Deciphering the rhizosphere microbiome of a bamboo plant in response to different chromium contamination levels

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PII: S0304-3894(20)31096-7
DOI: https://doi.org/10.1016/j.jhazmat.2020.123107
Reference: HAZMAT 123107
To appear in: Journal of Hazardous Materials

Received Date: 9 March 2020
Revised Date: 13 May 2020
Accepted Date: 1 June 2020

Deciphering the rhizosphere microbiome of a bamboo plant in response to different chromium contamination levels

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Graphical abstract

Highlights

- The effects of different Cr pollution levels on the bamboo rhizosphere microbiome were evaluated.
• Cr noticeably influences the bacterial and fungal communities of the bamboo rhizosphere.
• A wide range of bacterial and fungal Cr-sensitive taxa were found.
• The phylum Acidobacteria can be used as an indicator in the studied Cr-polluted soil.
• The microbial changes linked with increasing Cr indicate high environmental stress.

Abstract
Bamboo has been considered a potential plant species for phytoremediation due to its high biomass and heavy metal (HM) resistance. However, little is known about the interactions between bamboo and soil microbial activities in HM-contaminated soils. Here, we investigated the characteristics of microbial communities in the rhizosphere soil of Lei bamboo (Phyllostachys praecox) along a chromium (Cr) gradient. We found that the soil Cr content was positively correlated with the total organic carbon (TOC) and HCl-extractable Cr but negatively correlated with the pH and bacterial and fungal Shannon indices. Proteobacteria and Ascomycota predominated in the bamboo rhizosphere under Cr pollution. A co-occurrence network showed that two of the most Cr-sensitive bacterial genera and keystone taxa were from the Acidobacteria, indicating that this phylum can be as an indicator for the studied Cr-polluted soils. Redundancy analysis revealed that both the soil bacterial and fungal community compositions were significantly correlated (p < 0.05) with Cr, pH, TOC,
alkali-hydrolysable N (AN), and available phosphorus (AP). The increase in TOC as the Cr content increased can be ascribed to an adverse Cr effect on the soil microflora, probably because the microbial biomass was less effective in mineralizing soil C under Cr-polluted conditions.

**Keywords:** Chromium pollution; Bamboo; Plant rhizosphere; Microbial community; Phytoremediation

1. **Introduction**

Soil heavy metal (HM) contamination has become a major environmental issue because HMs are not biodegradable and can remain in the soil for a long time, potentially harming the soil ecological environment [1-3]. Chromium (Cr) is one of the most abundant HM contaminants due to its widespread use in numerous industrial processes, such as electroplating, pigmentation, corrosion control and leather-tanning [4, 5]. Thus, anthropogenic Cr contamination has frequently become a major environmental problem near industrialized areas [6]. A national survey of soil contamination in China found pollution at 16.1% of survey points, of which 1.1% were polluted with Cr [7]. Cr in the soil not only affects soil health and food safety [8-10] but also poses a large threat to human health [11, 12]. Therefore, it is necessary to take effective measures to remediate Cr-contaminated soil.

Phytoremediation is one of the bioremediation techniques and defined as the application of plants and associated microbes to minimize the concentration or toxic effects of potential contaminants in the environment [13, 14]. Compared with physical
and chemical techniques, phytoremediation technology has many advantages, such as the elimination of secondary pollution, low costs and suitability for use in large areas [15, 16]; thus, it is usually regarded as a green and effective method for the remediation of HM-contaminated soil [17-19]. However, most of the hyperaccumulator plant species identified to date, which cannot achieve the desired effects, grow slowly and have low biomass productivity [20, 21]. Wieshammer et al. [22] described the ideal phytoremediation model as not only demonstrating a strong tolerance to and effective absorption of HMs but also growing quickly and presenting some economic benefits.

Bamboo is the main forest type in tropical and subtropical areas, with a total area of more than 30 million ha globally, accounting for 0.8% of the world’s total forested area [23]. Recent studies found that some bamboo species have high tolerance to heavy metal-contaminated soils, enabling considerable uptake and accumulation of heavy metals [24]. Compared with woody plants, bamboo species are preferable for use in phytoremediation due to their fast growth, high absorption capacity, and easy cultivation. Lei bamboo (Phyllostachys praecox), widely distributed in southern China, is one of these species. This bamboo species is cultivated for its high shoot yield and economic benefits [25]. Bian et al. [26] found that this bamboo species can grow normally on heavy Zn/Cd polluted soil (Zn > 2900 mg/kg, Cd > 14.5 mg/kg) with no toxic symptoms; the values of the bioconcentration factor and translocation factor were 0.64-0.82 and 0.78-1.09, respectively. Therefore, Bian et al. [18] suggested that although Lei bamboo is not a hyperaccumulator, it can still be used for
phytoremediation in heavy metal-contaminated soils.

The rhizosphere is the interface between plant roots and the soil and is inhabited by highly diverse communities of macro- and microorganisms [27, 28]; these microbial species are particularly sensitive to environmental changes [29]. Researchers have confirmed that the rhizosphere microbiome plays vital roles during the phytoremediation process [30-32]. Additionally, root-associated microorganisms can promote plant biomass and metal availability and mobility in contaminated soils or enhance plant photosynthesis and metal tolerance, which help increase the level of HM accumulation [33, 34]. Thus, there is an urgent need to understand the microbial role in the plant-microbial interactions of Lei bamboo; this may help us design phytoremediation plans and improve remediation efficiency.

