

Mechanisms of response of rare and abundant species in rhizosphere soils of *Coriaria nepalensis* Wall. to heavy metal remediation of lead-zinc tailings

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Abstract

The increasing environmental pollution caused by metal tailings has become a global environmental problem. Rhizosphere microorganisms play a key role in phytoremediation of heavy metal pollution. However, the role of abundant and rare species in the phytoremediation of tailings remains to be investigated further. The rhizosphere and non-rhizosphere soils of *Coriaria nepalensis* Wall. in tailings and non-tailings areas were collected separately and Geochemical properties, soil enzyme activity and heavy metal content were measured. Differences between abundant and rare species were also exposed through macrogenomes and macro-metabolomes. The results show that due to the strong enrichment of heavy metals such as lead and zinc, heavy metals in the rhizosphere soil inhibited soil nutrient cycling. They exacerbated the resistance mechanism of rhizosphere microorganisms to heavy metals. The two ecological strategies of abundant and rare species to cope with heavy metal stress were elaborated through the joint analysis of metagenome and metabonomics. The abundance of species was significantly higher than that of rare species in most gene expressions, and they relied on gene expression to improve their tolerance and maintain their basal survival and reproduction. Rare species, on the other hand, play an important role in the expression of key genes (e.g., *cdhD*, *cdhE*, *CHS1*, *yesX*, *pelC*, *6GAL*, *PIGL*, *GES3_5*, *CES1*, *iaaM*, *czcA*, *NIT-6*, *sor*) as well as in the secretion of metabolites, responding to the dynamic stresses through inducible metabolites. We found that rare species play a more critical role in the phytoremediation of tailings.

Introduction

Metal tailings are solid wastes formed by the long-term accumulation of slag, waste liquids, and other wastes generated during mineral extraction and processing (Alvarez-Rogel et al. 2022). They are widely distributed and cause serious harm to the global ecosystem (Gallego et al. 2021). Due to their tiny particles, they generate large-scale air pollution under the wind (Gil-Loaiza et al. 2018; Ramdial et al. 2021). Precipitation and surface runoff can cause heavy metal pollutants to spread and seep into the ground, contaminating the soil (Geng et al. 2022; Martinez-Carlos et al. 2022). Soils in tailings areas are often characterized by poor structural stability, low nutrient and organic content, and high concentrations of heavy metals (Li et al. 2022b; Sun et al. 2018). As a result, most of the plants in the tailings area are difficult to survive, and the vegetation cover is low (Cross et al. 2017). The ecological restoration and management of tailings areas has become a hot global issue.

Traditional physical and chemical remediation techniques are subject to various limitations in addressing heavy metal contamination of soils in tailings areas (Saxena et al. 2020). Due to the super-enrichment ability of certain plants for heavy metal elements, the use of plants to remediate heavy metals in tailings soils has become a widely used, low-cost, and non-secondary pollution method (Hou et al. 2020a). Xin et al. found that manzanita (*Miscanthus sacchariflorus*) could colonize copper tailings and showed great potential for remediation (Xin et al. 2024). Su et al. also conducted a related study and found that improved oleander (*Nerium indicum*) has strong heavy metal remediation capacity in lead and zinc tailings (Su et al. 2022). *Coriaria nepalensis* Wall. is a non-leguminous nitrogen-fixing plant with a

strong osmotic adjustment function, drought tolerance, and nitrogen-fixing ability to restore nutrient-poor degraded land (Awasthi et al. 2022; Mourya et al. 2018; Zeng et al. 2021). Zhu et al.'s study also showed that *Masan* plays an important role in the self-remediation process of lead-zinc tailings (Zhu et al. 2025). In addition to enriching heavy metals in the soil, the plant improves soil quality, promotes soil nutrient cycling, and optimizes the soil microbial community (Beiyuan et al. 2021).

Rhizosphere microorganisms act as key players in the plant restoration of tailings soils (Li et al. 2022a; Santini et al. 2018; Wagg et al. 2018). They contribute to establishing stable and sustainable below-ground ecosystems, including pH regulation (Santini et al. 2016), accumulation of organic nutrients (Sun et al. 2020), and assisting plants in the uptake of heavy metal contaminants in the soil (Narayanan and Ma 2023). The abundance and distribution of microbial taxa in soil are heterogeneous and can be categorized into abundant and rare species according to their abundance (Sogin et al. 2006). Abundant species usually occupy core ecological niches and participate in more biogeochemical cycles, which gives them greater environmental resilience (Jiao et al. 2019; Liang et al. 2020). Rare species, on the other hand, are often neglected (Xu et al. 2021). However, it has recently been shown that rare species are highly genetically diverse and functionally redundant (Chen et al. 2020), allowing them to play an important role in maintaining microbial diversity and ecosystem functioning (Jousset et al. 2017; Lynch and Neufeld 2015), and even driving key ecosystem functions (Pedrós-Alió 2007). In addition, abundant and rare species respond to environmental change differently (Ji et al. 2020). Du et al. found that rare species responded more rapidly to environmental change than abundant species, ultimately becoming enriched species that maintained ecosystem stability and improved ecosystem function (Du et al. 2020). Of course, this response to environmental change is not constant (Liang et al. 2020; Liu et al. 2015). When the soil was contaminated with heavy metals, most of the rare species were wiped out, and microbial diversity decreased dramatically (Gans et al. 2005). However, some studies have presented opposite results, suggesting that rare species diversity and community composition are more stable when subjected to Cu stress (Kurm et al. 2019). These rare species may be dormant or extremely slow-growing. When the environment is transformed to favor their growth and reproduction, they are activated or even become abundant (Shade et al. 2014). Therefore, it is necessary to distinguish between the roles of abundant and rare species in our study of phytoremediation of soil heavy metal pollution in tailings areas.

Based on the above-mentioned lack of research, this study aimed to investigate the effects of abundant and rare species on nutrient cycling and the heavy metal content of inter-root and non-inter-root soils of *Morus alba* under different pollution concentrations of tailings. We collected inter-root and non-inter-root soils of *Matsan* from tailings and non-tailings areas, respectively, for the study. The main contents of this study are: (1) to reveal the effects of the inter-root microbial community of *Matsan* on the microenvironment of Pb-Zn tailings soils under different pollution concentrations of tailings; (2) to reveal the differences in gene expression and metabolite secretion between abundant and rare species on the nutrient cycling and heavy metal resistance related to tailings soils with different pollution concentrations through the combined analysis of macro-genomes and macro-metabolomes, and to

further reveal which taxonomic groups (abundant or rare) play a significant role in the process of tailings heavy metal remediation.

Materials and methods

Study sites and Experimental design

The lead-zinc tailing area (E104°34'16", N27°03'31") in Hezhang County, Bijie City, Guizhou Province, was selected for this study. The area, with an average elevation of 1995 m above sea level, has a subtropical continental monsoon climate, with an average annual temperature range of 12.5–13.8°C and annual precipitation between 790.4 and 910.3 mm. The regional soil types are mainly yellow and red soils, and the territory contains a variety of metallic and non-metallic minerals, totaling 25 species, of which the heavy metals with the highest content in the soil are copper, zinc, lead, cadmium, chromium, and arsenic.

After a field visit to the site, we found that the site of local lead smelting and the area of tailings accumulation are undergoing an ecological restoration. In the tailings dump area, *Coriaria nepalensis* Wall. has become a dominant population in the test area, with a wide variety of species and distribution. Previous studies have also shown that in the Pb-Zn tailings area, the plant not only contributes to the stabilization of the soil structure under the canopy but also increases the local resources of the soil, thus triggering the “fertilizer island” effect (Yang et al. 2023). Therefore, in this study, the rhizosphere soil of *Coriaria nepalensis* Wall. was selected as the research object, and two sampling sites were set up the tailings area (H), and non-tailings area (L) to collect soil samples from the rhizosphere (R) and non-rhizosphere (NR) areas, with three samples from each group, and a total of 12 soil samples were collected (Fig. S1). All soil samples were divided into two parts, and one part was stored at -4°C to detect soil physicochemical properties, soil heavy metal content, and soil enzyme activities. The other part was stored at -80°C for macro-genomic and macro-metabolomic assays.

