



Analysis of heavy metal content and microbial characteristics in the pioneer plant soil system of typical manganese tailing ponds in Guangxi

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Abstract

Aims Ecological restoration of mine tailing ponds plays a crucial role in managing heavy metal pollution and enhancing biodiversity. This study aimed to investigate the ecological restoration potential of pioneer plants under high manganese metal pollution, as well as the microbial community composition and functional characteristics of their rhizosphere soil.

Methods Sampling and analysis were conducted on seven plants of *Neyraudia reynaudiana* (LL), *Pueraria montana* (GT), *Bidens pilosa* (GZC), *Buddleja*

asiatica (BBF), *Pogonatherum crinitum* (JSC), *Crotalaria albida* (XND), *Thysanolaena maxima* (ZYL) and their rhizosphere soil in Daxin manganese tailing ponds of Guangxi Province.

Results Pioneer plants improved the physicochemical properties of rhizosphere soil, and increase soil enzyme activity and the abundance and diversity of microbial communities, and reduce soil available metal content. The BTF values of GZC, GT, BBF, and XND are close to or greater than 1 for Mn, Cd and Cu, indicating their strong ability to absorb and transfer heavy metals from underground to above-ground. *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* are the bacterial communities with the dominant abundance, showing a significant positive correlation with the BTF value and promoting the absorption of heavy metals by plants. FAPROTAX function prediction revealed that chemoheterotrophy, aerobic chemoheterotrophy, and chloroplasts were the main metabolic modes.

Conclusions GZC, GT, BBF, and XND can be utilized as habitat improvement plants in the preliminary ecological restoration of manganese tailing ponds. Rhizosphere soil microorganisms of pioneer plants respond to heavy metal pollution by regulating community structure and influencing metabolic function.

Keywords Pioneer plant · Bacterial community · Heavy metal content · Manganese tailing ponds · High-throughput sequencing

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Introduction

Manganese resources belong to the major strategic needs of the country and play an indispensable role in the development of metallurgy, chemical industry, iron and steel industry, aerospace, and other important fields in the construction of the national economy (Liu et al. 2019). Due to the low grade of manganese ore resources and poor technical processing performance, there is a contradiction between supply and demand, making China the world's largest consumer and importer of manganese resources (Shao 2017). The domestic distribution of manganese mineral resources is wide, with Guangxi being recognized as the hometown of non-ferrous metals due to its superior metallogenic geological conditions (Niu et al. 2021). It holds 55.8% of the total national manganese mineral reserves (Hou et al. 2019). However, the mining activities have led to serious geological disasters such as landslides, ground vibrations, explosions, as well as pollution of water environment, atmospheric environment, and surface vegetation through mining wastes like slag and tailings (Chen et al. 2023; Onifade et al. 2023).

Heavy metals in the surface soil of mining areas are mainly transported horizontally through rain-driven surface runoff and sediment transport (Qiao et al. 2023). These heavy metals accumulate in the soil through the food chain, causing harm to crops and posing a threat to human health (Worlanyo and Jiangfeng 2021). For example, prolonged exposure to excessive manganese can lead to symptoms like yellowing and necrotic leaf spots in plants (Schmidt and Husted 2019). Furthermore, excessive manganese, an essential trace metal for humans, can also exhibit neurotoxic effects (Bakulski et al. 2020). Long-term exposure to cadmium is linked to various cancers, including skin and lung cancer (Kubier et al. 2019; Okerefor et al. 2020), while excessive copper exposure can disrupt the normal function of multiple human systems, such as the respiratory, immune, and nervous systems (Taylor et al. 2020). Mining activities are recognized as a significant source of heavy metal pollution (Li et al. 2014), with the pollution caused by mining posing substantial potential harm to the environment (Wu et al. 2023). Ecological restoration of lands devastated by the mining industry has become an urgent environmental issue that needs to be addressed.

Vegetation restoration is a fundamental requirement for the recovery of degraded ecosystems and forms the basis for the development and succession of soil ecosystems in mining areas (Sarker et al. 2023; Barroso et al. 2023; Han et al. 2023). Over time, certain naturally occurring plants in mining areas have developed unique tolerance and absorption mechanisms for heavy metals under poor nutritional and harsh environmental conditions (Song et al. 2022; Mardonova and Han 2023), becoming pioneer plants in the restoration of vegetation in tailing ponds. These plants facilitate the creation of a conducive environment for the survival of diverse microorganisms within the rhizosphere and engage in interactions such as competition and cooperation between plants and microorganisms (Chepsergon and Moleleki 2023). Through root secretions and plant litter deposition, these pioneer plants directly improve the biogeochemical conditions in the root zone, providing favorable conditions for the growth of other plants and establishing a solid foundation for the succession of plant communities (Munford et al. 2022).

The restoration of soil microbial communities is the key to vegetation restoration and plays a positive role in realizing soil health, driving ecosystem versatility and sustainable use (Cai et al. 2022; Wang et al. 2023a; Matthews et al. 2023). Therefore, it is of great significance for the restoration of heavy metal pollution and the improvement of biodiversity to study the natural settlement pioneer plants and soil microbial communities and functional characteristics of manganese tailing ponds.

In recent years, scholars have continued to study the pioneer dominant plants in manganese mining areas. For instance, Nong et al. studied six pioneer plants, such as herbaceous plants *S. viridis* and *P. massonian* in Hunan Xiangtan manganese mine (Nong et al. 2023), and the soil quality and plant community diversity were promoted under the growth of the plants, which alleviated the heavy metal pollution. Luo Yang et al. studied 29 dominant plants (Luo et al. 2022), such as *Miscanthus floridulus* and *Buddleja lindleyana*, in manganese residue disposal sites located in eastern Guizhou Province and found that the nutrient composition of manganese slag was improved and the diversity of bacterial community was significantly increased by plant growth.

Currently, there is a limited amount of research on pioneer plants in manganese tailing ponds specifically in the Guangxi region. This study took seven pioneer plants of *Neyraudia reynaudiana*, *Pueraria montana*,

Bidens pilosa, *Buddleja asiatica*, *Pogonatherum crinitum*, *Crotalaria albida*, and *Thysanolaena maxima* grown naturally in the restoration area of Daxin manganese tailing ponds in Guangxi and analyzed the characteristics of manganese absorption, translocation, and tolerance mechanisms of pioneer plants, so as to clarify the ecological adaptability and ecological restoration potentials of the pioneer plants in manganese tailing ponds. Additionally, high-throughput sequencing was employed to explore the characteristics of soil microbial communities and their functions in the pioneer plants rhizosphere soil under conditions of high manganese metal pollution, in order to provide a theoretical basis for the restoration and management of manganese tailing ponds in Guangxi and the selection of vegetation species, and to lay a foundation for the formulation of tailing environmental management policies.

Materials and methods

Overview of the study area

Daxin manganese mine (Fig. 1), renamed as Xialei manganese mine, belongs to the state-run large-scale open-air manganese mine and is the largest manganese mine with the largest proven manganese ore reserves, with a total manganese ore reserve of 125 million tons, including 120 million tons of manganese carbonate. The manganese tailing pond restoration area belongs to the area to be restored artificially that no mining activities have been carried out within ten kilometers, and no amendment has been applied or any restoration has been carried out.

