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Effects of magnetic biochar-microbe composite on Cd remediation and microbial responses in paddy soil

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ABSTRACT

There is growing global interest in the bioremediation of cadmium (Cd) using combinations of biochar and microorganisms. However, the interactions among biochar, introduced and indigenous microorganisms remain unclear. Accordingly, a 90 day microcosm experiment was conducted to investigate this by adding *Bacillus* sp. K1 strain inoculated rice straw biochar (SBB) and magnetic straw biochar (MBB) into a Cd contaminated paddy soil from Hunan, China. All treatments were incubated aerobically (60% water holding capacity) or anaerobically for 90 d. During both soil incubations, *Bacillus* sp. K1 successfully colonized in soil with composites applications. Soil pH was significantly increased from acid to neutral, and available Cd decreased with the addition of both composites. The better remediation efficiency of MBB than SBB under anerobic conditions was attributed to the transformation of acetic acid-extractable Cd into the residual fraction, caused by Cd^{2+} bonding with crystal Fe_3O_4. The application of the two kinds of composites caused similar changes to both microbial communities. There was a slight decrease in indigenous microbial alpha diversity with the MBB aerobic application, while the total population number of bacteria was increased by 700%. Both the redundancy analysis and Mantel analyses indicated that pH and microbial biomass C contributed to the colonization of *Bacillus sp*. K1 with SBB under aerobic conditions, and with MBB under anerobic conditions, respectively. The research provides a new insight into interactive effects and investigates immobilization mechanisms involved of bacterial/biochar composites in anaerobic and aerobic soils.

1. Introduction

Due to increasing global industrial activities, contamination now occurs in most soil ecosystems. This causes persistently harmful effects on soil ecosystem functioning and human health (Bolan et al., 2014; He et al., 2019). In China, a nationwide survey indicated that inorganic pollutants are the main sources (82.4%) of soil contamination, with the Cd being the greatest inorganic pollutant (7.0%) in soils that exceed Chinese environment quality standards (Zhao et al., 2015). Cadmium could bioaccumulate readily in the human body via food chains and cause very serious diseases (e.g. prostatic carcinoma, liver and renal cancer) (Chen et al., 2008). Recently, in situ immobilization of the contamination by using combinations of biochar and bioremediation demonstrated its superiority over other techniques, in terms of its low cost, high efficiency and efficiency of soil remediation (Wu et al., 2019; Xiao et al., 2017).

Biochar is a carbonaceous material produced from the pyrolysis of agricultural residues. It has been widely used for contamination mitigation (Tan et al., 2020; Li et al., 2016). The mechanisms involved in Cd immobilization by biochar are well documented. Briefly, biochar contains abundant functional groups and an extensive surface area, so Cd is absorbed by cation exchange, complexation, and precipitation (Xu et al., 2016a). Biochar also alters soil characteristics (pH, CEC, etc.), modifying Cd movement and transformations in soil (Wang et al., 2017). Biochar pyrolyzed from different feedstocks have different abilities to immobilize Cd in soil. In order to enhance the efficiency of immobilization, modified magnetic iron oxides were tested in several studies (Zhang et al., 2020). The modifications significantly improved the adsorption ability of Cd by increasing the biochar specific surface area and functional groups. They also permit biochar reuse due to their magnetic

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properties (Wu et al., 2020a).

During recent decades, numerous strains of bacterial and fungal species which promote plant growth and absorb Cd have been isolated and used for remediation by direct inoculation into soils or with carriers such as biochar (Rahman, 2020). The inoculation of *Serratia marscens* PRE01 decreased Cd phytotoxicity by adsorption and increase the growth of *Pteris vittate* (Wang et al., 2018). *Sphingomonas* SaMR12 promoted Cd accumulation by increasing glutathione biosynthesis in *Sedum alfredii* (Pan et al., 2016). However, the efficiency of different strains is affected by soil properties, including pH, initial pollutant concentrations, temperature, carbon and nitrogen source (Bouabidi et al., 2019).

Biochar alleviates soil Cd toxicity to microbes and promotes their colonization by decreasing Cd mobility, it also alters soil characters such as pH and provides substrate. The combination of bacterial strains with biochar is a promising research area. It may help to avoid the limitations of direct inoculation, so achieving the efficient and sustainable remediation of Cd contaminations (Abu Talha et al., 2018). *Bacillus* sp. *TZ5* loaded onto biochar successfully colonized in soil and decreased Cd accumulation in ryegrass (Ma et al., 2020). *Pseudomonas* sp. NT-2 loaded onto straw biochar decreased the bio-availability of Cd in soil, and improved the diversity of soil microbial community (Tu et al., 2020). Previous studies focused on the remediation effects of bacterial/biochar composites. However, the interactions between inoculated microbes, biochar and indigenous microorganism in soil are poorly understood. Microorganisms can act as indicators of soil ecosystem disturbances because most bacteria are sensitive and not initially resilient to disturbance (Allison and Martiny, 2008).

Accordingly, to fill the research gaps mentioned above, a Cd contaminated soil was obtained from a paddy field in Hunan province, China. The field had a rotation cultivation with *Brassica napus* L and *Oryza sativa*. To simulate the field water conditions, both moist (aerobic, 60% water holding capacity) and flooded (anaerobic) soils were used under laboratory conditions (25 °C). Two types of bacterial/biochar composites were synthesized by loading *Bacillus* sp. K1 (Table S1) onto rice straw biochar and magnetic biochar. The study aimed to (1) determine whether the application of bacterial/biochar composites enhances the Cd immobilization by altering soil properties and promoting the bioremediation efficiency of bacteria under anaerobe and aerobic conditions; (2) elucidate the colonization of the added *Bacillus* sp. K1 and the influences on the indigenous microbial structure and community; (3) provide insight into the interactive effects among biochar, added bacterial strains and indigenous microorganism.

