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ORIGINAL PAPER

The effect of two different biochars on remediation of Cd-contaminated soil and Cd uptake by *Lolium perenne*

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Abstract Biochar can be widely used to reduce the bioavailability of heavy metals in contaminated soil because of its adsorption capacity. But there are few studies about the effects of biochar on cadmium uptake by plants in soil contaminated with cadmium (Cd). Therefore, an incubation experiment was used to investigate the effects of rice straw biochar (RSBC) and coconut shell biochar (CSBC) on Cd immobilization in contaminated soil and, subsequently, Cd uptake by *Lolium perenne*. The results showed that the microbial counts and soil enzyme activities were significantly increased by biochar in Cd-contaminated soil, which were consistent with the decrease of the bioavailability of Cd by biochar. HOAc-extractable Cd in soil decreased by 11.3–22.6% in treatments with 5% RSBC and by 7.2–17.1% in treatments with 5% CSBC, respectively, compared to controls. The content of available Cd in biochar treatments was significantly lower than in controls, and these differences were more obvious in treatment groups with 5% biochar. The Cd concentration in *L. perenne* reduced by 4.47–26.13% with biochar. However, the biomass of *L. perenne* increased by 1.35–2.38 times after adding biochar amendments. So, Cd uptake by whole *L. perenne* was augmented by RSBC and CSBC. Accordingly, this work suggests that RSBC and CSBC have the potential to be used as a useful aided phytoremediation technology in Cd-contaminated soil.

Keywords Cd-contaminated soil · *Lolium perenne* · Rice straw biochar · Coconut shell biochar · Aided phytoremediation technology · Bioavailability

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Introduction

During the past few decades, due to anthropogenic activities such as smelting activities, agricultural industry, and rapid development of industrialization and urbanization, large areas of soil have been polluted by heavy metals, organics, and so on (Bhattacharya et al. 2002; Hechmi et al. 2014a). Among all the contaminants, heavy metals (HM) are among the main elements that cause water and soil pollution (Sun

et al. 2011). In China, it is reported that over 16.76% of farmland soils are contaminated by heavy metals, especially cadmium (Cd) (Song et al. 2013). Cd is of great concern in agricultural ecosystems because it is quite difficult to be degraded by nature in soil (Huang et al. 2011). As a heavy metal of high toxicity to animal and human health (Pelfrêne et al. 2011), Cd in the soil may affect soil micro-ecosystems and inhibit plant growth in soil, even entering the human body through the food chain, causing various diseases, such as kidney dysfunction, pulmonary adenocarcinomas, and hypertension (Zukowska and Biziuk 2008; Bolan et al. 2014). Therefore, the remediation of Cd-contaminated soil is significant for environmental quality and our health.

It is imperative to establish an efficient and feasible method to stabilize heavy metals in soil. Previous work has illuminated many effective methods to decrease migration and bioavailability of heavy metals in contaminated soils (Chen et al. 2011). Common remediation technologies of heavy metals, such as electrokinetics, thermal treatment and excavation, are not suitable for large-scale deployment because of high costs and low efficiencies (Khan et al. 2004). Therefore, it is pressing to develop alternative materials that are low-cost and environment-friendly. In recent years, immobilization remediation agents for heavy metal-contaminated soil have received considerable attention because they are low-cost and highly effective (Liang et al. 2014; Nejad et al. 2017). As a highly effective and environmentally friendly agent for contaminated soil, biochar has become a research hotspot in recent years (Lucchini et al. 2014). Biochars that are produced by pyrolysis of biowaste are carbon-rich materials, which can be used to immobilize the heavy metal cations in soils because of the well-developed micropores, large surface area and large amounts of functional groups (Uchimiya et al. 2011). Moreover, the immobilization of HM by biochar increases soil enzyme activities and the quantity of soil microorganisms (Cui et al. 2013; Lehmann et al. 2011). Soil enzyme activities are a biological indicator for soil quality, especially dehydrogenase, urease and invertase (Paz-Ferreiro et al. 2014; Hossain et al. 2010). As one of the main enzymes in soil, dehydrogenase can be used as an indicator for simple toxicity detection and heavy metal pollution monitoring (Huang et al. 2012). Urease and invertase play an important role in soil, as they indicate the soil's potential to perform specific biochemical reactions (Moreno et al. 2001).

Furthermore, there is an increasing interest in application of biochar that was produced by different feedstocks to improve soil properties. Biochar can decrease the content of available Cd and alleviate its phytotoxicity in soil, which is beneficial to improving the soil microenvironment (Jin et al. 2011; Uchimiya et al. 2010). It has been reported that rice straw biochar (RSBC) is effective in reducing the concentration of the effective state of heavy metals (Lu et al. 2017), and application of crop straw biochar reduces Cd uptake by plants (Bian et al. 2014). Meanwhile, coconut shell biochar (CSBC) has been reported to have good potential as a low-cost and available adsorbent in Cd-contaminated soil (Paranavithana et al. 2016). These previous studies indicated that biochar was significant in reducing the phytoavailability and ecotoxicity of Cd and provides a theory for repairing heavy metal-polluted soil. However, various factors, such as raw material of biochar, initial metal concentration, and biochar usage amount, affect heavy metal adsorption in biochar-amended soil. So, rice straw biochar (RSBC) and coconut shell biochar (CSBC) have been used in this experiment to research the remediation effect of biochar on Cd contaminated soil. Biochar was conducive to enhance the endurance of plants by providing more nutrients (Waqas et al. 2014; Zhao et al. 2015), which accelerated the growth of plants indirectly in heavy metal-contaminated soil (Warnock et al. 2007). In addition, *Lolium perenne* (*L. perenne*) has a good tolerance to HM and can be used to study the effect of biochar on its Cd absorption capacity (Philippe et al. 2007). Until now, there are only few studies with regard to the effect of boichar on heavy metal accumulation in *L. perenne*.

The objective of this study was to investigate systematically the biochemical response of soil after adding biochar and evaluate the potential utilization of RSBC and CSBC as soil conditioners for remediation of Cd. This study was carried out (1) to evaluate the effects of RSBC and CSBC on Cd bioavailability; (2) to obtain the correlation between Cd concentration and soil enzyme activity under the influence of adding CSBC and RSBC; (3) to determine the Cd uptake by *L. perenne* under the influence of biochar.

