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Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil

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Abstract Biochar is a carbon-rich solid material derived from the pyrolysis of agricultural and forest residual biomass. Previous studies have shown that biochar is suitable as an adsorbent for soil contaminants such as heavy metals and consequently reduces their bioavailability. However, the long-term effect of different biochars on metal extractability or soil health has not been assessed. Therefore, a 1-year incubation experiment was carried out to investigate the effect of biochar produced from bamboo and rice straw (at temperatures ≥500 °C) on the heavy metal (cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn)) extractability and enzyme activity (urease, catalase, and acid phosphatase) in a contaminated sandy loam paddy soil. Three rates (0, 1, and 5 %) and two mesh sizes (<0.25 and <1 mm) of biochar applications were investigated. After incubation, the physicochemical properties, extractable heavy metals, available phosphorus, and enzyme activity of soil samples were analyzed. The results demonstrated that rice straw biochar significantly ($P<0.05$) increased the pH, electrical conductivity, and cation exchange capacity of the soil, especially at the 5 % application rate. Both bamboo and rice straw biochar significantly ($P<0.05$) decreased the concentration of $CaCl_2$-extractable heavy metals as biochar application rate increased. The heavy metal extractability was significantly ($P<0.01$) correlated with pH, water-soluble organic carbon, and available phosphorus in soil. The 5 % application rate of fine rice straw biochar resulted in the greatest reductions of extractable Cu and Zn, 97.3 and 62.2 %, respectively. Both bamboo and rice straw biochar were more effective at decreasing extractable Cu and Pb than removing extractable Cd and Zn from the soil. Urease activity increased by 143 and 107 % after the addition of 5 % coarse and fine rice straw biochars, respectively. Both bamboo and rice straw biochars significantly ($P<0.05$) increased catalase activity but had no significant impact on acid phosphatase activity. In conclusion, the rice straw biochar had greater potential as an amendment for reducing the bioavailability of heavy metals in soil than that of the bamboo biochar. The impact of biochar treatment on heavy metal extractability and enzyme activity varied with the biochar type, application rate, and particle size.

Keywords Application rate · Bioavailability · Biochar particle size · Contaminated soil · Heavy metal extractability · Soil enzyme

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Introduction

Waste emissions from industrial production, mining activities, wastewater irrigation, and other activities have increased the number of agricultural soils contaminated with heavy metals in many parts of the world (Houben et al. 2013; Zhang et al.

2013b). Heavy metals are non-degradable and easily accumulate in soils (Jiang et al. 2012a). Soils contaminated with heavy metals pose a risk to the environment and to human health (Jiang et al. 2012a; Lu et al. 2014) due to biomagnifications. Recently, studies have paid considerable attention to in situ remediation such as the addition of soil amendments (Xu et al. 2014).

Biochar is a carbonaceous solid derived from the pyrolysis of agricultural and forest residual biomass (Wang et al. 2010). Biochar amendment has been investigated as a potential remediation technology for heavy metal-contaminated soil based on its properties, such as having a highly porous structure, the presence of various functional groups, as well as a high surface pH and cation exchange capacity (Park et al. 2011; Jiang et al. 2012b; Zhang et al. 2013b, 2014). In addition, biochar has a potential use in long-term carbon sequestration (Lehmann 2007) and increasing soil fertility, as well as promoting plant growth (Laird et al. 2010). Notably, the addition of biochar could increase the concentration of available phosphorus (P) (Chintala et al. 2014) which is a vital nutrient for improving crop yield (Parvage et al. 2013). Some studies, at both lab-scale and field-scale, have demonstrated that biochar could significantly decrease the mobility and bioavailability of heavy metals in the soil (e.g., Jiang et al. 2012a; Abdelhafez et al. 2014; Lu et al. 2014). For example, Ahmad et al. (2014a) reported that biochar is able to decrease the bioavailability of lead (Pb) and Sb in contaminated soil. However, long-term research is still lacking. Controlled laboratory incubation study has been commonly used to evaluate the interaction between amendments and contaminants in soils effectively (e.g., Dai et al. 2013; Liang et al. 2014). Thus, this 1-year biochar incubation study was designed to examine the changes in soil properties and the extractability of heavy metals when heavy metal-contaminated soil was amended with different types of biochar.

Furthermore, there is increasing interest in the use of biochar to improve soil properties. Soil enzyme activity is an important bio-indicator for soil quality (Paz-Ferreiro et al. 2014), especially for evaluating the impact of heavy metal pollution in soil (Cui et al. 2013). Lehmann et al. (2011) reviewed the effect of biochar on microbial activity in soil. They stated that increasing microbial activity was mainly due to the improvement of the physicochemical nature of the soil. Cui et al. (2013) found that there was a correlation between the concentration of exchangeable heavy metals, soil enzyme activity, and pH. Urease, acid phosphatase, and catalase were used as bio-indicators in previous studies because they were sensitive to the heavy metal stress (Papa et al. 2010; Hu et al. 2014). Other studies have shown that heavy metal pollution can result in reduced enzyme activity in the contaminated soil (Li et al. 2009; Papa et al. 2010). Application of biochars from different feedstocks may result in different responses in soil enzyme activity due to their different physicochemical properties. Therefore, enzyme activity in heavy metal-contaminated soil, following the addition of biochars from different feedstocks, was investigated in this study.

The objectives of our work were to determine the (1) changes in heavy metal-contaminated soil caused by the addition of different rates of bamboo biochar or rice straw biochar; (2) influence of biochar particle size and application rate on extractability of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), and on the availability of phosphorus in contaminated soil; and (3) correlation between heavy metal concentration and soil physicochemical properties, as well as the correlation between enzyme activity and extractable heavy metals.

Materials and methods

Soil and biochar characterization

Surface soil (0–0.15 m), naturally co-contaminated with Cd, Cu, Pb, and Zn, was collected in June 2012 from an abandoned paddy field in the southwest of Hangzhou City, Zhejiang Province, China. The soil had developed from alluvial material and was a dense sandy loam consisting of 51 % sand, 38.9 % silt, and 9.6 % clay. It was classified as paddy soil according to the Chinese soil classification (Gong 1999). The bulk soil sample was air-dried and passed through a 2-mm sieve prior to the incubation experiment. The selected physicochemical properties of the soil are shown in Table 1.

Two biochar samples were investigated in this study; they were bamboo biochar and rice straw biochar. The bamboo biochar, purchased from a local producer, was produced via pyrolyzing bamboo at approximately 750 °C for 3 h using a batch pyrolysis facility. The rice straw biochar was produced from rice straw pyrolyzed at a final temperature of 500 °C with a retention time of 30 min using continuous slow pyrolysis in the laboratory. The biochar samples were ground and passed through 60- and 20-mesh sieves to obtain two different size fractions to use: fine (<0.25 mm) and coarse (<1 mm). The characteristics of the biochar samples are also presented in Table 1.

