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Lead contamination alters enzyme activities and microbial composition in the rhizosphere soil of the hyperaccumulator *Pogonatherum crinitum*

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ABSTRACT

Pogonatherum crinitum is a promising lead (Pb) hyperaccumulator; however, the effects of Pb contamination on *P. crinitum* rhizosphere soil enzymatic activities and microbial composition remain largely unexplored. Thus, an indoor experiment was conducted by cultivating *P. crinitum* seedlings and exposing them to four Pb concentrations (0, 1,000, 2000 and 3000 mg/kg Pb). Protease, urease, acid phosphatase and invertase activities were determined using standard methods while soil bacterial composition was determined by 16 S rDNA sequencing. The results showed that rhizosphere soil acid phosphatase activity significantly increased with increasing Pb concentration, while urease activity was significantly greater in rhizosphere soil contaminated with 1000 and 2000 mg/kg than in the control. There was a clear shift in bacterial composition during phytoremediation by *P. crinitum*. Compared to the control, Bacteroidetes was more abundant in all Pb-contaminated soils, Actinobacteria was more abundant in 1000 mg/kg Pb-treated soil, and Firmicutes was more abundant in 3000 mg/kg Pb-treated soil. Positive correlations were observed between dominant bacterial phyla and soil enzyme activities. Metabolic pathways, such as ABC transporter, quinine reductase, and ATP-binding protein were significantly increased in rhizosphere soil bacteria with Pb contamination. In conclusion, Pb contamination differentially influenced the activities of rhizosphere soil enzymes, specifically increasing acid phosphatase and urease activities, and alters the dominance of soil bacteria through up-regulation of genes related to some metabolic pathways. The strong correlations between dominant bacterial phyla and enzymatic activities suggest synergetic effects on the growth of *P. crinitum* during Pb contamination.

1. Introduction

Human activities, such as mining, are accompanied by the disposal of large amounts of waste (Edraki et al., 2014), which often contain high concentrations of heavy metals (Chileshe et al., 2020). In addition, mine waste lands have poor nutrient content, high soil compaction and high acidity (depending on the ore processing technique), limiting the growth of vegetation in post-mining landscapes (Chileshe et al., 2020; Festin et al., 2019). Therefore, reclamation of soils contaminated by heavy metals has become a major environmental problem of great urgency, as once heavy metals enter the food chain, they pose serious risks to human health. To address this environmental concern, a variety of remediation techniques have been tested with varying degrees of success (Festin et al., 2019). Biological methods (also known as bioremediation) are preferred because they are both eco-friendly and cost-effective. Phytoremediation involves the use of plants and microorganisms to reduce the toxic effects of heavy metals in the soil environment (Mendez and Maier, 2007). The success of phytoremediation depends on the ability of the plant to accumulate and translocate heavy metals while producing high biomass and tolerating potentially toxic heavy metal concentrations (Anning and Akoto, 2018).

The activities of enzymes extracted from roots, other soil organisms and rhizosphere-soil are sensitive indicators of nutrient cycling and soil contamination by heavy metals. Thus, they can be used to monitor the ecological health of the soil system under heavy metal contamination (Liang et al., 2014). In particular, rhizosphere enzyme activities reveal interactions between plant and microorganisms and may reflect the composition and function of the microbiome (Liu et al., 2017; Razavi

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et al., 2016). Previous studies have reported that soil extracted (and possibly rhizosphere-extracted) catalase, urease, dehydrogenase and phosphatase activities are sensitive to heavy metal contamination (Marzadori et al., 1996; Pattnaikand Equeenuddin, 2016; Duan et al., 2018). However, there is no uniformity in the changes in these enzyme activities in response to heavy metals; some studies have reported higher enzyme activity in contaminated soils (Hagmann et al., 2015), while others reported lower activity (Kuperman and Carreiro, 1997).

In addition, microorganisms play a pivotal role in influencing the success of polluted soil phytoremediation by producing substances beneficial for plant growth (Gouda et al., 2018) and boosting heavy metals absorption of by plants through the production of surfactants and chelating substances (Yang et al., 2012; Bahadur et al., 2017). Plant rhizosphere microorganisms can directly or indirectly affect plants under heavy metal stress (Ancona et al., 2017), by promoting plant growth, increasing antioxidant synthesis in the roots, and increasing or decreasing the valences of heavy metal ions (Moreira et al., 2016). Heavy metal stress can change the soil microbiome structure, affecting the quantities and activities of soil microorganisms, and impacting the normal function of the rhizosphere soil ecosystem (Macdonald et al., 2011; Boshoff et al., 2014). However, studies on the effects of heavy metal contamination on the composition and diversity of soil microbes have been inconsistent; some reported negative effects (Deng et al., 2015; Li et al., 2017), others found no correlation between heavy metal concentration and bacterial abundance (Grandlic et al., 2006) and others observed positive correlations between some bacteria and heavy metals (Liu et al., 2018). Thus, we still lack the evidence to be able to generalize the effects of heavy metals on the composition of microbial communities in heavy metal-contaminated soils.