Materials and methods

Soil and biochar

Soil samples used in this study were collected from topsoil (0–20 cm) on the campus of Sichuan University, Chengdu, China (31°24′N, 104°13′E). The soil samples were air-dried, ground, and sieved through a 2-mm steel sieve. The two biochars (RSBC and CSBC) in this experiment were purchased from Desheng Activated Carbon Company (Jiangsu, China). RSBC and CSBC were made form rice straw and coconut shell through pyrolysis at 700 °C for about 6 and 8 h in a lower oxygen environment. The morphology and microstructure of CSBC and RSBC were investigated by scanning transmission electron microscopy (STEM) with energy dispersive X-ray spectrometry (EDS) (JSM-5900LV, Japan). Fourier transform infrared spectroscopy (FTIR) of the CSBC and RSBC were detected using a Nicolet Nexus 670 spectrophotometer (Thermo Fisher Scientific, USA). The cation exchange capacity (CEC) was measured using a modified compulsive exchange method (Skjemstad et al. 2008). Total organic carbon (TOC) content in soil was determined by the $K_2Cr_2O_7$–H_2SO_4 digestion method (Sorrell et al. 1997; Bai et al. 2005).

Incubation experiments

In this study, measured concentrations (mg kg^{-1}) of Cd and the percentage of biochar (w/w) in different treatments were CK-2.5 (Cd2.5), CK-5 (Cd5), CK-10 (Cd10), $S_{2.5}$-1 (Cd2.5 + 2.5%RSBC), $S_{2.5}$-2 (Cd2.5 + 5%RSBC), $S_{2.5}$-3 (Cd2.5 + 2.5%CSBC), $S_{2.5}$-4 (Cd2.5 + 5%CSBC), S_5-1 (Cd5 + 2.5% RSBC), S_5-2 (Cd5 + 5%RSBC), S_5-3 (Cd5 + 2.5% CSBC), S_5-4 (Cd5 + 5%CSBC), S_{10}-1 (Cd10 + 2.5%RSBC), S_{10}-2 (Cd10 + 5%RSBC), S_{10}-3 (Cd10 + 2.5%CSBC), S_{10}-4 (Cd10 + 5%CSBC). All these groups were tested with three replications. The soil was air-dried and finely sieved through a 2-mm steel sieve. The solutions of Cd (Cd as $CdCl_2{\cdot}2.5H_2O$) were added to the soils to the Cd concentration at 2.5, 5, and 10 mg kg^{-1}. Then, the spiked soils were sieved again through a 2-mm steel sieve. The soil samples were placed in plastic pots (height 13 cm and diameter 18 cm). Six months later, RSBC and CSBC were added to the soil (0, 2.5 and 5%, w/w), respectively. A week later, seeds of *L. perenne* were sterilized in a 20% (v/v) solution of hydrogen peroxide for 20 min and then were placed in moist gauze on petri dishes. Next, the germinated seeds were subsequently transplanted to pots with 40 seedlings. During the period of cultivating, the temperature range was 22–28 °C during the day and 16–20 °C at night in the greenhouse. *L. perenne* was exposed to light for about 12 h every day during the whole incubation. Deionized water was added to the pots to compensate for the evaporation loss of water, and soil moisture content was maintained at approximately 70% of its water holding capacity. Soil samples were collected by the cutting-ring method and soaked with water for 8 h (Wang 1999). Then, the samples were weighed and recorded as W1. The soil samples were dried in an oven at 105 °C for about 10 h until reaching a constant weight (W2). Water holding capacity = W1–W2. Soil pH was measured in a soil/water slurry at a 1:2.5 (w/v) ratio and the electrical conductivity (EC) of soil was measured in a soil/water slurry at a ratio of 1:5 (w/v) (Corwin and Lesch 2003). After 2 months, the mature fruiting bodies of *L. perenne* were harvested, and washed by deionized water to measure the fresh weight. And then they were dried in an oven at 60 °C for 4 days in an oven to measure the dried weight. At each pot, five subsamples at depth of 5–10 cm were taken using a scoop and mixed as a composite sample to measure the soil properties (Xiao et al. 2009). The pH and $CaCl_2$-extractable Cd were measured at 7th, 21st, 42nd and 63rd day. Soil enzyme activities and microbial counts were measured at 7th and 63rd day, and Cd fractions and heavy metal contents were measured at 63rd day.

Heavy metals analysis

Metal speciation in soil samples were detected using the modified BCR procedure (Wu et al. 2016), which was used to extract HOAc-extractable, reducible, oxidizable and residual fractions of Cd. At the same time, a portion of the sub-sample was weighed and digested with HNO_3/$HClO_4$/HF (5:5:3, v/v) at 120 °C on an electric hot plate to determine Cd concentration (Zhu et al. 2016). In addition, the plant available Cd contents in soil were tested by 0.01 M $CaCl_2$ extraction procedures (Burgos et al. 2008). Five-gram samples of air-dry soil were shaken for 3 h at 25 °C with 100 mL of 0.01 M $CaCl_2$ solution. The suspension was centrifuged at 3000 r/min for 10 min and then was filtered by 0.45-μm filter film. The

concentration of Cd in *L. perenne* and soil samples was determined by flame atomic absorption spectrometry (FAAS, SpectrAA-220Fs) (Xiao et al. 2017). FAAS: Baseline drift: 0.004 A/30 min; Standard curve R^2 = 0.9997; Recovery: 95.7–105.0% with RSD of 1.0%; Detection limit: Cd 0.001 mg L^{-1}.

Microbial count analysis

Aqueous extracts of 3-g soil samples were serially diluted to estimate microbial counts (Cheema et al. 2009). Beef extract-peptone medium (pH 7.0 ± 0.2) was supplemented with natamycin (30 μg mL^{-1}) to culture bacteria under 37 °C for 1–3 days (Pedersen 1992), and potato dextrose agar medium was supplemented with streptomycin (50 μg mL^{-1}) to culture fungi under 28 °C for 3–5 days in the dark (Sutjaritvorakul et al. 2010). The colony forming units (CFU) of bacteria and fungi were determined by the series dilution plate count method.

