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Effects of biochars on the bioaccessibility of phenanthrene/pyrene/zinc/lead and microbial community structure in a soil under aerobic and anaerobic conditions

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ABSTRACT

The immobilization of co-contaminants of organic and inorganic pollutants by biochar is an efficient remediation strategy. However, the effect of biochar amendments on the bioaccessibility of the co-contaminants in dry *versus* flooded soils has rarely been compared. In batch experiments, bamboo-derived biochar (BB) had a higher sorption capacity for phenanthrene (Phe)/pyrene (Pyr)/zinc (Zn) than corn straw-derived biochar (CB), while CB had a higher sorption capacity for lead (Pb) than BB. After 150 days of incubation, the amendments of 2% CB, 0.5% BB and 2% BB effectively suppressed the dissipation and reduced the bioaccessibility of Phe/Pyr by 15.65%/18.02%, 17.07%/18.31% and 25.43%/27.11%, respectively, in the aerobic soils. This effectiveness was more significant than that in the anaerobic soils. The accessible Zn/Pb concentrations were also significantly lower in the aerobic soils than in the anaerobic soils, regardless of treatments. The Gram-negative bacterial biomass and the Shannon–Weaver index in the aerobic soil amended with 2% CB were the highest. The soil microbial community structure was jointly affected by changes in the bioaccessibility of the co-contaminants and the soil physiochemical properties caused by biochar amendments under the two conditions. Therefore, dry land farming may be more reliable than paddy soil cultivation at reducing the bioaccessibility of Phe/Pyr/Zn/Pb and enhancing the soil microbial diversity in the short term.

Introduction

Co-contamination of organic and inorganic pollutants has become a serious issue in agricultural soils located near manufacturing regions because of industrial waste production, wastewater irrigation, and pesticide and chemical leakage during agricultural production (Usman et al., 2016). The available fraction of these pollutants in soil can be taken up by crops and transferred through the food chain, eventually posing a threat to human health (Usman et al., 2016). Therefore, immobilization of the co-contaminants in soil, which seeks to reduce their transportation and bioavailability, is an effective soil remediation strategy to reduce these environmental risks (Ahmad et al., 2014; Mohan et al., 2014).

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The use of biochar in environmental remediation has been extensively investigated (Meyer et al., 2011). Biochars can reduce the mobility and bioavailability of the organic pollutants and heavy metals in soils through their large surface area and rich functional groups *via* physical adsorption, cation exchange, hydroxide formation and carbonate precipitation (Sohi et al., 2010; Beesley et al., 2010; Park et al., 2011; Cao et al., 2011). Soil-use (*e.g.*, dry land or paddy soil) can affect the environmental behavior of contaminants in soil and biochar-amended soil (Shankar, 2017). Studies have reported that biochar can reduce the availability of the co-contaminants of organic pollutants and heavy metals in soils under aerobic conditions (Beesley et al., 2010; Cao et al., 2011; Wu et al., 2016). For example, Beesley et al. (2010) reported that amendment with hardwood-derived biochar decreased the concentration of accessible polycyclic aromatic hydrocarbons (PAHs) by more than 50% and induced a 10-fold reduction in the available Cadmium (Cd) in the soil pore water of aerobic soils. The extractability of $CaCl_2$ after 1 hr of incubation was reduced by 25% for zinc (Zn) and 52% for lead (Pb) in the presence of 5% *Miscanthus* straw-derived biochar under aerobic conditions (Houben et al., 2013). Compared with the studies on the immobilizing potential of biochar in aerobic soils, those on the effect of biochar concerning the bioavailability of contaminants in anaerobic soils (*e.g.*, paddy soil) have been less reported. Chen et al. (2008) discovered that the PAH sorption was significantly enhanced in paddy soil amended with pine needles-derived biochar. The amendment of acidic paddy soil with sewage sludge biochar can reduce the amounts of bioavailable arsenic, chromium, cobalt, nickel, and Pb (Khan et al., 2013). Over five years, wheat straw-derived biochar consistently decreased the bioavailable Cd and Pb in a contaminated paddy field (Cui et al., 2016). However, these studies all investigated either organic pollutants or heavy metals. Whether biochar can simultaneously immobilize the co-contaminants of organic pollutants and heavy metals in anaerobic (flooded) soil has rarely been reported. The difference in the bioaccessibility of the co-contaminants in the biochar-amended soils under aerobic *versus* anaerobic conditions also remains unclear. Selection of a reasonable cultivation method for remediated soil amended with biochar is considerably important to ensure safe agricultural production.

The activity and diversity of soil microorganisms that are important for the ecological functions of agricultural soils might be decreased by the combined pollution of heavy metals and PAHs (Bourceret et al., 2016; Cao et al., 2008). The physical properties of biochars (*e.g.*, their pore structure, surface area and mineral content) support the functioning of soil biota (Lehmann et al., 2011). Thus, the relationships among co-contaminants, biochars and microorganisms should be deeply understood. Biochar amendment might increase the microbial diversity of PAH-contaminated soil (Liu et al., 2015). By contrast, Meynet et al. (2012) observed that the activity of PAH-degrading bacteria was more notable in unamended soil than in the soil amended with activated carbon. However, reports comparing the effects of biochar on the soil microbial community structure under aerobic *versus* anaerobic conditions are also lacking. Studying the changes in microbial community structure is helpful for selecting reasonable cultivation methods for contaminated soils amended with biochar.

This study aimed to (1) compare the effects of biochar on the bioaccessibility of the co-contaminants of organic and inorganic pollutants in a field soil under aerobic *versus* anaerobic conditions and (2) investigate the influence of biochar amendments on the microbial community structure under the two incubation conditions. Corn straw-derived biochar (CB) and bamboo-derived biochar (BB) were amended into an agricultural soil that was co-contaminated with phenanthrene (Phe)/pyrene (Pyr)/Zn/Pb. The soils were incubated under aerobic and anaerobic conditions to mimic upland and paddy soils, respectively. A batch sorption experiment was conducted to elucidate the Phe/Pyr/Zn/Pb sorption capacities of the biochars. A phospholipid fatty acid (PLFA) assay was conducted to analyze the soil microbial community structure. To the best of our knowledge, this is the first study to compare the effects of biochar amendments on the bioaccessibility of organic and inorganic co-contaminants and on the microbial community structure under aerobic *versus* anaerobic soils.