2. Materials and methods

2.1. Preparation of biochar and bacterial biochar composite

The methods for producing biochar and composites used in this study are described by Wang et al. (2021). The magnetic rice straw was immersed into a $FeSO_4$ and $FeCl_3$ mixture (molar ratio 2:1, Fe^{2+} 0.1 mol L^{-1}) and then loaded Fe_3O_4 through raising pH (11−12) with 0.1 M NaOH addition. After drying and milling, the modified straw and raw straw were pyrolyzed at 500 °C under an anerobic N_2 atmosphere for 2 h to synthesize magnetic biochar and straw biochar, respectively. The biochars were then separately added to the solution containing 10^8 cells mL^{-1} of *Bacillus* sp. K1 strain (Table S1) at a ratio of 1 g biochar and 40 mL bacterial solution at 25 °C, then shaken at 160 rpm for 16 h. The biochar bacteria mixtures were mixed with liquid sterilized sodium alginate and then dropped in 2% $CaCl_2$ solution to obtain the composites as bacterial/biochar alginate beads. The basic physiochemical properties of the biochar and composites are given in Table S2.

2.2. Soil incubation experiments

To determine the effects of the composites on Cd remediation and the indigenous microbial community in soil, a microcosm experiment was conducted under laboratory conditions for 90 days. The soil was collected from a paddy field (depth 0–20 cm) in Guiyang County, Hunan Province, China. The soil had a pH of 5.75 ± 0.08 and a total Cd concentration of 2.80 ± 0.10 mg kg^{-1}, the different composites were added to the amendments at a concentration of 1% according to Wu et al. (2020b). The different treatments each comprised 4 replicates the treatments were control (CK, no amendments), bacteria (B), straw biochar (SB), magnetic biochar (MB), magnetic bacterial/biochar composite (MBB), and straw bacterial/biochar composite (SBB). The soils were incubated anaerobically with 1–2 cm surface water and aerobically at 60% of field soil water-holding capacity, prepared by weight. The different soil treatments were then incubated in an incubator at 25 °C for 90 days. Separate soil replicates were each divided into two parts after 1, 30, and 90 days. At each sampling, one part was used to determine changes in soil chemical properties after the sample was freeze dried and ground < 2 mm. The other part was stored at −80 °C for microbial community analyses.

2.3. Change in soil properties and Cd concentrations

Soil pH values were determined using a pH meter (S470, SevenExcellence™) in a 1:2 (w/v) suspension of deionized water prepared by shaking at 200 rpm for 1 h. Available Cd concentrations were measured after suspending in a 10% (w/v) 0.1 mol L^{-1} $CaCl_2$ solution following shaking at 250 rpm for 2 h. Fractional analyses of Cd were determined by the Community Bureau of Reference method as described by Huang et al. (2016). A sequential extraction method to determine acetic acid (HOAc) extractable fractions (acid soluble, reducible, oxidizable and residual Cd fractions was used. It comprised, in brief: 1) An HOAc-extractable fraction (soluble and carbonate bonded Cd) was extracted by shaking with 0.1 M HOAc for 16 h; 2) Then, the reducible fraction (Fe and MnO fraction) was extracted by mixing the solid residue from step 1 with 0.5 M hydroxylamine hydrochloride (pH = 2) and shaking for 16 h; 3) The oxidizable fraction was subsequently obtained by mixing the solid residue from step 2 with hydrogen peroxide (30%) and extracting with 1 M ammonium acetate; 4) Finally, the residual fraction was obtained by digested the solid residue from step 3 with HF-HNO_3 (1:2) in a microwave digestion system. Cadmium concentrations in the extracts were determined by ICP-MS (Perkin Elmer 600X, USA).

2.4. Biomass C measurements

Soil microbial biomass C estimated by the chloroform fumigation extraction method was modified (Vance et al., 1987). Moist soil (equivalant to 10 g oven dry soil) was fumigated with ethanol-free $CHCl_3$ in darkness for 24 h in a glass desiccator. The same weights of nonfumigated soil were incubated under the same conditions. The soils were then extracted by shaking with 40 mL K_2SO_4 (0.5 mol L^{-1}) in centrifuge tubes for 30 min. The extracts were filtered and adjusted to pH 2–3 with HCl. Finally, the C concentrations in the extracts were determined using a TOC-V/CPN (Multi N/C 2100, Germany). Biomass C was calculated from:

$$\text{Biomass C} = \frac{(C_f - C_{nf})}{\text{kc}}$$

Where C_f is C concentration in fumigated soil; C_{nf} is C concentration in non-fumigated soil and kc is percentage of biomass C extracted following extraction (kc = 0.45).

2.5. Bacterial community and population

After incubation, the populations of bacteria in soil were determined by the dilution plate technique (Sun et al., 2012). The soil extracts were diluted to 10^{-5}–10^{-7} to provide inocula. The colonies were counted

after 72 h incubation at 28 °C under aerobic or anaerobic conditions (medium: beef extract 5 g, peptone 10 g, NaCl 5 g, distilled water 1000 mL, agar 18 g, pH 7).

The soil bacterial DNA was extracted using a Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. The bacterial hypervariable regions (V4 V5) of the 16S rRNA gene were PCR-amplified using a specific primer (515F-907R). The forward primer was 515F (50- GTG CCA GCM GCC GCG G −30), and the reverse primer was 907R (50- CCG TCA ATT CM TTT RAG TTT −30), purified and sequenced on the Illumina MiSeq PE250 platform, which were conducted by Genesky Biotechnology Inc. (Shanghai, China).

2.6. Data and statistical analysis

Data of soil characteristics and Cd were analyzed and imaged using Origin 9.0. The one-way and two-way ANOVA analysis was conducted using SPSS 22.0. The Sequence analyses were carried out by Qiime2 v2020.2 (Bolyen et al., 2019). 99% similarity were assigned to sequence variance. Alpha diversity was determined at even sequence depths for all samples (29,500 reads). The relationships between microbial communities and environmental factors were determined by redundancy analysis (RDA) in CANOCO 5.0 and the Mantel test from the Vegan package of R (ter Braak and Smilauer, 2012).

