ORIGINAL RESEARCH

Biochar alleviates metal toxicity and improves microbial community functions in a soil co-contaminated with cadmium and lead

Nahid Azadi[1] · Fayez Raiesi[1]

Abstract

Soil amendment with biochar alleviates the toxic effects of heavy metals on microbial functions in single-metal contaminated soils. Yet, it is unclear how biochar application would improve microbial activity and enzymatic activity in soils co-polluted with toxic metals. The present research aimed at determining the response of microbial and biochemical attributes to addition of sugarcane bagasse biochar (SCB) in cadmium (Cd)-lead (Pb) co-contaminated soils. SCBs (400 and 600 °C) decreased the available concentrations of Cd and Pb, increased organic carbon (OC) and dissolved organic carbon (DOC) contents in soil. The decrease of metal availability was greater with 600 °C SCB than with 400 °C SCB, and metal immobilization was greater for Cd (16%) than for Pb (12%) in co-spiked soils amended with low-temperature SCB. Biochar application improved microbial activity and biomass, and enzymatic activity in the soils co-spiked with metals, but these positive impacts of SCB were less pronounced in the co-spiked soils than in the single-spiked soils. SCB decreased the adverse impacts of heavy metals on soil properties largely through the enhanced labile C for microbial assimilation and partly through the immobilization of metals. Redundancy analysis further confirmed that soil OC was overwhelmingly the dominant driver of changes in the properties and quality of contaminated soils amended with SCB. The promotion of soil microbial quality by the low-temperature SCB was greater than by high-temperature SCB, due to its higher labile C fraction. Our findings showed that SCB at lower temperatures could be applied to metal co-polluted soils to mitigate the combined effects of metal stresses on microbial and biochemical functions.

Keywords Bagasse biochar · Microbial activity · Soil enzymes · Metal co-contamination

1 Introduction

Soil contamination with cadmium (Cd) and lead (Pb) is an important environmental concern because of their high toxicity and non-biodegradable nature, as well as different source contributions in the soil–plant system (Alloway 2013; Smolders and Mertens 2013; Steinnes et al. 2013; Wang et al. 2020). These highly mobile and potentially toxic elements enter into soils mainly through anthropogenic activities such as metal-mining/smelting activities and land application of sewage sludge (Alloway 2013; Palansooriya et al.

2020). Cadmium and Pb are those soil toxic metals that mostly exist together in contaminated areas due to their identical anthropogenic and natural sources, particularly through wastes produced during ore mining and industrial activities (Alloway 2013; Palansooriya et al. 2020). Therefore, it is anticipated that the potential toxicity of these metals would concomitantly affect soil microbial community and properties at metal-contaminated sites (Xu et al. 2018, 2019; Ni et al. 2019; Jiang et al. 2020). Numerous earlier studies have demonstrated a detrimental influence of Cd and Pb, both singly and jointly, on microbial and biochemical properties/processes, often with reduced microbial activity, microbial biomass and enzyme activity in polluted soils (Khan et al. 2010; Huang et al. 2017; Zhan et al. 2018; Nie et al. 2018; Xu et al. 2018, 2019). Metal co-contamination has a greater negative effect on soil microbial community and enzyme activity compared with single metals (Huang et al. 2017; Zhan et al. 2018; Xu et al. 2019). For example, it was reported that the co-contamination of Cd and Pb inhibited

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the activity and biomass of the soil microbial community as well as soil enzymatic activities more than soil contamination with single Cd or Pb (Khan et al. 2010; Zhan et al. 2018; Xu et al. 2018, 2019). However, responses of soil microbial communities and biogeochemical processes to metal contamination, alone or in combination, depend upon soil conditions and environmental factors (Vig et al. 2003; Abdu et al. 2017), which play a crucial role in the solubility and toxicity potential of metals (Bolan et al. 2014; Shaheen et al. 2019; Palansooriya et al. 2020). Amongst soil factors, pH and the content of soil organic matter (SOM) are important determinants of metal mobility and availability (Smolders and Mertens 2013; Bolan et al. 2014; Palansooriya et al. 2020), because of their impacts on metal adsorption, precipitation, surface complexation, ion exchange, their speciation in the soil solution and fractionation within the soil solid phases (Bolan et al. 2014; Palansooriya et al. 2020). Soil properties and processes, particularly pH and SOM, are known to improve with application of biochar (Spokas et al. 2010; Lehmann et al. 2011; Gul et al. 2015; Khadem and Raiesi 2017; Zhang et al. 2018; Song et al. 2019), a carbon-rich amendment produced during the pyrolysis of biomass under oxygen-limited conditions (Lehmann et al. 2011; Mukherjee et al. 2011). However, the direction and magnitude of changes in soil properties are largely biochar-specific and soil-specific as well (Lehmann et al. 2011; Gul et al. 2015; Pokharel et al. 2020).

In recent years, the use of biochar in metal-contaminated soils has gained widespread attention, because soil amendment with biochar was reported to immobilize and retain toxic metals in the soil (Ahmad et al. 2014; Bolan et al. 2014; Yuan et al. 2019; Palansooriya et al. 2020; Wang et al. 2020), thereby alleviating the potential toxicity of heavy metals to soil microbial community and biochemical functions (Park et al. 2011; Yang et al. 2016; Nie et al. 2018; Xu et al. 2018; Bashir et al. 2018, 2019). Biochar can also have a positive impact on microbial activity and enzymatic activity through its potential direct and indirect effects on soil physical, chemical and nutritional characteristics, particularly the build-up of SOM (Xu et al. 2018; Bashir et al. 2018; Song et al. 2019; Pokharel et al. 2020).

Nevertheless, very little is known about how biochar would influence the microbial and biochemical functions in soils simultaneously polluted with Cd and Pb (Xu et al. 2018). In co-contaminated soils, a metal may contribute to the release of other metals to soil solution and consequently would enhance the availability of the released metals (Ni et al. 2019; Fan et al. 2020; Jiang et al. 2020). In single-metal contamination, the biochar derived from digested sludge showed much greater adsorption capacity for Pb than for Cd from aqueous solution, and Cd adsorption on biochar was strongly reduced in metal co-contaminated systems (Ni et al. 2019). Application of biochar produced from wheat straw at 700 °C was shown to immobilize both Cd and Pb in co-contaminated soils and decrease their concentrations in ryegrass biomass (Jiang et al. 2020). Recently, Jiang et al. (2020) reported that Pb immobilization was higher than Cd immobilization in both single and binary contamination of metals, which was attributed to the strong affinity of Pb than Cd for binding to biochar surface sites when they coexist in the soil. Microbial activity and biomass were lower in Cd and Pb co-contaminated soils than in the soils spiked with Cd or Pb alone, and addition of macadamia nutshell biochar at 5% reduced metal toxicity (Xu et al. 2018).

