FEMS Microbiology Letters, 367, 2020, fnaa117

https://doi.org/10.1093/femsle/fnaa117
Advance Access Publication Date: 16 July 2020
Research Letter

RESEARCH LETTER – Environmental Microbiology

The recruitment of bacterial communities by the plant root system changed by acid mine drainage pollution in soils

Yang Li[1,2], Liang Yuan[2,3,*], Sheng Xue[1], Bingjun Liu[1] and Gang Jin[4]

[1]State Key Laboratory of Mining Response and Disaster Prevention and Control in Deep Coal Mines, Anhui University of Science & Technology, Huainan, Anhui province, China, [2]Key Laboratory of geological disaster prevention and control of mines in Anhui Province, Anhui University of Science & Technology, Huainan, Anhui province, China, [3]School of Earth and Environment, Anhui University of Science & Technology, Huainan, Anhui province, China and [4]Anhui Kunlang New Energy Technology Co. Ltd., Huainan, Anhui Province, China

[*]**Corresponding author:** State Key Laboratory of Mining Response and Disaster Prevention and Control in Deep Coal Mines, Anhui University of Science & Technology, No. 111 Taifeng Road, Huainan 232001, Anhui province, China. Tel: +86-0554-6668842; E-mail: yuanl_1960@sina.com

One sentence summary: Plants can recruit functional bacteria to the roots in response to AMD pollution.

Editor: Skorn Mongkolsuk

ABSTRACT

This study aims to better understand the relationship between the response to acid mine drainage (AMD) stress of tolerant plants and changes in root-related bacterial communities. In this study, reed stems were planted in AMD-polluted and unpolluted soils, and high-throughput sequencing was conducted to analyze the bacterial community composition in the soil, rhizosphere, rhizoplane and endosphere. The results showed that the effect of AMD pollution on root-associated bacterial communities was greater than that of rhizo-compartments. Proteobacteria were dominant across the rhizo-compartments between treatments. The microbiomes of unpolluted treatments were enriched by Alphaproteobacteria and Betaproteobacteria and depleted in Gammaproteobacteria ranging from the rhizoplane into the endosphere. However, the opposite trend was observed in the AMD pollution treatment, namely, Gammaproteobacteria were enriched, and Alphaproteobacteria and Deltaproteobacteria were mostly depleted. In addition, endophytic microbiomes were dominated by Comamonadaceae and Rhodocyclaceae in the unpolluted treatment and by Enterobacteriaceae in the AMD-polluted soils. PICRUSt showed that functional categories associated with membrane transport, metabolism and cellular processes and signaling processes were overrepresented in the endosphere of the AMD-polluted treatment. In conclusion, our study reveals significant variation in bacterial communities colonizing rhizo-compartments in two soils, indicating that plants can recruit functional bacteria to the roots in response to AMD pollution.

Keywords: AMD pollution; root-associated microbiome; rhizosphere compartments; *Phragmites communis*

INTRODUCTION

Acid mine drainage (AMD), caused by mining and smelting activities, has strong acidity and is rich in multiple heavy metals. Once AMD is discharged into the soil ecosystem, a large number of hydrogen ions cause soil acidification, which could inhibit plant respiration and nutrient absorption by the root system (Karaca, Cameselle and Reddy 2018). In addition, the increased heavy metals in soils caused by AMD are not easy

Received: 14 October 2019; **Accepted:** 14 July 2020

to remove. The increased potentially toxic metals (such Cd and Pb) and metalloid elements (such As), can contribute to great changes in the ecological dynamics of the rhizosphere niches (Gray and Smith 2005; Pajuelo *et al.* 2008; Chen *et al.* 2018). In addition, heavy metals also have negative effects on plant growth, biomass and photosynthesis (Nagajyoti, Lee and Sreekanth 2010), thereby affecting ecosystem development.

The most cost-effective and environmentally friendly remediation method to remove potentially toxic elements, the assisted phytoremediation technique, is widely applied to improve soil properties, preventing hydraulic erosion and wind dispersion and reducing pollution mobility (Adamo *et al.* 2015). The selection of remediation plants has always been an important part of phytoremediation, and native plants with high adaptability are preferred (Pandey 2015). Previous studies have focused on the mechanisms and physiological characteristics of plant tolerance. For example, plants can regulate the expression of gene(s) that are involved in signaling and regulatory pathways and in the synthesis of functional and structural proteins and metabolites (Singh *et al.* 2016). Plants can also change their photosynthetic efficiency, stomatal movement and root morphology to ameliorate the effects of heavy-metal toxicity (Nazir, Hussain and Fariduddin 2019). Recent studies have investigated the role of related functional microorganisms in the plant root zone. In particular, plant growth-promoting bacteria (PGPBs) play an important role in promoting plant growth and remediating soil pollution (Rajkumar *et al.* 2012; Chauhan *et al.* 2015; Chen *et al.* 2018; Khanna *et al.* 2019). These PGPBs effectively promote the migration of heavy metals by several mechanisms, such as dissolving metal minerals, acidifying the rhizosphere environment, improving the surface area of the root system and increasing the release of root secretions (Glick 2010; Rajkumar *et al.* 2012; Sessitsch *et al.* 2013). In recent years, some PGPBs tolerant of heavy metals are believed to promote the growth of plants in metal-contaminated fields and reduce the bioavailability of toxic metals. Many studies of PGPB heavy-metal resistance have shown a series of reactions to metal ions, such as metal biosorption, bioaccumulation, precipitation, complexation, reductive oxidation and enzymatic metal transformation, to reduce the toxicity of heavy metals to plants (Rajkumar *et al.* 2012; Ma *et al.* 2016). However, there is little direct evidence that plants can recruit specific functional microorganisms to enhance their resistance to and alleviate the negative effects of soil pollution. Bell *et al.* (2013) found that willow can preferentially recruit Pezizomycetes in the rhizosphere of hydrocarbon-contaminated soils. Knowledge of the compositional and functional differentiation of bacterial communities in different root-associated compartments and their roles in remediating plant growth, which can reflect the migration process of microorganisms affected by the plants, is scarce.

In this study, the reed, a resistant plant that can grow in AMD-polluted soils, was cultivated via cutting. The composition of bacterial communities in different soil rhizosphere compartments was analyzed after root growth of plants in AMD-polluted and unpolluted soils. The primary aim of this study was to determine whether plant growth would change the recruitment of soil microorganisms under soil pollution.

MATERIALS AND METHODS

Raw materials and experimental design

Phragmites communis (Cav.) Trin. ex Steud. samples were collected from a pond and utilized for cultivation. The AMD-polluted paddy soils and the unpolluted paddy soils were both collected from Zhongming town (Tongling, China), and the AMD-polluted paddy soils had been irrigated with AMD for a long time. Each soil was mixed completely after natural drying, and the roots were removed and milled. The thick reed stalks of *P. communis* were cut off from the roots, with the surface being sterilized, and then planted in the two soils and grown for 4 months. The plants growing in the AMD-polluted paddy soils were marked as the AMD pollution group (AMD-pollution) and the plants growing in the unpolluted paddy soils were marked as the control group (ck). The basic chemical properties of the two soils and the heavy metals in plant roots are shown in Table S1. The soils were irrigated with sterile deionized water every 2 or 3 days to maintain ~60% of the soil maximum field capacity. During plant growth, all weeds were removed from the pots. Each plant collected had all four rhizo-compartments sampled.