Determination of soil physical and chemical properties

Soil organic matter (SOM) was determined by the $K_2Cr_2O_7$ - H_2SO_4 oxidative external heating method. Soil pH was measured by a potentiometric method in 1:2.5 (w/v) soil-water suspension. After sulfuric acid digestion, total phosphorus (TP) was measured by molybdenum-antimony antimony spectrophotometry. Soil-effective phosphorus (AP) was measured by phosphomolybdenum blue spectrophotometry. Soil ammonium nitrogen (NH_4^+ -N) and nitrate nitrogen (NO_3^- -N) were analyzed using potassium chloride with the addition of sodium salicylate and hydrazine sulfate to produce a color reaction, and absorbance was measured spectrophotometrically at 660 nm and 550 nm. Total nitrogen (TN) was determined using the Kjeldahl method.

Determination of heavy metals in soil

Soil samples were laid flat in numbered order in a ventilated cool place to dry naturally. Impurities such as dead branches and debris were sieved, ground, passed through a 100-mesh nylon sieve and stored for

later use. Weighing 0.1 g of the sample was placed in a polytetrafluoroethylene airtight digestion jar, and 4 mL of HNO_3 , 1 mL of HF, and 1 mL of HClO_4 were added for digestion. After microwave treatment, the ablation jar was placed on an electric hot plate and heated to drive out the acid until the residue became a viscous colorless or light yellow liquid. Subsequently, 10 mL of aqua regia (HCl to HNO_3 ratio of 3:1) was added for secondary digestion. After digestion, the sample was transferred to a 50 mL volumetric flask, fixed, and filtered through a 0.22 μm filter membrane after sufficient shaking. Inductively coupled plasma emission spectroscopy (ICP-OES) determined the contents of Cu, Zn, Cd, Pb, and Cr. The As content of the soil was determined by atomic fluorescence method. The soil was ground until it passed through a 200-mesh nylon sieve, and 1 g of the sample was placed in a 50 mL stoppered colorimeter tube, 10 mL of aqua regia was added, and the sample was digested in a boiling water bath for 2 hours. After digestion, the sample was cooled and diluted with purified water. Pipette a small amount of digest into a 50 mL colorimetric tube and add 3 mL of HCl , 5 mL of 5% thiourea, and 5 mL of 5% ascorbic acid sequentially. After dilution with purified water, vortexing, and mixing for 30 seconds, the As content in the soil was measured.

Definition of different types of microbes in communities

Microbial communities are usually defined and categorized in two ways, one based on the relative abundance of microorganisms and the other based on the ecological role of microorganisms in the community (Geng et al. 2024). Based on regional differences in microbial relative abundance, we can categorize them into abundant species ($> 0.1\%$), intermediate species ($0.001\%-0.1\%$), and rare species ($< 0.001\%$) (Liang et al. 2020). In addition, we defined key microbial species based on their ecological roles in the community through covariate network analysis. Species weights were defined based on intra-module connectivity (Z_i) and inter-module connectivity (P_i) (Wu et al. 2023). Specifically, they were categorized into module hubs ($Z_i \geq 2.5$, $P_i < 0.62$), connectors ($Z_i < 2.5$, $P_i \geq 0.62$), and network hubs ($Z_i \geq 2.5$, $P_i \geq 0.62$), and peripherals ($Z_i < 2.5$, $P_i < 0.62$) (Deng et al. 2012).

Soil genome extraction and analysis

Total genomic DNA was extracted from soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-TEK) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using TBS-380 and NanoDrop2000, respectively. The quality of the DNA extracts was checked by 1% agarose gel. DNA-extracted fragments were constructed using Covaris M220 (Gene Company Limited, China), and the average fragment size was about 400 bp. Paired-end libraries were constructed using NEXTflex™ Rapid DNA-Seq (bioscience, Austin, TX, USA). Adapters containing the complete complement of sequencing primer hybridization sites were ligated to the blunt ends of the fragments. Paired-end sequencing was performed on Illumina NovaSeq/Hiseq Xten from Illumina Inc. in Shanghai, China, using the NovaSeq kit/Hiseq X kit according to the manufacturer's instructions. The full, uncropped gel and blot images are presented in Fig. S2.

Raw reads from macro genome sequencing were used to generate clean reads by removing adaptor sequences, trimming, and removing low-quality reads (N-base reads with a minimum length threshold of 50 bp and a minimum quality threshold of 20) using fast on the free online platform Majorbio Cloud platform (Cloud. major bio.com). These high-quality reads were then assembled into contigs using MEGAHIT (parameters: kmer_min = 47, kmer_max = 97, step = 10), which utilizes the concise de Bruijn plot. Contigs with a length greater than or equal to 300 bp were selected as the final assembly result. Open reading frames (ORFs) in the contigs were identified using MetaGene. ORFs greater than or equal to 100 bp in length were extracted and translated into amino acid sequences using the NCBI translation table. Non-redundant gene catalogs constructed using CD-HIT had 90% sequence identity and 90% coverage. Quality-controlled Reads were localized to the non-redundant gene catalog with 95% concordance using OAPaligner, and gene abundance was assessed in each sample. Representative sequences of non-redundant genes were tagged in the NCBI NR database using blastp implemented in DIAMOND and classified and annotated with DIAMOND with an e-value cutoff of $1e^{-5}$. Homologation of representative sequences was performed on the eggNOG database (version 4.5.1) using Diamond Protein group (COG) annotation was performed on the eggNOG database (version 4.5.1) using Diamond with an e-value cutoff of $1e^{-5}$. KEGG annotation was performed on the Kyoto Encyclopedia of Genes and Genomes database using Diamond with an e-value cutoff of $1e^{-5}$.

Soil metabolite extraction and determination

Metabolite extraction was carried out by taking 50 mg of soil sample in a 2 mL centrifuge tube, adding 6 mm grinding beads, and using 400 μ L of methanol: water (4:1 v:v) extract containing 0.02 mg/mL of internal standard (L-2-chlorophenylalanine). The extracts were ground in a frozen tissue grinder for 6 min (-10°C, 50 Hz), followed by ultrasonic extraction at 5°C for 30 min. The samples were allowed to stand at -20°C for 30 min and then centrifuged at 4°C for 15 min at 13,000 g. The supernatants were taken into the samples for analysis. Quality control (QC) samples were prepared, and a QC sample was inserted in every 5–15 samples during the analysis to monitor analytical reproducibility.

LC-MS/MS analysis was performed using a Thermo Fisher UHPLC-Q Exactive HF-X system. Chromatographic conditions were three μ L of sample separated on an HSS T3 column (100 mm $\times$ 2.1 mm i.d., 1.8 μ m) with mobile phase A of 95% water + 5% acetonitrile (with 0.1% formic acid) and mobile phase B of 47.5% acetonitrile + 47.5% isopropanol + 5% water (with 0.1% formic acid) at a flow rate of 0.40 mL/min and a column temperature of 40°C. The chromatographic conditions were as follows. Mass spectrometry conditions: positive and negative ion scanning, scanning range 70-1050 m/z, sheath gas 50 psi, auxiliary gas 13 psi, auxiliary gas heating 425°C, ion spray voltage 3500 V in positive mode and -3500 V in negative mode, transfer tube 325°C, collision energy 20-40-60 V cycles, primary mass spectrometry resolution of 60,000, secondary mass spectrometry resolution of 7,500, with a DDA mode acquisition.

Firstly, the LC-MS raw data were imported into Progenesis Q1 software for processing, baseline filtering, peak identification, and integration to obtain the data matrix of retention time, mass-to-charge ratio, and

peak intensity. After that, the mass spectrometry information was matched with HMDB, Metlin, and Meiji's self-built library, and finally, the metabolite information was obtained. The data were uploaded to the Meggie cloud platform for analysis, and the preprocessing included the 80% rule to remove missing values, the sum normalization method to normalize the mass spectrometry peak response intensities, deletion of variables with RSD > 30%, and log10 logarithmic processing.

Statistical Analysis

Statistical analysis of all data in this study was done through SPSS 27.0 software; for comparisons of differences between groups, a one-way ANOVA combined with Duncan's multiple range test was used, with the significance threshold set at $p < 0.05$. To ensure that the data met the prerequisites for parametric tests, the original data set was subjected to a Kolmogorov-Smirnov normality test (K-S test) with Levene's variance chi-square test (Levene's test) on the original data set. For variables that did not pass the variance chi-square test ($p < 0.05$), data were corrected using the natural logarithmic transformation ($\ln(x + 1)$), the arcsin $\sqrt{x}$ square root transformation ($\arcsin\sqrt{x}$), or the Box-Cox transformation (λ -values were optimized by maximum likelihood) until the variance chi-square requirement was met (corrected Levene's test $p > 0.05$). Visual analysis and interactive presentation of the macroeconomics data and metabolomics data were completed by the Majorbio Cloud platform (www.majorbio.com). The rest of the graphs were jointly drawn using Origin 2024, Gephi 0.9.2, and R language to ensure the accuracy and reproducibility of the scientific visualization results.