Pogonatherum crinitum, a confirmed lead (Pb) hyperaccumulator (i.e., it can accumulate more than 1000 mg/kg Pb). It has a wide distribution, fast propagation rate and relatively high biomass increment (Hou et al., 2012). The phytoremediation potential of *P. crinitum* has recently gained attention, and previous studies have examined its leaf chlorophyll fluorescence, anti-oxidative enzyme activities, and root organic acid contents in response to Pb stress, as well as its cellular compartmentalization of absorbed Pb (Hou et al., 2018, 2019). However, no available study has examined changes in enzyme activities and microbiome composition in the *P. crinitum* rhizosphere during the phytoremediation of Pb-contaminated soil. Such a study would provide insight on the mechanisms by which hyperaccumulators survive in highly polluted soils through interaction with soil microorganisms. In the present work, the activities of rhizosphere soil enzymes indicative of carbon (C) cycling (invertase), nitrogen (N) cycling (protease, urease) and phosphorus (P) cycling (acid phosphatase) were determined in the Pb-contaminated rhizosphere soil of *P. crinitum*. In addition, the bacterial composition and diversity in the rhizosphere soil was determined by high throughput 16 S rDNA sequencing.

The objectives of the study were to (1) determine changes in enzyme activities and bacterial composition in Pb-contaminated *P. crinitum* rhizosphere soil; (2) reveal relationship between microbiome and enzyme activities changes in Pb-contaminated rhizosphere soil, and (3) analyze changes in metabolic pathway of rhizosphere soil bacteria in relation to Pb contamination.

2. Materials and methods

2.1. Plant material and experimental design

P. crinitum seedlings were raised from seeds collected from the Youxi lead-zinc mining area in China. After germination in Petri dishes for seven days in a climate chamber set at 25 °C and 70% relative humidity, the germinates were transplanted into pots filled with nutrient-rich soil for further cultivation. Seedlings with a height of approximately 12 cm and uniform growth were used in the potted Pb stress experiment. The substrate was sandy loam soil (mixed forest land yellow soil and washed river sand with 1:3 v/v), which was air-dried and sieved through a 2 mm nylon sieve to remove impurities. The chemical properties of the substrate were as follows: pH = 5.3; organic matter = 15.4 g/kg; total N, P and K = 0.46, 0.37, and 37.62 g/kg, respectively; available N, P and K = 4.19, 1.44 and 38.19 mg/kg, respectively; Pb, Zn and Cu = 1.03, 2.14 and 0.35 mg/kg, respectively. The experiment was conducted in homemade plastic culture pots (30 cm in diameter and 25 cm in height), and the dry weight of the substrate in each pot was 8 kg.

The simulated Pb stress experiment was carried out by adding 0, 1,000, 2,000, 3000 mg/kg Pb in the form of $(CH_3COO)_2Pb$ solution. After addition of Pb solution, the substrate matrix was thoroughly mixed to ensure uniform distribution and left to equilibrate for 30 days. Then, seedlings were planted at the center of each pot (10 seedlings per pot and five replicates per treatment), and left to grow for 60 days (according to the growth characteristics of *P. crinitum*, the plants were in the vigorous growth period at 60 days). During the experiment, the plants were irrigated periodically with deionized water, and the soil moisture content was controlled by a soil moisture velocity measuring instrument to maintain the field water holding capacity at 60%–70% of the saturated soil water capacity.

The rhizosphere soil, defined as the soil adhering to the root hairs after gentle shaking (He et al., 2006), was sampled after 2 months. First, we shook off the large mass of soil surrounding the roots, and then used a plastic knife to gently scrape the soil around the roots and brushed off the rhizosphere soil (located 0–5 mm from the roots). The soil samples were quickly stored at 4 °C for determination of soil enzyme activities and microbial composition.

2.2. Analysis of soil Pb fractions

All rhizosphere soil samples were sieved through a 2 mm nylon sieve, and the speciation of Pb in these samples was determined by the Tessier continuous extraction method (Yin et al., 2017). The soil was digested with HF–$HClO_4$–HNO_3, and the Pb fractions were determined by the atomic absorption method (Jankowski et al., 2015).

2.3. Soil enzyme analysis

Soil enzyme activities were determined as described by Guan (1986). Soil protease activity was measured by ninhydrin colorimetry and determined using an ultraviolet–visible spectrophotometer at 560 nm (UV-2600, Shimadzu, Japan). The mass (μg) of NH_3–N released from the enzymatic protein per gram of soil at 37 °C for 2 h indicated protease activity. Urease activity was determined by indophenol colorimetry, and the ammonia nitrogen content was determined by spectrophotometer at 690 nm. Urease activity was expressed in milligram NH3–N released from urea per gram of soil in 1 h at 37 °C. Acid phosphatase activity was measured by the disodium phenyl phosphate method, and detected using the spectrophotometer at 510 nm. Acid phosphatase activity was indicated by the amount of milligrams of phenol released per gram of soil after 1 h at 37 °C. Invertase activity was measured by the 3,5-dinitrosalicylic acid colorimetric method, and detected using the spectrophotometer at 540 nm. The amount of glucose released per milligram of soil at 37 °C indicated invertase activity.