1. Materials and methods

1.1. Soil and biochar preparation

An agricultural upland soil co-contaminated with Phe/Pyr/Zn/Pb for more than 40 years was sampled at a depth of 0–20 cm in an area near a steel mill in the suburb of Nanjing, Jiangsu Province. The soil was air-dried and sieved through a 2 mm mesh. The soil had a pH of 7.36 and consisted of 27.60% sand, 63.52% silt, and 8.88% clay. The dissolved organic carbon (DOC) content of the soil was 309 mg/kg, and the total nitrogen (N), phosphorus (P), and potassium (K) contents of the soil were 1.3, 0.57, and 17.4 g/kg, respectively.

Phe and Pyr were chosen as model compounds in this study because they exist widely in PAH-contaminated soils, and in relatively higher concentrations than other PAH compounds (He et al., 2008). The Phe and Pyr concentrations in the soil samples were (258.59 ± 5.92) and (736.36 ± 18.10) μg/kg (dry weight), which exceeded the corresponding environmental quality standards for soils in China (GB 15618-2008) by one and two orders of magnitude. In addition, the Zn and Pb concentrations in the soil were (116.93 ± 14.36) and (38.64 ± 2.34) mg/kg (dry weight), respectively, which exceeded the corresponding environmental quality standards for soils in China (GB 15618-2008) by one order of magnitude.

CB was produced under oxygen-limiting conditions at a final temperature of 300°C using a patented biochar reactor (No. ZL2009 2 0232191.9) (Jia et al., 2013). BB, which is a representative of commercial biochar and produced at 700°C, was purchased from Shanghai Hainuo Charcoal Co., Ltd. The surface area, pore volume, and pore size of these biochars were measured *via* the Brunauer–Emmett–Teller (BET) method using a V-Sorb 2800P analyzer (Gold APP Instruments Corporation, Beijing, China). The elemental compositions were determined using an elemental analyzer (ANA1500, Carlo Erba, Milano, Italy) (Yuan et al., 2011). Table 1 details the physicochemical properties of the biochars.

The concentrations of the ∑16 PAHs in CB and BB were 778.72 and 413.17 μg/kg, respectively, which are both below the recommended maximum limit set by the International

Table 1 – Basic properties of corn straw-derived (CB) and bamboo-derived biochars (BB).

	CB	BB
pH	8.25	9.77
Dissolved organic matter (mg/kg)	425	83
BET surface area (m^2/g)	11.66	42.82
Pore volume (cm^3/g)	0.0219	0.0217
Pore size (nm)	6.34	4.28
Total phosphorus (g/kg)	2.95	0.88
Total potassium (g/kg)	32.89	8.27
C (%)	48.12	83.30
H (%)	3.94	2.67
O (%)	46.26	13.71
N (%)	1.50	0.24
S (%)	0.18	0.08
H/C	0.08	0.03
O/C	0.96	0.16
(N + O)/C	0.99	0.17
Phenanthrene (μg/kg)	109.81	74.63
Pyrene (μg/kg)	16.94	1.94
Zinc (mg/kg)	29.19	39.46
Lead (mg/kg)	1.55	4.31

BET: Brunauer–Emmett–Teller.

Biochar Initiative (IBI, 2012) for use in agriculture. The total concentrations of Zn/Pb/Cd/copper (Cu) in CB or BB (Table 1) did not exceed the maximum acceptable limit recommended by the State Environmental Protection Administration, China (SEPA, 1995).

1.2. Design of sorption experiment

A batch equilibrium experiment was conducted to verify the ability of the biochars used to sorb the contaminants and to compare the sorption capacity of the biochars for Phe/Pyr/Zn/Pb. The sorption of Phe/Pyr onto the biochars was determined using the batch equilibrium method (Song et al., 2013), and the Phe/Pyr concentrations were determined using a gas chromatography–mass spectrometry (GC–MS, Agilent 7890A/5975C, Santa Clara, CA). The sorption of Zn/Pb onto the biochars was also determined using the batch equilibrium method (Chen et al., 2011; Inyang et al., 2012), and the Zn/Pb concentrations were determined *via* inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700×, Agilent Technologies, Waldbronn, USA). All treatments were conducted in triplicates. The detailed experimental procedures are provided in the Supporting information (SI).

1.3. Amendment of soil with biochars

Briefly, 3 kg of soil was thoroughly and manually mixed with 15 (0.5%) or 60 g (2%) biochar in a storage box. The amended soil was subsequently transferred into a cylindrical polyvinyl chloride pot (20 cm in height and 20 cm in bottom diameter). The pots were subsequently divided into the Aerobic Group (under aerobic conditions) and the Anaerobic Group (under anaerobic conditions). The microcosms in the Aerobic Group were watered up to 60% of their water holding capacity and then equilibrated weekly, and the water levels of the microcosms in the Anaerobic Group were maintained at 2 cm above the soil surface. Therefore, two groups were established and 0.5% CB, 2% CB, 0.5% BB, 2% BB, and Control (without amendment), were performed in each group. All treatments were conducted in triplicates. All of the soils were incubated in a house under natural diurnal light conditions with a daytime temperature of 22–35°C and a nighttime temperature of 13–25°C from May to October 2015 to simulate actual field conditions. Every 30 days, 10 g of soil from each pot was sampled using a soil borer to measure the total and bioavailable concentrations of Phe/Pyr/Zn/Pb. At the end of the incubation, 5 g of soil was sampled and freeze-dried to detect PLFA.

1.4. Soil analysis

The total Phe/Pyr in the soils was extracted *via* accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA, USA) (Zhang et al., 2013). The accessible Phe/Pyr concentrations were extracted with hydroxypropyl-beta-cyclodextrin (HPCD) (Oleszczuk, 2009), and subsequently detected *via* GC–MS (Agilent 7890A/5975C, Santa Clara, CA).

The total Zn/Pb in soils was extracted using acid digestion. The fraction of available Zn/Pb was extracted using a reagent containing ethylene-diamine-tetra-acetic acid disodium ($EDTA\text{-}Na_2$: 0.05 mol/L), calcium chloride ($CaCl_2$: 0.01 mol/L), and tri-ethanolamine (TEA: 0.1 mol/L; Waqas et al., 2014). The Zn/Pb concentrations were subsequently determined *via* ICP-MS (Agilent 7700×, Agilent Technologies, Waldbronn, USA). The details of the above experimental procedures are provided in the SI.