The annual production of sugarcane bagasse, a major feedstock resource during the sugarcane refining process (Sarker et al. 2017), was estimated to be approximately 2.4 million tons in the Iranian sugarcane industry, Khuzestan province (Mohammadi et al. 2020). Previous works have demonstrated that biochar derived from sugarcane bagasse is an important source of organic matter and a valuable amendment to ameliorate the chemical, physical and biological fertility of soils with low organic matter content (Azeem et al. 2019; Bento et al. 2019; Zafar-ul-Hye et al. 2020; Rahman et al. 2021). Sugarcane bagasse biochar is characterized by high specific surface area, abundant micropores and high surface functional groups (Moradi-Choghamarani et al. 2019), which can sorb and immobilize heavy metal ions (Ding et al. 2014; Sarker et al. 2017; Bashir et al. 2018; Zahedifar and Moosavi 2020). Several studies have suggested that sugarcane bagasse-derived biochar could be used as a potential sorbent for metal removal and immobilization to remediate metal-contaminated soils, and to decrease metal toxicity to the soil microbial community (Bashir et al. 2018; Nie et al. 2018) and plants (Mohamed et al. 2019). However, limited information is available on how soil application of sugarcane bagasse biochar influences microbial properties and performance in metal-polluted soils (Nie et al. 2018). Such knowledge would be useful to use biochars generated from sugarcane bagasse for metal immobilization and to alleviation of metal toxicity to the soil microbial community and functions. Thus, the main aim of this research was to determine the response of microbial and biochemical properties to application of sugarcane bagasse biochar to a soil co-polluted simultaneously with Cd and Pb. The main hypotheses were that (1) the use of bagasse biochar would reduce the toxicity of Cd and Pb to the activity and biomass of microbial community and enzyme activity in co-contaminated soils, and (2) the efficacy of biochar on the microbial and biochemical functions would be lower in co-spiked soils than single-spiked soils, likely due to competitive sorption between metals.

2 Materials and methods

2.1 Soil and biochar preparation

A cropland soil sample (clay loam, Typic Calcixerepts, 0–20 cm depth) was collected from the Shahrekord Plain (32° 19' 41" N, 50° 47′ 37" E). The soil sample was sieved (< 2 mm) and analyzed for physicochemical properties with the following values: Clay = 315 g kg^{-1}; silt = 401 g kg^{-1}; sand = 284 g kg^{-1}; CaCO$_3$ = 280 g kg^{-1}; pH (1:2 ratio) = 7.9; electrical conductivity (EC, 1:2 ratio) = 0.31 dS m^{-1}; organic carbon (OC) = 4.7 g kg^{-1}; cation exchange capacity (CEC) = 30.1 cmol$_c$ kg^{-1}; field capacity (FC) = 21.2% (w/w); total Cd and Pb = 0.2 and 20 mg kg^{-1}, respectively. The measured concentrations of available Cd and Pb using DTPA-TEA (diethylene tri-amine penta acetic acid-triethanol amine) extractant were 0 and 0.6 mg kg^{-1}, respectively. The biochars were prepared from sugarcane bagasse feedstock by slow pyrolysis at 400 (B400) and 600 (B600) ^{o}C for 120 min under limited supply of oxygen using a muffle furnace (Khadem and Raiesi 2017). The physicochemical properties of the bagasse-derived biochars were determined following the methods described by Khadem and Raiesi (2017). The measured characteristics of the biochars are presented in Table 1.

2.2 Experimental layout and soil incubation

This laboratory experiment was conducted using a completely randomized factorial design with two factors. The factors used in the present study included: (i) metal contamination (10 mg Cd kg^{-1} as the single Cd contamination, 150 mg Pb kg^{-1} as the single Pb contamination and 10 mg Cd kg^{-1} + 150 mg Pb kg^{-1} as the co-contamination) and (ii) biochar application (CK, without biochar, B400 and B600 at 1%, w/w), each with three replications (n = 3). The study soil was polluted using Cd chloride and Pb chloride solutions to achieve the above concentrations. Metal-polluted soils were homogenized for the entire distribution of the metals in the soil matrix. The samples were remoistened at 60–70% of the FC, and kept at room tempreature (about 20 ± 5 °C) for 4 weeks. The polluted soils were then treated with the organic amendments. The soil-biochar mixtures were then mixed thoroughly for a uniform distribution of biochar particles in the soil matrix. To lessen the impact of soil preparation, and to reactivate the soil microorganisms, the treated soils were remoistened at 60–70% FC and pre-incubated at room temperature for 20 days. Ultimately, the soil samples were adjusted to 65 ± 5% of the FC and incubated for 4 months at 25 ± 1 °C.

Table 1 The basic properties of the bagasse biochars produced at 400 (B400) and 600 (B600) ^{o}C

Property	B400	B600
Yield (%)	38.8	27.2
Ash (%)	29.6	35.3
pH	9.20 (1:20)	9.90 (1:20)
Electrical conductivity (dS m^{-1})	1.0 (1:20)	1.9 (1:20)
Cation exchange capacity (cmol$_c$ kg^{-1})	4.00	2.60
Specific surface area (m^2 g^{-1})	10.4	97.3
Carbon (C) (g kg^{-1})	529	547
Nitrogen (N) (g kg^{-1})	4.8	5.6
Hydrogen (H) (g kg^{-1})	31	14
Sulfur (S) (g kg^{-1})	0.40	0.60
Oxygen (O) (g kg^{-1})	134	86
C:N molar ratio	127	113
O:C molar ratio	0.19	0.12
H:C molar ratio	0.70	0.31
H:O molar ratio	3.70	2.60
Volatile matter (%)	54.1	41.4
Fixed C (%)	23.1	36.0
Cadmium (mg kg^{-1})	0.18	0.20
Lead (mg kg^{-1})	5.00	5.50
Iron (mg kg^{-1})	680	741
Manganese (mg kg^{-1})	68.7	76.2
Copper (mg kg^{-1})	7.00	7.60
Zinc (mg kg^{-1})	88.1	79.6
Potassium (g kg^{-1})	5.95	8.01
Phosphorus (mg kg^{-1})	186	342
Sodium (g kg^{-1})	1.16	1.38
Calcium (g kg^{-1})	27.7	35.0
Magnesium (g kg^{-1})	8.06	11.6

2.3 Soil analysis for microbial properties

In this experiment, we used several microbial assays (i.e., C mineralization, basal respiration, substrate-induced respiration and microbial biomass) as the toxicological endpoints (Kuperman et al. 2014; Raiesi and Dayani 2021). Soil C mineralization was determined as the CO$_2$ evolved over a 120-day period using 0.5 M NaOH for trapping the respired CO$_2$ (Alef and Nannipieri 1995). The cumulative C mineralization (Cmin) was then calculated for the whole incubation period. The total biochar C mineralized (TCM, %) was calculated as the ratio of the Cmin values over the amount of C added initially and used as a measure of biochar C turnover rate. At the end of the incubation period, basal respiration (BR) was quantified as the amount of microbial CO$_2$ emitted from the treated soils over 4 weeks according to the method

described above, and substrate-induced respiration (SIR) as the evolved CO_2 from glucose-amended soils over 5–6 h following the method described in Lin and Brookes (1999). Microbial biomass carbon (MBC) was quantified using the fumigation-incubation method as described by Joergensen (1995). The microbial quotient (qM), the ratio of MBC to OC expressed as %, was calculated to provide an indicator of substrate availability for the microbial population or conversion efficiency of OC into microbial biomass. The metabolic quotient (qCO_2) was also calculated by dividing BR by MBC values (the CO_2–C respired per MBC per day) and used as an indicator of stress to the microbial community (Anderson and Domsch 2010).