Collection of rhizosphere, rhizoplane and endosphere compartments

Bulk soils were collected from uncultivated soil. The rhizosphere, rhizoplane and endosphere samples were collected according to the protocols described by Edwards *et al.* (2015). First, the loose soil was removed by shaking, and the soil attached to the roots (rhizosphere soil) was collected using sterile phosphate-buffered saline (PBS) solution by stirring with sterile forceps in a sterile flask. Second, the separated roots were washed with PBS solution and sonicated for 1 min at 50–60 Hz in a 50-mL sterile tube with 20 mL of PBS solution, and the soil pellet was considered the rhizoplane sample. Finally, the washed roots were cleaned with PBS solution and sonicated for 1 min at 50–60 Hz in a 50-mL sterile tube with 20 mL of PBS solution three times and were considered the endosphere samples. All samples were stored at −80°C before DNA extraction.

DNA extraction and bacterial community analysis

A total of 32 bulk soil, rhizosphere, rhizoplane and endosphere samples with four replicates for each component were prepared for DNA extraction. Total DNA from soil, rhizosphere, rhizoplane and endosphere was extracted from each sample with the FastDNA® SPIN Kit for soil (MP Biomedicals, Cleveland, OH, USA) according to the manufacturer's instructions. The bacterial communities in all samples were analyzed by pyrosequencing the 16S rRNA genes from the samples. The 515F/907R (515F: 5′-GTG CCA GCM GCC GCG G-3′; 907R: 5′-CCG TCA ATT CMT T TR AGT TT-3′) primer pair was used to amplify the V4–V5 region of the bacterial 16S rRNA gene.

The primers were tagged with unique barcodes for each sample. Negative controls using sterilized water instead of soil DNA extract were included to check for primer or sample DNA contamination. The qualities and concentrations of the purified barcoded polymerase chain reaction (PCR) products were determined by using a NanoDrop spectrophotometer. High-throughput sequencing was conducted with Illumina MiSeq from LC-Bio Technology Co., Ltd. (Hangzhou, China). The raw amplicon sequence data of the 16S rRNA gene have been deposited in the GenBank short-read archive (SRA) under the SRA accession code PRJNA641223.

Reads were merged and the raw sequences were quality filtered by QIIME pipeline. Operational taxonomic units (OTUs) were clustered at 97% similarity, and OTU selection and taxonomic assignment were performed according to the SILVA reference data (version 128). The reads that did not align to the anticipated region of the reference alignment were removed as

chimeras. Reads that were classified as 'chloroplast', 'mitochondria' or 'unknown' (could not be classified at the kingdom level) were removed.

Data analysis

The PICRUSt package was used to predict the metagenome functional context according to the bacterial 16S rRNA gene data. To determine the Shannon diversity and the OTU abundance, the valid reads in each sample were rarefied at 4650 counts. Clustering analysis according to Bray–Curtis dissimilarity was calculated in the Vegan package of R v 3.3.2. The unweighted pair group method with arithmetic mean (UPGMA) was applied based on Bray–Curtis dissimilarity. Permutational multivariate analyses of variance (PERMANOVAs) were used to compare the differences in bacterial community composition between treatments by Vegan's Adonis function in the R environment, and the proportion of the total variance explained by soil environments and rhizo-compartments was computed by Vegan's capsule function in the R environment. OTUs enriched in bacterial communities between rhizo-compartments in two soils were identified by the limma package in combination with linear statistics on their relative abundance by vcd's ternaryplot function in the R environment.

RESULTS

Bacterial diversity and community composition

A total of 748 843 high-quality sequences ranging from 4651 to 69461 were obtained from 32 samples. In terms of bacterial α-diversity, both the compartments and the soil environments affected the bacterial Shannon index and OTU richness ($P < 0.001$) (Table S2). In the AMD-polluted soils, the rhizosphere and rhizoplane samples reached the highest Shannon index (rhizosphere 8.98 ± 0.12; rhizoplane 9.06 ± 0.23) and OTU richness (rhizosphere 1859 ± 63; rhizoplane 1882 ± 143), and the endosphere samples had the lowest Shannon index (6.69 ± 0.17) and OTU richness (1150 ± 50) (Fig. 1). In the unpolluted soils, the rhizoplane sample possessed the highest bacterial α-diversity (Shannon index 10.48 ± 0.06; OTU richness 2706 ± 54), yet no consistent change in bacterial α-diversity indexes among soil, rhizosphere and endosphere samples was discovered (Fig. 1). In addition, the Shannon index and OTU richness of the rhizosphere and endosphere samples in the AMD-polluted soils were significantly lower than those in the unpolluted soils (Fig. 1). The UPGMA cluster of the bacterial communities in samples according to their Bray–Curtis dissimilarity showed that the bacterial communities in the samples were the most distinct within different compartments and soil environments (Fig. 2). In addition, the dissimilarity among different compartments was less than that between soil environments (Fig. 2), indicating the tremendous influence of soil environments on the root-associated bacterial communities. PERMANOVA (Table 1) using Bray–Curtis distance also supported the UPGMA cluster result in that soil environments exhibited the greatest variation (31.73%, $P < 0.001$) in bacterial communities.

The main bacterial phyla with relative abundance >1%, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Ignavibacteriae, Nitrospirae, Proteobacteria and Spirochaetae, accounted for 92.87% of the total sequences (Fig. 3A). Proteobacteria was the dominant phylum, with the highest abundance ranging from 44.18 to 90.83% (Fig. 3A). In addition, ANOVA with the LSD test indicated a significant difference in the relative abundance of these phyla and Proteobacteria classes between the compartments in each treatment (Fig. 3). Additionally, AMD pollution caused different distributions of these dominant phyla and Proteobacteria classes in different compartments. For example, in ck, the rhizoplane had a significantly greater proportion of the phyla Proteobacteria and Firmicutes and class Gammaproteobacteria than any other compartment and possessed the lowest proportions of the other dominant phyla and class Deltaproteobacteria; the endosphere had greater proportions of the classes Alphaproteobacteria and Betaproteobacteria, in which Gammaproteobacteria were significantly depleted. However, in AMD-pollution, the endosphere had greater proportions of the phylum Proteobacteria and class Gammaproteobacteria, and the other dominant phyla (except for Actinobacteria) and classes Alphaproteobacteria and Deltaproteobacteria were mostly depleted in the endosphere.