Soil enzyme activities analysis

Dehydrogenase, urease, and invertase activities were determined in this experiment. Dehydrogenase activity was indicated by assaying the formation of triphenylformazan (TPF) (Hechmi et al. 2014b). The formation of TPF was determined by the colorimetric method at 492 nm and the activity was expressed as TPF microgram per gram soil per 48 h.

Urease activity was determined spectrophotometrically at 578 nm by measuring the formation of ammonium which used urea as substrate (Gosewinkel and Broadbent 1984). The activity was expressed as NH_4^+–N microgram per gram soil per 24 h.

Invertase activity was determined by measuring the produced glucose using colorimetry, which used a sucrose solution as a substrate (Gu et al. 2009). One-gram fresh soil samples were incubated for 24 h at 37 °C with 3 mL of 8% sucrose solution and 1 mL phosphate buffer (pH 5.5). The amount of glucose in the supernatant was measured at 508 nm, and invertase activity was expressed as μg glucose per gram soil per 24 h.

Statistical analysis

The mean and standard deviation of the three replicates in the experiment were calculated. Statistical significance was performed using SPSS 18.0 package, and mean values were considered different when $P < 0.05$ using least significant difference (LSD). All statistics were performed using the Origin 8.0 software.

Results and discussion

Characterization of biochars and effects of biochars on soil

Table 1 shows the basic properties of the soil and biochars that were used in the experiment. There was little Cd (total Cd < 0.001 mg kg^{-1}) in soil and biochars, but the total contents of three heavy metal (Cu, Pb and Zn) and the content of CEC were higher. The pH values of RSBC and CSBC were 9.73, 10.55, respectively. RSBC and CSBC were prepared by pyrolysis at 700 °C that was a suitable range of reaction temperature. Pyrolysis biochar had more alkaline pH values compared to hydrochars derived from the same raw materials (Al-Wabel et al. 2013; Sun et al. 2014). The pyrolysis temperature was the critical factor influencing pH values of biochars, and pH values of biochar increased with the increase of pyrolysis temperature (Lehmann et al. 2011; Jiang et al. 2017). So, the biochars that were used in this study had a high pH and could be used to increase the soil pH. The pH value of soil was increased by adding biochar in treatment groups (Table 2). There was no significant difference in the effect of RSBC and CSBC on raising soil pH, and the effect was increased with the increase of the content of biochar. That may be because the soil pH value was relatively high and the proportion of biochar added in soil was small, so the effect of biochar on increasing the soil pH was not obvious.

Table 3 shows that the content of total organic carbon (TOC) was very small in controls. The minimum value for TOC was 3.74 g kg^{-1} in CK-10, but there was no obvious difference in the control groups with three Cd concentrations, which indicated that Cd concentration has little effect on soil organic carbon content. However, biochars significantly improved the content TOC in soil. Total organic carbon increased by 76.88–270.05% and 143.22–372.39% in RSBC and CSBC treatments compared with controls, respectively. The low TOC in soil may be on account of high content of sand in

Table 1 Basic properties of the soil and biochars used in the experiment

Property	Soil	Rice straw biochar	Coconut shell Bichar
Sand (%)	52	–	–
Silt (%)	22	–	–
Clay (%)	26	–	–
Water holding capacity (%)	61.75	–	–
pH (H_2O)	7.21	9.73	10.55
electrical conductivity (ds m^{-1})	0.019	0.18	0.26
Cation exchange capacity (cmol kg^{-1})	10.9	31.6	22.5
Total Cd (mg kg^{-1})	< 0.001	< 0.001	< 0.001
Total Cu (mg kg^{-1})	19.8	23.8	21.9
Total Pb (mg kg^{-1})	–	6.3	16.1
Total Zn(mg kg^{-1})	–	184	139.5

Values in each row followed by different letters indicate significant ($P < 0.05$) difference between different treatment systems. Values represent mean ± standard deviation

–, not measured

Table 2 Effects of different treatment systems on pH in soil

Treatments	pH value			
	Day 7	Day 21	Day 42	Day 63
CK-2.5	7.31 ± 0.01abc	7.28 ± 0.06abc	7.30 ± 0.03ab	7.30 ± 0.11a
CK-5	7.28 ± 0.06ab	7.27 ± 0.01ab	7.34 ± 0.05abc	7.32 ± 0.10ab
CK-10	7.25 ± 0. 10a	7.25 ± 0.01a	7.28 ± 0.01a	7.32 ± 0.06a
$S_{2.5}$-1	7.31 ± 0.11abc	7.35 ± 0.06cde	7.42 ± 0.06c	7.43 ± 0.08abcd
$S_{2.5}$-2	7.36 ± 0.01abc	7.40 ± 0.05def	7.44 ± 0.03cd	7.46 ± 0.02bcde
$S_{2.5}$-3	7.34 ± 0.12abc	7.55 ± 0.02h	7.57 ± 0.06de	7.58 ± 0.01defg
$S_{2.5}$-4	7.57 ± 0.04de	7.69 ± 0.01i	7.62 ± 0.01de	7.63 ± 0.07g
S_5-1	7.37 ± 0.04abc	7.34 ± 0.01bcd	7.44 ± 0.01cd	7.47 ± 0.06bcde
S_5-2	7.43 ± 0.01abcd	7.44 ± 0.01fg	7.44 ± 0.10cd	7.47 ± 0.05bcde
S_5-3	7.47 ± 0.13cde	7.54 ± 0.04h	7.57 ± 0.01de	7.61 ± 0.1fg
S_5-4	7.58 ± 0.14de	7.66 ± 0.04i	7.64 ± 0.04de	7.60 ± 0.01efg
S_{10}-1	7.41 ± 0.08abcd	7.36 ± 0.06cdef	7.39 ± 0.03bc	7.42 ± 0.08abc
S_{10}-2	7.41 ± 0.02abcd	7.42 ± 0.01ef	7.43 ± 0.03cd	7.49 ± 0.01cdef
S_{10}-3	7.44 ± 0.03bcd	7.50 ± 0.01gh	7.54 ± 0.08de	7.55 ± 0.02cdef
S_{10}-4	7.63 ± 0.01e	7.67 ± 0.15i	7.67 ± 0.04e	7.64 ± 0.04g

Values in each row followed by different letters indicate significant ($P < 0.05$) difference between different treatment systems. Values represent mean ± standard deviation

soil (Table 1), and sand content was an important influencing factor for the TOC content in soil, which was decreased with the increase of sand content (Wang et al. 2013). The TOC contents was proportional with the increase of application of biochar, but the effect of CSBC on improving TOC value was stronger than RSBC.