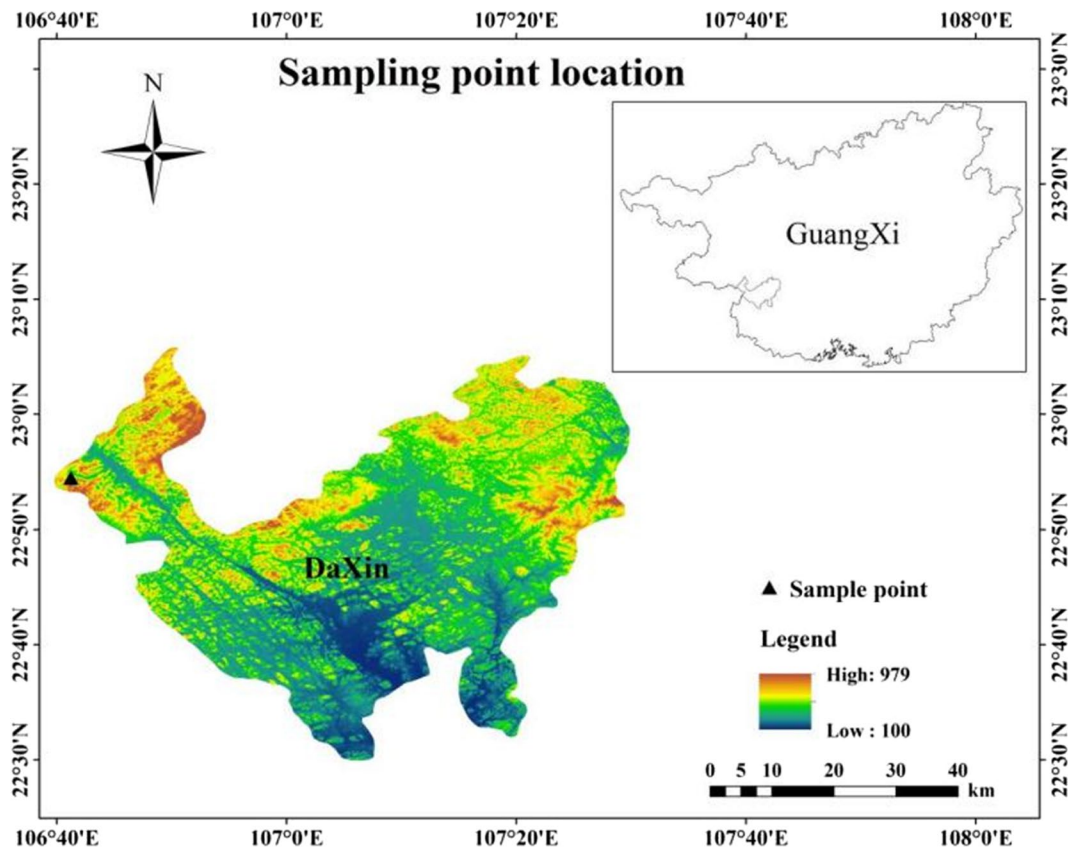


Fig. 1 Geographic location of the Daxin manganese mine sampling site. The sampling site is located in Xialei Town, Daxin County, Chongzuo City, Guangxi Province, China (lati-

tude: 22°54'58" N, longitude: 106°45'18" E), with an elevation of 241~845 m above sea level. It is a subtropical monsoon climate area with an annual rainfall of 135cm~192cm

Sample collection and processing

The 50 m×50 m area with no obvious height difference was artificially divided to reduce the influence of the uneven spatial distribution of soil. The main plants visible in the area were collected and recorded (Table 1), and the withered leaves, roots, gravel, biological debris, and other debris on the surface of the soil were removed. All samples were packed in clean polyethylene plastic sample bags, labeled with plant species, and preserved in ice packs for transport back to the laboratory. Wash the whole plant with running water and rinse with deionized water. The roots, stems, and leaves were individually excised, subjected to heat treatment at 100 °C for 30 min, and subsequently dried in an oven at 60 °C until a constant weight was achieved. The dry weight of each plant part was determined. The resultant dried plant samples were finely ground into a powder using a stainless steel grinder. This powdered material was then sieved through a 200 mesh (standard sieve) nylon sieve, homogenized, and stored in a desiccator for further analysis.

The S-shaped sampling method was applied to gather bare soil from a vegetation-free area (1 m×1 m), located at least 5 m away from any pioneer plants (Zhao et al. 2019). Five duplicate soil samples, with weights ranging from 500 to 1000 g, were collected. This particular plant-free location was designated as the blank sampling point CK. For rhizosphere samples, soil loosely adhered to the roots of excavated plants was delicately brushed off using a sterile brush. Rhizosphere soil samples obtained from the same plants were then combined into composite samples. These composite samples were gathered using the quartering method, carefully stored in ice bags, and transported back to the laboratory. Soil samples were frozen and stored for measurement of soil enzymes and microorganisms, while other samples were dried naturally, further removed from debris, crushed, ground, blended, passed through a 20-mesh (standard sieve) and 200-mesh (standard sieve) nylon sieve, homogenized and placed in a cool, dry place.

Sample measurement methods

The physicochemical properties and enzyme activities of soil samples were determined according to

the corresponding national standards (Table S1). Each sample was analyzed thrice for accurate determination. The total amount of heavy metals in soil and plant roots, stems, and leaves was determined by the microwave digestion method (GB HJ 832–2017) using the EXPEC 790 automatic microwave digestion instrument (Hangzhou Puyu Technology Development Co., Ltd.). Soil and plant samples were subjected to digestion using hydrochloric acid, nitric acid, hydrofluoric acid, hydrogen peroxide, and hydrogen peroxide-nitric acid methods, respectively. The available concentration of heavy metals was extracted by 0.1 mol/L hydrochloric acid solution. The concentrations of heavy metals were measured using an AA-6300 atomic absorption meter (Shimadzu Company, Japan).

Soil microbial community composition was sequenced using the 16S rDNA amplicon to detect microbial diversity. Soil samples were subjected to DNA extraction using the E.Z.N.A™ Mag-Bind Soil DNA Kit. The amplification library was generated with primers 341f (5'-C CTA CGG GNG GCW GCA G-3') and 805r (5'-GAC TAC HVG GGT ATC TAA TCC-3') targeting the V3-V4 hypervariable region of the bacterial 16S rRNA gene. Sequencing and data analysis were performed using the Illumina MiSeq platform of Sangon Biotech (Shanghai) Co., Ltd., and PCR amplification, purification, and quantification of PCR products were performed according to its standard protocol.

Data processing

The experimental data from three repeated samples were subjected to statistical analysis and processing using Microsoft Excel 2020, SPSS 26.0, and Origin 17.0 software. One-way analysis of variance (ANOVA) was employed to determine significant analysis of variance on the measured results. The Pearson correlation coefficient was utilized to evaluate the correlation between various factors. Canoco 5 software was applied to analyze and visually represent the association between community diversity and environmental factors.

Bio Enrichment Coefficient (BEC) refers to the ratio of heavy metal concentrations in plants to heavy metal concentrations in soil. A higher value indicates a stronger ability of the plant to accumulate heavy metal,

Table 1 Main pioneer plants in the manganese tailing ponds restoration area

	Latin name of plant	Plant abbreviation	Family	Plant life forms
	<i>Neyraudia reynaudiana</i>	LL	Poaceae	Perennial herb
	<i>Pueraria montana</i>	GT	Fabaceae	Robust vine
	<i>Bidens pilosa</i>	GZC	Asteraceae	Annual herb
	<i>Buddleja asiatica</i>	BBF	Scrophulariaceae	Small tree or shrub
Plant abbreviations in this study utilize the Pinyin transliteration of the plant's Chinese initials	<i>Pogonatherum crinitum</i>	JSC	Poaceae	perennial herb
	<i>Crotalaria albida</i>	XND	Fabaceae	Erect perennial herb
	<i>Thysanolaena maxima</i>	ZYL	Poaceae	perennial herb

while a lower value indicates a weaker ability (Torbaty et al. 2021). The calculation formula is as follows:

$$BEC = \frac{C_{\text{plant}}}{C_{\text{soil}}} = \frac{C_{\text{root}} \times M_{\text{root}} + C_{\text{stem}} \times M_{\text{stem}} + C_{\text{leaf}} \times M_{\text{leaf}}}{M_{\text{root}} + M_{\text{stem}} + M_{\text{leaf}}} \quad (1)$$

Bio Translocation Factor (BTF) refers to the ratio of heavy metal concentrations in the above-ground part of the plant to the heavy metal concentrations in the roots. A higher BTF value indicates a stronger ability of the plant to transfer heavy metal from the underground part to the above-ground part (Wu et al. 2022). BTF is calculated using the following formula:

$$BTF = \frac{C_{\text{aboveground}}}{C_{\text{root}}} = \frac{C_{\text{stem}} \times M_{\text{stem}} + C_{\text{leaf}} \times M_{\text{leaf}}}{M_{\text{stem}} + M_{\text{leaf}}} \quad (2)$$

Bio Accumulation Factor (BAF) is the ratio of heavy metal concentrations in the roots to the heavy metal concentrations in the soil. It is used to evaluate the potential of soil phytoremediation and to analyze the plant's tolerance to heavy metals (Aliyu et al. 2023). BAF is calculated using the following formula:

$$BAF = \frac{C_{\text{root}}}{C_{\text{soil}}} \quad (3)$$

Note: (1) (2) (3) where C_{plant} is the heavy metal concentrations in the body of each plant (mg/kg); $C_{\text{aboveground}}$ is the heavy metal concentrations of plant above-ground parts (mg/kg); C_{root} is the heavy metal concentrations of plant roots; C_{soil} is the heavy metal concentrations of the soil; C_{root} , C_{stem} , C_{leaf} is the heavy metal concentrations of the roots, stems and leaves of each plant, respectively (mg/kg); M_{root} , M_{stem} , M_{leaf} is the dry weight of the roots, stems and leaves of each plant, respectively (kg).

