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Alkaline amendments improve the health of soils degraded by metal contamination and acidification: Crop performance and soil bacterial community responses

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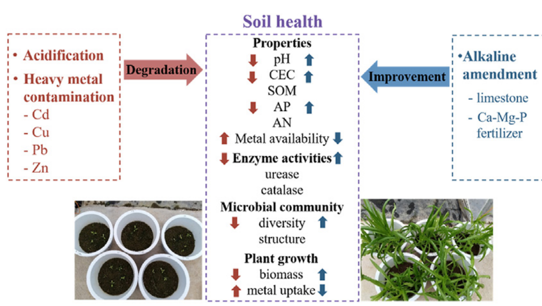
HIGHLIGHTS

- Soil recovery in the agroecological system was assessed in lettuce pot trials.
- Alkaline and organic amendments were investigated for their impacts on recovery.
- Bacterial diversity and community structures improved with alkaline amendments.
- Alkaline amendments performed better than the organic for soil health recovery.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil degradation due to heavy metal contamination and acidification has negative effects on soil health and crop growth. Many previous studies have tried to improve the growth of crops and decrease their metal uptake. The recovery of soil health, however, has rarely been focused in soil remediation. In this study, a pot trial was conducted with lettuce (*Lactuca sativa* L.) growing in heavy metal contaminated and acidic soils, to examine the effects of alkaline amendments (limestone, LS; calcium magnesium phosphate fertilizer, Pcm) and organic amendments (cow manure compost, CMC; biochar, BC) on the growth of lettuce and on the availability of heavy metals, enzyme activities, and bacterial community structures in the soils. The results showed that, in comparison with the CMC and BC treatments, LS and Pcm were more effective at improving lettuce growth and reducing metal concentrations in shoots. Urease and catalase activities in LS and Pcm amended soils were consistently higher than in those with CMC and BC. Additionally, the alkaline amendments dramatically improved the bacterial diversity and shaped more favorable bacterial community structures. Proteobacteria and Gemmatimonadetes were predominant in soils amended with alkaline treatments. The beneficial bacterial genera *Gemmatimonas* and *f_Gemmatimonadaceae*, which are vital for phosphate dissolution, microbial nitrogen metabolism, and soil respiration, were also enriched. The results suggest that alkaline amendments were superior to organic

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amendments, and thus may be useful for the future recovery of soil functions and health under heavy metal contamination and low pH.

1. Introduction

Soil degradation is an important issue affecting the environment globally and is usually associated with unsustainable anthropogenic activities. Soil degradation including contamination and acidification can lead to reductions in crop yields, and has received increasing attentions in recent decades (Kopittke et al., 2019). Soil contamination by multi-metals is ubiquitous in many polluted areas, due to practices such as mining, industrial operations, and the applications of agrochemicals. Electronic waste (e-waste) recycling activities were also identified as an important source of heavy metals in soils, where the concentrations of Cd, Cu, Pb, and Zn were dramatically enriched in farmland soils near e-waste recycling sites (Wu et al., 2018). The enrichment of both essential (e.g., Cu and Zn) and non-essential metals (e.g., Cd and Pb) is not only toxic to living organisms in farmland soils, but also causes metal contamination in crops, which is a main exposure pathway for human (León-Cañedo et al., 2019).

Another key cause of soil degradation is acidification. Soil acidification has been accelerating mainly due to over-usage of nitrogen fertilizers and waste-water irrigation. It is estimated that ~30% of the total ice-free land area of the earth has been affected by soil acidification, creating a global problem for agricultural sustainability (Sumner and Noble, 2003). Especially in China, it is common and severe, as there has been rapid urbanization and industrial development. For example, the surface farmland soils in southwest China were significantly acidified from 1981 to 2012, with a decline of 0.30 pH units (Li et al., 2019). More serious soil acidifications were reported in the Yangtze River Delta of China, where the mean decrements of the pH were 0.56 and 0.52 units, over the periods of 1980–2000 and 2000–2015, respectively (Xie et al., 2019).

Soil contamination and acidification usually occurred in hotspot areas, which can compound the issues. For example, it was reported that the topsoil pH of farmlands in China declined by 0.13–0.80 units between the 1980s and 2000s (Guo et al., 2010), and more than 30% of the farmland soils were polluted by heavy metals in South China (Zhang et al., 2015). It is known that soil acidification can strongly increase the bioavailability of toxic metals (e.g., Cd and Pb), and subsequently increase the exposure risks to human health (Novak et al., 2018; Zhu et al., 2016). Therefore, efficient and eco-friendly remediation methods are urgently required to mitigate these environmental challenges.

Two strategies (i.e., extraction and immobilization) are commonly employed to remediate metal contaminated soils. Some extraction technologies (e.g., excavation and washing) are effective but expensive and destructive to soil ecosystems (Bolan et al., 2014). Comparatively, *in-situ* immobilization via applying chemical amendments has been proposed as a promising approach. There are various materials available to achieve the *in-situ* immobilization of metals, including organic amendments (e.g., compost, biochar), alkaline amendments (e.g., limestone, phosphates), etc (He et al., 2019a; Liu et al., 2018; Wang et al., 2017). Most amendments can immobilize heavy metals and reduce bioavailability, but remediation effectiveness might be largely different amongst different types of amendments, due to their distinct properties. For instance, limestone immobilizes heavy metals mainly by high pH induced metal precipitation (Novak et al., 2018), while composts reduce metal availability by ligand complexation (Palansooriya et al., 2020). The application of these amendments to immobilize metals has been widely employed to remediate contaminated soils (Hou et al., 2020). During the last 20 years, there is a great scientific interest on the application of organic and inorganic amendments on metal mobility in contaminated soils with more than 3000 related articles published (Palansooriya et al., 2020).

The bioavailability of heavy metals and their effects on plant growth have frequently been used as indicators of immobilization effectiveness (Chen et al., 2019; Sevilla-perea et al., 2016). As soil enzymes and microorganisms play indispensable roles in nutrient cycling in soils (Giller et al., 2009; Luo et al., 2016), soil enzymes and bacterial community structures have recently been proposed as sensitive, early, and effective indicators of soil health during remediation (Liang et al., 2020; Mehmood et al., 2018; Novak et al., 2018). Nevertheless, the relationships between amendments and these biological properties are not well understood.

In the present study, two alkaline inorganic amendments (limestone, LS; calcium magnesium phosphate fertilizer, Pcm) and two neutral organic amendments (cow manure compost, CMC; biochar, BC), were studied for their potential contrasting remediation effectiveness in metal contaminated and acidic soils. We hypothesized that: (1) the alkaline and organic amendments have different remediation efficiencies due to their distinct chemical properties; (2) compared to the organic amendments, the alkaline amendments would more effectively reduce the heavy metal availabilities and consequently decrease the uptake by crops; and (3) alkaline amendment would increase the soil pH and thus better improve the bacterial community structures and soil enzyme activities.

2. Materials and methods

2.1. Soil and amendments

Topsoils (0–20 cm) were collected from a paddy field near an e-waste recycling site located in Qingyuan, a notorious recycling region in Southern China (Wu et al., 2018). The paddy field was irrigated with waste-water from the e-waste recycling sites since the 1990s, and the soil pH (4.8) was significantly lower than the average background value of 5.2 (CNEMC (China National Environmental Monitoring Centre), 1990). Prior to the trial, the soils were air-dried, screened using a 10-mesh stainless steel sieve for chemical analyses, and then screened using a 100-mesh stainless steel sieve for heavy metal determination.

Two alkaline inorganic amendments (limestone, LS; calcium magnesium phosphate fertilizer, Pcm), and two neutral organic amendments (cow manure compost, CMC; applewood biochar, BC) were tested. For comparison, a treatment without amendment (CK) was used as the control. The chemical properties of the tested soils and amendments are shown in Table S1.

