Effects of multi-heavy metal composite pollution on microorganisms around a lead-zinc mine in typical karst areas, southwest China

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ABSTRACT

Heavy metal pollution poses a serious hazard to the soil bacterial community. The purpose of this study is to understand the characteristics of soil heavy metal pollution in lead-zinc mines in karst areas and the response of Pb, Zn, Cd, and As-induced composite pollution to soil microorganisms. This paper selected soil samples from the lead-zinc mining area of Xiangrong Mining Co., Ltd., Puding County, Guizhou Province, China. The soil in the mining area is contaminated by multiple heavy metals such as Pb, Zn, Cd and As. The average levels of Pb, Zn, Cd and As in the Pb-Zn mining soil were 14.5, 7.8, 5.5 and 4.4 times higher than the soil background in this area, respectively. Bacterial community structures and functions were analyzed using 16 S rRNA high-throughput sequencing technology and the PICRUSt method. A total of 19 bacterial phyla, 34 classes and 76 orders were detected in the tested soil. At the phylum level, the *Proteobacteria* are the dominant flora of the soil in the tailings reservoir area of the lead-zinc mine, respectively GWK1 (49.64%), GWK2 (81.89%), GWK3 (95.16%); and for the surrounding farmland soil, the *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota*, *Chloroflexi* and *Firmicutes* are the most abundant in five bacterial groups. RDA analyses revealed that the heavy metal pollution of the lead-zinc mining area has a significant impact on the diversity of soil microorganisms. As the distance from the mining area increased, the heavy metal comprehensive pollution and potential risk value decreased, and the bacterial diversity increased. Additionally, various types of heavy metals have different effects on bacterial communities, and soil heavy metal content will also change the bacterial community structure. *Proteobacteria* positively related to Pb, Cd, and Zn, therefore, *Proteobacteria* were highly resistant to heavy metals. PICRUSt analysis suggested that heavy metals significantly affect the metabolic function of microorganisms. Microorganisms might generate resistance and enable themselves to survive by increasing the transport of metal ions and excreting metal ions. These results can be used as a basis for the microbial remediation of heavy metal-contaminated farmland in mining areas.

1. Introduction

Karst topography is a special landscape that forms on carbonate rocks including limestone, dolomite, or marble (Tang et al., 2019). Karst ecosystems, overlain by carbonate rocks (such as limestone or dolomite), account for about 15% of the world's land area (Chen et al., 2018). The karst region of southern China centered on Guizhou, amounts to 1.24 million km^2 and is the largest area of carbonate rocks and the strongest karst development among the three major continuous karst areas in the world (Song et al., 2022). Compared with that non-karst regions, karst ecosystems are extremely vulnerable due to the characteristics of a fragile rock-soil system and binary hydrological structure (Cheng et al., 2021). Carbonate rocks are unable to produce much soil for their extremely slow soil formation rate. This has resulted in the soils of the southwest China karst area being shallow (Guan et al., 2021). In addition, the carbonate rocks have developed broken karst surfaces through the long-term hydro-chemical process due to the highly soluble carbonate rocks, strong vertical and horizontal flow exchange of groundwater and surface water, and the steep landforms of southwest China (Bai et al., 2019; Guan et al., 2021). This makes karst ecosystems uniquely diverse in ecological and chemical properties, with relatively high soil pH (neutral or weakly alkaline soil), abundant organic matter,

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Ca and Mg content (Li et al., 2017b; Li et al., 2017a; Chen et al., 2018).

Additionally, more than half of China's metal mines and deposits are located in developed karst areas of South China (Qin et al., 2021). Studies have shown that soil heavy metal pollution in karst areas is characterized by multiple heavy metal complex pollution, with strong mobility, high potential ecological risks, and challenging governance (Wang et al., 2021b; Wang et al., 2021a). There are many types of heavy metal pollution elements. Most mines contain Cd, Pb, Zn, Hg, As, Cu and Ni, etc., and complex pollution occurs through complex chemical interactions between them. The most conspicuous regional geochemical anomaly element was Cd, with the largest excess area. The average content of cadmium in the soil parent material layer of Guizhou Province (1.24 mg·kg^{-1}) was much higher than the national average level (0.084 mg·kg^{-1}) (Zhang and Wang, 2020), which may be linked to the unique geochemistry of the regional soil formation process (Lu et al., 2018). Significant levels of heavy metal accumulation can change the soil composition, mineral recycling, and associated metabolic activities, and exert selective pressure on soil microbiota, leading to morphological and physiological changes in microbial population structures (Tseng et al., 2021).

As an important component of the soil ecosystem, soil microbes are widely involved in the energy flow and material cycle in ecosystems, and it is the primary driving force of geochemical cycles. Bacteria are the most abundant microbial group (Cui et al., 2018). They not only regulate the decomposition of soil animal and plant residues, soil organic matter and other harmful compounds, biochemical cycles, and the formation of soil structure. It is more sensitive to external disturbances and can predict the change process of soil environmental quality earlier (Luo et al., 2019). Increasingly, research has recently focused on soil bacteria inhabiting karst areas. Knáb et al. (2018) examined diversity in two distinct Hungarian karst areas and found that the difference in the composition of soil bacterial communities was related to geographic locations and soil types. Cheng et al. (2021) proved that the edaphic properties, weathering indices, such as Si concentration and the ratios of Ca/Al and Mg/Al significantly shaped bacterial communities in the karst area. Song et al. (2022) explored the effects of different restoration of cultivated pastures on soil properties and soil bacterial communities in karst ecosystems of southwest China. Li et al. (2021b); Li et al. (2021a) suggested that plant richness was the key factor determining soil bacteria community and soil microbial network complexity and stability. Xiao et al. (2022) found that diazotroph diversity and community composition significantly shifted in mature forests between karst and non-karst soils, with a higher diazotroph richness and Shannon index in karst mature forests than in non-karst soils. Previous studies report soil physicochemical properties (pH, soil organic matter (SOM), available potassium (AK), moisture content (MC), etc.) and heavy metals significantly affected the microbial community (Zhao et al., 2019; Deng et al., 2018; Risueño et al., 2020). Among them, heavy metals are essential factors affecting the abundance and structural diversity of microbial communities (Zhao et al., 2019). Li et al. (2017b); Li et al. (2017a) found that microbial community composition was significantly affected by long-term multiple heavy metal compound pollution, and multiple heavy metal elements were correlated with microbial community structure, including Cd, Ti, V, Cr, Co, Zn, As, Rb, Sr, Zr, Pb and Bi. Heavy metals differed in how they disturbed the microbial communities: As and Pb shifted the community composition and decreased microbial diversity; Cu reduced bacterial abundance in soil; Cd and Cr lowered the metabolic capabilities of bacteria (Shen et al., 2018). Li et al. (2020) found that elements Cr, Pb, and Zn showed uniformly negative associations with the relative abundance of bacteria *Nitrospirae* (including class *Nitrospira* and order *Nitrospirales*), *Bacteroidetes* (including class *Bucteroidiu*), and *Verracomicrobia*; Moreover, heavy metals affected predicted functions of microbial communities, including metabolic functions, genetic information processes, and functions related to the carbon cycle and the nitrogen cycle. In Xu's study (Xu et al., 2019), their results also supported that heavy metals led to the alteration of soil microbial community compositions and their activities, which consequently harmed soil microbial carbon. Although heavy metals could decrease the taxonomic species of microbes in the soil, their selection pressure can stimulate the growth of tolerant microbes, leading to changes in microbial community diversity (Feng et al., 2018; Liu et al., 2020). Under heavy metal pollution conditions, bacterial phyla such as *Proteobacteria* and *Bacteroidetes* have a strong tolerance to heavy metals (Lin et al., 2019; Yan et al., 2020a). However, there is less research that has been done on the changes in microbial community structure under heavy metal stress in karst mining areas, particularly, the mechanisms by which soil microbial communities in karst mines are resistant to heavy metals. In summary, karst soils are neutral-weakly alkaline soils, which naturally have a stronger sorption capacity for heavy metals such as Cd, Pb and Zn (except As), and accordingly have a buffer effect by reducing the risk of toxicity to crops. For then, karst soils are mostly non-zonal soils, and the microbial diversity or dominant populations are thought to differ from those of zonal soils, but the exact differences are not yet explicit. We hypothesize that the difference stems from the buffering effect of karst soils on heavy metals, which likewise reduces toxicity to the microbial community and heavy metal content caused changes in the composition of the microbial community. Therefore, exploring the response of bacterial communities and functions to heavy metal complex pollution in the soil in karst areas is of critical significance for guiding the safe use of soil in this area.

