# Structure and variation of root-associated bacterial communities of *Cyperus rotundus L.* in the contaminated soils around Pb/Zn mine sites

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# Abstract

Soil contamination due to mining activities is a great concern in China. Although the effects of mining pollution resulting in changes of soil characteristics and the microbiome have been documented, studies on the responses of plant root-associated microbial assemblages remain scarce. In this work, we collected bulk soil, rhizosphere soil, and root endosphere samples of *Cyperus rotundus* L (*Cyp*) plants from two Pb/Zn mines, of which, one was abandoned (SL) and the other was active (GD), to investigate the bacterial community responses across different site contamination levels and *Cyp* plant compartments. For comparison, one unpolluted site (SD) was included. Results revealed that soils from the SL and GD sites were seriously contaminated by metal(loid)s, including Pb, Zn, As, and Sb. Bacterial richness and diversity depended on the sampling site and plant compartment. All sample types from the SL site had the lowest bacterial diversities and their bacterial communities also exhibited distinct patterns compared to GD and SD samples. As for the specific sampling site, bacterial communities from the root endosphere exhibited different patterns from those in bulk and rhizosphere soil. Compared to the GD and SD sites, the root endosphere and the rhizosphere soil from the SL site shared core microbes, including *Halomonas*, *Pelagibacterium*, and *Chelativorans*, suggesting that they play key roles in *Cyp* plant survival in such harsh environments.


# 1. Introduction

Soil contamination due to mining activities is a cause of great concern worldwide, especially the mining of heavy metals, which diminish the availability of arable lands (Yang et al., 2018). In China, this problem in abandoned mining sites is unprecedented (Xu et al., 2019; Zhao et al., 2016). Long-term exposure to heavy metal contamination may pose serious threats to ecological safety and human health (Gao et al., 2015; Wang et al., 2017; Zhong et al., 2020). Previous studies reported distinct patterns of soil characteristics and microbiome in such harsh environments, which negatively affect soil ecosystems (Liu et al., 2019; Yun et al., 2018).

Soil microbes comprise the largest group of biodiversity in terrestrial ecosystems and play important roles in plant growth (Voges et al., 2019). Plant-microbe interactions allow microbes to adapt to various soil conditions, enabling them to resist or tolerate chemical toxicity and pathogen disease in contaminated environments (Berendsen et al., 2012; Mendes et al., 2011). However, long-term selective pressure may change soil or plant microbiomes, resulting in great differences in microbial structures and compositions between the root endosphere and external environments, including the rhizosphere and bulk soils (Gottel et al., 2011). The rhizosphere is a major hotspot for root-microbe interactions and consists of nutrient availability, growth promotion, and microbial enrichment (Henneron et al., 2020; Liu et al., 2020). Various factors, such as geographical site, plant species, developmental stage, soil type, and land management, affect the colonization pattern of rhizosphere microbes (Beckers et al., 2017; Edwards et al., 2015). Several studies have found that the root microbiome might be quite distinct from the external microbes in the soil (Gkarmiri et al., 2017; Hartman and Tringe, 2019). Changes in the soil microbial community due to external disturbances (i.e., mining activities) greatly influence the root-

associated microbiome. Distinct bacterial and fungal communities in the rhizosphere and root endosphere of *Populus deltoids* have been observed (Gottel et al., 2011). Peiffer et al. (2012) reported that geographical location had a significant effect on variations in the maize rhizosphere microbiome. Our previous study found that rhizosphere bacterial communities in the adjacent areas of Sb and Pb/Zn mines were crop-specific and strongly linked to concentrations of Cr and V (Sun et al., 2018a). Similarly, plant species and mine tailing pH were found to drive root-associated microbial community patterns in boreal trees and shrubs (Gagnon et al., 2020). A recent study by Li et al. (2020) found that plant growth changed the recruitment of soil microbes colonizing rhizosphere compartments in response to acid mine drainage pollution. However, the relative contributions of the soil characteristics, contamination due to mining activities, and variations in root-associated bacterial assemblages have not been well documented.

*Cyperus rotundus* L. (*Cyp*) is a popular perennial species distributing worldwide in tropical and subtropical regions. *Cyp* plants can grow well in soils contaminated by mining activities, suggesting that they are able to adapt to harsh environments. Previous studies reported that *Cyp* plants were suitable for use in diesel phytoremediation and heavy metal-contaminated soils (Ashraf et al., 2012; Bordoloi and Basumatary, 2016; Hou et al., 2016; Lum and Chikoye, 2018; Subhashini and Swamy, 2014). However, the responses of microbial communities to pollutant elimination have not been investigated and the contribution of root-associated microbes remains unknown.

In this study, we collected bulk soil, rhizosphere soil, and root endosphere samples of *Cyp* plants from two Pb/Zn mines, of which, one was abandoned (SL) and the other was actively mined (GD). Additionally, an unpolluted site (SD) was selected as a control for comparison. Geochemical factors, including soil pH, content of soil organic carbon (SOC), nitrate, and sulfate, as well as concentrations of metal(loid)s, including Cr, Cu, Cd, Pb, Zn, As, and Sb, were analyzed. The bacterial communities of plant compartments across different sampling sites were characterized. The aims of this work are to (1) explore root-associated bacterial community responses to mining activities; (2) compare bacterial distribution patterns in bulk soil, rhizosphere soil, and root endosphere of *Cyp* plants; and, (3) determine the core bacterial communities in *Cyp* plant compartments that are tolerant to heavy mining contamination.

# 2. Materials And Methods

## 2.1. Sampling campaign

*Cyp* samples together with the surrounding bulk soils were collected with a shovel from two Pb/Zn mines of SL and GD. Both mines were located in Liuzhou city of Guangxi Zhuang Autonomous Region in China. For comparison, the control samples were also collected from the unpolluted site in the Siding town (SD) in Liuzhou city in Guangxi Province. These three sampling sites and *Cyp* plants are shown in Fig. S1.

A total of 45 bulk and rhizosphere soils, as well as the root endosphere samples with five replicates for each component were collected from the GD, SL, and SD sites. Bulk soil was taken adjacent to each *Cyp*

plant and was kept on ice as soon as being transported back to laboratory for analysis. The *Cyp* roots were collected and the rhizosphere soil attached on the roots were washed with phosphate buffer saline (PBS) solution after the loose soils were shaken off. The remaining roots were cleaned with PBS solution for three times and were considered as endosphere samples. Bulk soil, rhizosphere soil, and root endosphere samples were stored at -20ºC prior to DNA extraction.

## 2.2. Soil physicochemical parameters analysis

Soil samples were freeze-dried, thoroughly grounded, and passed through a 200 mesh sieve prior to physicochemical characterization. Parameters of pH, sulfate, and nitrate were measured according to the standard methods, which were narrated previously (Sun et al., 2015). SOC was directly determined with a vario MACRO cube elemental analyzer (Elementar, Hanau, Germany). For measuring the concentrations of metal(loid)s including Cr, Cu, Cd, Pb, Zn, As, and Sb and their acid-soluble fractions, the dried soil samples were completely digested with $HNO_3$/HF at a volume ratio of 5:1 (Sun et al., 2018a) and 0.11 M acetic acid (Zhai et al., 2018), respectively, then determined using an Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS).

## 2.3. DNA extraction and 16r RNA amplicon sequencing analysis

Approximately 0.5 g each of bulk soil, rhizosphere soil, and root endosphere samples was individually prepared for extraction of the total bacterial DNA, using the Power Soil DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the protocol provided by the manufacturer. The quantity of the extracted DNA was determined with the NanoDrop ND-2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA), and the quality of DNA was determined with 1% (w/v) agarose gel electrophoresis.

Based on our previous optimization experiments, the V4-V5 hypervariable region of the 16S rRNA gene was amplified using the universal primers 515F (5'-GTG YCA GCM GCC GCG GTA A-3') and 907R (5'-CCY CAA TTC MTT TRA GTT T-3'). The obtained amplicons were barcoded uniquely in each sample and sent out for high-throughput sequencing on an Illumina MiSeq platform (Tianjin Novogene Bioinformatic Technology Co., Ltd, China). After sequencing, the raw sequence data were merged and filtered in accordance with the previously reported criteria. The remaining high-quality sequences within 97% similarity threshold were clustered into an operational taxonomic unit (OTU) and annotated against the Greengenes Database following our established pipelines (DeSantis et al., 2006; Sun et al., 2019a; Sun et al., 2019b).

