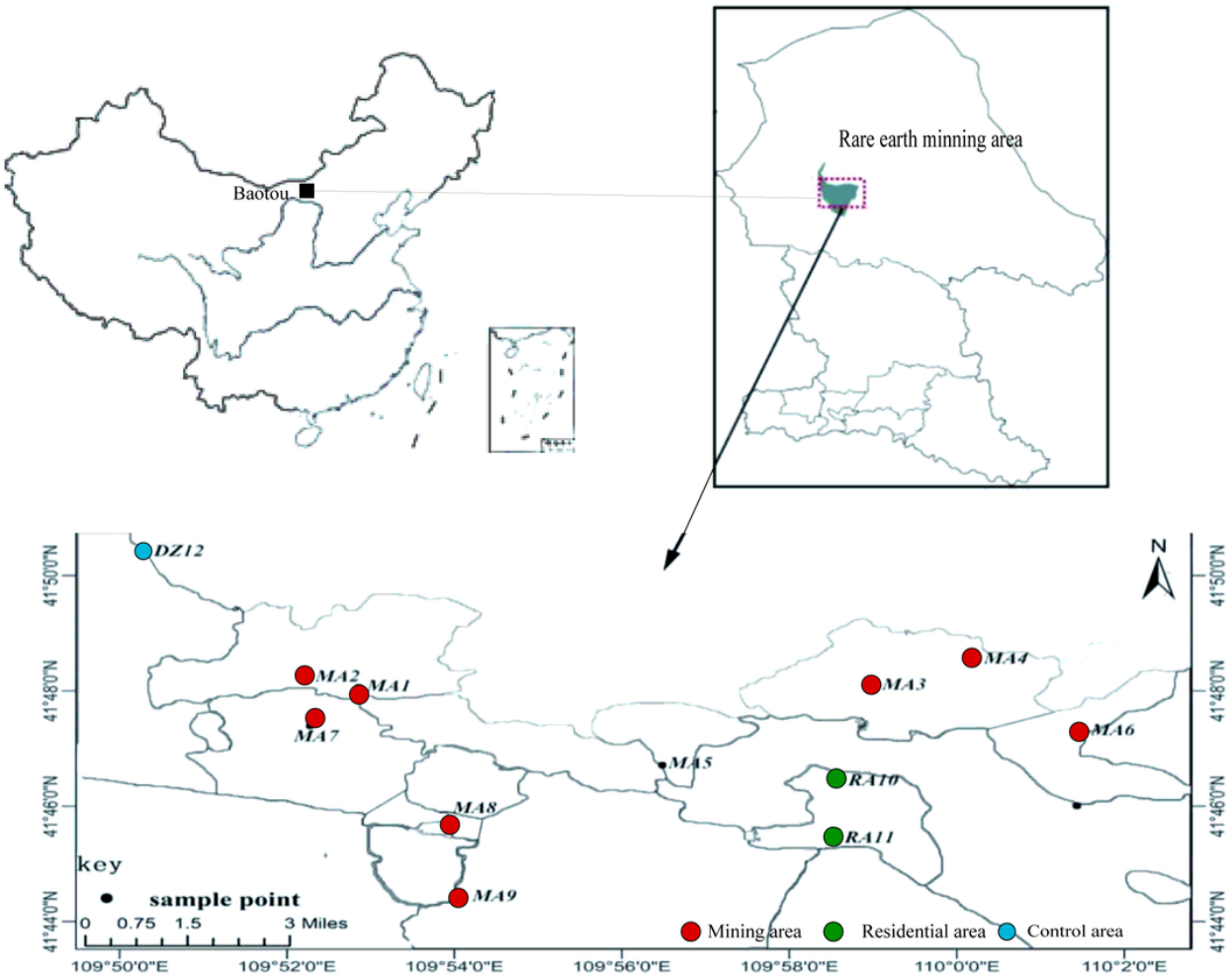

Figure 1

Map of the rare earth mining area and location of the sampling point.