To this end, soil samples from the lead-zinc mining area of Xiangrong Mining Co., Ltd., Puding County, Guizhou Province, one of the largest heavy metal mining areas in the representative karst area were collected and subjected to high-throughput sequencing of the 16 S rRNA gene. The main purposes of the study were as follows: (1) to characterize the distribution characteristics of heavy metal contaminated in different types of pollution in mining areas; (2) to provide new insights into the abundance and diversity of soil microbial communities around mining areas; (3) to explore the relationship between the types and concentrations of heavy metals and bacterial diversity. This research will provide a new reference for the subsequent use of microorganisms to study heavy metal pollution in mining areas, and supply theoretical guidance for environmental remediation of soil pollution in mining areas.

2. Materials and methods

2.1. Study area

The karst region in southern China, centered on the Guizhou Plateau, is one of the largest karst areas in the world. It is also the most typical and complex karst area (Chen et al., 2021). For the active biological and chemical processes of the CO_2-H_2O-$CaCO_3$ system, the karst region is a normal ecologically vulnerable area constrained by the geological environment (Huang et al., 2013). The Pb-Zn mine is located in the emblematic karst area of Puding Country, Anshun City, Guizhou Province in southern China, with an east longitude of 105° 27' 49"– 105° 58' 51" and north latitude of 26° 26' 36"– 26° 31' 42". Puding County is a typical subtropical monsoon humid climate zone, with an average annual temperature of 15.1 °C and a mean annual precipitation of 1378.2 mm (Zhang et al., 2019). The terrain is high in the south and north, and low in the middle. The karst landform is very typical, and the rocky desertification is serious (Wang et al., 2020). Moreover, mineral resources and biological resources are rich in variety and widely distributed. The study area is placed in Xiangrong Mining Co., Jichangpo township, Puding County. It is a dressing plant with a daily processing capacity of 4200 tons, covering an area of 3.36 km^2, which has a high content of heavy metals and great resource potential.

2.2. Sample collection and heavy metal analysis

The soil samples were collected from the mining area of Xiangrong Mining Co., LTD., Puding County, Guizhou Province. Sampling locations

are illustrated in Fig. 1, including the area near the tailings reservoir (labeled GWK) and the soil of nearby farmland (labeled GWT), where the major crop is corn. Fig. 1 shows the layout of the sampling sites well: the sampling sites in the north are close to the tailings pond (GWT1–3), mainly due to dust pollution; the GWT7, GWT8, GWT9, and GWT14 in the east are far away from the tailings pond (Among them, GWT14 is the south side of the ditch, which is to confirm that the groundwater pollution cannot cross this ditch; it can also be used as the geological background value of the region); the southern GWT10–19, may be contaminated by groundwater. Each soil sample was a mixture of five surface soil samples (5–20 cm depth), homogenized sub-samples collected at the four corners and the center of an area of 1 m × 1 m. All the collected soil samples were packed in special sterile sealed clean bags. They were stored at low temperatures until they were sent back to the laboratory. The samples were divided into two groups. The first of the soil samples was air-dried and stored for heavy metal analysis. The other soil samples were stored at − 80 °C for DNA extraction and high-throughput microbial sequencing.

Using an inductively coupled plasma mass spectrometer (ICP-MS) (NexION 300D, PE, USA), soil concentrations of Pb, Zn, Cd and As were determined after extracting with $HCl-HNO_3-HClO_4-HF$ extraction using a method by (Alsabhan et al., 2021).

2.3. Heavy metal pollution evaluation

The soil pollution status of the tailings reservoir and the nearby farmland in the Pb-Zn mining area is evaluated with the single pollution index (PI) method and the Nemerow comprehensive pollution index (P_N) (Wang et al., 2021b; Wang et al., 2021a), which are computed using the following formulas:

$$PI = \frac{C_s^i}{C_n^i} \tag{1}$$

$$P_N = \sqrt{\frac{(P_{max})^2 + (P_{ave})^2}{2}} \tag{2}$$

Where PI is the single factor pollution index of heavy metal pollutant i;

C_s^i is the measured concentration of the pollutant, $mg \cdot kg^{-1}$; C_n^i is the evaluation standard of pollutant, $mg \cdot kg^{-1}$. P_N is multifactor of the pollutant i at the sampling point; P_{ave} is the average pollution index of pollutants in the soil; P_{max} is the single pollution maximum index of pollutants in the soil.

The single pollution index and the Nemerow comprehensive pollution index evaluation criteria are shown in Table S1.

2.4. DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Microbial DNA was extracted from soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16 S rRNA gene were amplified with primers 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in a triplicate 20.0 μL mixture containing 4.0 μL of 5 ×FastPfu Buffer, 2.0 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5.0 μM), 0.4 μL of FastPfu Polymerase and 10.0 ng of template DNA. The resulting PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar and paired-end sequenced (2 ×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.5. Processing of sequencing data

Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (a) The reads were truncated at any

Fig. 1. Sampling locations of the study area.

3

site receiving an average quality score < 20 over a 50 bp sliding window. (b) Sequences whose overlap was longer than10 bp were merged according to their overlap with a mismatch of no more than 2 bp. (c) Sequences of each sample were separated according to barcodes (exactly matching)and Primers (allowing 2 nucleotide mismatching), and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version7.1 http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16 S rRNA gene sequence was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16 S rRNA database using a confidence threshold of 70%.