3. Results and discussion

3.1. The effectiveness of the bacterial/biochar composites in enhancing Cd immobilization

3.1.1. Changes in soil pH

During aerobic incubation, the pHs of the soils remained almost constant within treatments throughout the experiment, with most changes occurring at the start (Fig. 1). Application of all the amendments increased the soil pH in comparison with CK. The rapid increase in pH in biochar treatments (SB and MB), and in those with added bacteria treatments (B) and mixed treatments (SBB and MBB) were caused by the high pH of the added biochar, the presence of carbonates and hydroxides and the microbial release of extracellular polymeric substances and their interactions (Wang et al., 2017; Li et al., 2018). Due to this, the MBB and SBB treatments exhibited more pronounced effects on soil pH compared to treatments SB, MB or B, which increased soil pH from 5.60 ± 0.01–6.61 ± 0.02 and 6.68 ± 0.05 after incubation, respectively. The pH in anaerobic soils was higher than with the comparable treatments in the aerobic soils (Figs. 1 and S3). Increases in soil pH under anaerobic conditions were due to the continuous consumption of H^+, including the reduction process of iron oxide, manganese oxide and SO_4^{2-} (e.g. Fe $[OH]_3 + 3H^+ + e^- \leftrightarrow 4\ Fe^{2+} + 3H_2O$) (Li et al., 2020; Li and Xu, 2017). After incubation, the pH of SBB and MBB treatments were 6.88 ± 0.02 and 6.90 ± 0.01, which were 13.21% and 13.01% respectively higher than the pH of treatment CK (Fig. 1).

Both bacterial/biochar composites increased soil pH under aerobic and anaerobic conditions. Increasing the soil pH from acid to neutrality favored the deprotonation of functional groups including phenolic, hydroxyl and carbonyl, which increased negatively charged surfaces of soil colloids and promoted the Cd adsorption (Cui et al., 2019). The growth of bacteria was inhibited in the acidic conditions (West et al., 1985). Therefore, the neutral pH of the SBB and MBB treatments may favour the growth of *Bacillus* sp. K1.

3.1.2. Changes in Cd availability and fraction of soil

Changes in soil $CaCl_2$-extractable Cd concentrations (available Cd) in the different treatments were shown in Fig. 2a. Individual application of *Bacillus* sp. K1 decreased $CaCl_2$-extractable Cd concentrations by 44.7%

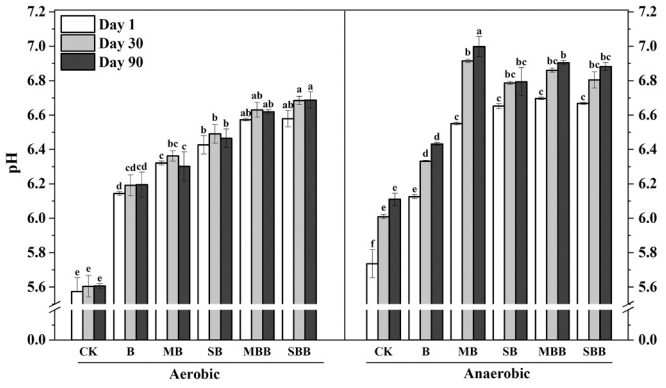

Fig. 1. Effects of different amendments on soil pH during incubation. CK, SB, MB, SBB, MBB and B represent the soil samples with none of amendments, straw biochar, magnetic biochar, magnetic bacterial/biochar composite, bacterial/biochar composite and bacteria inoculation, respectively. Lowercase letters above the error bars indicate significant differences among different treatments analyzed by Duncan test ($p < 0.05$).

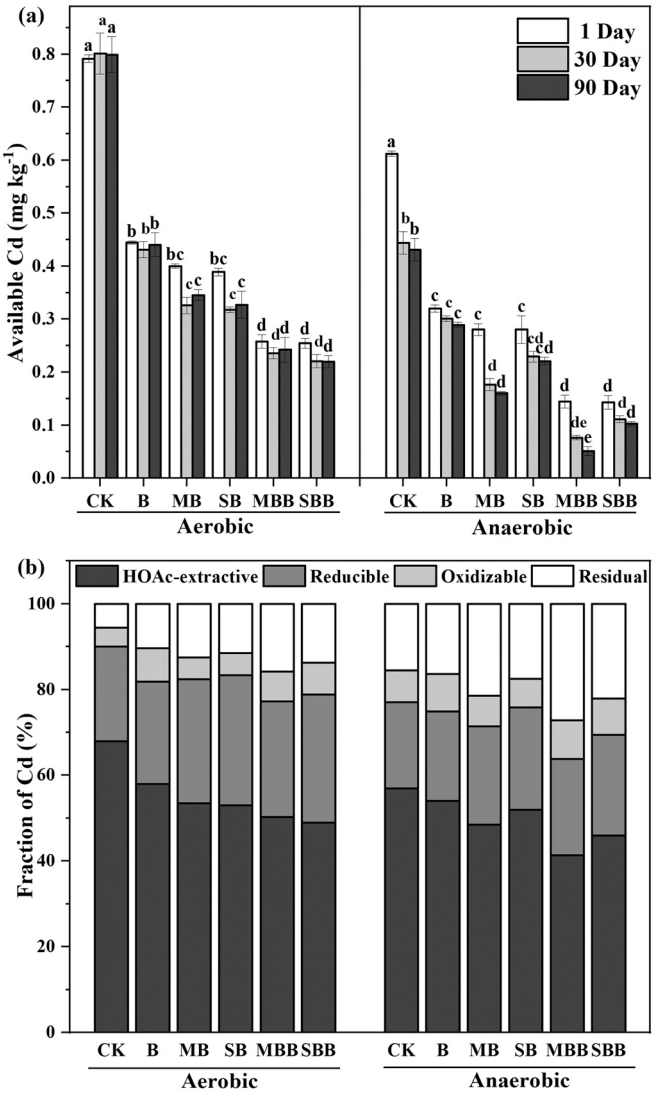

Fig. 2. Changes of a) $CaCl_2$ extracted available Cd during the incubation and b) Cd fractions after incubation. CK, SB, MB, SBB, MBB and B denotes the soil samples with none of amendments, straw biochar, magnetic biochar, magnetic bacterial/biochar composite, bacterial/biochar composite and bacteria inoculation, respectively. Lowercase letters above the error bars indicate significant differences among different treatments analyzed by Duncan test ($p < 0.05$).