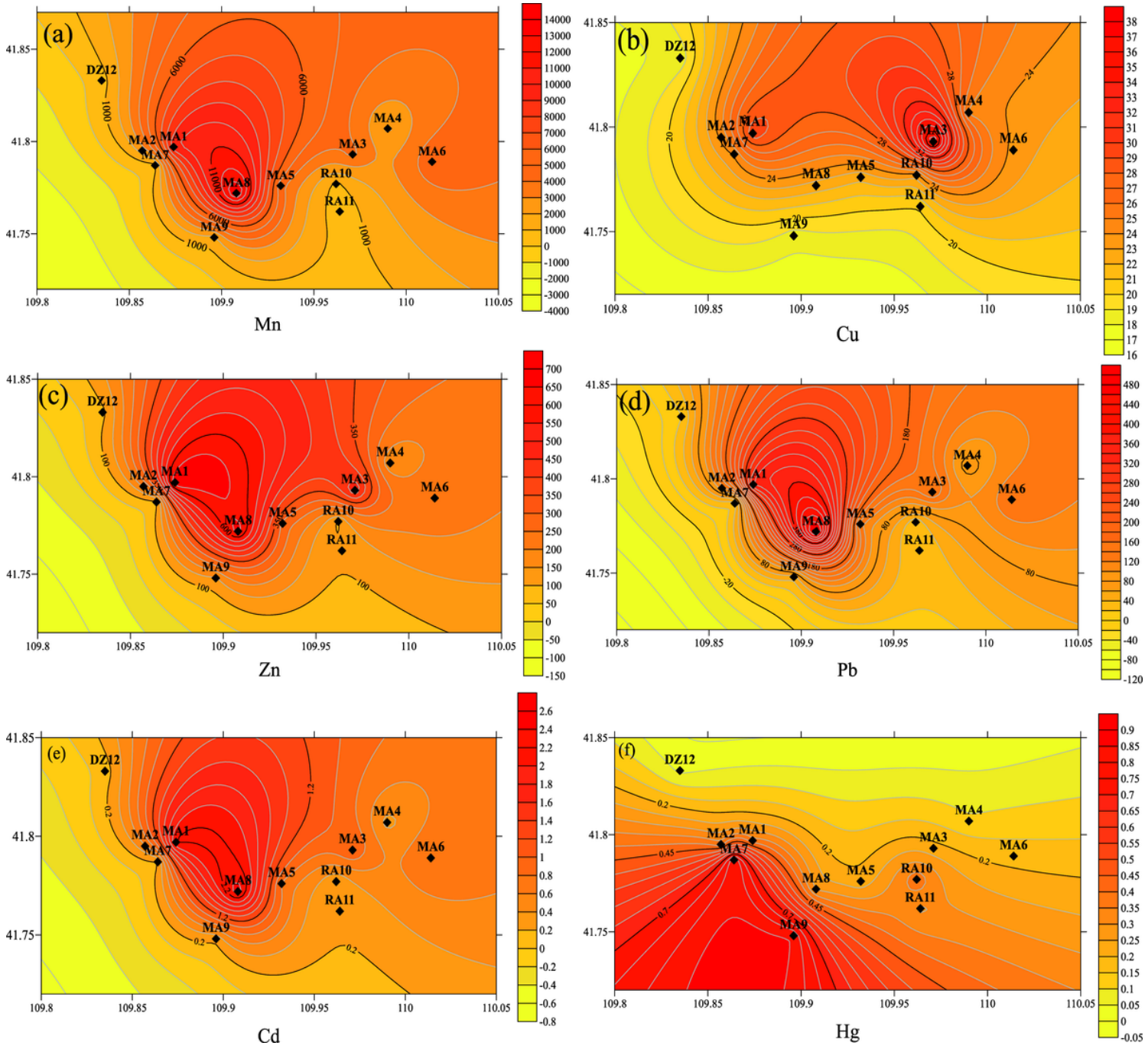

Figure 2

Change of heavy metal concentration in soil around rare earth tailings (Mg/kg)