2.2. Pot trial

The soil was ground to pass through a 4-mm mesh and was then mixed with the amendments (0.8% w/w). All of the treatments and the control were quintuplicated. Eight kilograms of the soils were placed into plastic pots (diameter 18 cm × height 30 cm), and were then equilibrated for six weeks prior to the experiment. Lettuce (*Lactuca sativa* L.) was selected as the test crop in this study, as it is widely consumed around the world and has a strong ability to absorb and accumulate heavy metals. Also, lettuce has been used as a bioindicator to evaluate soil contaminations (Wolf et al., 2017). Ten lettuce seeds were planted in each pot and the seedlings were thinned to five plants per pot after germination. During cultivation, the water-holding capacity of the soil was maintained at 60%, with the use of tap water in which Cd, Cu, Pb, and Zn were negligible.

The lettuces were harvested after 50-d cultivation, and were washed successively with tap water, and deionized water. In addition, the roots were washed with 0.1 M EDTA and deionized water again to remove the metals attached to the root surface. Afterwards, the shoots and roots were separated and dried at 60 °C to a constant weight and then weighed. The dried plants were then milled to pass through a 100-mesh sieve for the heavy metal analyses. The soils in the rhizosphere were collected, and one part was stored at −80 °C for DNA extraction. Another part of the soil samples was air-dried, sieved through 10-, 20-, and 100-mesh, for the measurement of the chemical properties, heavy metal availabilities, and enzyme activities, respectively.

2.3. Analyses of the soil chemical properties

The soil pH was measured after equilibrium in a soil-water suspension (soil: distilled water = 1:2.5) using a pH meter (model 744, Metrohm, Herisau, Switzerland). The cation exchange capacity (CEC) was determined with the ammonium acetate method (Kahr and Madsen, 1995). Soil available phosphate (AP), ammonium nitrogen (AN) and soil organic matter (SOM) were measured as described previously (Olsen et al., 1954; Murphy and Riley, 1962; Schulte, 1995; Wu et al., 2017; Nie et al., 2018).

2.4. Analyses of heavy metals and soil enzyme activities

Atomic absorption spectrometry (AAS, PinAAcle 900 T, PerkinElmer, Waltham, MA, USA) was used to measure the Cu, Pb, and Zn contents in soil or plant samples after $HCl-HNO_3-HClO_4-HF$ digestion. Graphite furnace atomic absorption spectrometry (GF-AAS, PinAAcle 900 T, PerkinElmer) was used to measure the contents of Cd. To estimate the metal bioavailability, soils were extracted by 0.1 M $CaCl_2$ for 2 h and the metals in the solutions were quantified. For quality assurance and control, two reference soils (GBW-07430 and GBW-07443) and one reference plant tissue (GBW-10048) from the Chinese National Research Center for Certified Reference Materials were analyzed during the measurement. The recoveries of the metals ranged from 93.8 to 105.0% (RSD = 2.5−5.4%, n = 4).

Soil catalase activity was determined by potassium permanganate titration following Sun et al. (2013). Urease activity was determined by colorimetric method with a spectrophotometer (LengGuang Tech 752sp, CHN) at 578 nm following Hu et al. (2014).

2.5. Bacterial community structure and diversity

Total bacterial DNA was extracted from 0.2 g sample using the Power Soil DNA Isolation Kit (MO BIO Laboratories), according to the manufacturer's protocol. DNA integrity and concentration were measured using agarose gel electrophoresis and Qubit fluorescence, respectively. To assess the bacterial community composition, V3−V4 variable region of 16 S rRNA gene was amplified with the common primer pair (5′-ACTCCTACGGGAGGCAGCA-3′ as the forward primer; and 5′-GGACTACHVGGGTWTCTAAT-3′ as the reverse primer) combined with the adapter sequences and barcode sequences. The PCR reaction system consisted of 10 μL buffer, 0.2 μL Q5 High-Fidelity DNA Polymerase, 10 μL High GC Enhancer, 1 μL dNTP, 10 μM of each primer, and 60 ng of genomic DNA. The amplified 16 S rRNA products were then purified using DNA Extraction Kit (Tiangen DNA extraction Kit, China). Further diversity analysis and significant difference analysis were performed after PCR amplification. The pipeline coupling of the Mothur (Kozich et al., 2013) and UPARSE (Edgar, 2013) softwares were used to processed the 16 S rRNA gene sequencing data with previously described method (Wu et al., 2017). The relative abundance of a taxon was the number of sequences affiliated with that specific taxon, divided by the total sequence number of the sample. Alpha diversity including observed OTU richness, Ace, Chao1, Simpson, and Shannon were calculated based on the 6400 sequences randomly selected from each sample.

2.6. Statistical analyses

All measurements were completed in at least triplicate and presented as the mean ± standard deviation (SD). One-way ANOVA and Student-Newman-Keuls tests were used for multiple comparisons to analyze the differences in chemical properties, heavy metal availabilities, and enzyme activities among the treatments. Redundancy analysis (RDA) was used to determine the major environmental variables related to the bacterial communities using CANOCO software (version 4.5). The significance of the variables was tested using the automatic selection of means with Monte Carlo permutations. All the statistical tests were performed using SPSS software (version 24.0).

3. Results

3.1. Soil chemical properties

The chemical properties of the soils with/without amendments are listed in Table 1. The alkaline amendments significantly increased the soil pH, as it was 2.95 and 2.25 units higher in the LS and Pcm treatments than that of the control ($p < 0.05$), respectively. In addition, the Pcm also greatly enhanced the soil CEC and AP ($p < 0.05$). While the application of the BC effectively improved the SOM and AN ($p < 0.05$). However, only weak increases of SOM and AN were observed in the CMC treatments.

Notably, the application of these amendments, especially the two alkaline amendments, could clearly reduce the bioavailabilities of heavy metals (Fig. 1). Specifically, the $CaCl_2$ extractable amounts of Cd, Cu, Pb, and Zn in LS treatment decreased by 89.3, 86.2, 75.8, and 99.9%, respectively; and those in Pcm treatment decreased by 82.1, 86.7, 86.7, and 99.2%, respectively, in comparison with CK. However, the application of the organic amendments (CMC and BC) was less effective in the reduction of the $CaCl_2$ extractable amounts of the metals.

3.2. Lettuce growth and metal uptake

The biomass and height of the lettuce are shown in Fig. 2a and (b). There was extremely poor lettuce growth in the soils without amendment (Fig. S1), yielding only 0.4 g of shoot biomass per pot after the 50-day growth (Fig. 2a). However, following the LS and Pcm applications, there was a significant increase of plant growth; shoot biomass reached 150−200 g per pot. Interestingly, shoot

Table 1
Effects of the amendments on the soil chemical properties.

Treatments	pH	CEC (cmol kg^{-1})	SOM (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})
CK	4.8 ± 0.01 e	8.3 ± 0.31 b	36.3 ± 0.49 b	3.1 ± 0.49 bc	21.6 ± 2.21 b
LS	7.7 ± 0.08 a	9.8 ± 0.07 a	34.6 ± 1.46 c	2.2 ± 0.54 c	23.0 ± 1.62 b
Pcm	7.1 ± 0.08 b	10.3 ± 0.57 a	35.6 ± 0.91 bc	2.2 ± 0.77 c	72.5 ± 10.80 a
CMC	5.4 ± 0.07 c	9.3 ± 0.54 a	36.9 ± 0.47 b	3.7 ± 0.76 b	21.7 ± 1.51 b
BC	5.0 ± 0.03 d	9.8 ± 0.67 a	38.3 ± 0.76 a	4.9 ± 0.29 a	21.2 ± 1.25 b

The data are mean ± SD, n = 5. Different letters in the same column represent significant differences at $p < 0.05$. CK, control; LS, limestone; Pcm, calcium magnesium phosphate fertilizer; CMC, cow manure compost; BC, biochar. CEC, cation exchange capacity; SOM, soil organic matter; AN, ammonium nitrogen; AP, available phosphate.