In this study, we investigated and compared the characteristics of bacteria and fungi in bamboo rhizosphere soil along a Cr gradient. The main aims of this study were: 1) to assess the impact of a long-term Cr pollution on the bacterial and fungal communities in rhizosphere soil under field conditions. We hypothesized that long-term contamination with high Cr concentrations in soils will impact both bacterial and fungal community structures and diversities as well as their ecological functions. The microbial communities in high Cr contaminated soils in studied plantation are assumed to have developed mechanisms to tolerate higher amounts of Cr. 2) to investigate the relationship among environmental parameters, Cr pollution level and microbial community structure; and 3) to explore the microbial mechanisms of studied bamboo species in adapting high Cr stress in soils. We assumed that most
previous studies of Cr bioremediation of bamboo species were conducted in the laboratory stage using synthetic Cr solutions, which may not reflect their real effects in the field conditions [35].

2. Materials and methods

2.1. Experimental site

The study sites were located in Lin’an (30°18’N, 119°34’E) in northwestern Hangzhou, Zhejiang Province, China (Fig.S1). The area has a subtropical monsoon climate, and the average precipitation is 1600 mm. The mean annual temperature is 15.4 °C, with extreme minimum and maximum temperatures of -13.3 and 40.2 °C, respectively. The mean annual sunshine duration is 1850-1950 h, and the mean annual frost-free period is 235 days.

The study area covered approximately 45 ha of Lei bamboo plantation. A stream approximately 1-2 m in width flows through the plantation. From 1995 to 2009, there was a chromium galvanizing plant near the upstream region (less than 10 m away) and approximately 80 m from the bamboo plantation. During that time, the stream was contaminated by the effluent from the Cr galvanizing plant. The contaminated water overflowed the bank during the rainy season, resulting in soil Cr pollution in the bamboo plantations. To determine whether the studied area had a high Cr concentration in the soil, a preliminary investigation was conducted. We found that a clear Cr pollution gradient was formed, i.e., the soil Cr content largely depended on the distance to the stream. Therefore, we selected one bamboo plantation (60 m × 60 m) next to the stream as a sampling area. The sampling area was flat and belonged to
one household. The soil was Ferralic Cambisols derived from river alluvium [36]. The selected sampling area had conditions very similar to the initial site conditions and had been managed using the same method since the bamboo plantation was established in the late 1980s.

Based on the distance from the bank of the stream, five treatments, i.e., low (L or control), low-moderate (LM), moderate (M), moderate-high (MH) and high (H) were implemented in the investigated bamboo plantation, each with 5 replicates. Each replicate consisted of a 3 m × 3 m plot, and the distance between the replicates of a treatment was greater than 6 m. The average total Cr concentrations in the five treatments (L, LM, M, MH and H) were 46.65±0.68, 77.64±2.53, 87.23±1.83, 133.10±1.56, and 357.38±19.72 mg/kg, respectively. According to Yang et al. (2018), the average total Cr in the agricultural area is 48.0 mg/kg; therefore, the L treatment was assumed to be an uncontaminated area (i.e., a control).

2.2. Soil sampling

In each sampling plot, three bamboo plants were selected and dug out. The rhizosphere soils were collected by shaking the roots. The fresh soils were sieved (mesh size of 2 mm) to remove any stones and roots and large organic residue and then divided into two parts: one part was air dried for use in chemical property analysis, and the other part was stored at −80 °C for soil microbiome analysis.

2.3. Soil chemical analysis

The soil pH was measured with a glass electrode with a soil:water extract at a 1:2.5 (w:v) ratio. The soil total organic carbon (TOC) was determined using a TOC
The soil alkali-hydrolysable N (AN, diffusion method), available phosphorus (AP, extracted with 0.5 M NaHCO₃), and available K (AK, extracted with 1 M NH₄OAc) were analyzed according to methods described by Lu [37]. For the total Cr (TCr) analysis, the soil samples were digested using an automatic graphite instrument (Auto Digiblock S60 UP, Lab Tech Inc., Hopkinton, MA, USA) with a mixture solution of concentrated HCl–HNO₃–HF–HClO₄. The available Cr (ACr) was extracted with 0.1 M HCl according to [38, 39]. The TCr and ACr were determined via atomic absorption spectrometry (AAS) (AAnalyst800, Perkin Elmer, USA).

2.4. DNA extraction, PCR, and high-throughput sequencing

Genomic DNA was extracted from the rhizosphere soils using an E.Z.N.A.® Soil DNA Kit (D5625, Omega, Inc., USA) following the manufacturer’s instructions. The bacterial 16S rRNA and fungal ITS genes were amplified using the 341F (5’-CCTACGGGNGGCWGCAG-3’)/805R (5’-GACTACHVGGGTATCTAATCC-3’) and ITS1FI2 (5’-GAACCWGCGGARGGA TCA-3’)/ITS2 (5’-GCTGCGTTCTTCCATCGATGC-3’) primers. PCR amplification and high-throughput sequencing were performed according to the standard protocols of the LC-Bio Technology Co., Ltd. (Hang Zhou, Zhejiang Province, China), and the details for these procedure are provided in previous studies [40, 41]. The libraries were sequenced on an Illumina NovaSeq 6000 (paired-end sequencing, 2 x 250 bp).