## 2.4. Bioinformatics and statistical analysis

OTU counts were normalized in R package (v3.5.2) and OTU-level alpha diversity indices (i.e., observed species number, Chao1 richness estimator, abundance-based coverage estimator (ACE), Simpson index, and Shannon diversity index) were calculated. To compare the richness and evenness of OTUs among samples, the rarefaction curves were generated by using Mothur program. Principal coordinate analysis

(PCoA) was performed using UniFrac distance metrics to investigate the structural variation of bacterial communities across samples and visualized by origin from either sampling sites or *Cyp* compartments. Taxa relative abundances at different levels were statistically compared among samples and visualized as stack plots. Linear discriminant analysis effect size (LEfSe) analysis was performed with the default parameters to determine differentially abundant taxa across *Cyp* compartments (Segata et al., 2011). Statistical analysis (i.e., two-sided *t*-test) was performed using IBM SPSS (v19.0) to evaluate the differences across samples, and it was considered statistically significant when *P* values less than 0.05.

# 3. Results

## 3.1. Soil characteristics

A total of 15 bulk soil samples (5 each from the GD, SL, and SD sites) were used for pH, SOC, nitrate, and sulfate measurements (Fig. 1). SD bulk soil had a lower pH (6.40 ± 0.04) and sulfate content (9.15 ± 1.70 mg/kg), but higher SOC (18.89 ± 4.10 g/kg) and nitrate content (20.83 ± 4.17 mg/kg) when compared to the GD and SL bulk soil. There were no significant differences in these characteristics between the GD and SL bulk soils. Metal(loid)s exhibited a wide variation of concentrations in the bulk soil samples (Fig. 2). The contents of all metal(loid)s, except Cr in the SD bulk soil, were significantly ($P < 0.05$) lower than those of the GD and SL bulk soil. In particular, the concentrations of Pb, Zn, and As in GD (908.8 ± 554.4, 5230 ± 2746, and 146.3 ± 85.1 mg/kg, respectively) and SL bulk soil (1842 ± 939, 6096 ± 2744, and 335.2 ± 146.7 mg/kg, respectively) were one and two orders of magnitude higher compared to the SD bulk soil (19.9 ± 5.7, 75.4 ± 23.1, and 10.7 ± 1.5 mg/kg, respectively). Surprisingly, the content of Sb in the SL bulk soil was significantly higher (1857 ± 1337 mg/kg) than that of the GD (19.8 ± 11.7 mg/kg) ($P = 0.04$) and SD bulk soil (1.1 ± 0.2 mg/kg) ($P = 0.04$). In contrast to the total metal(loid) contents, the concentrations of their acetic acid-soluble fractions were much lower (Fig. S2).

## 3.2. Bacterial diversity

Rarefaction curves were constructed using Mothur with 97% sequence similarity for bacterial samples (Fig. 3), which evaluated the species richness of each sample that approached saturation. Good's coverage scores were highly comparable across samples, ranging from 98.4–100%, indicating an adequate sequencing depth for analyzing the bacterial microbiome. Results revealed that the bacterial communities were heavily dependent on the sampling site and *Cyp* compartment. All SL sample types had a significantly ($P < 0.05$) lower richness (i.e., Chao1, ACE, and observed species number) and diversity (i.e., Simpson and Shannon indices) than the GD and SL samples (Fig. S3).

In the SD samples, the rhizosphere soil had the highest richness and diversity indices, while the root endosphere had the lowest values. Moreover, the richness and diversity indices between the bulk and rhizosphere soils were comparable, while the rhizosphere soil and the root endosphere were significantly different ($P < 0.05$). In the GD samples, the bulk soil had the highest richness and diversity indices, and were comparable to the rhizosphere soil. The root endosphere had the lowest values, but was not significantly different from the rhizosphere soil. The SL samples had the highest richness indices of the

rhizosphere soil and diversity indices of the root endosphere, while the lowest richness and diversity indices were observed in the bulk and rhizosphere soils, respectively. Significant ($P$ = 0.045) differences were detected in the richness indices between the bulk and rhizosphere soils. The bacterial richness indices revealed a decreasing gradient from the rhizosphere soil to the root endosphere (Fig. S3a–c), while the diversity indices showed a decreasing trend from the rhizosphere soil to the root endosphere in the GD and SD samples, but an increasing trend in the SL samples (Fig. S3d, e).

Similarities in the bacterial communities across various samples are visualized with a PCoA analysis (Fig. 4). PCo1 and PCo2 explained 28.4% and 20.4% of the total variation, respectively. All samples exhibited distinct clustering based on the sampling site (Fig. 4a). Within each cluster, the bacterial communities of bulk and rhizosphere soils overlapped considerably (Fig. 4b), but no consistent pattern was observed between rhizosphere soil and root endosphere. These findings were consistent with the alpha diversity results of site-dependent bacterial communities, which indicated that there were no significant differences in the bacterial richness or diversity indices between the bulk and rhizosphere soil samples within each site.

# 3.3. Bacterial community composition

The main bacterial phyla in all samples are presented in Fig. 5a and Fig. 6. *Proteobacteria* was the most abundant phylum with a relative abundance of 35.9% in the GD bulk soil and 91.7% in the SL rhizosphere soil, followed by *Acidobacteria* (3.2% and 31.7%, respectively) and *Actinobacteria* (1.7% and 7.9%, respectively). Bacterial phyla exhibited site-dependent effects, as the relative abundance of *Proteobacteria* in the SL samples (42.8–91.7%) was much higher when compared to GD (35.9–47.6%) and SD (36.9–57.8%) samples. Specifically, *Proteobacteria* in the SL rhizosphere (91.7%) and bulk soils (83.9%) were two times more abundant than in the GD (40.8% and 35.9%, respectively) and SD samples (39.4% and 36.9%, respectively). Similar effects were observed for *Acidobacteria* and *Actinobacteria*, which were much lower in the SL samples (3.2–5.3% and 1.7–3.3%, respectively) than in the GD (14.3–31.7% and 6.8–8.7%, respectively) and SD samples (9.6–29.1% and 5.9–7.9%, respectively).

As for specific sites, no significant differences were detected in the dominant phyla (i.e., *Acidobacteria*, *Proteobacteria*, and *Actinobacteria*) relative abundance patterns between the rhizosphere and bulk soil samples collected from the same site (Fig. 5a). Notably, overall distinct patterns in the relative abundances of the dominant phyla were observed between the rhizosphere soil and the root endosphere bacterial communities, including *Proteobacteria* and *Acidobacteria*. In the SD rhizosphere soil, a significant ($P$ = 0.001) enrichment of *Acidobacteria* was observed, while *Proteobacteria* was significantly ($P$ = 0.021) depleted when compared to the root endosphere. *Proteobacteria* were significantly ($P$ = 0.002) enriched in SL rhizosphere soil, while *Acidobacteria* were significantly ($P$ = 0.02) depleted in SL rhizosphere soil when compared to their respective root endosphere compartments. Moreover, other bacterial phyla, including *Deinococcus Thermus*, and *Tenericutes*, exhibited higher relative abundances in SL than in GD and SD, especially phylum *Tenericutes* of the *Mollicutes* subclass, which had a higher relative abundance in the SL root endosphere (14.1%) than other samples.

Within the most abundant phylum of *Proteobacteria* (Fig. 5b), *Gammaproeobacteria* (16.6–48.4%) dominated in SL samples when compared to GD (7.1–16.0%) and SD (2.4–5.9%) samples, followed by *Alphaproeobacteria*. Moreover, *Betaproteobacteria* (2.4–4.8%), *Actinobacteria* (1.7–3.3%), and *Acidobacteria* Gp6 (1.5–2.3%) had the lowest relative abundances. Root endosphere communities were heavily dominated by *Alphaproeobacteria*, followed by *Gammaproeobacteria*. *Alphaproeobacteria* was significantly enriched in GD ($P = 0.04$) and SD ($P = 0.0005$) root endosphere samples, but significantly depleted ($P = 0.01$) in the SL root endosphere samples when compared to their respective rhizosphere soil samples. A significant ($P = 0.0002$) reduction of *Gammaproeobacteria* in the SL root endosphere was observed, while similar significant depletions were found for *Acidobacteria* Gp6 in the SD root endosphere ($P = 0.003$) and *Acidobacteria* Gp4 in the GD root endosphere ($P = 0.008$).

