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# A novel calcium-based magnetic biochar is effective in stabilization of arsenic and cadmium co-contamination in aerobic soils

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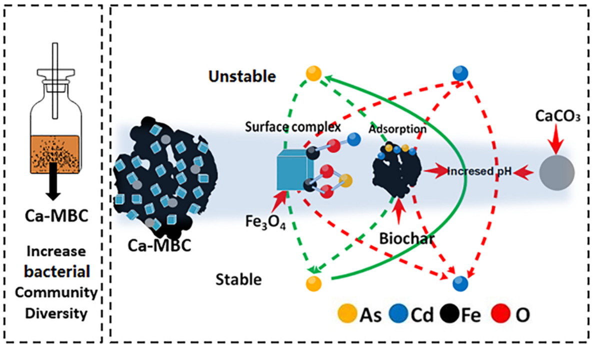
## GRAPHICAL ABSTRACT



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## ABSTRACT

This study developed a novel calcium-based magnetic biochar by pyrolysing rice straw mixed with calcium carbonate and iron oxide for stabilization of contamination of multiple metals. A 160-day incubation study was conducted to investigate its performance in stabilization of cadmium and arsenic co-contamination in soil. Both biochar and Ca-MBC treatments increased soil pH, decreased the bioavailability of cadmium. Ca-MBC decreased but biochar enhanced the bioavailability of arsenic. The BCR (European Community Bureau of Reference) sequential extraction confirmed Ca-MBC facilitated the transformation of the unstable fraction of arsenic to stable fractions. The stabilization mechanisms were explored through synchrotron-based micro X-ray fluorescence and X-ray absorption near edge structure. The results show that Ca-MBC remediated the dual contamination of arsenic and cadmium through (1) elevated pH and cation exchange capacity (for Cd); (2) the formation of bi-dentate chelate and ternary surface complexes on the surface of iron oxide; (3) enhanced adsorption ability of porous biochar. In addition, Ca-MBC increased the abundance and diversity of bacterial community, and modified the relative abundances of bacterial taxa, leading to a shift of the composition. These new insights provide valuable information for stabilization of co-contamination of arsenic and cadmium in soil using the potential material Ca-MBC.

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## 1. Introduction

Heavy metals and metalloids have long been known as hazards to threaten human health, among which cadmium (Cd) and arsenic (As) are the most toxic heavy metals (Ye et al., 2017). Cadmium has been widely reported to cause serious damage to both kidneys and bones (Awual et al., 2018), while As is poisonous to most multicellular lives (Li et al., 2018a; Jeon et al., 2018). Anthropogenic activities like electroplating, battery manufacturing industry (Luo et al., 2011), mining, fossil fuel combustion and pesticides usage (Awual et al., 2018) are the primary sources of Cd and As pollution.

These two elements can co-exist in smelting operations, metal–As bearing materials (such as Cd–As pesticides), and mine drainage (Gräfe et al., 2004), resulting in the co-contamination in soil and water. Research conducted in the co-contaminated areas such as those in Shantou (Yu et al., 2016) and Zhuzhou (Zhang et al., 2018a) reveals that the co-contamination of As and Cd made the rice grains produced in downstream paddy fields exceed the safe Cd and As limits. The contrasting chemical behaviors of anionic and cationic elements make the simultaneous stabilization difficult (Wang et al., 2019). Positively-charged Cd and negatively-charged As can be absorbed on different sites. The bioavailability of Cd will decrease while that of As will increase as pH increases (Qiao et al., 2018). In wet-dry cycles, Cd is less bioavailable under flooded conditions due to precipitation with sulfide (Honma et al., 2016) while As will be more bioavailable under reduced conditions because As adsorbed to Fe(III) oxides can be released into soil solution when the oxides are dissolved due to reduction reactions (Yu et al., 2017). Thus, it's hard to simultaneously lower the mobility of the two elements through modifying soil pH or Eh with one amendment or water management. Therefore, it is imperative to develop an efficient method that can simultaneously remediate the As and Cd co-contaminated soil and water.

Biochar is a carbon-rich product derived from biomass pyrolysis at relatively low temperatures (< 700 °C) under oxygen-limited conditions (Ahmad et al., 2014). Characteristics like porous structures, high surface area, and availability of abundant oxygen-containing functional groups, make biochar a widely-tested material for heavy metal stabilization (Dey et al., 2014; Beiyuan et al., 2017) and carbon sequestration (Lehmann and Joseph, 2009). Due to its negatively-charged surface and porous structure, previous studies have demonstrated the strong adsorption and immobilization ability of biochar for the cationic heavy metals such as Cd(II) (Kołodyńska et al., 2012; Bogusz et al., 2015; Bian et al., 2014), Pb(II) (Kołodyńska et al., 2017) and Cr(VI) (Rajput et al., 2011) but showed limited efficiency in the remediation of anions like phosphate (Knowles et al., 2011) and As (Wang et al., 2015). In comparison, Fe oxide-based materials have shown a considerable remediation ability for As in batch (Suda and Makino, 2016), soil incubation and pot experiments (Yin et al., 2017). Thus, it was proposed that combining Fe oxide with biochar would create a novel magnetic biochar material to immobilize co-contamination of As and Cd.

Previous studies have used magnetic Fe oxide impregnated materials to remediate contamination of heavy metals. However, the majority of these studies have focused on single-pollutant aqueous systems (Wang et al., 2015; Tian et al., 2011; Mohan et al., 2014; Zhang et al., 2013). For the application of magnetic biochars, it is necessary to study whether and how magnetic biochars work for stabilization of As and Cd co-contamination in soil systems. In addition, previous studies have demonstrated that the addition of biochars could have positive effects on soil bacterial community by increasing bacterial abundance and diversity (Gomeza et al., 2014; Xu et al., 2014; Zhang et al., 2018b), but it remains unknown how magnetic biochars would affect the soil bacterial community.

Our previous study (Wu et al., 2018) confirmed that a novel calcium-based magnetic biochar, produced through pyrolysis of rice straw with magnetite and calcium carbonate, could simultaneously remediate As and Cd in an aqueous system. In the current study, we aimed to further understand how the calcium-based magnetic biochar (Ca-MBC) would perform in stabilization of As and Cd co-contamination in soil and to elucidate associated mechanisms.