2.5. Bioinformatics and statistical analysis

The paired-end reads were merged by the fast length adjustment of short reads
(FLASH) software [42]. To obtain high-quality clean tags, the reads were processed using Fqtrim v0.94, and the chimeric sequences were filtered using Vsearch v2.3.4 [43]. After dereplication using DADA2 [44], we obtained an amplicon sequence variant (ASV) table. Taxonomy was assigned using the QIIME2 [45] plugin feature classifier based on the SILVA 132 [46] and Unite databases [47]. The bacterial ASVs (bASVs) and taxonomy tables were filtered to exclude the ASVs classified as chloroplasts and mitochondria and those in an unclassified kingdom. Similarly, the fungal ASVs (fASVs) were filtered and those classified as having an unclassified kingdom were removed.

To assess the alpha and beta diversity, we only kept the ASVs with more than two sequences in at least five samples (the number of replicates per group). The analyses of the alpha (Shannon diversity and Chao1 richness indices) and beta (Bray–Curtis dissimilarity metric) diversity were performed in the R package “phyloseq” [48]. Analysis of similarities (ANOSIM), redundancy analysis (RDA), and variation partitioning analysis (VPA) were carried out in the R package vegan [49]. The relationship between the alpha indices and the Cr levels was analyzed and visualized using the “ggpubr” package in R [50]. Linear discriminant analysis (LDA) effect size (LEfSe) was used to determine the differences in the abundances of taxa at the phylum and genus levels between the samples based on p < 0.05 and an LDA score > 4.0 [51].

To identify the Cr-sensitive ASVs and construct co-occurrence networks, only those bASVs and fASVs with average relative abundances greater than 0.1% across
all the soil samples were kept according to methods described by Hartman et al. [52]. Briefly, the communities were normalized by applying the trimmed mean of M-values (TMM) method and the values were expressed as the relative abundance counts per million (CPM) in the edgeR package [53]. The indicator species analysis was performed using the “multipatt” function with the “r.g” association function (9,999 permutations) in the indicspecies package [54]. Differences in the ASVs were also assessed by likelihood ratio tests (LRT) with the R package “edgeR” [53]. The Cr-sensitive ASVs (cs ASVs) were confirmed by both the indicator species analysis and the LRT, and visualized using R scripts on GitHub (https://github.com/YongxinLiu/Note/tree/master/WenTao/191124Maptree/V2.0/MapreeForMicrobiome.R) and the pheatmap package [55]. We merged the TMM-normalized CPM counts of the bacteria and fungi and calculated the Spearman rank correlations using the R package “Hmisc” [56]. The adjusted p-value was obtained using the R function “p.adjust” with the option method=“fdr”. The significant correlations (\( \rho > 0.7 \) and \( p < 0.001 \)) were retained as the edges of the network. The R package igraph [57] was used to calculate the network properties and identify the network modules. The keystone ASVs were identified as those ASVs with a high degree (top 1%) within each network.

3. Results

3.1. The Cr contents and other soil properties

The TCr and ACr concentrations in the rhizosphere soils are shown in Fig. 1. The TCr and ACr in the five treatments were significantly different (\( p < 0.05 \)) from each
other; the order of the differences is as follows: H > MH > M > LM > L. Within these treatments, the ACr accounted for 1.02%, 0.79%, 1.53%, 1.89%, and 1.45% of the TCr, respectively. The ACr to TCr ratios in the H, MH and H treatments were significantly higher than those in L and LM treatments.

The soil properties are listed in Table 1. The soil pH ranged from 4.66 to 5.45, showing a decreasing tendency with increasing Cr content. The pH in the L and M treatments was significantly lower (p < 0.05) than that in the LM, MH, and H treatments, and there were significant differences in pH among the LM, MH, and H treatments. In general, the TOC showed an increasing tendency with Cr accumulation. The TOC in the M, MH, and H groups was significantly higher (p < 0.05) than that in the L and LM groups. The soil AN increased significantly with the intensity of Cr pollution except that in the LM treatment. In the soils with higher levels of contamination (i.e., the M, HM and H treatments), the AP contents were significantly higher than those in soils with lower levels of contamination (i.e., the L and LM treatments). In contrast, there was no significant change in the AK with the increase in Cr pollution, except in the LM treatment. The AK in the LM treatment was significantly lower than that in the other treatments (L, M, MH and H).

The correlation analysis showed a positive correlation (p < 0.05) between the TCr and ACr. The TCr and ACr were both negatively (p < 0.05) correlated with pH and were positively correlated with TOC and AN, respectively (Table S1). The ACr was also positively (p < 0.05) correlated with the AP (Table S1).