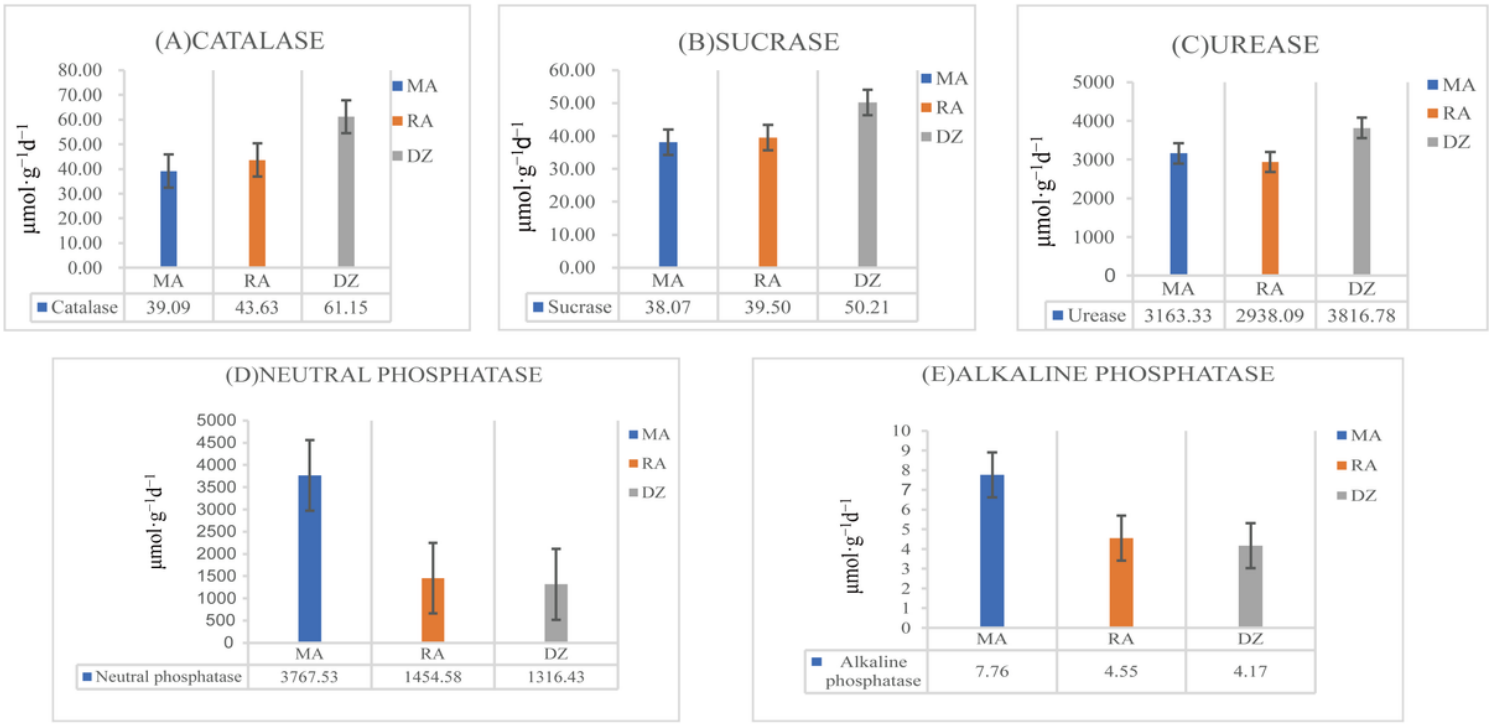

Figure 3

Soil enzyme activity under heavy metal-contaminated soil.