Results

Basic physicochemical properties and enzyme activities of soil

The study of soil condition is the primary task of ecological restoration, and the physicochemical properties of soil are an important reference for the study of soil health and fertility (Hao et al. 2023). The physicochemical properties of pioneer rhizosphere soil improved significantly relative to CK, with significant increases in pH, CEC, and AK (Fig. 2A, B). In general, among the seven pioneer plants, the rhizosphere soils of *Pueraria montana* (GT) and *Bidens pilosa* (GZC) had higher contents of CEC, OC, AP, and AK (Fig. 2A, B), and the contents of ammonium nitrogen were much less than those of CK (Fig. 2B), indicating that the colonization of these two pioneer plants was more conducive to the healthy development of soil. As shown in Fig. 2C, the CAT range of each rhizosphere soil was 3.33–3.84 mg·g⁻¹·h⁻¹, which got 55%–79% enhancement compared with CK soil, and S-SC range was 1.32–5.91 mg·g⁻¹·d⁻¹, and S-UE range was 4.20–11.72 mg·g⁻¹·d⁻¹. The enzyme activity of the rhizosphere soil of pioneer plants was generally increased, thus improving the ecological environment and soil fertility.

In order to further analyze the relationship between basic soil physicochemical properties and enzyme activities, Pearson correlation analysis was done as shown in Fig. 3. Soil pH had a greater influence on all environmental factors, and the positive correlation between soil pH, AK, and all other indexes except NH₃-N was shown to varying degrees. There was a highly significant positive correlation between pH and AK ($P < 0.01$, $R = 0.61$). pH was significantly positively correlated with the three enzymes (respectively,

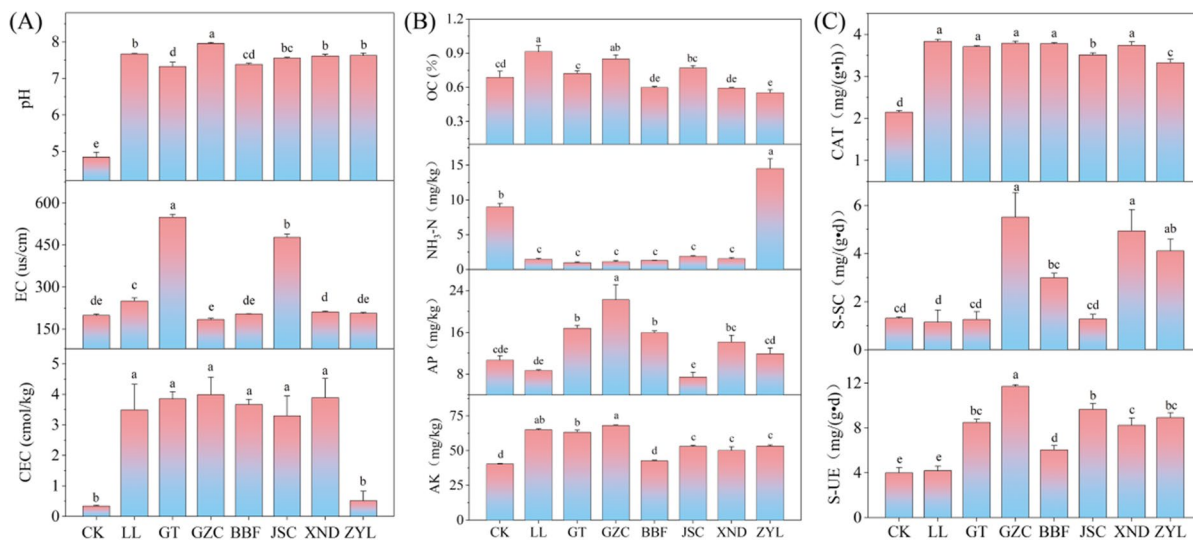


Fig. 2 Basic physicochemical properties and enzyme activities of different soil, **A** of the pH, EC, and CEC values for different soils, **B** of the values of the corresponding indicators OC, $\text{NH}_3\text{-N}$, AP, and AK for different soils with carbon, nitrogen, phosphorus, and potassium, **C** of the different soil enzyme

activities. Potassium permanganate titration was used for soil CAT, while the colorimetric method was utilized for soil S-SC and S-UE. Error lines are standard errors, and different lowercase letters on the columns indicate significant differences between treatments ($P < 0.05$)

$R=0.92$, $R=0.41$, and $R=0.60$). AK was significantly positively correlated with CAT ($R=0.59$) and S-UE ($R=0.45$), and highly significantly positively correlated with OC ($P < 0.01$, $R=0.63$). Soil $\text{NH}_3\text{-N}$ showed different degrees of negative correlation with all indicators except S-UE, and a highly significant negative correlation with CEC ($P < 0.01$, $R=-0.84$) and CAT ($P < 0.01$, $R=-0.61$). CEC and CAT were highly significant positively correlated with each other ($P < 0.01$, $R=0.73$). Soil AP was significantly positively correlated with S-SC ($R=0.53$) and S-UE ($R=0.45$). S-SC and S-UE exhibited a strong and statistically significant positive correlation ($P < 0.01$, $R=0.63$).

Soil available and total heavy metal content

In the tailing pond soil, heavy metal concentrations were found to be highest for Mn, followed by Cu and Cd (Fig. 4). The total heavy metal concentrations in the rhizosphere soil of pioneer plants in the tailings were measured to be 8210–14755 mg/kg for Mn, 1.25–4.58 mg/kg for Cd, and 61.9–77.8 mg/kg for Cu, respectively (Fig. 4B). These values exceeded the soil background values in Guangxi by 46.6–83.8 times for Mn, 17.1–62.7 times for Cd, and 2.8–3.5 times for Cu (Li et al. 2007). Specifically,

the rhizosphere soil of *Pueraria montana* (GT) had the highest levels of all three heavy metals, while *Bidens pilosa* (GZC) had lower levels of Cd compared to the other plant rhizosphere soils. CK soil showed a higher proportion of the three heavy metals in an effective state compared to the other plant rhizosphere soils (Fig. 4A), with manganese making up 18.7% of the total amount, Cd 33.8%, and Cu 12.0%. Within the rhizosphere soils of the plants, the available state of Mn ranged from 3.9% to 6.5% of the total amount, Cd from 3.8% to 13.8%, and Cu from 2.6% to 5.5%. The growth of pioneer plants was found to decrease the available states of heavy metals in the rhizosphere soil, thereby reducing the biotoxicity of the heavy metals (Li et al. 2023).