3. Results and discussion

3.1. Characteristics of heavy metal in soil

Table 1 and Table 2 summarize the basic statistics related to the heavy metal concentrations in soil samples collected from the Pb-Zn mining zone. Multiple heavy metals contaminate the soil in the mining area such as Pb, Zn, Cd and As. The average levels of Pb, Zn, Cd and As in the soil of the Pb-Zn mine exceeded the background soil levels in Guizhou Province (Pb: $35.20 mg \cdot kg^{-1}$, Zn: $99.50 mg \cdot kg^{-1}$, Cd: $0.66 mg \cdot kg^{-1}$, As: $20.00 mg \cdot kg^{-1}$) (Zhou et al., 2021) and China Agricultural Land Soil Pollution Risk Control Standard (GB 15618–2018). The data illuminated that agricultural soils in the Pb-Zn mining area have suffered from different degrees of heavy metal contamination. All metal concentrations varied significantly between the samples. The highest concentration of Pb, Zn, Cd and As recorded in the sample was 1476.00, 5036.00, 7.53 and $225.00 mg \cdot kg^{-1}$, while the lowest concentration was only 24.80, 155.00, 0.38 and $12.90 mg \cdot kg^{-1}$, respectively. The average levels of Pb, Zn, Cd and As in the Pb-Zn mining soil were 14.5, 7.8, 5.5 and 4.4 times higher than the soil background in this area, respectively. Additionally, the tailings reservoir area (GWK1–3) and the surrounding farmland soil (GWT1–3) that are closer to the tailings reservoir generally have higher heavy metal content. As the distance from the tailings reservoir increases, the concentration of heavy metals generally decreases. It should be noted that the concentrations of Pb ($359.5 mg \cdot kg^{-1}$), Zn ($1214 mg \cdot kg^{-1}$), Cd ($2.08 mg \cdot kg^{-1}$) and As ($57.03 mg \cdot kg^{-1}$) in the soil of this study were higher than those in other lead-zinc mining areas. According to previous studies, the concentrations of Pb, Zn, Cd and As in a lead-zinc mine in southeastern China were 157.17 $mg \cdot kg^{-1}$, 90.89 $mg \cdot kg^{-1}$, 0.8 $mg \cdot kg^{-1}$ and 18.24 $mg \cdot kg^{-1}$,

respectively (Huang et al., 2018). The concentrations of Pb, Zn, Cd and As in the soil of the Dexing lead-zinc mine in China were 58.4 $mg \cdot kg^{-1}$, 128.9 $mg \cdot kg^{-1}$, 1.5 $mg \cdot kg^{-1}$ and 45.3 $mg \cdot kg^{-1}$, respectively (Hu et al., 2019). The concentrations of Pb, Zn, Cd and As in the soil of a lead-zinc mining area in Nigeria were 18.49 $mg \cdot kg^{-1}$, 48.07 $mg \cdot kg^{-1}$, 1.56 $mg \cdot kg^{-1}$ and 11.19 $mg \cdot kg^{-1}$, respectively (Obasi and Akudinobi, 2019). High levels of heavy metal content aggravate the negative impact on the nearby environment and organisms exposed to the soil of the mining area. Therefore, there is an urgent need to explore a sustainable solution to reduce the impact of heavy metals and restore the balance of the ecosystem in the region.

According to the coefficient of variation of various heavy metals, Cd is a medium variation, and the coefficients of variation of Pb, Zn and As are all > 90%, which is a high degree of variation. It indicates that the distribution of heavy metals Pb, Zn and As in the soil is quite different and the content is uneven and the human activities in mining areas have a significant impact on the content and distribution of heavy metals.

3.2. Pollution index evaluation of heavy metal content in the soil

Table S2 presents the single pollution index (PI) method and the Nemerow comprehensive pollution index (P_N). The pollution degree of different heavy metals at various sampling points is diverse. The average values of the single-factor pollution average index, from highest to lowest, are Cd, Zn, Pb, As. It can be observed that the soil in the Pb-Zn mining area is seriously polluted by Cd and should be protected and repaired. The pollution of Pb and Zn is relatively severe, and the contamination of As is relatively light, but it cannot be ignored. Zhang et al. (2022) conducted a large-scale investigation on the content of cadmium (Cd) in farmland soils in Guizhou Province. Combined with the distribution of metal mines, they found that the Cd hotspots were mainly located around the mining areas, especially around the lead-zinc mines.

The comprehensive pollution index P_N of the soil in the mining area is from 1.00 to 20.77. Of these, soil samples with moderate and severe pollution represented 33.3% and 61.9% of the total number of samples, respectively. Furthermore, the closer the tailings reservoir is, the greater the enrichment of heavy metals in the soil and the greater the degree of soil pollution. It also shows that the development of lead-zinc mines has worsened soil pollution near the mining area. The soil throughout the study area was severely polluted and crops on surrounding agricultural land were also damaged. It should be taken seriously and effective action should be taken immediately to monitor it.

3.3. Sequence data and bacterial taxonomic richness

The dilution curve is randomly selects a certain number of individuals from the sample, counts the number of species represented by these individuals, and constructs the curve based on the number of individuals and species. It can be used to compare the abundance of species in samples with different amounts of sequencing data, and it can also be used to show whether the amount of sequencing data of a sample is reasonable. The method of random sampling of sequences is used to construct a dilution curve based on the number of sequences drawn and the number of taxonomic levels that they can represent. When the curve tends to be flat, it indicates that the amount of sequencing data is reasonable, and more data will be only a small number of new species (or OTUs) produced. On the contrary, it indicates that more new species may be produced by continuing in sequence. Therefore, by drawing the dilution curve, the sequencing depth of the sample can be obtained. The dilution curve of bacteria in the soil of the lead-zinc mining area is given in Fig. S1. The curve tends to be flat, indicating that the amount of sequencing data is reasonable and that the depth of sequencing has a relatively minor impact on species diversity.

Diversity index analysis provides information on the abundance, coverage and diversity of species in the community. The Alpha diversity

Table 1
Soil heavy metal contents in lead-zinc mining area ($mg \cdot kg^{-1}$).