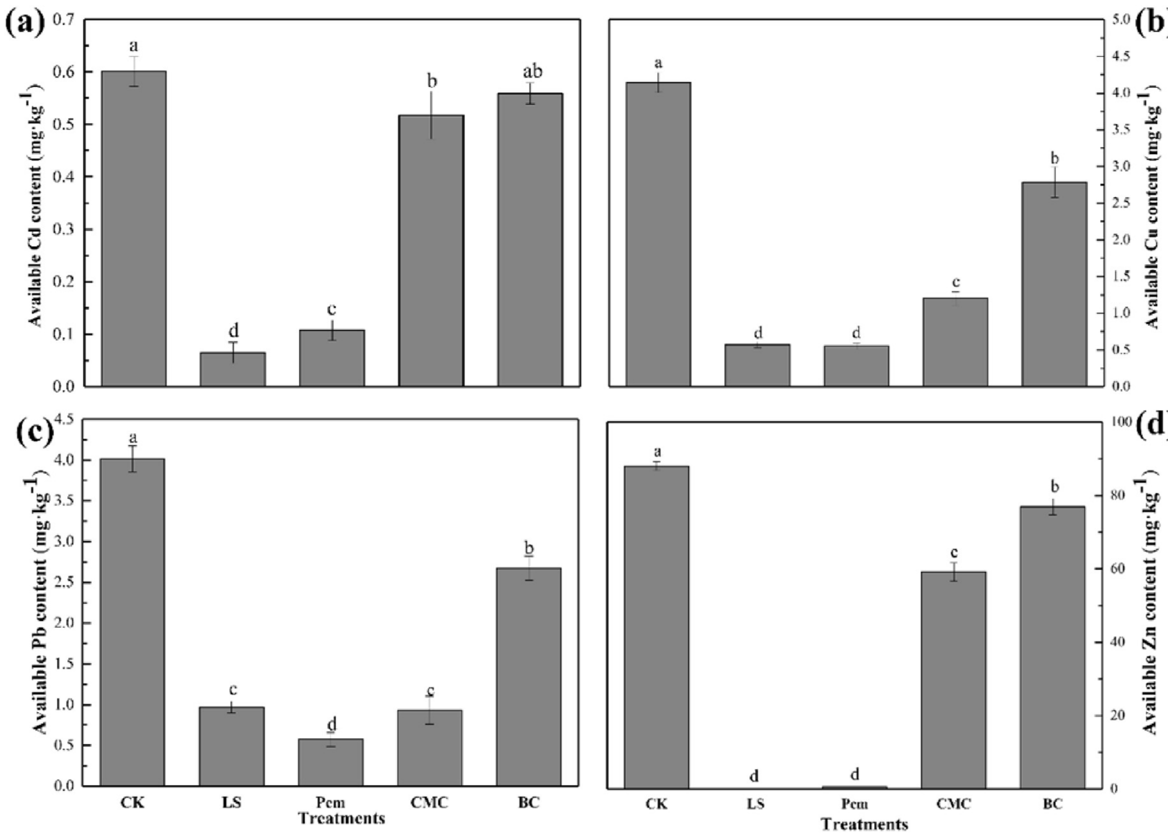

Fig. 1. Effects of the amendments on the available concentrations of heavy metals in the soils: (a) available Cd content; (b) available Cu content; (c) available Pb content; (d) available Zn content. CK, control; LS, limestone; Pcm, calcium magnesium phosphate fertilizer; CMC, cow manure compost; BC, biochar. Mean ± SD, n = 5. Different lowercase letters above the bars indicate significantly different results ($p < 0.05$).

biomass in Pcm treatment was higher than LS ($p < 0.05$). In contrast, shoot biomass in CMC and BC treatments was slightly higher than that of CK, and no significant difference was observed between the two organic amendments.

Consistently, the application of the alkaline amendments largely reduced concentrations of the metals in the lettuces (Fig. 2c ~ f). When compared with the CK, the Pcm and LS treatments were most effective in decreasing the metal concentrations in shoots (decreased 54.0–95.6% by Pcm, and 48.9–95.8% by LS), and there were no significant differences between the LS and Pcm treatments. On the other hand, the applications of CMC and BC were generally less effective in reducing the metals in both shoots and roots, although both treatments decreased the concentrations of heavy metals in lettuces. Interestingly, in the CMC treatment, the concentrations of Pb and Zn in the shoot decreased by 92.0% and 94.0%, respectively, compared to the CK.

3.3. Soil enzyme activity

The application of the amendments presented distinct effects on the activities of catalase and urease in the soil samples (Fig. 3a). In particular, the enhancement of urease and catalase activities with the LS treatments was the most significant, as they were 1.41 and 1.98 times higher than those in CK ($p < 0.05$), respectively. On the contrary, the application of CMC and BC also enhanced catalase activity, but only slightly enhanced urease activity.

3.4. Soil bacterial community structure and diversity

A total of 1609999 sequences were found across all the samples with the 16 S rRNA gene sequencing, ranging from 63144 to 71284 sequences per community. The most dominant phylum of bacteria found was Chloroflexi (33.4–37.6%), followed by Proteobacteria