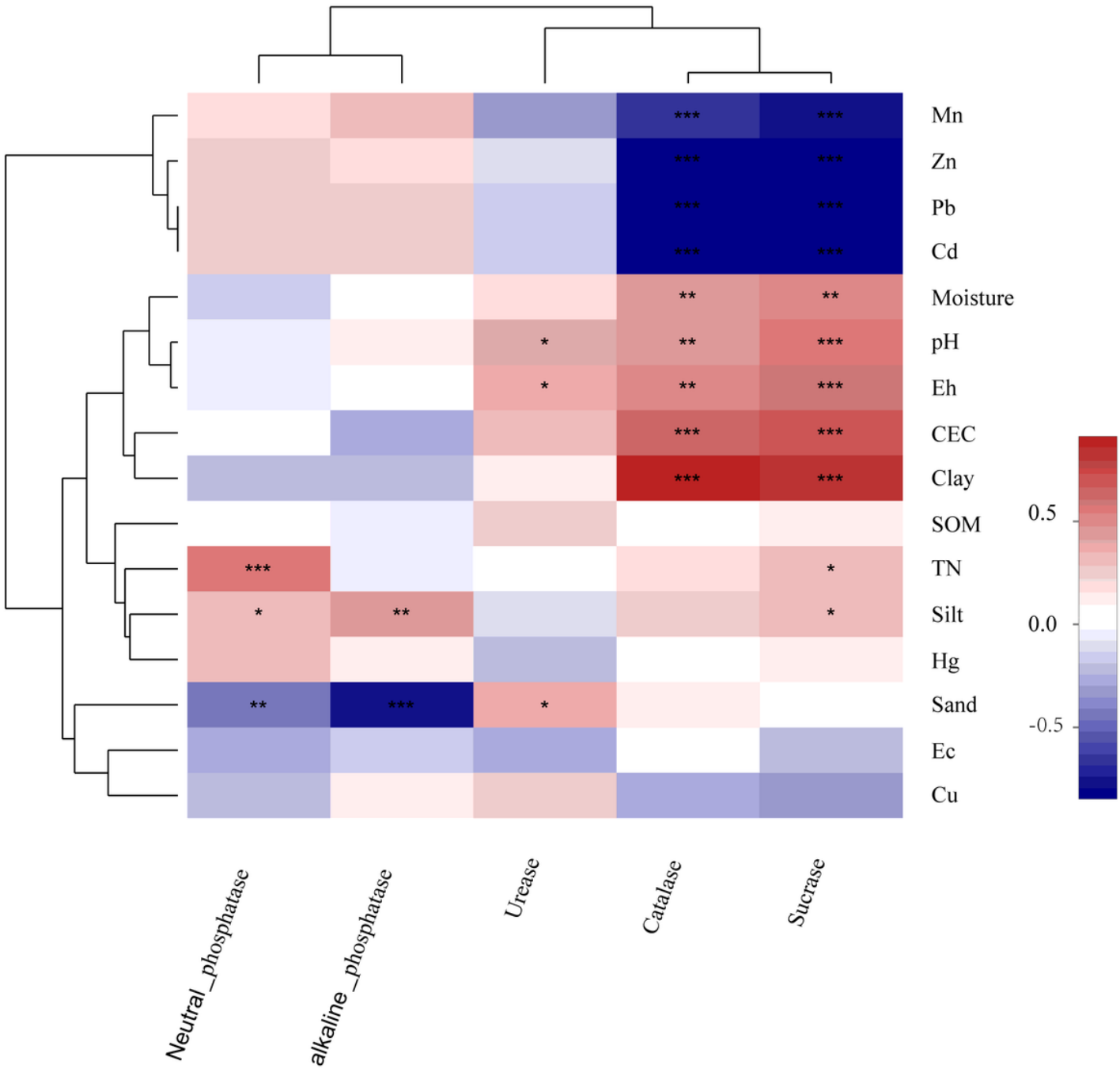

Figure 4

Heat map of the correlation between soil enzyme activities and properties in soil samples based on Pearson's correlation coefficients. Strong positive correlation (red); weak correlation (red); strong negative correlation (blue); *significant correlation ($P < 0.05$); **significant correlation ($P < 0.01$).

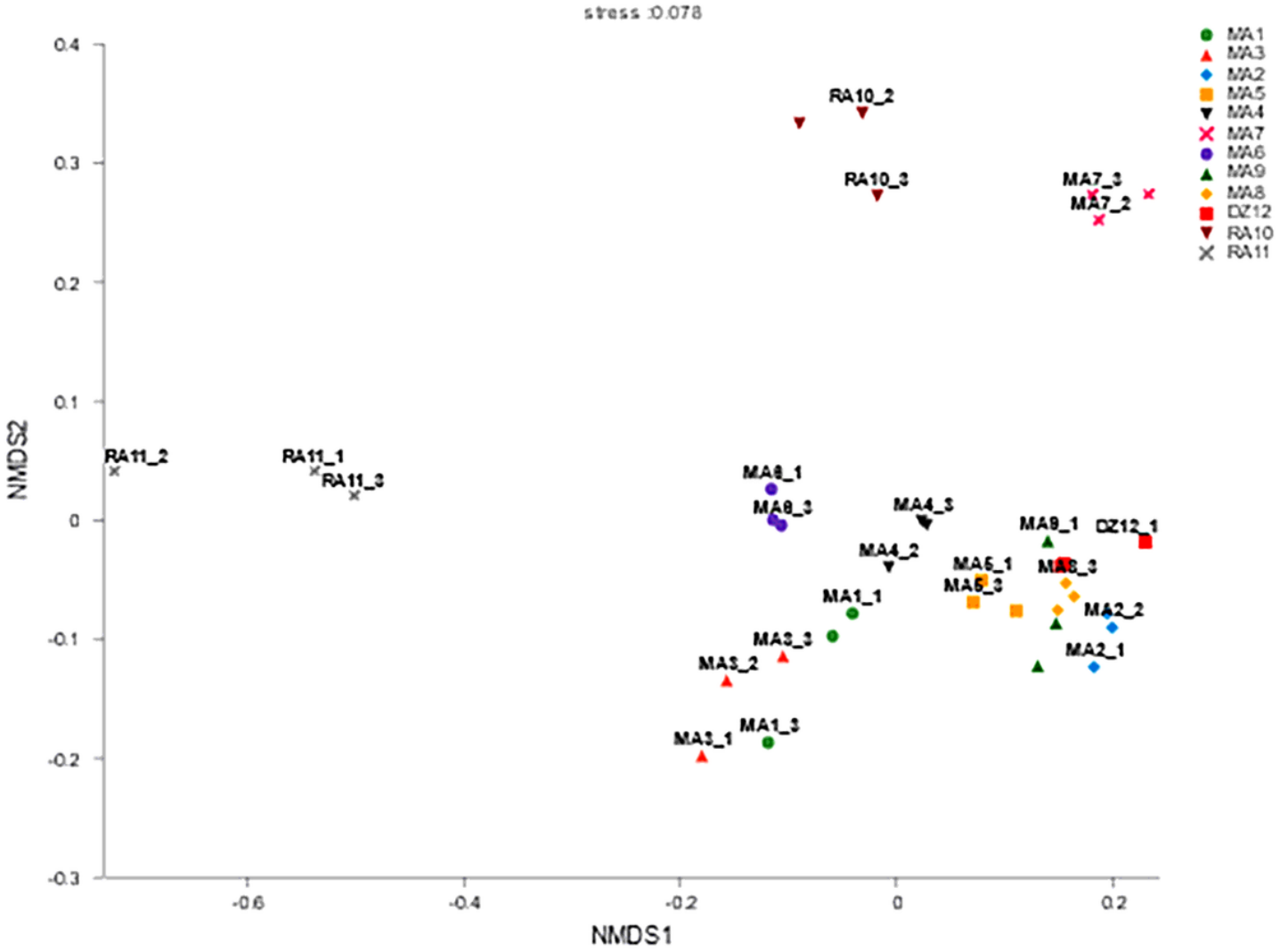

Figure 5

Non-metric multidimensional scale analysis of soil samples.