Scanning electron microscopy (SEM) pictures showed the structures of RSBC and CSBC (Fig. 1a, b). The SEM images of RSBC and CSBC exhibited their developed microporous structure and high specific surface. In this study, RSBC and CSBC had porous structure, and CSBC was more prominent than RSBC. The Fourier transform infrared (FTIR) spectra of RSBC and CSBC are shown in Fig. 2, which indicated that each characteristic absorption peak of CSBC was weaker than RSBC. The peaks were assigned to inorganic minerals Si–O–Si at 1014 cm^{-1}, 776 cm^{-1} and 463 cm^{-1}. The peaks at 2922 cm^{-1} and 2853 cm^{-1} represented the C–H bond of methyl and methylene groups. The band at 1567 cm^{-1} was assigned to the aromatic C=C, C=O vibration absorption. A broad peak was observed at 3400 cm^{-1} for CSBC, and this was indicative of O–H groups. And a broad peak of O–H groups at 3423 cm^{-1}. These sufficient active functional groups on biochar surfaces, including amino, hydroxyl and carbonyl, can form complexes with heavy metals, so the adsorption rate of

Table 3 Effects of different treatment systems on soil organic carbon in soil

Treatments	Total organic carbon (g kg^{-1})
CK-2.5	3.98 ± 0.16c
$S_{2.5}$-1	7.04 ± 0.31bc
$S_{2.5}$-2	13.66 ± 0.39ab
$S_{2.5}$-3	9.68 ± 0.51b
$S_{2.5}$-4	17.38 ± 0.43a
CK-5	4.02 ± 0.14c
S_5-1	7.40 ± 0.28bc
S_5-2	14.02 ± 0.35ab
S_5-3	10.14 ± 0.34b
S_5-4	18.99 ± 0.69a
CK-10	3.74 ± 0.21c
S_{10}-1	6.94 ± 0.34bc
S_{10}-2	13.84 ± 0.61ab
S_{10}-3	9.50 ± 0.46b
S_{10}-4	17.62 ± 0.57a

Values in each row followed by different letters indicate significant ($P < 0.05$) difference between different treatment systems. Values represent mean ± standard deviation

heavy metals can be increased by applying biochar (Jiang et al. 2012). In this study, microporous structure, high specific surface and sufficient active functional groups developed on the biochar surface so that the adsorption rate of heavy metals was increased by biochar in soil, which contributed to immobilizing Cd in soil (Baikousi et al. 2013).

Microbial counts in soil

Table 4 shows the total counts of bacteria and fungi in different treatments after the 7th and 63rd days

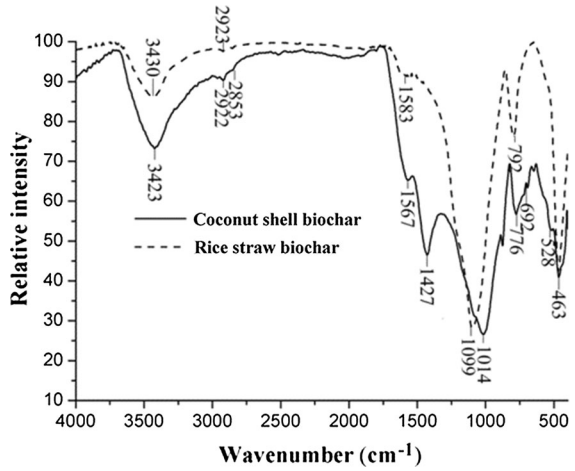

Fig. 2 FTIR-spectra of rice straw biochar (dashed line) and coconut shell biochar (full line)

incubation. The results showed that the counts of microorganisms decreased gradually with the increase of Cd concentration in soil, but microbial counts had a great increase in all treatments compared with the control. After day 7, fungi counts significantly increased by 27.9%, 29.4% and 31.6%, and the bacterial numbers increased by 23.6%, 26.2% and 27.3%, respectively, in $S_{2.5}$-3, S_5-3 and S_{10}-3 compared with the controls. At the 63rd day, there was no significant difference in the increase of microbial counts by adding RSBC and CSBC in low Cd concentration groups (2.5, 5 mg kg^{-1}). Nevertheless, at high Cd concentration (10 mg kg^{-1}), the most significant increase of fungi and bacteria counts was in S_{10}-3, which increased by 30.9% and 27.2%, respectively. However, the increase of fungi and bacteria counts was small in S_{10}-1 and S_{10}-2.

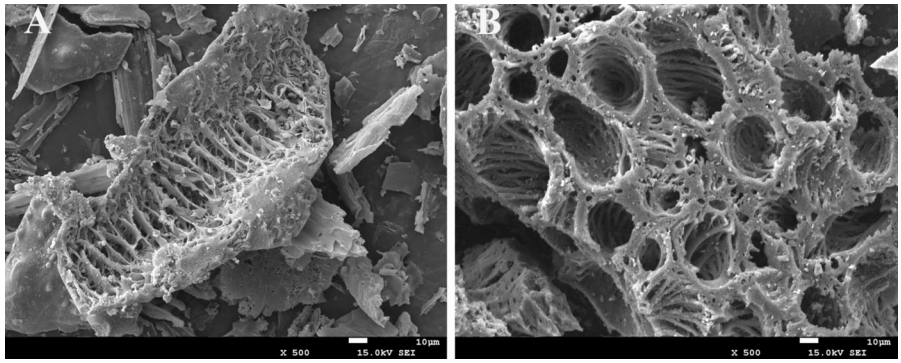

Fig. 1 Scanning electron microphotographs of rice straw biochar (**a**) and coconut shell biochar (**b**) samples