Results

Geochemical properties, soil enzyme activity and heavy metal content of the study area

The geochemical properties are shown in Fig. 1. pH was significantly higher in tailings than non-tailings areas, while there was no significant change between rhizosphere and non-rhizosphere soil. TN was significantly higher in tailings than non-tailings areas, where rhizosphere was significantly lower than non-rhizosphere in tailings areas, while the difference was not significant in the non-tailings areas. NO_3^- -N was significantly lower in tailings than non-tailings areas of rhizosphere soil, whereas the difference was insignificant in non-rhizosphere soil. NH_4^+ -N showed the opposite trend, with insignificant differences in the rhizosphere soil but significantly higher in tailings than non-tailings in the non-rhizosphere soil. TP was significantly lower in the non-tailings of rhizosphere soil than in the other three treatment groups. In contrast, AP was significantly higher in the non-tailings of rhizosphere soil in the other three treatment groups. SOM was significantly higher in tailings than non-tailings areas of non-rhizosphere soil, while the difference was insignificant in rhizosphere soil.

We examined the enzyme activities of soil samples from four treatment groups. We showed that S-NAG was higher in tailings than non-tailings and significantly lower in rhizosphere than non-rhizosphere soil. S-FDA was significantly higher in tailings areas of non-rhizosphere soil than in the other three treatment groups. S- α -GC was significantly lower in rhizosphere than non-rhizosphere soil of non-tailings areas,

whereas no significant difference was observed in tailings area. S-LAP was significantly different under the four treatments, highest in rhizosphere soil of non-tailings areas and lowest in the rhizosphere soil of tailings areas. S-CL was insignificantly different under the four different treatments. S-ACP in rhizosphere soils of tailings areas was significantly lower than the other three treatment groups. S-UE was significantly higher in non-tailings than tailings areas, and there was an insignificant difference between rhizosphere and non-rhizosphere soil. S-Phytase was significantly higher in non-tailings than tailings areas of rhizosphere soil and higher in the rhizosphere than non-rhizosphere soil, and there was no significant difference between tailings and non-tailings areas of non-rhizosphere soil. S- β -XYS was significantly higher in the rhizosphere than non-rhizosphere soil and in tailings than non-tailings areas. S-C1 was not significantly different under the four different treatments. S-SC was significantly higher in tailings than non-tailings areas and significantly higher in rhizosphere than non-rhizosphere soil of tailings areas.

In addition, we examined the heavy metal content of the four treatment groups and found that As, Cd, Pb, and Zn were significantly higher in tailings and non-tailings areas. As was significantly lower in rhizosphere than in non-rhizosphere soil of both tailings and non-tailings areas. Cd was significantly lower in rhizosphere than non-rhizosphere soil of tailings areas and insignificantly in non-tailings areas. Pb and Zn were significantly higher in rhizosphere than non-rhizosphere soil of tailings areas and insignificantly between rhizosphere and non-rhizosphere soil of non-tailings areas. Cr and Cu were both lower in tailings than non-tailings areas. Cr was significantly higher in rhizosphere than non-rhizosphere soil of tailings areas, and the opposite was true in non-tailings areas, while Cu was significantly lower in rhizosphere than non-rhizosphere soil of non-tailings areas, and the difference was not significant in tailings areas.

Microbial diversity and community structure

The PCoA analysis based on the Bray-Curtis distance matrix showed significant separation between tailings and non-tailings areas, while there was no significant separation between rhizosphere and non-rhizosphere soil (Fig. 2A). We analyzed species differences at the species level. We showed that the number of species in rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas, and non-rhizosphere soil of non-tailings areas were 28908, 22207, 28871, and 21667, respectively (Fig. 2B). We also performed α diversity indices (i.e., Sobs, Shannon, Pielou-e, and Chao1) analyses, which showed no significant difference between rhizosphere and non-rhizosphere soil (Fig. 2C-F). Species richness was significantly higher in tailings than non-tailings areas of rhizosphere and non-rhizosphere soil ($P < 0.001$; Fig. 2C, D). Species diversity was significantly higher in tailings than non-tailings areas of non-rhizosphere soil ($P < 0.05$; Fig. 2E). The evenness of community distribution did not differ significantly among the four treatments ($P > 0.05$; Fig. 2F).

The microbial community structure under the four treatment groups was analyzed based on macrogenome sequencing results. Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi were the dominant microorganisms at the phylum level (Fig. 3A). At the species level, *Acidobacteria*

bacterium, *Chloroflexi bacterium* and *Betaproteobacteria bacterium* were the dominant microorganisms (Fig. 3B). We constructed co-linear networks for all microorganisms and screened key species from them, including module hubs, connectors and network hubs (Fig. 3C). Subsequently, we further characterized the distribution of dominant bacterial species at the species level under different treatments (Fig. 3D-G). *Chloroflexi bacterium* and *Betaproteobacteria bacterium* were the dominant species in tailings areas. In contrast, *Acidobacteria bacterium* and *Candidatus Rokubacteria bacterium* were the dominant species in non-tailings areas. Screening was done for abundant and rare species under four treatments (Fig. 3H-K). The results showed that the highest relative abundance of abundant species (77.89%) was found in non-rhizosphere soil of non-tailings areas and the highest relative abundance of rare species (4.42%) was found in non-rhizosphere soil of tailings areas. We found that the relative abundance of rare species was higher in tailings than non-tailings areas of rhizosphere and non-rhizosphere soil.

The drivers of microbial community variation

To determine the effect of physicochemical factors and heavy metal pollution on microbial communities, we performed a redundancy analysis (RDA), in which there was a significant difference between the communities in tailings and non-tailings areas (Fig. 4A, B). The most significant physicochemical factors affecting soil microbial communities were pH, NO_3^- -N, NH_4^+ -N, SOM, and TP. As, Cr and Zn were the most significant heavy metal elements affecting soil microbial communities (Table S1). Afterward, we analyzed the linear correlation between the screened environmental factors and the microbial community diversity under the four treatments (Fig. 4C-J). The results showed that As ($R^2 = 0.68$, $P < 0.01$) and Cr ($R^2 = 0.75$, $P < 0.01$) were the most strongly correlated environmental factors with microbial diversity. Microbial diversity increased with increasing As and decreased with increasing Cr. High correlations were also found between NH_4^+ -N ($R^2 = 0.4$, $P = 0.026$), SOM ($R^2 = 0.44$, $P = 0.019$), and Zn ($R^2 = 0.56$, $P = 0.0051$) and microbial diversity, all of which increased with increasing levels. Based on the analysis of microbial communities in different treatments, we conducted a Mantel test analysis (Fig. 4K-N) of the correlation between environmental factors and abundant and rare species under the four treatments. The results showed that abundant and rare species' driving patterns differed among the four treatments.

Functional genes of abundant and rare communities

Based on macroeconomic analysis, we screened for functional genes related to carbon fixation, carbon decomposition, plant growth promotion, metal(loid) resistance, nitrogen cycle, phosphorus cycle, and sulfur cycle (Fig. 5). The results showed that the relative abundance of functional genes was higher in tailings than non-tailings among the abundant species. The relative abundance of functional genes was significantly higher in abundant species than in rare species, while specific functional genes were expressed only in rare species. The carbon fixation was *cdhD*, *cdhE*, and K15038 (Fig. 5A), carbon decomposition was *CHS1*, *yesX*, *pelC*, *6GAL*, *PIGL*, *GES3_5* and *CES1* (Fig. 5B), plant growth promotion

was *iaaM* (Fig. 5C), metal (loid) resistance was *czcA* (Fig. 5D), nitrogen cycle was NIT-6 (Fig. 5E), and sulfur cycle was *sor* (Fig. 5G).