The primary objectives of this study were (1) to examine the dynamics of As and Cd in a co-contaminated soil by quantifying changes over incubation time in various fractions of the heavy metals, (2) to evaluate the effect of Ca-MBC on speciation, spatial distribution and stabilization mechanisms of As and Cd using synchrotron-based techniques of X-ray absorption near-edge structure (XANES) and micro X-ray fluorescence (μXRF), and (3) to demonstrate how Ca-MBC would affect bacterial community diversity and composition in the soil.

## 2. Materials and methods

### 2.1. Experimented materials

#### 2.1.1. Soil preparation

A paddy soil was collected from the top layer (0 – 20 cm) of a paddy field located in Qingshuitang village, Zhuzhou, Hunan province, China (113°4′36″E, 27°55′37″N). The soil was co-contaminated by Cd and As, with total concentrations of 46.89 μg $g^{-1}$ and 3.01 μg $g^{-1}$, respectively. These levels are significantly higher than the environmental quality standard for agricultural soils (GB 15618-2018) by the Ministry of Ecology and Environment of the People's Republic of China. The soil had a pH of 4.65, cation exchange capacity of 7.1 cmol $kg^{-1}$, soil organic matter (SOM) of 12 g $kg^{-1}$. The content for clay, silt and sand particles is 12.05 %, 28.32 % and 59.63 %, respectively. The available K ($NH_4OAc$ extracted) and P ($NaHCO_3$ extracted) contents are 47.27 and 5.14 mg $kg^{-1}$. The total content of Cr, Cu, Pb and Zn are 27.80 ± 5.12, 13.47 ± 2.11, 29.45 ± 3.44 and 70.23 ± 9.18 mg $kg^{-1}$.

#### 2.1.2. Preparation of amendments

Two amendments, calcium-based magnetic rice straw biochar and rice straw biochar, were used in this study. The Ca-MBC was prepared by pyrolyzing the mixture of rice straw, Fe oxide and $CaCO_3$. The detailed producing procedure was given in our previous study (Gomeza et al., 2014). Briefly, dried rice straw (ground to pass 1 mm) was submerged into a mixed solution containing $Fe^{2+}$ (0.1 mol $L^{-1}$) and $Fe^{3+}$ (0.2 mol $L^{-1}$). The pH of the solution was pre-adjusted to 12 by adding 5 mol $L^{-1}$ NaOH solution. $CaCO_3$ was added at 3 % (mass percentage) into the suspension to avoid the drop in pH caused by the addition of iron oxide. The suspension was then dried and pyrolyzed under 400 °C with limited oxygen for 2 h. Biochar was also produced by the same type of rice straw in an oxygen-limited condition at 400 °C for 2 h. The product was then ground through a 0.15-mm sieve and stored.

The biochar and Ca-MBC used in the study had cation exchange capacity (CEC) of 40.2 and 32.4 $cmol_c$ $kg^{-1}$, respectively. The pH value for the biochar and Ca-MBC is 8.32 ± 0.07 and 8.51 ± 0.09, respectively.

### 2.2. Experiments design

#### 2.2.1. Incubation experiment

One kilogram of air-dried paddy soil (ground to pass 2 mm) was placed in a glass bottle, 220 ml of water was then added into the soil to maintain a moisture content which was equivalent to 40 % of field capacity. The Ca-MBC and biochar were added into the soil at the rates of 1 %, 2 % and 3 % (w/w). The bottles were placed in a constant-temperature illuminating chamber at 25 °C. Each treatment was triplicated including the no-amended control. Water was added every three days to maintain 40 % of field capacity. Soil was incubated for 160days.

#### 2.2.2. Measurements of physicochemical properties

50 g of the soil was collected and freeze-dried at Days 1, 3, 8, 16, 32, 72, 110 and 160 for the measurements of heavy metals and physicochemical properties. As were extracted with 0.01 mol $L^{-1}$ $CaCl_2$ (1:30

soil water ratio, 2 h) and 0.05 mol $L^{-1}$ NH4H$_2$PO$_4$ (1:25 soil water ratio,16 h) for proximate bioavailability measurement, respectively. The concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer 600X, USA). Soil pH was measured using a pH meter (Seven Excellence Cond meter S700, Mettler Toledo, USA) after shaking the soil in 2.5:1 water suspension for 2 h. Dissolved organic carbon was extracted in water at 1:15 soil: water ratio and measured using a TOC analyzer (Multi N/C 3100, Analytik Jena, Germany).

### 2.2.3. BCR sequential extraction

Various factions of As and Cd were extracted using the BCR sequential extraction on soil samples collected on Days 32 and 160. Five fractions of soil heavy metals were extracted according to the following procedure:

Step 1: 1.00 g freeze-dried soil was extracted with 40 ml HOAc (0.11 mol $L^{-1}$) for 16 h in a 100-mL centrifuge tube and then centrifuged at 1500 *g* for 5 min.

Step 2: The residual collected from Step 1 was extracted with 40 ml $NH_2OH·HCl$ (0.5 mol $L^{-1}$, pH = 2.2) for 16 h and centrifuged at 1500 *g* for 5 min.

Step 3: 10 ml of 30 % $H_2O_2$ was added into the residual collected from Step2. The suspension was kept under room temperature for 1 h (oscillating every 15 min). The tube was hot-water bathed (85 °C) till the volume of $H_2O_2$ was less than 3 mL. The procedure was repeated when the tube was cooled down to room temperature.

The residual was then extracted with $NH_4OAc$ (1 mol $L^{-1}$, pH = 2) for 16 h and centrifuged at 1500 *g* for 5 min.

Step 4: The residual collected from Step 3 was dried and digested with $HNO_3$-HF-$HClO_4$ using a microwave digestor (CEM MARS6, Matthews, NC, USA).

### 2.2.4. 16S RNA sequencing

Five grams of the soil was subsampled from each incubation bottle and immediately stored in −80 °C freezer at Days 1, 3, 8, 16, 32, 72, 110 and 160, respectively, for microbial measurement. DNA was extracted from 0.5 g soil using the FastDNA SPIN kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions for soil sample at Days 1, 32 and 160. The DNA was dissolved in 50 μL of sterilized deionized water free of nucleases, and stored at −80 °C till16 s RNA sequencing.

### 2.2.5. Synchrotron radiation analysis

The Ca-MBC was recovered from the freeze-dried soil in 3 % (w/w) Ca-MBC treatment using a magnetite at the end of the incubation. The recovered Ca-MBC was then ground to pass 0.075 mm to examine the distribution and speciation of heavy metals on the material. The synchrotron radiation analysis [micro X-ray fluorescence (μXRF) and X-ray absorption near edge structure (XANES)] was conducted in Shanghai Synchrotron Radiation Facility BL15U1 experimental station and ALS in Berkeley Lab beamline 10.3.2.