and 39.8% under aerobic and anaerobic condition, respectively, indicating great efficiency of Cd immobilization in soil. This contrasts with smaller decreases caused by inoculations of *Neorhizobium huautlense* T1–17 and *B.* 38 of 20.0% and 37.5% of available Cd of soil reported previously (Wang et al., 2014, 2016). However, the effects on decreases in available Cd concentration with the amendments differed between aerobic and aerobic soils. Both SBB and MBB significantly decreased available Cd under aerobic conditions, with the decrement of 83% and 85% of total available Cd concentrations, respectively. However, MBB caused the greatest decrease of available Cd under anaerobic condition. The available Cd concentration in SB, MB, SBB and MBB treatment decreased by 48.8%, 62.8%, 76.2% and 88.1% compared with the control, respectively.

Fractional analysis of total soil Cd that indicated the distribution of complexed soil Cd and the immobilization mechanisms involved were determined by BCR sequential extraction (Figs. 2b and S1). Under aerobic conditions, HOAc-extractable Cd in all amendments was converted to reducible and residual Cd. In the presence of *Bacillus sp.* K1, HOAc-extractable Cd was also converted to oxidizable Cd (organic Cd fractions) (i.e. with B, MBB and SBB) after incubation (Fig. 2b). Significant variations in percentages of the different Cd fractions occurred with bacterial/biochar composites treatments. HOAc-extractable, reducible, oxidizable and residual Cd changed by −17.6%, 4.9%, 2.5% and 10.4% with MBB, and −19.0%, 7.8%, 2.9% and 8.2% with SBB, respectively. The largest decrease of HOAc-extractable Cd with MBB was attributed to the strong binding of Cd to the inner Fe_3O_4 particles, which had a very low charge and would occur in the residual fraction (Li et al., 2019). Under anaerobic condition, HOAc-extractable Cd was probably converted to residual Cd. Relative to the CK, HOAc-extractable Cd with MBB and SBB decreased by 15.6% and 11.0% of total Cd concentrations, while the residual fraction increased by 11.7% and 6.6% at 90 days of incubation, respectively. The HOAc-extractable Cd was the most mobile and soluble soil fraction and comparable to the available Cd determined by $CaCl_2$ extraction. Both are used for evaluation of the concentrations and toxicity of Cd in soil (Wang et al., 2014; Zhou et al., 2018). Decreases in the available soil Cd concentrations were larger than the HOAc-extractable Cd concentrations, indicating that more water-soluble Cd was converted into $CdCO_3$ and organic matter rather than into the residual fraction, especially in the bacteria inoculation treatments (Nemati et al., 2011). Cadmium carbonate is mobilized below about pH 7. Therefore, bacterial inoculation may be not be effective in low pH soils. Increased reducible Cd in all treatments occurred under anaerobic conditions (Fig. 2b). This may be associated with the sulfide in the reducible fraction being converted to CdS (Zhou et al., 2018).

3.1.3. Changes in Cd transformations in soil by the composites

The inoculation of bacteria into soil decreases Cd toxicity by binding of Cd with soil functional groups (i.e., sulfhydryl, carboxyl and amide groups) and chelation by bacterial extracellular polymers and soil organic acids, which are all directly affected by soil properties (Shou et al., 2018; Ma et al., 2011). Bacterial/biochar composites increased the immobilization of Cd more than biochar alone, by increasing bacterial absorption capability in soil due to the alteration of soil properties. Thus, the bacterial/biochar composite was more effective than biochar alone. The MBB was the best treatment in converting Cd into the most stable residual fraction, containing metals bound in crystalline structures (Xu et al., 2016b). The BCR results (Fig. 2b) indicated a higher potential of the composites (SBB and MBB) for Cd immobilization in soil transforming soluble or mobile Cd into resistant forms, rather than the single application of biochars (SB and MB) or inoculation with bacteria (B) alone. The reduction of amorphous Fe(II) to crystalline forms Fe(III) occurs under anaerobic conditions, which are more associated with the available soil Cd than other Fe forms (Muehe et al., 2013). Under anerobic conditions, the greater Cd transformations by MBB than SBB can be explained by: (1) the process of reductions and redistributions of Fe under anaerobic condition, forming more large numbers of amorphous iron oxides, which have larger surface areas and more functional groups on their surface to immobilize Cd than crystalline forms; (2) Flooding with a higher pH in soil increased the negative charge on the surface of iron oxides and enhanced the adsorption capacity of Cd (Muehe et al., 2013; Zhang et al., 2012).

3.2. Changes in indigenous microbial community and colonization of *Bacillus sp. K1*

3.2.1. Increase of soil microbial biomass carbon by the composites

Biomass C contents were in the order MBB ≥SBB > MB > SB > B > CK after 90 days incubation (Fig. 3), indicated that the addition of bacterial/biochar composites increased biomass C under both aerobic and anaerobic conditions. During the incubation, biomass C decreased slightly under aerobic condition and decreased significantly under anaerobic conditions.

The addition of biochar into soil decreases CO_2 release, increases C sequestration and organic matter contents in soils, due to the release of the liable biochar fraction (Xu et al., 2018a). Thus, more suitable microorganism habitats were provided and the rates of bacterial metabolic processes increased in the biochar treatments (MB, SB, MBB and SBB), leading to the increase in biomass C. Biochar also alleviates the toxic effects of Cd on soil microorganisms, by decreasing Cd availability and extra energy cost of bacteria under toxic stress (Xu et al., 2015). Therefore, the composites (MBB and SBB) with significant Cd remediation effect and carbon substrate supply increased the biomass carbon of soil and were promising for microbial survival and colonization in soil.