The genera influenced by the plant compartments and soil environments were further determined. Fig. 4 shows a great difference in the relative abundance of the top 50 bacterial genera among soils. For example, some genera, *Enterobacter*, *Klebsiella*, *Pantoea*, *Serratia* and *Escherichia*, showed greater proportions in the rhizoplane of ck but enhanced their relative abundance in the endosphere of AMD-pollution. In addition, several genera, such as *Exiguobacterium*, *Vogesella*, *Fictibacillus*, *Uliginosibacterium*, *Anoxybacillus*, *Smithella*, *Candidatus Competibacter*, *Desulfatiglans*, *Syntrophus*, *Syntrophorhabdus*, *Aeromonas*, *Acinetobacter*, *Limnohabitans*, *Pseudomonas*, *Massilia*, *Dechloromonas* and *Rhizobacter*, showed significant differences among different compartments in ck but showed very low abundance in AMD-pollution. In contrast, the relative abundances of several genera, *Acidibacter*, *Bryobacter*, *Thermoanaerobaculum*, *Burkholderia*, *Acidiphilium*, *Thiomonas* and *Metallibacterium*, in AMD-pollution were significantly higher than that in ck.

OTUs colonized in specific compartments

Venn diagrams showed a total of 2649 and 4853 observed OTUs in AMD-pollution and ck, respectively (Fig. S1). Additionally, 347 OTUs (13.1%) were common in different compartments of AMD-pollution and 75 OTUs (1.5%) were common in four compartments of ck. These results showed that the soil environment led to tremendous changes in the bacterial species that coexist in different compartments. In addition, each compartment had specific OTUs, which also indicated that the compartments of each soil environment had unique bacterial population compositions.

To determine indicator OTUs that were enriched in each compartment across different soil environments, a linear model analysis was performed to identify taxa responsible for the differentiation of the observed bacterial community. According to this, the indicator OTUs in the soil, rhizosphere and endosphere were marked as soilOTUs, rhizoOTUs and endoOTUs, respectively (Fig. 5). The results showed that the rhizosphere and endosphere compartments shared some OTUs across both soil environments. In addition, the subcommunities retrieved from the rhizoOTUs and endoOTUs were defined by a variant taxonomic structure. Proteobacteria accounted for the vast majority of rhizoOTUs and endoOTUs (Fig. 5). In addition, Betaproteobacteria dominated the endoOTUs in ck, but Gammaproteobacteria were predominant in the endoOTUs of AMD-pollution (Fig. 5). In addition, the enrichment of Betaproteobacteria in the endosphere of ck was driven by two families, Comamonadaceae (10/15)

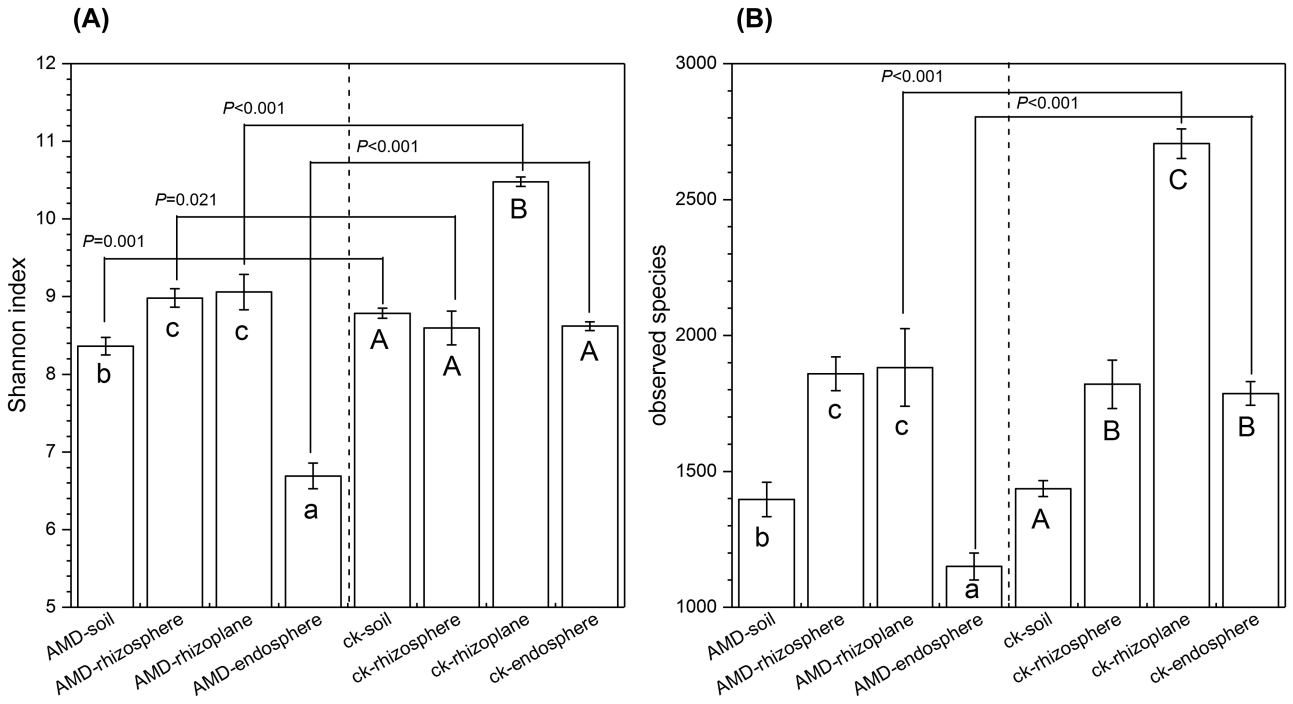

Figure 1. Shannon index **(A)** and OTU richness **(B)** across four root compartments of plants between AMD-polluted soils (AMD) and unpolluted soils (ck). The error bars show the standard deviations of the four subsamples for each sample. Different lowercase letters show significant differences from each other in the AMD-polluted treatment, and different capital letters show significant differences across the four compartments in the unpolluted treatment ($P < 0.05$, LSD). Connecting lines show significant differences of the Shannon index and OTU richness in the root compartments between AMD-pollution and ck ($P < 0.05$, *t*-test).

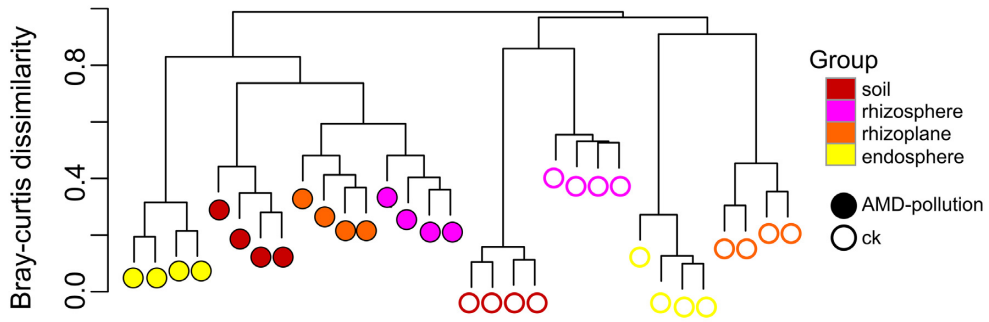

Figure 2. UPGMA tree showing clusters of bacterial communities based on Bray–Curtis dissimilarity of the whole OTU count data.