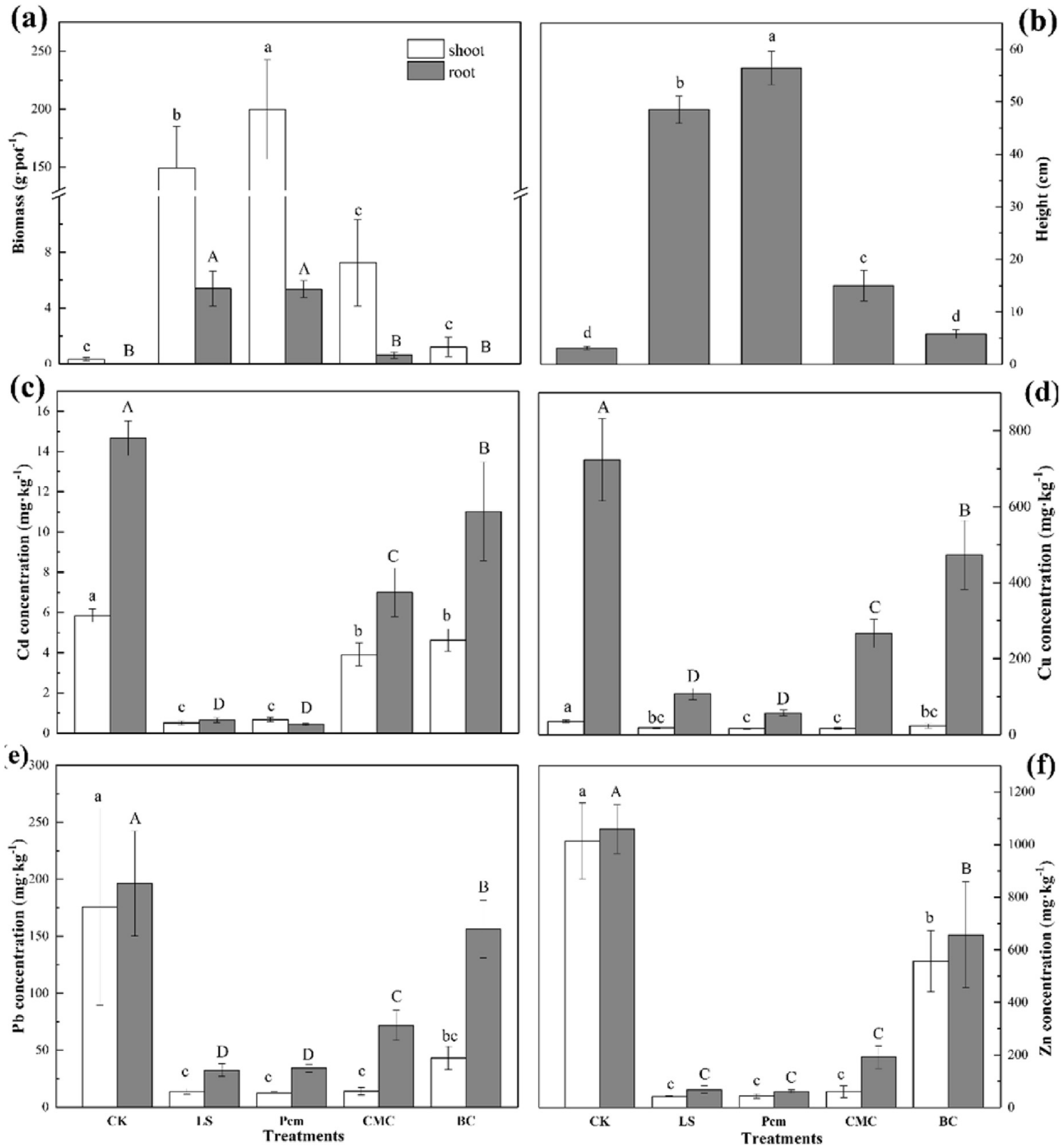

Fig. 2. Effects of the amendments on the lettuce growth: biomass (a) and height (b), and on the concentrations of heavy metals in the lettuce shoot and root (c ~ f). Mean ± SD, n = 5. Different letters above the bars indicate significantly different results ($p < 0.05$).

(22.6–24.2%), Actinobacteria (11.2–14.2%), and Acidobacteria (10.36–11.82%) (Fig. S2). It is notable that Proteobacteria and Gemmatimonadetes were the predominant phyla in the LS and Pcm treated soils, while Chloroflexi and Proteobacteria were the most dominant in the CMC, BC, and CK treatments.

At the genus level, significant shifts in the composition of community were observed after the applications of the amendments (Fig. 3b). In the LS treatments, the dominant genera were *f_Gemmatimonadaceae* and *f_Microscillaceae*, while the dominant genera in the Pcm treated soils were *f_Gemmatimonadaceae* and *Gemmatimonas*. On the contrary, the pattern of relative abundance for the dominant microbial genera in the CMC and BC amended soils and CK were similar, and *o_RBG-13-54-9*, *c_KD4-96*, and *f_JG*30-KF-*AS9* dominated in these communities.

Interestingly, the results show that the bacterial alpha diversity was significantly correlated with the amendments (Table S2). For instance, the soils with the alkaline amendments (Pcm and LS) had a higher number of OTUs and diversity (i.e. Chao1 and Ace). Nevertheless, only slight improvements of the alpha diversity were observed in the BC treated soils.

3.5. Relationships between the amendments, environmental factors, and primary genus

RDA was preformed to further quantify the relationships among the bacterial communities, the amendments and the soil chemical properties. The results are presented in Fig. 4. Environmental factors (i.e. pH and AP) on the first ordination axis explained 72.8% of the observed variations, and the second ordination axis explained 8.9%. The soils treated with different amendments were clearly

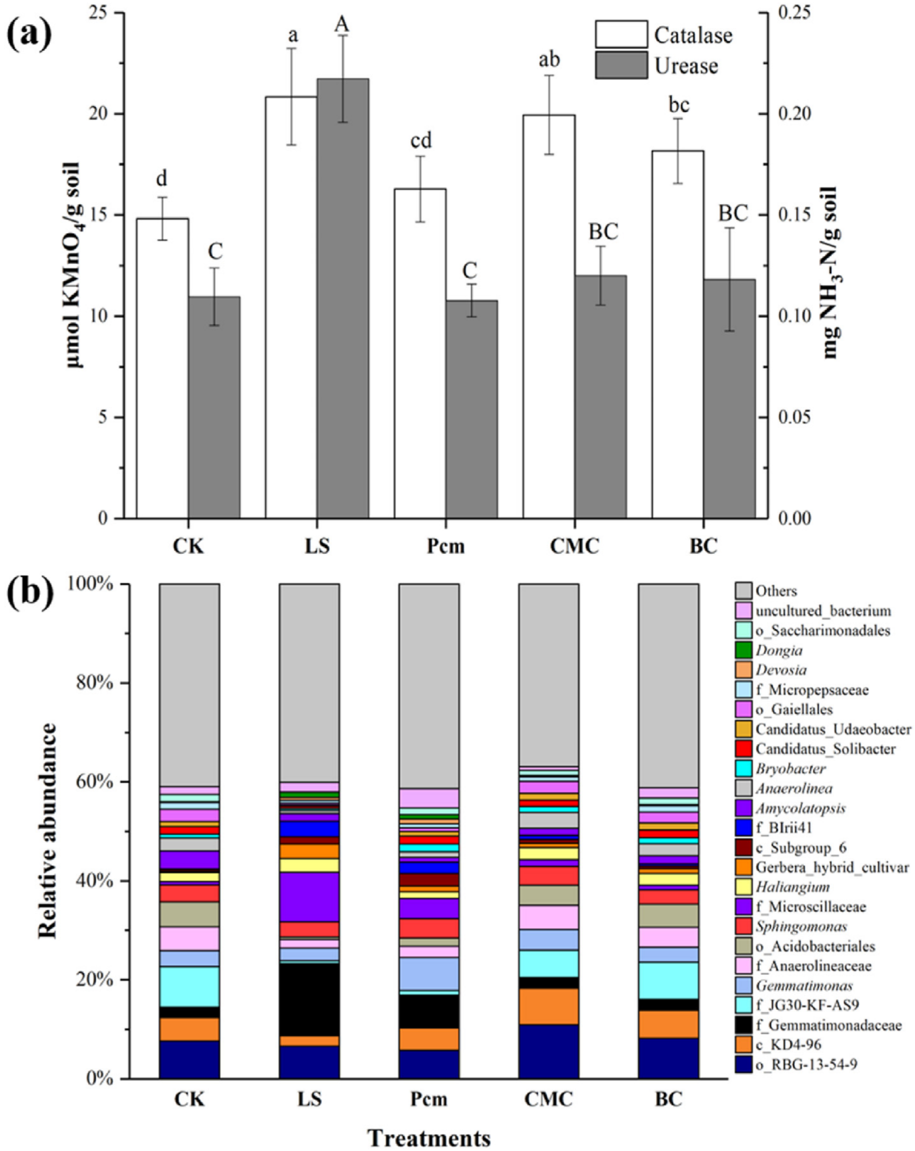

Fig. 3. (a) Effect of the amendments on the soil urease and catalase activities. Mean ± SD, n = 5. Different letters above the bars indicate significantly different results ($p < 0.05$). **(b)** Effects of the amendments on the relative abundance of the dominant (>0.5%) bacterial genera in the rhizosphere soils, as revealed by 16 S rRNA gene sequencing (p_, c_, o_, and f_ represent a certain genus under the phylum, class, order and family, respectively).

distributed in distinct parts of the ordinate axes. Notably, the LS and Pcm treatments were located at the right margin of the RD1 axis (quadrant I and II), which was associated with higher phosphorus content and pH, and lower availability of heavy metals. On the contrary, the CK, CMC, and BC treatments were mostly located at the left margin of the RD1 axis, due to the higher availability of the heavy metals and lower soil pH. The results of the Monte Carlo permutation test revealed that pH, available Cd, and available Zn (all $p < 0.05$) were the most predominant environmental variables affecting the soil bacterial community structures.

Most of the abundant bacterial genera, such as *Gemmatimonas*, *f_Gemmatimonadaceae*, *f_Microscillaceae*, *Devosia*, and *Dongia* were positively correlated with the available phosphorus content and soil pH, and negatively correlated with the heavy metal availability. In contrast, some genera such as *o_RBG-13-54-9*, *c_KD4-96*, *o_Acidobacterials*, and *Anaerolinea* presented negative correlations with the available phosphorus content and soil pH, but positive correlations with the available concentrations of the heavy metals and SOM.