Potential metabolites associated with key microbial taxa

Metabolomics analyses of 12 soil samples were performed on the UPLC-MS/MS platform. PCA was used to analyze the differences between the four treatment samples (Fig. 6A). The results showed that the metabolites of the four treatment groups were separated, indicating significant differences between treatments. We also screened for differential metabolites between rhizosphere and non-rhizosphere microorganisms of tailings and non-tailings areas (Fig. 6C-F). 392 up-regulated differential metabolites and 264 down-regulated differential metabolites were identified under RH vs. RL (Fig. 6C), 258 up-regulated differential metabolites and 145 down-regulated differential metabolites were identified under RH vs. NRH (Fig. 6D), 15 up-regulated differential metabolites and 40 down-regulated differential metabolites were identified under RL vs. NRL (Fig. 6E), and 229 up-regulated differential metabolites and 239 down-regulated differential metabolites were identified under NRH vs. NRL (Fig. 6F). Afterwards, the metabolites were clustered and analysed, and we found that the metabolites differed significantly among the four treatment groups (Fig. 6B). By VIP analysis, we screened out the top 30 clustered metabolites in terms of abundance, such as (5E,7E)-Undeca-2,5,7-trienedioylcarnitine, Salbutamol, 8-Hydroxy-5,6-octadienoic acid and Senecionine N-Oxide (Fig. 7). And further analysis of the correlation network between metabolites and seven types of functional genes revealed a significant synergistic or antagonistic regulatory relationship between the two.

Relationships between soil multifunction and microbial communities

Structural equation modeling (SEM) was carried out to account for the direct and indirect effects of the colonization of *Coriaria nepalensis* Wall. and heavy metal tailings on geochemical parameters, abundant and rare species, functional microbial genes, and microbial metabolites (Fig. 8, S3). The results showed that heavy metals had a significant positive effect (0.9902, $P < 0.001$) on soil environmental factors and a negative effect (0.0951, $P < 0.05$) at rhizosphere. Heavy metals had a negative effect on both abundant and rare species, while the opposite trend was observed in the rhizosphere, where both had a positive effect. Finally, we found that rare species exerted greater effects on microbial functional genes and microbial metabolites compared to abundant species. All of these effects were positive except for the negative effect of abundant species on microbial metabolites.

Discussion

Rhizosphere microorganisms influence the microenvironment of lead-zinc tailings soils

Navarro-Cano et al. found that pioneer plants could modulate the multifunctionality of soils in tailings areas under semi-arid conditions (Navarro-Cano et al. 2018). This is consistent with our results that show that rhizosphere microorganisms promote soil nutrient cycling in non-tailings areas. In contrast, nutrient cycling in rhizosphere soils of tailings areas was inhibited, in line with the trend of soil enzyme

activity changes (Fig. 1). This may be related to the degree of enrichment of heavy metals by *Coriaria nepalensis* Wall., which was significantly higher in rhizosphere than non-rhizosphere soils for As, Cd, Pb and Zn (Fig. 1). Plant colonization leads to changes in the microenvironment of the rhizosphere soil and the bioavailability of heavy metals is enhanced, thus promoting active uptake and accumulation of heavy metals by the root system (Zelaya-Molina et al. 2023). When the rhizosphere soil is enriched with heavy metals, it leads to poor soil nutrient status, which was confirmed by Geng et al. (Geng et al. 2023). In addition to the utilization of heavy metals by plants, pH also affects the soil organic matter content and the mobility of heavy metals, which ultimately leads to a decrease in soil fertility as well as the enrichment of heavy metals in rhizosphere soil (Feng et al. 2021; Warwick et al. 1998). The environmental constraints of tailings self-remediation mainly include physical factors, nutrient deficiencies, and negative impacts of pollutants (Wang et al. 2017). As, Cr, and Zn are the most critical environmental factors affecting tailings self-remediation (Fig. 4). Rhizosphere microorganisms have significant enrichment of heavy metals in the environment, but when the rhizosphere soil is overloaded with heavy metals then the diversity of microbial communities will be affected (Zhang et al. 2022).

Microorganisms significantly impact ecological processes and biogeochemical cycles (Hou et al. 2020b). In this study, we analyzed functional genes for nutrient cycling, heavy metal resistance, and plant growth promotion. The results showed that gene expression was significantly higher in rhizosphere than non-rhizosphere soil (Fig. 5). However, the results of gene expression for nutrient cycling showed an opposite trend to soil physicochemical properties, with significantly higher gene expression abundance in tailings than non-tailings areas, and significantly higher in rhizosphere than non-rhizosphere soil of tailings areas. This may be due to the accumulation of heavy metals in rhizosphere soil of plants. The plants were subjected to heavy metal stress, the soil nutrient cycling was inhibited, and the plants enhanced the expression of nutrient cycling genes through self-regulation to grow and develop (Zhao et al. 2023). When subjected to external stress, plants must grow and defend simultaneously to survive, thus creating a balance between growth and defense (Huot et al. 2014; Miao et al. 2018). This also explains that heavy metal resistance and plant growth-promoting genes were higher in rhizosphere than non-rhizosphere of tailings areas.

Rare species play a greater role in heavy metal remediation of lead-zinc tailings

We found that the composition of the rhizosphere microbial community of *Coriaria nepalensis* Wall. itself is altered during tailings self-remediation (Fig. 3). This is because plants subjected to heavy metal stress recruit dominant microorganisms through the root system, thus affecting the structure and composition of the rhizosphere bacterial community (Barbosa Lima et al. 2015). Further analyses showed abundant and rare species distribution characteristics differed in different treatment groups. The percentage of rare species was higher in tailings areas than non-tailings areas (Fig. 3). There were also differences in the responses of abundant and rare species to changes in environmental factors (Fig. 4). Enriched species have a great potential to promote cell movement and energy metabolism, which can help to build a stronger tolerance system under heavy metal stress (Qin et al. 2022). In addition, abundant species produce large amounts of hydrolytic enzymes that can help them utilize more

nutrients from the soil (Li et al. 2023). However, this function is not invariable, and Geng et al. found that rare species play an increasingly important role in the primary succession of tailings (Geng et al. 2024). We found that gene expression in nutrient cycling, heavy metal resistance, and promotion of plant growth remained significantly higher in abundant species than rare species, but rare species were expressed in key genes (Fig. 5). Covariance network analysis has been developed as an important tool in ecosystem studies (Li et al. 2021). Covariate network analysis of functional genes and key metabolites further revealed the differences between abundant and rare species during tailings self-remediation (Fig. 7). Most genes were significantly more expressed in the abundant species than rare species. In contrast, the rare species were regulated mainly by the secretion of key metabolites to resist heavy metal pollution. This difference reflects two ecological strategies, with abundant species relying on gene expression to improve their tolerance to maintain basal survival reproduction (Wei et al. 2019), whereas rare species respond to dynamic stress through inducible metabolites (Zhang et al. 2025). These findings suggest that abundant species dominate 'conservative' functions (e.g., basal metabolism), while rare species enhance system resilience through 'flexible' functions (e.g., secondary metabolite synthesis), and that the two synergistically maintain ecological stability during tailings remediation.

Although abundant species dominate in functional gene abundance (Fig. 5), several lines of evidence suggest that the contribution of rare species to tailings remediation is more critical. First, the relative abundance of rare species was significantly higher in tailings than non-tailings areas (Fig. 3), and their diversity was more sensitive to environmental factors (Fig. 4). Metabolomic data further supported the functional advantage of rare species. Rhizosphere soil of tailings areas is significantly enriched in rare species-associated metabolites (Fig. 6), which sequester heavy metals and act as signaling molecules to promote plant-microbe symbiosis (Chen et al. 2020). We predicted the functions of both enriched and rare species in the ecosystem (Fig. 8). The SEM results were consistent with previous findings, further suggesting that rare species play a greater role in the phytoremediation of tailings.

Conclusion

This study investigated microbial diversity, functional genes, and key metabolites in rhizosphere and non-rhizosphere soils of *Coriaria nepalensis* Wall., which had different tailing pollution concentrations. The results showed that *Coriaria nepalensis* Wall. has a significant enrichment potential for heavy metals, and its rhizosphere microbial community structure was altered, thus affecting soil nutrient cycling. Further screening of soil microbes showed that abundant and rare species applied two different ecological strategies in resisting heavy metal stress. Enriched species were significantly higher than rare species in most gene expression, relying on gene expression and increasing their tolerance to maintain basal survival and reproduction. In contrast, rare species played an important role in key gene expression (e.g., *cdhD*, *cdhE*, *CHS1*, *yesX*, *peIC*, *6GAL*, *PIGL*, *GES3_5*, *CES1*, *iaaM*, *czcA*, *NIT-6*, *sor*) as well as metabolite secretion, responding to the dynamic stress through inducible metabolites. Structural equation modeling suggests that rare species play more critical roles in the phytoremediation of tailings. These results help to broaden our understanding of the ecological roles of abundant, rare taxa in the phytoremediation of tailings.

Declarations

CRediT authorship contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Suxia Sun, Wei Zhao, Yutian Lv, and Junwei Zhang. Funding acquisition and supervision: Sixi Zhu. The first draft of the manuscript was written by Xianwang Du and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Kimi.ai for individual sentences to suggest individual words in order to improve language and readability. After using this tool, the authors reviewed and edited the affected sentences. AI was not used to produce content, and the authors take full responsibility for the content of the publication.

