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RESEARCH ARTICLE

Effects of lead pollution on soil microbial community diversity and biomass and on invertase activity

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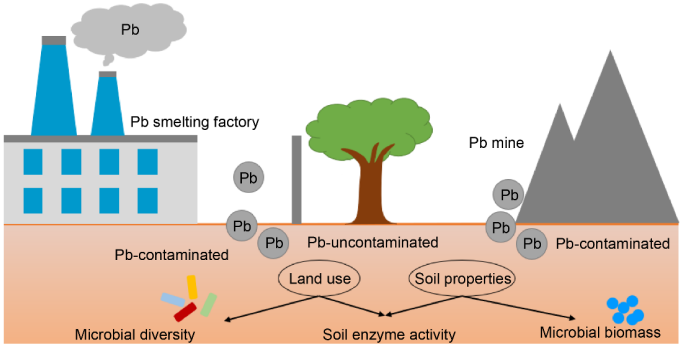
HIGHLIGHTS

- Pb pollution significantly affected the diversity of microbial community structure.
- Pb pollution reduced the soil microbial biomass-carbon and nitrogen.
- Pb pollution increased invertase but reduced catalase activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Lead (Pb) pollution is one of the most widespread and harmful environmental problems worldwide. Determination of changes in soil properties and microbial functional diversity due to land use is needed to establish a basis for remediation of soil pollution. This study aimed to investigate soils contaminated by Pb from different sources and to analyze the functional diversity and metabolism of soil microbial communities using Biolog technology. Pb pollution (> 300 mg kg^{-1}) significantly influenced the diversity and metabolic functions of soil microbial communities. Specifically, Pb contamination significantly reduced soil microbial biomass carbon (C) and nitrogen (N) levels and catalase activity while increasing invertase activity. Furthermore, Biolog EcoPlate assays revealed that Pb pollution reduced the general activities of soil microorganisms, suppressing their ability to utilize C sources. In Pb-contaminated areas lacking vegetation cover, Shannon, Simpson, and McIntosh diversity indices of soil microorganisms were significantly reduced. The microbial diversity and biomass C and N levels were affected by land use and soil properties, respectively, whereas soil enzyme activity was primarily affected by the interaction between land use and soil properties. Our results provide a reference and a theoretical basis for developing soil quality evaluation and remediation strategies.

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1 Introduction

Lead (Pb) is one of the heavy metals widely found in nature, with no known biological function. Further, as an important industrial raw material, Pb is used extensively, leading to high levels of accumulation of solid waste containing the element, such as in slag from mining, so that it has become a major threat to the environment (Cheng and Hu, 2010). Solid waste is commonly stacked and informally managed, whereby heavy-metal ions can easily leach out due to weathering, snow, and rain, and cause severe soil pollution (Zhang et al., 2012). Indeed, soil Pb pollution has become a critical environmental problem worldwide (Tang et al., 2019). Additionally, Pb smelters produce large amounts of dust containing Pb^{2+}, which eventually gets transferred to the soil (Han et al., 2017). Besides Pb from mining and smelting activities, Pb accumulation in the soil also results from combustion of leaded gasoline, sewage-sludge dumping, and disposal of batteries and other Pb-containing waste products (Huang et al., 2006; Zhang et al., 2019; Bidar et al., 2020). Thus, Pb can accumulate through the food chain and affect humans as well, causing irreversible damage, particularly in children (Liu et al., 2018; Kelepertzis et al., 2021). Many remediation techniques, including chemical precipitation, ion-exchange, evaporation, electrochemical treatment, and filtration, have been applied to Pb-polluted soil to reduce its negative effects on ecosystems and human health (Malik 2004; Yang et al., 2021). Regardless of the remediation methods used, investigation of Pb^{2+} levels in polluted soils and a thorough understanding of their physical, chemical, and biological characteristics remain a pending subject.

Soil heavy-metal pollution causes severe damage to microorganisms, reducing microbial activity, altering microbial community structure, and leading to changes in soil enzyme activity (Li et al., 2021). These changes may eventually reduce soil quality. Previous studies showed that heavy metals have adverse effects on soil microbial community structure (Aponte et al., 2021). In general, heavy-metal pollution reduces the efficiency of microbial transformation of organic substances (Bardgett and Saggar, 1994). Therefore, microbes need more energy to maintain their normal metabolism under adverse conditions. Soil type, vegetation, climate, and other factors are closely related to the content of C sources in the soil, which also affects microbial populations (Du et al., 2014). Previous studies have demonstrated that excessive quantities of heavy metals in soils cause a significant decrease in microbial community size (Li et al., 2020), with adverse effects and degrees influenced by heavy metal type, pollution level, and land use (Zhao et al., 2012). Notably, microbial activity is a crucial factor for maintaining the sustainability of soil productivity (Xu et al., 2020; Wang et al., 2021). The turnover and mineralization of soil organic matter, transformation of nutrients, and recycling of organic waste are all dependent on the metabolism of soil microorganisms (Yang et al., 2014). Therefore, microbial activity is an essential element in the stability of soil ecosystems (Liu et al., 2016). In recent years, technological advances have provided new methods for precise characterization of the diversity of microbial populations, taking more features into consideration. For example, biomarkers are essential tools for microbial classification in the research on soil ecological toxicology (Lima et al., 2018; de Oliveira et al., 2019). Biomarkers are organisms that are exposed to a variety of environmental conditions, their tissues, cells, and molecular structures responding to specific biological signals (Kumpiene et al., 2008). In soil ecosystems, biomarkers are used for the diagnosis of soil pollution, providing a basis for quality evaluation, and for the prevention and repair of damage caused by soil pollution (Ji et al., 2021; Wang et al., 2021). Microbial community structure in soils is an important parameter that needs to be considered when characterizing soil ecosystem and community structures and stability, as such a structure serves as a predictor of changes in soil nutrients and general environmental quality (Ma et al., 2016). For this purpose, various methods based on biological biomarkers, such as microbial quinones, fatty acids, and molecular biological markers, have been applied in recent years (Khalili et al., 2016). These methods do not involve the isolation and culture of microbes to reflect the community structure of microorganisms, nor can they yield information regarding the overall activity and metabolic function of microbial communities. However, quantitative analysis based on the carbon (C) source of Biolog microporous plates has provided a simple and rapid method for determining the functional diversity of microbial communities. This analysis has been extensively used to evaluate the functional diversity of soil microbial communities in different soil types, in the same soil type under different vegetation, in agricultural soils under different management strategies, and in rhizosphere soil under diverse vegetation covers (Garland, 1997).