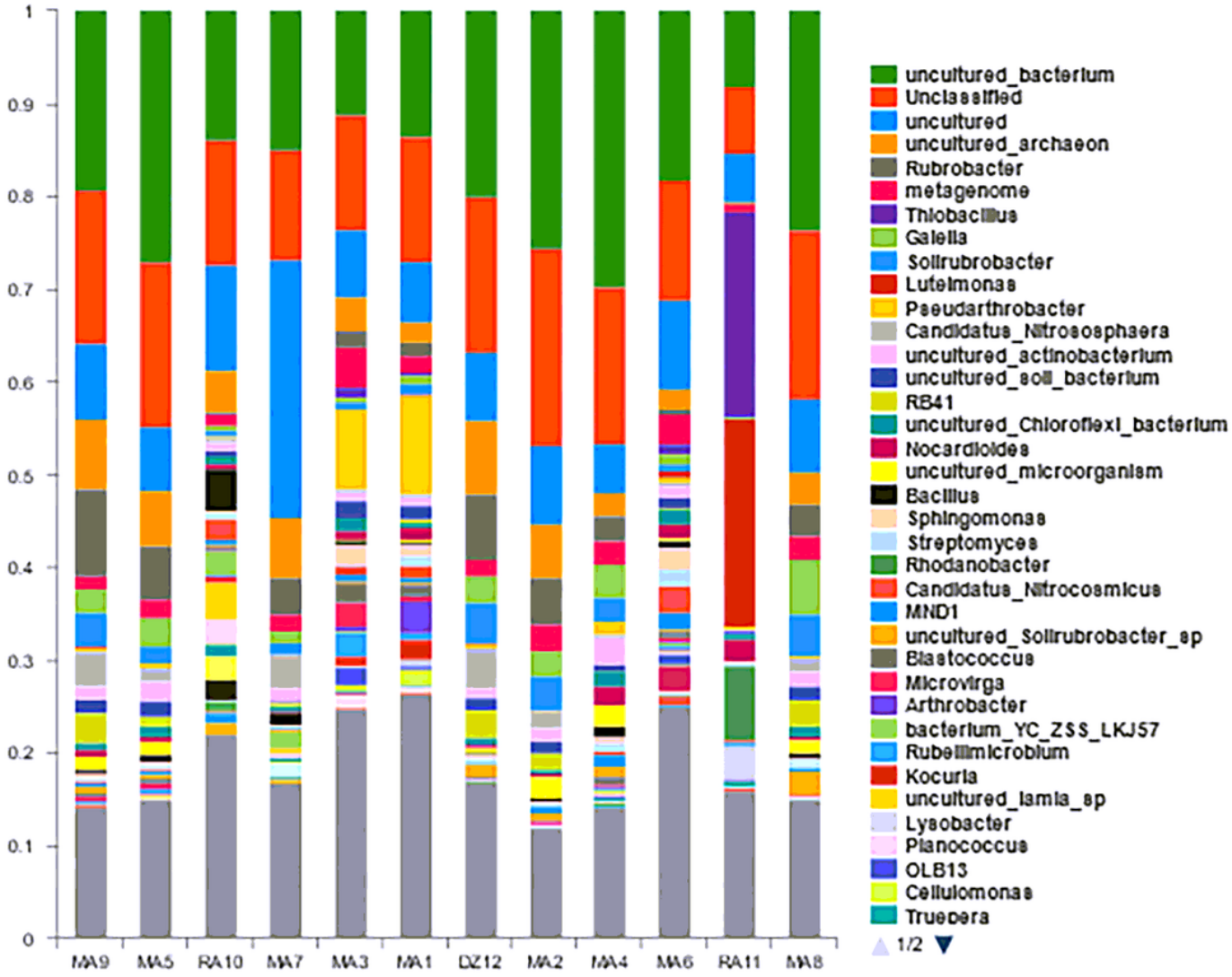

Figure 6

The relative abundance distribution bar chart of bacterial species (phylum level).