Declaration of competing interest

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

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Data availability

The raw data of macrogenome and macrometabolome will be Archived at a digital repository once accepted for publication.

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Figures

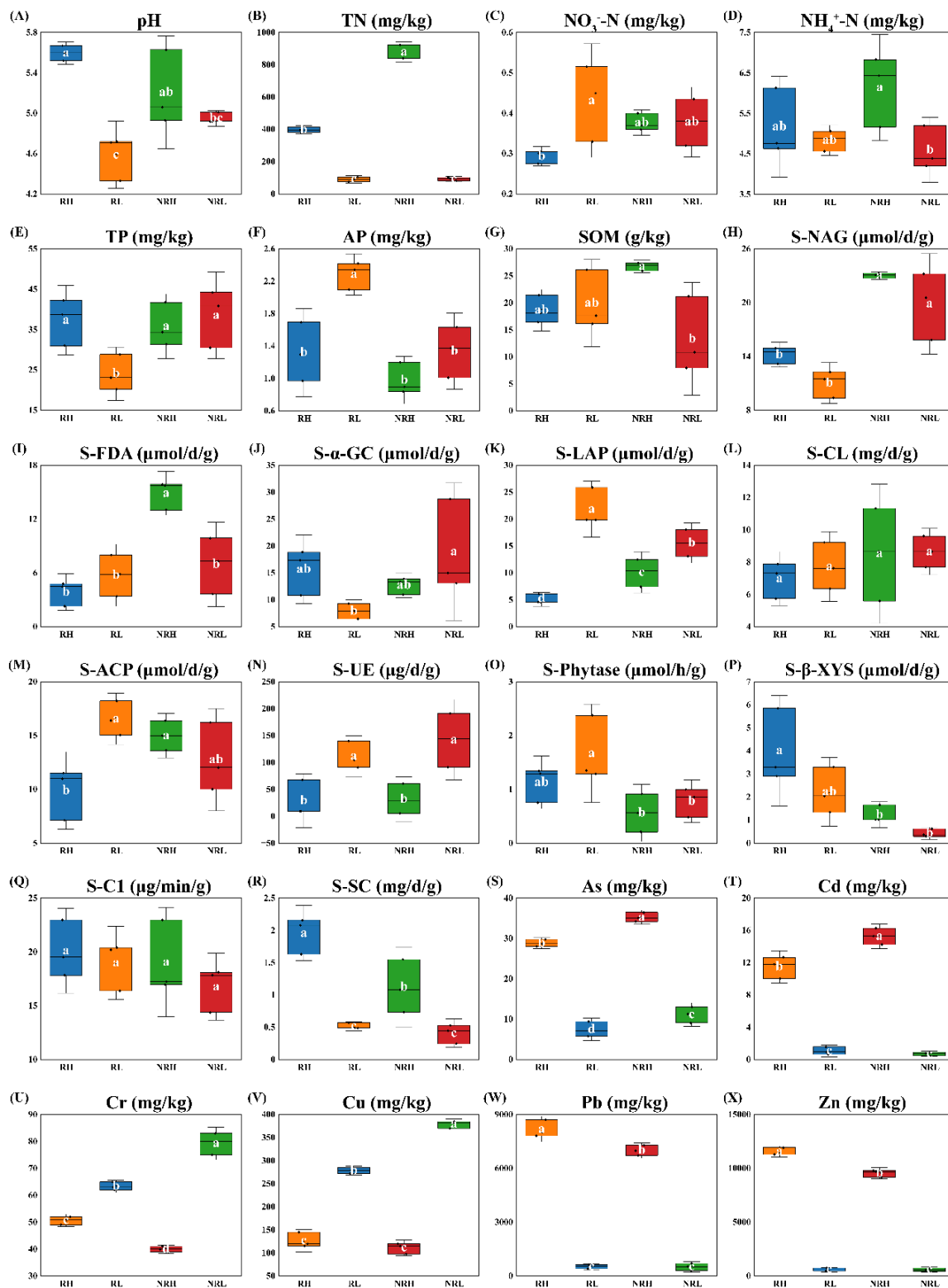


Figure 1

Geochemical properties, soil enzyme activity and heavy metal content in the study areas. Different letters indicate significant differences among four treatment groups. treatment groups. Geochemical properties are shown in Figures A–G, soil enzyme activity in Figures H–R and heavy metal content in Figures S–X. RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-

tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively. Significance is expressed in terms of a, b, c and d.

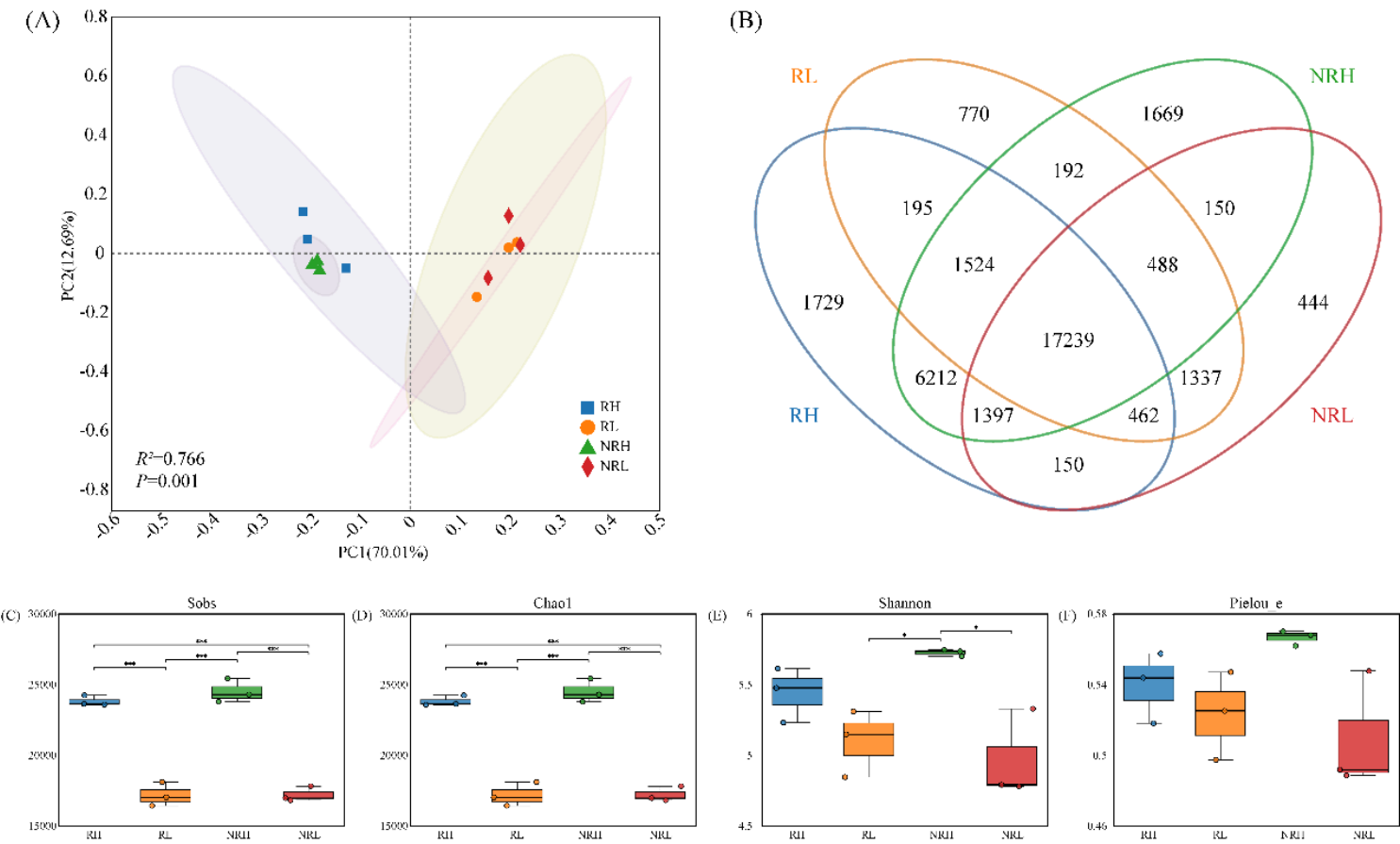


Figure 2

Principal component analysis (PCoA) results based on the Bray–Curtis distance matrix distance matrix for the four treatment groups (A). The ellipses denote 95 % confidence intervals. Venn diagram (B) of the shared species between the four treatment groups. Sobs (observed richness), Shannon (species diversity), Pielou-e (species evenness), and Chao1 (species richness) indices of the microbial (C, D, E and F) communities. The data are expressed as the mean ±standard error. RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively. *, P <0.05. **, P <0.01. ***, P <0.005.