Soil type	Sample number	Pb	Zn	Cd	As
surrounding farmland soil	GWT1	187	982	1.38	218
	GWT2	663	2028	3.75	225
	GWT3	1476	5036	7.53	103
	GWT4	307	1547	2.66	79.1
	GWT5	271	1248	1.82	95
	GWT6	510	1058	1.01	78.8
	GWT7	141	635	1.20	15.8
	GWT8	154	592	1.01	16.4
	GWT9	105	411	1.00	26.4
	GWT10	446	1826	3.54	28
	GWT11	115	200	0.87	25.6
	GWT12	154	677	1.84	31.1
	GWT13	149	708	1.81	21.2
	GWT14	24.8	155	0.38	12.9
	GWT15	76	352	1.16	14.4
	GWT17	156	324	0.97	17.9
	GWT18	35.1	257	0.82	20.7
	GWT19	49.7	372	0.91	21.8
tailings soil	GWK1	1090	2542	3.57	48.8
	GWK2	663	2159	2.79	51.5
	GWK3	777	2403	3.63	46.3

Table 2

Statistical characteristic values of soil heavy metal content in lead-zinc mining areas.

elements	Minimum	maximum	mean	standard deviation	Coefficient of Variation	GB15618-2018
Pb	24.80	1476.00	359.50	383.75	1.07	120.00
Zn	155.00	5036.00	1214.86	1164.61	0.96	250.00
Cd	0.38	7.53	2.08	1.65	0.79	0.30
As	12.90	225.00	57.03	61.15	1.07	30.00

Note: The national standard GB15618–2018 (China Agricultural Land Soil Pollution Risk Control Standard).

index of the bacterial community in the tested soil is shown in Table S3. The coverage of the sample reached 97.74%– 99.47%, indicating that the sequencing results can better reflect the characteristics of microbial community diversity. Soil heavy metal pollution has a crucial impact on the abundance and diversity of microorganisms. The higher the pollution degree, the lower the abundance and diversity of microorganisms. From Table S3, it can be seen that the tailings reservoir area (GWK2, GWK3) has the most serious pollution. The Simpson index is the largest, the Shannon index is the smallest, and the diversity is the lowest; the ACE and Chao indexes that characterize species richness are relatively the lowest, indicating that the number of species in the sample is relatively small; on the contrary, the sampling points with relatively light pollution (such as GWT14, GWT15, GWT17). The Simpson index is relatively small, the Shannon index is relatively large, and the diversity is the highest; the ACE and Chao indexes are relatively high, indicating that there are more species in this sample.

3.4. Bacterial community diversity and composition

Fig. 2a illustrates the composition of the bacterial community in the soil tested with regard to phylum. At the phylum level, a total of 19 bacterial phyla were detected in the test soil. The most abundant bacterial group in the tailings pond area with serious heavy metal pollution (GWK1, GWK2, GWK3) is the *Proteobacteria*, which are respectively

GWK1 (49.64%), GWK2 (81.89%), GWK3 (95.16%). It can be seen that the *Proteobacteria* are the dominant flora of the soil in the tailings reservoir area of the lead-zinc mine; and for the surrounding farmland soil, the *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota*, *Chloroflexi* and *Firmicutes* are the most abundant in five bacterial groups, while *Planctomycetota*, *Nitrospirota*, *Verrucomicrobiota* and *Cyanobacteria* and other relative abundance is low, not more than 2%. Differences exist in the distribution of microbial communities in soils with various types of pollution. Previous studies show that the dominant floras are the normal phyla in soil: When Wu et al. (2018) studied the soil microbial composition of the Kangjiawan lead-zinc tailings in Hunan Province, they found that *Firmicutes*, *Proteobacteria*, and *Actinobacteriota* are the dominant bacterial groups in the soil in this area. Hur and Park (2019) researched the soils of mines in the three regions of Hwaseong, Daegu, and Bonghua in South Korea. They investigated the distribution of microorganisms and found that at the phylum level, the *Proteobacteria* and *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi, Bacteroides*, etc. are the dominant flora, and *Proteobacteria* accounted for the highest proportion. Vishnivetskaya et al. (2018) found that *Proteobacteria, Actinobacteria, Chloroflexi,* and *Acidobacteria* were dominant in the soil of mercury-contaminated rice fields in Guizhou. These dominant phyla also exist in the soil of other mining areas contaminated by metals, such as manganese mines (Xiang et al., 2020), antimony mines (Wang et al., 2018) and gold mines (Sonthiphand et al., 2021), indicating that these
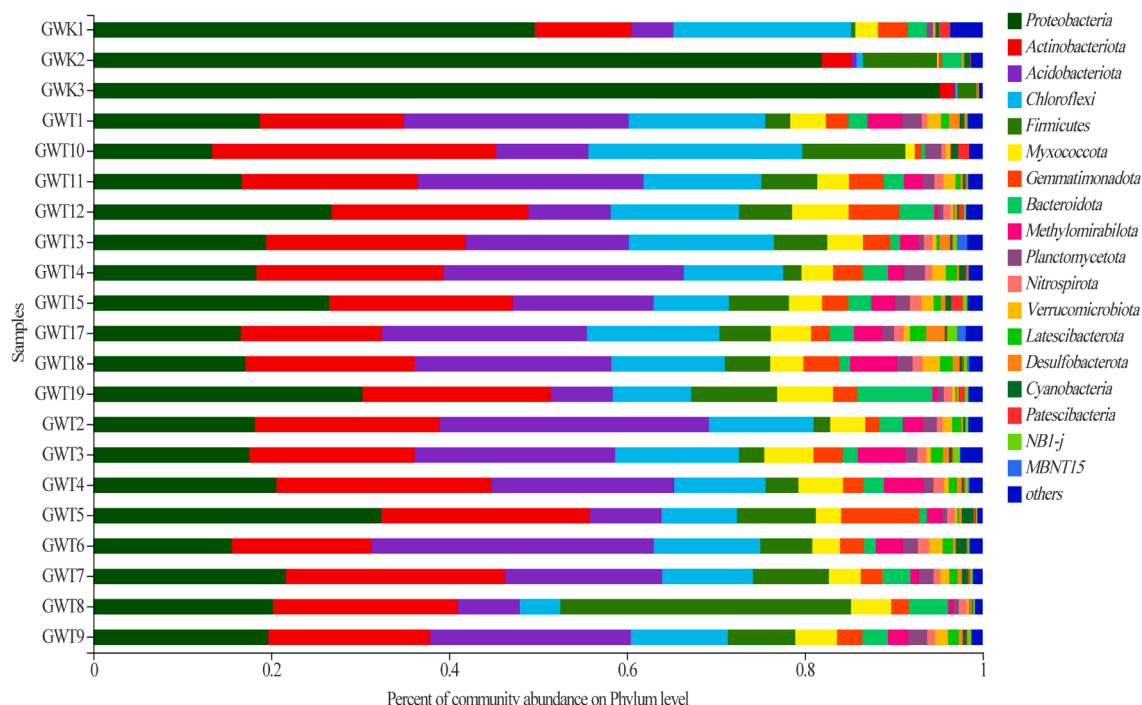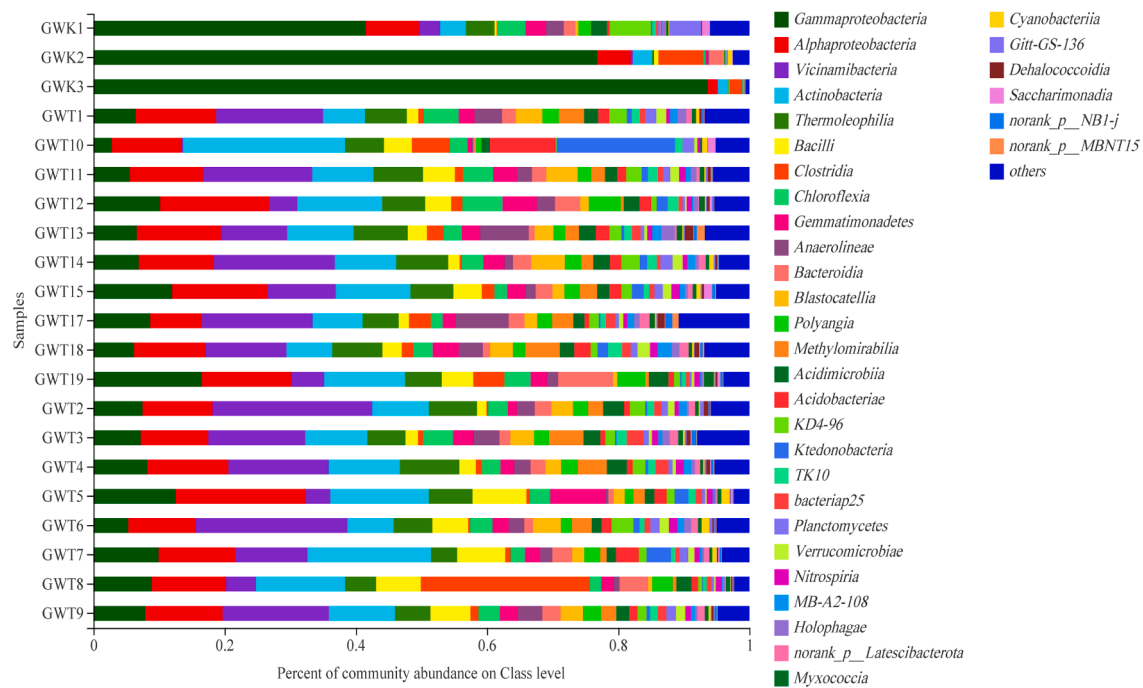
(a)