3.2. Soil bacterial and fungal community richness and diversity
After quality filtering, a total of 809,249 (range 19,249–46,640; median 32,786) and 1,283,464 (range 26,063–67,805; median 53,534) high-quality sequences were generated from all the soil samples. A total of 15,974 bacterial and 2,964 fungal ASVs were identified. Ten bacterial phyla with an average relative abundance > 1% were detected across all the soil samples (Fig. 2a): Proteobacteria (34.17%), Acidobacteria (25.51%), Actinobacteria (12.87%), Chloroflexi (8.00%), Planctomycetes (3.83%), Gemmatimonadetes (3.30%), Verrucomicrobia (3.22%), Bacteroidetes (3.06%), WPS-2 (1.39%), and Nitrospirae (1.03%). The soil fungal community was dominated by Ascomycota (64.62%), followed by Basidiomycota (28.00%) and Zygomycota (4.60%) (Fig. 2b).

The alpha diversity indices of the bacterial and fungal communities are shown in Fig. S2. The bacterial Shannon and Chao1 indices were significantly lower (p < 0.05) in the H treatment than in the four other treatments (L, LM, M, and MH). Both the TCr and ACr were significantly (p < 0.05) correlated with the bacterial Shannon and Chao1 indices (Fig. 3). For the fungal microbiome, there were significant differences (p < 0.05) in the Shannon index among the five treatments; the order of the Shannon index values is as follows: L>LM=M>H>MH. The correlation analysis showed that the fungal Shannon index was significantly related (p < 0.05) to the TCr and ACr.

3.3. Differences in the soil bacterial and fungal community compositions

The LEfSe analyses were used to reveal the taxa that contributed the most to the observed differences in the soil microbial communities under the different Cr pollution levels. As shown in Fig. 4a, no biomarkers were identified in the L treatment
at the phylum or genus level. The major bacterial group identified in the LM treatment was Acidobacteria; the M treatment had a relatively high abundance of Chloroflexi; the MH treatment had a relatively high level of Proteobacteria; and WPS-2, *Acidibacter, Acidothermus*, and *Candidatus Koribacter* were abundant in the H treatment. For the fungal communities (Fig. 4b), the highly abundant taxa in the L treatment included the *Exophiala, Pseudaleuria, Candida, and Trechispora; Hypocrea, Harpophora*, and *Cryptococcus* were abundant in the LM treatment; *Scutellinia* was highly represented in the M treatment; Ascomycota, *Verrucostoma*, and *Hydropus* were abundant in the MH treatment; and Basidiomycota, *Polyschema*, and *Auricularia* were underrepresented in the H treatment. As above, the microbes in the studied soil belonged to the Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, WPS-2, Ascomycota, and Basidiomycota. The comparison of the individuals of those phyla across all the samples indicated that the relative abundance of Proteobacteria in the MH treatment was significantly higher (p<0.05) than that in the LM and M treatments, while the abundance of Proteobacteria in the M treatment was significantly lower (p<0.05) than that in the L and H treatments (Fig. S3a). The relative abundance of Acidobacteria in the LM treatment was significantly higher (p<0.05) than that in the other four groups (L, M, MH, and H), and a significant difference (p<0.05) was also observed in the L vs M, L vs H, and MH vs H treatments (Fig. S3b). The relative abundance of Chloroflexi first increased and then decreased with the increasing Cr concentration; the abundance of Chloroflexi in the treatments was as follows: M>MH ≈LM>L>H (Fig. S3d). The abundance of WPS-2 was significantly higher in the H
group than in the other four groups, and its abundance in the LM treatment was significantly higher than that in the L, M, and MH treatments (Fig. S3e). However, no difference (p>0.05) was observed in the abundance of Actinobacteria among the five treatments (Fig. S3c). Among the fungal communities, the H treatment had a significantly lower (p<0.05) abundance of Ascomycota and a significantly higher (p<0.05) abundance of Basidiomycota than the other four groups (Fig. S3f and Fig. S3g). There was also a significant difference (p<0.05) in the abundance of Ascomycota in the L vs M, L vs MH, and LM vs MH treatments (Fig. S2f). We also found that the M and MH treatments had a significantly higher (p<0.05) abundance of Basidiomycota than the L and LM treatments, respectively (Fig. S3g).

The PCoA ordinations revealed that the structures of the bacterial and fungal communities also changed among the treatments with different Cr levels (Fig. 5). The first and second principal coordinates of the PCoA explained 59.4% (bacteria) and 52.7% (fungi) of the total variation. The ANOSIM statistically supported the significant separation among the five groups (bacteria, R = 0.98, p =0.001; fungi, R = 0.90, p =0.001).

### 3.4. Cr-sensitive ASVs and keystone taxa

Both indicator species analysis and likelihood ratio tests (edgeR) were used to identify the Cr-sensitive ASVs. A total of 204 and 101 ASVs were found to be Cr-sensitive ASVs (csASVs) for the bacteria and fungi, respectively (Fig. S4). The 204 csASVs in the bacterial communities of the rhizosphere soil were classified into at least 11 phyla (Fig. 6a), with Proteobacteria (36.03%), Acidobacteria (32.81%), and
Actinobacteria (12.17%) being the most abundant. Six bacterial genera can be identified from the top ten bacterial Cr-sensitive ASVs: *Acidothermus*, *Bryobacter*, *Granulicella*, *Sphingomonas*, *Dokdonella*, and *Gaiella* (Fig. 6c).

The 101 csASVs of the fungi can be classified into at least five different phyla (Fig. 6b), with the majority of the community sequences belonging to the Ascomycota (72.60%) and Basidiomycota (25.88%). Seven of the top ten fungal Cr-sensitive ASVs can also be identified at the genus level: *Exophiala*, *Gibberella*, *Pseudaleuria*, *Roussoella*, *Trechispora*, *Paracremonium*, and *Trichosporon* (Fig. 6d).