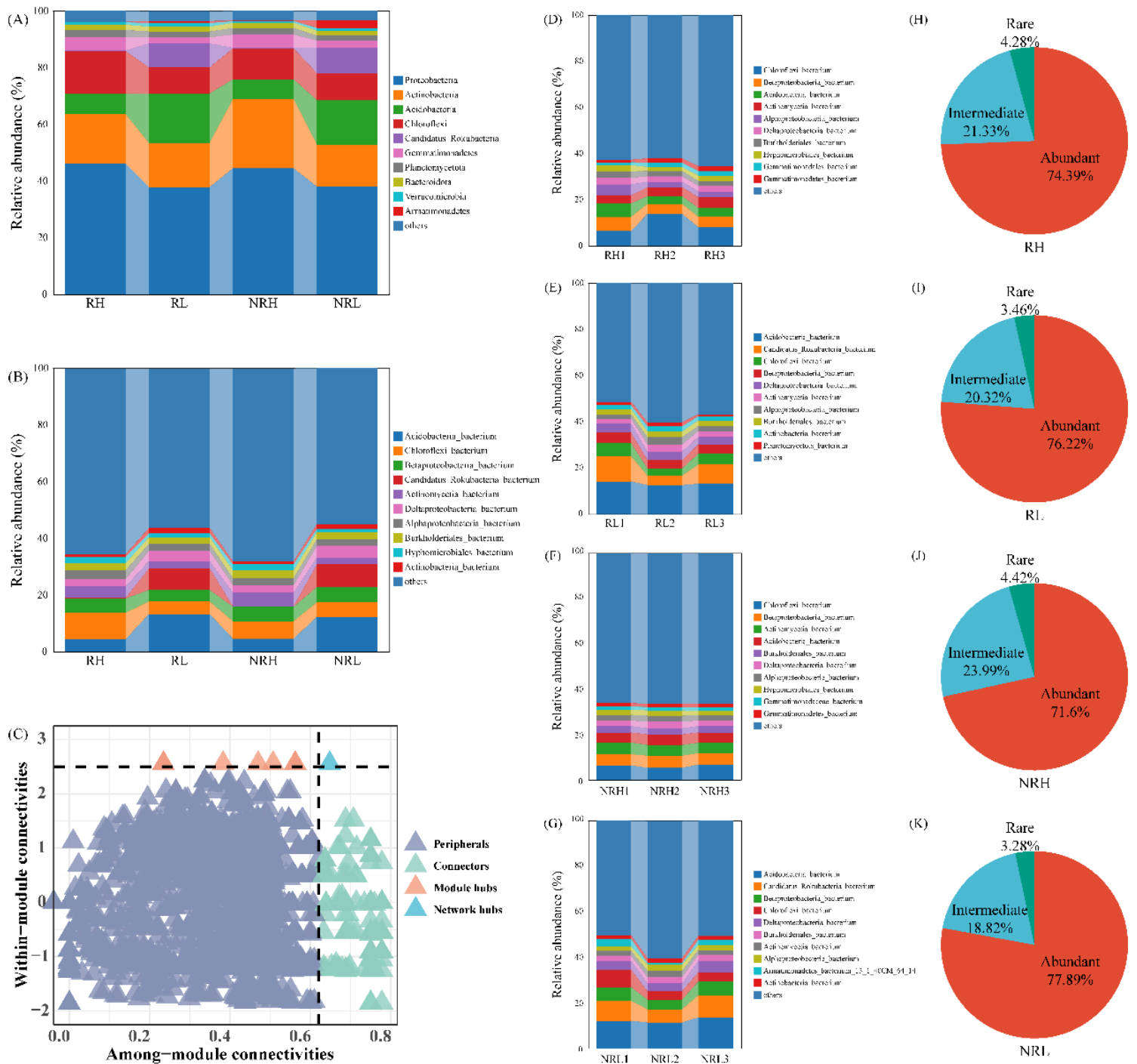


Figure 3

Microbial community characteristics under four treatments. Microbial community composition at the phylum level and species level, respectively. Figure C reveals the node topology of key species. Figures D–G represent the composition of dominant species in the microbial community, and Figures H–K represent the compositional structure of microbial species with different abundances in the community. RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively.

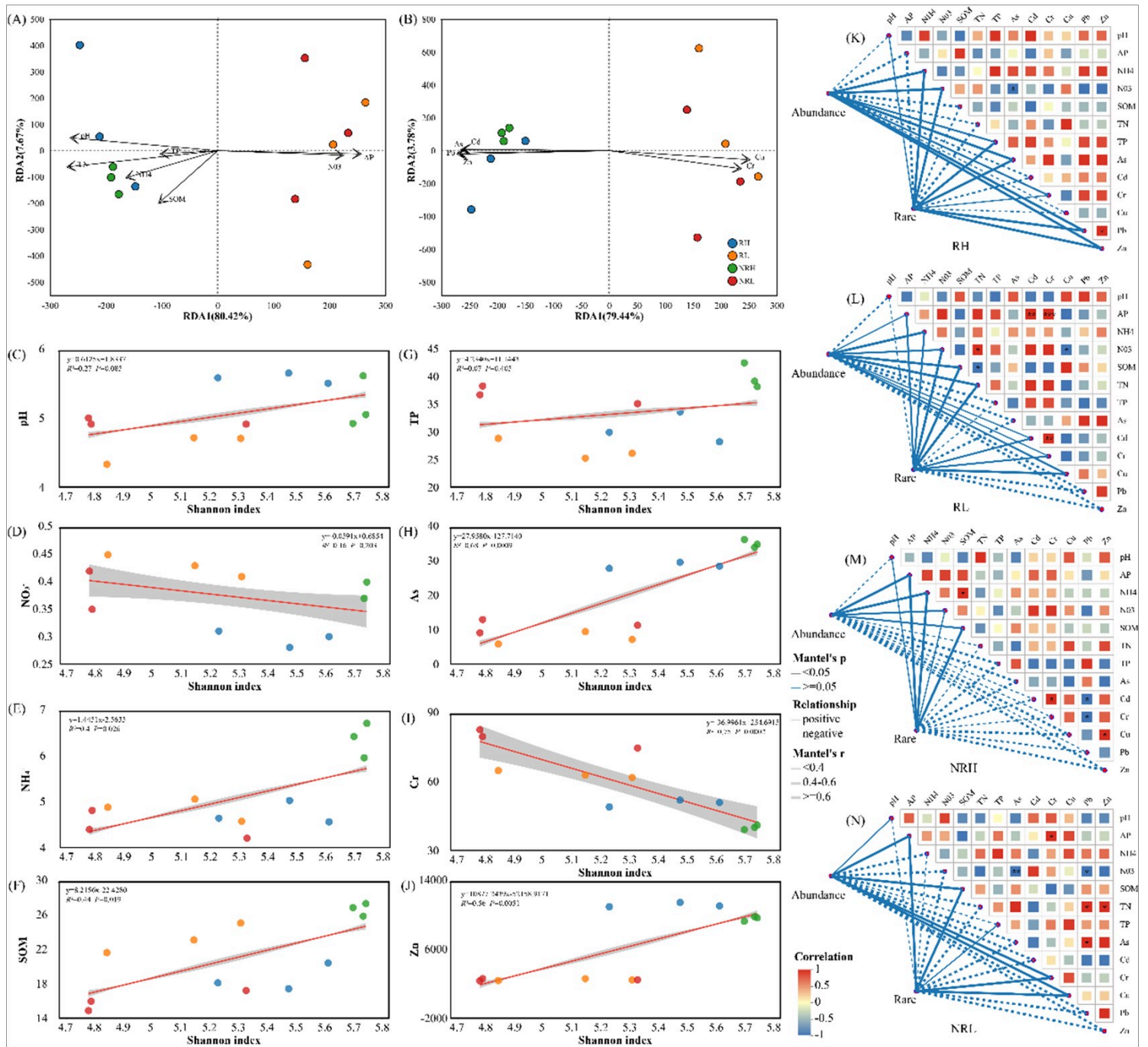


Figure 4

Driving effects of environmental factors on microbial communities involved in primary succession in tailings ponds in different states. Screening for key driving environmental factors of Geochemical properties (A) and heavy metal content (B). C–J, Linear regression analysis of key driving environmental factors and microbial diversity; K–N, Differences in the effects of environmental factors on abundant and rare species groups. RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively.