Incubation experiments

The experiment was conducted to determine the effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzymatic activity in soil. It was performed with 2 kg of air-dried co-contaminated sandy loam paddy soil enclosed in plastic pots, which were 18 cm in diameter and 14 cm in height. Different size fractions of bamboo or rice straw biochar were amended into the pot at the rate of 0, 1, and 5 % of total dry soil weight and homogenously mixed. Four replicates were run. A nutrient solution was mixed with deionized

Table 1 Physicochemical properties of the soil and biochars

Property	Soil	Bamboo biochar	Rice straw biochar
Olsen-P (mg kg^{-1})	18	–	–
Total P (mg kg^{-1})	–	2.3	2.6
Electrical conductivity (ds m^{-1})	0.02	0.08	0.18
pH (H_2O)	5.7	9.5	10
Total C (g kg^{-1})	–	860	508
Total N (g kg^{-1})	2.5	4.5	16.6
Total H (g kg^{-1})	–	14.9	17.2
C_{org} (g kg^{-1})	8.7	839	470
C_{org}/N	–	186	28
Atomic H/C_{org}	–	0.21	0.44
Ash (%)	–	11.9	42.7
Cation exchange capacity (cmol kg^{-1})	5.3	15	45
Surface alkalinity (cmol kg^{-1})	–	123	152
Specific surface area (BET) (m^2 g^{-1})	–	907.4	36.7
Total Cd (mg kg^{-1})	1.4	Not detected	Not detected
Total Cu (mg kg^{-1})	693	19	47
Total Pb (mg kg^{-1})	527	Not detected	4.8
Total Zn (mg kg^{-1})	1471	33	197

– not measured

water and blended thoroughly with the solids to ensure the base dose of N, P, and K at 156, 125, and 156 kg ha^{-1}, respectively. Then, deionized water was added to 70 % of the water-holding capacity of the soil, and water was added every 5 days to maintain constant moisture contents during the incubation period (from July 12, 2012 to July 12, 2013). After 1 year of incubation at 25 °C, the soil sample was retrieved from each pot. Then, sub-samples (100 g) were air-dried and ground to pass 20- and 100-mesh sieves for analysis.

Measurement of biochar properties

The pH was measured on a 1:20 (*w*/*v*) water suspension of the biochar samples after stirring for 1 h. The electrical conductivity (EC) was measured using a 1:10 biochar:solution ratio after shaking for 30 min at 25 °C. The cation exchange capacity (CEC) was assessed using the compulsive exchange method with 1 M ammonium acetate (pH 7). The biochar alkalinity was determined with a back titration method (Lu et al. 2014). The ash content of biochar was determined by following the ASTM D1762-84 method. The total C, H, and N contents were measured by an elemental analyzer (Flash EA1112, Thermo Finnigan, Italy). The inorganic C concentration in the biochar was determined by following the ASTM D4373-02 method. Organic C was calculated from the inorganic C, as described in the IBI (2013) Biochar Standards. Total P was determined using the molybdate-ascorbic acid procedure at 700 nm (Lu 1999). The Brunauer–Emmett–Teller (BET) surface area of the biochar was determined by N_2 sorption analysis at 77 K in a surface analyzer (TristarII3020, Micromeritica Instrument Corporation, USA) after degassing. Two biochar samples were examined under the scanning electron microscope (SEM) (Sirion-100, FEI, Poland). X-ray diffraction (XRD), which reflected the differences in mineral crystals between the two biochars, was carried out on a computer-controlled diffractometer (X'Pert PRO, PANalytical, Netherlands). Chemical analysis of the biochars was determined using the energy dispersive X-ray spectrometry (EDS) elemental mapping. Functional groups on the biochar surface were identified by Fourier transform infrared (FTIR) spectrometer. The KBr pellets, for FTIR, were prepared with 2 wt% biochar.

Soil sample analyses

The pH and EC of the soil were measured in a soil/water slurry at 1:2.5 (*w*/*v*) and 1:5 (*w*/*v*) ratio, respectively. The CEC of the soil was determined by the ammonium acetate (pH 7.0) method. The available P was extracted using 0.5 M $NaHCO_3$ and measured by colorimeter (UVA 132122 spectrophotometer, Thermo electron corporation, England) at 650 nm (Lu 1999). For the measurement of the water-soluble organic carbon (WSOC), 10 g of fresh soil sample (passed through a 2-mm sieve) was extracted with deionized water at a 1:2 (*w*/*v*) ratio and shaken for 2 h. The resulting water extract was transferred into a clean auto-sampler-fitted vial, and the WSOC concentration was determined on a TOC-V CPH/CPN total carbon analyzer (Shimadzu, Japan).

The total heavy metal content of the experimental soil and biochars was determined by the HF–$HClO_4$–HNO_3 method (Carignan and Tessier 1988). The samples were completely dissolved with HF–$HClO_4$–HNO_3 and analyzed using an inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 2000, PerkinElmer Co., USA) after filtering. The amount of 0.1 M $CaCl_2$-extractable heavy metals was determined in the soil samples collected from each pot. An air-dried soil sample (1 g) was put into a plastic centrifuge tube, shaken with 25 mL 0.1 M $CaCl_2$ for 2 h on an orbital shaker, and then centrifuged at 3500 rpm for 15 min. The supernatant was filtered and collected in a plastic vial, then analyzed for metals with ICP-OES.

Urease activity in the soil was determined by spectrophotometry at 578 nm as the NH_4–N released from 1.0 g of soil after a 24-h incubation at 37 °C with 10 % (*w*/*v*) urea solution, in 20 mL of 1 M citrate buffer at pH 6.7 (Kandeler and Gerber 1988). Catalase activity in the soil was measured by back titration residual H_2O_2 added to soil with 0.1 M $KMnO_4$ described by Xu and Zheng (1986). Acid phosphatase activity was measured by spectrophotometer at 400 nm of the p-nitrophenol released from 1.0 g of soil after a 60-min incubation at 37 °C with a 0.025 M p-nitrophenyl phosphate substrate, in 4 mL of 0.17 M universal buffer at pH 5 (Tabatabai and Bremner 1969).

Statistical analysis

A SPSS 17.0 statistical package program was used to perform statistical analysis of the data. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to assess the statistical significance of the biochar treatment based on soil properties, solubility of heavy metals, and enzymatic activity. Variability in the data was expressed as the standard error, and the level of significance was set at P value <0.05. The correlation matrix between the extractable heavy metal concentrations, the enzymatic activity, and available P was based on the Pearson's correlation coefficients ($P<0.01$ and $P<0.05$), and the significant correlation coefficients are reported.