A co-occurrence network was constructed to visualize the positions of the csASVs associated with each Cr level in the soil microbial community (Fig. 7a). We found three bacteria-sensitive ASVs (Table S2) defined as keystones in the network, and the most detailed classifications were Subgroup_6 (Acidobacteria), *Acidothermus* (Actinobacteria), and *Candidatus Koribacter* (Acidobacteria). The comparison of individuals of those keystones across all the samples (Fig.S5) indicated that, compared with the L, M, and MH treatments, in the H treatment, the relative abundances of *Acidothermus* and *Candidatus Solibacter* was significantly increased and the relative abundance of Subgroup_6 was significantly decreased. We also found that *Acidothermus* and *Candidatus Solibacter* were significantly higher in the LM treatment than in the L, M, and MH treatments. Among the five groups, a significant difference (p<0.05) was also observed in Subgroup_6; the order of the abundance of Subgroup_6 in the treatments was as follows: MH≈M>L>LM>H. We also visualized the three modules with the highest interactions in the network. These three modules
were separated from each other (Fig. 7a). Module 4 in the largest module in the network (Fig. 7a) and is considered the central module. The highest cumulative relative abundances of the ASVs belonging to module 1, module 2, and module 4 were in the M, H, and L treatments, respectively, and were also significantly (p < 0.05) higher than those in the other groups (Fig. 7b). We also found variations in the bacterial and fungal phyla in the different modules (Fig. 7c), indicating that different Cr concentrations may inhabit specific microbial lineages.

3.5. Relationships between microbial community structures and soil variables

We performed a redundancy analysis (RDA) to reveal the relationship between the microbial community structures and soil variables. The soil parameters explained 71.04% and 66.34% of the bacterial and fungal community variation in the first two RDA axes, respectively (Fig. 8a, b). The results showed that the TCr (R² = 0.82, p = 0.001; R² = 0.92, p = 0.001), ACr (R² = 0.77, p = 0.001; R² = 0.85, p = 0.001), pH (R² = 0.47, p = 0.002; R² = 0.70, p = 0.001), TOC (R² = 0.89, p = 0.001; R² = 0.59, p = 0.001), AN (R² = 0.79, p = 0.001; R² = 0.71, p = 0.001), and AP (R² = 0.32, p = 0.015; R² = 0.44, p = 0.002) were significantly correlated with the bacterial and fungal community structures. The soil AK (R² = 0.53, p = 0.001) was also significantly correlated with the bacterial community structure. The Cr and soil properties explained 22.4% and 6.2% of the variations in the bacterial communities, respectively (Fig. 8c). The interaction between the soil properties and Cr explained 20% of the variation (Fig. 8c). For the fungal communities, the VPA showed that a total of 51.4% of the variation could be explained (Fig. 8d). The Cr and soil properties explained 7%
and 22.8% of the variation, respectively, and their interaction explained 21.6% of the variation (Fig. 8d).

4. Discussion

The rhizosphere is the root-soil interface, where a range of key biological functions of plant roots, such as uptake, respiration and exudation, can considerably alter the soil biochemical properties, further influencing whole reactions at the soil solid/soil solution interface [58]. Therefore, in this study, we used rhizosphere soil instead of bulk soil to study the changes in microbial community structure and function along a chromium pollution gradient and more accurately and sensitively reflect the changing processes in response to Cr stress [59]. Additionally, since the pollution source (a Cr galvanizing plant) was closed in 2009, the pollution in the studied soils, which were exposed to Cr stress, had to be more than 10 years old. This is enough time for the soil microorganisms to adapt to the changed soil conditions. Moreover, while most of the studies on the effect of Cr in soil are conducted as laboratory experiments, long-term studies in the field are still scare [35]. This study was designed to evaluate the responses of rhizosphere microbiome of a bamboo plant to different chromium contamination levels, giving scientific guidance regarding the application of bamboo plants for Cr remediation in the field.