At the genus level, the core bacterial microbiome was identified, including *Gp6*, *Halomonas*, *Bradyrhizobium*, *Pelagibacterium*, *Gp16*, *Chelativorans*, *Gaiella*, and *Spartobacteria genera incertae sedis* (Fig. 7). We detected site effects on these core bacterial communities. In the SD samples, core communities in the rhizosphere soil exhibited significant ($P < 0.05$) differences when compared to root endosphere communities, of which, *Halomonas*, *Bradyrhizobium*, *Pelagibacterium*, and *Chelativorans* were enriched and *Gp6*, *Gp16*, *Gaiella*, and *Spartobacteria genera incertae sedis* were depleted. In the SL samples, significant ($P < 0.05$) enrichment of *Halomonas*, *Pelagibacterium*, and *Gaiella* were observed in the rhizosphere soil, while only *Chelativorans* was significantly ($P = 0.05$) enriched when compared to the root endosphere. In contrast, only *Bradyrhizobium* was significantly ($P = 0.035$) enriched and *Gp16* was significantly ($P = 0.006$) depleted in the rhizosphere soil of GD samples. No significant differences in the relative abundances of the core bacterial communities were observed between the rhizosphere and bulk soils, regardless of the sampling site, except *Bradyrhizobium* in the SD samples ($P = 0.01$).

# 4. Discussion

Mining activities and contamination introduced by these activities are severe environmental issues in China. Bioremediation mediated by plants and microorganisms is an effective method for alleviating mining contamination. The roots connecting soils and plants are hotspots for interactions between microorganisms and the environments. Although microbial communities in mining-contaminated sites have been extensively characterized, less attention has been focused on the roots, especially the root endosphere. In this study, microbial communities in plant compartments, including the bulk soil, rhizosphere soil, and the root endosphere, were characterized and compared. Their interactions with geochemical conditions were also analyzed and discussed.

## 4.1. Bacterial diversity was influenced by soil contamination level

Endosphere species richness was lower than the bulk and rhizosphere soil richness, indicating the non-uniform and selective colonization of plant roots (Beckers et al., 2017). It is well known that the root microbiome is primarily assembled from external soil microbes (Edwards et al., 2015), which may migrate to the rhizosphere due to the attraction of rich nutrients like root exudates (Zhalnina et al., 2018), resulting

in the rhizodeposition of various microbes. However, root-colonizing bacteria are highly competitive in order to ensure successful colonization. Therefore, root-colonizing bacteria possess traits that favor resistance to harsh environments, such as chemical toxicity and pathogen disease (Mendes et al., 2011; Berendsen et al., 2012). Thus, bacteria inside the root endosphere are much less rich and diverse than in the rhizosphere soil, as confirmed in this study, with the exception of the SL root endosphere samples (Fig. S3), which had the highest Shannon and Simpson diversity indices when compared to the bulk and rhizosphere soils (Fig. S3d, e). These findings may be attributed to the long-term impact pollution on *Cyp* roots in the surrounding SL tailing, which may affect *Cyp* development, shape root-associated microbes, and change acquisition patterns in the root endosphere (Edwards et al., 2015; Chaparro et al., 2014).

Based on the bacterial richness and diversity indices, we found that the alpha diversity was highly dependent on the sample site. The SL samples had the lowest richness and diversity, regardless of sample type, followed by the GD samples, while the SD samples possessed the highest alpha diversity (Fig. S3). These results implied that the contamination level of the Pb/Zn mines greatly affected and shaped plant-associated microbes. The concentrations of metal(loid)s, including Cu, Cd, Pb, Zn, As, and Sb, in the SL bulk soil were significantly higher than those in SD bulk soil, which exerted persistent selection pressure on the assemblage of soil microbes (Fig. 2b). Additionally, soil characteristics, including pH and the nitrate and sulfate contents in the SL bulk soil, were significantly higher compared to those of the SD bulk soil (Fig. 2a), which have been reported to closely correlate with soil bacterial communities (Gagnon et al., 2020; Xiao et al., 2016; Chen et al., 2013). In contrast, the GD site was being actively mined and its contamination of the surrounding soils was less severe. The concentrations of metal(loid)s in the GD bulk soil were lower compared to the SL bulk soil, but were still significantly higher than in the SD bulk soil (Fig. 2b). Moreover, a significantly higher pH and lower SOC were also observed (Fig. 2a). These factors likely modulate the population and composition of plant-associated microbes, resulting in the loss of bacterial richness and diversity. Additionally, the PCoA analysis showed site-dependent clustering of the bulk soil, rhizosphere soil, and root endosphere communities (Fig. 4a). This result is consistent with the alpha diversity results, confirming that contamination conditions greatly affected microbial assemblages. As for specific sites (i.e., the GD, SL, and SD), the majority of bulk and rhizosphere soil samples clustered together (Fig. 4b), suggesting that bacterial diversity was comparable between the bulk and rhizosphere soil samples. In contrast, the root endosphere samples were sporadically distributed, indicating distinct patterns of bacterial communities.

# 4.2. Structural distribution of the dominant bacterial communities

Bacterial structures and compositions were dependent on the sampling site and *Cyp* compartment. However, phylum *Proteobacteria* (mostly *Alphaproteobacteria* and *Gammaproteobacteria*) consistently dominated bulk soil, rhizosphere soil, and root endosphere samples, followed by *Acidobacteria* and *Actinobacteria*. These phylotypes have been commonly identified in the soil microbiome surrounding mining areas (Gao et al., 2019; Sun et al., 2018b). It was previously reported that the ratio of *Proteobacteria* and *Acidobacteria* could be an indicator of soil trophic levels (Castro et al., 2010; Smit et

al., 2001; Gottel et al., 2011; Beckers et al., 2017), in which, *Acidobacteria* and *Proteobacteria* were associated with nutrient-rich and nutrient-poor soils, respectively. The relative abundance of *Proteobacteria* (mostly *Alphaproteobacteria*) increased from rhizosphere soil to the root endosphere, while *Acidobacteria* decreased in the GD and SD samples, indicating nutrient-rich conditions of the root endosphere compared to rhizosphere and bulk soils. Similar results were observed in other plants, including poplar (Beckers et al., 2017; Gottel et al., 2011; Shakya et al., 2013) and rice (Edwards et al., 2015). In the SL samples, however, contrasting results were observed, where the relative abundance of *Proteobacteria* (mostly *Alphaproteobacteria* and *Gammaproteobacter*) decreased remarkably from rhizosphere soil to the root endosphere, while *Acidobacteria* increased slightly, which was similar to previous reports on grasslands (Singh et al., 2007) and soybeans (Xu et al., 2009).

These findings suggested that heavy contamination (i.e., high contents of metal(loid)s) in the SL site likely changed the soil nutrient status and thereby shaped bacterial assemblages. Similar results were previously reported by Sun et al. (2018a) who found that plant rhizosphere communities strongly correlated with Cr and V in Pb/Zn mining sites. Moreover, *Acidobacteria* and *Proteobacteria* in the bulk and rhizosphere soil samples from the GD and SD sites were comparable, indicating an intermediate nutrient level. This result is consistent with the alpha diversity results (Fig. S3). As for the SL samples, *Proteobacteria* were enriched in the rhizosphere soil when compared to the bulk soil, which may be attributed to the nutrient-rich conditions of the rhizosphere soil. This result is likely due to the production of root exudates under the selection of metal(loid)s in polluted soils (Kozdrój and van Elsas, 2000; Qin et al., 2007), as well as the increased pollution resistance and/or tolerant *Proteobacteria*, such as *Alphaproteobacteria*, in this study (Sandaa et al., 1999). In addition to metal(loid)s, soil pH was previously found to correlate with overall bacterial community composition and phylogenetic diversity, which affect bacterial relative abundances, such as *Acidobacteria* (Lauber et al., 2009; Qi et al., 2018). Unfortunately, the pH values of the rhizosphere soil and the root endospheres were not directly measured in this study due to a lack of samples. Rhizosphere pH is mediated by plant roots (i.e., exudates of organic acids) in response to environmental constraints (Hinsinger et al., 2003; Sasse et al., 2018). As a result, plant-associated microbial communities in contaminated mining sites are largely shaped by various factors, including plant genotype and soil conditions.