Results and discussion

Characteristics of the biochar

The biochars had high C contents, and most of the carbon was classified as organic carbon; the content of N and H was relatively low in both biochars (Table 1). The rice straw biochar had a slightly higher pH of 10.0, compared to bamboo biochar of 9.5. Rice straw biochar had a slightly higher EC and surface alkalinity than bamboo biochar and had a CEC three times that of the bamboo biochar. The H/C of 0.02 and 0.04, respectively for bamboo biochar and straw biochar, indicated that the biochars formed a highly aromatic structure with the carbonization (Chun et al. 2004). The ash content of straw biochar was approximately four times that of the bamboo biochar, which was related to the feedstock. There is abundant lignin in bamboo, while rice straw mainly consists of hemicellulose and cellulose (Yang et al. 2007).

The XRD spectrum (Fig. 1a) demonstrated that there was SiO_2 or other silicon oxides in the bamboo biochar. There was also silicon oxide observed in the XRD spectrum of straw biochar, and the presence of sylvite, quartz, cristobalite, and calcite was confirmed by the peaks in the spectrum. Figure 1b shows that the rice straw biochar had relatively higher contents of Si, K, Cl, Na, Mg, and Fe when compared with bamboo biochar. According to the previous studies, the presence of P, Si, Al, and O in soil may be associated with the immobilization of Pb (Ahmad et al. 2012a; Moon et al. 2013). The carbonate and chloride from straw biochar may contribute to the formation of stable $CdCO_3$ (Mousavi et al. 2010), $PbCO_3$ (Cao et al. 2002), and pyromorphite-like phases (Moon et al. 2013). The FTIR spectrum of the biochars (Fig. 1c) contained a number of adsorption peaks; the peak at 798.80 cm^{-1} for bamboo biochar and at 794.58 cm^{-1} for rice straw biochar were assigned to C–H bending aromatic CH out-of-plane deformation (Wu et al. 2012). The peaks around 1100 cm^{-1} were assigned to out-of-plane bending of carbonates (CO_3^{2-}) (Yuan et al. 2011) or C–OH and C–C stretching, and the band around 1590 cm^{-1} to –COO^- anti-symmetric stretching (Yuan et al. 2011) or aromatic C=C (Luo et al. 2011). The band intensities at 3400 cm^{-1} indicated the presence of hydroxyl (–OH) groups (Chen et al. 2008). In general, the intensity of these peaks was stronger for the rice straw biochar than those of bamboo biochar. The SEM of the biochars (Fig. 1d) showed many porous structures on their surface, but the sizes and shapes were different, which indicated potentially different capabilities for adsorption (Jiang et al. 2012a). The bamboo biochar had a much larger BET surface area of 907.43 m^2 g^{-1} than rice straw biochar of 36.70 m^2 g^{-1}, which indicated different surface areas for different feedstocks and different pyrolysis conditions (Park et al. 2011).

Effect of biochar on soil properties

Changes in the physicochemical properties of soils, after incubation with bamboo biochar and rice straw biochar at two different particle sizes and two application rates, are presented in Fig. 2. The addition of rice straw biochar significantly ($P<0.05$) increased the soil pH (Fig. 2a). Compared to the control, the addition of coarse rice straw biochar at 1 and 5 % application rates increased soil pH by 0.4 and 0.6 units, respectively. However, in the case of fine rice straw biochar, the soil pH increased by 0.7 and 0.8 units, respectively. Soil pH increased with increasing addition rate of rice straw biochar, and the fine rice straw biochar was more effective at increasing soil pH than the coarse one, which is in agreement with Lu et al. (2014). However, application of bamboo biochar made no significant difference in the soil pH compared with control. This result is not unexpected as the rice straw biochar had a higher pH, ash content, and surface alkalinity than bamboo biochar (Table 1). The results indicate that rice straw biochar could be used as a substitution for lime material to increase soil pH value. It is also likely that an increased pH affects the metal mobility in soil (Lu et al. 2014), by promoting the formation of precipitates, such as $CdCO_3$ (Mousavi et al. 2010), $Cu(OH)_2$, and $Pb_5(PO_4)_3OH$ (Cao et al. 2011). Compared to the control, the electrical conductivity (EC) of the soil did not increase significantly in the bamboo biochar treatments (Fig. 2b). The EC values of two fine rice straw treatments at 1 and 5 % application rates were significantly greater than that of the control, whereas there were no significant differences between the coarse rice straw biochar treatments and the control. This indicates that EC increased with the increasing application rate of rice straw biochar, and the fine particle size was more effective. The results were in line with Abdelhafez et al. (2014) who found that soil EC