3.2.2. Changes in microbial community structure and diversity

The bacterial community compositions were determined to evaluate further mechanisms involved in Cd immobilization by different amendments and more detailed elucidation of the possible effect of MBB and SBB on the soil microbial community (Fig. 4). The bacterial community structure varied between soils under different treatments and conditions. Under aerobic conditions, *Bacillus*, *Streptomyces*, and *Micromonospora*, which are representative metal-reducing bacterial genera (Sahmoune, 2018; Selvin et al., 2009), dominated the bacterial community in all treatments, with a relative abundance ranging from 41.13% to 54.62%, 5.90 – 8.51% and 3.79 – 7.85%, respectively. *Bacillus* have been used previously in Cd remediation due to their wide

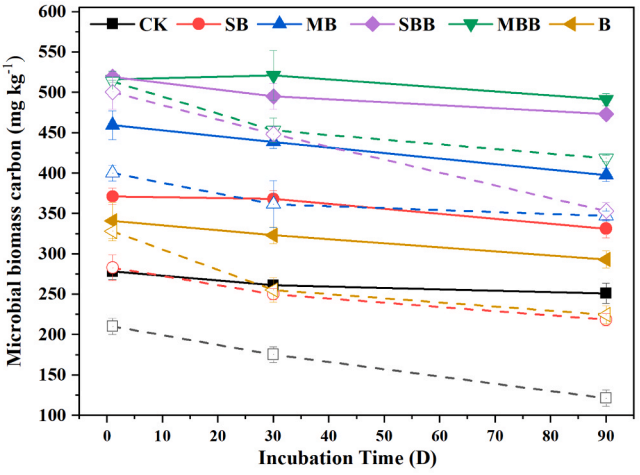

Fig. 3. Changes of soil biomass carbon during the incubation. Treatments under aerobic and anaerobic condition were shown as solid and dashed line, respectively. CK, SB, MB, SBB, MBB and B denotes the soil samples with none of amendments, straw biochar, magnetic biochar, magnetic bacterial/biochar composite, bacterial/biochar composite and bacteria inoculation, respectively. The significant differences among different treatments was analyzed by Duncan test ($p < 0.05$).

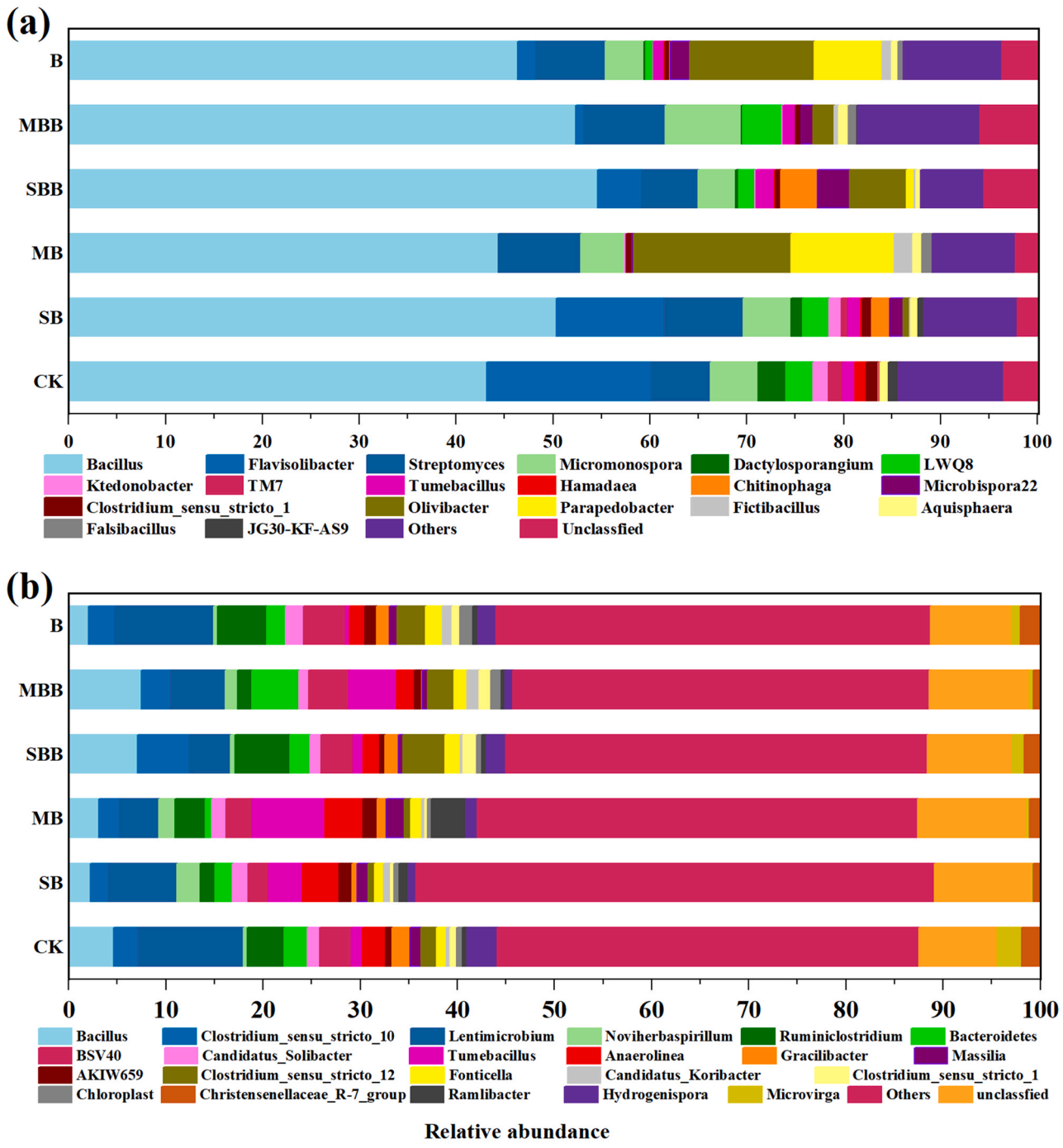

Fig. 4. Relative abundances of soil microbial community at the genus level revealed by 16S rRNA high-throughput sequencing after a) aerobic and, b) anaerobic incubation. CK, SB, MB, SBB, MBB and B represent the soil samples with none of amendments, straw biochar, magnetic biochar, magnetic bacterial/biochar composite, bacterial/biochar composite and bacteria inoculation, respectively.