Additionally, phylum *Tenericutes* was exclusively found in the SL root endosphere samples with a relative abundance of 14.1%, which was exclusively consisted of genus *Acholeplasma*. Previous studies found that *Acholeplasma* belongs to wall-less, saprophytic, and free-living bacteria (Zhao et al., 2015; Mitter et al., 2017), which is transmitted to plants and colonize in the vascular tissues as a sap-sucking endophyte (Blain et al., 2017). Phylum *Deinococcus Thermus* (mostly *Meiothermus*) was remarkably richer in SL samples than in GD and SD samples. This result can be ascribed to the heavy contamination conditions of SL sites, as *Meiothermus* species are always present in extreme environments (Thokchom et al., 2017). Consistently, the dominance of *Meiothermus* has been observed in mining tailings (Sun et al., 2018b). Thus, it was proposed that *Meiothermus* may have the potential to oxidize and reduce As.

# 4.3. Core members of the root bacterial microbiome

Within the core bacterial microbiome, similar site-specific distributions were observed. At the genus level, the GD and SD bulk and rhizosphere soil communities were dominated by *Gp6*, *Spartobacteria genera incertae sedis*, *Bradyrhizobium*, *Gp16*, and *Gaiella*, of which, *Gp6* and *Bradyrhizobium* also dominated root endosphere assemblages. This finding suggested that the root endosphere communities consisted of a subset of specific microbes from the corresponding rhizosphere soil, which was consistent with the findings of previous reports (Hallmann et al., 1997; Miliute et al., 2015). Similar results were observed in SL rhizosphere soil and root endospheres, which were both primarily dominated by *Halomonas*, *Pelagibacterium*, and *Chelativorans*, and their rhizosphere relative abundances were almost one order of magnitude higher. *Bradyrhizobium* is a common endophyte with higher abundance in the root endosphere than in the bulk and rhizosphere soils, which has been reported to improve plant growth in the heavy metal-contaminated environment (Wani et al., 2008; Seneviratne et al., 2016). In contrast, overall distinct compositions of root core microbes between SD and SL samples, which could be ascribed to the selection pressures of the surrounding soil conditions (described above). *Halomonas* of class *Gammaproteobacteria* is capable of indole acetic acid production and phosphate solubilization in the presence of high concentrations of heavy metals and high-salinity, which thereby promotes plant growth (Desale et al., 2014). Additionally, *Halomonas* species isolated from mangrove *Avicennia marina* rhizosphere soil increased exopolysaccharide production under arsenic stress, thereby enhancing rice growth (Mukherjee et al., 2019). *Pelagibacterium* of class *Alphaproteobacteria* has been found in *Tamarix ramosissima* root-associated communities and is tolerant to salinity (Taniguchi et al., 2015). Some species have been found to grow well in high-salinity environments, such as seawater (Li et al., 2013; Xu et al., 2011; Wang et al., 2017), lake water (Lu et al., 2018), and deserts (Yang and Sun, 2016). Several *Chelativorans* species of class *Alphaproteobacteria* have been isolated from EDTA-enriched soils (Bohuslavek et al., 2001). These species grew with EDTA as carbon, nitrogen, and energy sources (Doronina et al., 2010). This microbe was relatively abundant in SL rhizosphere soil, which may be due to EDTA pollution from mining. *Chelativorans* species have also been found in seawater (Evans et al., 2018). A recent study reported that *Chelativorans* species were abundant in marine sponge-associated microbes in the Palk Bay of India, which has suffered from sewage wastewater, salt pan, and heavy metal pollution (Meenatchi et al., 2020). In this study, it was interesting to find that the core bacterial microbes in SL rhizosphere soil (i.e., *Halomonas*, *Pelagibacterium*, and *Chelativorans*) were salinity- and metal-tolerant communities, which could be ascribed to long-term selection under heavy soil pollution in the SL site.

# 5. Conclusions

In this study, we performed physicochemical analyses to assess the contamination level of bulk soils in unpolluted and polluted Pb/Zn mining sites, and 16S rRNA amplicon high-throughput sequencing to characterize and compare bacterial community variations in *Cyp* plants across different sampling sites and plant compartments. Both site- and compartment-dependent patterns of bacterial communities were detected based on the alpha and beta diversity results. Soil nutrient status and contamination level affected the core bacterial microbiome in the rhizosphere and root endosphere compartments, though many abundant microbes were shared among compartments. There was little variation in the dominant

bacterial phyla in the bulk and rhizosphere soils between unpolluted and active mining sites. Overall, these findings improve our understanding of soil-plant-microbe interactions under pollution selection and provide insight into microbial associations for potential phytoremediation applications.

# Declarations


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### Compliance with ethical standards

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Data availability** All data related to this publication are made available from the corresponding author on reasonable request.

**Competing interests** The authors declare that they have no conflict of interest.

**Authors' contributions** Pin Gao and Benru Song wrote the original draft, Weimin Sun conceptualized and designed the study. Benru Song, Rui Xu, Xiaoxu Sun and Hanzhi Lin performed the experiment and data analysis. Fuqing Xu, Baoqin Li, Pin Gao, and Weimin Sun commented on and revised the manuscript. All authors read and approved the final manuscript.

# References


1. Ashraf MA, Maah MJ, Yusoff I (2012) Assessment of phytoextraction efficiency of naturally grown plant species at the former tin mining catchment. Fresen Environ Bull 21(3):523–533. https://doi.org/10.1080/10643389.2011.556553
2. Beckers B, De Beeck MO, Weyens N, Boerjan W, Vangronsveld J (2017) Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar

trees. Microbiome 5:25. https://doi.org/10.1186/s40168-017-0241-2

3. Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486. https://doi.org/10.1016/j.tplants.2012.04.001
4. Blain NP, Helgason BL, Germida JJ (2017) Endophytic root bacteria associated with the natural vegetation growing at the hydrocarbon-contaminated Bitumount Provincial Historic site. Can J Microbiol 63:502–515. https://doi.org/10.1139/cjm-2017-0039
5. Bohuslavek J, Payne JW, Liu Y, Bolton H, Xun L (2001) Cloning, sequencing, and characterization of a gene cluster involved in EDTA degradation from the bacterium BNC1. Appl Environ Microb 67(2):688–695. https://doi.org/10.1128/AEM.67.2.688-695.2001
6. Bordoloi S, Basumatary B (2016) A Study on degradation of heavy metals in crude oil-contaminated soil using *Cyperus rotundus*. Phytoremediation Springer International Publishing. https://doi.org/10.1007/978-3-319-41811-7_4
7. Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil microbial community responses to multiple experimental climate change drivers. Appl Environ Microb 76(4):999–1007. https://doi.org/10.1128/AEM.02874-09
8. Chaparro JM, Badri DV, Vivanco JM (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8(4):790–803. https://doi.org/10.1038/ismej.2013.196
9. Chen L, Li J, Chen Y, Huang L, Hua Z, Hu M, Shu W (2013) Shifts in microbial community composition and function in the acidification of a lead/zinc mine tailings. Environ Microbiol 15(9):2431–2444. https://doi.org/10.1111/1462-2920.12114
10. Desale P, Patel B, Singh S, Malhotra A, Nawani N (2014) Plant growth promoting properties of *Halobacillus* sp. and *Halomonas* sp. in presence of salinity and heavy metals. J Basic Microb 54(8):781–791. https://doi.org/10.1002/jobm.201200778
11. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microb 72(7):5069–5072. https://doi.org/10.1128/AEM.03006-05
12. Doronina NV, Kaparullina EN, Trotsenko YA, Nörtemann B, Bucheli-Witschel M, Weilenmann HU, Egli T (2010) *Chelativorans multitrophicus* gen. nov., sp. nov. and *Chelativorans oligotrophicus* sp. nov., aerobic EDTA-degrading bacteria. Int J Syst Evol Micr 60(5):1044–1051. https://doi.org/10.1099/ijs.0.003152-0
13. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. P Natl Acad Sci USA 112(8):E911–E920. https://doi.org/10.1073/pnas.1414592112
14. Evans JS, Erwin PM, Shenkar N, López-Legentil S (2018) A comparison of prokaryotic symbiont communities in nonnative and native ascidians from reef and harbor habitats. FEMS Microbiol Ecol 94(9):fiy139. https://doi.org/10.1093/femsec/fiy139
15. Gagnon V, Rodrigue-Morin M, Tremblay J, Wasserscheid J, Champagne J, Bellenger JP, Greer CW, Roy S (2020) Life in mine tailings: microbial population structure across the bulk soil, rhizosphere, and

roots of boreal species colonizing mine tailings in northwestern Québec. Ann Microbiol 70(1):1–18. https://doi.org/10.1186/s13213-020-01582-9