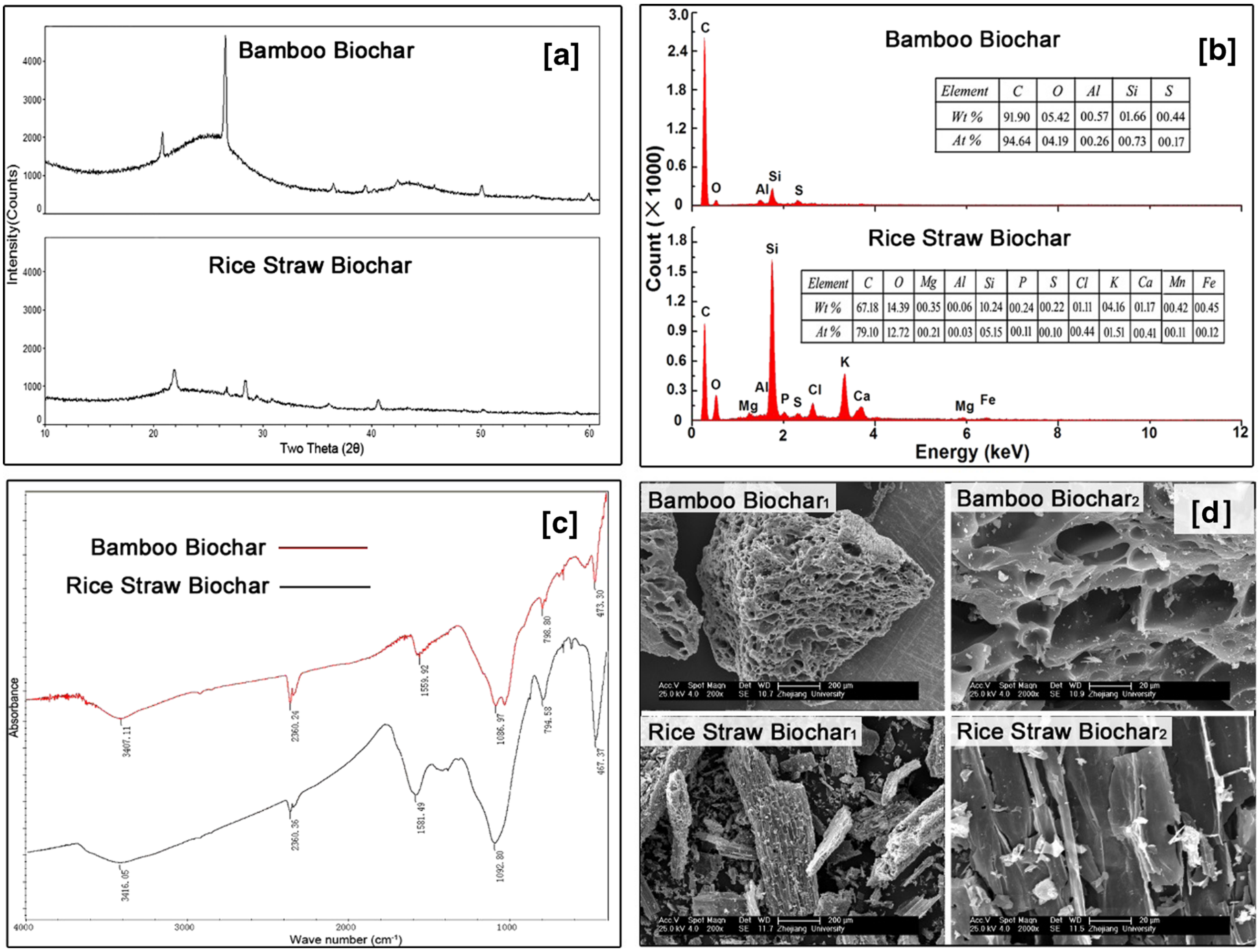

Fig. 1 X-ray diffraction (XRD) (**a**), energy dispersive X-ray spectrometry (EDS) (**b**), Fourier transform infrared (FTIR) spectrometry (**c**), and scanning electron microscope (SEM) (**d**) results of bamboo and rice straw biochars

increased with increasing application rate of biochar and that biochar derived from different materials had a distinct effect on soil EC. Rice straw biochar was more effective in increasing soil EC than bamboo biochar at the same application rate, which was mostly related to the higher ash content and surface alkalinity of the rice straw biochar. WSOC is the most mobile fraction of organic ligands and may promote the formation of heavy metal-organic complexes (Cao et al. 2003). Biochar is known to have a high content of WSOC. However, as Fig. 2c shows, only the coarse straw biochar treatment at 5 % amendment rate significantly increased the WSOC content in soil; the other treatments had no significant effect on WSOC. This result may be due to the WSOC released by biochar being adsorbed by soil during the incubation experiment. When Kuiters and Mulder (1993) added WSOC to the soil, they found that the amount of WSOC decreased by more than 80 % after 4 weeks. With respect to the CEC for bamboo biochar treatments (Fig. 2d), only the fine biochar treatment at 5 % amendment rate significantly increased the CEC of soil. Whereas in the case of rice straw biochar treatments, only the 1 % fine biochar treatment rate had no significant effect on soil CEC. The CEC increased with the increasing addition rate of rice straw biochar, and overall, the fine particle size treatments were more effective than coarse ones. Previous studies indicated that soil CEC increases with biochar addition (Ahmad et al. 2012b; Almaroai et al. 2014; Beesley et al. 2010; Zhang et al. 2013b). The pH, EC, WSOC, and CEC were key factors in evaluating the quality of soil and affected the heavy metal behavior in soil.

Influence of biochar on P availability

Phosphorus is an essential macronutrient for plants in soil. In general, the concentration of available P would increase after the addition of biochar, because abundant soluble P was forming during the biochar process (Angst and Sohi 2013), and the P can be released when biochar was used as soil amendment. In our study, compared to the control, the application of both fine and coarse rice straw biochar at 5 % amendment rate led to a significant (P<0.05) increase of available P

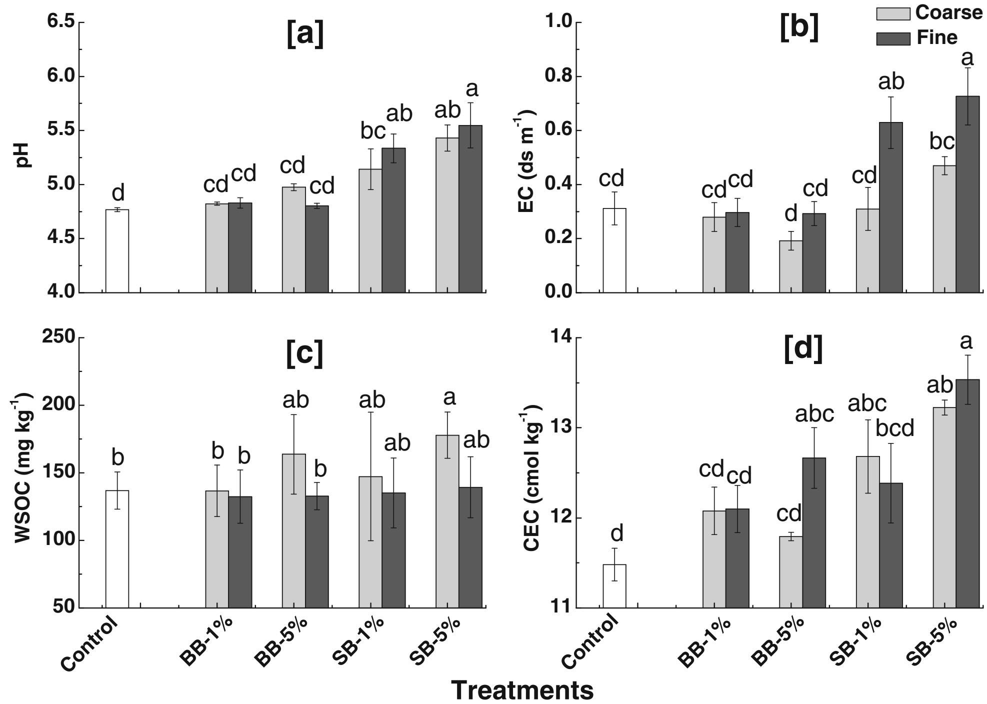

Fig. 2 Effect of biochar application on soil properties: pH (**a**), electrical conductivity (EC) (**b**), water-soluble organic carbon (WSOC) (**c**), and cation exchange capacity (CEC) (**d**). Treatments: control, 1 % and 5 % bamboo biochar (BB) and rice straw biochar (SB) with two particle sizes (coarse and fine). *Error bars* are standard error of the means (n=4). *Different letters* indicate significant differences between treatments at P<0.05 level

in the soil, as the former had a slightly higher concentration than the latter (Fig. 3). Overall, the high application rate (5 %) and fine size (<0.25 mm) were more effective in increasing soil P availability for rice straw biochar. The application of fine bamboo biochar at the rate of 5 % increased the available P by 13.8 %. Considerable research (DeLuca et al. 2009; Chintala et al. 2014) has been conducted to find the potential mechanism of biochar on the availability of P in soil but the mechanisms remain unclear. It has been reported that biochar can enhance the availability of P through surface adsorption (Chintala et al. 2014) and also by increasing the pH and CEC of soil (DeLuca et al. 2009). Addition of rice straw biochar, with its higher pH and CEC was more effective than bamboo biochar in enhancing the availability of P.