4. Discussion

Paddy fields near e-waste recycling sites commonly suffer from heavy metal contaminations and acidification, because wastewater related with recycling activities is used for irrigation, which are regional problems (Wu et al., 2018). In this study, the growth of lettuce was considerably inhibited in these degraded soils, while the application of two types of amendments (alkaline amendments including LS and Pcm and organic amendment including CMC and BC) showed distinct effects on lettuce growth. Generally, the performance of the LS and Pcm on the lettuce growth was superior to that of the CMC and BC, which could be attributed to their effects on the recovery of the soil health, including improved chemical properties (i.e. higher pH value and available P content), reduced

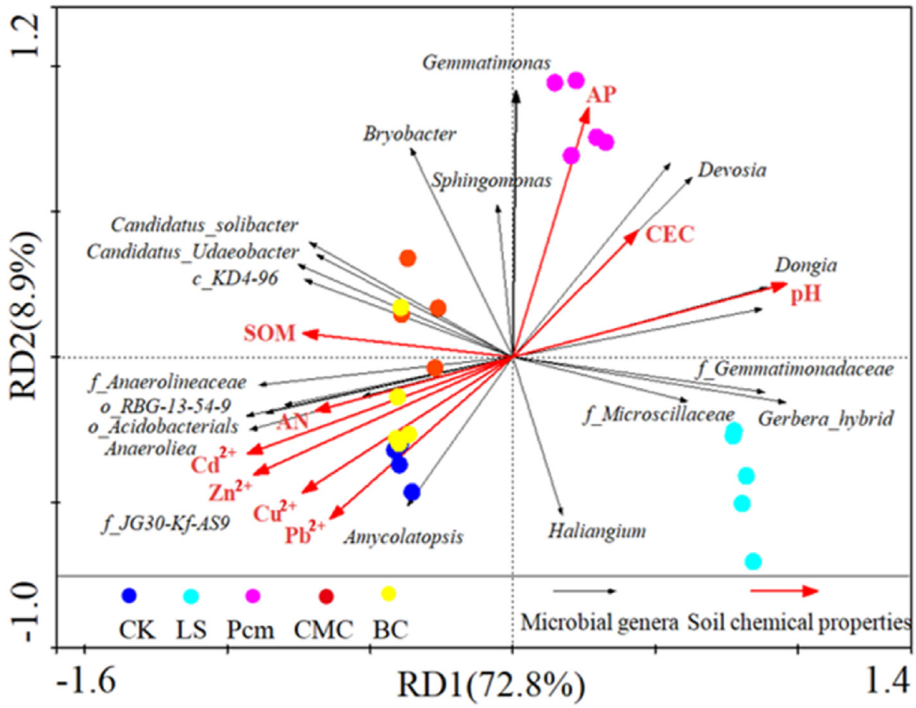

Fig. 4. Redundancy analysis of the chemical properties and available concentrations of the heavy metals on the bacterial community structures in the soils.

heavy metal availabilities, and favored bacterial community structures.

The present study showed that the soil chemical properties during remediation were closely related to the properties of the amendments. As the increase of soil pH in LS treatment, the Pcm treatment resulted in an increase in the soil pH and the AP contents, and AN and SOM were consistently increased in the BC amended soils. According to the correlation analysis, the lettuce biomass was positively correlated with pH, CEC, and AP, and negatively correlated with the availabilities of Cd, Cu, Pb, and Zn (all $p < 0.05$, Table S3). This result indicates that the growth of the lettuce was directly ameliorated by the increasing soil pH and reduced heavy metal availability in the amended soils. Similarly, a previous study found that the production of crops was positively correlated with the soil pH value (ranging from 4.4 to 8.0) (Holland et al., 2019). On the other hand, it is well known that heavy metal availability primarily depends on the soil chemical properties (Smith, 2009). Specifically, the pH value is the most important factor, with regard to the effects on the solution and the surface complexation reactions of the cations, ion-exchange, and other metal-binding processes (Kumpiene et al., 2008; Peng et al., 2009). When soil pH increases, the mobility and solubility of the heavy metal ions such as Cd^{2+} and Pb^{2+} tend to decrease. On the contrary, the concentrations of H^+ and Al^{3+} ions are higher in acidic soils, and thus competition happens between H^+/Al^{3+} ions and heavy metal cations, which decreased the adsorption of heavy metals by the soil matrix (Palansooriya et al., 2020). Therefore, one important strategy for remediating heavy metal contaminated soils is to regulate the soil pH (Bolan et al., 2014; Palansooriya et al., 2020). The shifted availability of the heavy metals observed in this study supports this mechanism, as the applications of LS and Pcm resulted in significant increases in soil pH, and hence decreased heavy metal availability. In addition, the average shoot biomass with the Pcm treatments was higher than that with the LS treatments. It is well known that P is an essential element during plant growth, and thus the exogenous input of P might promote plant growth and yields. However, the available P content in the CK (21.6 mg kg^{-1}) is much higher than the critical value for crops (8 mg kg^{-1}) (Wen et al., 2016), so the increased AP level in the Pcm treated soils might not be the main factor ameliorating the lettuce growth. Notably, the soil AP in this study was negatively correlated with the availability of the heavy metals (e.g., Cd, Cu, and Zn), suggesting that the higher biomass of the lettuce in the Pcm treated soils could be partly due to the precipitation of metal ions by the phosphates (Saavedra et al., 2018).

Based on the distinct influences on the heavy metal availability and observed plant growth in this study, it is suggested that the remediation effectiveness of the alkaline amendments in contaminated and acidic soils is stronger than that of the organic amendments. However, compared to the CK, the application of the CMC and BC could also significantly reduce the heavy metal availability. The organic amendments (i.e., CMC and BC) tested in the present study have been proposed as immobilizing agents. Cow manure compost, which contains high levels of humic substances, can form stable organometallic complexes with metal ions, thus reducing the metal mobility in soils (Shaheen et al., 2017; Clemente et al., 2018). For example, Houben et al. (2012) reported that the applications of cow manures in contaminated soils significantly reduced Cd and Zn availabilities by 63.1 and 89.0%, respectively. On the other hand, the immobilization capacity of biochar depends on the negatively charged functional groups on its surfaces, which absorb metal ions in the soil (He et al., 2019b).

Due to the fact that soil degradation, caused by heavy metal contamination and acidification, can negatively affect the soils function and its biochemical properties (Wyszkowska et al., 2009), the recovery of soil physicochemical and biological properties was considered an important indicator, defining the success of the remediation process (Liang et al., 2020). Soil enzymes play fundamental roles in the regulation of biochemical transformations. Specifically, catalase is a type of oxidoreductase related to the activity of aerobic microorganisms (Kang et al., 2018), and urease plays an important role in the nitrogen cycle in soils (Hu et al., 2014). Therefore, these enzyme activities can also be used to monitor the degradation or recovery of soil functions (Burns et al., 2013; Rao et al., 2014). This study showed that urease and catalase activities dramatically increased after the application of LS and Pcm, suggesting a significant improvement in the soil function due to the alkaline amendments. Moreover, the Pearson's correlation analysis revealed that urease activity was positively correlated with pH ($r = 0.626$, $p < 0.01$), but negatively correlated with the availability of the heavy metals, e.g., Cd ($r = -0.576$, $p < 0.01$), and Zn ($r = -0.516$, $p < 0.01$) (Table S4). Similar trends were also observed for the catalase activity. Therefore, it seems that the elevating soil pH reduced the metal availability, and subsequently promoted root exudation and improved enzyme activities (Li and Xu, 2018; Wu et al., 2017). In addition, the present study showed that the application of BC apparently enhanced the catalase activity but did not enhance the urease activity. The effects of biochar on soil enzyme activities were somewhat inconsistent with previous studies. Tang et al. (2020) found that the activities of eight enzymes including catalase, but not urease, were inhibited by biochar. Another study, however, reported that biochar resulted in large increases in the activities of urease and catalase in soils (Wu et al., 2020). These conflicting results might be ascribed to other unknown complicated interactions between biochar and soil (He et al., 2019b).