16. Gao P, He S, Huang S, Li K, Liu Z, Xue G, Sun W (2015) Impacts of coexisting antibiotics, antibacterial residues, and heavy metals on the occurrence of erythromycin resistance genes in urban wastewater. Appl Microbiol Biot 99(9):3971–3980. https://doi.org/10.1007/s00253-015-6404-9
17. Gao P, Sun X, Xiao E, Xu Z, Li B, Sun W (2019) Characterization of iron-metabolizing communities in soils contaminated by acid mine drainage from an abandoned coal mine in Southwest China. Environ Sci Pollut R 26:9585–9598. https://doi.org/10.1007/s11356-019-04336-6
18. Gkarmiri K, Mahmood S, Ekblad A, Alstrom S, Hogberg N, Finlay R (2017) Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. Appl Environ Microb 83(22):e01938–e01917. https://doi.org/10.1128/AEM.01938-17
19. Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher ED, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW (2011) Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl Environ Microb 77(17):5934–5944. https://doi.org/10.1128/AEM.05255-11
20. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43(10):895–914. https://doi.org/10.1177/0095244305054674
21. Hartman K, Tringe SG (2019) Interactions between plants and soil shaping the root microbiome under abiotic stress. Biochem J 476(19):2705–2724. https://doi.org/10.1042/BCJ20180615
22. Henneron L, Kardol P, Wardle DA, Cros C, Fontaine S (2020) Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies. New Phytol 228:1269–1282. https://doi.org/info:doi/10.1111/nph.16760
23. Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59. https://doi.org/10.1023/A:1022371130939
24. Hou Y, Liu X, Zhang X, Hu X, Cao L (2016) Rhizosphere phytoremediation with *Cyperus rotundus* for diesel-contaminated wetlands. Water Air Soil Poll 227(1):26. https://doi.org/10.1007/s11270-015-2728-4
25. Kozdrój J, van Elsas JD (2000) Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches. Soil Biol Biochem 32(10):1405–1417. https://doi.org/10.1016/S0038-0717(00)00058-4
26. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microb 75(15):5111–5120. https://doi.org/10.1128/AEM.00335-09
27. Li Q, Xu Y, Liu K, Cai L, Fu Y, Sun J, Zhang R (2013) *Pelagibacterium nitratireducens* sp. nov., a marine *Alphaproteobacterium* isolated from the East China Sea. Curr Microbiol 66:450–455. https://doi.org/10.1007/s00284-012-0299-9

28. Li Y, Yuan L, Xue S, Liu B, Jin G (2020) The recruitment of bacterial communities by the plant root system changed by acid mine drainage pollution in soils. FEMS Microbiol Lett 367(15):fnaa117. https://doi.org/10.1093/femsle/fnaa117
29. Liu J, Yao J, Wang F, Min N, Gu J, Li Z, Sunahara G, Duran R, Solevic-Knudsen T, Hudson-Edwards KA, Alakangas L (2019) Bacterial diversity in typical abandoned multi-contaminated nonferrous metal(loid) tailings during natural attenuation. Environ Pollut 247:98–107. https://doi.org/10.1016/j.envpol.2018.12.045
30. Liu M, Adl S, Cui X, Tuan Y, Xu X, Kuzyakov Y (2020) In situ methods of plant-microbial interactions for nitrogen in rhizosphere. Rhizosphere 13:100186. https://doi.org/10.1016/j.rhisph.2020.100186
31. Lu H, Xing P, Phurbu D, Tang Q, Wu Q (2018) *Pelagibacterium montanilacus* sp. nov., an alkaliphilic bacterium isolated from Lake Cuochuolong on the Tibetan Plateau. Int J Syst Evol Micr 68(7):2220–2225. https://doi.org/10.1099/ijsem.0.002812
32. Lum AF, Chikoye D (2018) The potential of *kyllinga erecta* Schumach and *Cyperus rotundus* Linn. to remediate soil contaminated with heavy metals from used engine oil in cameroon. Int J Phytoremediat 20(13):1346–1353. https://doi.org/10.1080/15226514.2018.1501339
33. Meenatchi R, Brindangnanam P, Hassan S, Rathna K, Kiran GS, Selvin J (2020) Diversity of a bacterial community associated with *Cliona lobata* Hancock and *Gelliodes pumila* (Lendenfeld, 1887) sponges on the South-East coast of India. Sci Rep-UK 10(1):11558. https://doi.org/10.1038/s41598-020-67717-9
34. Mendes R, Kruijt M, De Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100. https://doi.org/10.1126/science.1203980
35. Miliute I, Buzaite O, Baniulis D, Stanys V (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste 102(4):465–478. https://doi.org/10.13080/z-a.2015.102.060
36. Mitter EK, de Freitas JR, Germida JJ (2017) Bacterial root microbiome of plants growing in oil sands reclamation covers. Front Microbiol 8:849. https://doi.org/10.3389/fmicb.2017.00849
37. Mukherjee P, Mitra A, Roy M (2019) *Halomonas* rhizobacteria of *Avicennia marina* of Indian Sundarbans promote rice growth under saline and heavy metal stresses through exopolysaccharide production. Front Microbiol 10:1207. https://doi.org/10.3389/fmicb.2019.01207
38. Qi D, Wieneke X, Tao J, Zhou X, Desilva U (2018) Soil pH is the primary factor correlating with soil microbiome in karst rocky desertification regions in the Wushan County, Chongqing, China. Front Microbiol 9:1027. https://doi.org/10.3389/fmicb.2018.01027
39. Qin R, Hirano Y, Brunner I (2007) Exudation of organic acid anions from poplar roots after exposure to Al, Cu and Zn. Tree Physiol 27:313–320. https://doi.org/10.1093/treephys/27.2.313
40. Sandaa RA, Torsvik V, Enger Ø, Daae FL, Castberg T, Hahn D (1999) Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. FEMS Microbiol Ecol 30(3):237–251. https://doi.org/10.1111/j.1574-6941.1999.tb00652.x