Table 1. Determining the soil environment and compartment affecting the bacterial community composition using PERMANOVA with 999 permutations.

	Sums of squares	Mean squares	%	F value	P value
Soil environment	3.53	3.53	31.73	21.82	<0.001
Compartment	1.71	1.71	15.33	10.54	<0.001
Soil environment:compartment	1.36	1.36	12.23	8.41	<0.001
Residuals	4.53	0.16	40.71	0.41	
Total	11.13	1			

and Rhodocyclaceae (5/15), and the enrichment of Gammaproteobacteria in the endosphere of AMD-pollution was driven by the family Enterobacteriaceae (10/12). These results suggested that the soil environment led to differences in unique enrichment community members. The potential role of AMD pollution in the bacterial profiles could be analyzed by comparing the indicator OTUs in each rhizo-compartment between the two soils. Of the soilOTUs and rhizoOTUs, no OTU was enriched in both soil environments, while of the endoOTUs, only a single OTU was enriched in both soil environments.

Predictive metagenome of the bacterial community

To predict the effect of rhizo-compartments and soil environments on metagenome functional content, PICRUSt was performed based on the functional annotation of the KEGG

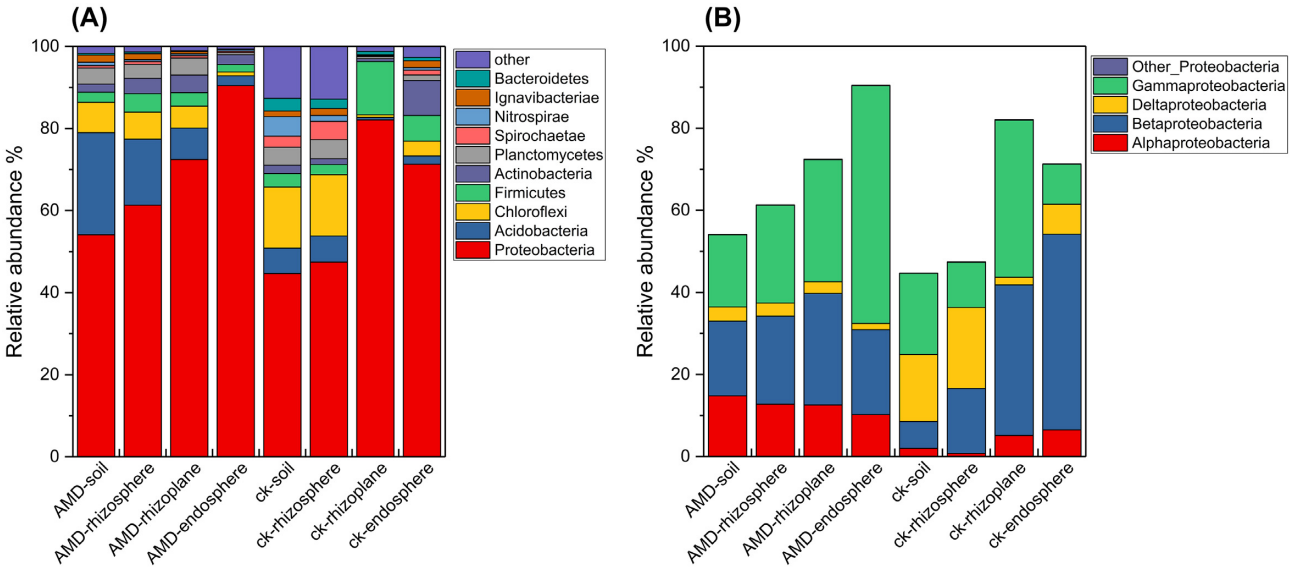

Figure 3. Relative abundances (percentages) of the main identified bacterial taxonomic groups, i.e. **(A)** the phyla Proteobacteria, Acidobacteria, Chloroflexi, Firmicutes, Actinobacteria, Planctomycetes, Spirochaetae, Nitrospirae, Ignavibacteriae and Bacteroidetes and **(B)** the classes Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria and Gammaproteobacteria (within the phylum Proteobacteria). For each sample, the relative abundances of the sequences assigned to a given taxonomic unit were calculated for each of the four subsamples, and the average value was then used to represent the relative abundance of each sample.