Furthermore, the activities of soil urease (URE), alkaline phosphomonoesterase (ALP) and arylsulphatase (ARY) were determined (Alef and Nannipieri 1995). Soil catalase (CAT) activity was measured according to the method described by Liu et al. (2008), by the back titration of residual hydrogen peroxide (H_2O_2) with $KMnO_4$ and the activity of dehydrogenase (DEH) was assayed by determining the amount of triphenyl formazan released from the reduction of the triphenyl tetrazolium chloride (Alef and Nannipieri 1995). The fluorescein diacetate hydrolysis (FDA) activity was measured colorimetrically using fluorescein diacetate as substrate according to the method described by Green et al. (2006). Soil enzyme activities are not considered as the toxicological endpoints, since the enzymatic activities are measured at conditions that are not representative for in situ conditions (Kuperman et al. 2014), and, therefore, their ecological relevance is uncertain for soil ecotoxicological risk assessment of heavy metals.

Soil pH, EC, OC and DTPA-extractable Cd and Pb concentrations (Lindsay and Norvell 1978) were also determined at the end of the incubation period. Soil dissolved organic carbon (DOC) content was determined by following the procedure as outlined by Guo et al. (2015).

2.4 Statistical analysis

We used two-way analysis of variance (ANOVA) to examine the impact of metal contamination, biochar application, and their interaction on soil properties. The soil datasets were tested for the homogeneity of variance and normality before statistical analysis using the procedure described by Kozak and Piepho (2018). The mean values were compared based on the Tukey's test at 5% level of significance ($P \leq 0.05$). The software Minitab (Minitab 18.1) was used for all statistical analyses. Soil datasets were also subjected to a redundancy analysis (RDA) as a constrained ordination model to evaluate the correlation of microbial properties and enzyme activities as response variables with soil chemical properties as explanatory variables using the CANOCO 4.5

software for Windows. For the RDA model, each RDA axis and explanatory variables were tested using a Monte Carlo permutation test in CANOCO.

3 Results

3.1 Soil chemical properties

The effects of biochar addition on soil pH and EC were statistically ($P \leq 0.05$) significant (Fig. 1). Soil pH increased by 0.1 units and EC decreased by 0.1 units only with addition of B400. Although significant, the effects of B400 on soil pH and EC were small and these changes are unlikely to be a factor to influence the availability of metals in the current experiment. Generally, the DTPA-extractable metal (Cd and Pb) concentrations were considerably higher with the co-presence of metals compared with the single metals, regardless of the biochar application (Fig. 1). The results of two-way ANOVA indicated that the DTPA-extractable metal concentrations were significantly affected by the main effects of pollution treatment ($P \leq 0.001$), biochar addition ($P \leq 0.001$) and their interaction ($P \leq 0.001$). Biochar application decreased the DTPA-extractable Cd concentration by 14–18% in Cd-contaminated soil and by 16–18% in co-contaminated soil compared with the control (Fig. 1). The reduction of Cd availability with addition of B600 and B400 was 18% and 14%, respectively, in Cd-contaminated soils but did not differ between the two biochars in co-contaminated soils. With addition of B400, the declined Cd availability was greater in co-spiked soils (16%) than in single-spiked soils (14%). Similarly, biochar addition decreased the DTPA-extractable Pb by 10–22% in Pb-contaminated soils and 12–16% in Cd + Pb co-contaminated soils when compared with the control without biochar, with greater reductions in B600 than B400 treatments (Fig. 1). In B400-treated soils, the decreased Pb availability was greater in co-contaminated soils (12%) than in single-contaminated soils (10%) while a reverse trend was observed in B600-treated soils (16 vs. 22%). Furthermore, the decrease of Cd availability (16–18%) with biochar addition was larger than that of Pb availability (12–16%) in co-spiked soils compared with the corresponding control. The amount of soil OC was significantly increased by 94–100% after addition of both biochars across contaminated soils compared to the control, from 4.61 g kg^{-1} in the unamended soil to 8.93 g kg^{-1} in B400-amended soils and 9.23 g kg^{-1} in B600-amended soils (Fig. 1). The increased soil OC pool was greater in B600 (100%) than B400 (94%) treatments. When compared with the unamended control, B400 biochar increased the content of DOC under individual Cd-contamination. The increased DOC content was much higher with B400 (42–50%) than with B600 (17–19%) under both individual Pb and co-contamination.
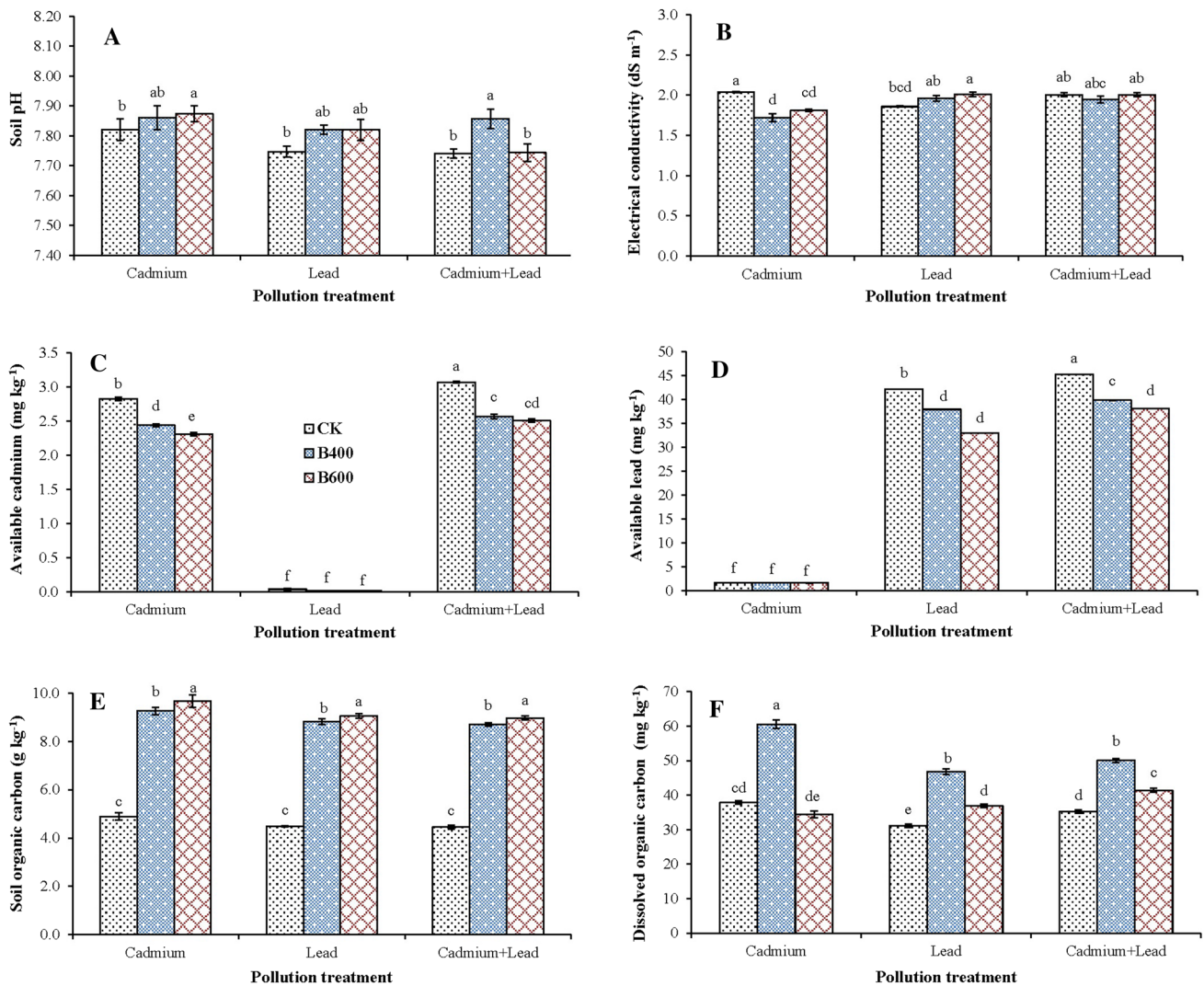
Fig. 1 Effect of sugarcane bagasse biochar on soil pH (**A**), electrical conductivity (**B**), the concentrations of DTPA-extractable cadmium (**C**) and lead (**D**), soil organic carbon (**E**) and dissolved organic carbon (**F**) in metal-polluted soils. Values are mean ($n=3$) and the vertical bars represent standard error of the mean. Different letters above bars indicate significant differences among the treatment means by Tukey's post hoc test at $P \leq 0.05$ (CK, control soil; B400 and B600, soils amended with biochars produced at 400 and 600 °C, respectively)