1.5. PLFA extraction and analyses

The PLFA assay was used to analyze the microbial community structure in the soil (Lazcano et al., 2013). The detailed experimental procedure is provided in the SI. Fatty acid methyl esters (FAMEs) were identified and quantified using MIDI software with MIDI microbial calibration standards (MIDI, Inc., Newark, DE, USA). The characterization of microbial groups (*e.g.*, Gram-negative bacteria, Gram-negative bacteria, actinobacteria and fungi) followed the reported rules (Frostegård et al., 1993; Huygens et al., 2011).

Microbial diversity was characterized using the Shannon–Wiener (H') and Simpson indices (D):

$$H' = -\sum P_i \ln P_i \quad (1)$$

$$D = 1 - \sum P_i^2 \quad (2)$$

where, P_i is the ratio of a characteristic PLFA number in treatment i to the total characteristic PLFAs number in the experiment.

Canonical correspondence analysis (CCA) was performed using the concentrations of all detectable PLFAs to compare the microbial community structures among the treatments as well as the difference in the microbial community structure between the Aerobic and Anaerobic Groups to reveal the relationship between species abundance and environmental variables, such as soil pH, DOC, organic matter (OM) and cation exchange capacity (CEC).

1.6. Quality control and statistical analysis

Recovery studies of Phe/Pyr/Zn/Pb in the sorption experiment and in the soil were conducted to control the analysis quality. The detailed experimental procedure is provided in the SI. The Phe, Pyr, Zn, and Pb recovery rates in the sorption experiment were (78.17 ± 2.9)%, (84.57 ± 8.7)%, (91.38 ± 4.5)%, and (102.79 ± 7.4)%, respectively. The average recoveries of Phe, Pyr, Zn, and Pb in soil were (91.65 ± 8.1)%, (99.25 ± 2.4)%, (89.58 ± 5.5)% and (86.09 ± 6.2)%, respectively.

Microbial diversity analysis and CCA were carried out using the Vegan ecological function package in R. The data were analyzed *via* one-way Analysis of variance (ANOVA) in SPSS 14.0 at a significance level of $p < 0.05$.

2. Results and discussion

2.1. Sorption of Phe/Pyr/Zn/Pb to the biochars

The sorption isotherms of Phe/Pyr/Zn/Pb onto the biochars are shown in Fig. 1. The sorption isotherms of Phe/Pyr onto CB or BB fit well to the Freundlich and Langmuir equations (Fig. 1A and B). The sorption isotherms of Phe/Pyr onto CB or BB were nonlinear, as confirmed by the coefficient n values higher than 1 (Table S1). This nonlinear sorption isotherm is primarily caused by surface adsorption (Jia et al., 2013). Based on the Q_m values in the Langmuir equation, BB clearly demonstrated a higher sorption capacity for Phe/Pyr than CB. The isotherms for the sorption of Zn/Pb onto CB or BB fit well to the Freundlich and Langmuir equations (Fig. 1C and D) and were nonlinear (Table S1). The Q_m values in the Langmuir equation indicated that BB had a higher sorption capacity for Zn than CB, whereas CB had a higher sorption capacity for Pb than BB.

2.2. Dynamic change in the total Phe/Pyr concentrations

The dissipation trends of Phe/Pyr in the soils amended with/without biochars are presented in Fig. 2. Although CB and BB contained a certain level of Phe/Pyr, the amendment of soil with CB or BB did not significantly enhance the initial Phe/Pyr concentrations in the soils (contributing to less than 1%).

Under aerobic conditions, the Phe concentration in the Control treatment significantly decreased after 150 days of incubation, resulting in 48.48% of the initial residue in the soil (Fig. 2A). No significant difference in the total Phe concentration was observed between the Control and 0.5% CB treatments throughout the incubation period. After 60 days of the incubation, the total Phe concentrations in the 2% CB, 0.5% BB, and 2% BB treatments were higher than that in the Control treatment ($p < 0.05$). At the end of the incubation, the remaining Phe residues in the 0.5% CB, 2% CB, 0.5% BB, and 2% BB treatments were 50.19%, 65.61%, 66.84%, and 69.98% of the initial values, respectively. Similarly, 71.87% of the Pyr residues remained in the Control treatment after 150 days of

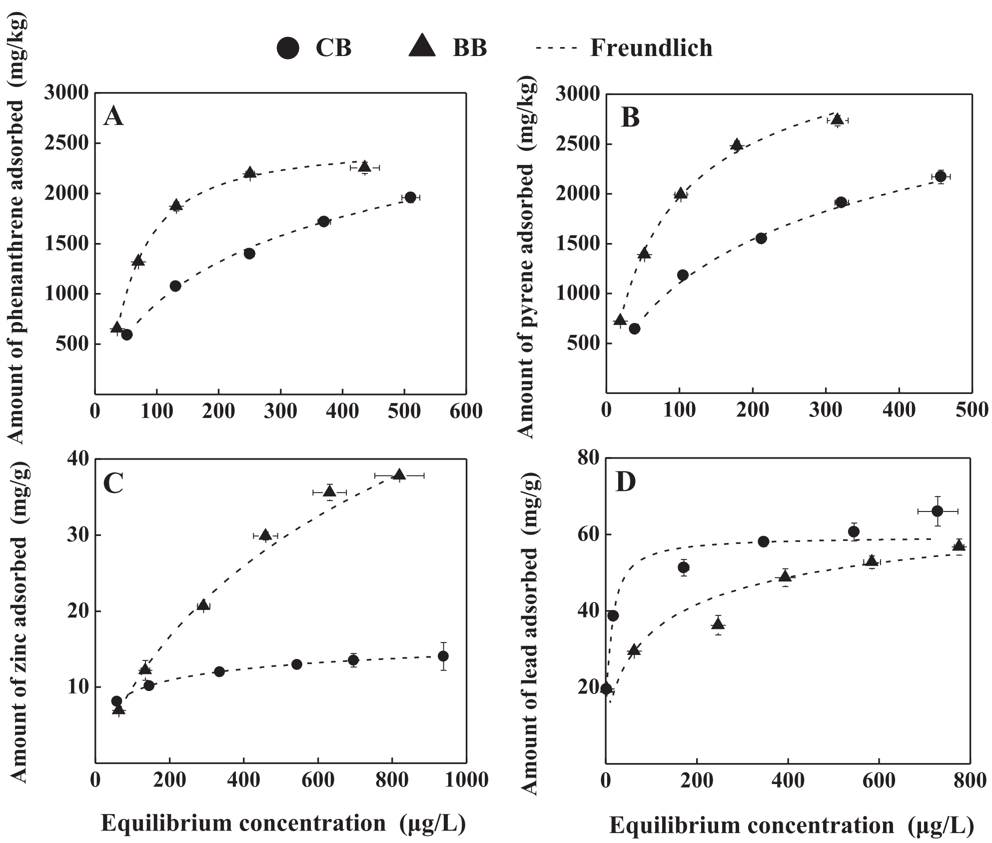

Fig. 1 – Sorption isotherms of phenanthrene (A), pyrene (B), zinc (C), and lead (D) onto corn straw-derived (CB) and bamboo-derived biochars (BB). Dots: measured data; dotted line: modified Freundlich equation fitted.