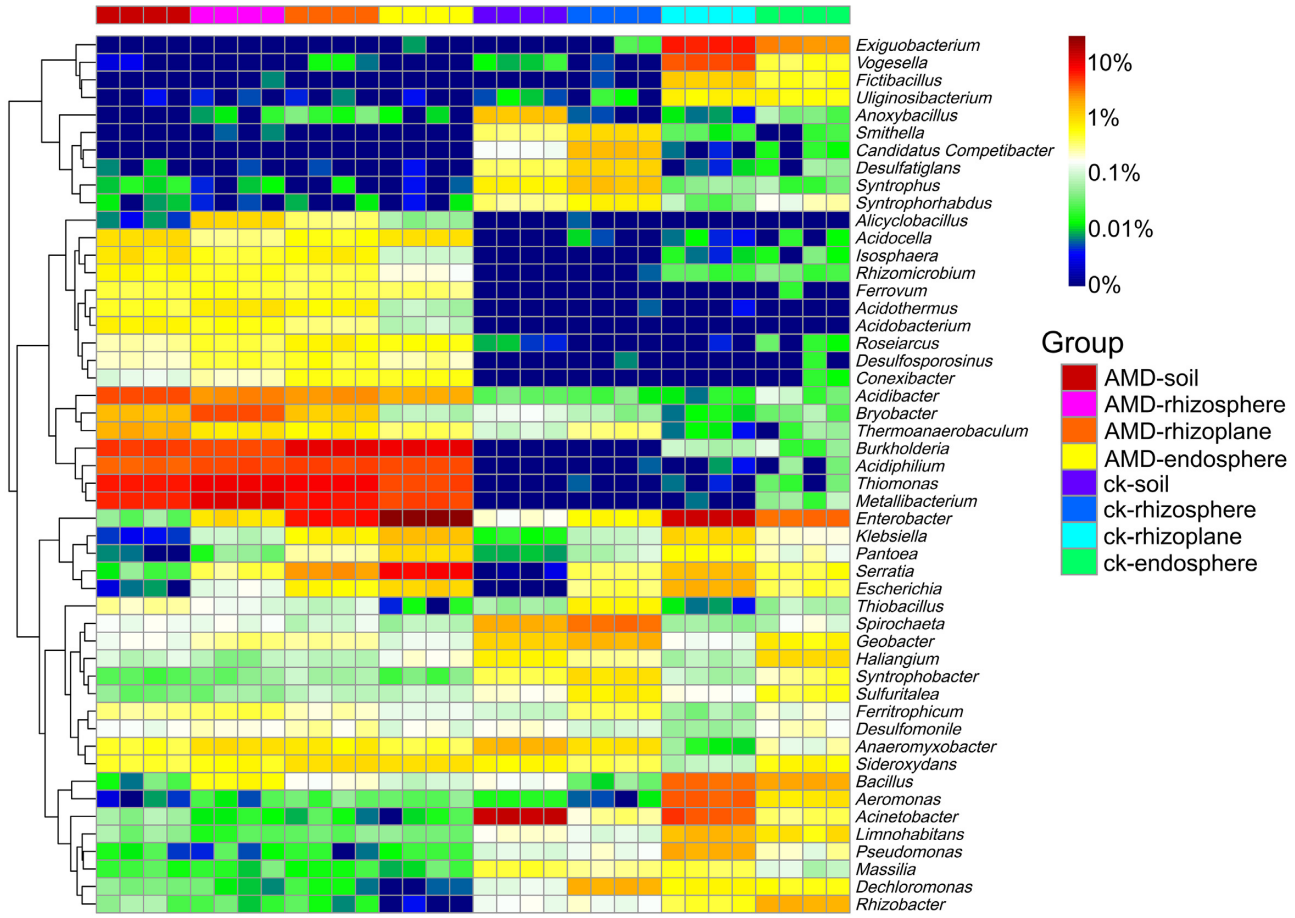

Figure 4. Heat map of the top 50 genera in samples. The eight groups are marked with different colors.

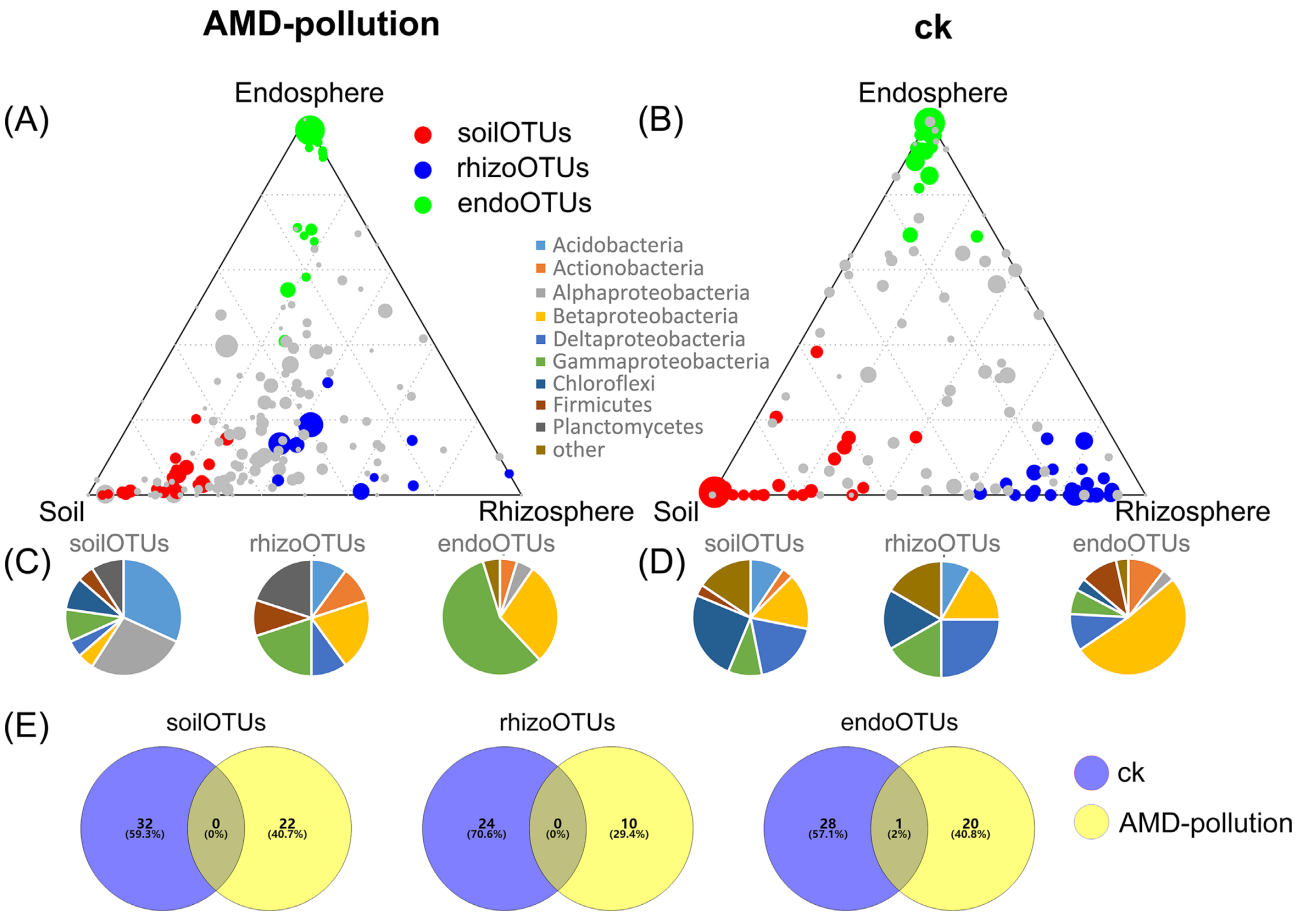

Figure 5. OTU enrichment in different compartments across the two treatments. Ternary plot depicting relative abundance and proportional contribution of OTUs with a relative abundance >0.5% across AMD-polluted **(A)** and unpolluted treatments **(B)**. Each circle represents one OTU. The size of each circle reflects the relative abundance. The position of each circle was determined by the contribution of the indicated compartment to the relative abundance. The dotted grid and numbers inside the plot showed 20% increments of contribution from each compartment. The pie charts show the taxonomic composition of soilOTU, rhizoOTU and endoOTU subcommunities in AMD-polluted **(C)** and unpolluted **(D)** treatments. The size of each segment in the chart was proportional to the cumulative relative abundance of OTUs assigned to the indicated taxa. **(E)** Venn diagrams display OTU numbers that significantly ($P < 0.05$) differentiate soil, rhizosphere and endosphere compartments in the two treatments. The numbers shared by the two treatments indicate the co-occurrences of the compartment-specific OTUs.

pathway. The PERMANOVA results (Table S3) showed that the rhizo-compartment ($F = 4.3227$, $P = 0.015$) and soil environment ($F = 3.8132$, $P = 0.030$) significantly affected the metagenome functional content, but their interaction had no effect ($F = 1.7754$, $P = 0.145$). This was also confirmed by the change in the predictive metabolic functions among samples at the level of 2–3 (Fig. S2). In ck, several KEGG orthologs (KOs) assigned to infectious diseases, immune system, enzyme families, cancers, digestive system, genetic information processing, metabolic diseases, energy metabolism, metabolism of cofactors and vitamins, replication and repair, nervous system, cell growth and death and folding, sorting and degradation showed a visual increase in abundance across the soils into the endosphere. Among them, some KOs assigned to infectious diseases, immune system, cancers and digestive system were also increased in abundance from soils to the endosphere of AMD-pollution, but their abundances were much lower than those in ck. In addition, some KOs assigned to membrane transport, metabolism and cellular processes and signaling showed a visual increase in abundance across soils into the endosphere of AMD-pollution. In addition, some KOs, including transcription, immune system diseases and metabolism of other amino acids, decreased in abundance from the soils into the endosphere of AMD-pollution, but their abundances in the endosphere were also higher than that of ck.