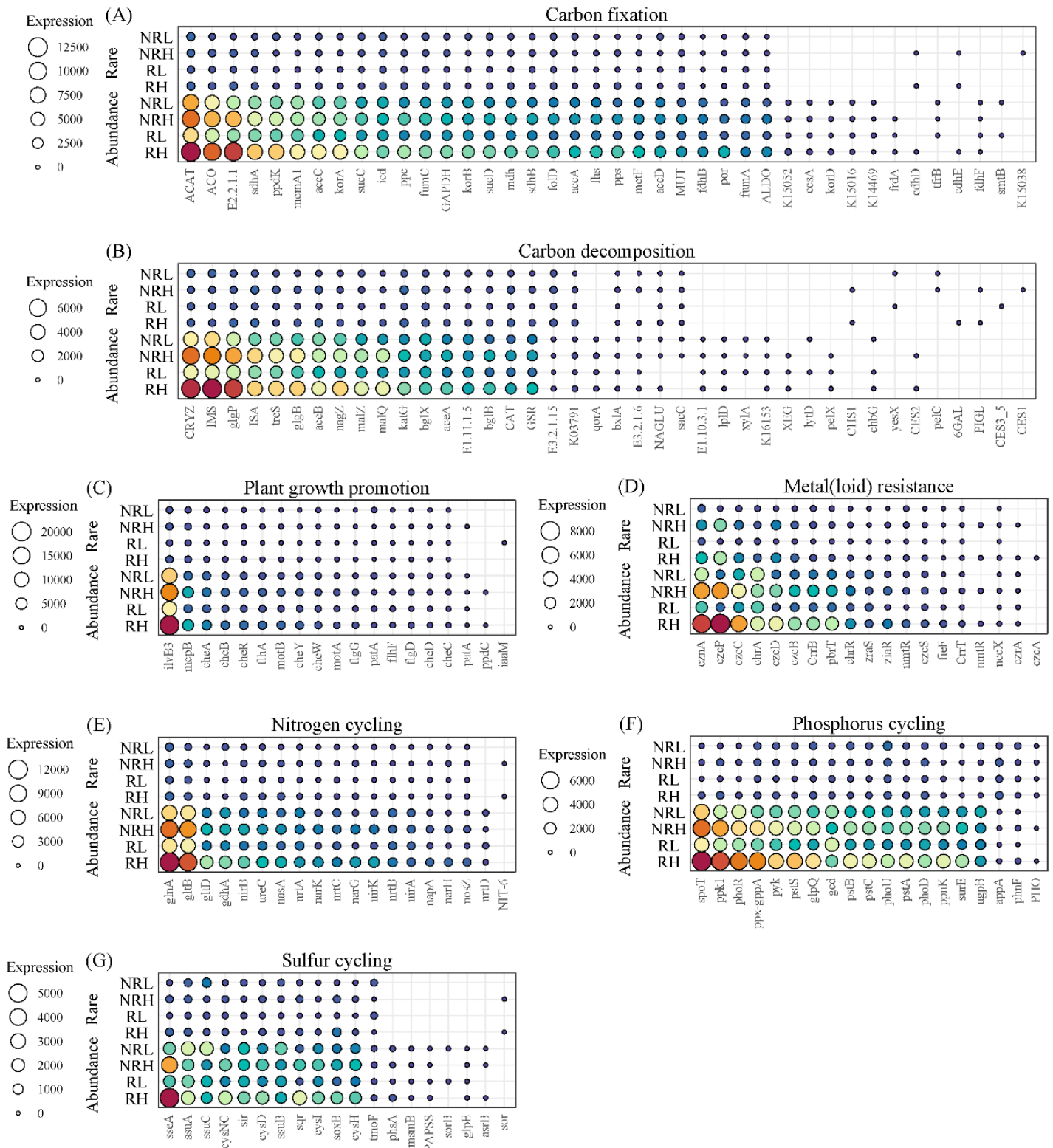


Figure 5

Bubble chart indicating differences in the relative abundance of genes associated with the cycling of carbon fixation(A), carbon decomposition (B), plant growth promotion (C), metal(loid) resistance (D), nitrogen (E), phosphorus (F) and sulfur (G). RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively.

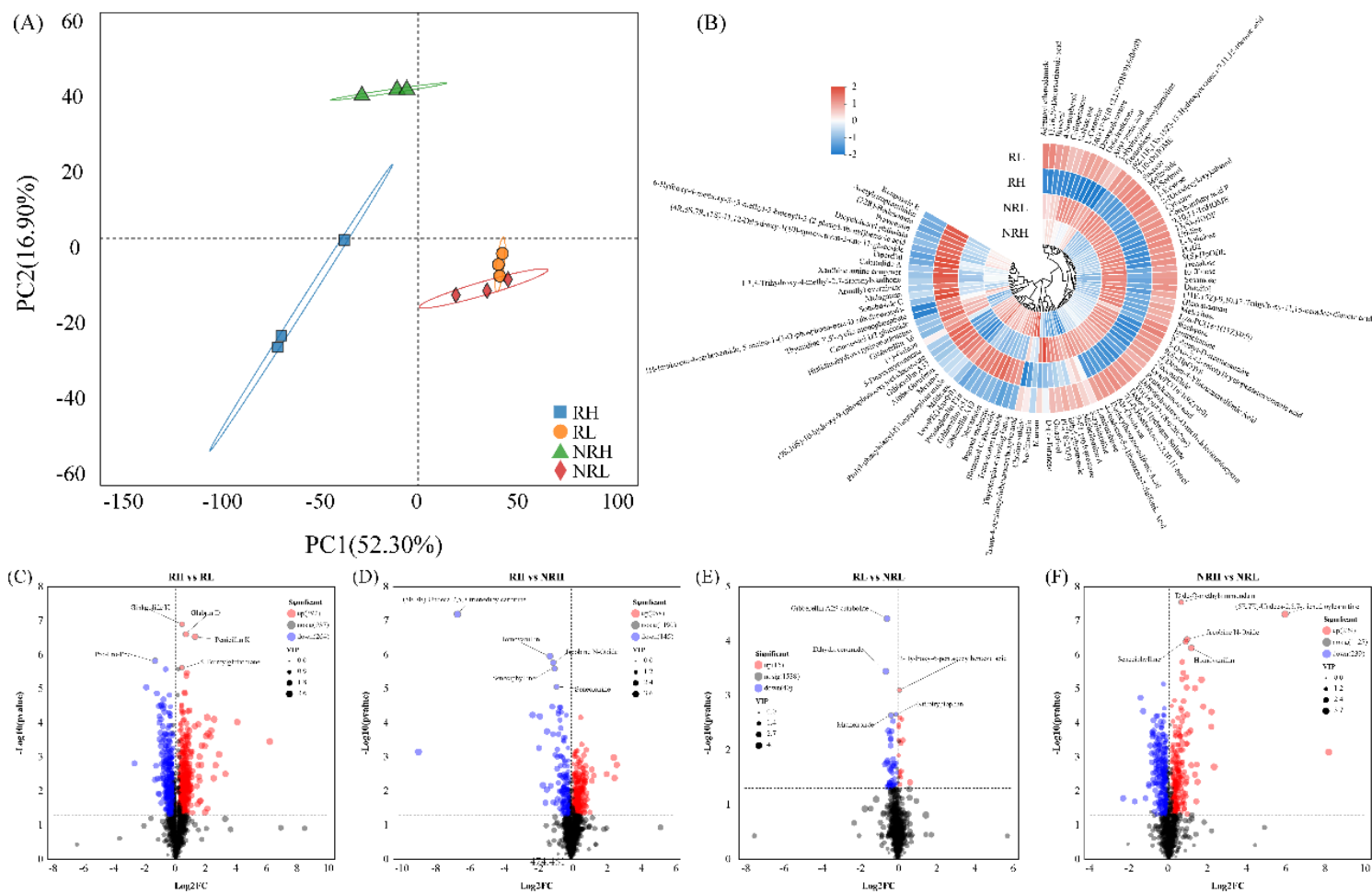


Figure 6

Principal component analysis (PCoA) based on Bray-Curtis distance matrix for metabolites of the four treatment groups (A). Metabolite clustering analysis of the four treatments (B). Volcano plot analysis of differential metabolites between the two groups, RH vs RL (C), RH vs NRH (D), RL vs NRL (E) and NRH vs NRL (F). RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively.