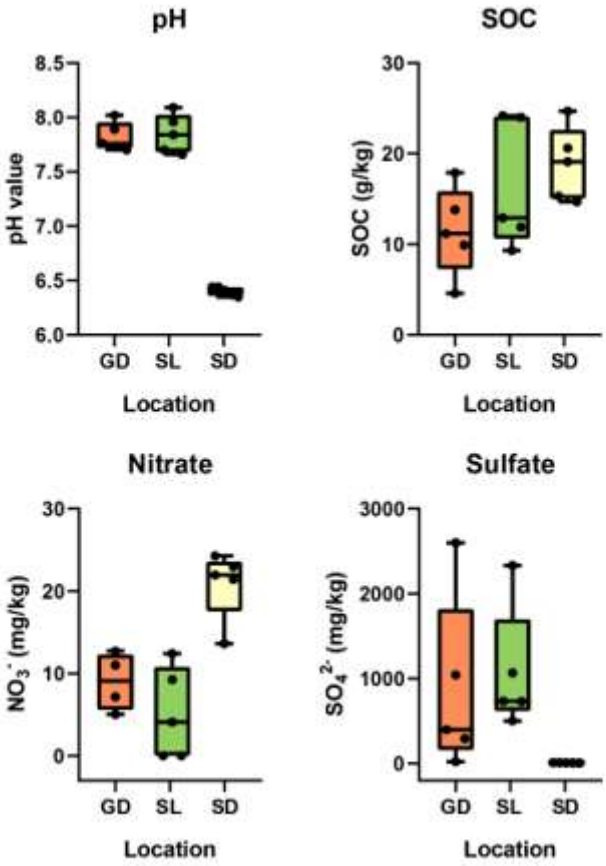
41. Sasse J, Martinoia E, Northen T (2018) Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci 23(1):25–41. https://doi.org/10.1016/j.tplants.2017.09.003
42. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12(6):R60. https://doi.org/10.1186/gb-2011-12-6-r60
43. Seneviratne M, Gunaratne S, Bandara T, Weerasundara L, Rajakaruna N, Seneviratne G, Vithanage M (2016) Plant growth promotion by *Bradyrhizobium japonicum* under heavy metal stress. S Afr J Bot 105:19–24. https://doi.org/10.1016/j.sajb.2016.02.206
44. Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbé J, Muchero W, Bonito G, Vilgalys R, Tuskan G, Podar M (2013) A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. PloS one 8(10):e76382. https://doi.org/10.1371/journal.pone.0076382
45. Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36(2–3):147–155. https://doi.org/10.1016/j.apsoil.2007.01.004
46. Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microb 67(5):2284–2291. https://doi.org/10.1128/AEM.67.5.2284-2291.2001
47. Subhashini V, Swamy AVVS (2014) Phytoremediation of cadmium and chromium contaminated soils by *Cyperus rotundus* L. Am Int J Res Sci Technol Eng Math 6:97–101
48. Sun W, Sun X, Li B, Häggblom MM, Han F, Xiao E, Zhang M, Wang Q, Li F (2019a) Bacterial response to antimony and arsenic contamination in rice paddies during different flooding conditions. Sci Total Environ 675:273–285. https://doi.org/10.1016/j.scitotenv.2019.04.146
49. Sun W, Xiao T, Sun M, Dong Y, Ning Z, Xiao E, Tang S, Li J (2015) Diversity of the sediment microbial community in the Aha watershed (Southwest China) in response to acid mine drainage pollution gradients. Appl Environ Microb 81(15):4874–4884. https://doi.org/10.1128/AEM.00935-15
50. Sun W, Xiao E, Krumins V, Häggblom MM, Dong Y, Pu Z, Li B, Wang Q, Xiao T, Li F (2018a) Rhizosphere microbial response to multiple metal(loid)s in different contaminated arable soils indicates crop-specific metal-microbe interactions. Appl Environ Microb 84(24):e00701–e00718. https://doi.org/10.1128/AEM.00701-18
51. Sun W, Xiao E, Häggblom M, Krumins V, Dong Y, Sun X, Li F, Wang Q, Li B, Yan B (2018b) Bacterial survival strategies in an alkaline tailing site and the physiological mechanisms of dominant phylotypes as revealed by metagenomic analyses. Environ Sci Technol 52:13370–13380. https://doi.org/10.1021/acs.est.8b03853
52. Sun X, Li B, Han F, Xiao E, Xiao T, Sun W (2019b) Impacts of arsenic and antimony co-contamination on sedimentary microbial communities in rivers with different pollution gradients. Microb Ecol 78:589–602. https://doi.org/10.1007/s00248-019-01327-5

53. Taniguchi T, Imada S, Acharya K, Iwanaga F, Yamanaka N (2015) Effect of soil salinity and nutrient levels on the community structure of the root-associated bacteria of the facultative halophyte, *Tamarix ramosissima*, in southwestern United States. J Gen Appl Microbiol 61:193–202. https://doi.org/10.2323/jgam.61.193
54. Thokchom E, Thakuria D, Kalita MC, Sharma CK, Talukdar NC (2017) Root colonization by host-specific rhizobacteria alters indigenous root endophyte and rhizosphere soil bacterial communities and promotes the growth of mandarin orange. Eur J Soil Biol 79:48–56. https://doi.org/10.1016/j.ejsobi.2017.02.003
55. Voges MJEEE, Bai Y, Schulze-Lefert P, Sattely ES (2019) Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. P Natl Acad Sci USA 116(25):12558–12565. https://doi.org/10.1101/485581
56. Wang G, Yu K, Wang Y, Su H, Wu H, Li T, Liang J, Huang W, Xiang W (2017) *Pelagibacterium lentulum* sp. nov., a marine bacterium from the culture broth of *Picochlorum* sp. 122. Int J Syst Evol Micr 67(9):3182–3185. https://doi.org/10.1099/ijsem.0.002054
57. Wang Y, Wang R, Fan L, Chen T, Bai Y, Yu Q, Liu Y (2017) Assessment of multi exposure of chemical elements and health risks among residents near Huodehong lead-zinc mining area in Yunnan, Southwest China. Chemosphere 174:613–627. https://doi.org/10.1016/j.chemosphere.2017.01.055 ()
58. Xiao E, Krumins V, Dong Y, Xiao T, Ning Z, Xiao Q, Sun W (2016) Microbial diversity and community structure in an antimony-rich tailings dump. Appl Microbiol Biot 100:7751–7763. https://doi.org/10.1007/s00253-016-7598-1
59. Xu D, Shi L, Qu X, Tian J, Wang K, Liu J (2019) Leaching behavior of heavy metals from the coal gangue under the impact of site Ordovician limestone karst water from closed Shandong coal mines, North China. Energ Fuel 33(10):10016–10028. https://doi.org/10.1021/acs.energyfuels.9b01928 ()
60. Xu X, Huo Y, Wang C, Oren A, Cui H, Vedler E, Wu M (2011) *Pelagibacterium* halotolerans gen. nov., sp. nov. and *Pelagibacterium luteolum* sp. nov., novel members of the family *Hyphomicrobiaceae*. Int J Syst Evol Micr 61(8):1817–1822. https://doi.org/10.1099/ijs.0.023325-0
61. Xu Y, Wang G, Jin J, Liu J, Zhang Q, Liu X (2009) Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. Soil Biol Biochem 41(5):919–925. https://doi.org/10.1016/j.soilbio.2008.10.027
62. Yang N, Sun C (2016) *Pelagibacterium lixinzhangensis* sp. nov., a novel member of the genus *Pelagibacterium*. Curr Microbiol 72(5):551–556. https://doi.org/10.1007/s00284-016-0989-9
63. Yang Q, Li Z, Lu X, Duan Q, Huang L, Bi J (2018) A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. Sci Total Environ 642(15):690–700. https://doi.org/10.1016/j.scitotenv.2018.06.068
64. Yun SW, Baveye PC, Kim DH, Kang DH, Lee SY, Kong MJ, Park CG, Kim HD, Son J, Yu C (2018) Analysis of metal(loid) contamination and their continuous input in soils around a zinc smelter:

development of methodology and a case study in South Korea. Environ Pollut 238:140–149. https://doi.org/10.1016/j.envpol.2018.03.020 ()