DISCUSSION

Previous studies have shown that different compartments in plants lead to changes in the structure and function of bacterial communities (Edwards *et al.* 2015; Luo *et al.* 2017; Singer *et al.* 2019), but it is still unknown whether this change trend could be affected by soil pollution. This study proved that the effect of AMD pollution on root bacterial communities was greater than that of rhizo-compartments. Luo *et al.* (2017) reported that different soil types led to differences in the bacterial community, but their plants were transplanted directly. Their work has not considered the effect of the abundance and composition of original endophytes in plants on the bacterial communities and function in rhizo-compartments after transplantation. In addition, differences in the soil properties are not caused by environmental pollution. In this study, the bacterial community composition and function in rhizo-compartments also showed great changes, and the difference in the bacterial community distribution might be related to the rhizodeposition process (Paterson *et al.* 2007). In addition, plants can secrete

organic compounds (Dakora and Phillips 2002) in roots, which could alter rhizosphere microhabitats, resulting in changes in the ecological niches of some special groups. Most strikingly, the new endophytic bacterial communities from the same plant species reconstructed in soils showed considerable differences between two soils, which might be related to the migration of soil microorganisms (Hao, Ma and Qiao 2015; Schulz-Bohm *et al.* 2018). In unpolluted soils, the selected and established bacterial communities preferentially colonized the rhizoplane and then infiltrated the roots (Edwards *et al.* 2015; van der Heijden and Schlaeppi 2015). Once the soil was polluted by the AMD, this phenomenon changed, and the majority of the bacterial communities showed an increasing trend from the soils across the rhizosphere and into the endosphere (Fig. 4).

In this study, the changes in bacterial community composition in different rhizo-compartments may be related to their specific life-history strategies (Luo *et al.* 2017). For example, similar to previous studies on Arabidopsis and rice, the abundance of Proteobacteria in rhizo-compartments was significantly higher than that in soils, and the abundance of Acidobacteria significantly decreased from the soils into the roots (Edwards *et al.* 2015). This result suggests that the root-associated distribution pattern of different bacteria at the phylum level might be similar for most plants in different soil environments (Fig. 3). However, for bacterial classes, the soil environment might be an important influencing factor. For example, the abundance of Betaproteobacteria in the unpolluted soils increased significantly from the soil across the rhizosphere into the root, but this change trend only occurred in the class Gammaproteobacteria in the AMD-polluted soils. In the unpolluted soils, most bacteria were mainly concentrated in the rhizoplane, and the abundance and diversity of bacterial communities within the root were similar to those in the rhizosphere and soils (Fig. 1). This was because the root exudate supported the growth of many bacterial communities, and the generated bacteria recruited by the root can thrive in the root surroundings and subsequently colonize the endosphere (Edwards *et al.* 2015). Once the soils are polluted, the organic compounds secreted by roots could protect the bacterial communities in the rhizosphere and rhizoplane from the AMD pollution (Wang *et al.* 2018), but the high concentration of heavy metals in roots also inhibited the survival of most bacteria (Xu *et al.* 2019). In the environment with high nutrient availability, r-strategists in the Proteobacteria phylum (mainly the classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) had great advantages in the competition for root nutrients (Nemergut *et al.* 2010). However, the class Gammaproteobacteria, which had a universal tolerance to heavy metals (Ivanova *et al.* 2002; Feris *et al.* 2009), showed good competitiveness once their survival was under the pressure of heavy metals. These results showed that the colonization of bacteria in roots is not a random process, and plants can select specific or certain bacteria that are suitable for root-associated niches.

The changes in the root-associated bacterial communities caused by soil AMD pollution were also shown at the genus level in different compartments. The genera *Enterobacter*, *Klebsiella*, *Pantoea*, *Serratia* and *Escherichia* were widespread in unpolluted soils and plant roots (Fig. 4), but they also became the dominant groups of the AMD-polluted rhizo-compartments. The existence of these unique endophytic bacteria in the different rhizo-compartments might be related to some of their own plant growth-promoting traits, such as nitrogen fixation (Banik, Mukhopadhaya and Dangar 2016), dissolved P (Chen *et al.* 2014; Ibort *et al.* 2018) and IAA production (Carlos *et al.* 2016; Yu *et al.* 2018). However, in soils that are polluted by AMD for a long time, the presence of these bacteria in plants is likely due to their heavy-metal tolerance (Subrahmanyam *et al.* 2018; Moretto *et al.* 2019; Shahid *et al.* 2019). In the soils polluted by AMD, most acidophilic bacteria with unique functions became the main taxa in plant rhizo-compartments. For example, the genera *Acidibacter* (Falagán and Johnson 2014), *Bryobacter* (Hao *et al.* 2018), Burkholderia (Gao *et al.* 2019; Huang *et al.* 2019; Khanna *et al.* 2019; Lu *et al.* 2019), *Acidiphilium* (Sun *et al.* 2016; Gao *et al.* 2019), *Thiomonas* (Valentín-Vargas *et al.* 2018) and *Metallibacterium* (Brantner and Senko 2014; Sun *et al.* 2016) had special heavy metal-reducing capacity. Among them, the genus *Burkholderia* carrying plant growth-promoting traits contributed to plant growth and inhibited the effects of plant pathogens (Mittal *et al.* 2019). In parallel, several PGPBs with high abundance in the unpolluted soils, such as *Exiguobacterium* (Chauhan *et al.* 2015), *Aeromonas* (Vaikuntapu *et al.* 2014), *Acinetobacter* (Syed-Ab-Rahman *et al.* 2018) and *Pseudomonas* (Ul Hassan and Bano 2019), were restricted in the root zone that was polluted by the AMD due to the harsh environment.