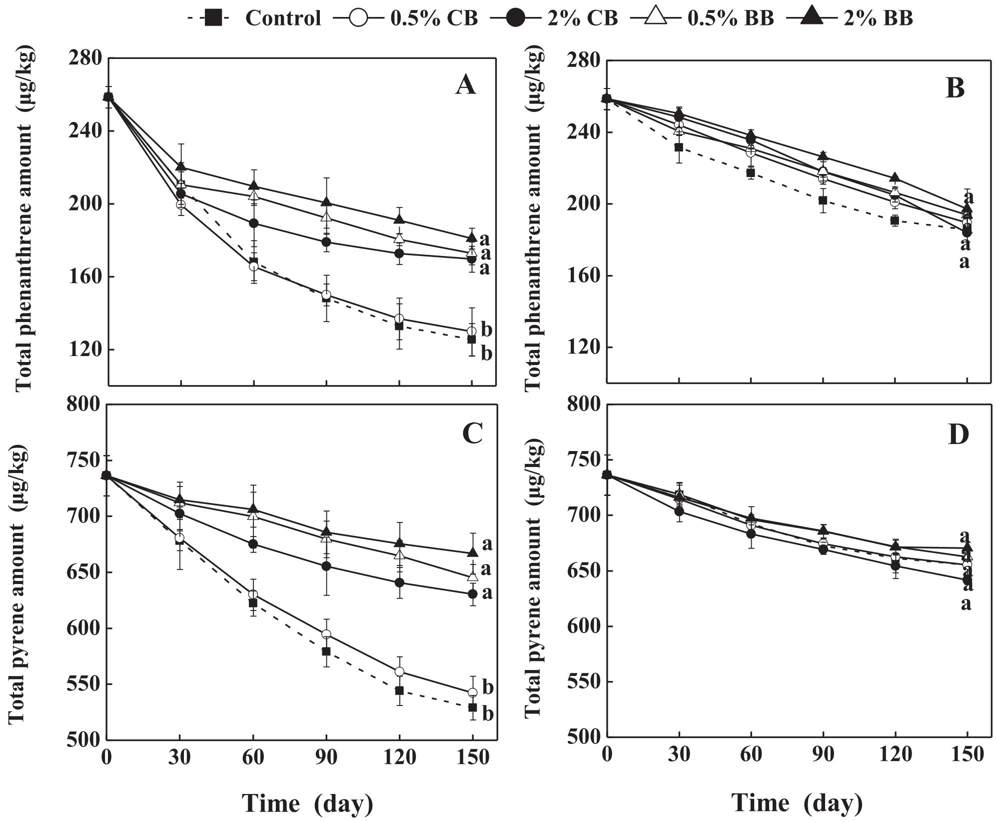

Fig. 2 – Time course of total phenanthrene concentrations in soils amended with/without corn straw-derived biochar (CB) and bamboo-derived biochar (BB) under aerobic (A) and anaerobic conditions (B), and that of pyrene under aerobic (C) and anaerobic condition (D). The different letters indicate the significant differences of total phenanthrene/pyrene concentrations among the treatments after 150 days of incubation at $p < 0.05$.

incubation (Fig. 2C). No significant differences in the total Pyr concentrations were found between the Control and 0.5% CB treatments. The Pyr residues in the 2% CB, 0.5% BB, and 2% BB treatments were 85.61%, 87.63% and 90.56% of the initial values, respectively, which were significantly higher than that in the Control treatment at the end of the incubation. The total Phe/Pyr concentrations were higher in the CB and BB amendments at a certain ratio than those in the Control treatments, which indicates that the CB and BB used in this study effectively suppressed Phe/Pyr dissipation in the aerobic soil. Considering that volatilization and photolysis were minor, the dissipation of Phe/Pyr in the aged contaminated soil may be ascribed to microbial degradation. Thus, the above results also suggest that due to the strong sorption of PAHs onto the biochars, their bioavailability to microorganisms may be decreased, reducing their degradation rates. Similarly, Jones et al. (2011) discovered that herbicide degradation was weakened by the addition of hardwood-derived biochar.

After 150 days of incubation under anaerobic conditions, no significant differences in Phe/Pyr residues were observed between the Control and biochar-amended treatments, suggesting that the biochars did not remarkably suppress Phe/Pyr dissipation in the anaerobic soils (Fig. 2B and D). The total Phe and Pyr concentrations in the Control treatments decreased over the 150 days (Fig. 2B and D), resulting in 71.79% of the initial Phe concentration and 88.99% of the initial Pyr concentration in the soils, which were significantly higher than the total Phe/Pyr concentrations in the Control treatments under aerobic conditions. This result indicates that PAH dissipation, primarily involving biodegradation, occurred more easily in aerobic soils (Musat et al., 2009) than in anaerobic soils. No significant differences in the total Phe/Pyr concentrations between the two incubation conditions were observed in the 2% CB, 0.5% BB, and 2% BB treatments potentially because the retention of Phe/Pyr in the aerobic soil amended with 2% CB, 0.5% BB, and 2% BB decreased the microbial degradation to a level similar in effectiveness to the anaerobic degradation of Phe/Pyr in the anaerobic soil.

2.3. Bioaccessible PAH concentrations in soil amended with or without biochars

As shown in Fig. S1, the HPCD-extracted concentrations of Phe/Pyr decreased with the increases in the incubation time and the amount of biochar amended to the soil. Based on the different total residue concentrations of Phe/Pyr in the soils (Fig. 2), the HPCD-extracted PAH percentages were calculated to precisely express the bioaccessibility of PAHs in the soils (Fig. 3).