Table 4 Effects of different treatment systems on fungal and bacterial counts in soil

Treatments	Microbial counts (log CFU/g soil)			
	Fungus		Bacteria	
	Day 7	Day 63	Day 7	Day 63
CK-2.5	3.59 ± 0.04b	3.92 ± 0.03b	5.25 ± 0.01c	5.64 ± 0.03b
CK-5	3.50 ± 0.03b	3.71 ± 0.03a	5.12 ± 0.01b	5.49 ± 0.05b
CK-10	3.29 ± 0.10a	3.63 ± 0.01a	5.02 ± 0.02a	5.29 ± 0.08a
$S_{2.5}$-1	4.42 ± 0.07cd	4.28 ± 0.23c	6.42 ± 0.07hij	6.91 ± 0.11f
$S_{2.5}$-2	4.52 ± 0.05fg	4.47 ± 0.08de	6.30 ± 0.01fg	6.81 ± 0.15ef
$S_{2.5}$-3	4.59 ± 0.06g	4.64 ± 0.07def	6.49 ± 0.02j	6.88 ± 0.08f
$S_{2.5}$-4	4.52 ± 0.05fg	4.65 ± 0.03ef	6.42 ± 0.02hij	6.64 ± 0.01cde
S_5-1	4.25 ± 0.07c	4.52 ± 0.06de	6.36 ± 0.004gh	6.65 ± 0.05cde
S_5-2	4.43 ± 0.06ef	4.55 ± 0.04de	6.25 ± 0.04ef	6.63 ± 0.07cde
S_5-3	4.53 ± 0.03fg	4.59 ± 0.02def	6.46 ± 0.02ij	6.80 ± 0.04ef
S_5-4	4.37 ± 0.14de	4.63 ± 0.04def	6.36 ± 0.04gh	6.54 ± 0.01cd
S_{10}-1	4.22 ± 0.04ef	4.58 ± 0.07def	6.22 ± 0.05e	6.57 ± 0.02cd
S_{10}-2	4.24 ± 0.09cd	4.48 ± 0.06de	6.06 ± 0.06d	6.55 ± 0.07cd
S_{10}-3	4.33 ± 0.06cde	4.75 ± 0.03f	6.39 ± 0.08hi	6.73 ± 0.02def
S_{10}-4	4.25 ± 0.07cd	4.45 ± 0.041d	6.37 ± 0.03h	6.45 ± 0.07c

Values in each row followed by different letters indicated significant ($P < 0.05$) difference between different treatment systems. Values represent mean ± standard deviation

It can be concluded from the results that the growth of microorganisms was stimulated by the presence of biochar in the soil, especially CSBC. Soil microorganism was very sensitive to the changes of soil environment and played an important role in soil structure (Ma et al. 2013). So, the increase of microbial counts indicated that the addition of biochar in soil improved the soil environment, indicating that RSBC and CSBC had a positive remediation effect on Cd contaminated soil. Firstly, biochar provided the soil with mineral nutrients and stable organic matter to improve soil physicochemical characteristics, which promoted the reproduction of microorganisms in soil (Steiner et al. 2010; Lehmann and Joseph 2009). Just as RSBC and CSBC were added in soil, the content of TOC and microbial counts were increased. Moreover, a large amount of uniform pore structures of biochar were generated from biomass cracking in the process of biochar production, which provided a good "shelter" for microbial reproduction (Kolb et al. 2009). The micropore structure of CSBC was almost spherical in shape (Fig. 1), which contributed to the soil water retention and microbial growth (Kauffman et al. 2009). These reasons might indirectly explain why the microbial counts were positively correlated with biochar in soil.

Soil enzyme activities

In order to further reflect the soil microecology after remediation, we tested soil enzyme activities (Fig. 3). Activities of soil enzymes were remarkably increased by the presence of biochar and *L. perenne* compared with control. After 7 days of incubation, dehydrogenase and urease activities were slightly decreased by the addition of biochar compared to the control (Fig. 3a, b), and invertase activities had no significant change (Fig. 3c). As an important indicator of pollution levels in soil, dehydrogenase activities declined by 23.30–25.70% in treatments compared with the controls. The lowest value of dehydrogenase (1.38 μg TPF g^{-1} dry soil h^{-1}) and urease activities (12.1 μg NH_4^+–N g^{-1} dry soil h^{-1}) were found in the S_{10}-2 group. These results are similar to the change trend of bacteria and fungi counts, suggesting that high Cd levels inhibited soil microbial quantity and soil enzyme activities. After 63 days of incubation, the activities of dehydrogenase, urease and invertase in these groups with biochar amendments markedly increased by 37.78–60.16%, 20.37–37.22%, 105.56–144.12% compared with controls, respectively. Especially, urease activity in $S_{2.5}$-3, S_5-3 and S_{10}-3 increased by 6.35, 3.9, 4.07 μg NH_4^+–N g^{-1} - dry soil h^{-1}, respectively, compared to the controls.