High throughput sequencing was used to further evaluate the responses of the bacteria to the amendments in terms of community structures and diversity. Consistent with previous studies (Liu et al., 2014), this study showed that the predominant bacteria phyla in the unamended soils were Chloroflexi, Proteobacteria, Acidobacteria, Actinobacteria, and Gemmatimonadetes. The bacterial community structure was clearly altered following the application of the amendments. To be specific, the most abundant bacteria phylum found in the LS and Pcm treatments were the Proteobacteria, followed by Gemmatimonadetes and Chloroflexi, while the bacterial community structure in the CMC and BC treatments was similar to the CK, as the most dominant bacteria phyla was Chloroflexi, followed by Proteobacteria, Actinobacteria, and Acidobacteria.

It is known that most Chloroflexi bacteria are facultative anaerobes, and are the most active in metal contaminated soils (Kim et al., 2013). Consistently, the abundance of Chloroflexi in this study was negatively correlated with the soil pH ($r = 0.955$, $p < 0.01$), and positively correlated with the availability of the heavy metals, such as Cd ($r = 0.951$, $p < 0.01$) and Zn ($r = 0.941$, $p < 0.01$) (Table S5). In addition, Acidobacteria are regarded as a highly diverse bacterial phylum, and widely distributed in the environment, especially in acidic and metal-enriched soils (Lopez et al., 2019). A number of characteristic lipids in this phylum may be responsible for the adaptation to harsh environmental conditions. Therefore, in accordance with previous studies (Wang et al., 2018; Lauber et al., 2009; RouskBååth et al., 2010), the abundance of Acidobacteria in this study was negatively correlated with soil pH ($r = 0.876$, $p < 0.01$) (Table S5). The relative abundance of Acidobacteria in the soils of high pH (LS and Pcm treatments) was dramatically decreased. On the other hand, Proteobacteria were significantly enriched after the applications of the LS and Pcm, and they had a positive correlation with the soil pH but a negative correlation with the availabilities of the heavy metals. This result suggested that the predominant microorganisms from this phylum were metal-sensitive. Moreover, Proteobacteria represent one of the largest phyla of soil bacteria, include many nitrogen-fixing bacteria, which play an important role in the cycling of nitrogen and energy in soil ecosystem. Salam and Varma (2019) investigated the soil bacterial community structures in e-waste recycling sites and revealed that e-waste related contamination (e.g., heavy metals) altered the soil bacterial compositions, and the abundance of Proteobacteria decreased.

Furthermore, taxonomic classifications revealed that the bacterial structures were notably shifted among the treatments. The genera of *Gemmatimonas*, *f_Gemmatimonadaceae*, and *f_Microscillaceae* were most frequently detected in the LS and Pcm treated soils. *Gemmatimonas* belongs to the phylum Gemmatimonadetes, which is known as one of the essential genera in phosphate dissolution (Takaichi et al., 2010). It can dissolve inert phosphorus and convert into more bioavailable phosphorus for plant growth (Zhang et al., 2019). The Pearson's correlation analysis revealed that the abundance of *Gemmatimonas* was positively correlated with AP (Table S6). Thus, the proportion of *Gemmatimonas* was significantly higher in the Pcm treatments with higher AP content than the other treatments, suggesting a symbolic genus responded to the P contents. In addition, *f_Gemmatimonadaceae* are commonly found in agricultural soils (Kim et al., 2008), and previous studies have demonstrated that these microorganisms have strong effects on microbial N metabolism and soil respiration, which can be used as bioindicators of the microbial nutrition limitations (Cui et al., 2018; Huang et al., 2019). However, as for the predominant genera of *o_RBG-13-54-9*, *c_KD4-96*, and *f_JG*30-KF-*AS9* from the CMC, BC, and CK treatments, little is known about their ability to adapt in contaminated and acidic soil environments, due to a lack of isolated pure culture studies. This study suggested that these genera are probably tolerant to heavy metals. In addition, the effects of heavy metals on soil microorganisms can be reflected by the shifts in bacterial diversity (Mucha et al., 2013). The present study revealed that the applications of LS and Pcm presented higher alpha diversity, including OTUs, Chao1, and Ace in the soils. This result further suggested that the applications of alkaline amendments could be more effective at enriching beneficial bacterial groups and consequently enhancing soil functions in contaminated and acidic soils.

The results of the RDA further demonstrated that the bacterial communities were largely affected by the amendments, due to the regulated soil chemical properties and heavy metal availabilities. Specifically, soil pH, available Cd, and available Zn were identified as the three most important factors in structuring soil community. Cd is known as a non-essential metal for living organisms, it is one of the most mobile and bioavailable metals in soils (Jones et al., 2019; Khan et al., 2015). On the other hand, although Zn is an essential micronutrient for living organisms, it can be toxic above specific thresholds (Navarrete et al., 2017). As revealed by previous studies (Touceda-González et al., 2015; Xiao et al., 2009), the results of the RDA identified a significantly negative relationship between Zn availability and the relative abundance of *f_Gemmatimonadaceae*, suggesting that Zn had inhibitory effects on these microorganisms. Soil pH was also found to be more effective in influencing bacterial community compositions, compared to the other environmental variables. In addition to the indirect influence on reducing heavy metal availability, soil pH can also directly regulate microbial community structures by controlling the carbon source utilization (Ren et al., 2018). Also, when soil pH < 5.5, rhizosphere bacteria were inhibited and bacterial community functions, including nutrient cycling related enzymes and proteins that were down regulated (Jones et al., 2019; Wan et al., 2020).

5. Conclusion

Soil degradation caused by heavy metal contamination and acidification has serious negative impacts on crop yields. This study showed that, in comparison to organic amendments (i.e., CMC and BC), the applications of alkaline amendments on the degraded soils had better amelioration effects. This was evidenced by the greater increases in soil pH, CEC, and P content, the reduction in the bioavailability of Cd, Cu, Pb, and Zn, and the promotions of lettuce growth. High throughput sequencing further demonstrated that the applications of LS and Pcm could result in favored bacterial community structures.

As cost-effective amendments, *in-situ* remediation with LS and Pcm appears to be a promising strategy for the recovery of soil functions and soil health after degradation by heavy metal contamination and acidification. To further develop this strategy, a long-term field experiment should be carried out to assess how to maintain soil pH within appropriate ranges, and the changes in soil nutrient levels should also be monitored.

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.

CRediT authorship contribution statement

Huilin Lu: Methodology, Writing - original draft. **Yingxin Wu:** Validation, Writing - review & editing. **Puxing Liang:** Data curation, Investigation. **Qingmei Song:** Methodology. **Huixi Zhang:** Investigation. **Jiahui Wu:** Data curation, Software. **Wencheng Wu:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **Xiaowen Liu:** Funding acquisition. **Changxun Dong:** Project administration, Supervision.

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Appendix A. Supplementary data

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References

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metal(loid)s contaminated soils — to mobilize or to immobilize? J. Hazard Mater. 266, 141—166.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216—234.

Chen, G.N., Shah, K.J., Shi, L., Chiang, P.C., You, Z.Y., 2019. Red soil amelioration and heavy metal immobilization by a multi-element mineral amendment: performance and mechanisms. Environ. Pollut. 254, 112964.

Clemente, R., Arco-L_azaro, E., Pardo, T., Martín, I., S_anchez-Guerrero, A., Sevilla, F., Bernal, M.P., 2018. Combination of soil organic and inorganic amendments helps plants overcome trace element induced oxidative stress and allows phytostabilisation. Chemosphere 223, 223—231.

CNEMC (China National Environmental Monitoring Centre), 1990. The Background Values of Elements in Chinese Soils. Environmental Science Press of China, Beijing (in Chinese).