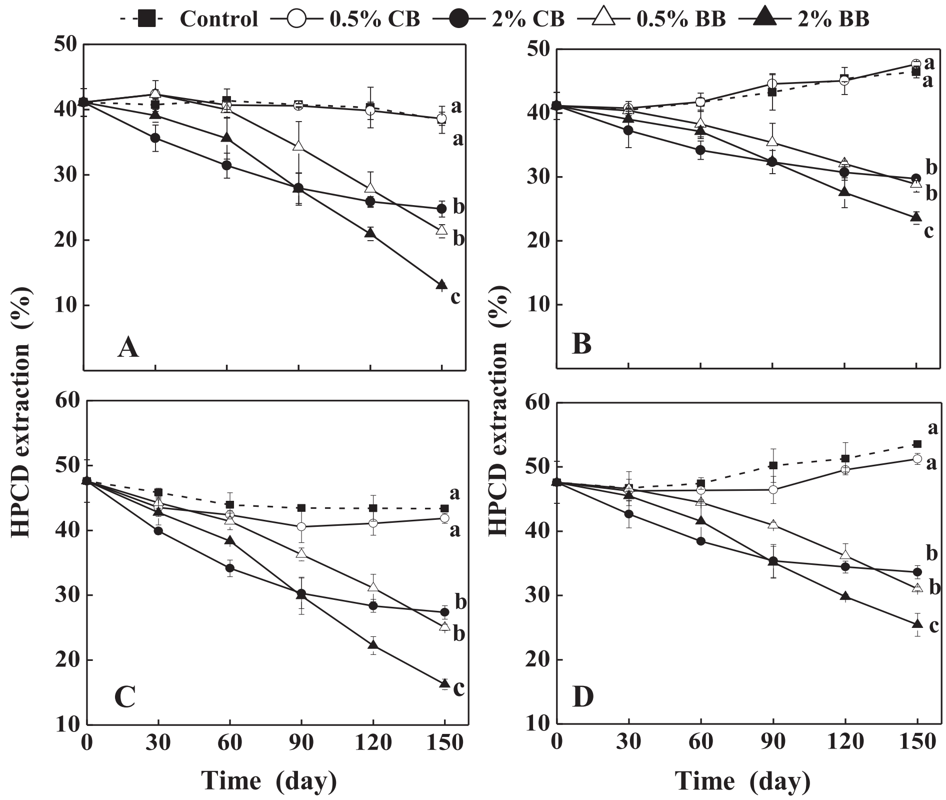

Fig. 3 – Time course of HPCD-extracted percentages of phenanthrene in soils amended with/without corn straw-derived biochar (CB) and bamboo-derived biochar (BB) under aerobic (A) and anaerobic (B) condition, and that of pyrene under aerobic (C) and anaerobic (D) conditions. The different letters indicate the significant differences of bioaccessible phenanthrene/pyrene concentrations among the treatments after 150 days of incubation at $p < 0.05$. HPCD: hydroxypropyl-beta-cyclodextrin.

Under aerobic conditions, the HPCD-extracted percentages of Phe/Pyr in the Control treatment did not significantly change throughout the 150 days of incubation (Fig. 3A and C). After 150 days, the HPCD-extracted percentages of Phe/Pyr in the 2% CB, 0.5% BB and 2% BB treatments were significantly lower than that in the Control treatments. Simultaneously, the Phe/Pyr residues in the three amendments were higher than those in the Control treatments (Fig. 2A and C). These results suggest that the biochars can reduce the bioaccessibility of Phe/Pyr in the soil *via* immobilization when applied at appropriate dosages. No significant differences in the HPCD-extracted percentages of Phe/Pyr were observed between the Control and 0.5% CB treatments, verifying that the 0.5% CB amendment did not immobilize PAHs in the soil. Compared with the Control treatments, the HPCD-extracted percentages of Phe/Pyr in the 2% CB treatments rapidly decreased before day 60 and gradually decreased thereafter, suggesting that the sorption capacity of CB gradually became saturated during the incubation. Over the first 60 days, no significant differences in the HPCD-extracted percentages of Phe/Pyr were observed among the Control and BB treatments. After 60 days, the HPCD-extracted percentages of Phe/Pyr in the 0.5% BB and 2% BB treatments were significantly lower than those in the Control treatments. At the end of the incubation, the HPCD-extracted percentages of Phe/Pyr in the 2% BB treatments were less than those in the 2% CB treatments, indicating that BB had a higher sorption capacity than CB. This finding was consistent with the results of Phe/Pyr sorption onto CB and BB (Fig. 1A and B). Table 1 shows that BB has larger surface area, higher C content (83.30%) and lower polarity (defined by the O/C ratio of 0.16) than CB. These results are related to the high frequency of PAH sorption sites per gram, assuming that these sites are C-rich, low-polarity surfaces (Khan et al., 2015). Therefore, BB exhibited a stronger ability to immobilize Phe/Pyr in the soil than CB. On the 150th day, the HPCD-extracted percentages of Phe in the Control, 0.5% CB, 2% CB, 0.5% BB, and 2% BB treatments were 38.46%, 38.62%, 22.81%, 21.39%, and 13.03%, respectively, and the HPCD-extracted percentages of Pyr in the Control, 0.5% CB, 2% CB, 0.5% BB, and 2% BB treatments were 43.38%, 41.85%, 25.36%, 25.07%, and 16.27%, respectively.

Under anaerobic conditions, the HPCD-extracted percentages of Phe/Pyr in the Control and 0.5% CB treatments slightly increased (Fig. 3B and D) potentially because the Phe/Pyr in the anaerobic soil, which were originally difficult to be dissolved, was solubilized by DOC over time. DOC might promote the migration of PAHs from soil to the aqueous phase and increase the apparent dissolved concentration of PAHs, thereby enhancing the bioaccessibility of PAHs in soil (Smith et al., 2011). These trends in the HPCD-extracted percentages of Phe/Pyr in the anaerobic 2% CB, 0.5% BB, and 2% BB treatments were similar to those observed under aerobic conditions (Fig. 3A and C), indicating that the three amendment levels reduced the bioaccessibility of PAHs though without any obvious suppression of PAH dissipation in the anaerobic soils (Fig. 2B and D). On the 150th day, the HPCD-extracted percentages of Phe in the Control, 0.5% CB, 2% CB, 0.5% BB, and 2% BB treatments were 46.39%, 47.09%, 32.46%, 28.87% and 23.61%, respectively. The HPCD-extracted percentages of Pyr in the Control, 0.5% CB, 2% CB, 0.5% BB, and 2% BB treatments were 53.56%, 51.25%, 33.64%, 31.05%, and 25.43%, respectively.