Soil microbial activity is closely related to soil enzyme activity (Tan et al., 2021), as many soil enzymes are secreted by microbes and are involved in the recycling of substances and energy in the soil (Wang et al., 2013). Soil enzymes are bioactive substances secreted in the soil and serve as an important index of soil biology (Siczek et al., 2020). As bioactive molecules, enzymes can be affected by heavy-metal contamination (Wu et al., 2017). Kandeler et al. (1996) reported that heavy-metal stress affects soil enzyme activity, eventually inhibiting enzymes related to soil C, nitrogen (N), phosphorus (P), and sulfur cycles, and reducing their activity.

This study aimed to evaluate the chemical and physical characteristics of the soil, the Pb pollution level, microbial diversity, and soil enzyme activity in Pb mines and Pb smelters. Understanding the differences between the effects

of these two different human activities on soil microorganisms could provide a fundamental theoretical basis for the remediation of Pb-polluted soils.

2 Materials and methods

2.1 Study site and sample collection

Two types of Pb-polluted areas (a Pb smelting factory and a Pb mine) in Shandong Province, China were selected as sampling sites. Three sampling areas (immediately outside the factory perimeter, named factory wall-out area [F1]; inside the factory perimeter, named factory-in areas [F2]; and an area 10 km away from the factory, as the unpolluted reference point [UF]) were selected for the Pb smelting factory. Four sampling areas (mine opening [M1]; new waste-residue stacking area [M2]; outdated waste-residue stacking area [M3]; and an area 10 km away from the mine, as the unpolluted reference point [UM]) were selected for the Pb mine. Three sample replicates per site were collected using sterile tools and secured in valve bags. Five samples from within a distance of 50 m of each other were mixed to form each replicate. All samples were transported to the laboratory on ice and sieved through a stainless-steel mesh (2 mm). The samples were stored in kraft paper bags after removing impurities by air drying. Soil organic C (SOC) was measured by the potassium dichromate external heating method. Total N, available P, and available K contents of the soil were measured using methods described by Lu (1999). For Pb detection, the soil samples were digested using a microwave digestion apparatus (Multiwave PRO, Anton Paar, Graz, Austria), and Pb^{2+} content was measured using an atomic absorption spectrophotometer (AAS, AA-700, Japan).

2.2 Functional diversity of microorganisms

Functional diversity of soil microorganisms was identified using Biolog EcoPlates (Biolog Inc., Hayward, CA, USA), with the collected samples utilizing 31 different C sources. Approximately 10 g of fresh soil (dry weight equivalent) from each sample was dispersed with 90 mL of 0.05 mol sterilized phosphate buffer (pH = 7.0) in a pre-sterilized Erlenmeyer flask, which was then sealed with a rubber stopper and mixed at 200 r min^{-1} for 60 min and at 25°C in the dark. Subsequently, samples were centrifuged for 10 min at 3000 × *g*. The supernatant was collected and diluted, and 150 µL from the 10^{-3} dilution was inoculated into Biolog EcoPlates. Each of the 96-well plates contained one of the 31 different C sources and a blank well, and each was replicated thrice. All plates were maintained in an incubator (25°C), and utilization of each carbon source by soil microorganisms was monitored by determining absorbances at 590 nm after 4, 24, 48, 72, 96, 120, 144, and 168 h. In addition, a 72-h microplate culture assay was performed to measure the soil microbial activity index based on average well-color development (AWCD), as described by Garland (1997).

Data obtained from the Biolog EcoPlates and the 72-h microplate culture assays were statistically analyzed. Four diversity indices, i.e., Substrate evenness (I), Shannon (H), Simpson (Ds), and McIntosh (U) indices were used to calculate the diversity of C-source utilization by the microbial community in the soil samples and to assess the heterogeneity of species richness and dominance of the most common species in the soil microbial community. As the best resolution occurred at the shortest incubation time of 72 h, statistical analyses were performed using the 72-h microplate culture and Shannon diversity index values (Lewis et al., 2010).

Principal component analysis (PCA) was used to transform the multivariate vectors of the different processed samples into uncorrelated principal component vectors (PC1 and PC2 as principal sub vectors). In PCA, the metabolic characteristics of different microbial communities are intuitively reflected by their location in space. In this study, the 31 C sources in the plates were divided into six categories, namely amino acids, sugars, carboxylic acids, amines, alcohols, and esters.

2.3 Soil enzyme activity, and soil microbial biomass carbon and nitrogen

Soil enzyme activity was determined from fresh soil samples using the method described by Guan et al. (1986). Invertase, urease, and dehydrogenase activities were determined by the 3,5-dinitrosalicylic-acid colorimetric method, the sodium phenolate method, and the Johnson-Temple method, respectively. Neutral phosphatase activity was measured by the disodium phenyl phosphate colorimetric method, based on the amount of phenol produced by hydrolysis of disodium phenyl phosphate under the action of phosphatase. Soil microbial biomasses of C and N were extracted using the chloroform fumigation-K_2SO_4 extraction method (Vance et al., 1987).

2.4 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS software (version 22.0; IBM, Armonk, NY, USA), and two-way ANOVA was used when considering the various sources and their interactive effects on soil microbial diversity, biomass, and enzymes. Fisher's least significant difference test at $p < 0.05$ was used for multiple comparisons of means.

3 Results

3.1 Soil Pb content and nutrient characteristics

Soil Pb contamination in the Pb-smelter area was more

severe than that in the mining area; for example, the soil Pb content was approximately 6.86-fold higher in F2 than in M1 (Table S1). In the Pb-smelter area, Pb contents in F2 and F1 were 70.02- and 510.68-fold higher, respectively, than in UF. Notably, soil total N, and available P and K contents decreased with increasing Pb level.

3.2 Functional diversity of microbial communities

Higher AWCD was observed in both Pb-polluted and unpolluted soils collected from the Pb smelter than in those collected from the mining area (Fig. 1). Notably, the AWCD values in contaminated soil from the mining areas (M1 and M2) were significantly lower than those in the contaminated soil of the Pb-smelter sampling areas (F1 and F2), after 72 h. Similarly, the AWCD value in the uncontaminated soil from the mining area was lower than that from the Pb-smelter area.