3.2 Soil C mineralization

The pattern of soil C mineralization during the 120 days of incubation is shown in Fig. 2. The soil C mineralization was very high during the first 50 days of incubation in all treatments, but the rates of increase in C mineralization tended to decrease subsequently. About 62–82% of the soil C was mineralized during the first 50 days of incubation when compared with that after 120 days. The C mineralization in biochar-amended soils was generally higher than that in unamended soils in all metal-spiked soils throughout the incubation with greater values in B400 than B600 treatments after 50 days (Fig. 2). Significant interactions ($P \leq 0.001$) in the cumulative C mineralization (Cmin)

occurred between metal pollution and biochar treatments (Fig. 3). At the end of the 120-day soil incubation, the Cmin significantly increased (75–135%) by biochar in all metal-spiked soils. The lowest Cmin values (186–196 mg C kg^{-1}) were recorded in all metal-spiked soils without amendment. The highest Cmin values (442–460 mg C kg^{-1}) were observed with B400 in single metal-spiked soils, which were 126–135% higher than that of the corresponding control soil without biochar (195–196 mg C kg^{-1}). In biochar-amended soils, the increase in Cmin was lower under Cd + Pb (78–93%) relative to individual (75–135%) metals (Fig. 3). The Cmin values were highly ($P \leq 0.001$) correlated with soil OC ($r=0.89$) and DOC ($r=0.70$) contents (Table S1). After 120 days of
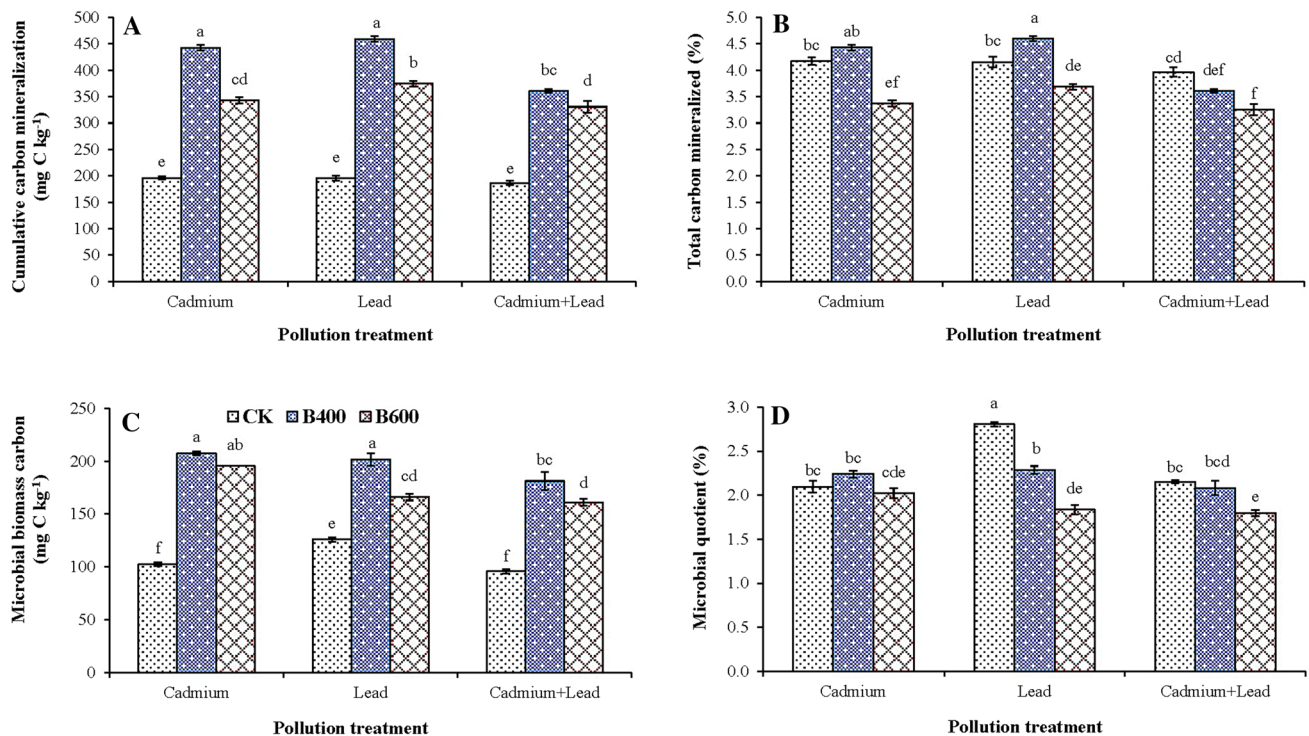
Fig. 2 Effect of sugarcane bagasse biochar on carbon (C) mineralization in soils spiked with cadmium (Cd) and lead (Pb) over 120 days of incubation. Values are mean ($n=3$) and the vertical bars represent standard error of the mean (CK, control soil; B400 and B600, soils amended with biochars produced at 400 and 600 °C; respectively)
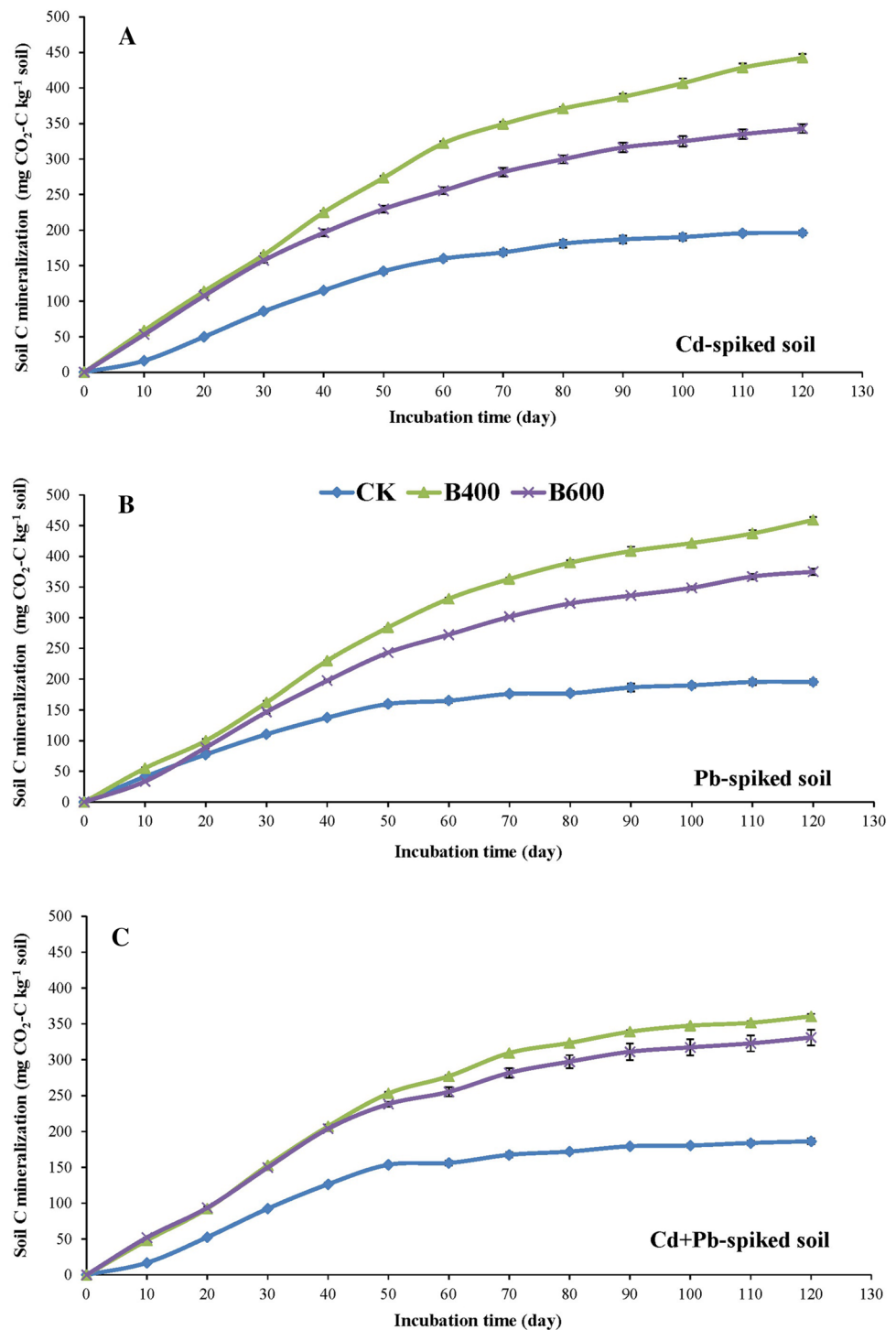
the laboratory incubation, the total biochar C mineralized (TCM) averaged across polluted soils was greater in B400 (3.6–4.6%) than B600 (3.2–3.7%) treatments (Fig. 3).

3.3 Soil microbial activity and biomass

There was also a significant interaction between metal pollution and biochar treatments on soil BR (Table 2). Soils polluted with Cd and Pb alone displayed the highest BR values (5.71–5.93 mg C kg^{-1} day^{-1}) in B400 treatments, while all metal-contaminated soils showed the lowest BR