### 2.3. Statistical analysis

Means of different treatments were compared by one-way ANOVA at a significance level of $P < 0.05$ through Origin 9.0 software. The 16sRNA data was analyze and charting with R studio.

## 3. Results

### 3.1. Changes in soil properties

The addition of biochar and Ca-MBC increased soil CEC from 7.1 $cmol_c\,kg^{-1}$ to 11.5 $cmol_c\,kg^{-1}$ at most at Day 32.

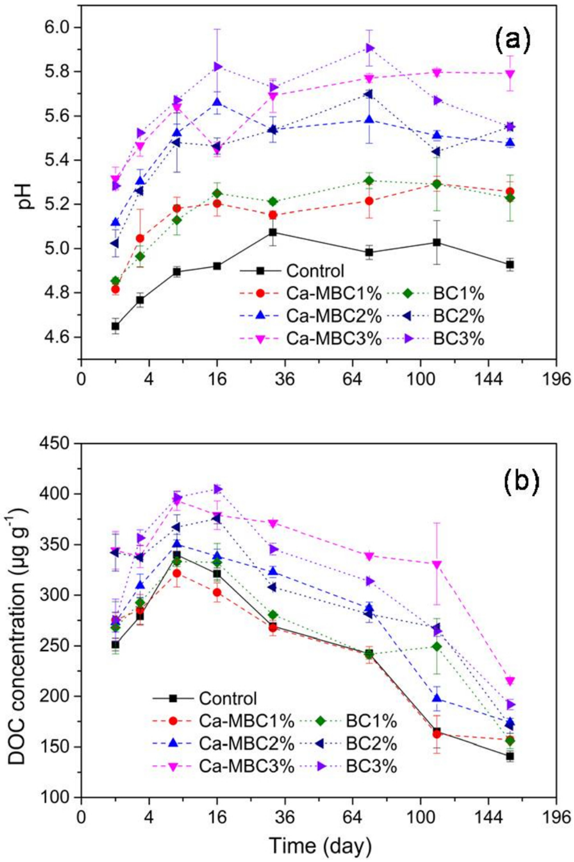

**Fig. 1.** Changes over time in soil pH and dissolved organic C (DOC) concentration in soils amended without biochar (Control), and with magnetic biochar (Ca-MBC) and biochar addition (BC) at addition rate of 1 %–3 % of soil weight. Error bars represent ± standard error.

Comparing to the no-amended control, the addition of biochar and Ca-MBC increased soil pH by 0.3-0.6 units and 0.3-0.9 units, respectively (Fig. 1a). A significant pH increase was observed at Day 1. With incubation time, soil pH first increased and peaked at Day 32 or 72, and then decreased. The pH increased with increasing addition rate. After incubated for 160 d, soil pH of Ca-MBC3 % treatment increased the most by as much as 0.9 units.

For both biochar and Ca-MBC, 2 % or 3 % of amendments addition increased the DOC concentration dramatically comparing to the control (Fig. 1b). Soil DOC concentration first increased and peaked at Day 16, and then decreased. After incubated for 180 days, soil DOC concentration was reduced by 30–50 % by treatments.

### 3.2. Spatial distribution and speciation of heavy metals on the recovered magnetic biochar

The μ -XRF analysis was conducted to investigate the spatial distribution of elements on the recovered Ca-MBC particles (Fig. 2). Iron was distributed in certain blocks rather homogeneously over the whole surface. Arsenic was detected over the whole area with hotspots highly correlated to the Fe hotspots ($R^2$ = 0.789) (Fig. 3). The distribution pattern of Cd was more similar to that of Ca with a relatively higher correlation($R^2$ = 0.498) than between Cd and Fe ($R^2$ = 0.413). To further verify the speciation of arsenic, XANES was conducted on selected spots. As shown in Fig. 4, the As was mainly as As(V) on the surface of the Ca-MBC.

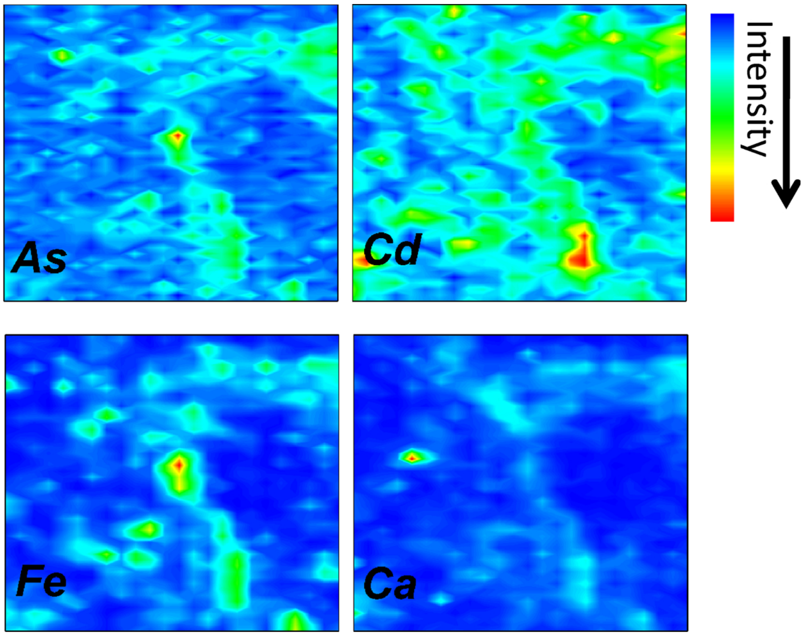

**Fig. 2.** Micro X-ray fluorescence (µXRF) images of the spatial distribution of arsenic (As), cadmium (Cd), iron (Fe) and calcium (Cd) on the surface of recovered magnetic biochar after incubated with soil for 160 d.

### 3.3. *Changes in BCR fractions*

#### 3.3.1. *Arsenic*

For the soil collected on Day 32, increasing addition rate significantly increased the soluble fraction of As by up to 17 %, and the Fe/Mn oxyhydroxide fraction by 23 % in biochar treatments (Fig. 5a, b) while Ca-MBC decreased the Fe/Mn oxyhydroxide fraction. For the organically-bound and sulfide fraction, difference ($P < 0.05$) was only observed in BC1 % and BC2 % with an increase from 17.4%–37.5% compared with the control. Over 97 % of total As was in the residual fraction.