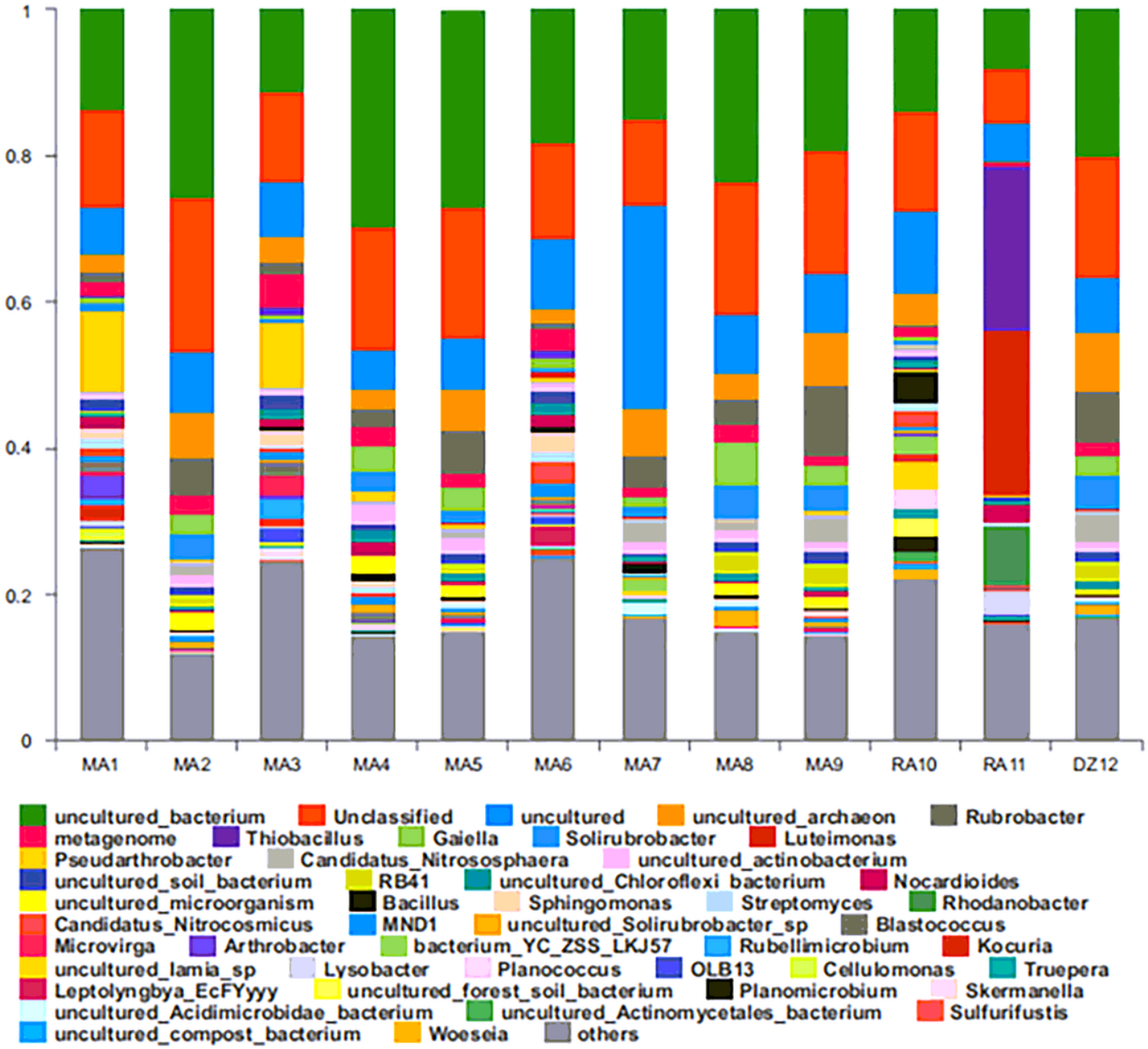

Figure 7

The relative abundance distribution bar chart of bacterial species (genus level).