Fig. 3 Effect of biochar on cumulative carbon mineralization (**A**) and total C mineralized (**B**) over 120 days of incubation, microbial biomass carbon (**C**) and microbial quotient (**D**) in soils spiked with cadmium (Cd) and lead (Pb). Values are mean ($n = 3$) and the vertical bars represent standard error of the mean. Different letters above bars indicate significant differences among the treatment means by Tukey's post hoc test at $P \leq 0.05$ (CK, control soil; B400 and B600, soils amended with biochars produced at 400 and 600 °C, respectively)

values (3.37–4.03 mg C kg^{-1} day^{-1}) in unamended treatments (Table 2). The BR increased significantly with biochar addition (by 30–70%) and this effect was greater in B400 (44–56%) than B600 (30–47%) treatments in both Cd-spiked and co-spiked soils. However, the substrate-induced respiration (SIR) was affected only by pollution ($P \leq 0.05$) and biochar ($P \leq 0.001$), and was lower in co-spiked than Pb-spiked soils (Table 3). Biochar addition increased the SIR with greater increases in B600 (67%) than B400 (53%). The BR was strongly correlated with OC and DOC, while the SIR was highly correlated only with DOC (Table S1).

The MBC content increased in biochar-amended soils by 32–103% in comparison with unamended soils (Fig. 3). However, the extent to which MBC was stimulated by biochar depended on the pyrolysis temperature and metal treatment. The increases in MBC were not different between B400 and B600 treatments in Cd-spiked soils while they were greater in B400-amended soils than B600-amended soils under Pb and Cd + Pb spiked treatments. The microbial quotient (qM) remained unaffected by biochar in Cd-spiked soils, decreased by 18–35% in Pb-spiked soils, and decreased only by B600 (17%) in co-spiked soils (Fig. 3). The MBC was positively correlated ($P \leq 0.001$) with OC, DOC, BR, SIR and Cmin, whereas the qM negatively

correlated ($P \leq 0.01$) with OC and SIR (Table S1). The qCO$_2$ was highest (36.3 µg C mg^{-1} MBC day^{-1}) in the unamended co-spiked soil (Table 2). This microbial parameter significantly decreased by biochar application (2–17%) and did not vary with pyrolysis temperature. The qCO$_2$ was negatively correlated with OC, DOC and other microbial properties (Table S1).