The HPCD-extracted percentages of Phe/Pyr in the Control treatments were significantly higher under anaerobic conditions than those under aerobic conditions after 150 days of incubation, indicating that the aerobic conditions were more beneficial to the predominant group of soil microorganisms that biodegrade complex organic compounds (Ren et al., 2016) than the anaerobic conditions. In addition, the DOC contents in the aerobic soils were significantly lower than those in the anaerobic soils (Table S2) and therefore more PAHs were solubilized in the anaerobic soils. In the biochar-amended treatments before day 60, no significant differences in the HPCD-extracted percentages of Phe/Pyr were observed between the two incubation conditions. After 90 days, however, the HPCD-extracted percentages of Phe/Pyr in the 2% CB and 2% BB treatments under aerobic conditions were remarkably lower than those observed under anaerobic conditions. After 120 days, the HPCD-extracted percentages of Phe/Pyr in the aerobic 0.5% CB and 0.5% BB treatments were also lower than those in the anaerobic amendments ($p < 0.05$). These results suggest that dry land farming, especially using crops with 2- to 5-month growth cycles, and rice cultivation may be feasible for agricultural production in Phe/Pyr-contaminated soil, with dry land farming expected to be more secure.

2.4. Accessible Zn/Pb concentrations

The CB and BB amendments had little influence on the total Zn/Pb concentrations in the soils after 150 days of incubation (Fig. S2), so the dispersion of these metals into the water out of the soil could be ignored. Under aerobic conditions, compared to the Control, the extractability of Zn after 150 days of the incubation was reduced by 16.56%, 20.17%, 38.22% and 64.76% in the presence of 0.5% CB, 2% CB, 0.5% BB and 2% BB, respectively (Fig. 4A). BB adsorbed Zn better than CB, as reported elsewhere (Chen et al., 2011), which was consistent with the sorption isotherm of Zn onto CB and BB in this study (Fig. 1C). The pore size of BB is smaller than that of CB (Table 1), and the hydration radius of Pb^{2+} is larger than that of Zn^{2+}. Thus, Zn might more readily enter the pores of BB and become fixed than Pb (Inyang et al., 2012). The extractability of Pb in the 0.5% CB, 2% CB, 0.5% BB and 2% BB amendments was reduced by 38.68%, 67.58%, 21.35% and 27.77%, respectively (Fig. 4B). The preferential sorption of Pb by CB over BB was consistent with the Pb sorption onto CB and BB (Fig. 1D). Pb was also reported to have greater affinity for carboxylic and phenolic functional groups situated on the surface of oxidized biochar particles (Houben et al., 2013). The detection of Pb minerals (hydrocerussite and cerussite) on biochar *via* X-ray diffraction (XRD) after sorption has been used to confirm Pb precipitation (Inyang et al., 2011). CB, with abundant mineral components and O-containing functional groups (Table 1 and Fig. S3), was more capable of Pb stabilization, binding and precipitation (Cao et al., 2011; Uchimiya et al., 2012) in this study.

Similarly, under anaerobic conditions, BB sorbed Zn better than CB, while CB sorbed more Pb than BB (Fig. 4A and B). At the end of the incubation, the bioaccessible Zn concentrations in the 0.5% CB, 2% CB, 0.5% BB and 2% BB amendments were reduced by 14.41%, 24.31%, 38.64% and 60.05%, respectively, and the bioaccessible Pb concentrations in the 0.5% CB, 2% CB, 0.5% BB and 2% BB amendments were reduced by 33.18%, 49.71%, 22.01% and 25.41%, respectively. The accessible Zn/Pb concentrations under aerobic conditions were significantly lower than those under anaerobic conditions ($p < 0.05$) because of the higher DOC contents in the anaerobic soil than in aerobic soil (Table S2). DOC has been found to be responsible for the dissolution equilibria of metals in the soil solution, especially at neutral pH (Harter and Naidu, 1995). DOC reduces metal adsorption to soil surfaces by either competing more effectively for the free metal ion and forming soluble organo-metallic

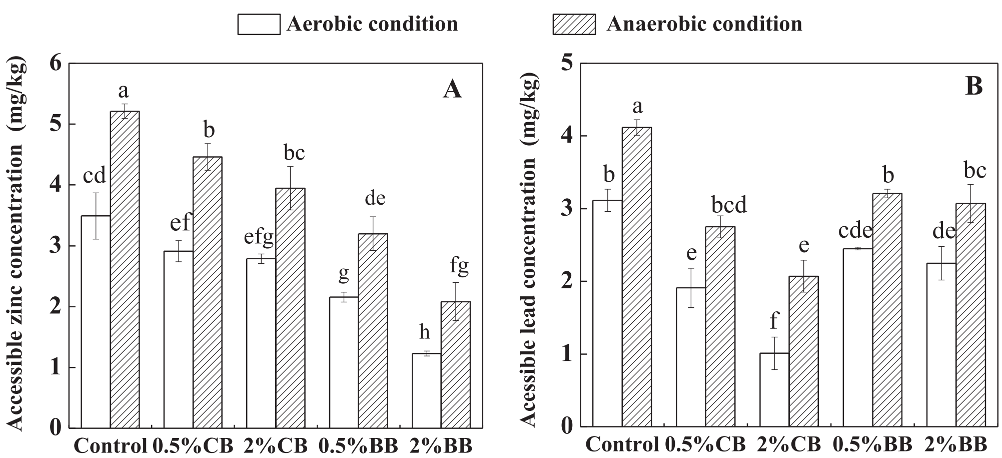

Fig. 4 – Concentration of accessible zinc (A) and lead (B) in soils amended with/without corn straw-derived biochar (CB) and bamboo-derived biochar (BB) under aerobic and anaerobic conditions. The different letters indicate the significant differences of accessible zinc and lead concentrations among the treatments at $p < 0.05$.

complexes or being preferentially adsorbed on the surfaces instead of the metals with which it is competing (Guisquiani et al., 1998). These mechanisms have been successively demonstrated in many studies (Antoniadis and Alloway, 2002; Venegas et al., 2016; Xu et al., 2016).