Effect of biochar on heavy metal extractability

The concentrations of $CaCl_2$-extractable Cd, Cu, Pb, and Zn in soil were determined after 1 year of incubation (Fig. 4). The concentration of $CaCl_2$-extractable heavy metals decreased significantly (P<0.05) with an increasing addition of both bamboo biochar and rice straw biochar. Compared to the control, only coarse and fine rice straw biochar at the 5 % addition rate significantly reduced the concentration of extractable Cd, 17.7 and 25.8 %, respectively. In addition, both bamboo biochar and rice straw biochar were effective in reducing the concentration of extractable Cu and Zn in the soil. For the soil amended with bamboo biochar, the concentration of extractable Cu decreased by 26.8 and 47.7 % for coarse treatment, 31.9 and 66.0 % for fine treatment, with 1 and 5 % of addition rate, respectively. Whereas, for the soils amended with rice straw biochar, the highest reductions of extractable Cu (97.3 %) and Zn (62.2 %) were achieved with the 5 % application rate for fine particle size. The coarse rice straw biochar treatment, at the same addition rate, decreased the Cu and Zn extractability by 94.8 and 52.9 %, respectively. There was no significant effect on extractable Pb with the addition of bamboo biochar at a dose of 1 %, while the extractability of Pb in soil amended with coarse and fine rice straw biochar (at the same dose) decreased by 29.0 and 48.1 %, respectively. By increasing the application rate of bamboo and rice straw

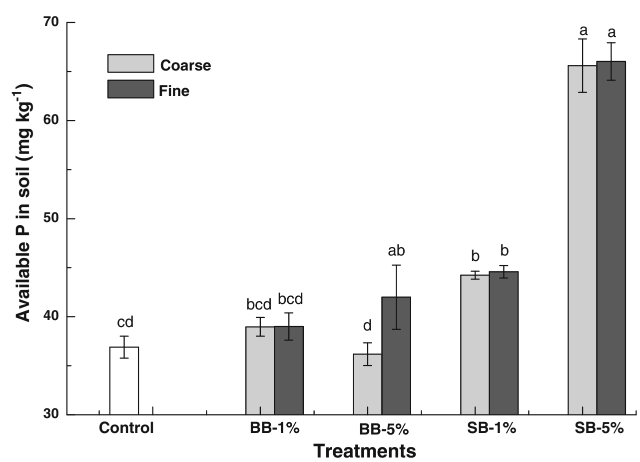

Fig. 3 Effect of biochar treatments on the concentration of available P in soil. Treatments: control, 1 and 5 % bamboo biochar (BB) and rice straw biochar (SB) with two particle sizes (coarse and fine). *Error bars* are standard error of the means (n=4). *Different letters* indicate significant differences between treatments at P<0.05 level

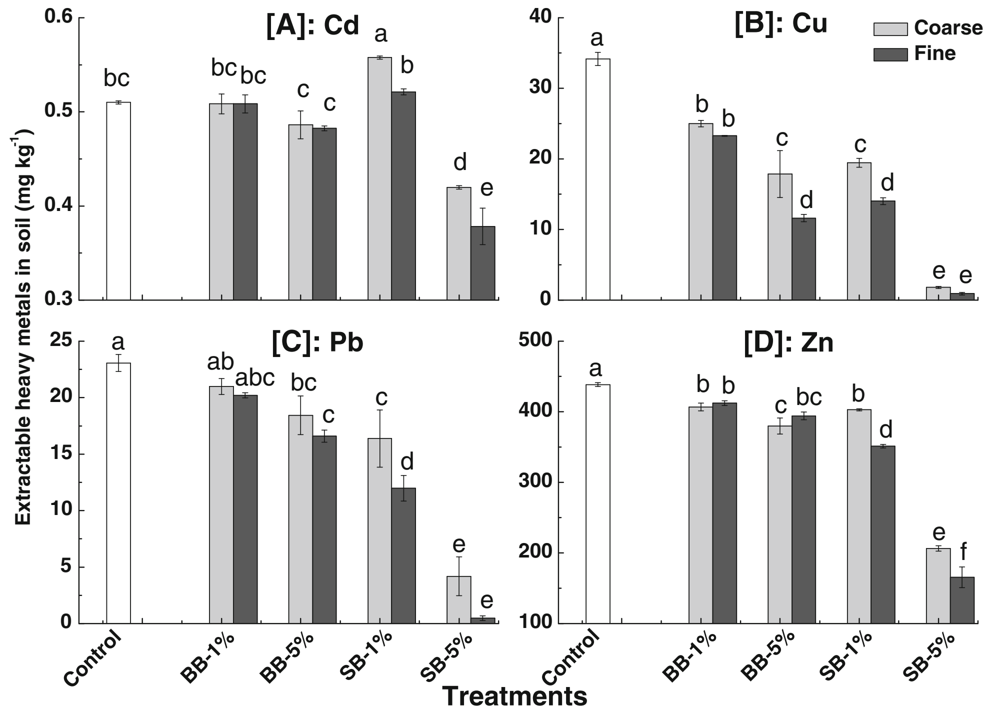

Fig. 4 Effect of biochar addition on the concentration of extractable heavy metals: Cd (**a**), Cu (**b**), Pb (**c**), and Zn (**d**) in soil. Treatments: control, 1 and 5 % bamboo biochar (BB) and rice straw biochar (SB) with two particle sizes (coarse and fine). *Error bars* are standard error of the means (n=4). *Different letters* indicate significant differences between treatments at P<0.05 level

biochar to 5 %, the concentration of Pb significantly decreased by 20.1 and 81.9 % for coarse treatments and 28.1 and 97.9 % for fine treatments, respectively. In short, after incubation, there was almost no significant difference between $CaCl_2$-extractable Cd and Pb concentrations in soil amended with 1 % bamboo biochar relative to the untreated soil, whereas a significant effect was observed at the higher dose of bamboo biochar. Application of rice straw biochar significantly altered the extractability of heavy metals; the higher application rate and finer particle size were more effective because of the higher surface area. The presence of both bamboo biochar and rice straw biochar was more effective in decreasing extractable Cu and Pb than Cd and Zn in soil, and rice straw biochar was more effective than bamboo biochar.