3.4 Biochar effects on soil enzyme activity

Results showed that only the main effects of metal and biochar treatments on CAT activity were significant (Table 3), while the activities of other enzymes were interactively affected by metal and biochar treatments (Table 2). The activity of CAT was greater in Pb-spiked soil than Cd or Cd + Pb spiked soils, and stimulated equally by both biochars (Table 3). Biochar application improved DEH, ALP, URE, ARY and FDA activities in heavy metal-contaminated soils, depending on the pyrolysis temperature and metal treatment (Table 2). Addition of biochar significantly increased the activities of enzymes with a greater increase in DEH, ALP and ARY activities in B400 soils under individual Cd treatment, and in URE and FDA activities in B400 soils under individual Pb treatment. The increases in the DEH and ALP activities

Table 2 Effect of bagasse biochar (CK, control soil; B400 and B600, soils amended with biochars produced at 400 and 600 °C, respectively) on basal respiration, metabolic quotient and the activities of soil enzymes in metal-contaminated soils

Biochar treatment	Pollution treatment			ANOVA summary	
	Cadmium (Cd)	Lead (Pb)	Cd + Pb	Effect	P
Basal respiration (mg C kg^{-1} day^{-1})					
CK	3.37 ± 0.01f	4.04 ± 0.03e	3.48 ± 0.09f	Pollution (P)	<0.001
B400	5.71 ± 0.03a	5.83 ± 0.03a	5.43 ± 0.07bc	Biochar (B)	<0.001
B600	5.64 ± 0.03ab	5.26 ± 0.06 cd	5.13 ± 0.05d	P×B	<0.001
Metabolic quotient (µg C mg^{-1} MBC day^{-1})					
CK	32.9 ± 0.53b	32.1 ± 0.37b	36.3 ± 0.35a	Pollution (P)	<0.001
B400	27.5 ± 0.39d	29.0 ± 0.95 cd	30.0 ± 1.04bcd	Biochar (B)	<0.001
B600	28.9 ± 0.15 cd	31.6 ± 0.24bc	31.8 ± 0.45b	P×B	<0.05
Dehydrogenase activity (µmol TPF g^{-1} h^{-1})					
CK	0.167 ± 0.0005f	0.181 ± 0.0001e	0.148 ± 0.0012 g	Pollution (P)	<0.001
B400	0.369 ± 0.0010a	0.372 ± 0.0012a	0.292 ± 0.0007d	Biochar (B)	<0.001
B600	0.348 ± 0.0002c	0.362 ± 0.0006b	0.290 ± 0.0015d	P×B	<0.001
Alkaline phosphomonoesterase activity (µmol PNP g^{-1} h^{-1})					
CK	9.80 ± 0.18e	14.2 ± 0.08b	10.5 ± 0.11e	Pollution (P)	<0.001
B400	13.5 ± 0.14bc	16.6 ± 0.17a	14.0 ± 0.15bc	Biochar (B)	<0.001
B600	12.0 ± 0.30d	13.7 ± 0.23bc	13.1 ± 0.13bc	P×B	<0.01
Urease activity (µmol NH$_4$-N g^{-1} h^{-1})					
CK	2.47 ± 0.01e	2.37 ± 0.05e	2.36 ± 0.02e	Pollution (P)	<0.001
B400	4.20 ± 0.01a	4.16 ± 0.03ab	3.02 ± 0.04d	Biochar (B)	<0.001
B600	4.01 ± 0.21b	4.20 ± 0.05a	3.21 ± 0.06c	P×B	<0.001
Arylsulphatase activity (µmol PNP g^{-1} h^{-1})					
CK	2.36 ± 0.08f	3.18 ± 0.10d	2.74 ± 0.04e	Pollution (P)	<0.001
B400	4.80 ± 0.03b	5.47 ± 0.06a	3.44 ± 0.06d	Biochar (B)	<0.001
B600	4.22 ± 0.04c	4.79 ± 0.05b	3.21 ± 0.06d	P×B	<0.001
Fluorescein diacetate hydrolysis activity (µmol fluorescein g^{-1} h^{-1})					
CK	0.163 ± 0.0012 g	0.161 ± 0.0012 g	0.142 ± 0.0011 h	Pollution (P)	<0.001
B400	0.296 ± 0.0010b	0.310 ± 0.0012a	0.240 ± 0.0011e	Biochar (B)	<0.001
B600	0.262 ± 0.0020d	0.283 ± 0.0013c	0.228 ± 0.0040f	P×B	<0.001

Values are mean ± standard error of the mean ($n = 3$)

Different letters represent significant differences by Tukey test at $P \leq 0.05$

Table 3 Effect of bagasse biochar (CK, control soil; B400 and B600, soils amended with biochars produced at 400 and 600 °C, respectively) on substrate-induced respiration and catalase activity in metal-contaminated soils

Pollution treatment		Biochar treatment		ANOVA summary	
				Effect	P
Substrate-induced respiration (mg C kg^{-1} h^{-1})					
Cadmium (Cd)	44.2 ± 2.94ab	CK	31.1 ± 1.23c	Pollution (P)	<0.05
Lead (Pb)	46.2 ± 4.04a	B400	47.5 ± 1.28b	Biochar (B)	<0.001
Cd + Pb	40.3 ± 3.00b	B600	52.1 ± 1.66a	P×B	>0.05
Catalase activity (µmol O$_2$ g^{-1} h^{-1})					
Cadmium (Cd)	2.43 ± 0.03b	CK	2.30 ± 0.02b	Pollution (P)	<0.001
Lead (Pb)	2.52 ± 0.05a	B400	2.55 ± 0.03a	Biochar (B)	<0.001
Cd + Pb	2.39 ± 0.04b	B600	2.49 ± 0.03a	P×B	>0.05

Values are mean ± standard error of the mean ($n = 9$)

Different letters represent significant differences by Tukey test at $P \leq 0.05$

were more pronounced with B400 than B600 addition only under individual metals, and in stimulating the ARY and FDA activities in all metal treatments. The increased

URE activity was much greater in B400 than B600 treatments only in single Cd and co-contaminated soils. In addition, enzyme activities were lower under Cd + Pb than

under individual metals, except ALP (Table 3). The ALP activity did not show a clear trend with metal treatments across the biochar treatment. The ALP, ARY and CAT activities were negatively correlated with the concentration of available Cd but not with available Pb (Table S1). All enzyme activities were positively correlated with OC, BR, SIR and MBC. Generally, enzyme activities were highly correlated with each other (Table S1).