Fig. 2. Bacterial composition and abundance for each pollution level at phylum (a), class (b) and order levels(c).
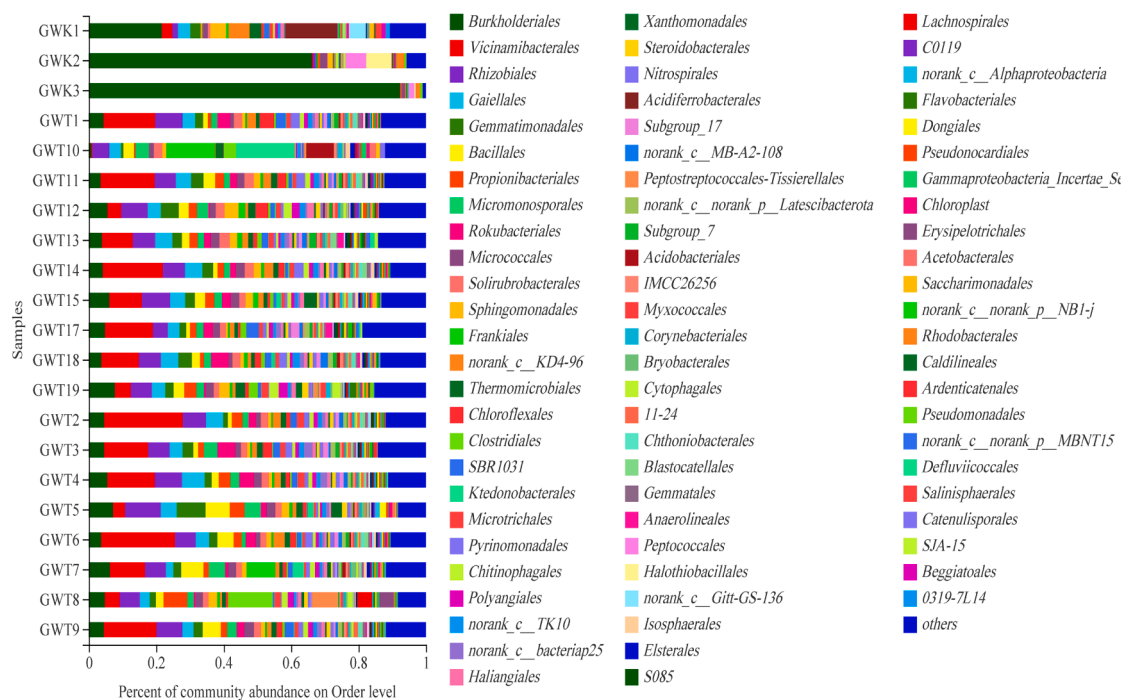
(b)

(c)

Fig. 2. (*continued*).

gates may be closely related to metal-contaminated soil. However, there were differences in relative abundance, with each level having its unique bacterial populations. Overall, the results of this study are similar to the results reported above but slightly different, indicating that although the regions are different, the microbial community composition of heavy metal contaminated soils in lead-zinc mining areas is similar, but the

bacterial distribution of different soil types responds differently to heavy metal pollution. In addition, in the dominant species (*Proteobacteria*) of the GWK1-K2-K3 microflora (phylum level), there is a gradient of changes. However, from a heavy metal content standpoint, the relationship between the change in heavy metal content and microorganisms is invisible. Judging from our actual investigation, the stacking time of K1-K2-K3 is getting shorter and shorter. Time may be a very important factor. Time offered the possibility of microflora reconstruction and self-repair.

Proteobacteria, one of the most abundant phyla in the soils investigated. The main function of *Proteobacteria* is to decompose organic matter and promote plant growth. Most of the bacteria in *Proteobacteria* are oligotrophic bacteria, which have the ability to absorb nutrients from the atmosphere and can use carbon and nitrogen sources in the atmosphere to maintain normal growth. The phylum *Proteobacteria* was found to be abundant in many high heavy metal content environments, such as sediments (Zhang et al., 2018), groundwater (Zou et al., 2021) and heavy metal polluted soils (Tseng et al., 2021). *Proteobacteria* were also shown to be the predominant phylum in antimony (Sb) tailings piles in Guizhou Province, southwest China, accounting for 60.5% of the total effective sequences (24.3–83.8% per sample) (Xiao et al., 2016). In the present study, within the *Proteobacteria*, *Gammaproteobacteria* were the most abundant class in all samples, followed by *Alphaproteobacteria* in the tailings pond area (Fig. 2b). For the surrounding farming soil, *Alphaproteobacteria* generally have a higher abundance. This result is consistent with Radeva et al. (2013), who also found that the abundance of *Alphaproteobacteria* in contaminated soil was lower than that of *Gammaproteobacteria*. The dominance of *Gammaproteobacteria* may be due to the capacity of some of its members to form biofilms on stone surfaces, providing potential resistance of bacterial colonies to the toxic effects of heavy metal (Radeva et al., 2013).