Cui, Y.X., Fang, L.C., Guo, X.B., Wang, X., Wang, Y.Q., Li, P.F., Zhang, Y.J., Zhang, X.C., 2018. Responses of soil microbial communities to nutrient limitation in the desert—grassland ecological transition zone. Sci. Total Environ. 642, 45—55.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996—998.

Giller, K.E., Witter, E., Mcgrath, S.P., 2009. Heavy metals and soil microbes. Soil Biol. Biochem. 41 (10), 2031—2037.

Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., Goulding, K.W.T., Vitousek, P.M., Zhang, F.S., 2010. Significant acidification in major Chinese croplands. Science 327, 1008—1010.

He, D.Y., Cui, J., Gao, M., Wang, W.Q., Zhou, J., Yang, J., Wang, J.J., Li, Y., Jiang, C.S., Peng, Y., 2019a. Effects of soil amendments applied on cadmium availability, soil enzyme activity, and plant uptake in contaminated purple soil. Sci. Total Environ. 654, 1364—1371.

He, L.Z., Zhong, H., Liu, G.X., Dai, Z.M., Brookes, P.C., Xu, J.M., 2019b. Remediation of heavy metal contaminated soils by biochar: mechanisms, potential risks and applications in China. Environ. Pollut. 252, 846—855.

Holland, J.E., White, P.J., Glendining, M.J., Goulding, K.W.T., McGrath, S.P., 2019. Yield responses of arable crops to liming — an evaluation of relationships between yields and soil pH from a long-term liming experiment. Eur. J. Agron. 105, 176—188.

Houben, D., Pircar, J., Sonnet, P., 2012. Heavy metal immobilization by cost-effective amendments in a contaminated soil: effects on metal leaching and phytoavailability. J. Geochem. Explor. 123, 87—94.

Hou, D., Bolan, N.S., Tsang, D.C.W., Kirkham, M.B., O'Connor, D., 2020. Sustainable soil use and management: an interdisciplinary and systematic approach. Sci. Total Environ. 729, 138961, 2020.

Hu, B., Liang, D.L., Liu, J.J., Lei, L.M., Yu, D.S., 2014. Transformation of heavy metal fractions on soil urease and nitrate reductase activities in copper and selenium co-contaminated soil. Ecotoxicol. Environ. Saf. 110, 41—48.

Huang, X.F., Li, S.Q., Li, S.Y., Ye, G.Y., Lu, L.J., Zhang, L., Yang, L.Y., Qian, X., Liu, J., 2019. The effects of biochar and dredged sediments on soil structure and fertility promote the growth, photosynthetic and rhizosphere microbial diversity of Phragmites*communis (Cav.). Trin. exSteud*. Sci. Total Environ. 697, 134073.

Jones, D.L., Cooledge, E.C., Hoyle, F.C., Griffiths, R.I., Murphy, D.V., 2019. pH and exchangeable aluminum are major regulators of microbial energy flow and carbon use efficiency in soil microbial communities. Soil Biol. Biochem. 138, 107584.

Kahr, G., Madsen, F.T., 1995. Determination of the cation exchange capacity and the surface area of bentonite, illite and kaolinite by methylene blue adsorption. Appl. Clay Sci. 9, 327—336.

Kang, H.Z., Gao, H.H., Yu, W.J., Yi, Y., Wang, Y., Ning, M.L., 2018. Changes in soil microbial community structure and function after afforestation depend on species and age: case study in a subtropical alluvial island. Sci. Total Environ. 625, 1423—1432.

Khan, A., Khan, S., Khan, M.A., Qamar, Z., Waqas, M., 2015. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environ. Sci. Pollut. Res. 22, 13772—13799.

Kim, H.M., Chae, N., Jung, J.Y., Lee, Y.K., 2013. Isolation of facultatively anaerobic soil bacteria from Ny-Ålesund, Svalbard. Polar Biol. 36, 787—796.

Kim, J.S., Dungan, R.S., Crowley, D., 2008. Microarray analysis of bacterial diversity and distribution in aggregates from a desert agricultural soil. Biol. Fertil. Soils 44, 1003—1011.

Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environ. Int. 132, 105078.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the Miseq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112—5120.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments — a review. Waste Manag. 28, 215—225.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111.

León-Cañedo, Jesús, A., Alarcón-Silvas, Suammy, G., Fierro-Sañudo, Juan, F., Rodríguez-Montes de Oca Gustavo, A., Partida-Ruvalcaba, L., Díaz-Valdés, Tomás, Páez-Osuna Federico, 2019. Mercury and other trace metals in lettuce (*Lactuca sativa*) grown with two low-salinity shrimp effluents: accumulation and human health risk assessment. Sci. Total Environ. 650, 2535—2544.

Li, J., Xu, Y.M., 2018. Effects of clay combined with moisture management on Cd immobilization and fertility index of polluted rice field. Ecotoxicol. Environ. Saf. 158, 182—186.

Li, Q.Q., Li, S., Xiao, Y., Zhao, B., Wang, C.Q., Li, B., Gao, X.S., Li, Y.D., Bai, G.C., Wang, Y.D., Yuan, D.G., 2019. Soil acidification and its influencing factors in the purple hilly area of southwest China from 1981 to 2012. Catena 175, 278—285.

Liang, J., Tang, S.Q., Gong, J.L., Zeng, G.M., Tang, W.W., Song, B., Zhang, P., Yang, Z.X., Luo, Y., 2020. Responses of enzymatic activity and microbial communities to biochar/compost amendment in sulfamethoxazole polluted wetland soil. J. Hazard Mater. 385, 121533.

Liu, L.W., Li, W., Song, W.P., Guo, M.X., 2018. Remediation techniques for heavy metal-contaminated soils: principles and applicability. Sci. Total Environ. 633, 206—219.

Liu, Y.R., Wang, J.J., Zheng, Y.M., Zhang, L.M., He, J.Z., 2014. Patterns of bacterial diversity along a long-term mercury-contaminated gradient in the paddy soils. Microb. Ecol. 68, 575—583.

Lopez, S., Goux, X., Echevarria, G., Calusinska, M., Morel, J.L., Benizri, E., 2019. Community diversity and potential functions of rhizosphere-associated bacteria of nickel hyperaccumulators found in Albania. Sci. Total Environ. 654, 237—249.

Luo, X.S., Fu, X.Q., Yang, Y., Cai, P., Peng, S.B., Chen, W.L., Huang, Q.Y., 2016. Microbial communities play important roles in modulating paddy soil fertility. Sci. Rep. 6, 20326.

Mehmood, S., Saeed, D.A., Rizwan, M., Khan, M.N., Aziz, O., Bashir, S., Ibrahim, M., Ditta, A., Akmal, M., Mumtaz, M.A., Ahmed, W., Irshad, S., Imtiaz, M., Tu, S.X., Shaheen, A., 2018. Impact of different amendments on biochemical responses of sesame (*Sesamum indicum* L.) plants grown in lead-cadmium contaminated soil.