Pioneer plant uptake, transfer, and tolerance of heavy metals

The concentrations of heavy metals in the pioneer plant species varied across different parts of the plants (Fig. 5). In the body of the plants, the concentrations ranged from 245.4 to 594.7 mg/kg for Mn, 0.06 to 0.42 mg/kg for Cd, and 3.6 to 8.9 mg/kg for Cu. In the aboveground parts of the plants, the concentrations were between 146.5 and 490.3 mg/kg for Mn, 0.06 and 0.41 mg/kg for Cd, and 3.6 to 9.2 mg/kg

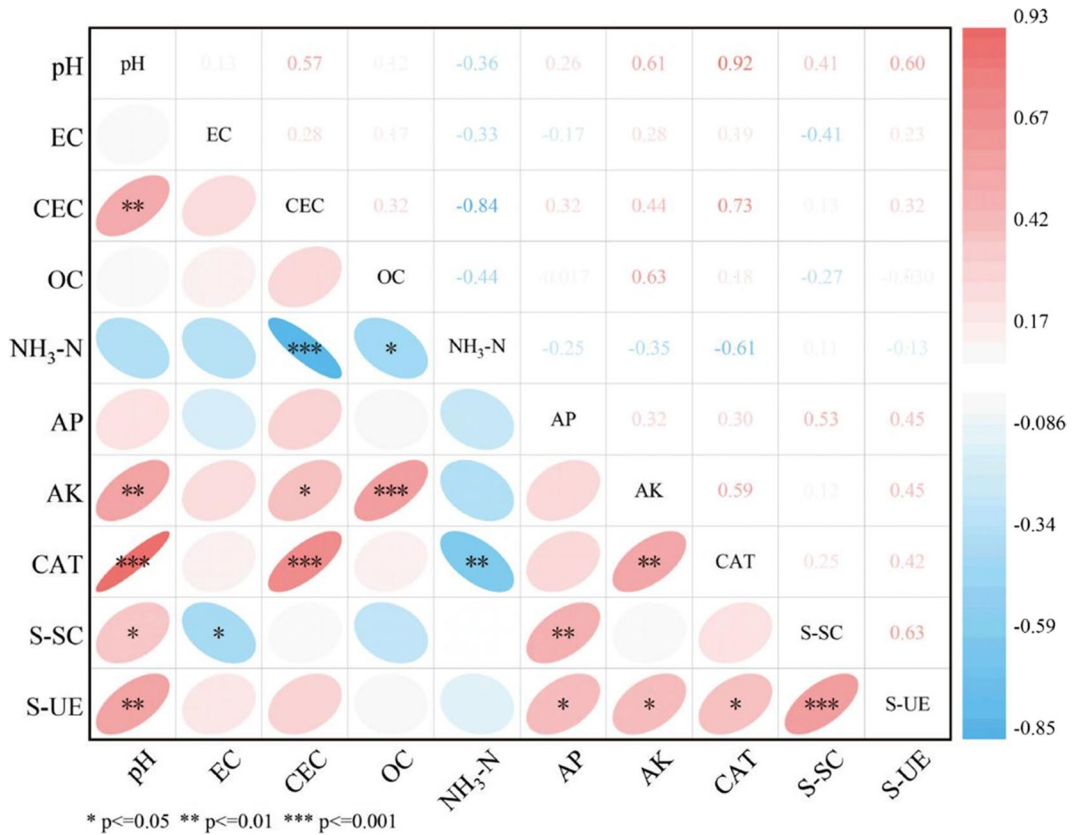


Fig. 3 Pearson correlation analysis between basic soil physicochemical properties and enzyme activity. Significant differences were analyzed by *t*-test at the 0.05 level (*), 0.01 level (**), and 0.001 level (***). Red indicates positive correlation,

while blue indicates negative correlation; the flatter and darker the circle, the greater the absolute value of the correlation coefficient

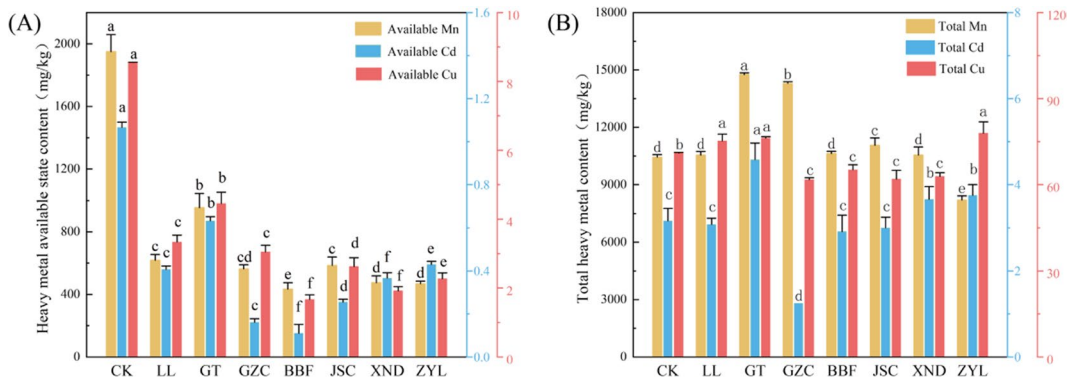


Fig. 4 Manganese (Mn), cadmium (Cd), and copper (Cu) content in different soils: available state content (A) and total content (B). The content of Mn (color yellow) is plotted on the left side, while the content of Cd (color blue) and Cu (color red) is plotted on the right side, consistent with the color. Error

lines are standard errors, and different lowercase letters on the columns indicate significant differences between treatments ($P < 0.05$). Among them, the content of lead (Pb) and zinc (Zn) has not been detected and has not been analyzed and compared

kg for Cu. The underground parts of the plants had the highest concentrations, with values ranging from 125.0 to 1541.9 mg/kg for Mn, 0.08 to 0.46 mg/kg for Cd, and 3.5 to 17.6 mg/kg for Cu.

Diversity analysis of soil microbial community structure

The average number of effective sequences obtained from different plant rhizosphere soil samples by Illumina Miseq sequencing ranged from 51,725.0 to 69,386.0 in size order, and the sequence coverage was above 99% (Table 2). In the dilution curve analysis

(data not shown), the curves tended to be stable after 10,000 sequences, and the amount of sequencing data was asymptotically reasonable, with a similarity degree of 97%. Additional sequencing did not affect species diversity. Among them, the number of soil microorganisms in CK was small, and the number of amplifications did not meet the requirements for on-line sequencing. Combined with each diversity index in Table 2, the Shannoneven index was around 0.80, and species evenness within the community was basically the same in different samples. The sequence with the highest diversity of bacterial communities was *Pueraria montana* (GT), *Thysanolaena maxima*