distribution and high resistance to harmful external conditions (i.e., high toxicity metal concentrations, high temperature and dry soils) (Vijayaraghavan and Yun, 2008). As a hazardous metal, Cd generally suppresses the growth and colonization of soil bacteria. However, *Bacillus* sp. K1 survives better, reproduces more and remains metabolically active under the stress of high Cd concentrations (Fig. 4). The relative abundance of *Bacillus* in the B, MBB and SBB treatments were increased by 3.24%, 9.18% and 11.47% respectively. This suggests that it may survive and colonization better using biochars as a carrier (MBB and SBB) than direct inoculation (B). The biochar and alginate of the composite would provide nutrition from available organic matter for the bacteria and alleviate the effects of other adverse conditions, accounting for the high abundance of *Bacillus* sp. Palansooriya et al., (2019). Under anaerobic condition, *Clostridium, Lentimicrobium* and *Ruminiclostridium* were the dominant bacterial genera accounting for 22.61–35.45% in soil. The relative abundance of *Bacillus* ranged from 2.4% to 7.6% in anaerobic condition, much lower than under aerobic conditions. The increased spatial isolation of microhabitats in drier soil decreased the competition among species, thus resulting in better inoculation of exogenous bacteria under aerobic condition (Xu et al., 2015; Carson et al., 2010). Moreover, some of the *Bacillus* grew well on a range of carbon sources under aerobic condition, while only fermented glucose

provided energy under anaerobic condition (Shariati et al., 1995). The fermentation supplies less ATP than respiration, leading to adverse effects of exogenous *Bacillus* strain on the competition with indigenous microorganism and decease the relative abundance of *Bacillus* under anerobic condition.

The total bacterial plate counts increased with the biochar and bacterial/biochar composites applications (Table S3). In previously study, the addition of biochar was proved to increase microbial community (Avanthi et al., 2017). The maximum plate counts of bacteria under aerobic and anerobic conditions occurred with SBB and MBB, at 6.42 and 7.39 more than CK. However, adverse effects occurred with MBB and SBB, as shown by the decreased alpha-diversity analysis (Fig. 5). Under anaerobic condition, the alpha-diversity from the Chao1 index was in the order of SB > B > CK > MBB > SBB > MB. Difference in the other two indexes among the treatments were not significant.

Addition of biochar prepared as a as a slow-release fertilizer could continually supply nutrients (N, P and K, etc.) and provide substrate for microbial growth. This would lead to increased α-diversity in biochar treated soils. Zhu et al. (2017). The lowest alpha-diversity was found in the MB treatment. Though, the Fe_3O_4 could stimulate the growth of some bacteria in soil by supplying available iron ions with organic compounds, the excessive Fe(II) ionized following addition of Fe_3O_4