Plant Physiol. Biochem. (Paris) 132, 345–355.
Mucha, A.P., Teixeira, C., Reis, I., Magalhães, C., Bordalo, A.A., Almeida, C.M.R., 2013. Response of a salt marsh microbial community to metal contamination. Estuar. Coast Shelf Sci. 130 (2), 81–88.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Navarrete, A.A., Mellis, E.V., Escalas, A., Lemos, L.N., Junior, J.L., Quaggio, J.A., Zhou, J.Z., Tsai, S.M., 2017. Zinc concentration affects the functional groups of microbial communities in sugarcane-cultivated soil. Agric. Ecosyst. Environ. 236, 187–197.
Nie, Y.X., Wang, M.C., Zhang, W., Ni, Z., Hashidoko, Y., Shen, W.J., 2018. Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment. Sci. Total Environ. 624, 407–415.
Novak, J.M., Ippolito, J.A., Ducey, T.F., Watts, D.W., Spokas, K.A., Trippe, K.M., Sigua, G.C., Johnson, M.G., 2018. Remediation of an acidic mine spoil: miscanthus biochar and lime amendment affects metal availability, plant growth, and soil enzyme activity. Chemosphere 205, 709–718.
Olsen, S.R., Cole, C.V., Watanable, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. USDA. Circular. 939, 1–19.
Palansooriya, K.N., Shaheen, S.M., Chen, S.S., Tsang, D.C.W., Hashimoto, Y., Hou, D., Bolan, N.S., Rinklebe, J., Ok, Y.S., 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: a critical review. Environ. Int. 134, 105046.
Peng, J.F., Song, Y.H., Yuan, P., Cui, X.Y., Qiu, G.L., 2009. The remediation of heavy metals contaminated sediment. J. Hazard Mater. 161, 633–640.
Rao, M.A., Scelza, R., Acevedo, F., Diez, M.C., Gianfreda, L., 2014. Enzymes as useful tools for environmental purposes. Chemosphere 107, 145–162.
Ren, B.H., Hu, Y.M., Chen, B.D., Zhang, Y., Thiele, J., Shi, R.J., Liu, M., Bu, R.C., 2018. Soil pH and plant diversity shape soil bacterial community structure in the active layer across the latitudinal gradients in continuous permafrost region of Northeastern China. Sci. Rep. 8, 5619.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4, 1340–1351.
Saavedra, F., Liu, Y.J., Southam, G., Huang, L.B., 2018. Phosphate treatment alleviated acute phytotoxicity of heavy metals in sulfidic Pb-Zn mine tailings. Environ. Pollut. 250, 676–685.
Salam, M., Varma, A., 2019. Bacterial community structure in soils contaminated with electronic waste pollutants from Delhi NCR, India. Electron. J. Biotechnol. 41, 72–80.
Schulte, E.E., 1995. Recommended soil organic matter test. In: Horton, M.L. (Ed.), Recommended Soil Testing Procedures for the Northeastern United States, second ed. Northeastern Regional Publication, Newark, DE, pp. 52–61.
Sevilla-perea, A., Romero-puertas, M.C., Mingorance, M.D., 2016. Optimizing the combined application of amendments to allow plant growth in a multi element-contaminated soil. Chemosphere 148, 220–226.
Shaheen, S.M., Shams, M.S., Khalifa, M.R., Ei-Dail, M.A., Rinklebe, J., 2017. Various soil amendments and environmental wastes affect the (im) mobilization and phytoavailability of potentially toxic elements in a sewage effluent irrigated sandy soil. Ecotoxicol. Environ. Saf. 142, 375–387.
Smith, S.R., 2009. A critical review of the bioavailability and impact of heavy metals in municipal solid waste composts compared to sewage sludge. Environ. Int. 35, 142–156.
Sumner, M.E., Noble, A.D., 2003. Soil acidification: the world story. In: Rengel, Z. (Ed.), vols. 1–28. Handbook of Soil Acidity.
Sun, Y.B., Sun, G.H., Xu, Y.M., Wang, L., Liang, X.F., Lin, D.S., 2013. Assessment of sepiolite for immobilization of cadmium-contaminated soils. Geoderma 193–194, 149–155.
Takaichi, S., Maoka, T., Takasaki, K., Hanada, S., 2010. Carotenoids of *Gemmatimonasaurantiaca* (*Gemmatimonadetes*): identification of a novel carotenoid, deoxyoscillol 2-rhamnoside, and proposed biosynthetic pathway of oscillol 2, 2'-dirhamnoside. Microbiology 156, 757–763.
Tang, J.Y., Zhang, L.H., Zhang, J.C., Ren, L.H., Zhou, Y.Y., Zheng, Y.Y., Yang, Y., Huang, H.L., Chen, A.W., 2020. Physicochemical features, metal availability and enzyme activity in heavy metal-polluted soil remediated by biochar and compost. Sci. Total Environ. 701, 134751.
Touceda-González, M., Brader, G., Antonielli, L., Ravindran, V.B., Waldner, G., Friesl-Hanl, W., Corretto, E., Campisano, A., Pancher, M., Sessitsch, A., 2015. Combined amendment of immobilizers and the plant growth-promoting strain *Burkholderia phytofirmans* PsJN favours plant growth and reduces heavy metal uptake. Soil Biol. Biochem. 91, 140–150.
Wan, W.J., Tan, J.D., Wang, Y., Qin, Y., He, H.M., Wu, H.Q., Zuo, W.L., He, D.L., 2020. Responses of the rhizosphere bacterial community in acidic crop soil to pH: changes in diversity, composition, interaction, and function. Sci. Total Environ. 700, 134418.
Wang, H., Zeng, Y.F., Guo, C.L., Bao, Y.P., Lu, G.N., Reinfelder, J.R., Dang, Z., 2018. Bacterial, archaeal, and fungal community responses to acid mine drainage-laden pollution in a rice paddy soil ecosystem. Sci. Total Environ. 616–617, 107–116.
Wang, T., Sun, H.W., Ren, X.H., Li, B., Mao, H.J., 2017. Evaluation of biochars from different stock materials as carriers of bacterial strain for remediation of heavy metal-contaminated soil. Sci. Rep. 7, 12114.
Wen, Z.H., Shen, J.B., Blackwell, M., Li, H.G., Zhao, B.Q., Yuan, H.M., 2016. Combined applications of nitrogen and phosphorus fertilizers with manure increase maize yield and nutrient uptake via stimulating root growth in a long-term experiment. Pedosphere 26, 62–73.
Wolf, M., Baretta, D., Becegato, V.A., Almeida, V.C., Paulino, A.T., 2017. Copper/zinc bioaccumulation and the effect of phytotoxicity on the growth of lettuce (*Lactuca sativa* L.) in non-contaminated, metal-contaminated and swine manure-enriched soils. Water, Air, Soil Pollut. 228, 1573–2932.
Wu, W.C., Wu, J.H., Liu, X.W., Chen, X.B., Wu, Y.X., Yu, S.X., 2017. Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure. Ecotoxicol. Environ. Saf. 143, 322–329.
Wu, W.C., Wu, Y.X., Wu, J.H., Liu, X.W., Chen, X.B., Cai, X.D., Yu, S.X., 2018. Regional risk assessment of trace elements in farmland soils associated with improper e-waste recycling activities in Southern China. J. Geochem. Explor. 192, 112–119.
Wu, S.W., Zhang, Y., Tan, Q.L., Sun, X.C., Wei, W.H., Hu, C.X., 2020. Biochar is superior to lime in improving acidic soil properties and fruit quality of Satsuma Mandarin. Sci. Total Environ. 714, 136722.
Wyszkowska, J., Kucharski, M., Kucharski, J., Borowik, A., 2009. Activity of dehydrogenases, catalase and urease in copper polluted soil. J. Elementol. 14, 605–617.
Xiao, S.M., Xie, X.H., Liu, J.S., 2009. Microbial communities in acid water environments of two mines, China. Environ. Pollut. 157, 1045–1050.
Xie, E.Z., Zhao, Y.C., Li, H.D., Shi, X.Z., Lu, F.Y., Zhang, X., 2019. Spatio-temporal changes of cropland soil pH in a rapidly industrializing region in the Yangtze River Delta of China, 1980–2015. Agric. Ecosyst. Environ. 272, 95–104.
Zhang, C., Zhou, T., Zhu, L., Juhasz, A., Du, Z., Li, B., Wang, J., Wang, J., Sun, Y., 2019. Response of soil microbes after direct contact with pyraclostrobin in fluvo-aquic soil. Environ. Pollut. 255, 113164.
Zhang, X.Y., Zhong, T.Y., Liu, L., Ouyang, X.Y., 2015. Impact of soil heavy metal pollution on food safety in China. PloS One 10, e0135182.
Zhu, H.H., Chen, C., Xu, C., Zhu, Q.H., Huang, D.Y., 2016. Effects of soil acidification and liming on the phytoavailability of cadmium in paddy soils of central subtropical China. Environ. Pollut. 219, 99–106.