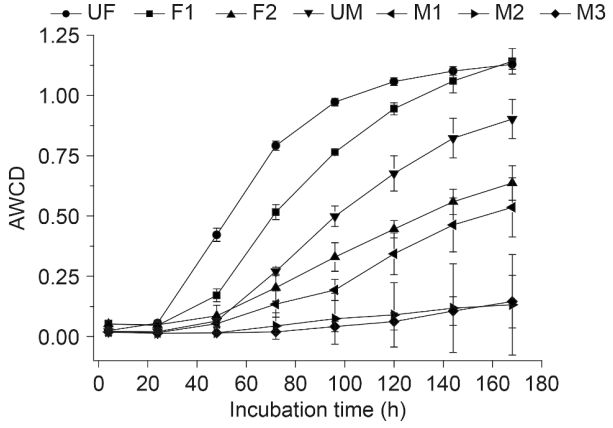

Fig. 1 Changes in average well-color development (AWCD) with soil incubation time. Error bars represent standard deviations of the means (n = 3). UF, unpolluted reference point (10 km away from the factory); F1, factory wall-out area (immediately outside the factory perimeter); F2, factory-in area (inside the factory perimeter); UM, unpolluted reference point (10 km away from the mine); M1, mine opening; M2, new waste-residue stacking area; M3, outdated waste-residue stacking area.

Overall, the ability for utilization of the six C sources by soil microbes was lower in the soil from the mining area than for utilization in the soil from the smelter area (Fig. 2). Regarding ability to utilize sugars, carboxylic acids, esters, and amino acids, the highest was observed in UF and F1, whereas that of amines was observed in F1 and UM, and that of alcohols was recorded in areas UF, F1, UM, and M1. For the Pb-smelter area, the ability to utilize sugars, carboxylic acids, and amino acids as C sources in F2 was lower than in UF and F1, and no significant difference was found between F1 and UF. Similarly, no difference in the ability to use alcohols and esters as C sources was detected across the UF, F1, and F2 areas. The ability for the utilization of amine C sources decreased in the following order: F1 > UF > F2, and no significant difference in the ability to utilize esters was observed between F1 and F2 areas. However, the ability to use alcohol was higher in F1 than in F2. In the mining area, the abilities to use the six types of C source were lower in M2 and M3 than in UM, whereas ability to use carboxylic acids, amines, and amino acids was significantly lower in M1 than in UM. Additionally, the ability to utilize sugars, alcohols, esters, and amino acids was lower in M2 and M3 than in M1, and no significant difference was observed in the ability for use of carboxylic acids and amines as C sources across the areas M1, M2, and M3.

The McIntosh index was higher in soil samples from the smelting area than in samples from the mining area (Table 1). For the smelting area, McIntosh index in F2 was lower than that in UF and F1, and no significant difference was found between F1 and UF areas for this index. Similar values of the Shannon richness index, Substrate evenness, and Simpson index were observed across UF, F1, and F2 areas. In the mining area, Shannon richness index, Substrate evenness, and Simpson index were lower in areas M2 and M3 than those in areas UM and M1, and no significant

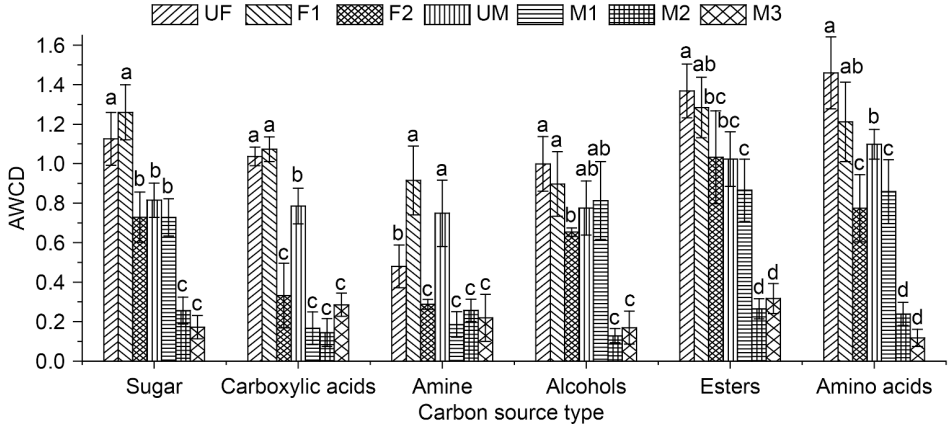

Fig. 2 Utilization of six types of carbon sources by soil microbial communities from different sampling areas. Error bars represent standard deviations of the means (n = 3). Different letters on the error bars indicate significant differences among treatments at $p < 0.05$.

Table 1 Diversity index of soil microbial community and soil microbial biomass carbon and nitrogen in different sampling areas.

Sampling areas	Shannon richness index (*H*)	Substrate eventness (*I*)	Simpson index (*Ds*)	McIntosh index (U)	SMBC (mg kg^{-1})	SMBN (mg kg^{-1})	SMBC/SMBN
UF	3.25 ± 0.02 a	0.950 ± 0.012 a	0.959 ± 0.001 a	5.95 ± 0.14 a	179.63 ± 5.71 a	33.02 ± 2.71 b	5.52 ± 0.52 a
F1	3.32 ± 0.01 a	0.988 ± 0.008 a	0.962 ± 0.001 a	5.92 ± 0.05 a	95.02 ± 4.22 d	23.71 ± 1.74 d	4.03 ± 0.13 c
F2	2.95 ± 0.03 a	0.892 ± 0.021 a	0.942 ± 0.003 a	4.44 ± 0.25 b	42.91 ± 4.83 e	9.52 ± 1.22 e	4.62 ± 0.54 bc
UM	3.16 ± 0.04 a	0.929 ± 0.009 a	0.953 ± 0.002 a	5.29 ± 0.24 a	173.12 ± 4.32 a	38.63 ± 2.13 a	4.52 ± 0.13 bc
M1	3.01 ± 0.23 a	0.913 ± 0.115 a	0.934 ± 0.004 a	4.06 ± 0.46 bc	49.83 ± 4.53 e	11.83 ± 1.42 e	4.24 ± 0.22 c
M2	2.26 ± 0.29 b	0.792 ± 0.049 b	0.861 ± 0.037 b	3.52 ± 0.11 c	122.34 ± 6.22 c	22.34 ± 1.53 d	5.54 ± 0.13 a
M3	1.95 ± 0.40 b	0.624 ± 0.078 c	0.782 ± 0.073 c	2.01 ± 0.84 d	135.51 ± 6.23 b	27.62 ± 2.71 c	4.92 ± 0.32 b