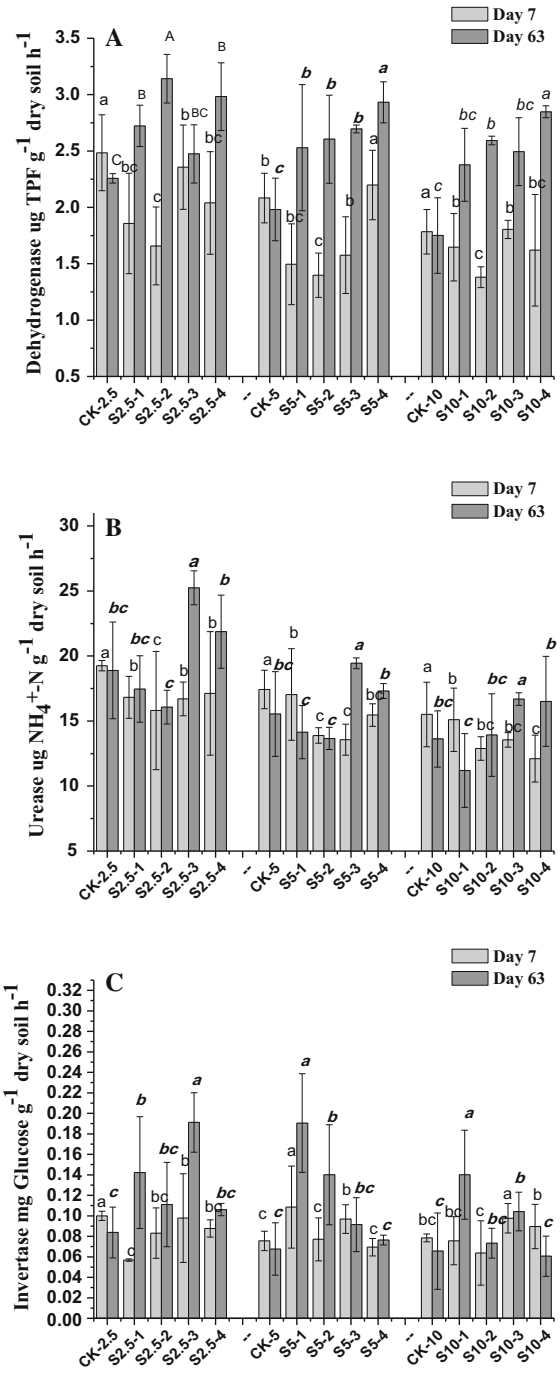

Fig. 3 Effects of different treatment systems on the soil enzyme activities. **a** Dehydrogenase activities, **b** urease activities, **c** invertase activities. Error bars represent the standard deviation of three sampled pots ($P < 0.05$)

Dehydrogenase and urease activities in these treatments with addition of CSBC were increased more dramatically than those in treatments with addition of RSBC in Cd-contaminated soil. In particular, the urease activity was increased significantly with the application of 2.5% CSBC.

The results showed that the soil enzyme activities decreased gradually with the increase of Cd concentration, but adding biochar could significantly enhance soil enzyme activities in soil. In heavy metal-contaminated soil, biochar not only increased the amount of soil microorganisms, but also promoted the soil enzyme activities (Cui et al. 2013). The improvement effect of CSBC on soil enzyme activities was consistent with the changes trend of microbial counts. Obviously, the microbial counts were closely related to soil enzyme activities. The change trend of soil enzymes activities was consistent with microbial counts which indicated a positive correlation between microbial counts and soil enzyme activities, and the same conclusion was found in previous studies (Liu et al. 2015; Wu et al. 2015). Obviously, biochars weakened the negative effect of Cd on soil enzyme activities. In the meantime, the addition of CSBC was more effective on improving soil biochemical characteristic than RSBC.

Cd fractions in soil and Cd content in *L. perenne*

The fractions of Cd in soil were shown in Fig. 4. The HOAc-extractable and the reducible of Cd were the most main two fractions of Cd. In this study, the proportion of these two forms was greater than 90%. The addition of biochar significantly changed the distribution of Cd formations in soil and effectively promoted the conversion of HOAc-extractable Cd into other stable forms. The conversion effect of biochars was increased with the increase of adding of biochar and was different in three concentrations of Cd in soil. When the Cd concentration in soil was 2.5 mg kg^{-1}, the percentage of HOAc-extractable Cd decreased by 11.3% and 7.2%, respectively, in $S_{2.5}$-2 and $S_{2.5}$-4 compared with the control. When the Cd concentration was 5 mg kg^{-1}, the percentage of HOAc-extractable Cd decreased by 22.6% and 10.4%, respectively, in S_5-2 and S_5-4 groups compared with the control. When the Cd concentration was 10 mg kg^{-1}, the percentage of HOAc-extractable Cd reduced by 11.5% and 17.1%, respectively, in S_{10}-2 and S_{10}-4 groups compared with the control.

RSBC and CSBC effectively reduces the content of HOAc-extractable Cd in soil, because biochar as a

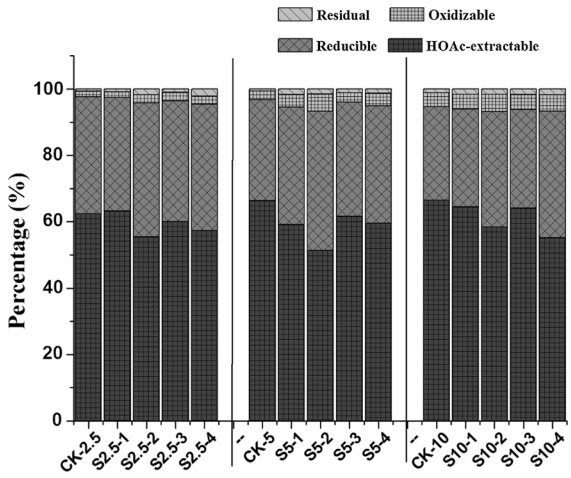

Fig. 4 Effects of different treatment systems on the Cd fractions in soil. Error bars represent the standard deviation of three sampled pots ($P < 0.05$)

fundamental fixative forms a complex compound with heavy metals to reduce the bioavailability of heavy metals in soil (Bolan et al. 2014). The different conversion rates of HOAc-extractable Cd demonstrated that CSBC was more suitable for immobilizing Cd in contaminated soil with high-concentration Cd, and RSBC was more applicable to immobilize Cd in low concentrations of Cd in contaminated soil. These might tightly relate to the different biochar properties. Biochar has the capacity to adsorb heavy metals in the soil depending on the cation exchange mechanism and the complexation of surface functional groups (Uchimiya et al. 2011). The surface functional groups of RSBC and CSBC (carboxylic, carbonylic, phenolic, and other oxygen-containing groups) affect the absorption capacity of heavy metals and the extent of the biochar to which it can immobilize heavy metals in the long term (Mao et al. 2012). Some studies showed that CEC, specific area and high pH are the important factors that influenced the adsorption of metal ions (Yuan et al. 2011; Lundberg and Sundqvist 2011). The CEC and the surface functional groups of RSBC were higher than those of CSBC (Table 1; Fig. 2), but the microporous structure of CSBC was more developed than RSBC (Fig. 1). In our study, the effect of CEC and surface functional groups on the adsorption of heavy metals was stronger than the microporous structure in contaminated soil with low Cd concentration. But, the effect of microporous structure was stronger in contaminated soil with high Cd concentration. So, the difference of CEC, surface functional groups and microporous structure of CSBC and RSBC led to the distinction of reducing HOAc-extractable Cd contents in different treatments.