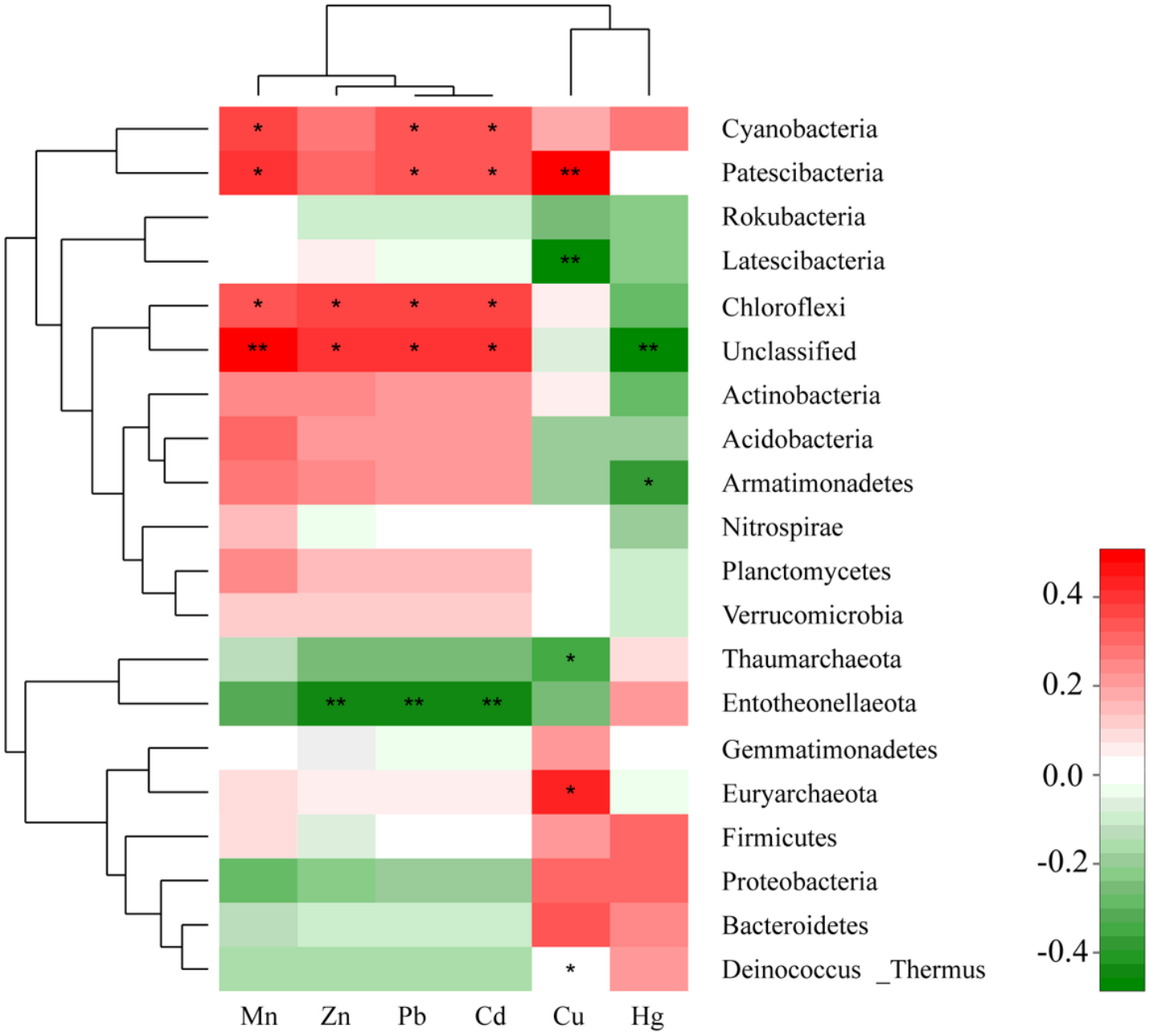

Figure 8

Spearman rank correlation to study the environmental factors and microbial species richess.