3.5 RDA ordination of soil dataset

The variations in soil microbial attributes and enzyme activities among the treatments were also analyzed by RDA ordination method (Fig. 4). The RDA results showed that soil chemical attributes (OC, DOC, DTPA-extractable Cd and Pb) explained a significant portion of soil microbial and biochemical properties. Two RDA axes were significant and together were responsible for 95% of the total variability in soil microbial properties. The first axe accounted for 94.5% and the second axe explained only 0.5% of the total variation in soil microbial properties (Fig. 4A). The soil OC ($F = 101.6$, $P = 0.001$) was the most highly significant variable explaining 80% of the variance in microbial properties, followed by available Cd concentration (8%, $F = 32.5$, $P = 0.001$) and DOC (7%, $F = 13.6$, $P = 0.001$) across treatments (Fig. 4A). RDA results showed that the first axe was associated with about 79% of the total variation in enzyme activities, and the second axe accounted for 5.5% of the variability (Fig. 4B). Soil OC ($F = 25.6$, $P = 0.001$), available Cd ($F = 25.1$, $P = 0.001$), DOC ($F = 7.25$, $P = 0.006$) and available Pb ($F = 3.25$, $P = 0.049$) were significantly correlated with enzyme activities and explained 51, 25, 6% and 2% of the total variability in enzyme activity, respectively (Fig. 4B).

4 Discussion

Application of biochars (400 and 600 °C) derived from sugarcane bagasse lowered the availability of both Cd and Pb in singly and jointly contaminated soils. This confirms that the potential risk of soil Cd and Pb biotoxicity could be lowered with addition of bagasse biochars to metal-contaminated soils. Other short-term laboratory studies also found that addition of biochar derived from sugarcane bagasse had the potential to immobilize Cd and Pb in soil (Lu et al. 2017; Bashir et al. 2018, 2019; Nie et al. 2018) and remove Pb from aqueous solution (Ding et al. 2014). Metal immobilization with biochar application is mainly attributed to different chemical reactions (Ding et al. 2014; Palansooriya et al. 2020; Wang et al. 2020). Biochar application to soil can immobilize metals through increasing binding sites for metal ions (Ahmad et al. 2014; Yuan et al. 2019). In this study,
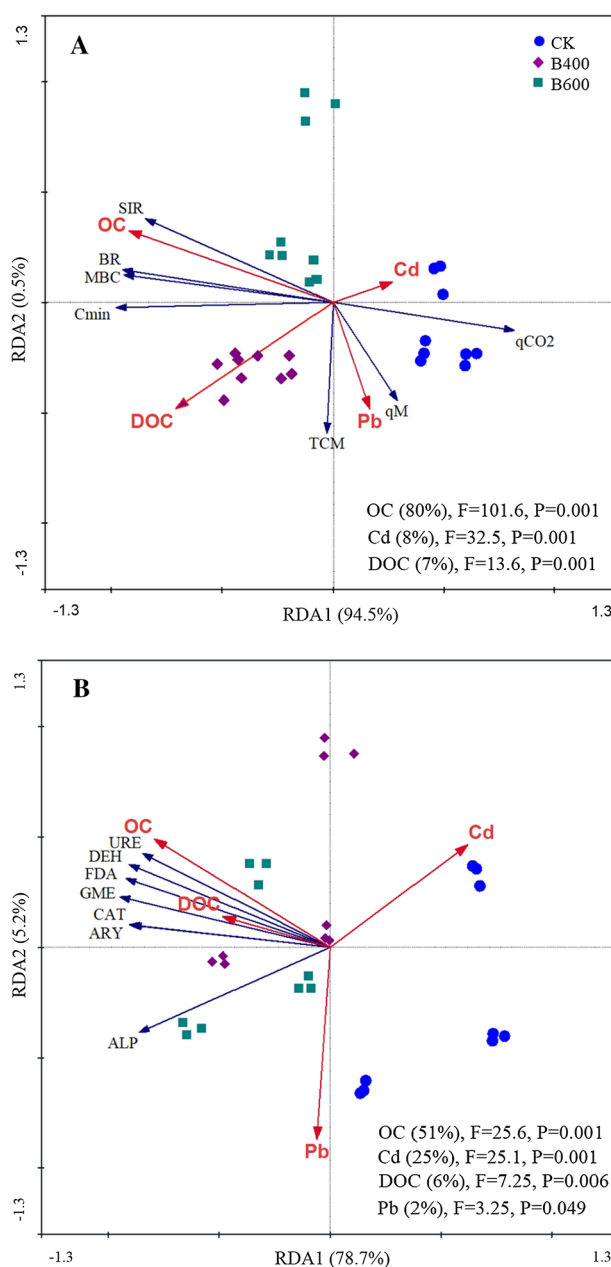
Fig. 4 Redundancy analysis (RDA) ordination of the microbial properties (**A**) and enzyme activities (**B**) constrained by soil chemical properties in metal-contaminated soils amended with biochar. The red arrows display explanatory variables (soil chemical properties) and response variables (microbial properties and enzyme activities) are shown in blue vectors. The corresponding proportion of the variability explained F and P values are shown on the figures. Filled circles show soil samples (abbreviations: *Cd* available cadmium; *Pb* available lead; *OC* soil organic carbon; *DOC* dissolved organic carbon; *BR* basal respiration; *SIR* substrate-induced respiration; *Cmin* cumulative C mineralization; *TCM* total biochar C mineralized; *MBC* microbial biomass carbon; *qM* microbial quotient; *qCO2* metabolic quotient; *DEH* dehydrogenase activity; *FDA* fluorescein diacetate hydrolysis; *ALP* alkaline phosphomonoesterase activity; *URE* urease activity; *ARY* arylsulphatase urease activity; *CAT* catalase activity, *GME* geometric mean of enzyme activity)

despite the high pH (9.2–9.9) and alkalinity of the biochars (Table 1), biochar application did not have a major effect on soil pH, which is known to control the solubility and mobility of heavy metals in biochar-amended soils (Bolan et al. 2014; Yuan et al. 2019; Wang et al. 2020). This is consistent with previous studies (Khadem and Raiesi 2017; Bashir et al. 2018), which reported the small changes in the pH of neutral and alkaline soils following addition of biochars derived from sugarcane bagasse. Bashir et al. (2018) reported that increases in soil pH were not always significant in slightly alkaline (pH 7.5) soils amended with sugarcane bagasse-derived biochar. We observed that the availability of Cd and Pb was higher in the metal co-contaminated soils than the single metal-contaminated soils either unamended or amended with both biochars; consistent with earlier reports (Khan et al. 2010; Xu et al. 2018, 2019; Fan et al. 2020). This suggests a possible competition between Cd and Pb for the adsorption sites on biochar and soil surfaces when both metals coexisted in the soil (Park et al. 2016; Ni et al. 2019). Surprisingly, the reduction of Cd availability (16%) was greater than that of Pb availability (12%) in co-contaminated soils amended with low-temperature bagasse biochar. This finding suggests a greater immobilization of Cd than Pb by low-temperature biochar under competitive adsorption conditions. Similarly, addition of macadamia nutshell biochar (5% w/w) to Cd and Pb co-spiked soils reduced metal availability during 49 days of incubation, and the decline of Cd availability was greater than that of Pb (Xu et al. 2018). In a recent experiment (Fan et al. 2020), addition of modified rice straw biochar to a soil naturally co-contaminated with Cd (9.18 mg kg^{-1}) and Pb (1182 mg kg^{-1}) reduced the DTPA-extractable Cd concentrations (34.8–39.2%) more than the DTPA-extractable Pb concentrations (8.6–11.1%) during a 28-day incubation period. In contrast, other studies (Park et al. 2011, 2016; Inyang et al. 2012; Jiang et al. 2020) reported greater Pb than Cd adsorption on biochar surfaces under competitive adsorption conditions. Nevertheless, Inyang et al. (2012) indicated that biochar type is an important factor under competitive adsorption conditions. They reported that the removal of Cd from aqueous solution was greater than that of Pb for digested whole sugar beet biochar, but the removal of Pb was greater than that of Cd for digested dairy waste biochar (Inyang et al. 2012). For these cases, surface electrostatic interaction might not be an overriding mechanism for metal removal, and other possible mechanisms, such as surface co-precipitation, surface complexation and intraparticle diffusion, should also be involved in metal immobilization (Inyang et al. 2012; Ding et al. 2014). Nonetheless, further studies are needed to validate this conclusion using the well-established adsorption isotherms such as Langmuir and Freundlich isotherms in soil–biochar mixtures. Our finding suggests that Pb toxicity and thus the potential risk to microbial population and