The results above were consistent with a previous report, that the application of rice straw biochar resulted in a more significant effect than bamboo biochar on toxicity characteristic leaching procedure (TCLP)-extractable heavy metals in a pot experiment (Lu et al. 2014). Previous studies also investigated the transformation of heavy metals, in the contaminated soil, after the addition of biochar derived from a variety of biomasses at different application rates (Jiang et al. 2012a; Houben et al. 2013; Xu et al. 2014). Xu et al. (2014) concluded that enhancement of Cd adsorption by sandy soil amended with swine manure-derived biochar increased with increasing biochar addition. The effect was attributed to the different composition of surface functionalities in biochar and biochar amended soil. Jiang et al. (2012a) added rice straw biochar to three different kinds of soils at different application rates. They demonstrated that the reduction of available Pb increased with increasing rice straw biochar addition rate, and the addition of biochar was more effective on the immobilization of Cu and Pb than that of Cd (Jiang et al. 2012b), which is similar to our study. Moreover, a recent study reported that the reduction of $CaCl_2$-extractable Cd, Pb, and Zn reached 71, 92, and 87 %, respectively, in the presence of 10 % Miscanthus biochar (Houben et al. 2013).

pH is an important parameter controlling $CaCl_2$-extractable heavy metals in soil (Rieuwerts et al. 2006). Uchimiya et al. (2010) reported that addition of biochar could decrease the adsorption of Cd and Ni through increasing the pH of the soil. Therefore, in our work, the decrease in concentrations of extractable heavy metals may be mainly attributed to the increasing soil pH due to the addition of rice straw biochar (Table 2). There were strong and significant (P<0.01) negative correlations between extractable heavy metal concentrations and soil

Table 2 Correlation coefficients between soil physicochemical properties and concentrations of extractable heavy metals

	$CaCl_2$-extractable heavy metals			
	Cd	Cu	Pb	Zn
pH	−0.749[b]	−0.464[b]	−0.920[b]	−0.953[b]
WSOC	−0.408[a]	−0.464[b]	−0.568[b]	−0.558[b]
Available P	−0.462[b]	−0.631[b]	−0.894[b]	−0.922[b]

WSOC water-soluble organic carbon

[a] Correlation is significant at the 0.05 level

[b] Correlation is significant at the 0.01 level

pH, indicating that pH is an important factor in decreasing extractable heavy metals in soil. The biochars were alkaline, thereby able to exert a liming effect when amended in soil (Ahmad et al. 2014b). Interestingly, a significant negative correlation was found between extractable heavy metal concentrations and WSOC, which was in contrast to previous studies (Beesley et al. 2010; Park et al. 2011). In addition, Table 2 showed the negative correlation between the content of extractable heavy metals and available P. Increasing available P induced by biochar led to a reduced extractability of heavy metals, potentially by precipitation and complexation with phosphate (Ahmad et al. 2014b). Besides the three factors above, the higher surface alkalinity, Si content (Lu et al. 2014), and O-containing functional groups in the biochar (Ahmad et al. 2014a, b) made rice straw biochar more effective than bamboo biochar at heavy metal immobilization.

Effect of biochar on enzyme activities

It is well-established that the enzyme activity is one of the most important indicators to monitor the effect of soil management, agricultural practices, or contamination on soil health (Oleszczuk et al. 2014). Enzyme activity also reflects the capacity to self-purify the soil contamination indirectly (Cui et al. 2013). Thus, after incubation with the bamboo biochar and rice straw biochar, urease, catalase, and acid phosphatase activities of soil samples were measured in our study, and the results are shown in Fig. 5.

Urease plays an important role on the transformation of soil nitrogen. A previous study indicated that urease activity related to soil pH and soil texture (Makoi and Ndakidemi 2008). In our study, after incubation, the activity of urease significantly (P<0.05) increased with the 5 % addition rate of rice straw biochar, and the fine biochar treatment was more effective than the coarse one. The urease activity increased by 143 and 107 % respectively, compared to the control. Wang et al. (2007) showed that reduction in the availability of heavy metals enhances the growth of soil microorganisms and thus results in an increase in enzyme activity per gram of soil. Catalase is an important enzyme for indicating the oxidation-reduction potential of soil (Cui et al. 2013). Compared to the control, the catalase activity was significantly increased for all eight biochar treatments, regardless of whether they were coarse or fine biochar treatments. The maximum catalase activity (as measured by $KMnO_4$ consumption) was 0.63 mL g^{-1} with 5 % coarse rice straw biochar addition, which was 2.2 times greater than the control treatment. In a similar study by Masto et al. (2013), catalase activities increased significantly with an increasing dose of biochar. In addition, phosphatase, another important enzyme, was evaluated. Phosphatase is an enzyme involved in the mineralization of organic P (Spiers and McGill 1979). The acid phosphatase activity decreased significantly with the application of

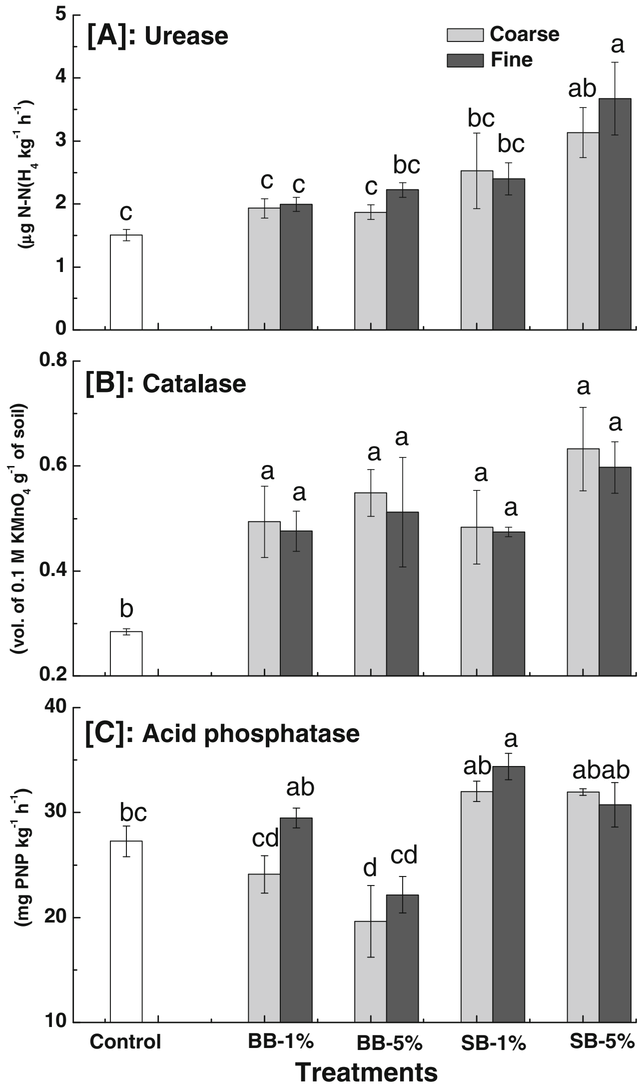

Fig. 5 Effect of bamboo and rice straw biochar on the activity of urease, catalase, and acid phosphatase in soil. Treatments: control, 1 and 5 % bamboo biochar (BB) and rice straw biochar (SB) with two particle sizes (coarse and fine). *Error bars* are standard error of the means (n=4). *Different letters* indicate significant differences between treatments at P<0.05 level

bamboo biochar at the 5 % level. For rice straw biochar, a marked increase was observed only in the 1 % fine biochar