The addition of Ca-MBC significantly increased while biochar decreased As concentrations in the residual fraction although increasing the addition rate did not show a clear pattern.

By Day 160, As concentration in the soluble fraction decreased (Fig. 5b), which was attributed to the transformation to the residual fraction over time. Biochar greatly enhanced while Ca-MBC decreased or did not affect the soluble and the Fe/Mn oxyhydroxide fractions of As. Compared to the control, the Fe/Mn oxyhydroxide fraction in the Ca-MBC1 % and Ca-MBC2 % treatments decreased but increased for all biochar treatments. Both biochar and magnetic biochar addition had no significant changes on the organically-bound and sulfide fraction.

The residual fraction was increased in all treatments except Ca-MBC3 % compared to that on Day 32. The addition of Ca-MBC at 1 % and 2 % increased while biochar decreased the residual fraction of As compared to the control. The high content of Fe oxide in the Ca-MBC3

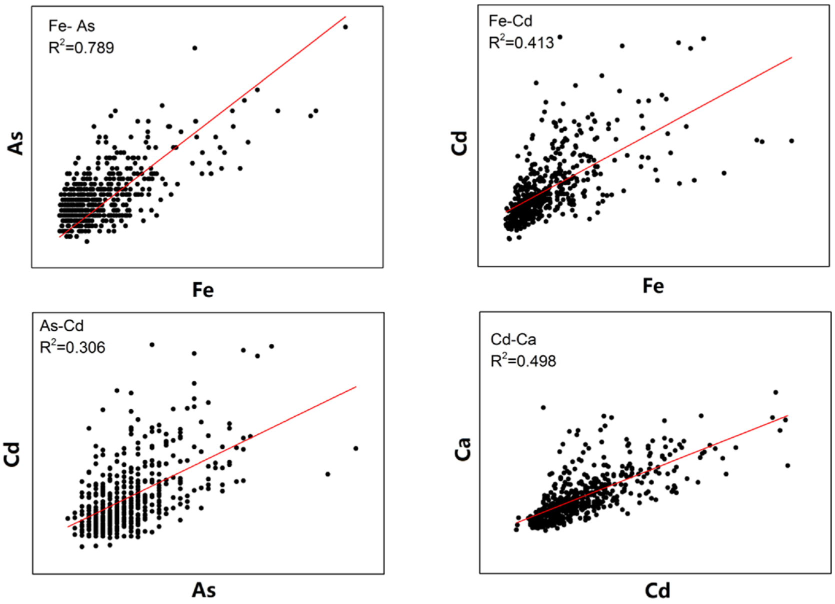

**Fig. 3.** Correlations of spatial distribution between different metal elements.

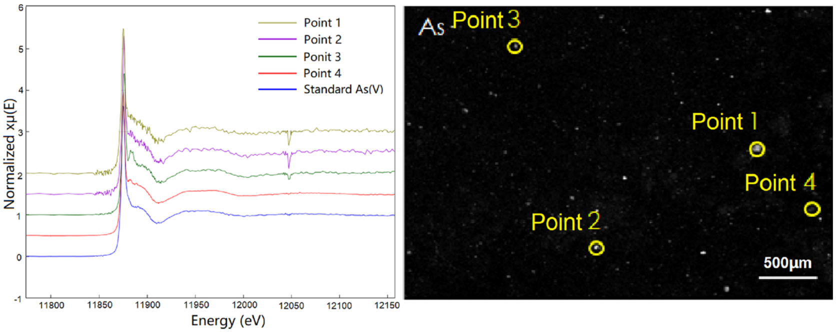

**Fig. 4.** Normalized X-ray absorption near-edge structure (XANES) spectra of arsenic (As) on the surface of recovered magnetic biochar after incubated with soil for 160 d.

% treatment led to an increase in the Fe/Mn oxyhydroxide fraction, which relatively decreased the residual fraction by the end of incubation.

### 3.3.2. Cadmium

As shown in Fig. 5c and d, the Cd concentrations of the four fractions at both Days 32 and 160 were in the order of soluble fraction > residual fraction > Fe/Mn oxyhydroxide fraction > organically-bound and sulfide fraction.

Except for Ca-MBC1 % treatment, all amendments significantly decreased the soluble fraction of Cd compared with the control at Days 32 and 160. Increasing amendment quantity significantly decreased the soluble fraction at Day 32 but this effect diminished by Day 160. In addition, the amendments dramatically dropped the soluble and the Fe/Mn oxyhydroxide fractions of Cd at both Days 32 and 160. The high content of $Fe_3O_4$ in the Ca-MBC was attributed to elevation in the Fe/Mn oxyhydroxide fraction of Cd at Day 160 when Ca-MBC was applied at 2 % and 3 %. The addition of Ca-MBC at 2 % and 3 % slightly decreased the organically-bound and sulfide fraction ($P < 0.05$) at Day 30. By Day 160, the concentration of Cd in Fraction 3 was 3 %–16 % higher in the 2 % and 3 % biochar than the rest treatments. Both amendments increased the concentration of Cd in the residual fraction with the residual fraction increasing over time.

## 3.4. Effects on proximate bioavailability of As and Cd

### 3.4.1. Arsenic

The Ca-MBC treatments had lower concentration of $NH_4H_2PO_4$-extractable As than that the control, while biochar addition elevated that by up to 26 % (Fig. 6b). During Days 1–8, biochar additions lightly