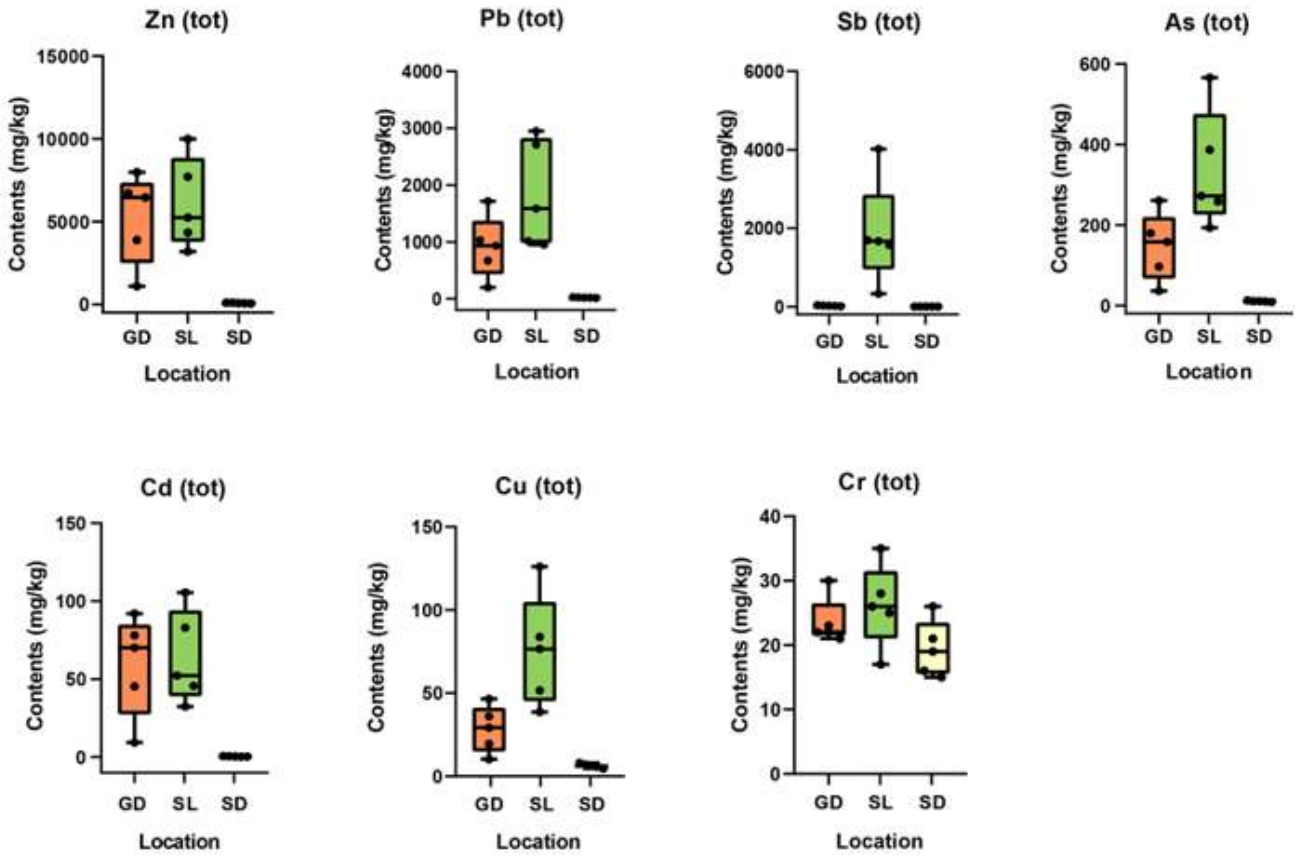
65. Zhai X, Li Z, Huang B, Luo N, Huang M, Zhang Q, Zeng G (2018) Remediation of multiple heavy metal-contaminated soil through the combination of soil washing and in situ immobilization. Sci Total Environ 635:92–99. https://doi.org/10.1016/j.scitotenv.2018.04.119
66. Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP, Firestone MK, Northen TR, Brodie EL (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480. https://doi.org/10.1038/s41564-018-0129-3
67. Zhao L, Qiu G, Anderson CWN, Meng B, Wang D, Shang L, Yan H, Feng X (2016) Mercury methylation in rice paddies and its possible controlling factors in the Hg mining area, Guizhou province, Southwest China. Environ Pollut 215:1–9. https://doi.org/10.1016/j.envpol.2016.05.001 ()
68. Zhao Y, Davis RE, Wei W, Lee M (2015) Should '*Candidatus Phytoplasma*' be retained within the order *Acholeplasmatales*? Int J Syst Evol Micr 65(Pt 3):1075–1082. https://doi.org/10.1099/ijs.0.000050
69. Zhong X, Chen Z, Li Y, Ding K, Liu W, Liu Y, Yuan Y, Zhang M, Baker AJM, Yang W, Fei Y, Wang Y, Chao Y, Qiu R (2020) Factors influencing heavy metal availability and risk assessment of soils at typical metal mines in Eastern China. J Hazard Mater 400:123289. https://doi.org/10.1016/j.jhazmat.2020.123289 ()

# Figures



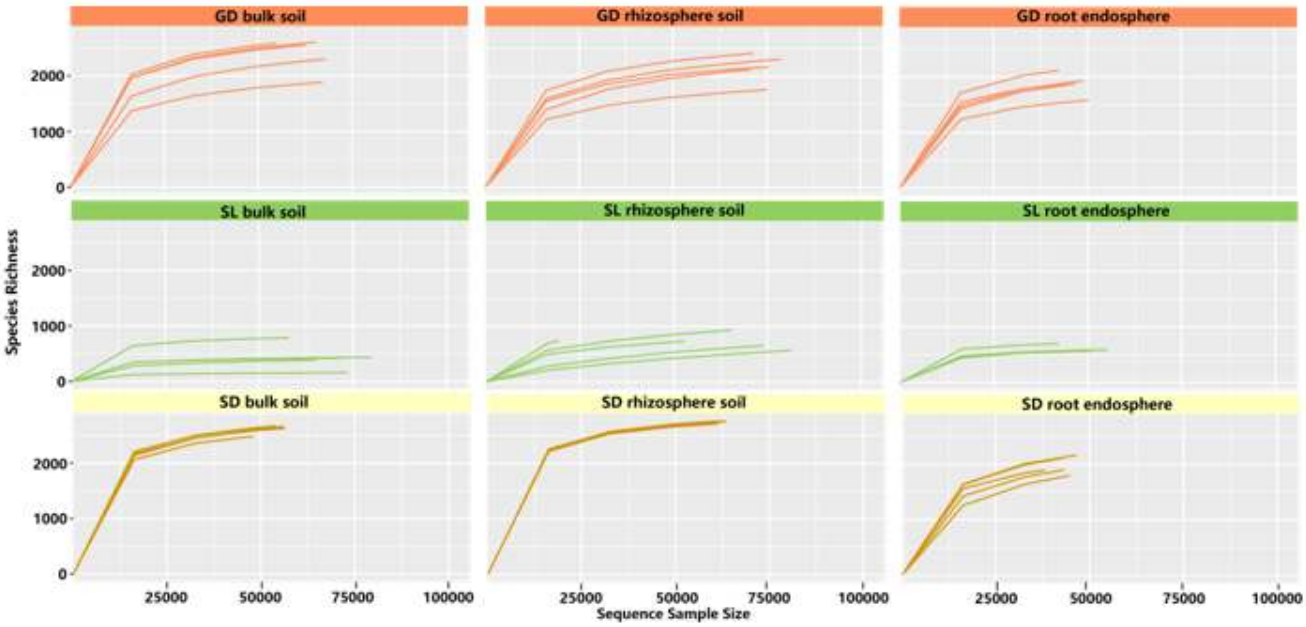

## Figure 1

Boxplots of the physicochemical parameters of bulk soil collected from the active Pb/Zn mine (GD), the abandoned Pb/Zn mine (SL), and an unpolluted site (SD).



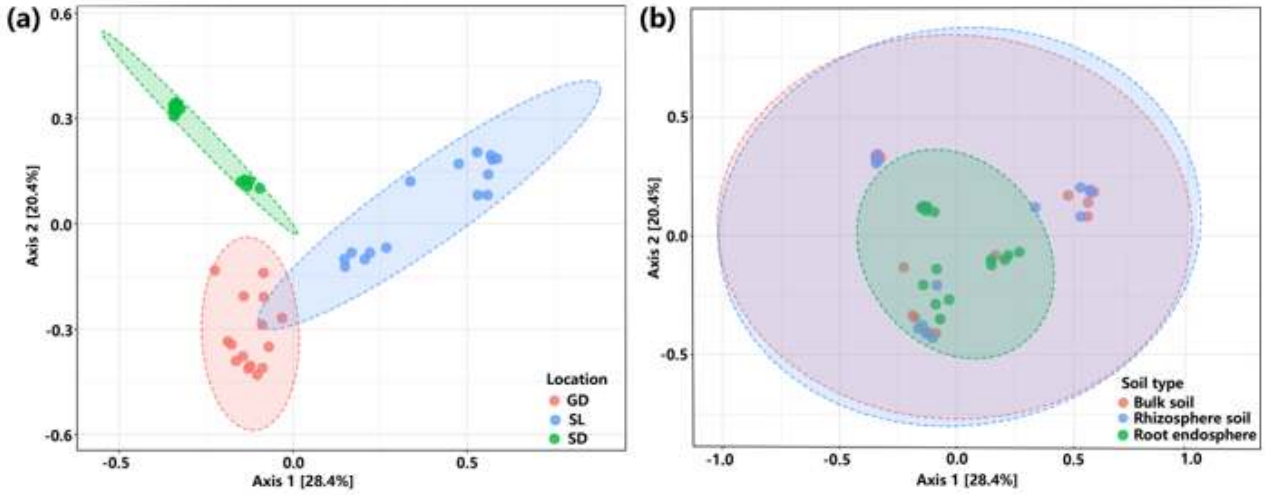

## Figure 2

Total concentrations (tot) of heavy metal(loid)s in the bulk soil collected from the active Pb/Zn mine (GD), the abandoned Pb/Zn mine (SL), and an unpolluted site (SD).




## Figure 3

Rarefaction curves of bacterial OTUs at 97% sequence similarity relative to the total identified sequences.