2.5. Microbial community structure based on PLFA analysis

The identified PLFAs were assigned to four main groups of soil microorganisms (*i.e.*, Gram-positive bacteria, Gram-negative bacteria, actinobacteria, and fungi). The concentrations of these groups in the microbial community were calculated for all treatments after 150 days of incubation (Table 2). Under aerobic conditions, no significant differences in the total PLFAs among the Control, 0.5% CB, 0.5% BB and 2% BB treatments were observed after 150 days. The 2% CB amendment remarkably increased the total PLFAs by 21.89%. Biochar addition did not affect the abundance of Gram-positive bacteria, actinobacteria or fungi. However, the biomass of Gram-negative bacteria in the 2% CB treatments increased by 29.47%. Gomez et al. (2014) also discovered that oak-derived biochar addition proportionally altered the community composition towards a more Gram-negative bacteria-dominated community. Similar results were found in the anaerobic soils (Table S2). Compared to the Control treatment, the total PLFAs and Gram-negative bacterial biomass in the 2% CB treatment were increased by 13.20% and 29.08%, respectively.

The total and individual PLFAs in each treatment were higher under aerobic conditions than under anaerobic conditions potentially because the water content in the aerobic soil provided a more stable environment for the growth of soil microorganisms (Niederberger et al., 2015). The Gram-negative bacterial biomass in the 2% CB treatment was higher under aerobic conditions than under anaerobic conditions, which may have led to the lower Phe/Pyr/Zn/Pb bioaccessibility in the 2% CB treatments in the aerobic soils than in the anaerobic soils (Figs. 3 and 4) because most of the bacteria (*e.g.*, *Pseudomonas*, *Bacillus* and *Arthrobacter*) that degrade organic compounds (*i.e.*, hydrocarbons) are reportedly Gram-negative bacteria (Liu et al., 2015; Song et al., 2016). The formation of bacterial membrane vesicles loaded with toxic compounds or the metabolism of toxic hydrocarbons contributed to their transformation into nontoxic compounds (Coppotelli et al., 2010). Additionally, more Gram-negative bacteria may be tolerant of Zn and Pb (*e.g.*, *Acidithiobacillus ferrooxidans*, *Pseudomonas fluorescens* BM07 and *Bacillus subtilis* DBM) *via* extracellular precipitation, biosorption onto cell surfaces and intracellular detoxification (Donati et al., 2009; Noghabi et al., 2007; Bai et al., 2014).

The microbial diversity index is a bio-indicator of soil ecosystem restoration (Anand et al., 2015). A higher Shannon–Weaver index represents better richness and evenness of the microbial population, and a higher Simpson index is often used to quantify the larger number of species present and the greater relative abundance of each species (Garland, 1996). As shown in Fig. 5, these two indices did not change significantly in the 0.5% CB, 0.5% BB and 2% BB treatments under either aerobic or anaerobic conditions. The 2% BB amendment did not increase nor reduce the soil microbial diversity, which indicates that the significant reduction of bioaccessible Phe/Pyr/Zn in this treatment was not directly associated with the microbial diversity. The 2% CB amendment remarkably increased the Shannon–Weaver index after 150 days of incubation (Fig. 5A). Correlation analysis revealed a positive relationship between the Shannon–Weaver index and the amount of CB added ($r = 0.927$, $p < 0.05$). Increasing amounts of CB added to the soil were associated with decreasing bioaccessible Phe/Pyr/Pb (Figs. 3 and 4B). In addition, compared to BB, CB contained more abundant nutrients (Table 1) that would have been released into the soil. These results suggest that high nutrient contents and the reduction of bioaccessible Phe/Pyr/Pb both played vital roles in improving the soil microbial diversity after the application of 2% CB. Similarly, Cao et al. (2016) observed that wheat straw-derived biochar promoted soil microbial activity when associated with biochar remediation. No significant differences were observed in these two indices for the Control and biochar-amended treatments between the two incubation conditions in the present study.

The relationship between the species composition and environmental variables was estimated *via* CCA using the

Table 2 – Microbial biomass indicated by phospholipid fatty acid assay (PLFA) concentrations in all treatments after 150 days of the incubation.

Incubation condition	Treatment	Microbial biomass indicated by PLFA concentrations (nmol/g)				
		Gram-positive bacteria	Gram-negative bacteria	Actinobacteria	Fungi	Total PLFAs
Aerobic	Control	2.89 ± 0.1 a	3.97 ± 0.19 bc	1.79 ± 0.05 a	0.67 ± 0.03 a	9.69 ± 0.16 bc
	0.5% CB	3.11 ± 0.01 a	4.54 ± 0.17 b	1.73 ± 0.22 a	0.78 ± 0.12 a	10.82 ± 0.25 b
	2% CB	3.07 ± 0.03 a	5.61 ± 0.18 a	1.85 ± 0.03 a	0.83 ± 0.09 a	11.99 ± 0.21 a
	0.5% BB	3.26 ± 0.36 a	3.82 ± 0.08 bc	1.80 ± 0.03 a	0.72 ± 0.04 a	10.06 ± 0.46 bc
	2% BB	2.64 ± 0.09 a	3.88 ± 0.09 bc	1.59 ± 0.80 a	0.73 ± 0.21 a	8.91 ± 0.27 cd
Anaerobic	Control	2.26 ± 0.03 a	2.82 ± 0.05 d	1.65 ± 0.17 a	0.62 ± 0.02 a	7.87 ± 0.22 f
	0.5% CB	2.61 ± 0.10 a	3.07 ± 0.17 d	1.55 ± 0.34 a	0.70 ± 0.04 a	8.41 ± 0.39 ef
	2% CB	2.54 ± 0.06 a	3.64 ± 0.06 c	1.39 ± 0.11 a	0.75 ± 0.03 a	8.66 ± 0.16 de
	0.5% BB	2.87 ± 0.10 a	2.84 ± 0.01 d	1.54 ± 0.22 a	0.65 ± 0.06 a	8.62 ± 0.25 ef
	2% BB	2.19 ± 1.09 a	3.09 ± 0.10 d	1.38 ± 0.02 a	0.65 ± 0.11 a	7.96 ± 0.22 f

Control: no biochar addition; 0.5% CB: 0.5% corn straw-derived biochar addition; 2% CB: 2% corn straw-derived biochar addition; 0.5% BB: 0.5% bamboo-derived biochar addition; 2% BB: 2% bamboo-derived biochar addition. Mean values with the same letter in column are not significantly different among treatments by the least significant difference (LSD) at the 5% level.