UF, unpolluted reference point (10 km away from the factory); F1, factory wall-out area (immediately outside the factory perimeter); F2, factory-in area (inside the factory perimeter); UM, unpolluted reference point (10 km away from the mine); M1, mine opening; M2, new waste-residue stacking area; M3, outdated waste-residue stacking area. Data are presented as the mean ± SD. No significant difference was found between means with the same lowercase letter ($p < 0.05$).

difference between the Simpson index was verified between areas UM and M1. U values in Pb-polluted soils (M1, M2, and M3) were lower than in UM.

3.3 PCA of the utilization of soil microbial carbon sources in different Pb-polluted areas

AWCD data measured at 72 h were used for PCA (Fig. 3), and seven principal components were extracted whose corresponding cumulative contribution rate reached 86.3%. The microbial community patterns in the UF, F1, and UM samples were significantly distinct from those in the F2, M1, M2, and M3 samples (Fig. 3A). Fifteen C sources influenced PC1, among which correlation between 2-hydroxy benzoic acid and PC1 was the highest (Fig. 3B). All C sources were correlated with PC2, although the impact of 2-hydroxy benzoic acid was the weakest.

3.4 Soil enzyme activity

Invertase activity was higher in Pb-polluted soils than in unpolluted soils, both in smelting and mining areas. However, higher invertase activity was observed in soil samples from mining areas than in those from smelting areas, both in unpolluted and Pb-polluted soils (Fig. 4A). Invertase activity in F2 was 62.44% lower than that in F1. Interestingly, no significant differences were observed in invertase activity among M1, M2, and M3.

Urease activity was 2.05-fold higher in F1 than in UF and 48.67% lower in F2 than in F1. Meanwhile, lower urease activity was detected in M2 and M3 compared to that in UM and M1, and no significant difference was found in this regard between UM and M1.

Phosphatase activity was 2.61-fold and 1.75-fold higher in F1 than in UF and F2, respectively, whereas no difference was observed between UF and F2. In the mining area, lower levels of phosphatase activity were recorded in M1 and M2 than in UM and M3, and no significant difference was found between UM and M3 or between M1 and M2.

Dehydrogenase activities in F1 and F2 were 82.49% and 94.00% lower than in UF, respectively, and 65.71% lower in F2 than in F1. In the mining area, dehydrogenase activity was

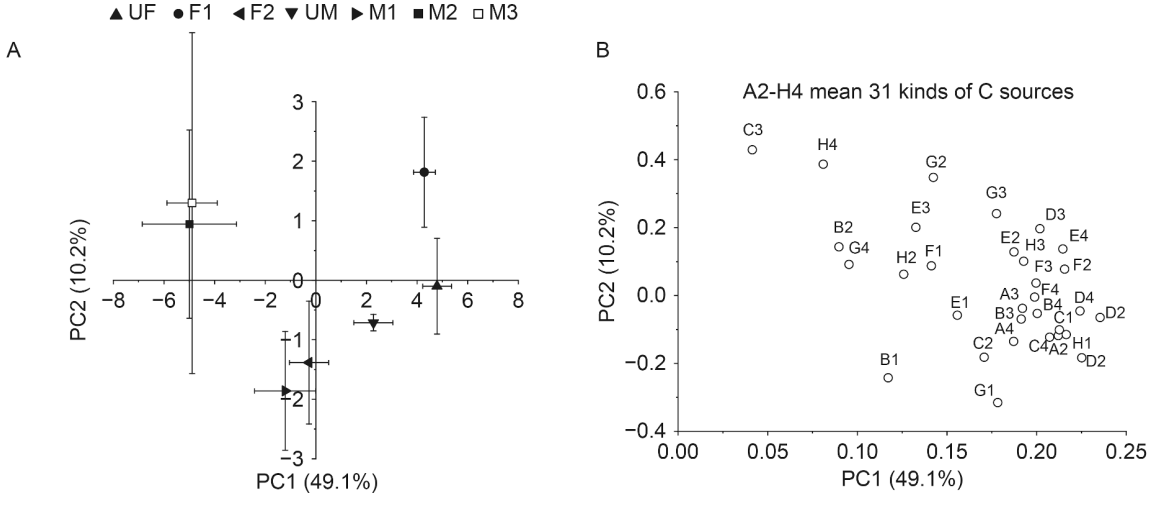

Fig. 3 Principal component analysis of soil microbial communities for carbon substrate utilization (A) and the eigen vector of each carbon substrate for PC1 and PC2 (B) in different sampling areas. Error bars represent standard deviations of the means ($n = 3$).