2.4. DNA extraction and sequencing

DNA was extracted from approximately 0.25 g rhizosphere soil using Fast Prep nucleic acid extractor and FastDNA Spin Kit for Soil according to the manufacturer's protocol (Bio101, Vista, CA, USA). The V3–V4 variable region of the 16 S rDNA gene was amplified by polymerase chain reaction (PCR) using the primer 338 F (5′ -ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′ -GGACTACHVGGGTWTCTAAT-3′). The PCR amplification conditions were 98 °C for 3 min, followed by 30 cycles of 98 °C, 55 °C and 72 °C for 45 s separately, then 72 °C for 7 min. The PCR products were resolved on 1% agarose gel at 150 V at 4 °C for 40 min

and purified. Based on the Illumina HiSeq sequencing platform, a small fragment library was constructed by the Paired-End method. The library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit and the constructed library was quantified by Qubit and quantitative-PCR. Thereafter, the DNA was sequenced by HiSeq2500 PE250. Data were processed and analyzed using Mothur (https://www.mothur.org; Schloss et al., 2009) after splicing and filtering of ambiguous base. The sequences were then assigned to operational taxonomic units (OTUs) at a 97% similarity level.

2.5. Metabolic pathway analysis

Metabolic pathways were analyzed to standardize the OTU abundance of the 16 S rDNA and then compare it to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/). Metabolic pathway information was obtained from the KEGG database, and the abundance of each functional category was calculated according to the OTU abundance. Three levels of metabolic pathway information and the abundance at each level were calculated using PICRUSt software (Langille et al., 2013).

2.6. Statistical analysis

OTUs were analyzed for abundance, Ace, Chao, Shannon and Simpson diversity, Venn diagrams were used to analyze overlap, and petal diagrams were used to obtain species richness and uniformity information within samples, and common and unique OTUs between different samples or groups. Soil enzyme activities and bacterial alpha diversity were analyzed by one-way analysis of variation (ANOVA), and significant differences were compared by Duncan's multiple comparison test ($p < 0.05$). All data represent the mean ± standard error of five replicates. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and all figures were generated in Origin Pro 8.5 (OriginLab, Northampton, MA, USA). As detrended correspondence analysis revealed that the gradient lengths were shorter than 3.1, redundancy analysis was used to examine the relationships between the relative abundances of dominant bacterial phyla and soil enzyme activities using Canoco (version 4.5).

3. Results

3.1. Pb fractions in P. crinitum rhizosphere soil

Exchangeable, carbonate-bound, iron manganese oxide-bound, organic matter (OM) bound, and residual Pb contents significantly increased in *P. crinitum* rhizosphere soil with increasing Pb concentration (Table 1). Carbonate and iron manganese oxide-bound Pb constituted the largest proportion, and more than 50% of the total Pb content. The proportions of different Pb fractions were carbonate-bound > Fe/Mn-bound > residual fraction > OM-bound > exchangeable fraction.

Table 1
Fractions of Pb in rhizosphere soils of *P. crinitum* under different Pb treatments (mean ± SD). Means followed by the different letter across the columns are statistically significant ($p < 0.05$).

Treatment	Pb content mg/kg				
	F1	F2	F3	F4	F5
CK	–	–	–	–	–
Pb.1000	43.27 ± 1.97c	497.19 ± 35.92c	266.49 ± 19.64c	53.93 ± 3.51c	139.12 ± 17.96c
Pb.2000	89.51 ± 5.54 b	1026.17 ± 38.61 b	485.73 ± 14.17 b	121.05 ± 2.43 b	277.53 ± 10.30 b
Pb.3000	112.78 ± 5.60a	1761.15 ± 53.84a	604.25 ± 26.42a	165.63 ± 5.29a	356.18 ± 20.50a

Note: Exchangeable fraction (F1), bound to carbonate (F2), bound to Fe/Mn (F3), bound to organic matter (F4), Residual fraction (F5).

3.2. Enzyme activities

Protease, urease, acid phosphatase and invertase activities significantly varied among treatments (Fig. 1). Protease activity in the rhizosphere soil of *P. crinitum* was twice higher in the control than in the Pb stress treatments. The urease activity was higher in 1000 and 2000 mg/kg Pb treatments than in the control and in 3000 mg/kg Pb treatment; the latter being significantly lower than in the control. Acid phosphatase activity was higher in Pb-contaminated soils than in the control soil, and the highest activity was observed in 2000 mg/kg Pb treatment, followed by 1000 mg/kg Pb contaminated rhizosphere soil. Invertase activity was significantly lower in rhizosphere soil contaminated with 3000 mg/kg Pb than in the control or contaminated with 1000 and 2000 mg/kg Pb; the invertase activity of the latter three did not significantly differ among each other.

3.3. Bacterial diversity

The average number of effective sequences obtained from the rhizosphere soil samples by Illumina HiSeq sequencing analysis were 41,487, 42,044, 32,719, and 41,886 for the control, 1,000, 2000 and 3000 mg/kg Pb-contaminated rhizosphere soil, respectively, with up to 99% sequence coverage (Table 2). Shannon rarefaction analysis showed that the curve tended to plateau after 20,000 sequences at 97% similarity (data not shown); thus, additional sequencing had little effect on species diversity. The number of effective sequences in rhizosphere soil contaminated with 2000 mg/kg Pb was significantly lower than that in the other Pb treatments and the control. Shannon's diversity index was significantly lower in the 2000 and 3000 mg/kg Pb treatments than in the control and 1000 mg/kg Pb treatments, which were not significantly different from each other. There were no significant differences in the Simpson's dominance, Chao l and abundance-based coverage between the control and the Pb treatments.