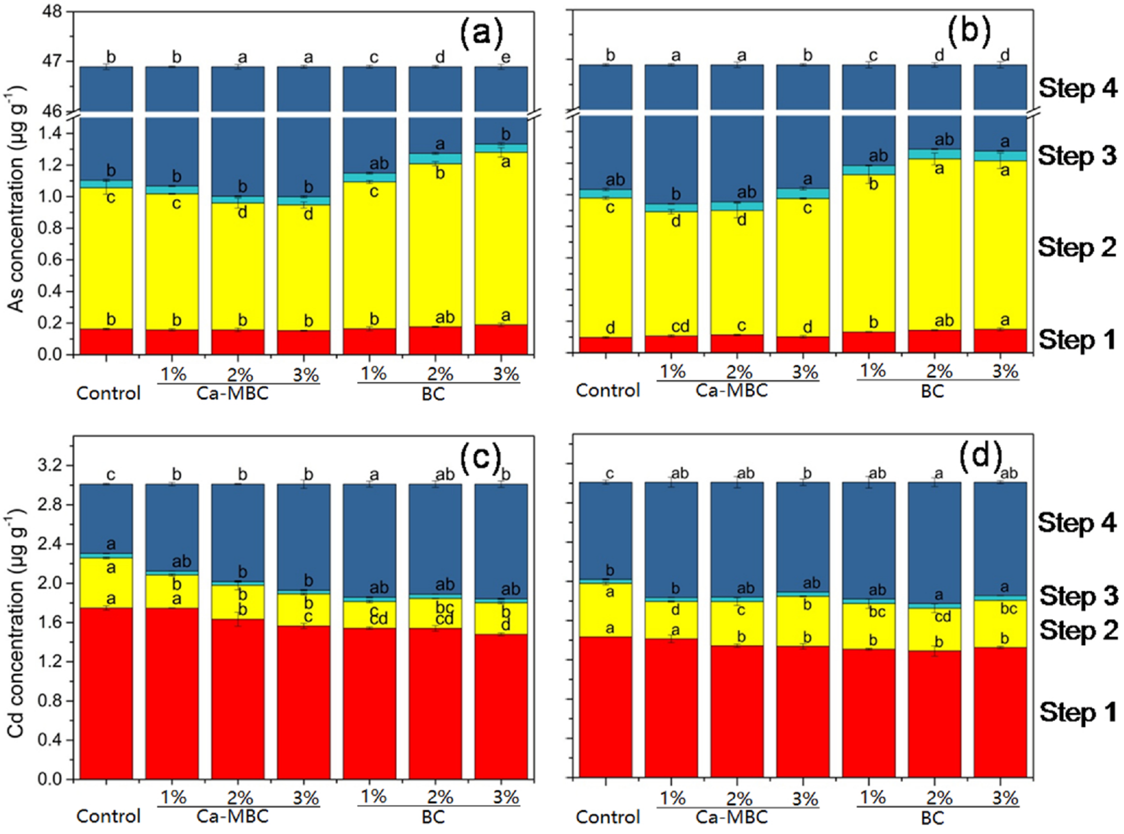

**Fig. 5.** BCR sequential extraction fractions for a) arsenic on Day 32, b) arsenic on Day 160, c) cadmium on Day 32, and d) cadmium on Day160 in soil after incubated without biochar (Control), and with magnetic biochar (Ca-MBC) and biochar addition (BC) at addition rate of 1 %–3 % of soil weight. Error bars represent ± standard error. Step 1, soluble fraction; Step 2, Fe/Mn oxyhydroxide fraction; Step 3, organically-bound and sulfide fraction; Step 4, residual fraction. Heavy metal concentration for each step under different treatments followed by a same letter are not statistically significant at $P < 0.05$.

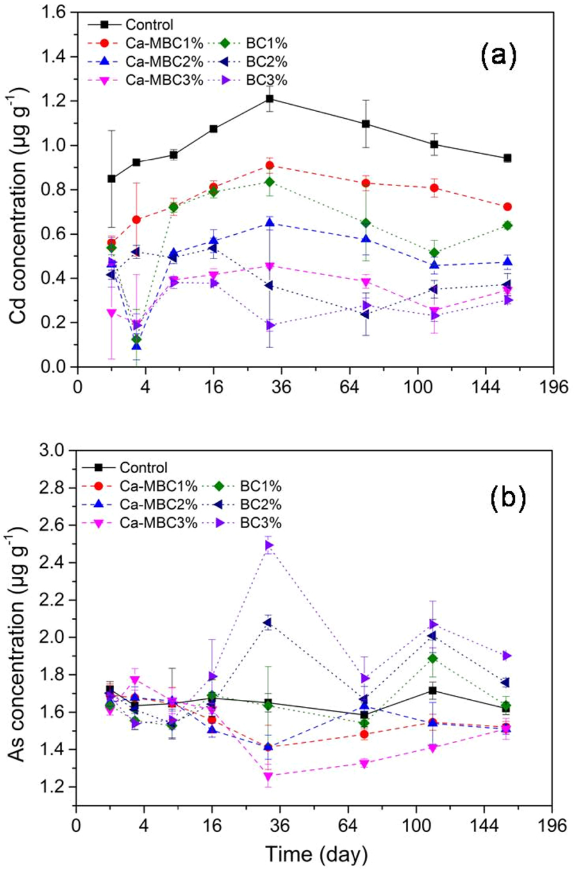

**Fig. 6.** Changes in the concentrations of proximate bioavailable a) cadmium (Cd) and b) arsenic (As) over 160 days without biochar (Control), and under amendments with magnetic biochar (Ca-MBC) and biochar addition (BC) at addition rate of 1 %–3 % of soil weight. Error bars represent ± standard error.

reduced the concentration of $NH_4H_2PO_4$-extractable As but then greatly elevated the $NH_4H_2PO_4$-extracted As concentration and peaked at Day 30, and plateaued thereafter. Increasing addition amount of biochar significantly increased the concentration of $NH_4H_2PO_4$-extractable As.

The addition of Ca-MBC hardly affected the concentration of $NH_4H_2PO_4$-extractable As during Days 1–16 and then dramatically decreased from Day 32. Different from biochar, the addition ratio of Ca-MBC had little effect on the concentration of $NH_4H_2PO_4$-extracted As at the end of incubation (Day 160). $NH_4H_2PO_4$-extractable As concentration was reduced by 12 % comparing to the control at the end of the incubation.

### 3.4.2. *Cadmium*

Compared with the control, amendments decreased the concentration of $CaCl_2$-extractable Cd by 23–68 % (Fig. 6a). For both Ca-MBC and biochar, increasing the addition amount decreased the concentration of $CaCl_2$-extractable cadmium. A very sharp drop in the concentration of $CaCl_2$-extracted Cd could be seen on Day 3 for BC3 % and Ca-MBC2 % treatments. At the end of incubation, the $CaCl_2$-extracted Cd concentration was decreased by 23%–64% for Ca-MBC and 33%–68% for BC.

## 3.5. *Effects on bacterial community*

### 3.5.1. *Overall changes of bacterial community*

The amendments increased the abundance of the bacterial community as indicted by Chao 1 index (Fig. 7). Compared to the control, the Ca-MBC increased but biochar decreased Shannon and InvSimpon indexes which reflected the diversity of the bacterial community.