In this study, a linear model analysis was applied to determine some bacterial taxa whose abundance in the endosphere and rhizosphere was significantly higher than that in soils (Fig. 5). These niche-specific bacterial taxa were the determining indicator of OTUs in each rhizo-compartment. Both in unpolluted and AMD-polluted soils, the bacterial taxonomic composition of the three subcommunities was consistently significantly different, and these differences were evident in specific phyla and their families, which is the cause of the proteobacterial differentiation in the rhizosphere and endosphere. In addition, this study found that the Comamonadaceae and Rhodocyclaceae belonging to the class Betaproteobacteria and the Enterobacteriaceae belonging to the class Gammaproteobacteria were dominant in the endosphere communities of the unpolluted and AMD-polluted groups, respectively, which indicated that they were a better indicator of pollution. Comamonadaceae and Rhodocyclaceae were significantly enriched in the endosphere of plants growing in unpolluted soil, which might have an impact on the growth and survival of the host plants and bacteria–bacteria interactions. Rhodocyclaceae is a common family of endophytic bacteria. The Comamonadaceae in roots are closely related to nitrogen uptake in plants (Pagé *et al.* 2019) and usually possess plant growth-promoting traits (Cope-Selby *et al.* 2017; Chen *et al.* 2018) and inhibit plant pathogens (Cretoiu *et al.* 2013; Cretoiu *et al.* 2014; Li *et al.* 2015). The Enterobacteriaceae is also known to be equipped with PGPBs (Chen *et al.* 2018), and its divergent nitrogen fixation paralogs play a role in different ecological contexts (Sharaf *et al.* 2019). In addition, the survival of those bacteria assigned to the family Enterobacteriaceae in the roots of plants growing in AMD-polluted soils might be related to their heavy-metal tolerance and heavy metal-reducing capacity (Chaturvedi and Pandey 2014).

PICRUSt results showed that some KOs related to infectious diseases, immune system and metabolic diseases, etc. are enriched from the soils into the roots in unpolluted soils, and several bacteria are also involved in nutrient metabolism and energy metabolism in rhizo-compartments (Fig. S2). This suggests that bacterial communities in roots are closely related to plant immunity and plant growth (Berendsen *et al.* 2018; Singer *et al.* 2019). Once the soil is polluted by AMD, the KOs involved in the plant immune response remain, most of which are assigned to membrane transport, unclassified metabolism and unclassified cellular processes and signaling processes. The abundance of KOs assigned to membrane transport such as transporters,

ABC transporters and the phosphotransferase system showed a significant increase from the soil to endosphere, indicating that endosphere bacteria in AMD-pollution have a strong ability to absorb and/or accumulate heavy metals (Rincón-Molina *et al.* 2019). It also provides indirect evidence that plants can improve their resistance to harsh environments by changing the migration of functional bacteria.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGMENTS

This study was funded by the National Natural Science Foundation of China (31800456), the Key Research and Development Projects in Anhui Province (201904a020027), and the talent Research Startup Foundation of Anhui University of Science and Technology.

Conflict of Interest. None declared.

REFERENCES

Adamo P, Mingo A, Coppola I *et al.* Plant colonization of brownfield soil and post-washing sludge: effect of organic amendment and environmental conditions. *Int J Environ Sci Te* 2015;**12**:1811–24.

Banik A, Mukhopadhaya SK, Dangar TK. Characterization of N_2-fixing plant growth promoting endophytic and epiphytic bacterial community of Indian cultivated and wild rice (*Oryza* spp.) genotypes. *Planta* 2016;**243**:799–812.

Bell TH, El-Din Hassan S, Lauron-Moreau A *et al.* Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *ISME J* 2013;**8**:331–43.

Berendsen RL, Vismans G, Yu K *et al.* Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J* 2018;**12**:1496–507.

Brantner JS, Senko JM. Response of soil-associated microbial communities to intrusion of coal mine-derived acid mine drainage. *Environ Sci Technol* 2014;**48**:8556–63.

Carlos MHJ, Stefani PVY, Janette AM *et al.* Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting bacteria. *Microbiol Res* 2016;**188-189**:53–61.

Chaturvedi N, Pandey PN. Phylogenetic analysis of gammaproteobacterial arsenate reductase proteins specific to enterobacteriaceae family, signifying arsenic toxicity. *Interdiscip Sci* 2014;**6**:57–62.

Chauhan H, Bagyaraj DJ, Selvakumar G *et al.* Novel plant growth promoting rhizobacteria—Prospects and potential. *Appl Soil Ecol* 2015;**95**:38–53.

Chen Y, Ding Q, Chao Y *et al.* Structural development and assembly patterns of the root-associated microbiomes during phytoremediation. *Sci Total Environ* 2018;**644**:1591–601.

Chen Y, Fan JB, Du L *et al.* The application of phosphate solubilizing endophyte *Pantoea dispersa* triggers the microbial community in red acidic soil. *Appl Soil Ecol* 2014;**84**:235–44.

Cope-Selby N, Cookson A, Squance M *et al.* Endophytic bacteria in Miscanthus seed: implications for germination, vertical inheritance of endophytes, plant evolution and breeding. *GCB Bioenergy* 2017;**9**:57–77.

Cretoiu MS, Kielak AM, Schluter A *et al.* Bacterial communities in chitin-amended soil as revealed by 16S rRNA gene based pyrosequencing. *Soil Biol Biochem* 2014;**76**:5–11.

Cretoiu MS, Korthals GW, Visser JHM *et al.* Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the Actinobacterial and Oxalobacteraceal communities in an experimental agricultural field. *Appl Environ Microbiol* 2013;**79**:5291–301.

Dakora FD, Phillips DA. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 2002;**245**:35–47.

Edwards J, Johnson C, Santos-Medellín C *et al.* Structure, variation, and assembly of the root-associated microbiomes of rice. *P Natl Acad Sci USA* 2015:201414592.

Falagán C, Johnson DB. *Acidibacter ferrireducens* gen. nov., sp. nov.: an acidophilic ferric iron-reducing gammaproteobacterium. *Extremophiles* 2014;**18**:1067–73.

Feris KP, Ramsey PW, Gibbons SM *et al.* Hyporheic microbial community development is a sensitive indicator of metal contamination. *Environ Sci Technol* 2009;**43**:6158–63.

Gao P, Sun X, Xiao E *et al.* Characterization of iron-metabolizing communities in soils contaminated by acid mine drainage from an abandoned coal mine in Southwest China. *Environ Sci Pollut R* 2019;**26**:9585–98.

Glick BR. Using soil bacteria to facilitate phytoremediation. *Biotechnol Adv* 2010;**28**:367–74.

Gray EJ, Smith DL. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biol Biochem* 2005;**37**:395–412.

Hao B, Ma L, Qiao X. Quantitative analysis of the migration and accumulation of *Bacillus subtilis* in *Asparagus officinalis*. *Curr Microbiol* 2015;**71**:357–62.

Hao L, Zhang B, Feng C *et al.* Microbial vanadium (V) reduction in groundwater with different soils from vanadium ore mining areas. *Chemosphere* 2018;**202**:272–9.

Huang Y, Wang Y, Feng H *et al.* Genome-guided identification and characterization of bacteria for simultaneous degradation of polycyclic aromatic hydrocarbons and resistance to hexavalent chromium. *Int Biodeter Biodegr* 2019;**138**:78–86.