4.1. Changes in rhizosphere soil properties along the Cr pollution gradient

In this study, a decreasing pH was found with Cr accumulation. The decrease in pH may be caused by heavy metal cations displacing base cations, such as Ca\(^{2+}\) and Na\(^{+}\), from the soil exchange complex or binding sites [5, 60]. According to Rinklebe
et al. [61], pH was negatively correlated to the redox potential (E<sub>H</sub>), which can explain the concentration of Cr in soil solution increase with rising E<sub>H</sub>. The release of organic acids in root exudates can partially contribute to the decrease in pH. According to UdDin et al. [62], the release of organic acids in root exudates of Solanum nigrum was significantly increased with increasing levels of Cr. Interestingly, the TOC and AN showed increasing tendencies in the soils with higher levels of Cr-contamination (i.e., the M, MH and H treatments). This may be attributed to an adverse heavy metal effect on the soil microbes when the soil Cr content reached a toxic degree. Studies have indicated that microbes are less effective in mineralizing soil organic matter under heavy metal stress [63, 64]. Azarbad et al. [63] found that microbial basal and substrate-induced respiration decreased with an increasing toxicity index. The mobility and availability of Cr usually increased with increasing soil pH due to the reduction in the ratio of soluble Cr(VI) to insoluble Cr(III) under acidic conditions [9]. Thus, a positive correlation is expected between the available Cr and pH values in acidic soil. However, a significantly negative correlation of HCl-extractable Cr content and soil pH was observed in this study, indicating that other soil parameters, such as soil organic matter content, deeply influence the availability of Cr in the studied soils. Organic matter plays a dual function in the availability and mobility of heavy metals in soil. Since SOM is rich in functional groups and has strong complexation ability, it can be a major sink for heavy metals [65]. On the other hand, soil organic matter can supply organic chemicals, which can serve as chelates to increase heavy metal solubility in soil [66]. Luo et al. [67] also found that some Cr(III)
organic complexes can be soluble in soils. As previously found by Shaheen and Rinklebe [68], SOC, cation exchange capacity (CEC), and total sulphur (St), as well as the various forms of Fe–Mn oxides positively affected the potential mobility and total concentration of Cr. The positive influence of organic matter on the availability and solubility of Cr in the studied soil could be a potential danger to the soil environment. Additionally, our study found that the soil bacterial and fungal compositions were different among the five levels of Cr. We speculate that the variation in the rhizosphere soil pH, TOC, Cr, and other properties caused by the different Cr concentrations may be partly explained by the shifts in microbial community structures along the Cr pollution gradient.

4.2. Effects of Cr pollution on rhizosphere soil microbial communities and functions

Adaptations by the soil microbial community to the long-term pollution of high concentrations of heavy metals included changes in diversity, abundance and structure and uniformity [69]. In our study, the results showed that the rhizosphere soil bacterial and fungal Shannon indices were negatively correlated with the Cr concentrations. Obviously, the increasing level of Cr damaged the soil microbial richness. Similar results were observed by other studies [70, 71], which found that there were significant negative correlations between the abundances and diversities of bacteria and fungi and HM levels in agricultural soils. However, Liu et al. [72] reported that the bacterial Shannon index was positively correlated with total Hg in paddy soils. They suggest that through direct interaction with HMs, the rhizosphere soil microbial communities could reduce the toxicity of HMs to plants, resulting in changes in their
bioavailability [69, 73, 74]. However, this phenomenon was not observed herein. In this study, the number of bacterial taxa (15,974 ASVs) was much greater than that of fungi (2,964 ASVs). In contrast, we found that the bacterial Chao1 index was significantly reduced with increasing Cr levels, but this was not the case for the fungi. This result may support the viewpoint that fungi are more resistant than bacteria to HMs [75, 76].

The PCoA ordinations clearly indicated that the structures of the bacterial and fungal communities changed significantly among the different Cr levels (Fig. 5). Among the bacteria, the largest changes were observed in the Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and WPS-2 phyla. More than 80% of the Cr-sensitive ASVs were from Proteobacteria, Acidobacteria, and Actinobacteria (Fig. 6). Generally, Proteobacteria was the most abundant phylum in the studied soils. Proteobacteria have been described as copiotrophs and can grow quickly when labile substrates are available [77, 78]. Member of Proteobacteria favors soil with relatively high levels of available carbon [79, 80]. Some proteobacterial strains are plant growth-promoting bacteria, which can symbiotically fix nitrogen [81, 82] and are the most tolerant taxa to HMs [83, 84]. In this study, we also found that 36.03% of the Cr-sensitive genera were from this phylum, such as Sphingomonas and Dokdonella, both of which are very important genera in C and N cycling [85-87]. Acidobacteria was the second most abundant taxon in the studied soils. Previous studies found that Acidobacteria generally prefer an oligotrophic environment [88, 89]. Cui et al. [90] found that the abundance of the phylum Acidobacteria may be significantly negatively
correlated with HM concentrations. In contrast, Guo et al. [91] found that Acidobacteria was positively correlated with Zn, Pb and As (P < 0.05) and soil organic matter (SOM) (P < 0.01). Rowe et al. [92] suggested that some members of the phylum Acidobacteria can survive in nutrient-limited conditions due to their large number of anion/cation symporters. In this study, two of the most Cr-sensitive genera (Bryobacter and Granulicella) and two keystone taxa (Acidobacteria Subgroup_6 and Candidatus Koribacter) were from this phylum, suggesting that this phylum can be used as an indicator in Cr-polluted soils. Moreover, two of the most Cr-sensitive genera (Acidothermus and Gaiella) were members of the Actinobacteria. Previous studies have shown that Actinobacteria exhibited an ability to reduce Cr(VI) [93, 94]. Ellis et al. [95] found that two genera within the phylum Actinobacteria, Iamia and Arthrobacter, were sensitive to Cr. Significantly decreased abundance of Chloroflexi was observed in the H treatment. Genomic analyses have indicated that the phylum Chloroflexi plays roles in carbon cycling, such as the respiration of sugars, fermentation, and CO₂ fixation [96]. Researchers have also isolated some nitrite-oxidizing bacterial strains belonging to the phylum Chloroflexi [97, 98]. Thus, the marked reduction in the abundance of the phylum Chloroflexi in the H treatment might indicate a reduction in the C and N cycles. Interestingly, there was a sharp increase in WPS-2 in the H treatment. This bacterial phylum was recently discovered and is known to be capable of anoxygenic photosynthesis using compounds such as sulfide, molecular hydrogen, ferrous iron, or arsenic as electron donors [99]. Our result was consistent with findings from Zhang et al. [100], who found that the
phylum WPS-2 was negatively ($p < 0.05$) correlated with pH and positively ($p < 0.05$) correlated with TOC and AN. The relatively low pH and high TOC and levels of other nutrients in the highly Cr contaminated soil (H) might be favorable for this phylum.