Table 3 Correlation coefficients between concentrations of extractable heavy metals and enzyme activities

	Enzyme activities		
	Urease	Catalase	Acid phosphatase
Cd	−0.448[a]	−0.632[a]	0.182
Cu	−0.669[a]	−0.810[a]	−0.052
Pb	−0.705[a]	−0.648[a]	−0.258
Zn	−0.664[a]	−0.668[a]	−0.216

[a] Correlation is significant at the 0.01 level

treatment; increasing by 26.09 %. Kumar et al. (2013) observed a decreased acid phosphatase activity after the dose of *Parthenium hysterophorus* biochar was increased. In the present study, both bamboo biochar and rice straw biochar significantly increased catalase activity. Bamboo biochar was more effective at increasing the activity, which may be attributed to bamboo biochar having a lower pH, EC, and CEC than rice straw biochar (Fig. 1a). Whereas acid phosphatase activity decreased with the addition of 5 % bamboo biochar, which may be due to the increase in soil pH (Acosta-Martínez and Tabatabai 2000). Chen et al. (2013) also found that increased soil pH led to increasing alkaline phosphatase but decreasing acid phosphatase activities. However, enzyme activity may also change under heavy metal stress (Zhang et al. 2013a). In our study, the addition of biochars may change the heavy metal stress level in the soil, which is another factor that can influence enzyme activity.

Table 3 shows the Pearson's correlation coefficients between enzyme activity and the concentration of extractable Cd, Cu, Pb, and Zn. The activities of urease and catalase were negatively correlated ($P<0.01$) with extractable heavy metals in soil. Urease and catalase activities increased with the decreasing concentrations of extractable heavy metals. These results were in line with previous studies that reported that heavy metal content may affect soil enzyme activity by direct and indirect mechanisms (Cui et al. 2013; Hu et al. 2014; Li et al. 2009; Papa et al. 2010). For instance, Ahmad et al. (2012a) found that there was a negative correlation between enzyme activities and concentration of TCLP-Pb. The significant negative correlation between activities of urease and catalase and content of heavy metals was also reported by Hu et al. (2014). However, the responses of different enzymes to heavy metals have been shown to vary (Li et al. 2009). In this study, in contrast to urease and catalase, the acid phosphatase activity showed no significant correlation with the concentration of extractable heavy metals. Hu et al. (2014) also observed that acid phosphatase activity was only slightly affected by heavy metal pollution in the paddy field. Based on urease and catalase activity, we can conclude that the application of biochars, in particular, rice straw biochar, during the 1-year period incubation, improved soil microbial activity and soil quality.

Conclusions

Our incubation experiment clearly showed significant ($P<0.05$) changes in soil properties, including increases in soil pH, EC, CEC, and available P, after application of rice straw biochar, especially at the higher application rate and finer particle size. Significant increases in the soil CEC and available P were also observed at the 5 % fine bamboo biochar addition rate. These results indicated that rice straw biochar was more suitable as a soil amendment than bamboo biochar. Rice straw biochar was more effective than bamboo biochar in decreasing extractable heavy metals in soil, which were significantly ($P<0.01$) correlated with soil pH, WSOC, and available P. In addition, the biochars investigated in this study also had the potential to significantly affect the activities of urease and catalase in the contaminated soil. Significant correlations were observed between the concentration of extractable heavy metals and activity of enzymes. Overall, the influence of biochar on heavy metal extractability and enzyme activity varied owing to the feedstock, application rate, and particle size of the biochars. In the present study, biochar derived from rice straw was more effective in improving soil quality than bamboo biochar; the higher application rate and finer particle size were more effective than the lower application rate and coarser particle size of the biochar.

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References

Abdelhafez AA, Li J, Abbas MH (2014) Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. Chemosphere 117:66–71

Acosta-Martínez V, Tabatabai MA (2000) Enzyme activities in a limed agricultural soil. Biol Fertil Soils 31:85–91

Ahmad M, Hashimoto Y, Moon DH, Lee SS, Ok YS (2012a) Immobilization of lead in a Korean military shooting range soil using eggshell waste: an integrated mechanistic approach. J Hazard Mater 209–210:392–401

Ahmad M, Lee SS, Yang JE, Ro HM, Lee YH, Ok YS (2012b) Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil. Ecotox Environ Safe 79:225–231

Ahmad M, Lee SS, Lim JE, Lee SE, Cho JS, Moon DH, Hashimoto Y, Ok YS (2014a) Speciation and phytoavailability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and biochar: EXAFS spectroscopy and chemical extractions. Chemosphere 95:433–441

Ahmad M, Rajapaksha AU, Lim JE, Zhang M, Bolan N, Mohan D, Vithanage M, Lee SS, Ok YS (2014b) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99:19–33

Almaroai YA, Usman ARA, Ahmad M, Moon DH, Cho JS, Joo YK, Jeon C, Lee SS, Ok YS (2014) Effects of biochar, cow bone, and eggshell on Pb availability to maize in contaminated soil irrigated with saline water. Environ Earth Sci 71:1289–1296

Angst TE, Sohi SP (2013) Establishing release dynamics for plant nutrients from biochar. GCB Bioenergy 5:221–226

Beesley L, Moreno-Jiménez E, Gomez-Eyles JL (2010) Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ Pollut 158:2282–2287

Cao X, Ma LQ, Chen M, Singh SP, Harris WG (2002) Impacts of phosphate amendments on lead biogeochemistry at a contaminated site. Environ Sci Technol 36:5296–5304

Cao X, Ma LQ, Shiralipour A (2003) Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, Pteris vittata L. Environ Pollut 126:157–167

Cao X, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45:4884–4889

Carignan R, Tessier A (1988) The co-diagenesis of sulfur and iron in acid lake sediments of southwestern Québec. Geochim Cosmochim Ac 52:1179–1188

Chen B, Zhou D, Zhu L (2008) Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. Environ Sci Technol 42:5137–5143

Chen J, Liu X, Zheng J, Zhang B, Lu H, Chi Z, Pan G, Li L, Zheng J, Zhang X, Wang J, Yu X (2013) Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. Appl Soil Ecol 71:33–44

Chintala R, Schumacher TE, McDonald LM, Clay DE, Malo DD, Papiernik SK, Clay SA, Julson JL (2014) Phosphorus sorption and availability from biochars and soil/biochar mixtures. CLEAN–Soi Air Water 42:626–634

Chun Y, Sheng G, Chiou CT, Xing B (2004) Compositions and sorptive properties of crop residue-derived chars. Environ Sci Technol 38: 4649–4655