At the order level, 76 orders were identified in the soils. The bacterial community structures showed significant differences among the soils (Fig. 2c). Changes in dominant communities may indicate differences in soil function in the area. In the tailings pond area, the most dominant order was *Burkholderiales*, which are 21.64% (GWK1), 66.17% (GWK2), 92.34% (GWK3), respectively. For the surrounding farmland soil, *Vicinamibacterales* and *Rhizobiales* are the most abundant. *Burkholderiales* belong to *Proteobacteria*, which are capable of degrading diverse organic carbons including starch, cellulose, gelatin, chitin, and humic acids (Guo et al., 2015). *Rhizobiales* were reported to degrade diverse organic matter and are typical symbiotic rhizobia, some bacteria in the order *Rhizobia* have nitrogen fixation function (Guo et al., 2015). Notable, *Micromonosporales* and *Bacillales* were only detected in farmland soils. *Micromonosporales* belong to *Actinobacteriota*. Because of its particular morphology and cellular structure and ability to produce hyphae and spores. *Actinobacteriota* sporogenes decompose organic matter to produce a large number of hydrolases and secondary metabolites, which not only have essential functions such as improving soil structure and promoting material circulation but also have a more significant impact on soil fertility and activity (Tian and Zhang, 2017). *Bacillales* belong to *Firmicutes*, many *Firmicutes* can produce spores, which are resistant to dehydration and extreme environments and are reported to have antagonistic activity against soilborne pathogenic fungi (Li et al., 2014). That may be one of the major reasons for the difference in soil function between the tailings and the surrounding agricultural land. We also found similarities at the phylum and class levels in the same type of soil samples, possibly underlying differences at the order level, such as GWT10 had a very little abundance of *Burkholderiales* and *Vicinamibacterales* compared to other farmland soil samples whereas the abundance of *Frankiales* and *Ktedonobacterales* higher. *Frankiales* belong to *Actinomycetes* that can symbiotically nodulate and fix nitrogen with non-leguminous woody plants. It has extremely high nitrogen-fixing efficiency, can promote the growth of plant roots, improve the absorption of mineral elements by plants, and enhance the adaptation of plants to drought and cold conditions (Ghodhbane-Gtari et al., 2013). Besides,

the abundance of *Acidobacteriales* in GWT10 was higher than that in other farmland soil samples. The function of *Acidobacteriales* is less studied, but it plays an important role in the ecosystem, helping to convert the organic matter in the humus into nutrients in the soil, actively interacting with plants, and playing the role of bacteria that promote plant growth, and Produces exopolysaccharides, supports bacterial adhesion to root surfaces, etc (Kielak et al., 2016). *Sphingomonadales* are prevalent in various soil samples except for GWK3, which may be related to the characteristics of *Sphingomonadales*. Some bacteria in *Sphingomonadales* can improve the cold tolerance, heat tolerance and drought tolerance of plants due to their ability to produce heat shock proteins. Some bacteria in *Sphingomonadales* can also participate in iron metabolism and a small amount of potassium and sulfur metabolism, affecting the physiological metabolism of plants and enhancing plant disease resistance and insect resistance (Patel et al., 2004).

The Heatmap chart uses color gradients to characterize the size of the data in a two-dimensional matrix or table and presents community species composition information. The abscissa is the sample names, and the ordinate is the species names. The similarity and difference in the community composition of different samples at each classification level are reflected by the color change and similarity. The right side of the figure is the value represented by the color gradient, as shown in Fig. 3.

It is found from Fig. 3 that the dominant bacterial phyla of tailings ponds (GWK1, GWK2, GWK3) and surrounding farmland (GWT1~19) are quite different, and they rarely cross. Compared with tailings ponds, surrounding farmland soil samples have advantages. There are more bacteria phyla, including *Proteobacteria, Chloroflexi, Actinobacteriota, Acidobacteriota,* etc., a total of 9 bacteria. Bacteria adapt to high heavy metal pollution by changing their community abundance and structure (Deng et al., 2015; Li et al., 2020). It can be seen from the species similarity (horizontal clustering) of each sample point that the soil samples in the heavily polluted tailings ponds (GWK2, GWK3) are the most different from other samples, and they are clustered separately, indicating that the soil is polluted by heavy metals. Later, the difference in the distribution of bacterial communities in the soil increased. The pollution of GWK2 and GWK3 samples was the most serious, and the dominant bacteria phyla were also the least. Sampling sites with less pollution have more dominant bacteria phyla, the bacterial population is relatively evenly distributed, and the bacterial community is more stable.