The fungal phyla Ascomycota and Basidiomycota were the most abundant phyla in the studied rhizosphere soils, and together accounted for more than 90% of the total relative abundance of fungi. Similar results have been found by Narendrula-Kotha and Nkongolo[101], who revealed that Ascomycota and Basidiomycota were the most abundant taxa in HM-contaminated soils. The LEfSe results also showed significant differences in the soil fungal community structure under different Cr contamination levels, especially for the phyla Ascomycota and Basidiomycota (Fig. 4b). According to Lin et al. [102], Ascomycota, Basidiomycota and Zygomyctota showed strong tolerance to HMs and can be beneficial for creating a healthy soil environment for crop growth. Likar and Regvar [103, 104] indicated that Ascomycota had a greater stress tolerance than Basidiomycota. In this study, we found that the relative abundance of Ascomycota dominated the fungal profile in the soils with moderate Cr pollution levels (i.e., the M and MH treatments). When Cr pollution reached a high level (the H treatment), the relative abundance of Basidiomycota was higher than that of Ascomycota, indicating that changes in the richness of both phyla largely depended on the Cr pollution level. The phylum Basidiomycota has important roles in plant residue degradation, especially for lignocellulosic organic matter [105, 106]. This result suggested that basidiomycetes played a more important role than ascomycetes in driving the C cycle in highly Cr-contaminated soil. The high variation in the
relative abundances of Ascomycota and Basidiomycota with increasing soil Cr content may partially contribute to changes in the abundances of Cr-sensitive taxa, because most of the Cr-sensitive taxa found in this study belong to the Ascomycota (e.g., Exophiala, Gibberella, Pseudaleuria, Roussoella and Paracremonium) and Basidiomycota (e.g., Trechispora and Trichosporon).

4.3 Effects of Cr pollution on the interactions between the rhizosphere soil microbial communities and environmental parameters

Keystone taxa are considered microbes that frequently co-occur with many other microbes and may play ecologically important roles by determining community dynamics and microbiome functioning [52]. The co-occurrence network analysis in this study found three keynote taxa (Acidothermus, Acidobacteria Subgroup_6, and Candidatus Koribacter) in the bacterial community. The highest abundance of Acidothermus and Candidatus Solibacter and the lowest abundance of Subgroup_6 were found in the H treatment. Acidothermus species can efficiently degrade cellulose [107]. Candidatus Koribacter is involved in degradation, e.g., of cellulose, hemicellulose, and chitin [108], and can be negatively affected by pH [109]. Acidobacteria Subgroup_6 may be associated with a relatively slow turnover of organic carbon in the low-N-input rhizosphere [110] and had a significant negative correlation with soil pH [111]. In contrast, we did not find keynote taxa in the fungi. However, there was a large increase in fungal species, such as Auricularia and Polyschema, in the treatments with high Cr pollution levels. For example, the genus
Auricularia, belonging to the phylum Basidiomycota, is one of the very important wood-decaying fungi [112]. These results demonstrated that the increase in Cr was associated with the increase in soil TOC and other nutrient (e.g., AN and AP) contents. Moreover, an increase in the microbial taxa (both bacteria and fungi) involved in C and N cycling occurred; however, the soil TOC still showed an increasing tendency. These contradictory phenomena may indicate that the input of Cr has led to a high ecological risk associated with sustainable land use.

In the current study, RDA indicated that Cr (TCr and ACr) and other soil properties (pH, TOC, AN, and AP) were significantly related to bacterial and fungal community structures (Fig. 8). Zhang et al. [113] found that pH, TOC, AN, and AP were significantly related to bacterial communities, while soil pH and AK were highly correlated with fungal communities in soils. The correlation analysis showed that both TCr and ACr were negatively (p < 0.05) correlated with pH and positively correlated with TOC and AN (Table S1), indicating that Cr input can affect the C and N cycles. The RDA further confirmed that the Cr addition could have a direct or indirect impact via affecting other soil properties such as TOC, pH etc. in shaping the bacterial and fungal communities. The indirect impact could be more influential.

5. Conclusion

The present study showed shifts in the bacterial and fungal community structures in the Lei bamboo rhizosphere along a Cr gradient. Cr accumulation in the soil appeared to cause an alteration in the soil C cycle, which may be caused by the
decrease in the net mineralization of soil C. Soil Cr (both TCr and ACr), pH, TOC, AN, and AP were significantly correlated with the bacterial and fungal communities. The positive influence of organic matter on the availability and solubility of Cr in the studied soil could be a potential danger to the soil environment. Based on the sequencing data, our results identified some Cr-sensitive bacterial and fungal taxa. Acidobacteria can be used as an indicator of soil changes because both of the top two Cr-sensitive genera and two of the three keystone taxa were from this phylum. However, we did not find keystone taxa in the fungi. The shift in microbial communities indicated that the soil bacteria were more sensitive than the fungi to Cr pollution. These results will help understand the complex interactions among soil properties, Cr pollution, and shifts in bacterial and fungal community structures and will provide a scientific reference for the remediation and management of Cr-contaminated soils.