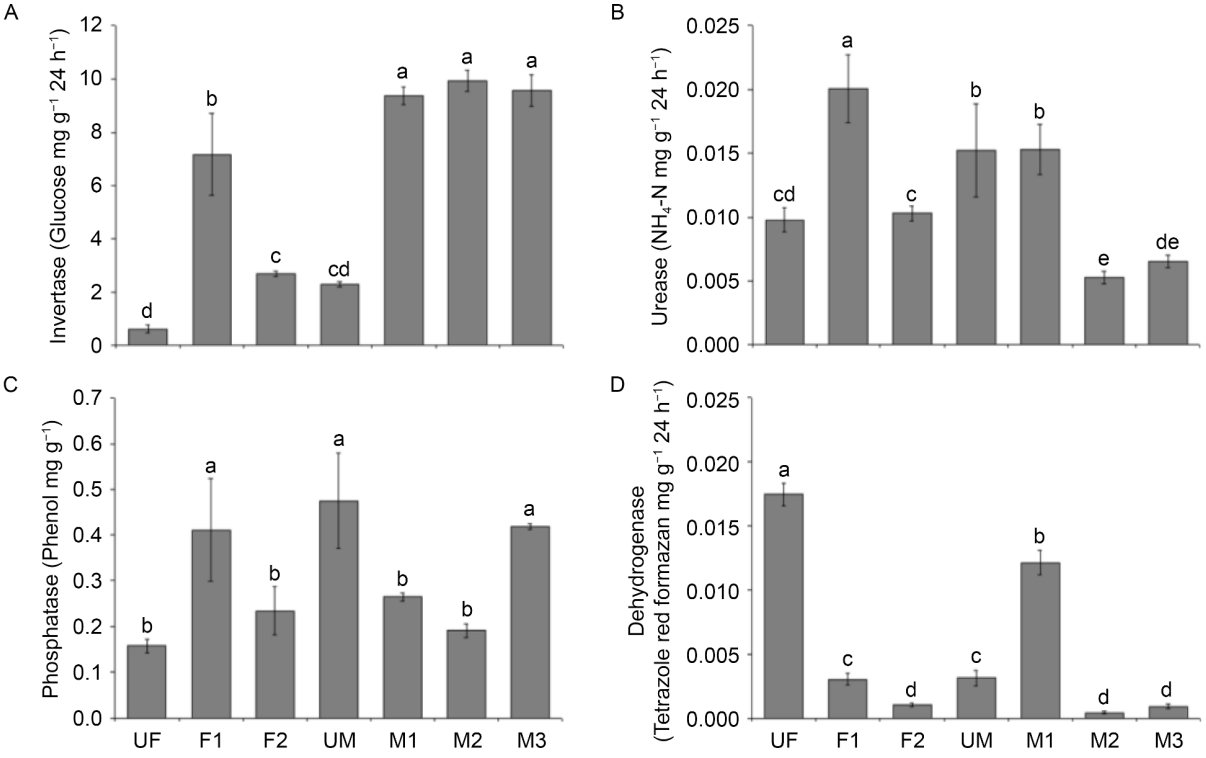

Fig. 4 Enzyme activity in soils from different Pb-polluted sites. Error bars represent standard deviations of the means (n = 3). Different letters on the error bars indicate significant differences among treatments at $p < 0.05$.

3.85-fold higher in M1 than in UM and 86.00% and 70.34% higher in UM than in M2 and M3, respectively (Fig. 4D). Similarly, dehydrogenase activity was 96.36% and 92.29% lower in M2 and M3 than in M1, respectively. In this regard, there was a positive relationship between dehydrogenase activity and Pb concentration in the mining area and between soil total N and available K (Fig. 5).

3.5 Soil microbial-biomass carbon (SMBC) and soil microbial-biomass nitrogen (SMBN)

In Pb-polluted soils, SMBC and SMBN were lower than in unpolluted soils (UF and UM) in both smelting and mining areas, and the difference ranged from 21.72% to 76.11% (Table 1). For the smelting area, SMBC and SMBN were 54.84% and 59.85% lower, respectively, in F2 than in F1, and no significant difference was found in SMBC/SMBN ratio between F1 and F2. For the Pb-polluted mining area, the highest SMBC was observed in M3, and the lowest SMBN in M1. The highest SMBC/SMBN ratio was detected in M2, whereas the lowest was detected in M1.

3.6 Effects of land use and lead pollution level on microbial biomass and enzyme responses

Significant effects of land use (smelting factory area as opposed to mining area) on the four microbial diversity indices were detected (Table 2). However, only Shannon richness and McIntosh indices were significantly affected by Pb pollution level. The interaction between land use and Pb pollution level had no significant effect on soil microbial diversity.

Urease activity was significantly affected by land use and Pb pollution level; furthermore, phosphatase activity was significantly affected by Pb pollution level. Interestingly, all four soil enzyme activities were significantly affected by the interaction between land use and Pb pollution level. Lastly, SMBC and SMBN were significantly affected only by Pb pollution level.

4 Discussion

In our study, AWCD in Biolog EcoPlates was correlated with Pb concentration, thereby indicating that the richness and diversity of microorganisms decreased as Pb concentration increased owing to the sensitivity of the different microorganisms to Pb toxicity. Unexpectedly, microbial communities from the Pb-polluted smelter area showed higher C utilization rates than those from the Pb-polluted mining area. The former contained the highest concentrations of heavy metals. Based on these results, we hypothesized that the Pb-polluted smelter area contained metal-resistant microbial communities that could use various C sources, such as sugar, alcohols, esters, and amino acids.

Our study indicated that Pb pollution reduced the functional diversity of microbial communities and the number of microorganisms that could utilize C-source substrates and