The alpha diversity index Chao 1 decreased while InvSimpon decreased and then increased with incubation time (Fig. 7b). In addition, the Shannon index was highest at Day 1 and lowest at Day 160.

### 3.5.2. *Detailed change in bacterial composition*

The composition of the bacterial community under different

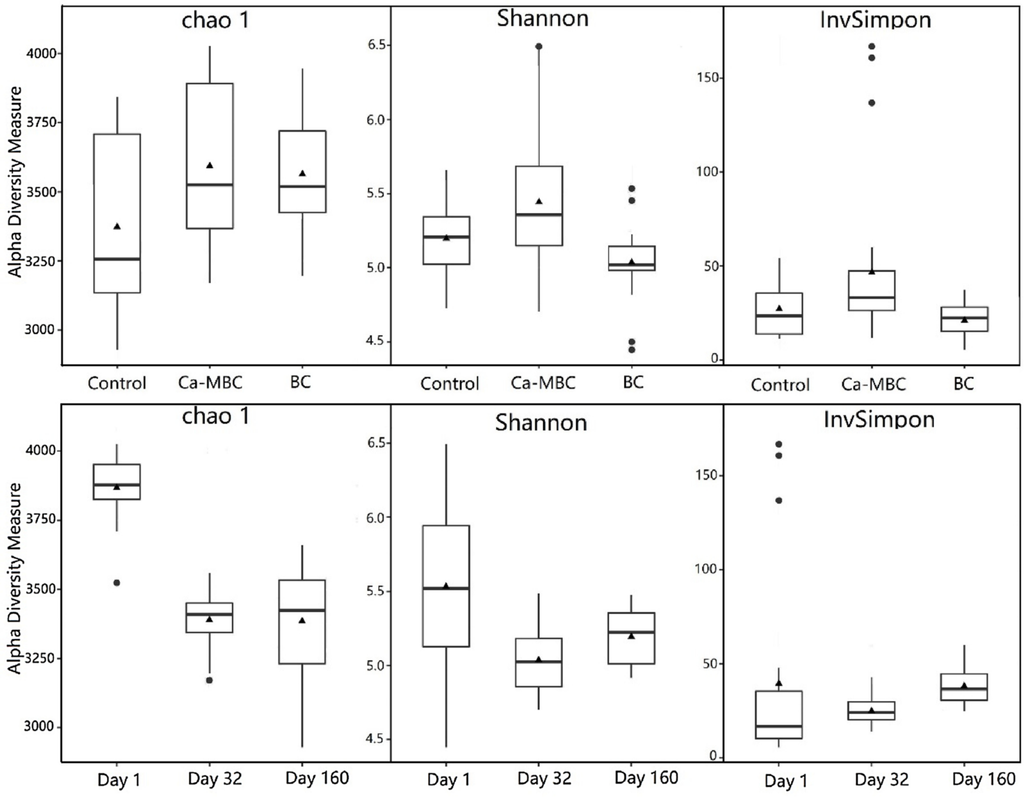

**Fig. 7.** The alpha diversity indexes in soil amended without biochar (Control) and with biochar (BC) and calcium-based magnetic biochar (Ca-MBC) (top), and at Days 1, 32 and 160 after incubation (bottom). Bars show the maximum (top edge) and minimum (lower edge) percentiles, the lines at the top, bottom, and middle of the box correspond to the 75th, 25th, and 50th percentiles (median), respectively. The solid triangle represents the means value.

treatments and incubation times at the phylum level was displayed in Fig. 10a. Under all treatments, *Firmicutes*, *Actinobacteria*, *Chloroflexi* and *Proteobacteria* were the dominating taxa which contributed to the most relative abundance (> 90 %). The addition of Ca-MBC decreased while biochar treatments increased the abundance of *Firmicutes* on Day 1. The abundance of *Firmicutes* had a sharp drop over time and the addition of amendments somehow ease the drop. Over the incubation time, the abundance of *Chloroflexi* was greatly elevated in the control, slightly increased in biochar treatments, and decreased in Ca-MBC treatments. The relative abundance of *Actinobacteria* and *Bacteroidetes* increased under all treatments but was elevated more with the addition of Ca-MBC and biochar. The abundance of *Acidobacteria* decreased over time and the addition of amendments amplified the decrease.

## 4. Discussion

### *4.1. Changes in soil properties affect proximate bioavailability of heavy metals*

Soil cation exchange capacity (CEC) reflects the negative charges of soil that can be neutralized by exchangeable cations including nutrients such as ammonium, calcium and heavy metals like Cd and Cu. In this study, the addition of Ca-MBC and biochar significantly increased soil CEC leading to a stronger adsorption ability for Cd which could explain the decrease in Cd proximate bioavailability.

Soil pH can greatly influence the concentration of bioavailable heavy metals. As soil pH increases, most soil particles and amendments become more negatively-charged due to deprotonation of phenolic, hydroxyl, carboxylic and other acidic functional groups of the amendments and soil. Thus, more negatively-charged sites lead to an increased attraction for Cd but repulsion for the As anions. Besides, the change of pH can affect the existing form of Cd and As. As soil pH increases, Cd can be gradually transformed into $Cd(OH)_2$, a more stable and non-bioavailable form which becomes the dominant species when pH > 8 (Reddy and Lee, 2014). On the contrary, more As exists as negatively-charged free state rather than molecular form, and As becomes more bioavailable as soil pH increases. In our present study, the relatively high correlation between Cd and calcium further confirmed that Cd was distributed on surface sites corresponding to high-pH spots on the surface of magnetic biochar, which had mainly resulted from biochar and calcium carbonate.

In this present study, the soil amended with Ca-MBC and biochar had pH below 6 (5.2–5.8) which is lower than the pH to cause fully precipitation of Cd (8.86) (Qian et al., 2013). Thus, the dramatic drop in the concentration of proximate bioavailable Cd was probably also driven by co-precipitation of Cd with other hydroxide precipitates and adsorption by magnetic biochar/biochar or soil colloids.