Ibort P, Imai H, Uemura M *et al.* Proteomic analysis reveals that tomato interaction with plant growth promoting bacteria is highly determined by ethylene perception. *J Plant Physiol* 2018;**220**:43–59.

Ivanova EP, Kurilenko VV, Kurilenko AV *et al.* Tolerance to cadmium of free-living and associated with marine animals and eelgrass marine Gamma-Proteobacteria. *Curr Microbiol* 2002;**44**:357–62.

Karaca O, Cameselle C, Reddy KR. Mine tailing disposal sites: contamination problems, remedial options and phytocaps for sustainable remediation. *Rev Environ Sci Bio* 2018;**17**:205–28.

Khanna K, Jamwal VL, Gandhi SG *et al.* Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in *Lycopersicon esculentum* under metal toxicity. *Sci Rep* 2019;**9**:5855.

Li X, Yn Z, Ding C *et al.* Declined soil suppressiveness to *Fusarium oxysporum* by rhizosphere microflora of cotton in soil sickness. *Biol Fert Soils* 2015;**51**:935–46.

Lu L, Li J, Hu A *et al.* Microbial community structure analysis and isolation of vanadium-resistant strains in vanadium mining–impacted soil. *J Soil Water Conserv* 2019;**74**:296–308.

Luo J, Tao Q, Wu K *et al*. Structural and functional variability in root-associated bacterial microbiomes of Cd/Zn hyperaccumulator Sedum alfredii. *Appl Microbiol Biot* 2017;**101**:7961–76.

Ma Y, Oliveira RS, Freitas H *et al*. Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. *Front Plant Sci* 2016;**7**:918.

Mittal D, Shukla R, Verma S *et al*. Fire in pine grown regions of Himalayas depletes cultivable plant growth promoting beneficial microbes in the soil. *Appl Soil Ecol* 2019;**139**:117–24.

Moretto JAS, Braz VS, Furlan JPR *et al*. Plasmids associated with heavy metal resistance and herbicide degradation potential in bacterial isolates obtained from two Brazilian regions. *Environ Monit Assess* 2019;**191**:314.

Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett* 2010;**8**:199–216.

Nazir F, Hussain A, Fariduddin Q. Interactive role of epibrassinolide and hydrogen peroxide in regulating stomatal physiology, root morphology, photosynthetic and growth traits in *Solanum lycopersicum* L. under nickel stress. *Environ Exp Bot* 2019;**162**:479–95.

Nemergut DR, Cleveland CC, Wieder WR *et al*. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biol Biochem* 2010;**42**:2153–60.

Pagé AP, Tremblay J, Masson L *et al*. Nitrogen- and phosphorus-starved *Triticum aestivum* show distinct belowground microbiome profiles. *PLoS One* 2019;**14**:e0210538.

Pajuelo E, Rodríguez-Llorente ID, Dary M *et al*. Toxic effects of arsenic on *Sinorhizobium–Medicago sativa* symbiotic interaction. *Environ Pollut* 2008;**154**:203–11.

Pandey VC. Assisted phytoremediation of fly ash dumps through naturally colonized plants. *Ecol Eng* 2015;**82**:1–5.

Paterson E, Gebbing T, Abel C *et al*. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytol* 2007;**173**:600–10.

Rajkumar M, Sandhya S, Prasad MNV *et al*. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnol Adv* 2012;**30**:1562–74.

Rincón-Molina CI, Hernández-García JA, Rincón-Rosales R *et al*. Structure and diversity of the bacterial communities in the acid and thermophilic crater-lake of the volcano "El Chichón", Mexico. *Geomicrobiol J* 2019;**36**:97–109.

Schulz-Bohm K, Gerards S, Hundscheid M *et al*. Calling from distance: attraction of soil bacteria by plant root volatiles. *ISME J* 2018;**12**:1252–62.

Sessitsch A, Kuffner M, Kidd P *et al*. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biol Biochem* 2013;**60**:182–94.

Shahid M, Javed MT, Masood S *et al*. *Serratia* sp. CP-13 augments the growth of cadmium (Cd)-stressed *Linum usitatissimum* L. by limited Cd uptake, enhanced nutrient acquisition and antioxidative potential. *J Appl Microbiol* 2019;**126**:1708–21.

Sharaf H, Rodrigues RR, Moon J *et al*. Unprecedented bacterial community richness in soybean nodules vary with cultivar and water status. *Microbiome* 2019;**7**:63.

Singer E, Bonnette J, Kenaley SC *et al*. Plant compartment and genetic variation drive microbiome composition in switchgrass roots. *Env Microbiol Rep* 2019;**11**:185–95.

Singh S, Parihar P, Singh R *et al*. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Front Plant Sci* 2016;**6**:1143.

Subrahmanyam G, Sharma RK, Kumar GN *et al*. *Vigna radiata* var. GM4 plant growth enhancement and root colonization by a multi-metal-resistant plant growth-promoting bacterium *Enterobacter* sp. C1D in Cr(VI)-amended soils. *Pedosphere* 2018;**28**:144–56.

Sun W, Xiao E, Krumins V *et al*. Characterization of the microbial community composition and the distribution of Fe-metabolizing bacteria in a creek contaminated by acid mine drainage. *Appl Microbiol Biot* 2016;**100**:8523–35.

Syed-Ab-Rahman SF, Carvalhais LC, Chua E *et al*. Identification of soil bacterial isolates suppressing different *Phytophthora* spp. and promoting plant growth. *Front Plant Sci* 2018;**9**:1502.

Ul Hassan T, Bano A. Construction of IAA-deficient mutants of *Pseudomonas moraviensis* and their comparative effects with wild type strains as bio-inoculant on wheat in saline sodic soil. *Geomicrobiol J* 2019;**36**:376–84.

Vaikuntapu PR, Dutta S, Samudrala RB *et al*. Preferential promotion of *Lycopersicon esculentum* (Tomato) growth by plant growth promoting bacteria associated with tomato. *Indian J Microbiol* 2014;**54**:403–12.

Valentín-Vargas A, Neilson JW, Root RA *et al*. Treatment impacts on temporal microbial community dynamics during phytostabilization of acid-generating mine tailings in semiarid regions. *Sci Total Environ* 2018;**618**:357–68.

van der Heijden MGA, Schlaeppi K. Root surface as a frontier for plant microbiome research. *P Natl Acad Sci USA* 2015;**112**:2299–300.

Wang C, Jiang K, Zhou J *et al*. Responses of soil N-fixing bacterial communities to redroot pigweed (*Amaranthus retroflexus* L.) invasion under Cu and Cd heavy metal soil pollution. *Agr Ecosyst Environ* 2018;**267**:15–22.

Xu Y, Seshadri B, Bolan N *et al*. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. *Environ Int* 2019;**125**:478–88.

Yu Z, Xia K, Liu H *et al*. Endophytes isolated from ginger rhizome exhibit growth promoting potential for *Zea mays*. *Arch Agron Soil Sci* 2018;**64**:1302–14.