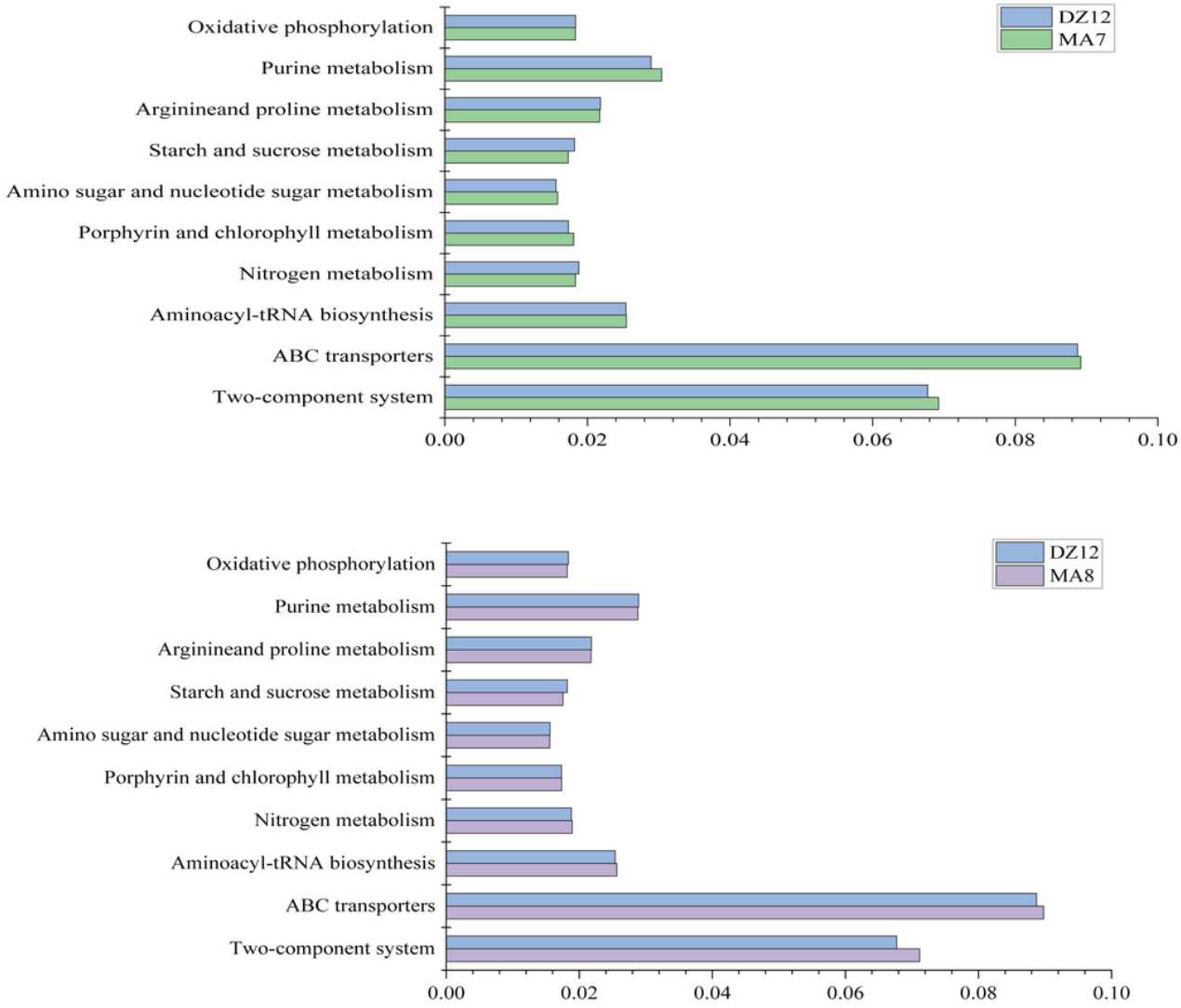

Figure 9

Functional prediction of microbial community in mining area.

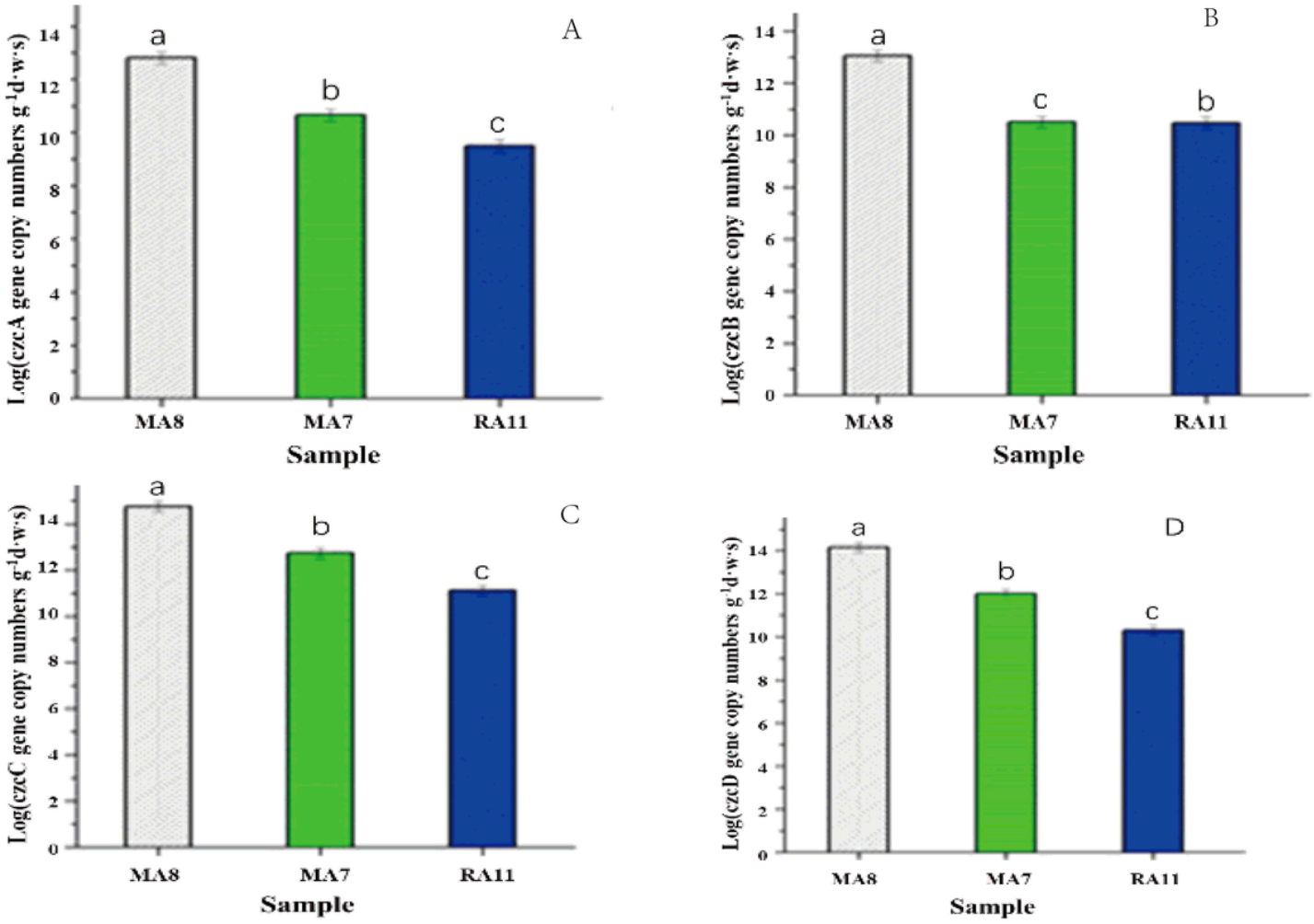

Figure 10

Abundance of heavy metal resistance genes.

Figure 11

Correlation analysis between environmental factors and heavy metal resistance genes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2Bacterialalphadiversityindex.docx
- GraphicalAbsaract.png