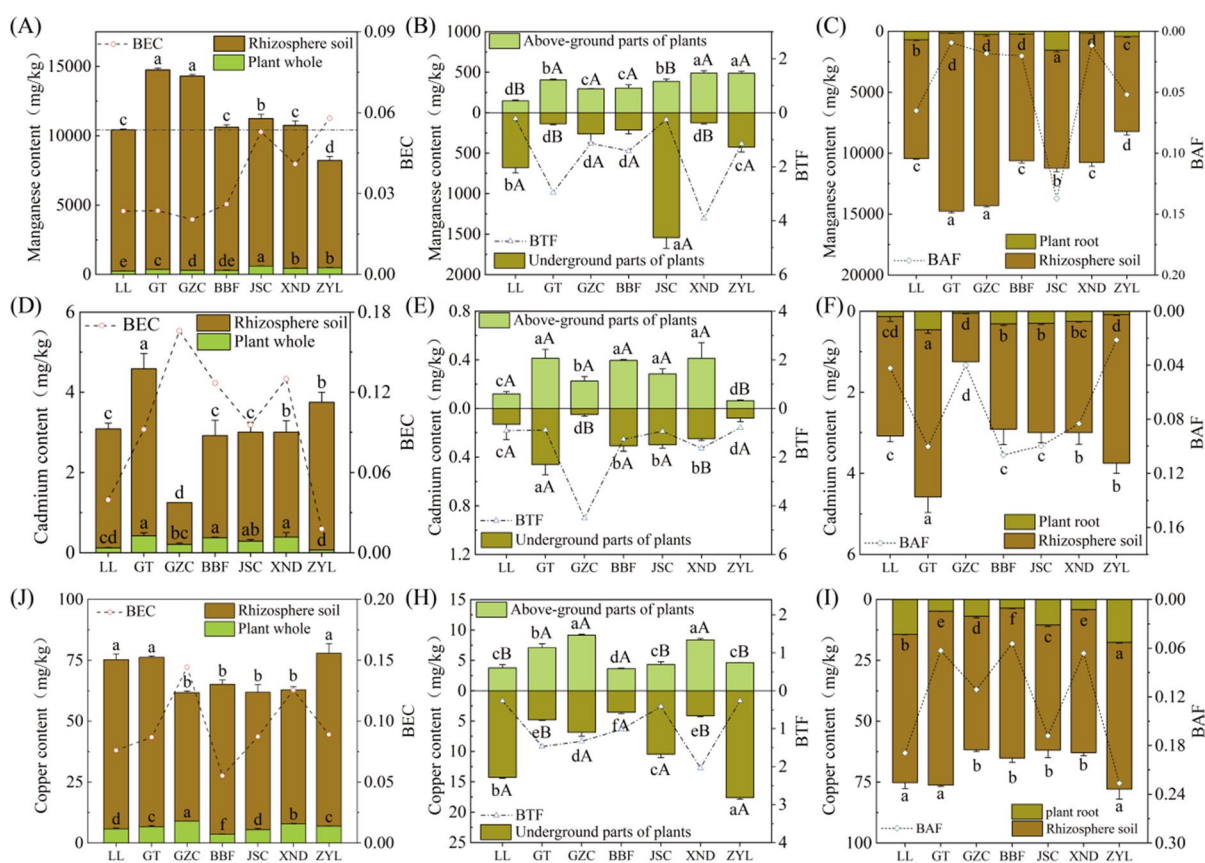


Fig. 5 Corresponding indicators of absorption, transfer, and tolerance to heavy metal in different pioneer plants: BEC for Mn (A), Cd (D), and Cu (J); BTF for Mn (B), Cd (E), and Cu (H); and BAF for Mn (C), Cd (F), and Cu (I). Error lines are standard errors. The auxiliary line in (A) is the total Mn content of CK soil $10,420.6 \text{ mg}\cdot\text{kg}^{-1}$, and different lowercase letters on the columns indicate that the differences between the Mn contents in the rhizosphere soil and plant body of different plants are significant ($P < 0.05$); B Different lowercase letters

on the middle column indicated significant differences in Mn content between above-ground and underground parts of different plants ($P < 0.05$), and different uppercase letters indicated significant differences in Mn content between above-ground and underground parts of the same plants ($P < 0.05$). (C) Different lowercase letters in the middle column indicate significant differences among treatments ($P < 0.05$). The graphical plotting of the relevant indicators for Cd and Cu is consistent with that for Mn

Table 2 Effective sequences and diversity of soil bacterial communities in the plant rhizosphere (mean value)

Sample	Effective sequence	Shannon	Chao	Ace	Simpson	Shannoneven	Coverage
LL	59,509.0	5.93	2780.51	2850.30	0.02	0.76	0.99
GT	53,929.0	6.54	3039.72	3118.88	4.6e-03	0.82	0.99
GZC	51,725.0	6.20	2901.95	3015.75	0.01	0.78	0.99
BBF	65,498.0	6.41	2857.47	2904.00	5.3e-03	0.81	0.99
JSC	54,956.0	6.04	2715.69	2809.85	0.01	0.77	0.99
XND	69,386.0	6.21	3096.53	3158.55	6.8e-03	0.78	0.99
ZYL	59,177.0	6.51	3238.38	3339.79	5.1e-03	0.81	0.99

Analyzing the diversity and richness of rhizosphere microorganisms in pioneer plants using the Shannon index, Shannoneven index, Simpson index, Chao index, and Ace index. The higher the value of the Shannon index and Simpson index, the lower the value of the Simpson index, and the higher the bacterial diversity. The higher the Chao index and Ace index values, the higher the abundance of bacterial communities

(ZYL), *Buddleja asiatica* (BBF). The order of higher bacterial community richness was *Thysanolaena maxima* (ZYL), *Crotalaria albida* (XND), *Pueraria montana* (GT), *Bidens pilosa* (GZC).

Based on the 97% similarity clustering level, the size order of the total OTUs (data not shown) for each sample was ZYL (3007), GT (2876), XND (2856), BBF (2726), GZC (2725), LL (2557) and JSC (2530). The number of OTUs common to the seven samples was 899, and the number of OTUs specific to each sample ranged from 223 to 473 (Fig. S1), with the ranking of the proportion of total OTUs being ZYL (15.7%), BBF (14.7%), GT (14.7%), JSC (13.7%), GZC (13.7%), XND (9.4%), and LL (8.7%).

Soil bacterial community composition

Sequencing readings sorted by phylum level from different rhizosphere soil samples were associated with 12 bacterial phyla, as shown in Fig. 6A. The dominant phyla were mainly *Proteobacteria* (relative abundance ranging from 26.9% to 38.53%), *Actinobacteria* (17.8% to 35.3%), *Acidobacteria* (5.10% to 9.97%), *Cyanobacteria Chloroplast* (1.10% to 15.50%) and *Bacteroidetes* (2.49% to 5.63%). As shown in Fig. 6B, in the study based on class level, *Actinobacteria* (17.44% to 34.44%) had the highest percentage of the seven rhizosphere soil samples in the phylum *Actinobacteria*. Other dominant classes included *Alphaproteobacteria* (13.77% to 24.45%), *Betaproteobacteria* (5.61% to 9.04%), *Gammaproteobacteria* (1.84% to 7.56%) in the phylum *Proteobacteria*, and *Chloroplast* (From 0.92% to 13.4%) in the phylum *Cyanobacteria Chloroplast*.

Correlation between soil bacterial communities and environmental factors

To further investigate the correlation between soil microorganisms and environmental factors, as well as to analyze the effect of rhizosphere soil microbial communities on plant uptake of heavy metals, 12 dominant phyla with high abundance were used as microbial species data, and the basic physicochemical properties of the soil (pH, SOC, NH₃-N, AP, and AK) as well as the accumulation capacity of heavy metals in pioneer plants (BTF-Mn, BTF-Cd, BTF-Cu, BAF-Cd, and BEC-Cu) as environmental variables were plotted with Canoco 5 software for redundancy analysis, as shown in Fig. 7.

The eigenvalues of axes 1, 2, 3, and 4 in Fig. 7A were 0.53, 0.41, 0.02, and 0.02, respectively, as shown in Table S2. The cumulative interpretation amount of all microbial community data changes was 98.29%, and the cumulative interpretation amount of microbial fraction changes—soil environmental factors was 99.78%. As shown in Fig. 7A, the correlations between *Candidate division WPS-I* and *Chloroflexi* showed a highly significant positive correlation. *Verrucomicrobia* showed a significant negative correlation with the abundance of *Gemmatimonadetes*, and *Cyanobacteria Chloroplast* showed a significant negative correlation with the abundance of *Proteobacteria*. Soil bacteria showed a significant correlation with basic physicochemical properties, and the abundance of *Candidate division WPS-I* showed a significant positive correlation with soil pH, SOC, and AK. The abundance of *Candidatus Saccharibacteria* showed a significant

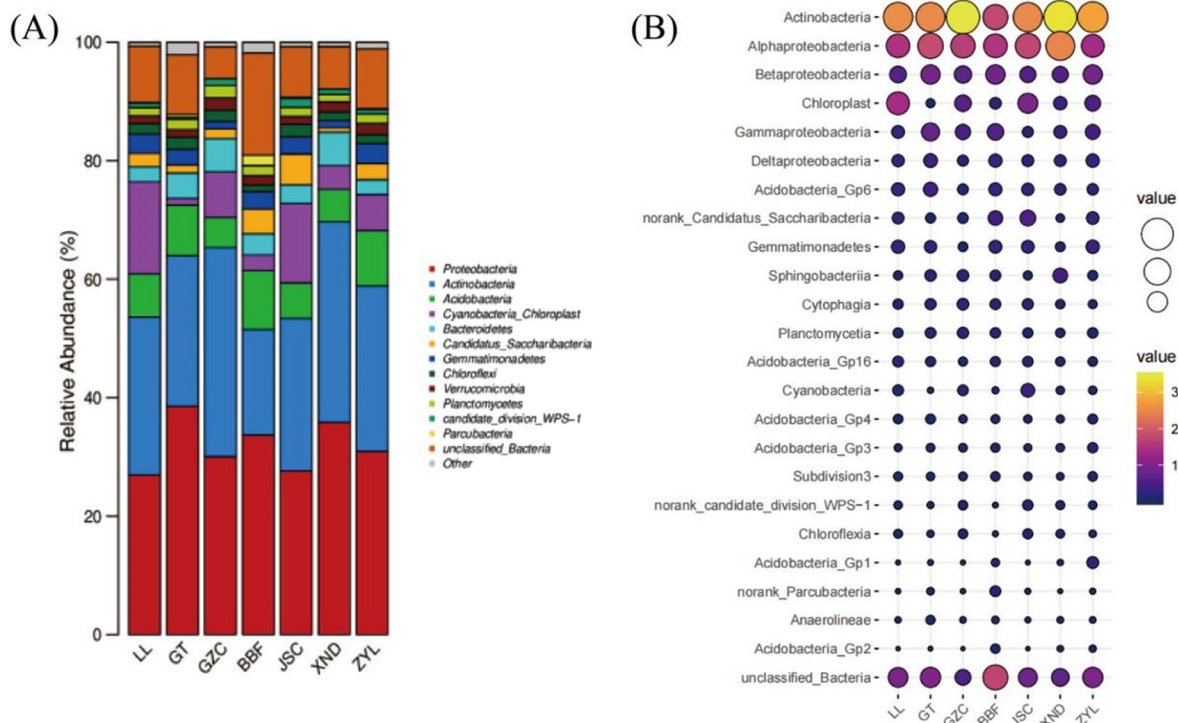


Fig. 6 Bacterial community composition in different rhizosphere soils. **A** shows the relative abundance of each bacterial community at the phylum level. At least one sample had a relative abundance greater than 0.01, and bacterial phylum species with a relative abundance less than 0.01 were combined into