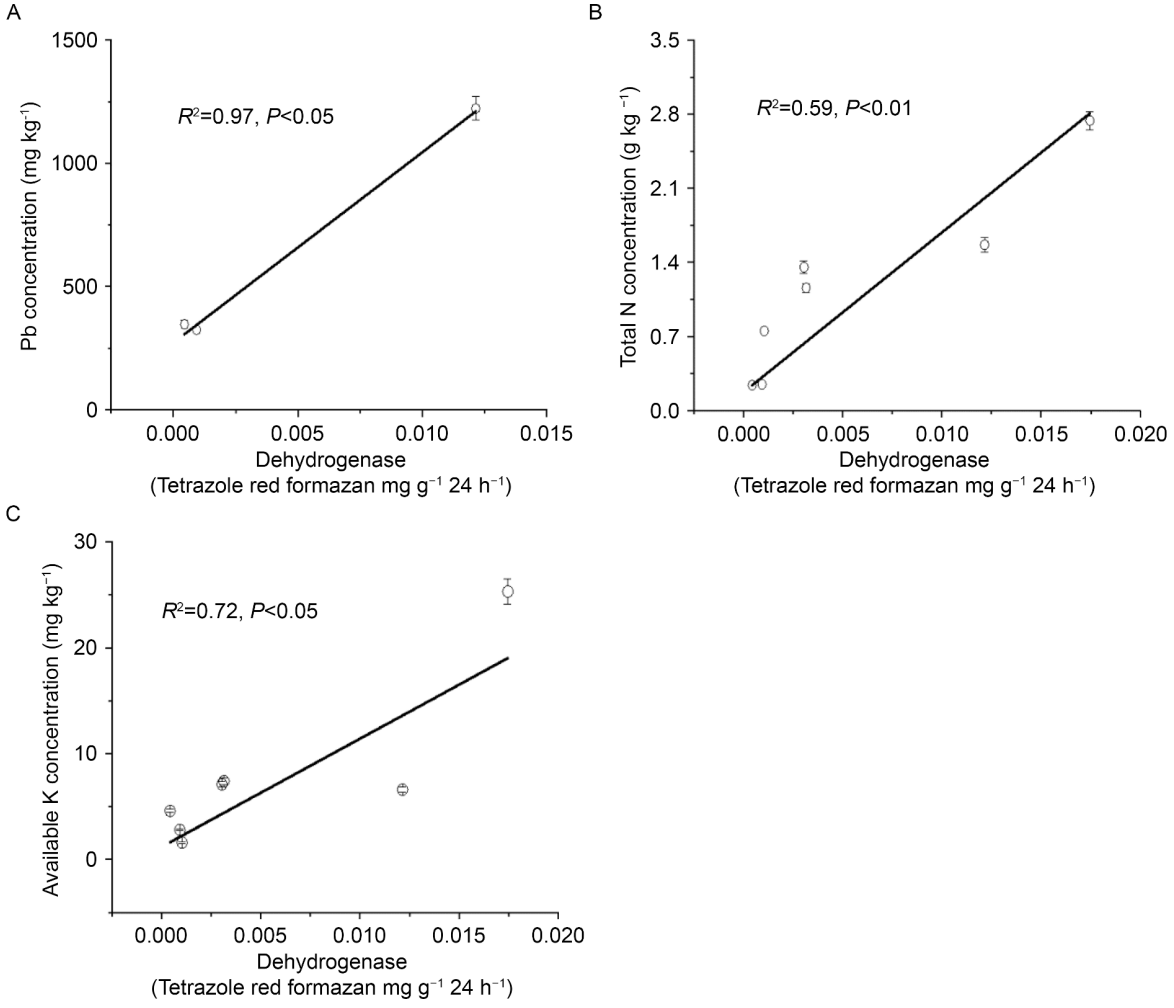

Fig. 5 Relation of dehydrogenase activity with Pb (A), soil total N concentration (B), and soil available K concentration (C). Pb concentrations were attributed to polluted mining areas. Error bars are standard deviations of the means (n = 3).

Table 2 Statistical analysis of various sources and their interactive effects on soil microbial diversity, biomass, and enzymes (p values).

Source of variation*	Shannon richness (H)	Substrate eventness (I)	Simpson (Ds)	McIntosh (U)	Invertase	Urease	Phosphatase	Dehydrogenase	SMBC	SMBN
Land use	<0.01	<0.05	<0.01	<0.001	<0.001	0.189	0.146	0.107	0.320	0.318
Pb pollution level	<0.05	0.109	0.055	<0.01	<0.001	0.754	0.703	<0.01	<0.001	<0.001
Land use × Pb pollution level	0.114	0.179	0.114	0.087	<0.05	<0.05	<0.01	<0.001	0.213	0.802

* Land use refers to Pb smelters and Pb mines. Pb pollution level refers to unpolluted and Pb-contaminated soils.

the ability of microorganisms to utilize single C-source substrates. Similar to the results obtained by Kelly and Tate (1998), the functional diversity of microbial communities was reduced by Pb. In this study, we detected differences in the utilization intensities of different C sources across the sample sites, indicating that metabolism of the soil microbial communities was influenced by Pb pollution. C use by soil microbes reflects differences in the choice of C source by soil microorganisms in a polluted environment (Siczek et al., 2020). The function of soil microbial community diversity changed when the soil ecosystem lost the required microbial community functional normalization characteristics.

Many studies have demonstrated the decrease of microbial diversity with increasing heavy-metal toxicity (Yang et al., 2015; Montiel-Rozas et al., 2016). Unpolluted soils with low heavy-metal concentrations have higher microbial diversity than polluted soils (Alguacil et al., 2011; Ahmad et al., 2018). Tu et al. (2020) also reported that heavy-metal pollution might account for the decreased microbial diversity observed in severely polluted areas. Studies have also indicated that microbial diversity is positively influenced by soil nutrients, such as P and N (Ohtomo and Saito 2005; Zarei et al., 2010). Therefore, the negative effects of Pb on soil microbial diversity may result from decreased nutrient

content. SMBC and SMBN are important microbiological indicators reflecting soil quality and degradation. Corresponding changes in these indicators can directly reflect changes in the soil environment, thus they are recognized as sensitive indicators of soil contamination due to exogenous heavy metals.

Therefore, the activities of invertases, ureases, phosphatases, and dehydrogenases can reflect the soil microbial activity under different pollution levels. Low concentrations of heavy metals stimulate soil enzymatic activities, whereas high concentrations reduce them. The inflection points of enzymatic activities vary with the type of soil enzyme. A previous study indicated that an increase in soil heavy-metal content resulted in a decrease in urease activity, as heavy metals inhibit soil microbial activity (Huang et al., 2017). Interestingly, we observed a positive correlation between Pb content and dehydrogenase enzyme activity. However, invertase activity increased with soil Pb accumulation.

N, P, and K are the main elements required for microbial metabolism (Zhang et al., 2020). In this study, dehydrogenase activity was positively correlated with soil N and K contents. Further, a previous study showed that soil nutrients interact with soil enzymes; for example, increased N availability correlates with increasing activity of C metabolism enzymes in the field (Bowles et al., 2014). Similarly, Xie et al. (2016) suggested that there may be a positive correlation between soil nutrients and soil enzyme activity in coastal saline soils as well.

Based on our results, we concluded that the soil enzyme activity in Pb-polluted environments increased, whereas the microbial biomass content decreased significantly. The results of the Biolog EcoPlates showed that changes in microbial community structure in Pb-polluted soils reduced the amount of microbial C substrate utilized and the microbial utilization of a single C substrate. Moreover, the driving factors of microbial diversity and biomass were different; the former was primarily affected by land use, whereas the latter was affected by Pb pollution level. Additionally, soil enzyme activity was primarily driven by the interaction between land use and Pb pollution level.