Increasing DOC concentration can suppress the adsorption of Cd onto soil particles and increase the formation of soluble metal-organo complexes (Antoniadis and Alloway, 2002). Besides, DOC can compete with As for the retention sites on soil particles (Hartley et al., 2009) and hence elevated DOC concentration results in an increase in the concentration of soluble arsenic. This could also be a reason for the elevation in proximate bioavailable As concentration in the biochar treatments because the addition of biochar increased DOC concentration comparing to the control in our present study. With an increased DOC concentration, the proximate bioavailability of As still decreased in the Ca-MBC treatments, further confirming the stability of Ca-MBC for As. Meanwhile, with elevated DOC concentration in the biochar and Ca-MBC treatments, the concentration of proximate bioavailable Cd decreased, implying other factors such as increased pH upon addition of amendments dominate the process.

A lag could be found in the change of proximate bioavailable As comparing with the changes in pH and DOC. That was the reason why As concentration was low at the beginning period of the incubation and highest pH and DOC values occurred on Day 16 while the highest As concentration occurred on Day 32. For Cd, as water was added into the soil at the very beginning of incubation, stronger microbial activities in the incubation system were expected comparing to the air-dried soil. Part of the Cd precipitation might be dissolved due to the strong microbial activities leading to a slight increase in proximate bioavailable Cd concentration during the first 32-day incubation.

The concentration of proximate bioavailable Cd decreased over time, which was likely attributed to the aging effect (Li et al., 2018b). The aging effect was usually driven by the complexation, surface adsorption and precipitation of metal in soil matrix (Huang et al., 2015), which was also dependent on the microbial activity. As discussed before, the decreased DOC concentration over time could results in decreased concentrations of proximate bioavailable cadmium.

### *4.2. Ca-MBC remediate heavy metal co-contamination*

As discussed above, the Ca-MBC can absorb and complex the As in soil. Thus, the addition of Ca-MBC reduced the soluble fraction and increased the residual fraction of As. The high content of Fe on the surface of Ca-MBC did not elevate but rather decreased the Fe/Mn oxyhydroxide fraction of As. This indicates that the Ca-MBC could bind with the Fe/Mn oxyhydroxide fraction of As, and that the adsorption and surface complex between As and Ca-MBC was strong enough to not be extracted in the second step of BCR extraction.

On the contrary, biochar addition led to the transformation of the stable As (residual fraction) into unstable As (soluble and Fe/Mn oxyhydroxide fractions). As discussed above, the elevated pH and DOC concentration resulted in an increase of soluble As. It is noticeable that the Fe/Mn oxyhydroxide fraction of As increased and this increased part was mainly transferred from the residual fraction.

Both biochar and Ca-MBC treatments increased the transformation of the unstable Cd (soluble and Fe/Mn oxyhydroxide fractions) into stable Cd (residual fraction), which had resulted from the elevated pH.

Unlike Cd, As may be mobilized following an increase in pH (Beesley et al., 2014) upon the application of alkaline amendments such as biochar, lime and compost. The results in the present study are in agreement with previous researches, suggesting that the application of biochar would elevate As proximate bioavailability. Comparing to the biochar, Ca-MBC successfully decreased the concentration of proximate bioavailable As after incubation in the present study. Iron oxide has been reported to have a strong sorption for As, while As can form mono-(with As(III) or bi-dentate (with As(V) chelate on the surface of Fe oxide (Ohtsuka et al., 2013), which would mainly be bi-dentate chelate. The XANES spectrum confirmed the domination of As(V) on the surface of magnetic biochar. The μ-XRF results indicated that As was mainly distributed on Fe oxide whereas Cd bound to $CaCO_3$, biochar and Fe oxide, which is in agreement with our hypothesis.

Our previous study (Wu et al., 2018) on the co-adsorption of Cd and As in an aqueous system also showed that As was strongly adsorbed on the Ca-MBC and competed for adsorption sites with Cd but meanwhile formed ternary surface complexes to promote the adsorption of Cd. The high correlation between As and Fe distributed on the surface of Ca-MBC particles in the present study further proved the strong ability of Fe oxide to absorb or bind As. In addition, As and Cd co-localized on the Fe hot-spots, which also suggests the formation of ternary surface complexes on Fe oxide distributed on the surface of Ca-MBC. With this respect, biochar had a better removal efficiency for Cd, meaning that the competition for adsorption sites surpassed the synergistic effect caused by ternary surface complexes.

### *4.3. Ca-MBC increased the diversity of bacterial community*

The PCoA PERMANOVA analysis showed that amendments and incubation time had significant effects on the bacterial community (Fig. 8). A canonical analysis of principal coordinates (CAP) was conducted to further investigate the effects of environmental factors on the

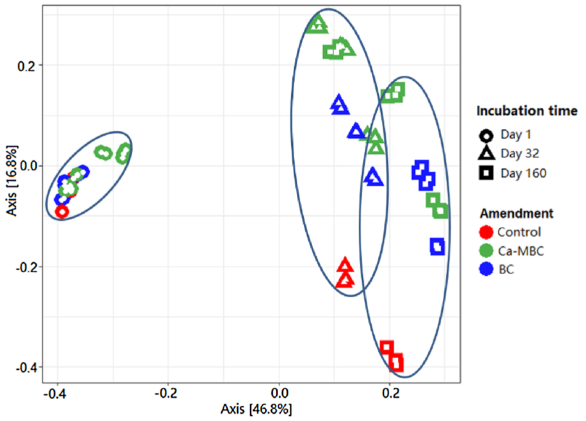

**Fig. 8.** Principal coordinate analysis (PCoA) permutational multivariate analysis of variance (PERMANOVA) tests for the microbial community in soil at Days 1, 32 and 160. Control, with no biochar addition; Ca-MBC, with magnetic biochar addition; BC, with biochar addition.

bacterial community with the effect being in the order of pH > DOC > addition ratio of amendments > bioavailable Cd concentration > bioavailable As concentration (Fig. 9). The angle between the addition rate vector and DOC/ pH vector was less than 90°, meaning that the addition rate positively correlated with both DOC and pH. On the contrary, it negatively correlated with the proximate bioavailable concentration of the two elements which all conformed to the results from the soil chemical analyses.

Generally, the addition of Ca-MBC had a very similar effect on soil bacterial community compared with biochar treatments in terms of the abundance, diversity and composition. The abundance and diversity of bacterial community were increased after incubation with the amendments which agreed well with previous studies (Zhang et al., 2018c). Changes in soil physicochemical properties and the properties of Ca-MBC were the main reason for the increase in abundance and diversity of soil bacterial community. Soil pH has been proven to be an important factor affecting the bacterial abundance and diversity by many studies (Sheng and Zhu, 2018; Murray et al., 2015; Feng et al., 2010). The bacterial diversity could be higher as soil pH increased from acid to neutral. Besides, the porous structure of magnetic biochar/biochar could have provided an appropriate environment for the survival of soil microbes (Zhang et al., 2018c).