A total of 1948 soil bacterial OTUs were obtained from all samples based on a 97% similarity clustering level at a genetic distance of 3% (Fig. 2). The number of soil bacterial OTUs was lowest in *P. crinitum* rhizosphere soil contaminated by 2000 mg/kg Pb, accounting for 4.7% of the total OTUs, and the highest being in rhizosphere soil contaminated with 3000 mg/kg Pb, accounting for 16.2% of the total OTUs. The number of OTUs unique to *P. crinitum* rhizosphere soil during the different Pb treatments was 799, accounting for 41.1% of the total OTUs, which was not significantly different from the control.

3.4. Bacterial composition

The sequencing reads obtained from the rhizosphere soils classified at the phylum level were associated with 10 bacterial phyla (Fig. 3a). Dominant phyla (accounting for more than 1% of the overall community in each rhizosphere soil sample) were Proteobacteria (67.14–778.89%), Bacteroidetes (6.83–719.87%), Actinobacteria (4.27–79.18%) and Firmicutes (1.31–74.44%). A clear shift in bacterial composition occurred during *P. crinitum* phytoremediation. While the relative abundance of Proteobacteria was slightly higher in the control soil than in Pb-contaminated soil, the relative abundance of Bacteroidetes were 1.88, 1.91 and 1.57 times higher in the 1000 mg/kg, 2000 mg/kg, and 3000 mg/kg Pb treatments, respectively, compared to the control. Actinobacteria was more abundant in the control and 1000 mg/kg Pb-treated soils than in the other Pb treatments, while Firmicutes was more abundant in the 3000 mg/kg Pb-treated soil than in the other Pb treatments and the control.

Similarly, the sequencing reads were affiliated with 10 bacterial classes. The dominant bacterial classes were Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Sphingobacteriia, Bacteroidia and Epsilonproteobacteria in both the control and Pb-treated soils (Fig. 3b). Among them, the relative abundances of Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, and Sphingobacteriia

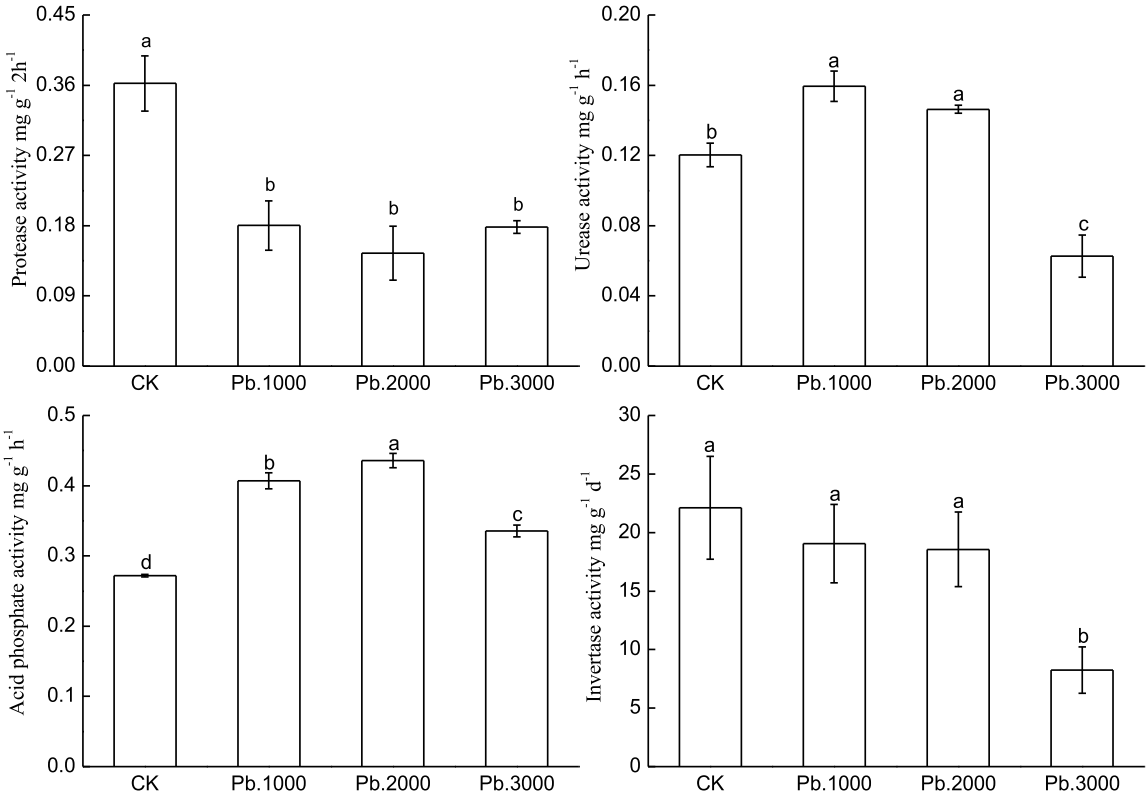

Fig. 1. Enzyme activities of rhizosphere soil of *P. crinitum* under different Pb treatments. Bars with different letter are significantly different ($p < 0.05$).