5 Conclusions

Pb pollution significantly affected the diversity of the soil microbial community structure and soil microbial metabolism. Moreover, Pb pollution reduced the content of microbial biomass-C and N in the soil, whereas it increased invertase activity and reduced catalase activity. The main types of C sources used by soil microorganisms changed as the ability for the utilization of all C sources by microorganisms changed under Pb stress. The overall activity and diversity indices of soil microorganisms decreased in Pb-polluted soils. Microbial diversity was primarily affected by land use, whereas microbial biomass-C and N contents were primarily affected by soil properties; in turn, soil enzyme activity was primarily affected by the interaction between land use and Pb pollution level. Although we only evaluated two types of human activity, namely, Pb mining and smelting, the production, use, and disposal of a large number of Pb-containing industrial products can also cause severe soil Pb pollution. Therefore, the impact of different Pb sources on the environment warrants further research.

Authors contributions

Y.H. Lou and Y.P. Zhuge conceived the idea and designed the study. X Sun and MJ Sun collected the data. X. Sun, M.J. Sun, Y. Chao, and X.Y. Shang did the analysis and drafted the manuscript. H. Wang, H. Pan and Q.G. Yang edited the earlier versions of the manuscript. Y.H. Lou and Y.P. Zhuge edited the final versions of manuscript. All the authors contributed to the final draft of the manuscript.

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Competing interests

The authors declare no competing interests.

Electronic supplementary material

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References

Ahmad, M., Usman, A.R.A., Al-Faraj, A.S., Ahmad, M., Sallam, A., Al-Wabel, M.I., 2018. Phosphorus-loaded biochar changes soil heavy metals availability and uptake potential of maize (*Zea mays* L) plants. Chemosphere 194, 327–339.

Alguacil, M.M., Torrecillas, E., Caravaca, F., Fernández, D.A., Azcón, R., Roldán, A., 2011. The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. Soil Biology & Biochemistry 43, 1498–1508.

Aponte, H., Mondaca, P., Santander, C., Meier, S., Paolini, J., Butler, B., Rojas, C., Diez, M.C., Cornejo, P., 2021. Enzyme activities and microbial functional diversity in metal(loid) contaminated soils near to a copper smelter. Science of the Total Environment 779, 146423.

Bardgett, R.D., Saggar, S., 1994. Effects of heavy metal contamination on the short-term decomposition of labelled [^{14}C] glucose in a pasture soil. Soil Biology & Biochemistry 26, 727–733.

Bidar, G., Pelfrêne, A., Schwartz, C., Waterlot, C., Sahmer, K.,

Marot, F., Douay, F., 2020. Urban kitchen gardens: Effect of the soil contamination and parameters on the trace element accumulation in vegetables–A review. Science of the Total Environment 738, 139569.

Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology & Biochemistry 68, 252–262.

Cheng, H., Hu, Y., 2010. Lead (Pb) isotopic fingerprinting and its applications in lead pollution studies in China: a review. Environmental Pollution 158, 1134–1146.

de Oliveira, F.G., Lirola, J.R., Salgado, L.D., de Marchi, G.H., Mela, M., Padial, A.A., Guimarães, A.T.B., Cestari, M.M., Silva de Assis, H.C., 2019. Toxicological effects of anthropogenic activities in *Geophagus brasiliensis* from a coastal river of southern Brazil: A biomarker approach. Science of the Total Environment 667, 371–383.

Du, Z., Xie, Y., Hu, L., Hu, L., Xu, S., Li, D., Wang, G., Fu, J., 2014. Effects of fertilization and clipping on carbon, nitrogen storage, and soil microbial activity in a natural grassland in southern China. PLoS One 9, e99385.

Garland, J.L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiology Ecology 24, 289–300.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57, 2351–2359.

Guan, S., Zhang, D., Zhang, Z., 1986. Soil Enzyme and its Research Methods. Beijing: Agriculture Press , 274–297.

Han, J., Liu, W., Qin, W., Zhang, T., Chang, Z., Xue, K., 2017. Effects of sodium salts on the sulfidation of lead smelting slag. Minerals Engineering 108, 1–11.

Huang, D., Liu, L., Zeng, G., Xu, P., Huang, C., Deng, L., Wang, R., Wan, J., 2017. The effects of rice straw biochar on indigenous microbial community and enzymes activity in heavy metal-contaminated sediment. Chemosphere 174, 545–553.

Huang, D.L., Zeng, G.M., Jiang, X.Y., Feng, C.L., Yu, H.Y., Huang, G.H., Liu, H.L., 2006. Bioremediation of Pb-contaminated soil by incubating with *Phanerochaete chrysosporium* and straw. Journal of Hazardous Materials 134, 268–276.

Ji, X., Abakumov, E., Chigray, S., Saparova, S., Polyakov, V., Wang, W., Wu, D., Li, C., Huang, Y., Xie, X., 2021. Response of carbon and microbial properties to risk elements pollution in arctic soils. Journal of Hazardous Materials 408, 124430.

Kandeler, F., Kampichler, C., Horak, O., 1996. Influence of heavy metals on the functional diversity of soil microbial communities. Biology and fertility of soils 23, 299–306.

Kelepertzis, E., Chrastný, V., Botsou, F., Sigala, E., Kypritidou, Z., Komárek, M., Skordas, K., Argyraki, A., 2021. Tracing the sources of bioaccessible metal(loid)s in urban environments: A multidisciplinary approach. Science of the Total Environment 771, 144827.

Kelly, J.J., Tate, R.L. III, 1998. Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter. Journal of Environmental Quality 27, 609–617.

Khalili, B., Ogunseitan, O.A., Goulden, M.L., Allison, S.D., 2016. Interactive effects of precipitation manipulation and nitrogen addition on soil properties in California grassland and shrubland. Applied Soil Ecology 107, 144–153.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments–a review. Waste Management (New York, N.Y.) 28, 215–225.

Lewis, D.E., White, J.R., Wafula, D., Athar, R., Dickerson, T., Williams, H.N., Chauhan, A., 2010. Soil functional diversity analysis of a bauxite-mined restoration chronosequence. Microbial Ecology 59, 710–723.

Li, C., Quan, Q., Gan, Y., Dong, J., Fang, J., Wang, L., Liu, J., 2020. Effects of heavy metals on microbial communities in sediments and establishment of bioindicators based on microbial taxa and function for environmental monitoring and management. Science of the Total Environment 749, 141555.