positive correlation with the content of soil $\text{NH}_3\text{-N}$. The abundance of *Planctomycetes* showed a significant positive correlation with soil AP content. The abundance of *Parcubacteria* showed a significant negative correlation with soil pH and OC content. Similar to the data in this study, Vargas et al. found that *Acidobacteria* showed a negative correlation with AK as well as with pH, and soil pH is an important feature in shaping the microbial community (Vargas et al. 2023).

The eigenvalues of axes 1, 2, 3, and 4 in Fig. 7B were 0.53, 0.41, 0.02, and 0.02, respectively, as shown in Table S2. The cumulative interpretation amount of all microbial community data changes was 98.18%, and the cumulative interpretation of microbial component changes—soil environmental factors was 99.39%. As shown in Fig. 7B, it is evident that among the nine indexes reflecting the absorption of Mn, Cd, and Cu by plants, five indexes displayed a clear correlation with the root

other. **B** shows the relative abundance of each bacterial community at the class level. The larger the circle, the higher the relative abundance of bacterial communities at the corresponding class level

soil bacterial community. Specifically, the BTF indexes of the three heavy metals were involved. The abundance of *Proteobacteria* exhibited a significant positive correlation with BTF-Mn and BTF-Cu, while the abundance of *Actinobacteria* was significantly positively correlated with BEC-Cu and BTF-Cd. Additionally, the abundance of *Bacteroidetes* showed a significant positive correlation with all four indicators mentioned above. On the other hand, the abundance of *Acidobacteria* and *Parcubacteria* demonstrated a significant positive correlation with the abundance of BAF-Cd.

Prediction of soil bacterial community function

K03088 (rpoE; RNA polymerase sigma-70 factor, ECF subfamily) is the dominant gene at higher Mn concentrations in this repair region in Fig. 8A, and K03088 is also dominant at high Al concentrations (Zhang et al. 2023), verifying the role of K03088

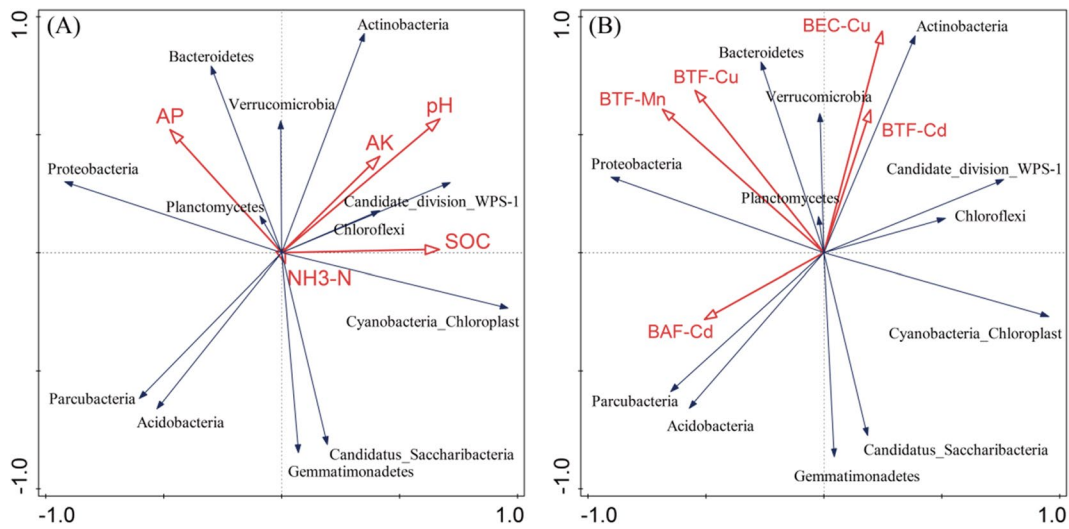


Fig. 7 Redundancy Analysis (RDA) of soil bacteria with basic physical and chemical properties (A) and the ability of plants to absorb heavy metals (B) based on phylum level. Blue arrows represent soil bacteria at the phylum level, and red arrows represent environmental factors. The cosine of the angle between

two blue arrows represents the correlation between two microbial groups, and the cosine of the angle between a blue arrow and a red arrow represents the relationship between the microbial and environmental factors

in inducing gene transcription in response to environmental stress (Guo et al. 2023). Dominant genes K01692 and K00059 were associated with carbon metabolism, glycolysis, and methane metabolism (Cao et al. 2022), suggesting that higher Mn concentrations have an effect on microbial carbon

metabolism. There is a close relationship between the iron complex outer membrane receptor protein K02014 and iron carriers, which can be synthesized by microorganisms to obtain iron from insoluble iron oxides and promote microbial metabolism (Lu et al. 2022). ABC transporter proteins can be strictly

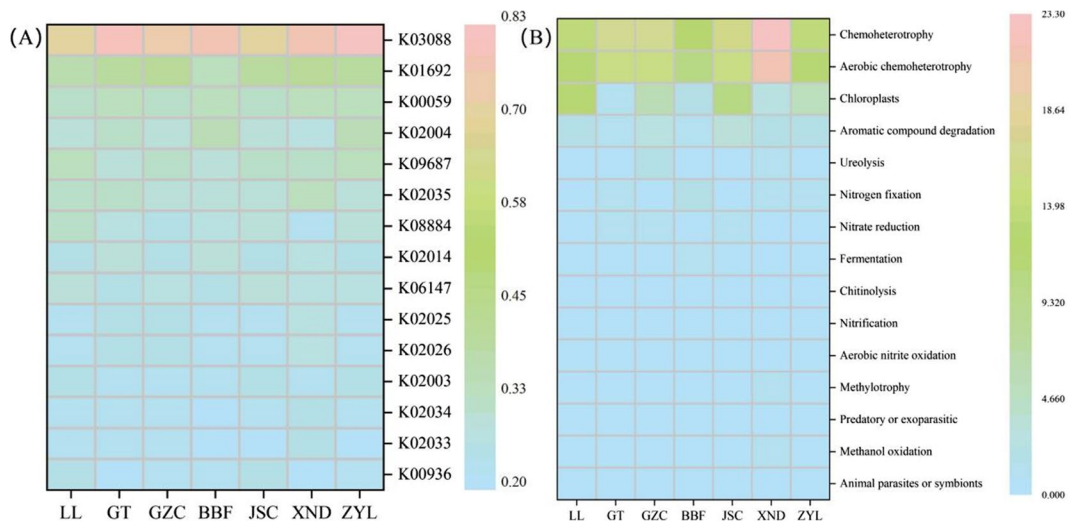


Fig. 8 KEGG relative abundance heatmap (A) and FAPROTAX-based heatmap (B) for the prediction of bacterial ecological functions. Select the top 15 relative abundances for plotting

involved in plant metal transport, and most of the functional gene in other dominant genes, such as the hypothetical ABC transporter system osmolytic enzyme protein K02004, the antibiotic transporter system ATP-binding protein K09687, the Peptide/Nickel transporter system substrate binding protein K02033, K02034, and K02035, and polysaccharide transporter system permease proteins K02025 and K02026, etc., belong to the membrane transport pathway in environmental information processing (Fajardo et al. 2019; Sami et al. 2021; Wang et al. 2023b; Yu et al. 2023). As shown in Fig. 8B, the main functional models of rhizosphere soil bacteria of pioneer plants were analyzed based on FAPROTAX. Chemoheterotrophy, aerobic chemoheterotrophy, chloroplasts synthesis, aromatic compound degradation, and aromatic compound degradation were the main metabolic modes.