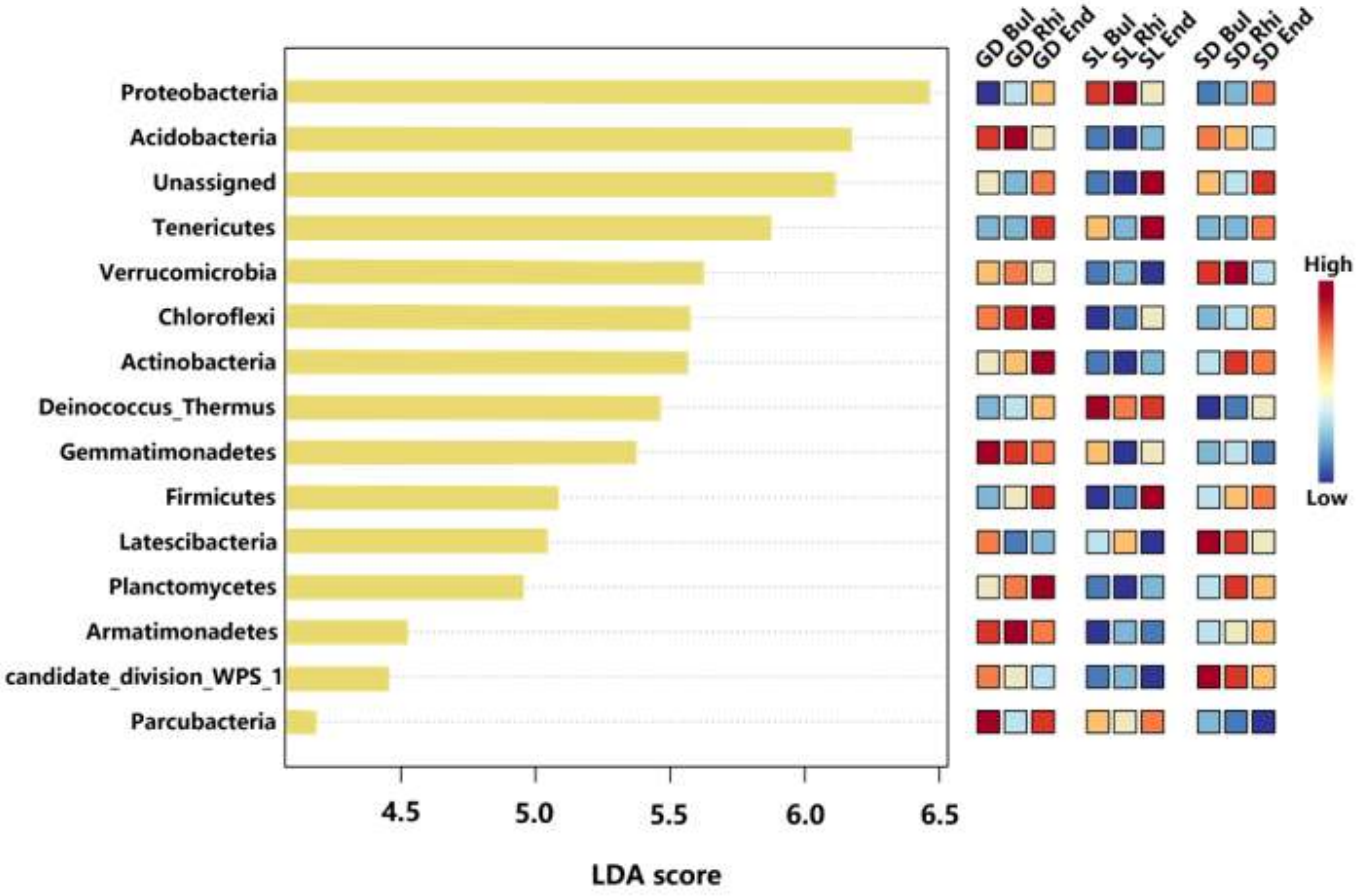



## Figure 4

PCoA of the bacterial communities depicting the samples obtained from (a) the GD (red), SL (blue), and SD (green) sampling sites, and (b) the bulk soil (red), root endosphere (green), and rhizosphere soil (blue) sample types.


## Figure 5

Relative abundances of the most abundant bacterial communities at the phylum (a) and class (b) levels in bulk soil, rhizosphere soil, and the root endosphere from the GD, SL, and SD sites. Five replicates of each sample are displayed in separate stacked bars.




## Figure 6

LefSe program showing the differences in relative abundances of the dominant phyla in bulk soil, rhizosphere soil, and root endosphere.

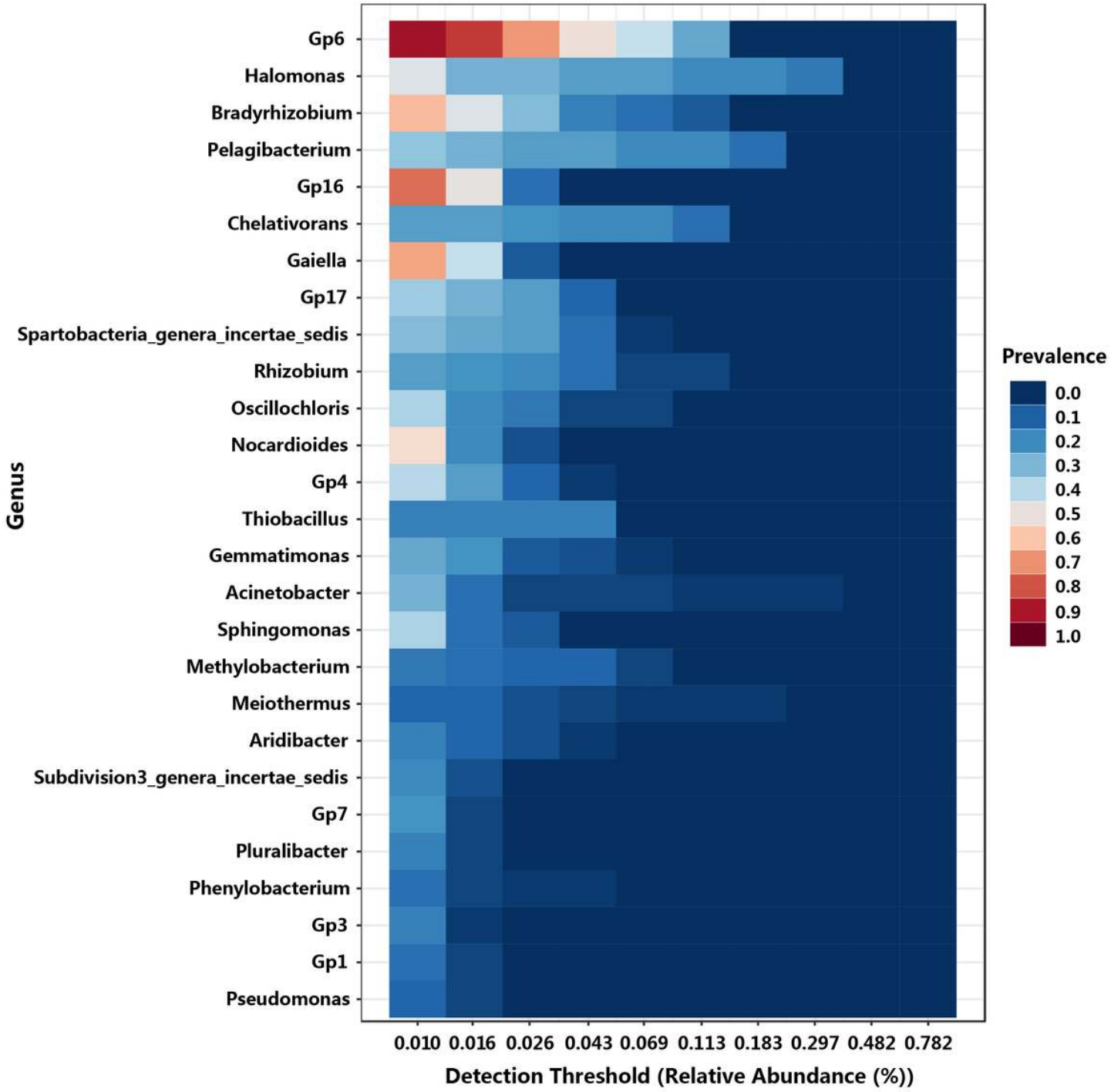



**Figure 7**

Core bacterial microbiome at the genus level across all samples.

# Supplementary Files

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