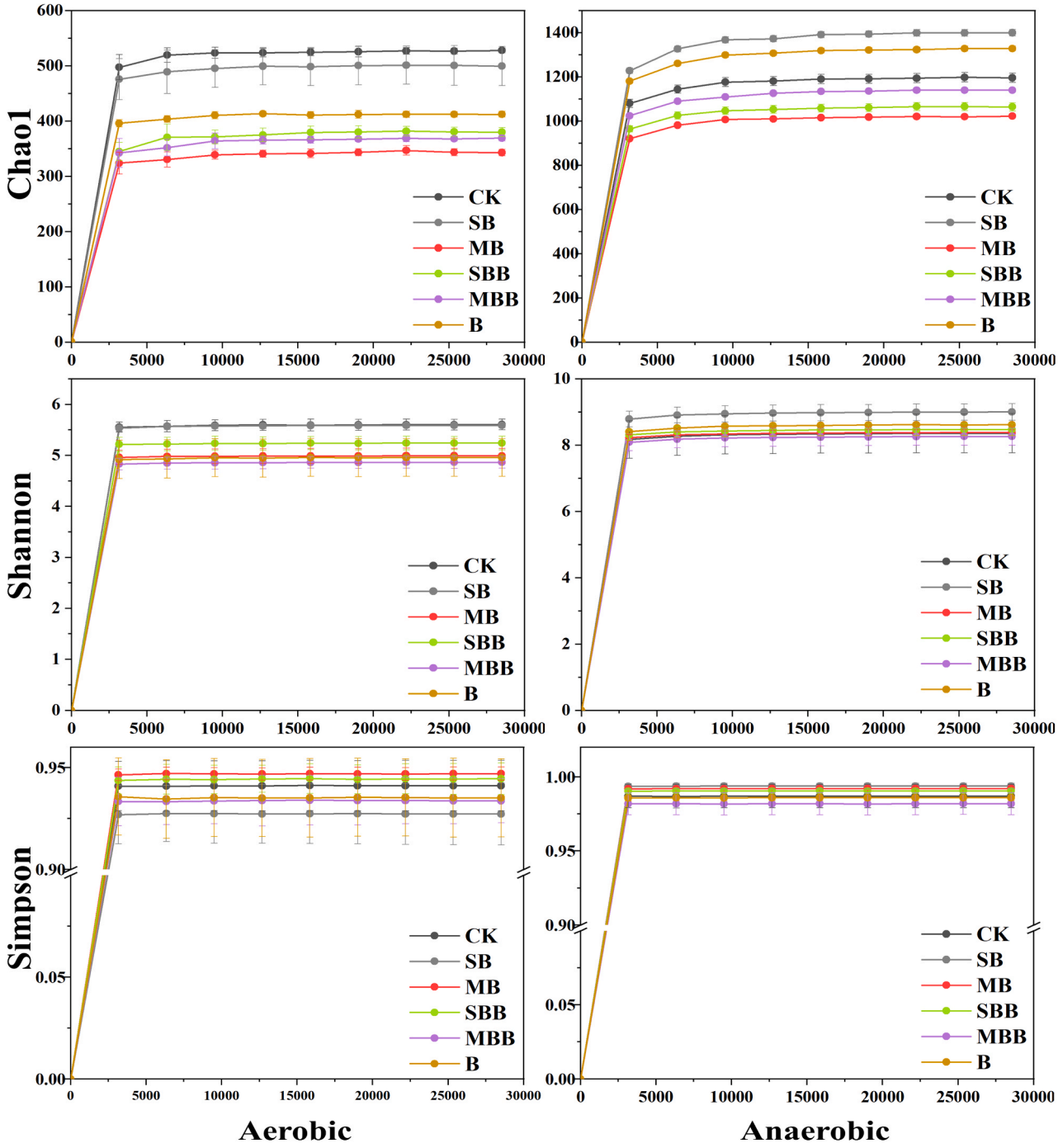

Fig. 5. Changes in microbial alpha diversity (Chao1; Shannon; Simpson) after aerobic and anaerobic incubation in soils (n = 10). All the changes in Shannon and Simpson index were not significant (T-test; two tails). CK, SB, MB, SBB, MBB and B represent the soil samples with none of amendments, straw biochar, magnetic biochar, magnetic bacterial/biochar composite, bacterial/biochar composite and bacteria inoculation, respectively.

could lead to cellular damage via oxidative stress and inhibit of bacterial growth (Ren et al., 2018). In contrast, the MBB application may alleviate the toxic effects by encapsulating Fe_3O_4 into alginate, which slowed its interaction with soil clay and microbes, resulting in higher bacteria alpha-diversity than with MB. In MBB and SBB, the inoculation of *Bacillus* sp. K1 faced competition for nutrients and habitats with indigenous microbes. This would result in the elimination of some indigenous bacterial species, shown the lower alpha-diversity of the soils receiving composites than CK (Palansooriya et al., 2019).

3.3. Interactive relationships between the composites and indigenous microorganisms

Redundancy discriminant analysis (RDA, Fig. 6) and Mantel analysis (Table 1) were conducted to identify the effects of environmental factors on the bacterial community at the genus level. The first axis of RDA explained 58.2% and 47.5% of the cumulative variance of the relationship under aerobic and anaerobic conditions, respectively. This indicates that environmental factors and samples were separated by the first axis. The Mantel statistic r of cumulative environmental factors was also strongly correlated with the microbial community (0.53, $p < 0.01$ and 0.31, $p < 0.01$ for aerobic and anaerobic incubations respectively). This implies that the selected environmental factors were important in explaining the disturbances in the microbial community. The angles between the pH vector with available Cd vector were both over 90° under both aerobic and anaerobic conditions. This indicates that pH was negative correlated with available Cd, ($R^2 = -0.91$, p, 0.001) (Fig. S2). Application of biochar and bacterial/biochar composites in the soil could increase the range of Eh and pH changes, leading to changes in metal mobility (El-Naggar et al., 2018). Soil Cd is converted from highly soluble Cd^{2+}into insoluble forms (i.e. $CdCO_3$, CdS and $Cd(OH)_2$) with increasing pH (Wang et al., 2017). Therefore, of amendments with a higher pH should be preferred to decrease Cd solubility.

Under aerobic condition, available Cd and pH were mainly responsible for changing soil microbial community structure at the genus level following amendment applications. This was shown from the vertical intercept of pH and the available Cd vector on the first axis in RDA analysis (Fig. 6a) and the high Mantel statistic r between the factors and microbial community ($r = 0.79$, $p < 0.01$ and $r = 0.78$, $p < 0.01$, respectively). The *Bacillus* variation was also highly correlated with available Cd and pH according to the RDA image and Mantel analysis ($r = 0.88$, $p < 0.01$ and $r = 0.84$, $p < 0.01$, respectively). Under anaerobic conditions, the distribution of soil microbial community (genus level) and *Bacillus* with the application of amendment were the main factors affected by the biomass C contents. This is supported by the longest vertical intercept of biomass C on the first axis (Fig. 6b) and the highest r value ($r = 0.35$, $p < 0.01$ and $r = 0.40$, $p < 0.01$, respectively). Both available Cd and HOAc-extractable Cd were both indicators to evaluate the mobility and toxicity of Cd in soil to microbes (Ma et al., 2020). *Bacillus* was more sensitive to changes in available Cd ($r = 0.84$, $p < 0.01$) than HOAc-extractive Cd ($r = 0.71$, $p < 0.01$) under aerobic condition, while the opposite occurred under anaerobic conditions. The reasons for these opposite results remain unclear and need further investigation.

Previous studies indicated that the abundance of bacteria was positively correlated with increasing soil pH from acid to neutral (Rousk et al., 2010). Soil pH even determined the growth of the soil bacterial community within a narrow acid pH interval (Fernandez-Calvino and Baath, 2010). Thus, treatment SBB, with the highest pH increment and lowest available Cd altered the soil microbial community under aerobic condition, especially with *Bacillus sp*. The pH of all treatments increased under anaerobic condition, especially in SB, MB, SBB and MBB which pH reached neutral (Fig. 1). Though the difference of pH among treatments was not significant, the microbial communities still shown significant variations. It indicated that pH value was no longer the vital factor for the variation under anaerobic condition. Biomass C is a valid indicator of soil quality changes. The process of fermentation of organic compounds and anaerobic respiration which provides energy for bacteria survival and colonization under anaerobic condition are closely related to soil nutrient availability. Soil microbial processes such as substrate release and uptake are also affected by the application of minerals (Xu et al., 2018b). The highest biomass C contents were in MBB under anaerobic conditions. This may due to the organic carbon and Fe release of the composite and therefore significant altered the microbial community and the distribution of *Bacillus* (Wang et al., 2021).