3.5. Influence of heavy metal pollution on bacterial communities distribution and ecological function

Redundant analysis (RDA) was performed on the different heavy metal content (Pb, Zn, Cd, As) of the tested soil and the relative abundance at the bacterial phylum level, and the results are shown in Fig. 4. The results of RDA further demonstrated that the heavy metals Pb, Zn, Cd and As played an important role in the establishment of the bacterial community. It is found that different types of heavy metals also have different effects on bacterial communities: Pb, Cd, and Zn are positively correlated with *Proteobacteria*, and with *Actinobacteriota, Acidobacteriota,* and *Chloroflexi* and other microbial groups are negatively correlated; while As is positively correlated with *Acidobacteriota* and *Chloroflexi*, and negatively correlated with *Firmicutes* and *Proteobacteria*. *Proteobacteria* had significant tolerance to Zn, Pb, and Cd, a result similar to that found by Zhao et al. (2020). *Acidobacteriota* and *Chloroflexi* had significant tolerance to As. In addition, soil's heavy metal content will also change the structure of the bacterial community. There is a negative correlation between *Myxococcota* and heavy metal content, indicating that this microbial group is more sensitive to heavy metal pollution and has poor resistance to heavy metals. The content of heavy metals is positively correlated, indicating that *Proteobacteria* can survive well in heavily polluted soil. It has also been found in previous studies that *Proteobacteria* is also a dominant phylum in many mine soils and *Proteobacteria* species are highly resistant to some heavy metals (Liu et al.,
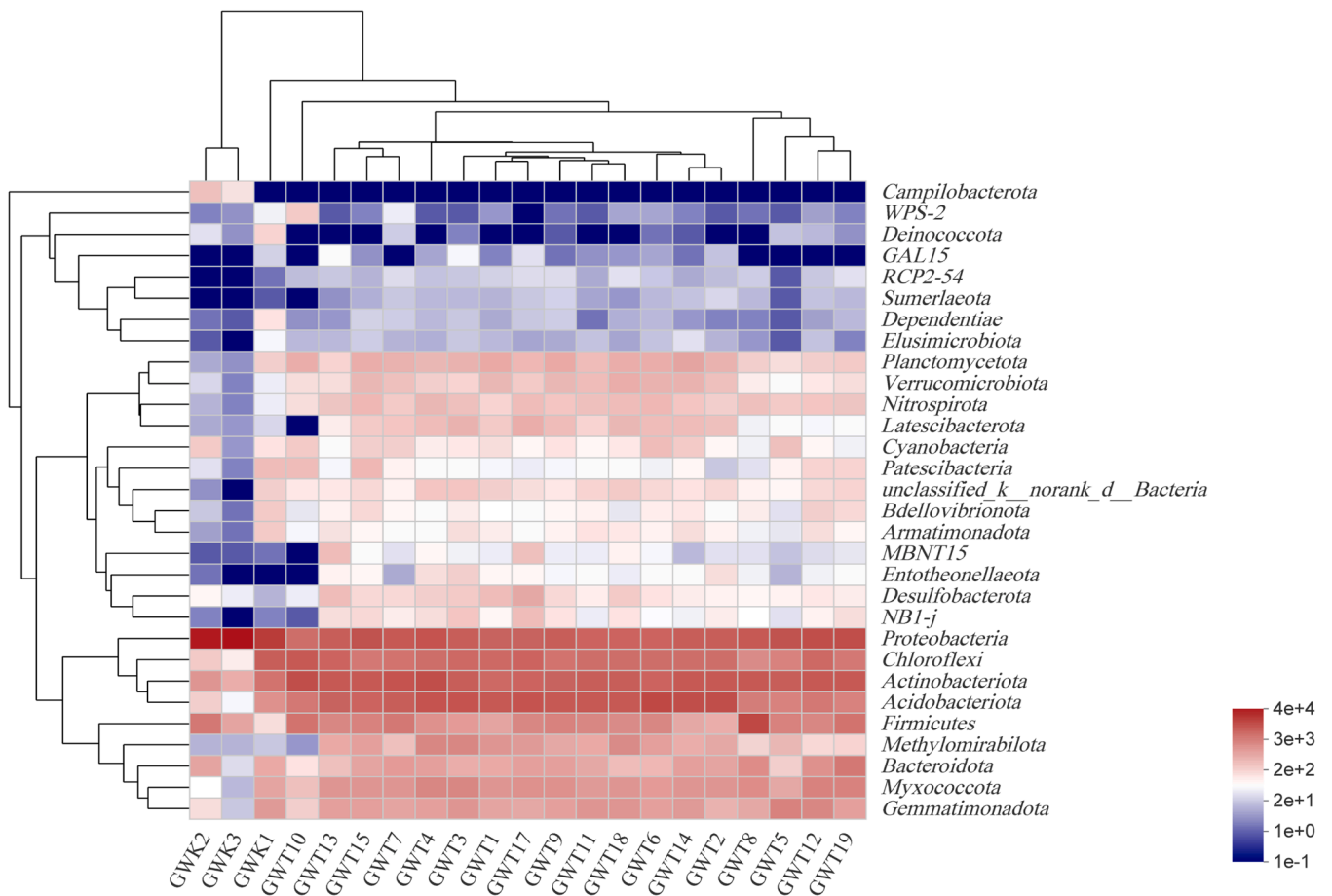
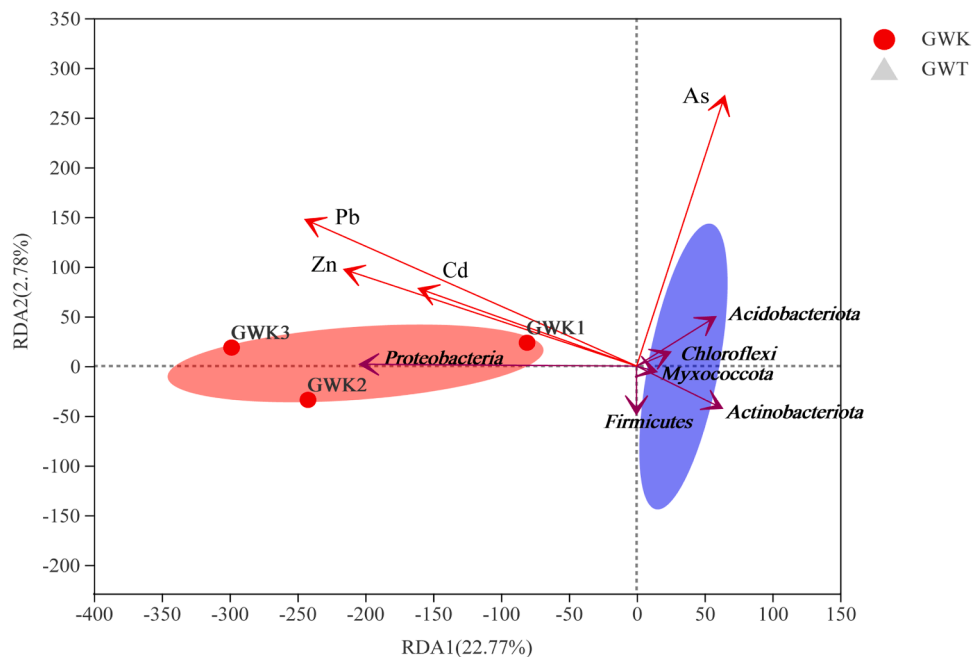**Fig. 3.** Community Heatmap analysis on phylum level.

Fig. 4. Redundancy analysis (RDA) of soil heavy metals and bacterial communities.

2021; Yan et al., 2020b). The dominant bacteria found in the polluted mine soil may be due to their possessing tolerant gene groups, which are less sensitive to these heavy metals and related to the extensive degradation metabolic properties of *Proteobacteria* and its ability to inhabit a wide range of habitats. Therefore, *Proteobacteria* may live in extreme environments and are considered capable of repairing

broad-spectrum heavy metal pollution (Singh et al., 2014; Zhao et al., 2019).

The method of PICRUSt was used to predict the potential functions of microbial communities in soil (Fig. 5). It can be seen that in soils contaminated by heavy metals, the functions of the top 5 genes with the largest proportions are RNA processing and modification, Amino acid transport and metabolism, General function prediction only, Function unknown and Signal transduction mechanisms. Soils contaminated by heavy metals have reduced microbial carbohydrate transport and metabolism and cell motility; On the contrary, the ability of microorganisms to transport and metabolize inorganic ions has enhanced, and intracellular trafficking, secretion, vesicular transport and energy production and conversion have also been up-regulated. It is further indicated that microorganisms might generate resistance and enable themselves to survive by increasing the transport of metal ions and excreting metal ions (Li et al., 2020). Specifically, heavy metal resistance in bacteria involves four mechanisms: (i) Extracellular sequestration, where the cell wall of microorganisms is a very important defense system against the toxicity of heavy metals. There are numerous cationic and anionic functional groups on the cell wall, such as hydroxyl, amine, carboxyl and phosphate groups. Generally speaking, heavy metal ions can be adsorbed on the polysaccharide slime layers of bacteria via functional groups, which could avoid heavy metal ions from entrancing into the intracellular environment by extracellular metal sequestration (Martins et al., 2008; Yin et al., 2019). (ii) Intracellular sequestration. Once heavy metal ions have crossed the cell wall and entered the microorganisms, intracellular traps within the cytoplasm can sequestrate these heavy metal ions and prevent them from reaching toxic levels. Thus, sensitive cellular components can be protected from exposure to heavy metal ions. Many microorganisms can transform heavy metal ions with the help of sulfides, cytosolic polyphosphates and cysteine-rich proteins (Yin et al., 2019; Prabhakaran et al., 2016). (iii) Transporting heavy metal ions away from the intracellular environment is another process to defend against heavy metal stress, which can efficiently regulate intracellular concentrations of heavy metal ions with efflux systems (Silver, 2003; Yin et al., 2019). (iv) Enzymatic detoxification. The redox state change of heavy metal ions through reduction or oxidation reactions can efficiently decrease their toxicity (Yin et al., 2019; Prabhakaran et al., 2016). Detoxification enzymes, which are also controlled by special resistance genes of microorganisms, can regulate this defense pathway. The defense mechanism may first increase and then decrease with the degree of heavy metal pollution, such as GWT14, GWT10, and GWK2 are 2.14%, 2.32%, and 1.44%, respectively. This may be due to heavy metals affecting the physiological changes of microorganisms, such as the temporary slowdown or cessation of cell division cycles, changes in cell morphology, or the development of resistance to stress factors. When the unfavorable stimulus is prolonged or strengthened, and the components of the cell structure may be damaged, the activation of the defense mechanism becomes impossible, resulting in the reduction of defense mechanism genes (Prabhakaran et al., 2016). Severe heavy metal stress can cause cell death. Metal-resistant microorganisms can serve as bioremediation agents, supporting the application of bioremediation.