Table 2
Effective sequences and diversity of bacterial community of rhizosphere soil of *P. crinitum* under different Pb treatments (mean ± SD). Means followed by the same letter across the columns are not statistically significant ($p > 0.05$).

Treatment	Effective sequence	Shannon	Simpson	Chao1	ACE	Coverage
CK	41,487 ± 5019a	6.66 ± 0.45a	0.96 ± 0.03a	878.19 ± 10.00a	919.50 ± 22.78a	0.99 ± 0.00a
Pb.1000	42,044 ± 1358a	6.26 ± 0.09 ab	0.97 ± 0.00a	919.74 ± 12.86a	931.05 ± 18.34a	0.99 ± 0.00a
Pb.2000	32,719 ± 2839 b	5.99 ± 0.18 b	0.96 ± 0.02a	928.02 ± 29.35a	851.36 ± 31.51a	0.99 ± 0.00a
Pb.3000	41,886 ± 1995a	5.98 ± 0.36 b	0.94 ± 0.06a	899.29 ± 43.79a	947.53 ± 50.56a	0.99 ± 0.00a

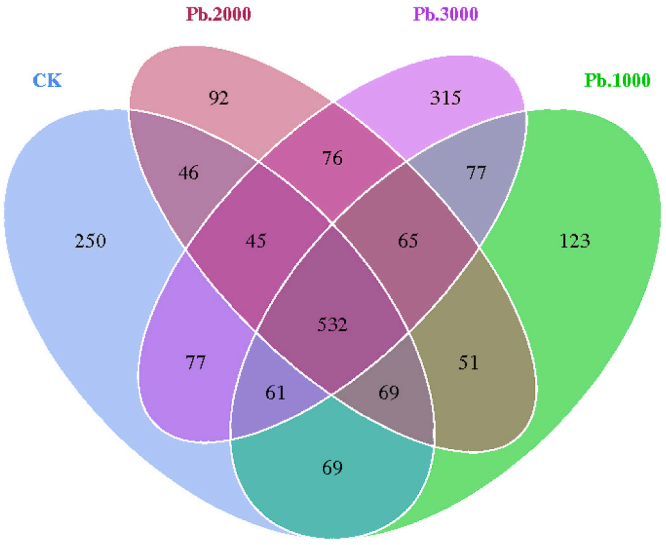

Fig. 2. Venn diagram depicting common and unique OTU in different Pb treatments.

were the highest; accounting for 77.76%, 85.11%, 89.09%, and 80.93% of species in the control, 1000 mg/kg, 2000 mg/kg and 3000 mg/kg Pb-treated soils, respectively. In the 3000 mg/kg Pb-treated soil, Betaproteobacteria was dominant, while Gammaproteobacteria was less abundant. Alphaproteobacteria was more abundant in the control soil than in Pb-treated soils, while Sphingobacteriia was less abundant in the control soil. The relative abundance of Bacteroidia increased with increasing Pb concertation, while the relative abundance of Epsilonproteobacteria showed the opposite trend. Clostridia and Bacilli had relatively higher abundance in the 3000 mg/kg Pb-treated soils than in the other treatments.

3.5. Relationships between enzymatic activities and bacterial composition

Redundancy analysis of the OUT (97%) composition of bacteria in control and Pb-contaminated rhizosphere soils is shown in Fig. 4. Acidobacteria and uncultured eubacterium WD272 were strongly correlated with rhizosphere invertase activity. Candidate phylum SHA-109 positively correlated with rhizosphere soil protease activity. Actinobacteria, Cyanobacteria and Gemmatimonadetes were positively correlated with invertase and protease activity. Bacteroidetes and Saccharibacteria were most positively correlated with acid phosphatase activity. Conversely, acid phosphatase, urease, invertase and protease