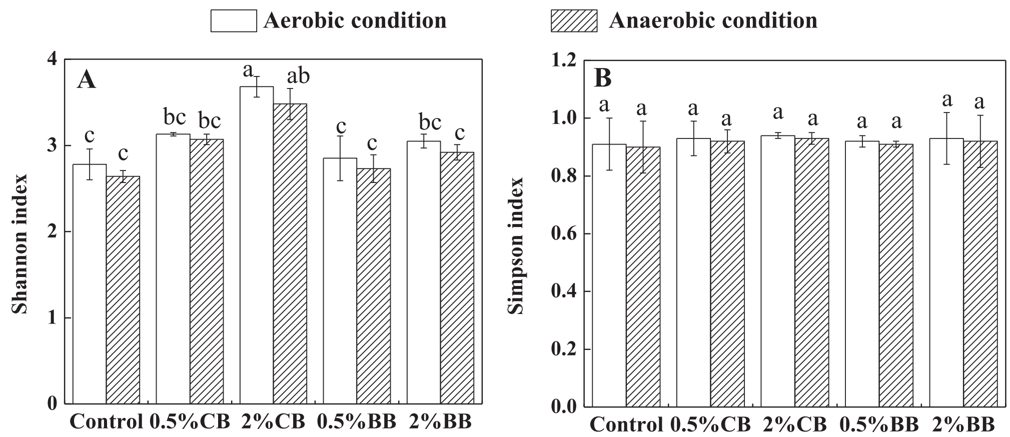

Fig. 5 – Microbial diversity expressed as Shannon (A) and Simpson (B) indexes indicated by phospholipid fatty acid (PLFA) assay concentrations in soils amended with/without corn straw-derived biochar (CB) and bamboo-derived biochar (BB)under aerobic and anaerobic conditions after 150 days of the incubation. The different letters indicate the significant differences of Shannon index among the treatments at $p < 0.05$.

concentrations of all detectable PLFA across all treatments after 150 days of the incubation (Fig. 6). Under aerobic conditions, the microbial community compositions in the 0.5% BB and 2% BB treatments were similar to that in the Control, while the 0.5% CB and 2% CB amendments significantly changed the soil microbial community composition (Fig. 6A). Among the soil physiochemical properties and the bioaccessible co-contaminants determined in this study, the pH and bioaccessible Pyr and Zn concentrations were the top three soil attributes that influenced the microbial community structure in the aerobic soils (Fig. 6A). Under anaerobic conditions, the microbial community structures in the CB and BB treatments differed from one another, and both amendment groups differed from the Control treatment (Fig. 6B). The pH, OM content, and bioaccessible Zn concentration were the top three soil attributes that influenced the microbial community structure in the anaerobic soils (Fig. 6B). Thus, the microbial community structure may be jointly affected by changes in co-contaminant bioaccessibility and soil physiochemical properties caused by biochar addition in aerobic and anaerobic soils. The results of the present study agree with previous reports that the toxicity of the co-contaminants to microbes is reduced in biochar-amended soils and that changes in soil physicochemical properties associated with the biochar may shift the soil microbial community structure (Anderson et al., 2011).

3. Conclusions

CB and BB, when applied at appropriate doses, effectively immobilized Phe/Pyr/Zn/Pb to reduce their bioaccessibility in

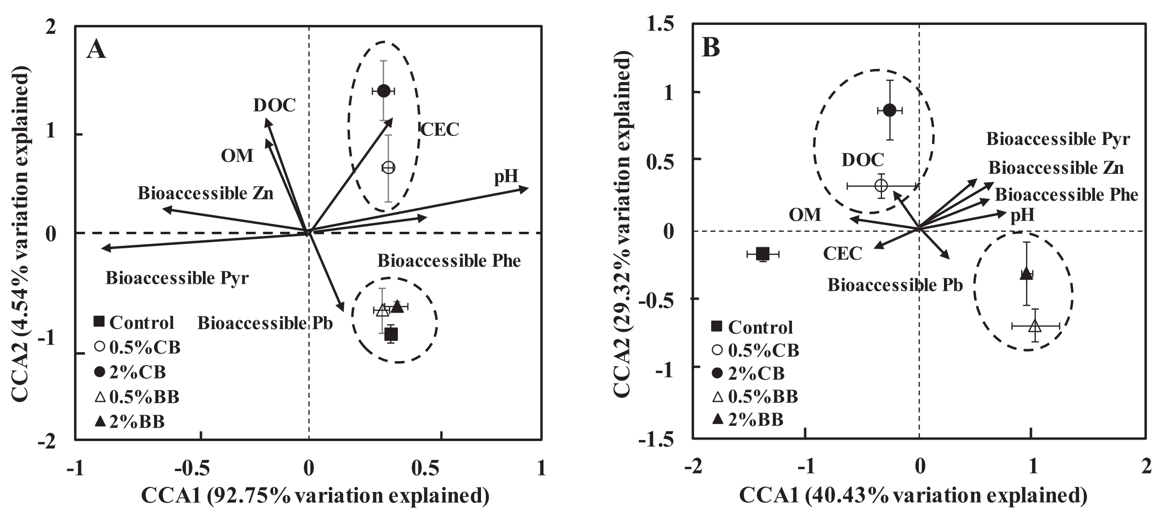

Fig. 6 – Canonical correspondence analysis (CCA) of microbial community and environmental variables in soils amended with/without corn straw-derived biochar (CB) and bamboo-derived biochar (BB) under aerobic condition (A) and anaerobic condition (B) after 150 days of the incubation.

soils, and this effectiveness was more significant in aerobic soils than in anaerobic soils. The ecological reconstruction of contaminated soil was promoted by CB amendment under aerobic conditions. Thus, the results of this study have reference value for the use of biochar and determination of soil-use methods in Phe/Pyr/Zn/Pb-contaminated soil. In the short term, dry land farming may be more reliable than paddy soil cultivation for reducing the bioaccessibility of Phe/Pyr/Zn/Pb. Future research monitoring the change trend of bioaccessible Phe/Pyr/Zn/Pb concentrations in biochars-amended soils over a longer culture time under the two conditions is necessary to verify the safety of the long-term use of biochar in soils. Simultaneously, the influence of the interactions and interaction mechanisms among organic pollutants and heavy metals on their bioaccessibility in biochar-amended soils needs to be elucidated.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2017.05.023.

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