Figure 5 shows the concentration of Cd in *L. perenne* (dried weight) in all groups. The Cd concentration in *L. perenne* in treatment groups was slightly less than controls. The Cd concentration in *L. perenne* in these treatments of three Cd levels decreased by 4.47–8.07%, 14.20–24.27%, 11.20–26.13%, respectively, compared with controls. To further understand of effects of biochar on total Cd accumulation in *L. perenne*, we have weighed the biomass of *L. perenne* (Table 5). In these groups of different Cd concentrations, the highest dried weights of *L. perenne* were in $S_{2.5}$-4 (5.26 g), S_5-4 (6.27 g), and S_{10}-4 (5.77 g), which increased to 1.87, 2.38, and 2.27 times, respectively, compared with controls. It suggested that the treatments of adding 5% CSBC in polluted soil had the best effect on improving the growth of *L. perenne*.

The Cd concentration in *L. perenne* was decreased because the inactivation of Cd by biochar amendment prevented the transportation of Cd from soil to plant and consequently reduced the content of Cd in plants (Puga et al. 2015). The accumulation of Cd in the whole plant in the treatment group was increased by adding biochar compared with the control group (Figs. 3, 4). This was because, although high

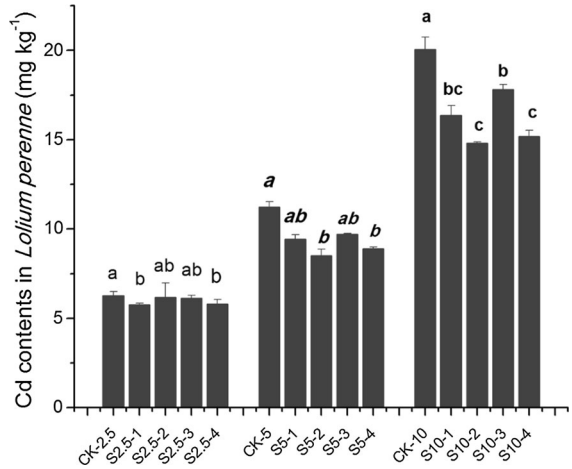

Fig. 5 Effects of different treatment systems on Cd concentration in *Lolium perenne* (dried weight). CK-2.5, CK-5 and CK-10 represent 2.5, 5 and 10 mg kg^{-1} of Cd in soil without biochar. Error bars represent the standard deviation of three sampled pots ($P < 0.05$). Values represent means ± standard deviation

Table 5 The fresh and dried weight of *L. perenne* in different treated soils

Treatments	Fresh weight (g)	Dried weight (g)
CK-2.5	13.30 ± 0.31ab	2.94 ± 0.17abc
CK-5	12.50 ± 0.82a	2.63 ± 0.24ab
CK-10	12.13 ± 0. 92a	2.54 ± 0.21a
$S_{2.5}$-1	17.02 ± 0.97bcde	4.64 ± 0.19bcdef
$S_{2.5}$-2	18.77 ± 2.03abc	5.50 ± 1.09def
$S_{2.5}$-3	15.36 ± 1.58abc	3.96 ± 0.86abcde
$S_{2.5}$-4	19.11 ± 0.53de	5.26 ± 0.19def
S_5-1	15.58 ± 1.40abcde	4.23 ± 0.41abcdef
S_5-2	18.11 ± 3.76cde	5.45 ± 1.73def
S_5-3	14.75 ± 0.59abcd	3.85 ± 0.53abcde
S_5-4	20.59 ± 0.70e	6.27 ± 0.46f
S_{10}-1	16.23 ± 0.68abcde	4.50 ± 0.15abcdef
S_{10}-2	17.75 ± 1.96bcde	4.87 ± 0.64cdef
S_{10}-3	14.49 ± 4.14abc	3.51 ± 1.71abcd
S_{10}-4	19.26 ± 2.23de	5.77 ± 1.36ef

Values in each row followed by different letters indicated significant ($P < 0.05$) difference between different treatment systems. Values represent mean ± standard deviation

adsorption capacity of biochar reduced Cd concentration in *L. perenne*, the biomass of *L. perenne* was significantly increased by biochar. The high specific surface area and developed microporous structure of biochar increased nutrient stocks in the rooting zone and reduced nutrient leaching to improve plant yields (Baronti et al. 2010). As shown in Tables 3 and 5, the content of TOC was increased by the adding of biochars, which increased the soil nutrients in treatment groups. CSBC had a more distinguishable porous structure than RSBC (Fig. 1a, b), which provided CSBC an advantage in promoting *L. perenne* growth. Therefore, the adding of biochar decreased the Cd concentration in *L. perenne*, but the Cd uptake in the whole *L. perenne* was increased compared with controls.

Availability of Cd and its relationship to bioaccumulation by biochar

In this study, 0.01 M $CaCl_2$ -extractable Cd concentration is represented in Fig. 6. Figure 6a–d respectively represents the concentration of $CaCl_2$-extractable Cd in soil at the 7th, 21st, 42nd, and 63rd reaction day after applying the biochar. When the biochar was added to Cd-polluted soil, the content of $CaCl_2$-extractable Cd was effectively reduced. With the increase of biochar application, the content of $CaCl_2$-extractable Cd was decreased significantly ($P < 0.05$). Furthermore, CSBC was more effective in reducing the content of available Cd than RSBC. At 63rd day, $CaCl_2$-extractable Cd reduced in soil by 27.2%, 49.9% and 43.7% respectively, in $S_{2.5}$-4, S_5-4, and S_{10}-4 compared with controls. Similarly, $CaCl_2$-extractable Cd decreased by 19.1%, 40.6%, and 35.4% in $S_{2.5}$-2, S_5-2, and S_{10}-2.