Discussion

The positive impact of pioneer plant growth on soil in manganese mine areas

The soil in the Daxin Mn tailing ponds in Guangxi was found to be acidic and nutrient-poor (Fig. 2). However, the natural growth of pioneer plants has been found to be effective in enhancing the soil's physical and chemical properties (Fig. 2), as well as microbial activity (Fig. 6). These pioneer plants, belonging to 7 genera and 4 families, are mostly perennial herbs (Table 1), which are often selected as starting plants for ecosystem restoration due to their fast growth, erosion prevention, and facilitation of early topsoil formation (Doyama et al. 2024).

The pioneer plants that thrive in such extreme environments demonstrate strong environmental adaptability and pollution resistance (Li et al. 2024). The rhizosphere microbial community leverages the plant litter, decayed above-ground parts, roots, stems, and root secretions to promote the reproduction of microbial masses and release extracellular enzymes into the surrounding environment (Canarini et al. 2019). Additionally, the release of intracellular enzymes through cellular autolysis after the plants' demise further promotes the increase of enzyme activity and available nutrients (Santoyo et al. 2021).

These processes impact the nutrient content required for plant growth and the diversity of the community in the tailing ponds, as they are influenced by the long-term effects of natural factors (Chaudhary et al. 2023). Notably, there was no significant correlation found between rhizosphere soil enzyme activity and microbial community, suggesting that changes in soil enzyme activity caused by heavy metals might be primarily attributed to the biological toxicity of heavy metals and soil nutrient content (Tang et al. 2019). It's important to note that the positive effect of nutrient stimulation may not completely offset the inhibitory effect caused by the increase of heavy metals (Pinto-Poblete et al. 2022).

Soil pH plays a crucial role in influencing various environmental factors (Figs. 3 and 7A). It has been observed that vegetation restoration tends to have a significant neutralizing effect on soil pH (Goulding 2016). The presence of negatively charged groups (such as carboxyl and phenol groups) in the growth of pioneer plants provides additional adsorption sites for cations (Yang et al. 2024), resulting in an increase in rhizosphere soil CEC (Fig. 2A). Plants can impact processes such as nutrient cycling, capture of acid deposition, and litter input by absorbing exchangeable cations, thereby affecting the production and consumption of soil hydrogen ions (Raza et al. 2020). pH is also a critical factor in shaping the soil microbial community (Fig. 7A), which greatly influences microbial activity. The process of microbial metabolic activity is very susceptible to the influence of a high or low pH value (Hong et al. 2018). Pioneer plants regulate the acidity of the soil to maintain the soil in a pH environment suitable for microbial growth, which directly or indirectly enhances microbial activity (Naz et al. 2022). Furthermore, soil pH can significantly affect the solubility and availability of heavy metal ions (Meng et al. 2023). In this study (Fig. S3), the pH value was found to be negatively correlated with the content of three exchangeable heavy metals ($R_{Mn} = -0.95$, $R_{Cd} = -0.85$, and $R_{Cu} = -0.90$). Soil acidification leads to a significant increase in the mobility of metals in the soil, and the rise of pH value due to the growth of pioneer plants is shown to have a certain activating and inhibitory effect on heavy metals (Kicińska et al. 2022). This reduces the competition between hydrogen ions and metals for surface adsorption sites. In addition, electrical conductivity and

organic matter content were found to be positively correlated with the contents of the three exchangeable heavy metals (Fig. S3). The increase in rhizosphere soil electrical conductivity enhances the competitiveness of water-soluble salts, such as Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , and other strong electrolytes (Huang et al. 2023), to compete with heavy metal ions for adsorption sites, thereby reducing the specific adsorption of heavy metals (Zhong et al. 2020). Furthermore, functional groups in organic matter can form organometallic complexes with heavy metals through chelation or complexation (Zeng et al. 2011), thereby improving the solubility of heavy metals (Wang et al. 2021).

Pioneer plant root interactions can enhance soil physicochemical and microbiological properties through processes such as respiration, secretion, and absorption (Wang et al. 2017). The abundant microorganisms proliferating in the rhizosphere can facilitate the adsorption of heavy metals in the soil through mechanisms such as extracellular precipitation, complexation, and intracellular binding (Ma et al. 2016). Additionally, the bacterial communities of *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* were found to have a significant positive correlation with the BTF values in this study (Fig. 7B). This correlation promotes the transport of heavy metals from the underground parts of the plant to the aboveground parts. Plants have evolved specific tolerance mechanisms to cope with heavy metal stress in their environment (Fig. 5). Plants that are adapted to heavy metal stress can enhance their ability to compete for limited nutrient resources, consequently increasing their uptake of heavy metals (Feng et al. 2021). This establishes a beneficial cycle among pioneer plants, microorganisms, and soil quality under heavy metal pollution conditions. This cycle promotes the development of the soil environment in a positive direction, fostering soil health and sustainability.

Analysis of heavy metal absorption, transfer, and tolerance in pioneer plants

In this restoration area, the content of heavy metals in pioneer plants is Mn, Cu and Cd in order (Fig. 5). These characteristics are consistent with the heavy metal content in the soil (Fig. 4). Although the proportion of available Cd in the soil of mining areas is relatively high, and the BEC for Cd in the pioneer

plant is also higher than that for Cu and Mn, the proportion of available Cu and Mn content does not align with the BEC value of the pioneer plant (Fig. 5). Additionally, the levels of the three heavy metals in the pioneer plants do not follow the same trend as the total amount of heavy metals in the soil, indicating that the absorption of heavy metals by plants is influenced by both the total amount of heavy metals in the soil and the form in which the heavy metals occur (Zakaria et al. 2021).

Currently, based on different mechanisms of heavy metal tolerance, plants can be classified into accumulator, indicator and exclusion types (Vaculík et al. 2012). The BEC values of the seven pioneer plants were all less than 1 (Fig. 5), which is inconsistent with the characteristics of accumulator-type plants that actively absorb and accumulate heavy metals from the soil and transfer them to above-ground parts (Malayeri et al. 2013). While there were no hyperaccumulators with strong phytoextract abilities identified in the restoration area (Kumar et al. 2021), the growth of the seven pioneer plants did not exhibit obvious toxic symptoms such as leaf chlorosis or necrotic leaf spots (Santos et al. 2017; Dey et al. 2023). This could be attributed to the BTF of Mn, Cd, and Cu being greater than 1 for *Bidens pilosa* (GZC), *Buddleja asiatica* (BBF) and *Crotalaria albida* (XND) (Fig. 5). Additionally, The BTF-Mn and BTF-Cu of *Pueraria montana* (GT) are greater than 1, with the BTF-Cd is close to 1. The BTF-Mn of *Thysanolaena maxima* (ZYL) is greater than 1, indicating some phytoextraction potential (Sheoran et al. 2016). These pioneer plants are capable of transferring heavy metals from their underground parts to the above-ground parts, sequestering the heavy metals to cell wall or vacuole compartmentalization to avoid physiological toxicity (Parrotta et al. 2015). Conversely, *Neyraudia reynaudiana* (LL) and *Pogonatherum crinitum* (JSC) exhibit low absorption capacities for Mn, Cd, and Cu (Fig. 5), making them suitable for thriving in heavily metal-polluted environments. This ability of pioneer plants to tolerate and manage heavy metal contamination is crucial for enhancing ecosystem diversity and promoting ecosystem stability in polluted mining areas (Li et al. 2014).

Rhizosphere microorganisms play a crucial role in promoting plant growth and health (Hakim et al. 2021), with some possessing the ability to resist heavy metals and support plant growth (Shen et al.