Cui H, Zhou J, Zhao Q, Si Y, Mao J, Fang G, Liang J (2013) Fractions of Cu, Cd, and enzyme activities in a contaminated soil as affected by applications of micro- and nanohydroxyapatite. J Soils Sediments 13:742–752

Dai Z, Meng J, Muhammad N, Liu X, Wang H, He Y, Brookes PC, Xu J (2013) The potential feasibility for soil improvement, based on the properties of biochars pyrolyzed from different feedstocks. J Soils Sediments 13:989–1000

DeLuca TH, MacKenzie MD, Gundale MJ (2009) Biochar effects on soil nutrient transformations. In: Lehmann J, Joseph S (eds) Biochar for environmental management: science and technology. Earthscan, London, pp 251–270

Gong ZT (1999) Chinese soil taxonomy: theory approaches and application. China Science Press, Beijing (in Chinese)

Houben D, Evrard L, Sonnet P (2013) Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (Brassica napus L.). Biomass Bioenerg 57:196–204

Hu XF, Jiang Y, Shu Y, Hu X, Liu L, Luo F (2014) Effects of mining wastewater discharges on heavy metal pollution and soil enzyme activity of the paddy fields. J Geochem Explor 147:139–150

Jiang TY, Jiang J, Xu RK, Li Z (2012a) Adsorption of Pb (II) on variable charge soils amended with rice-straw derived biochar. Chemosphere 89:249–256

Jiang J, Xu RK, Jiang TY, Li Z (2012b) Immobilization of Cu (II), Pb (II) and Cd (II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. J Hazard Mater 229:145–150

Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 6:68–72

Kuiters A, Mulder W (1993) Water-soluble organic matter in forest soils. Plant Soil 152:225–235

Kumar S, Masto RE, Ram LC, Sarkar P, George J, Selvi VA (2013) Biochar preparation from Parthenium hysterophorus and its potential use in soil application. Ecol Eng 55:67–72

Laird D, Fleming P, Wang B, Horton R, Karlen D (2010) Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158:436–442

Lehmann J (2007) A handful of carbon. Nature 447:143–144

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota—a review. Soil Biol Biochem 43:1812–1836

Li YT, Rouland C, Benedetti M, Li FB, Pando A, Lavelle P, Dai J (2009) Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. Soil Biol Biochem 41:969–977

Liang Y, Cao X, Zhao L, Arellano E (2014) Biochar-and phosphate-induced immobilization of heavy metals in contaminated soil and water: implication on simultaneous remediation of contaminated soil and groundwater. Environ Sci Pollut Res 21:4665–4674

Lu K, Yang X, Shen J, Robinson B, Huang H, Liu D, Bolan N, Pei J, Wang H (2014) Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to Sedum plumbizincicola. Agr Ecosyst Environ 191:124–132

Lu RK (1999) Analytical methods for soil agrochemistry. Chinese Agricultural Science and Technology Publishing. House, Beijing (in Chinese)

Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes P (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol Biochem 43:2304–2314

Makoi JHJR, Ndakidemi PA (2008) Selected soil enzymes: examples of their potential roles in the ecosystem. Afr J Biotechnol 7:181–191

Masto RE, Kumar S, Rout TK, Sarkar P, George J, Ram LC (2013) Biochar from water hyacinth (Eichornia crassipes) and its impact on soil biological activity. Catena 111:64–71

Moon DH, Park JW, Chang YY, Ok YS, Lee SS, Ahmad M, Koutsospyros A, Park JH, Baek K (2013) Immobilization of lead in contaminated firing range soil using biochar. Environ Sci Pollut Res 20:8454–8471

Mousavi ZH, Hosseinifar A, Jahed V (2010) Removal of Cu (II) from wastewater by waste tire rubber ash. J Serb Chem Soc 75:845–853

Oleszczuk P, Jośko I, Futa B, Pasieczna-Patkowska S, Pałys E, Kraska P (2014) Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. Geoderma 214–215:10–18

Papa S, Bartoli G, Pellegrino A, Fioretto A (2010) Microbial activities and trace element contents in an urban soil. Environ Monit Assess 165:193–203

Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348:439–451

Parvage MM, Ulén B, Eriksson J, Strock J, Kirchmann H (2013) Phosphorus availability in soils amended with wheat residue char. Biol Fert Soils 49:245–250

Paz-Ferreiro J, Fu S, Méndez A, Gascó G (2014) Interactive effects of biochar and the earthworm Pontoscolex corethrurus on plant productivity and soil enzyme activities. J Soils Sediments 14:483–494

Rieuwerts J, Ashmore M, Farago M, Thornton I (2006) The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils. Sci Total Environ 366:864–875

Spiers GA, McGill WB (1979) Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. Soil Biol Biochem 11:3–8

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Uchimiya M, Lima IM, Thomas Klasson K, Chang S, Wartelle LH, Rodgers JE (2010) Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil. J Agr Food Chem 58:5538–5544

Wang H, Lin K, Hou Z, Richardson B, Gan J (2010) Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars. J Soils Sediments 10:283–289

Wang Y, Shi J, Lin Q, Chen X, Chen Y (2007) Heavy metal availability and impact on activity of soil microorganisms along a Cu/Zn contamination gradient. J Environ Sci 19:848–853

Wu W, Yang M, Feng Q, McGrouther K, Wang H, Lu H, Chen Y (2012) Chemical characterization of rice straw-derived biochar for soil amendment. Biomass Bioenerg 47:268–276

Xu D, Zhao Y, Sun K, Gao B, Wang Z, Jin J, Zhang Z, Wang S, Yan Y, Liu X (2014) Cadmium adsorption on plant-and manure-derived biochar and biochar-amended sandy soils: impact of bulk and surface properties. Chemosphere 111:320–326

Xu GH, Zheng HY (1986) Handbook of analysis of soil microorganisms. Agriculture Press, Beijing (in Chinese)

Yang H, Yan R, Chen H, Lee DH, Zheng C (2007) Characteristics of hemicellulose, cellulose and lignin pyrolysis. Fuel 86: 1781–1788

Yuan JH, Xu RK, Zhang H (2011) The forms of alkalis in the biochar produced from crop residues at different temperatures. Bioresource Technol 102:3488–3497

Zhang X, He L, Sarmah A, Lin K, Liu Y, Li J, Wang H (2014) Retention and release of diethyl phthalate in biochar-amended vegetable garden soils. J Soils Sediments 14:1790–1799

Zhang X, Li F, Liu T, Xu C, Duan D, Peng C, Zhu S, Shi J (2013a) The variations in the soil enzyme activity, protein expression, microbial biomass, and community structure of soil contaminated by heavy metals. Int Sch Res Not

Zhang X, Wang H, He L, Lu K, Sarmah A, Li J, Bolan NS, Pei J, Huang H (2013b) Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci Pollut Res 20: 8472–8483