We know that the high content of sand in soil was an important factor for the low TOC content, which was a key reason for the high $CaCl_2$ -extractable Cd in soil (Mohamed et al. 2015). The organic matter content was increased by applications of the biochars (RSBC and CSBC) while reducing the concentrations of $CaCl_2$ -extractable Cd, which revealed a negative correlation between TOC and the available Cd concentrations. Moreover, soil pH and the mobility of heavy metal in soil had a close relationship, because the immobilization of heavy metal was governed by the increase of soil pH, which induced a greater retention of metals on soil particles (Rees et al. 2014). In this study, RSBC and CSBC with high pH value was used to increase the soil pH to relatively alkaline, and the effect of CSBC on improving soil pH value was more significant (Table 2). The results have confirmed that these biochars had the ability to reduce $CaCl_2$-extractable Cd in soil, and CSBC was more efficient than RSBC.

Conclusions

The soil pH, total organic carbon, microbe quantity and enzyme activities were increased by biochar treatments. The soil biological properties in Cd-polluted soils were remarkably improved by CSBC and RSBC, especially CSBC. The application of RSBC and CSBC significantly decreased the percent of HOAc-extractable Cd in soil. The diminution of Cd toxicity was positively related to Cd immobilization, and the decrease of Cd bioavailability in soil improved the soil microbiology. Furthermore, *L. perenne* showed a high tolerant ability for all Cd levels in this experiment. Although the concentration of Cd in *L. perenne* was reduced by biochar, RSBC and CSBC

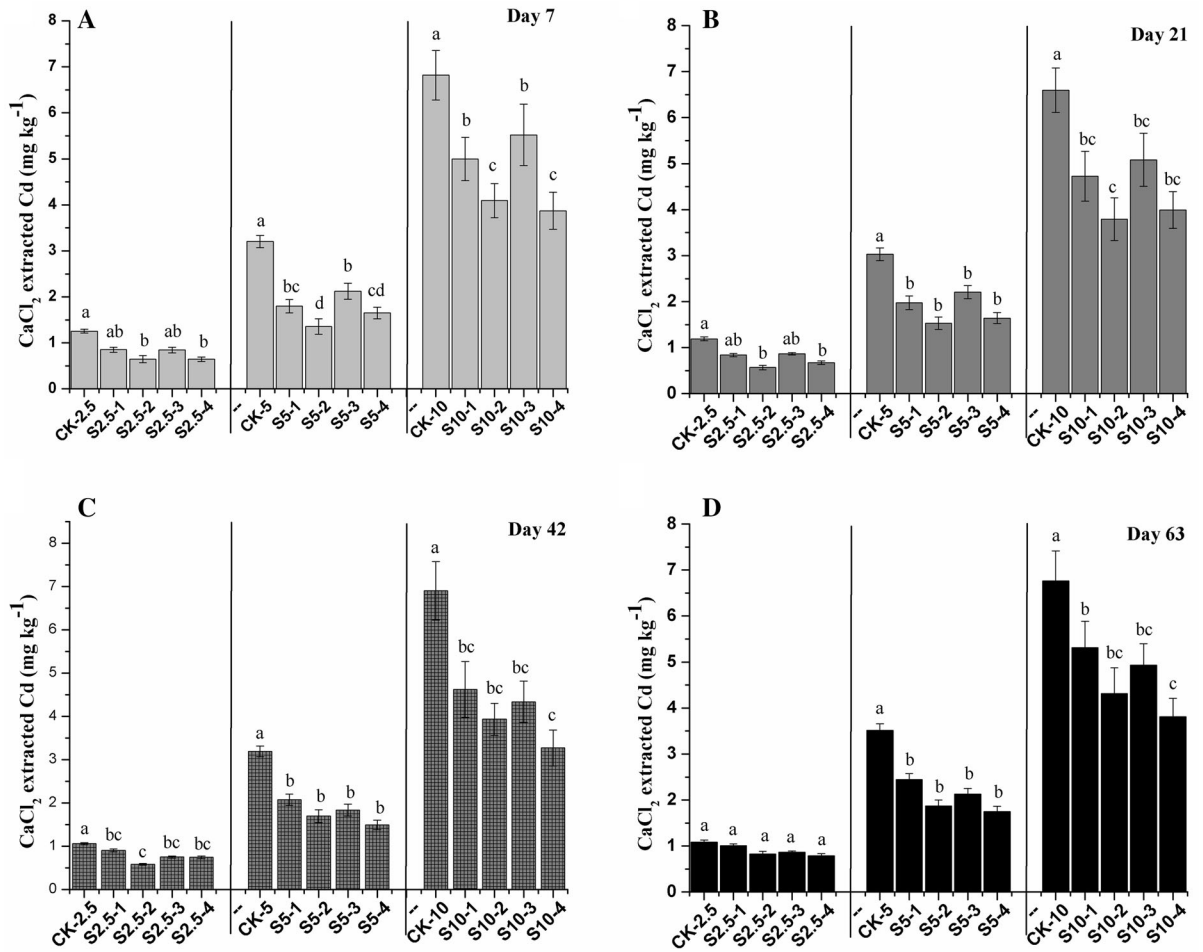

Fig. 6 Effects of different treatment systems on the $CaCl_2$-extractable Cd in soil. **a**–**d** respectively represent the concentration of $CaCl_2$-extractable Cd in soil at the 7th, 21st, 42nd, and 63rd reaction day after applying the biochar. Error bars represent the standard deviation of three sampled pots. Columns denoted by different letters indicated significant ($P < 0.05$) differences among different treatments

greatly increased the biomass of *L. perenne* to enhance the Cd uptake in whole *L. perenne* compared with controls. Our results suggested that the presence of biochar was effective in promoting the remediation of soil contaminated with Cd and impacting the Cd uptake by *L. perenne*, especially the CSBC for the soil with high level of Cd.

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