4. Conclusions

The treatment MBB is a promising amendment for Cd remediation, both under aerobic and anerobic soil conditions. The addition of MBB and SBB both immobilized Cd in soil under aerobic conditions, due to the alkaline natural of biochar and absorption by the added bacteria.

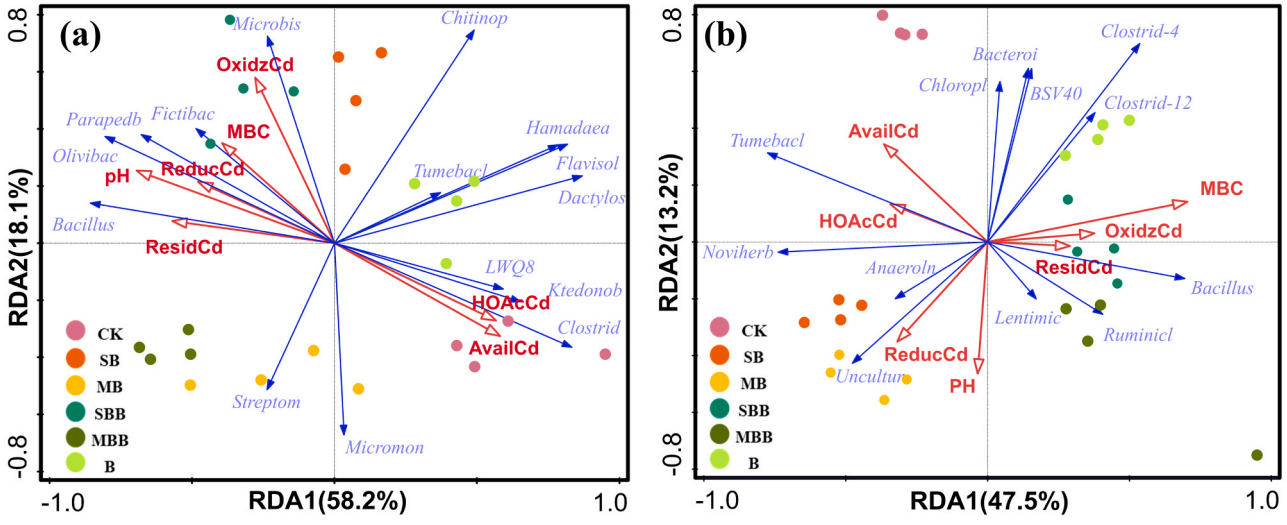

Fig. 6. Environmental explanation of the changes in microbial community (Genus level) and the sample distributions under a) aerobic incubation and b) anaerobic incubation by RDA analysis. Environmental factors in red vectors includes pH, AvailCd (available Cd), HOAcCd (HOAc-extractable Cd), ReducCd (Reductive Cd), OxidzCd (Oxidization Cd), ResidCd (Residual Cd) and MBC (Biomass C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mantel analysis determined the correlation between environmental factors with microbial community (top genera (relative abundant over 1%) and *bacillus*) of soil under aerobic and anaerobic conditions.

	Aerobic				Anaerobic			
	Top genera		*Bacillus*		Top genera		*Bacillus*	
	r value	P value	r value	r value	P value	r value	P value	r value
pH	0.79	>0.01	0.88	>0.01	0.32	0.08	0.15	0.06
Available Cd	0.78	>0.01	0.84	>0.01	0.32	0.02	0.31	0.04
HOAc-extractable Cd	0.61	>0.01	0.71	>0.01	0.17	0.02	0.32	0.02
Reducible Cd	0.31	0.02	0.4	0.04	0.09	0.11	-0.07	0.88
Oxidizable Cd	0.29	0.08	0.29	0.05	0.15	0.1	0.05	0.23
Residual Cd	0.72	>0.01	0.66	>0.01	0.11	>0.01	0.27	0.04
MBC	0.51	>0.01	0.48	>0.01	0.35	>0.01	0.4	>0.01
Total factors	0.53	>0.01	0.6	>0.01	0.31	>0.01	0.4	>0.01

The MBB composite performed better under anaerobic condition because the residual fraction of Cd contained more complexed crystalline iron oxides. The MBB also increased soil biomass C, increased the survival and colonization of *Bacillus sp*. K1 strain, and altered the soil microbial community structure. The pH and biomass C content were the most crucial factor associated with the disruption changes in the microbial community (especially *Bacillus sp*) under aerobic and anerobic conditions, respectively. Therefore, the highest pH value, in SBB caused the largest increase of *Bacillus sp*. K1 colonization under aerobic conditions. In contrast, under anaerobic conditions, the largest increase in biomass C was with MBB. However, the inoculation of exogenic microorganisms with biochar applications may have adverse effect on microbial diversity due to the competition between indigenous microbes and inoculated bacterial strains. The efficiency and mechanisms of soil remediation, and activity changes in microbial community structure ecological risk should be considered in future research on application of bacterial/biochar materials.

CRediT authorship contribution statement

Lu Wang: Investigation, Data curation, Writing- Original draft preparation. **Xingmei Liu**: Conceptualization, Writing - Review & Editing. **Hanrui Chen**: Investigation. **Jizi Wu**: Methodology. **Laibin Huang**: Software and Visualization. **Jorge L. Mazza Rodrigues**: Writing - Review & Editing. **Philip C. Brookes**: Methodology and Resources. **Jianming Xu**: Supervision.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125494.

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