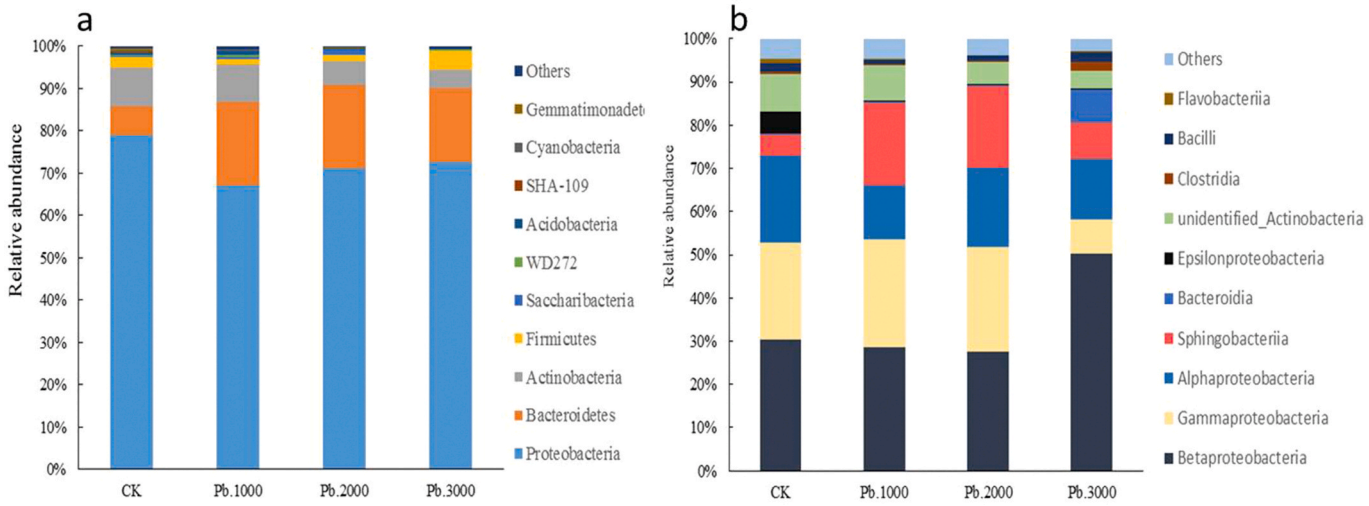

Fig. 3. Relative abundance of bacterial phyla (a) and class (b) in rhizosphere soil exposed to different Pb treatments.

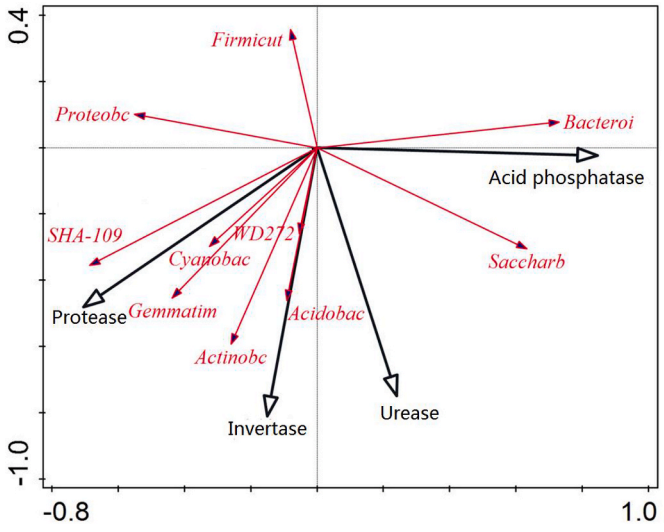

Fig. 4. RDA analysis depicting the relationship between bacterial community and enzyme activity in rhizosphere soil of *P. crinitum*.

activities were negatively correlated with Proteobacteria and Firmicutes.

3.6. Metabolic pathway predictions

A cluster heat map of the relative abundances of KEGG metabolic pathways in the microbial composition of the Pb-treated rhizosphere soil samples is shown in Fig. 5. Soils treated with 3000 mg/kg Pb clustered into one class, soils contaminated with 1000 and 2000 mg/kg Pb into another, and the control soil into another. At level 3, there were 35 metabolic pathways that were significantly differed between the different Pb treatments (Fig. 5a). The abundance of the pyruvate metabolism, energy metabolism, propanoate metabolism, valineleucine- and isoleucine degradation, fatty acid metabolism, butanoate metabolism, glyoxylate- and dicarboxylate metabolism and ABC transporter pathways were significantly upregulated in 3000 mg/kg Pb treatment compared to the other Pb treatments and the control. Compared to the control, glycolysis/gluconeogenesis, amino sugar- and nucleotide sugar metabolism and peptidases were upregulated in 1000 mg/kg Pb treatment, and membrane and intracellular structural molecules, peptidases, and oxidative phosphorylation were upregulated in 2000 mg/kg Pb treatment. There were significant differences in the upregulated metabolic pathways related genes of rhizosphere soil microorganisms under different Pb treatments (Fig. 5b). Putative ABC transport system, ATP-binding protein, and lacI family transcriptional related metabolic pathways genes were significantly upregulated in 1000 mg/kg Pb treatment, while hydrophobic/amphiphilic exporter-1 (mainly G-bacteria) and F420H(2)-dependent quinone reductase related metabolic pathway genes were significantly increased in 2000 mg/kg Pb treatment. There were 17 metabolic pathways genes upregulated in 3000 mg/kg Pb treatment, including amino acid transport protein, sugar transport system permease protein, NitT transport protein, enoyl-CoA hydratase, and aspartyl-tRNA/glutamyl-tRNA amidotransferase subunit.

4. Discussion

Heavy metal absorption by plant roots is closely related to the heavy metal concentrations of heavy metals of the soil medium (Rosenfeld et al., 2018). In soil, these metals are found in five forms: the exchangeable, carbonate bound, iron manganese oxide-bound, OM bound and residual states. Exchangeable Pb has the highest activity and toxicity to plants, while the residual state has the lowest toxicity (Alirzayeva et al., 2017). Our study showed that during Pb stress, the Pb in *P. crinitum* rhizosphere soil was mainly carbonate and iron manganese oxide-bound (Table 1). These states indicate that during Pb stess the Pb in *P. crinitum* rhizosphere soil may form complex states through chelating that are not easily absorbed by roots, thus reducing damage to the plant. It might be so that roots of *P. crinitum* produce chelating compounds that bound the excess Pb in the rhizosphere.