Li, M., Zhang, J., Yang, X., Zhou, Y., Zhang, L., Yang, Y., Luo, L., Yan, Q., 2021. Responses of ammonia-oxidizing microorganisms to biochar and compost amendments of heavy metals-polluted soil. Journal of Environmental Sciences (China) 102, 263–272.

Lima, L.B.D., Morais, P.B., Andrade, R.L.T., Mattos, L.V., Moron, S.E., 2018. Use of biomarkers to evaluate the ecological risk of xenobiotics associated with agriculture. Environmental Pollution 237, 611–624.

Liu, S., Maimaitiaili, B., Joergensen, R.G., Feng, G., 2016. Response of soil microorganisms after converting a saline desert to arable land in central Asia. Applied Soil Ecology 98, 1–7.

Liu, Y., Tie, B., Li, Y., Lei, M., Wei, X., Liu, X., Du, H., 2018. Inoculation of soil with cadmium-resistant bacterium *Delftia* sp. B9 reduces cadmium accumulation in rice (Oryza sativa L.) grains. Ecotoxicology and Environmental Safety 163, 223–229.

Lu, R.K., 1999. Analytical methods of soil agrochemistry. Chinese Agriculture Science and Technology Press, Beijing.

Ma, T., Pan, X., Liu, W., Christie, P., Luo, Y., Wu, L., 2016. Effects of different concentrations and application frequencies of oxytetracycline on soil enzyme activities and microbial community diversity. European Journal of Soil Biology 76, 53–60.

Malik, A., 2004. Metal bioremediation through growing cells. Environment International 30, 261–278.

Martley, E., Gulson, B.L., Pfeifer, H.R., 2004. Metal concentrations in soils around the copper smelter and surrounding industrial complex of Port Kembla, NSW, Australia. Science of the Total Environment 325, 113–127.

Montiel-Rozas, M. del M., López-García, Á., Kjøller, R., Madejón, E., Rosendahl, S., 2016. Organic amendments increase phylogenetic diversity of arbuscular mycorrhizal fungi in acid soil contaminated by trace elements. Mycorrhiza 26, 575–585.

Ohtomo, R., Saito, M., 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. New Phytologist 167, 571–578.

Siczek, A., Frąc, M., Gryta, A., Kalembasa, S., Kalembasa, D., 2020. Variation in soil microbial population and enzyme activities under faba bean as affected by pentachlorophenol. Applied Soil Ecology 150, 103466.

Tan, X., Nie, Y., Ma, X., Guo, Z., Liu, Y., Tian, H., Megharaj, M., Shen, W., He, W., 2021. Soil chemical properties rather than the abundance of active and potentially active microorganisms control soil enzyme kinetics. Science of the Total Environment 770, 144500.

Tang, J., Zhang, J., Ren, L., Zhou, Y., Gao, J., Luo, L., Yang, Y., Peng, Q., Huang, H., Chen, A., 2019. Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution. Journal of Environmental Management 242, 121–130.

Tu, C., Wei, J., Guan, F., Liu, Y., Sun, Y., Luo, Y., 2020. Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environment International 137, 105576.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.

Wang, G., Zhang, Q., Du, W., Ai, F., Yin, Y., Ji, R., Guo, H., 2021a. Microbial communities in the rhizosphere of different willow genotypes affect phytoremediation potential in Cd contaminated soil. Science of the Total Environment 769, 145224.

Wang, Q., Xiao, F., He, T., Wang, S., 2013. Responses of labile soil organic carbon and enzyme activity in mineral soils to forest conversion in the subtropics. Annals of Forest Science 70, 579–587.

Wang, S., Miltner, A., Muskus, A.M., Nowak, K.M., 2021b. Microbial activity and metamitron degrading microbial communities differ between soil and water-sediment systems. Journal of Hazardous Materials 408, 124293.

Wu, W., Wu, J., Liu, X., Chen, X., Wu, Y., Yu, S., 2017. Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure. Ecotoxicology and Environmental Safety 143, 322–329.

Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., Fu, J., 2016. Effect of heavy metals pollution on soil microbial diversity and Bermudagrass genetic variation. Frontiers in Plant Science 7, 755.

Xu, Y., Ge, Y., Song, J., Rensing, C., 2020. Assembly of root-associated microbial community of typical rice cultivars in different soil types. Biology and Fertility of Soils 56, 249–260.

Yang, D., Deng, W., Tan, A., Chu, Z., Wei, W., Zheng, R., Shangguan, Y., Sasaki, A., Endo, M., Chen, H., 2021. Protonation stabilized high As/F mobility red mud for Pb/As polluted soil remediation. Journal of Hazardous Materials 404, 124143.

Yang, J., Huang, J.H., Lazzaro, A., Tang, Y., Zeyer, J., 2014. Response of soil enzyme activity and microbial community in vanadium-loaded soil. Water, Air, and Soil Pollution 225, 1–10.

Yang, Y., Song, Y., Scheller, H.V., Ghosh, A., Ban, Y., Chen, H., Tang, M., 2015. Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. Soil Biology & Biochemistry 86, 146–158.

Zarei, M., Hempel, S., Wubet, T., Schäfer, T., Savaghebi, G., Jouzani, G.S., Nekouei, M.K., Buscot, F., 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environmental Pollution 158, 2757–2765.

Zhang, X., Lou, X., Zhang, H., Ren, W., Tang, M., 2020. Effects of sodium sulfide application on the growth of *Robinia pseudoacacia*, heavy metal immobilization, and soil microbial activity in Pb-Zn polluted soil. Ecotoxicology and Environmental Safety 197, 110563.

Zhang, X., Yang, L., Li, Y., Li, H., Wang, W., Ye, B., 2012. Impacts of lead/zinc mining and smelting on the environment and human health in China. Environmental Monitoring and Assessment 184, 2261–2273.

Zhang, Y., Hou, D., O'Connor, D., Shen, Z., Shi, P., Ok, Y.S., Tsang, D.C.W., Wen, Y., Luo, M., 2019. Lead contamination in Chinese surface soils: Source identification, spatial-temporal distribution and associated health risks. Critical Reviews in Environmental Science and Technology 49, 1386–1423.

Zhao, H., Xia, B., Fan, C., Zhao, P., Shen, S., 2012. Human health risk from soil heavy metal contamination under different land uses near Dabaoshan Mine, Southern China. Science of the Total Environment 417–418, 45–54.