### *4.4. Ca-MBC altered the composition of bacterial community*

This study showed that heavy metal pollution altered bacterial composition in soil, as indicated by the abundance of phylum *Proteobacteria*. *Proteobacteria* was often found to be an abundant phylum in the environments highly polluted with heavy metals, for example in neutral mine drainage (Zhang et al., 2014), As mine sediments (Chen et al., 2016) and soils high in heavy metals (Gołebiewski et al., 2014). In this present study, *Proteobacteria* was highly abundant in a paddy soil co-contaminated with Cd and As, consistent with the finding of Lorenz et al. (Lorenz et al. (2006)).

In addition, the Ca-MBC had shaped the bacterial composition to a certain extent through elevated pH and DOC. As soil pH increases, a more gram-negative bacterium-dominated community would be observed (Feng et al., 2010). Besides, the increased DOC means a more labile-carbon pool. Thus, the addition of Ca-MBC significantly increased the abundance of copiotroph phylum such as *Bacteroidetes*. Furthermore, the addition of Ca-MBC sharply lowered the abundance of *Acidobacteria* which was reported to be abundant in acid soils (Hanada et al., 2014; Verastegui et al., 2014).

*Actinobacteria* were reported to have a strong ability to degrade the recalcitrant polymers which were introduced into the soil by the addition of magnetic biochar, such as quinolone, benzoic acid and phthalic acid (Zheng et al., 2016), leading to an increase in the relative abundance of *Actinobacteria*. In contrast, the addition of Ca-MBC decreased the relative abundance of *Chloroflexi*, which is consistent with the results of increased pH and DOC. Similar results were observed in other studies (Xu et al., 2016; Zhang et al., 2018b), however, the specific mechanisms are unknown.

The results from our present study indicate that the Ca-MBC could be an efficient amendment for the stabilization of Cd and As co-contamination and also change bacterial abundance, increase soil bacterial α-diversity and modify relative abundances of taxa leading to a shift of the bacterial community composition in soil.

## 5. Conclusions

The Ca-MBC and biochar amendments can increase both soil pH and DOC concentration. The addition of Ca-MBC decreased the concentrations of both proximate bioavailable As and Cd while biochar decreased the concentration of proximate bioavailable Cd but failed to lower that of As. The BCR extraction further confirmed that the Ca-MBC could transform the unstable As faction into the stable fraction. Ca-MBC could simultaneously remediate As and Cd mainly through (1) elevated pH and CEC (for Cd); (2) the formation of bi-dentate chelate and ternary surface complexes on the surface of Fe oxide (for As and Cd); and (3) the great adsorption ability of porous Ca-MBC surface (for As and Cd). Additionally, the Ca-MBC changed bacterial abundance, increased soil bacterial α-diversity and modified relative abundances of taxa leading to a shift of the bacterial community composition. It appears that the Ca-MBC is an efficient and environmentally-friendly amendment to remediate As and Cd co-contamination in soil.

## CRediT authorship contribution statement

**Jizi Wu:** Conceptualization, Writing - original draft, Methodology, Software, Validation, Formal analysis, Visualization, Investigation. **Zhangtao Li:** Resources, Investigation, Data curation. **Dan Huang:** Resources, Investigation. **Xingmei Liu:** Project administration, Resources. **Caixian Tang:** Writing - review & editing. **Sanjai J. Parikh:** Writing - review & editing. **Jianming Xu:** Conceptualization, Resources, Supervision, Writing - review & editing, Project administration.

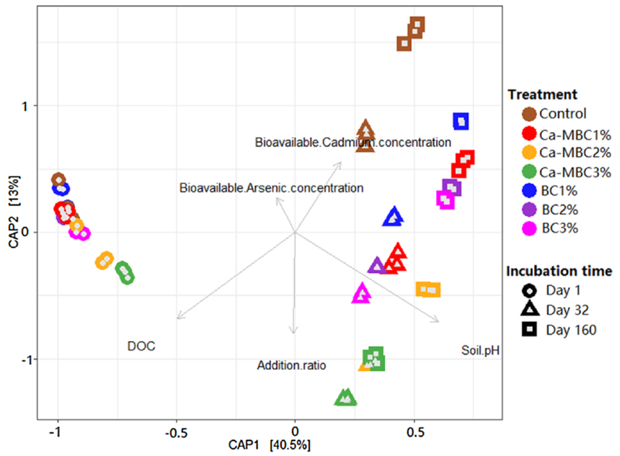

**Fig. 9.** Canonical analysis of principal coordinates (CPA) of bacterial community and environmental variables (pH, bioavailability of heavy metals, dissolved organic C (DOC)) for individual samples across addition rates of 1.0 %, 2.0 % and 3.0 % on Days 1, 32 and 160 after incubation. Control, with no biochar addition; Ca-MBC, with magnetic biochar addition; BC, with biochar addition.

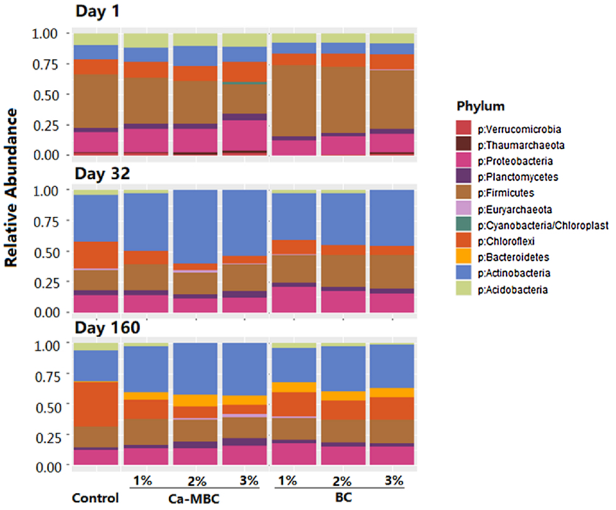

**Fig. 10.** Relative abundances of soil microbial taxa at the phylum level revealed by 16S rRNA high-throughput sequencing, at Days 1, 32 and 160 after incubated without biochar (Control), and with calcium-based magnetic biochar (Ca-MBC) and biochar addition (BC) at addition rate of 1 %–3 % of soil weight.

## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.122010.

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