Soil enzymes and microorganisms are important parts of the soil ecosystem and are sensitive to environmental changes (Pang et al., 2009). Soil enzymes are mainly derived from soil microbial activities, exudates of plant root systems, and the decomposition of plant and animal residues, and are bioactive catalytic polymers (Ahmed et al., 2018). There are a linear relationships between soil microbial attributes, and enzyme activity, and heavy metal concentrations in contaminated soil (Raiesi and Sadeghi, 2019). Our study revealed a decreased in protease activity, relatively stable invertase activity, and increased urease and acid phosphatase activities in the 1000 and 2000 mg/kg Pb-treated *P. crinitum* rhizosphere soil compared to the control (Fig. 1). This may be due to interactions between the protease active site and excess Pb ions which block the original functional group competitive inhibition. The increased available P may promote its exchange from soil binding sites to enhance its mobilization, thereby increasing the Pb

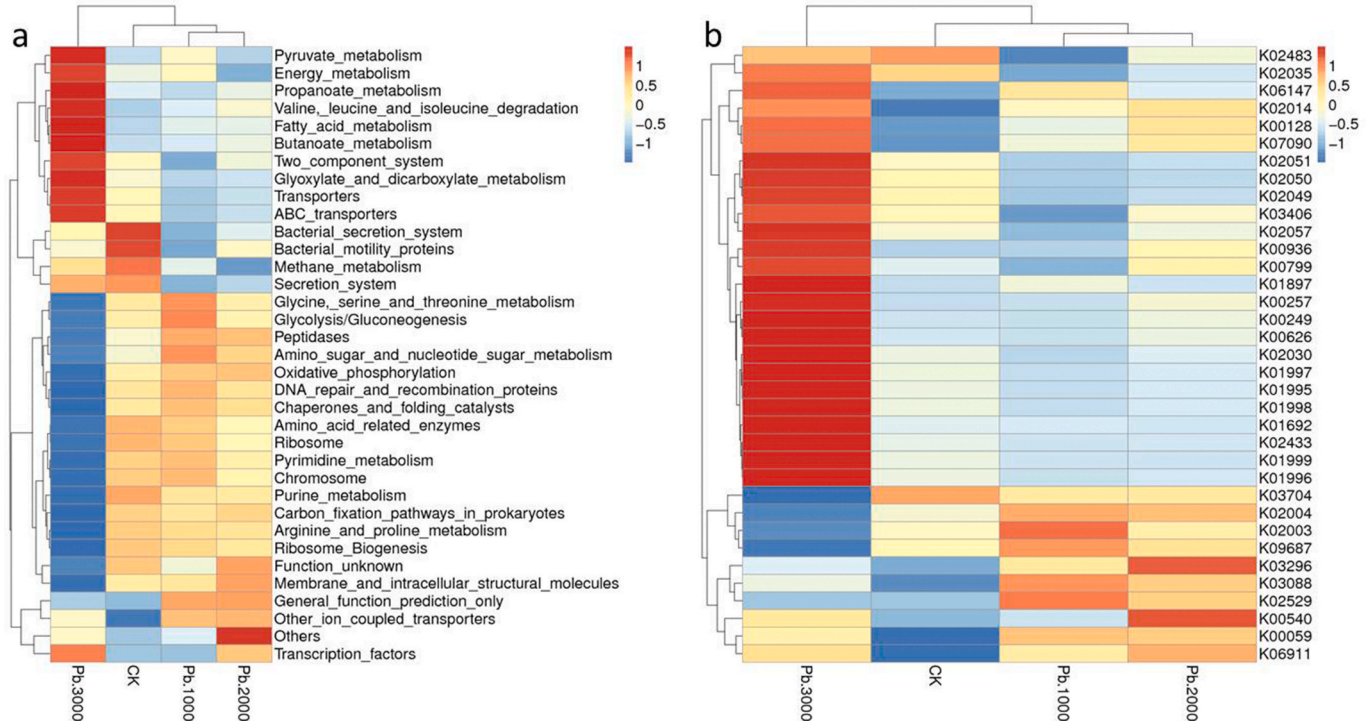

Fig. 5. KEGG metabolic pathway relative abundance cluster heat map in Level3 (a) and Gene Ontology (b) of soil bacteria in rhizosphere of *P. crinitum* exposed to different Pb treatments.

uptake efficiency of *P. crinitum* grown in Pb-contaminated soil, as reported in other studies (Hou et al., 2012; Das et al., 2017). As a whole, our results are consistent with previous studies that demonstrated increase phosphatase activity in metal-contaminated sites (Hagmann et al., 2015), and an increase in urease and phosphatase activities in response to arsenic enrichment of rhizosphere soil (Das et al., 2017).