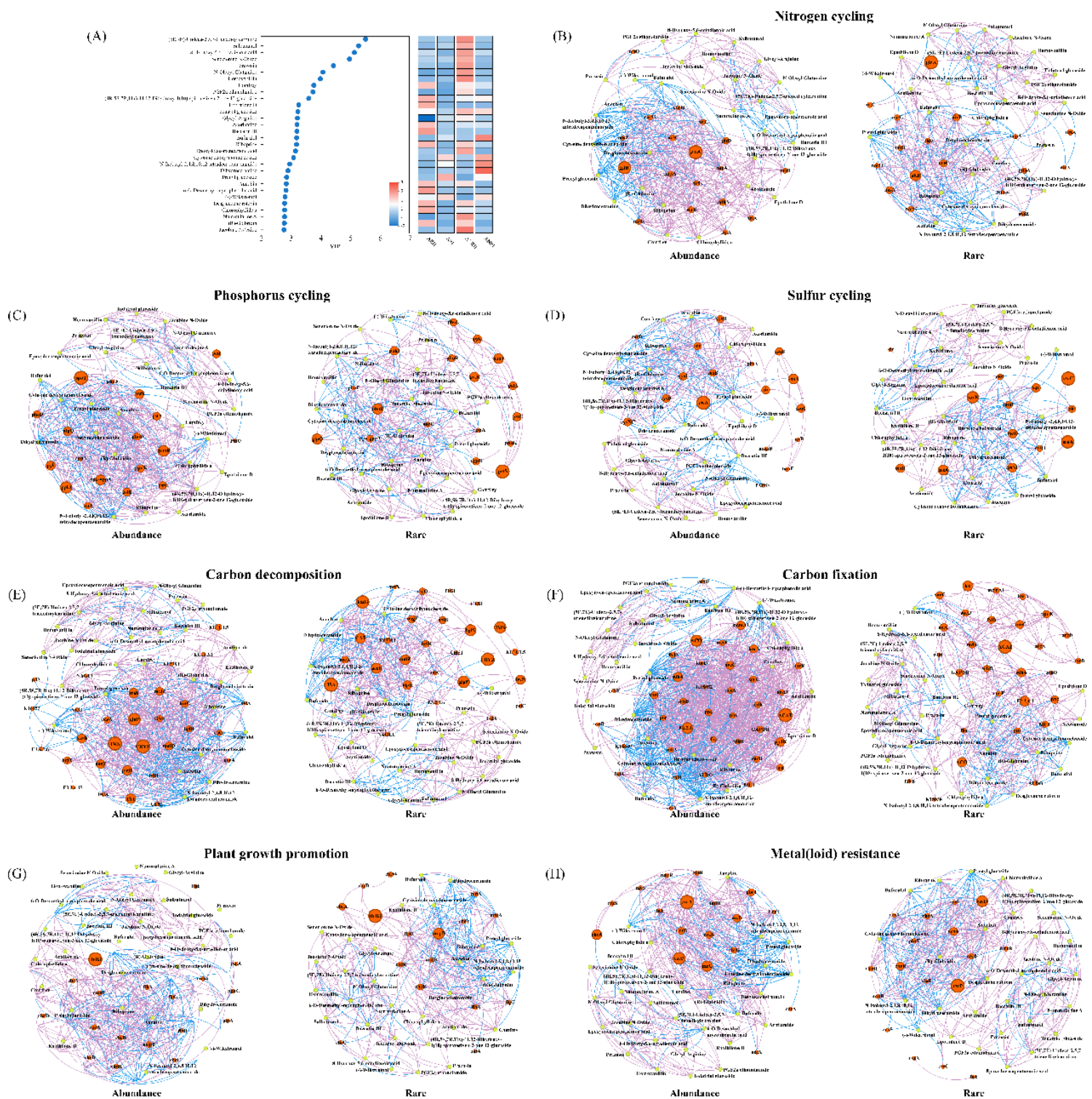


Figure 7

Key metabolite screening and covariance network analysis with genes. VIP-based clustering of metabolites (A). Covariance network analysis of key metabolites with genes for nitrogen cycling (B), phosphorus cycling (C), sulfur cycling (D), carbon decomposition (E), carbon fixation (F), plant growth promotion (G) and metal(loid) resistance (H). RH, RL, NRH and NRL represent rhizosphere soil of tailings areas, rhizosphere soil of non-tailings areas, non-rhizosphere soil of tailings areas and non-rhizosphere soil of non-tailings areas, respectively.

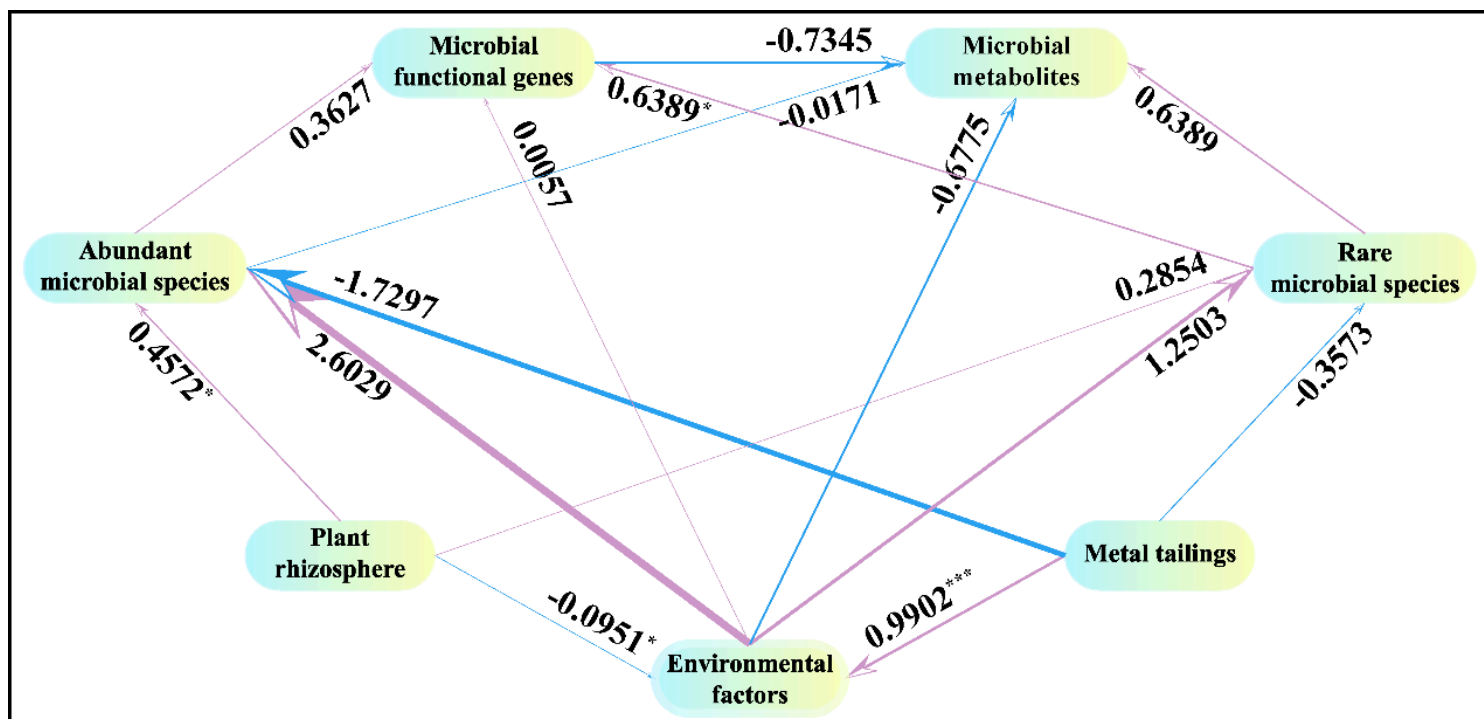


Figure 8

The direct and indirect effects of plant rhizosphere, metal tailings, environmental factors, abundant microbial species, and rare microbial species network on microbial functional genes and microbial metabolites according to the structural equation model (SEM). The pink as well as blue arrows show positive as well as negative path coefficients, respectively. Path width is proportional to the path factor. The number adjacent to the arrows is the path coefficient, representing the directly standardized influence size of the relationship. The significance levels of each predictor are * ($P < 0.05$), ** ($P < 0.01$), as well as *** ($P < 0.001$).

Supplementary Files

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

- [Supportinginformation.docx](#)
- [TableS2.xlsx](#)