2022). In the study, it was found that *Cyanobacteria_Chloroplast* in the rhizosphere soil of *Neyraudia reynaudiana* (LL) and *Pogonatherum crinitum* (JSC), which have the lowest heavy metal absorption capacity (Fig. 5), exhibited significantly higher levels (13.4% and 15.5%, respectively) compared to other pioneer plants (1.1%–7.7%). This suggests that *Cyanobacteria_Chloroplast* may play a key role in protecting plants from heavy metal toxicity. Furthermore, rhizosphere microorganisms can enhance the absorption of heavy metals by plants and improve the efficiency of phytoremediation in heavy metal-contaminated soil (Qin et al. 2024). The study also revealed a positive correlation between the abundance of *Bacteroidetes* and the BEC value of the three heavy metals. Additionally, the root microorganisms of *Bidens pilosa* (GZC) and *Pueraria montana* (GT), which have high total Mn content in plant rhizosphere soil, also demonstrated higher levels of *Bacteroidetes*, indicating their potential tolerance to Mn. This finding is consistent with other studies that have shown *Bacteroidetes* to display higher resistance to Cd (Fu et al. 2023; Pan et al. 2023). At the same time, *Proteobacteria* and *Actinobacteria*, with the higher abundance, exhibited a significant positive correlation with the BEC values of Mn and Cu (Fig. 7B). This suggests that *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* play a crucial role in promoting the absorption of heavy metals by the pioneer plants in the restoration area.

Pueraria montana (GT) can tolerate high concentrations of heavy metal due to its well-developed vines and symbiosis with rhizobia (Mensah et al. 2021), thereby improving soil quality (Fig. 2). Similarly, *Bidens pilosa* (GZC) has been shown to effectively enhance soil fertility (Fig. 2) and is known as a Cd superaccumulator plant (Dai et al. 2021). It is worth noting that there are limited studies on the phytoremediation of mine metals by *Crotalaria albida* (XND), possibly due to its smaller biomass and a lesser role in the remediation of heavy metal soils in mining areas. However, the exceptional capacity of *Bidens pilosa* (GZC), *Pueraria montana* (GT), *Buddleja asiatica* (BBF), and *Crotalaria albida* (XND) to absorb heavy metals (Fig. 5) and enrich soil microbial communities (Fig. 6) makes them highly suitable choices for ecological restoration in mining areas.

Bacterial communities and functional characteristics of rhizosphere soil in pioneer plants

Proteobacteria, *Actinobacteria*, and *Acidobacteria* are the bacterial communities with the highest abundance in plant rhizosphere soil (Fig. 6A). In the Mn mining area of Xiangtan, Hunan Province studied by Nong (Nong et al. 2023), *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were also the three most abundant bacterial communities, and the average abundance of *Proteobacteria* was also over 30%. The results indicated that *Proteobacteria* had strong tolerance to Mn metal. *Actinobacteria* has the ability to bioremediate heavy metals through the secretion of biosurfactants, iron carriers, and organic acids (Behera and Das 2023). The high tolerance shown by its metabolic diversity and high content ratio makes *Actinobacteria* a potential microorganism for bioremediation process in this restoration area. The dominant bacteria *Alphaproteobacteria* class (Fig. 6B), particularly the orders Sphingomonadales and Rhizobiales, significantly influence nitrogen fixation (Veerasamy et al. 2023). Certain bacteria belonging to *Betaproteobacteria* actively participate in the Mn redox cycle, influencing the mobilization and fixation of arsenic in groundwater (Chakraborty et al. 2020), and also play a certain role in the biological filtration of Mn (McCormick et al. 2023). The dominance of *Gammaproteobacteria* may be attributed to its adaptability to arid environments with low humidity (Radeva et al. 2013). Additionally, some members of this group have the capability to form biofilms on stone surfaces, providing bacterial colonies with potential resistance against the toxic effects of heavy metals (Zuo et al. 2023). The phylum *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were found to be prevalent in the rhizosphere soil as major bacterial groups (Dai et al. 2018). Previous studies have demonstrated that *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* are key endophytic plant growth-promoting rhizosphere bacteria. These bacteria stimulate plant growth and development, enhance resistance to biotic and abiotic stress, and perform various functions such as internal environmental protection and carbon compound metabolism (Upadhyay et al. 2022). These factors may contribute to their high abundance within the bacterial community in plant rhizosphere soil.

According to Fierer et al., microorganisms can be classified into copiotrophic and oligotrophic phyla (Fierer et al. 2007), with the copiotrophic organisms exhibiting high growth rates in nutrient-rich environments, while oligotrophic organisms dominate in conditions of low nutrient availability. Fierer et al. observed that *Betaproteobacteria* and *Bacteroidetes* were more inclined to display copiotrophic characteristics, whereas *Acidobacteria* showed a tendency towards oligotrophic properties. Additionally, studies indicated that *Actinobacteria* also tended to exhibit copiotrophic traits (Dopheide et al. 2023). Figure 7A, depicting indicators of soil bacteria and fertility status, reveals a positive correlation between higher abundance of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* showed a certain positive correlation with soil fertility, indicating copiotrophic attributes. Simultaneously, *Acidobacteria* with the same high abundance exhibited a negative correlation with pH (Fig. 7A). The pH of rhizosphere soil increased during the growth of pioneer plants (Fig. 2A), resulting in a decrease in the abundance of *Acidobacteria*, which tend to manifest oligotrophic properties. These findings collectively demonstrate that pioneer plant growth directly or indirectly influences microbial activity, enhancing soil health and increasing the richness and diversity of soil microbial communities.

Most of the functional gene (Fig. 8) have an impact on the rate of heavy metal enrichment and translocation to the plant roots (Sheoran et al. 2016; Steingräber et al. 2022; Feng et al. 2023), and participate in the process of controlling heavy metals in Mn tail mining area. The region exhibited a high abundance of genes involved in metabolic function, reflecting the adaptability of soil microorganisms to the stress of heavy metal pollution (Harindintwali et al. 2020; Qureshi et al. 2024). Among the highly abundant bacteria phyla, *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* have chemoheterotrophic functions, which affect the metabolism modes of chemoheterotrophic and aerobic chemoheterotrophy (Fig. 8B). Excessive Mn(II) can disrupt chloroplast structure, leading to decreased chlorophyll synthesis and photosynthetic rate (Kobayashi et al. 2016). It also affects the abundance of genes related to the metabolism of chemoheterotrophy, aerobic chemoheterotrophy, and chloroplasts. Additionally, functions such as urea catabolism and lignin decomposition were found to be associated with the soil nitrogen cycle (Bani et al. 2018; Zhang

et al. 2019), and their functional abundance showed a positive correlation with the phylum *Actinobacteria* (Elframawy et al. 2022). In this study, the abundance of urea decomposition functional groups in GZC (2.04%) and XND (1.32%) were higher than other pioneer plants (0.30%-0.77%), and the abundance of cellulose hydrolysis functional groups in GZC (0.11%) and XND (0.10%) were also higher than other plants (0.004%-0.04%). The abundance of GZC (35.3%) and XND (33.9%) was also higher than that of other pioneer plants (17.8%~27.9%), which confirmed the correlation between the two and demonstrated the role of soil microorganisms in the biogeochemical cycle of nutrients (Yang et al. 2022).

Conclusion

The soil in Daxin Mn tailing ponds in Guangxi was acidic and nutrient poor. However, the natural settlement growth of pioneer plants has been found to increase soil pH, positively impacting a variety of environmental factors and microbial activities. Additionally, it leads to improvements in the physical and chemical properties of the manganese tailings pond soil, enhanced soil enzyme activity, and a reduction in the content of available metals in the soil. Notably, the BTF values of *Bidens pilosa* (GZC), *Pueraria montana* (GT), *Buddleja asiatica* (BBF), and *Crotalaria albida* (XND) are close to or greater than 1 for the three heavy metals, indicating their strong ability to absorb and transfer heavy metals from underground to above-ground. Furthermore, these pioneer plants have been shown to effectively increase the richness and diversity of bacterial communities, making them suitable for use as habitat improvement plants during the initial ecological restoration of manganese tailings ponds. At the phylum level, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were found to be the dominant bacterial communities that are positively correlated with soil nutrient levels and significantly associated with the BTF values of Mn, Cd, and Cu, thereby promoting the absorption of heavy metals by plants. Rhizosphere soil microorganisms of pioneer plants respond to heavy metal pollution by regulating community structure and influencing metabolic function. This community participated in the process of heavy metal control by regulating environmental information processing in membrane transport

metabolism. Additionally, it induced gene transcription to cope with the stress of Mn metal pollution. Functional prediction analysis using FAPROTAX revealed that chemoenergetic chemoheterotrophy, aerobic chemoheterotrophy, and chloroplast synthesis were the main metabolic modes observed.

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Data availability Data are contained within the article.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose that could have appeared to influence the work reported in this paper.

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