The effects of heavy metal stress on soil microorganisms are multifaceted and complex, and can significantly alter the number of microorganisms and their composition (Yu et al., 2003; Fatnassi et al., 2015). We found that Pb contamination of *P. crinitum* rhizosphere soil induced the presence of rhizosphere-specific bacteria, with significantly more unique bacterial types than observed in the control. While Proteobacteria dominated in the rhizosphere of *P. crinitum* grown in both Pb-contaminated and control soil, Bacteroidetes, Actinobacteria and Firmicutes were dominant in Pb-contaminated rhizosphere soil (Fig. 3a). Proteobacteria have been identified as the most stress-tolerant bacteria in heavily contaminated soils (Burkhardt et al., 2011; Xavier et al., 2019), most likely due to their copiotrophic nature i.e., their ability to grow in high organic substrate conditions such as the rhizosphere soil (note that the OM content of the substrate in our study was 15.4 g/kg). The high relative abundances of Bacteroidetes and Actinobacteria in Pb-contaminated rhizosphere soil may stimulate P mineralization and degradation by producing extracellular enzymes. Consistent with this, Bacteroidetes and Actinobacteria were positively correlated with acid phosphatase and invertase activities. Want et al. (2019) found that Bacteroidetes was the dominant phylum in Cd-contaminated soils while Liu et al. (2018) found higher relative abundances of Bacteroidetes after phytoremediation of Pb-contaminated soil by *Trifolium repens*. Similarly, Xavier et al. (2019) found that Bacteroidetes and Firmicutes were the most abundant phyla in heavy metal contaminated soil in Riacho dos Macacos, Brazil.

Among bacterial classes, the relative abundance of Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, and Sphingobacteriia were the highest (Fig. 3b). The relative abundance of Bacteroidia increased with increasing Pb concentration. This suggests that Pb pollution most likely affects microbial diversity by stimulating metal-resistant species while inhibiting metal sensitive species. Betaproteobacteria has been shown to rapidly respond to C availability (Das et al., 2016), which may explain its high relative abundance in Pb-contaminated *P. crinitum* rhizosphere soil in our study. Note that the growing media in our study has a large amount of C.

Our study also showed strong correlations between enzyme activities and dominant bacterial phyla (Fig. 4). This suggests that rhizosphere soil enzymes and dominant bacteria promote each other to the plant's benefit, as increased urease and invertase activities promote root growtht and antioxidant synthesis (Moreira et al., 2016), while increased acid phosphatase activity improves P availability. Generally soils from contaminated study sites (like the substrates used in our study) have limited available P, because of their acidic nature and P fixation by aluminum and iron (Chen et al., 1996). Thus, the increased acid phosphatase activity in Pb-contaminated rhizosphere soils which was associated with Bacteroidetes abundance, increases P availablity through chelation. The increase in available P is beneficial for the growth of *P. crinitum* in Pb-contaminated soil. Our results are consistent with previous studies that demonstrated a significant correlation between soil microorganisms and soil enzyme activities (Pang et al., 2009) and a positive correlation between the number of bacteria in the rhizosphere soil of radish seedlings and soil urease activity under mercury stress (Li, 2018).

We found that the metabolic pathways of rhizosphere soil bacteria of *P. crinitum* were significantly different between the various Pb treatments and the control. The relative abundances of metabolic pathways related to amino acid, ABC transport protein, quinone reductase, acyl-ACP dehydrogenase, and ATP-binding protein were increased in Pb-treated rhizospheres compared to the control (Fig. 5). Genes encoding ATP-binding cassette transporters were upregulated in the rhizosphere after inoculation with *Mesorhizobium loti* HZ76 (Fan et al., 2018). Amino acid metabolism and ABC transporters are related to cellular metabolism and being dominant in the prediction of microbial metabolic function (Lin et al., 2019), which is consistent with our study. Overall, the results indicate that ATP-binding protein, ABC transporter, and amino acid metabolism pathways play key roles in rhizosphere heavy metal

detoxification.

5. Conclusions

Based on these findings, the following conclusions can be drawn: (1) Pb contamination adversely affects protease activity but it increases the activities of acid phosphatase and urease (depending on the concentration in the rhizosphere soil), however, soil invertase activity remains relatively stable; (2) Pb contamination alters the soil microbial composition compared to untreated soil, with Bacteroidetes being the most abundant phylum in Pb-contaminated soils; (3) strong correlations between dominant bacterial phyla and enzymatic activities suggest synergetic effects on *P. crinitum* growth in Pb-contaminated soil and hyperaccumulation of Pb; and (4) the relative abundances of metabolic pathways related to amino acids, ABC transport protein, quinone reductase, and ATP-binding metabolism were increased in Pb-contaminated rhizosphere soil compared to the control. Overall, these results indicate that the increased activities of acid phosphatase and urease and the abundance of Bacteroidete play important roles in rhizosphere Pb decontamination by *P. crinitum*.

Credit authors statement

Xiaolong Hou, designed and performed the study, wrote the manuscript, read and approved the final manuscript. Hang Han, contributed to the DNA extraction and sequencing, data analysis and chart making, read and approved the final manuscript. Mulualem Tigabua, supervised the statistical analysis and language edit, read and approved the final manuscript. Qiyan Li, contributed the soil enzyme measured, read and approved the final manuscript. Zongxun Li, contributed the soil enzyme measured, read and approved the final manuscript. Chenlu Zhu, contributed the chart making and Pb concentration measured, read and approved the final manuscript. Siqi Huang, contributed the chart making and Pb concentration measured, read and approved the final manuscript. Liping Cai, supervised the experimental analytical methods and soil enzyme measured, read and approved the final manuscript. Aiqin Liu, contributed to the research design and supervised the project, read and approved the final manuscript.

Declaration of competing interest

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

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