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4. Conclusions

This field study demonstrated that soil from lead and zinc extraction in the karstic region poses a significant potential ecological risk. On the whole, various heavy metals polluted the soil, of which the pollution of Pb, Zn and Cd was the most serious.

The heavy metal pollution of the lead-zinc mining area has a significant impact on the diversity of soil microorganisms. As the distance from the mine zone increased, heavy metal pollution and the value of the potential risk decreased, and bacterial diversity increased. The statistical analysis further revealed significant differences in microbial communities across soil types, *Proteobacteria* are the dominant flora of the soil in the tailings reservoir area of the lead-zinc mine, and for the surrounding farmland soil, the *Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi* and *Firmicutes* are the most abundant in five bacterial groups.
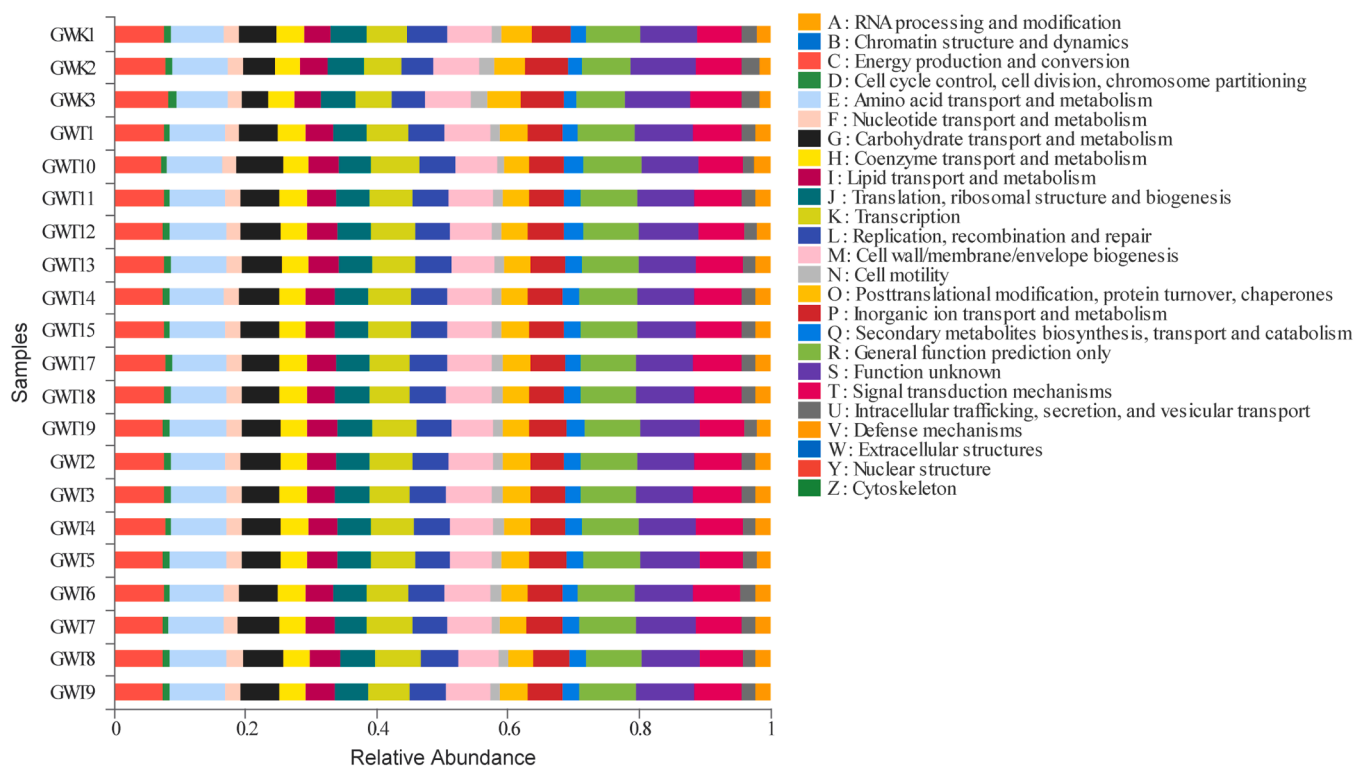

Fig. 5. Metabolic functions of the bacterial communities prediction.

Then, the study found that various types of heavy metals have different effects on bacterial communities, and soil heavy metal content will also change the bacterial community structure. Soils with a slight degree of heavy metal contamination have more dominant bacterial phyla, more evenly distributed and more stable bacterial populations. In addition, heavy metals significantly affect the metabolic function of microorganisms. Microorganisms might generate resistance and enable themselves to survive by increasing the transport of metal ions and excreting metal ions. It is worth noting that highly tolerant bacteria were found under heavy metal contamination, *Proteobacteria*, which are the most dominant bacteria in this region.

The research results are of great significance for understanding the specific functions and ecological effects of soil microorganisms in lead-zinc mining areas, and provide theoretical support for the restoration of soil heavy metal pollution in mining areas. In the future, the response of other physicochemical properties of soils in the region to microbial diversity should be explored in conjunction with relevant predictive models, and the potential use of tolerant microorganisms should be explored to give full play to their unique functions and provide strains and theoretical basis for the bioremediation of heavy metals in karst areas.

CRediT authorship contribution statement

Yingying Zuo: Formal analysis, Investigation, Software, Writing – original draft. **Ying Li**: Investigation, Validation. **Hu Chen**: Supervision, Project administration. **Gang Ran**: Investigation. **Xiuming Liu**: Investigation, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xiuming Liu reports financial support was provided by the Strategic Priority Research Program of the Chinese Academy of Science.

Data Availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115190.

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