Credit Author Statement

Xiaoping Zhang: Conceptualization, Software, Formal analysis, Writing -Original Draft

Fangyuan Bian: Investigation, Formal analysis

Zhezhe Zhong: Conceptualization, Writing-Review & Editing, Supervision, Funding acquisition

Xu Gai: Investigation

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

Acknowledgments
This research was supported by the Fundamental Research Funds for the Central Non-profit Research Institution of CAF (CAFYBB2018ZD002).

References


doi:https://doi.org/10.1016/j.gexplo.2005.08.041
[72] Y.-R. Liu, M. Delgado-Baquerizo, L. Bi, J. Zhu, J.-Z. He, Consistent responses of soil microbial taxonomic and functional attributes to mercury pollution across China,
Microbiome, 6 (2018) 1-12.
[86] H.P. Bacosa, C. Inoue, Polycyclic aromatic hydrocarbons (PAHs) biodegradation


[112] Y. Yuan, F. Wu, J. Si, Y.-F. Zhao, Y.-C. Dai, Whole genome sequence of *Auricularia heimuer* (Basidiomycota, Fungi), the third most important cultivated mushroom worldwide, Genomics, 111 (2019) 50-58.


**Fig.1.** The Cr concentrations in the bamboo rhizosphere soils. The different letters indicate significant differences among the five Cr levels based on a one-way ANOVA (LSD, p < 0.05). TCr, total Cr; ACr, available Cr; L, low; LM, low-moderate; M, moderate; MH, moderate-high; H, high.
**Fig.2.** Relative abundances of the dominant bacterial (a) and fungal (b) phyla in the bamboo rhizosphere soils under different Cr contamination levels.

**Fig.3.** Linear relationships between the soil Cr and the bacterial and fungal alpha indices in the bamboo rhizosphere soils. Blue line, bacteria; red line, fungi.
Fig. 4. Linear discriminant analysis (LDA) effect size (LEfSe) of the bacterial (a) and fungal (b) taxa, which identifies the most differentially abundant taxa among the different Cr pollution levels. Only the phyla and genera identified as meeting a significant LDA threshold of > 4 are shown.
**Fig. 5.** Principal coordinate analysis based on Bray–Curtis distance for the soil bacterial (a) and fungal (b) community structures of the bamboo rhizosphere under different Cr pollution levels.

**Fig. 6.** Cr-sensitive taxa in the bamboo rhizosphere. a and b. Overview of the bacterial (a) and fungal (b) Cr sensitivity at the phylum level; c and d, the top ten most Cr-sensitive bacteria (c) and fungi (d) at the genus level.
Fig. 7. Co-occurrence patterns of the Cr-sensitive ASVs. a, Co-occurrence networks visualizing the significant correlations ($r > 0.7$, $p < 0.001$) between the bacterial and fungal ASVs with an average relative abundance $> 0.1\%$ across all the samples. b, Cumulative relative abundances (as counts per million, CPM; y-axis: $\times 1000$) of the bacterial and fungal OTUs belonging to the respective module of each Cr pollution level. c, The bacterial and fungal phyla composition for each module.
Fig. 8. Redundancy ordinations (a, b) and variation partitioning analysis (c, d) of the selected soil properties for the bacterial (a, c) and fungal (b, d) community structures in the bamboo rhizosphere soil along a Cr pollution gradient.

Table 1 Soil chemical properties at different levels of soil Cr contamination

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TOC (g/kg)</th>
<th>AN (mg/kg)</th>
<th>AP (mg/kg)</th>
<th>AK (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>5.45±0.04a</td>
<td>36.02±2.37c</td>
<td>203.70±4.56c</td>
<td>115.16±1.45c</td>
<td>242.98±9.23a</td>
</tr>
<tr>
<td>LM</td>
<td>4.84±0.04c</td>
<td>35.45±1.10c</td>
<td>194.60±3.99d</td>
<td>101.11±1.36d</td>
<td>139.41±6.03b</td>
</tr>
<tr>
<td>M</td>
<td>5.40±0.02a</td>
<td>38.05±1.06b</td>
<td>209.30±3.83c</td>
<td>129.42±1.27a</td>
<td>257.31±3.62a</td>
</tr>
<tr>
<td>MH</td>
<td>5.30±0.07b</td>
<td>46.39±1.49a</td>
<td>232.40±3.99b</td>
<td>129.36±1.40a</td>
<td>251.35±28.63a</td>
</tr>
<tr>
<td>H</td>
<td>4.66±0.03d</td>
<td>47.35±1.10a</td>
<td>263.20±5.19a</td>
<td>123.57±7.59b</td>
<td>237.07±22.48a</td>
</tr>
</tbody>
</table>

The different letters indicate significant differences among the five Cr levels based on a one-way ANOVA (LSD, p < 0.05). TOC, total organic carbon; AN,
alkali-hydrolysable nitrogen; AP, available phosphorus; AK, available K; L, low; LM, low-moderate; M, moderate; MH, moderate-high, H, high.