activity could be greater than Cd toxicity in Cd + Pb co-contaminated soils.

In this incubation experiment, the immobilization capacity of the high-temperature biochar for Pb (16%) was greater than that of the low-temperature biochar (12%) in co-spiked soils, and both biochars equally affected Cd immobilization. This finding suggests that Pb showed greater affinity to the high-temperature bagasse biochar, which could be attributed to the transformation of Pb into more stable Pb-phosphate fractions (Ding et al. 2014). In the present study, the P content in biochars increased substantially with the increase of pyrolysis temperature (Table 1).

Soil microbial communities are the main driving agents responsible for the long-term sustainability of terrestrial ecosystems because they regulate the formation and decomposition of SOM and plant residues, the cycling and availability of essential nutrients, C cycling and sequestration, biodegradation of harmful organic pollutants as well as biotransformation of toxic metals in the soil (Abdu et al. 2017; Nannipieri et al. 2017; Xu et al. 2019). Microbial and biochemical properties are sensitive indicators of soil health and ecological risks in polluted environments (Khan et al. 2010; Abdu et al. 2017; Xu et al. 2018), and are improved by biochar amendment (Lehmann et al. 2011; Gul et al. 2015; Khadem and Raiesi 2017). The results presented here suggest that biochar addition promotes microbial functions in metal-contaminated soils, as evidenced by significant increases in microbial and biochemical indicators. Previous experiments have similarly illustrated significant positive influences of sugarcane bagasse biochar on microbial activity and biomass, and enzyme activities in metal-contaminated soils under field and laboratory conditions (Nie et al. 2018; Bashir et al. 2018, 2019). These results suggest that the use of sugarcane bagasse biochar promotes microbial growth and metabolism, enhances nutrient (P, N and S) cycling, and hence improves soil fertility and quality under metal stress. The promotion of the microbial and biochemical performances in contaminated soils with addition of bagasse biochar is an indicator of improvements in the functioning and quality of the soil due to development of a more active microbial community (Nie et al. 2018; Xu et al. 2018). The improved soil microbial and biochemical indicators under metal stress resulted from biochar application could be attributed to lower metal toxicity, higher soil OC content and addition of labile C and nutrients (Fig. 1). The RDA analysis in the present study confirmed that OC, DOC and extractable Cd were dominant factors affecting soil microbial properties (Fig. 4A) while the four soil chemical parameters (OC, DOC and both extractable Cd and Pb concentrations) played a major role in enzyme activity. In metal-contaminated soils, application of biochar could reduce the negative effect of metal stress on microbial community with adsorbing free cationic metal ions from the soil solution and

their toxicity (Park et al. 2011; Yang et al. 2016; Xu et al. 2018). Soil microbial biomass, activity and enzyme activities were increased with the application of bagasse biochar produced at 500 °C in Cd-polluted soils, which was due to Cd stabilization (Bashir et al. 2018, 2019). Enzyme (urease, catalase and invertase) activities were improved after addition of sugarcane bagasse-derived biochar (produced at 450 °C) to a soil contaminated with Cd and Pb, which was attributed to enhanced heavy metal immobilization following biochar application (Nie et al. 2018). Most soil microbial properties were positively correlated with OC and DOC contents (Table S1), which suggests the added substrate C by biochar also plays an important role in promoting the microbial communities and thus maintaining their functionalities in metal-contaminated soils, in accordance with the RDA results (Fig. 4). The input of C compounds from biochar into the soil, especially DOC, is an important driver for stimulating microbial activity and biomass C immobilization as an immediate source of microbial substrate (Ouyang et al. 2014; Bashir et al. 2018; Song et al. 2019), and for the increased enzyme activity as an enzyme substrate (Khadem and Raiesi 2017; Song et al. 2019). Bashir et al. (2018) found that addition of sugarcane bagasse-derived biochar stimulated URE and DEH activities by increasing microbial biomass in metal co-contaminated soils. Soil amendment with biochar would lessen the unfavorable effects of toxic metals on soil microbial community by providing C substrates for microbial consumption, making DOC and nutrients available for microbes and enhancing soil water retention and supply to microorganisms (Yang et al. 2016; Huang et al. 2017; Nie et al. 2018; Xu et al. 2018; Bashir et al. 2018, 2019). The ratio of microbial respiration to microbial biomass, also known as the specific respiratory quotient or metabolic quotient (qCO_2), is often used to demonstrate metal stress in the microbial community (Anderson and Domsch 2010). We observed that the qCO_2 was positively correlated with Pb availability but not with Cd availability (Table S1), indicating the potential Pb stress to microbial community. In contrast, we noted that addition of biochar lowered the qCO_2 value, which is similar to other studies (Khadem and Raiesi 2017; Liu et al. 2019). This indicates an active microbial community under less stress conditions to produce more enzymes under metal stress conditions when more substrate C is available for microbial assimilation. This positive impact of the biochar specifically explained the reduction in extractable metal contents in the soils treated with biochar, in particular Pb availability. The decrease in qCO_2 would mean soil microorganisms would metabolize a smaller amount of C and respire less CO_2 per unit microbial biomass per unit time as stress decreases, and a shift of energy and substrate to growth rather than maintenance of the microbial population (Anderson and Domsch 2010; Liu et al. 2019). The negative correlations of enzyme

activities with the qCO_2 values (Table S1) suggest that the accelerated enzyme activity following biochar application is closely linked to the metal stress and the formation of microbial biomass to synthesize enzymes in contaminated soils.

Our findings confirm that the improvements of soil microbial functions were not only because of the declined metal toxicity and stress as a consequence of their immobilization on bagasse biochar surfaces, but also because of the substrate C provided by biochar for the metabolically active microorganisms. Redundancy analysis (RDA) results further indicated that better soil microbial conditions and enzyme activities were associated with lower metal availability and higher substrate availability in biochar-treated soils. However, the use of RDA indicated that a large portion of the total variability observed in microbial properties (80%) and enzyme activities (51%) was only explained by soil OC (Fig. 4), as is supported by the Pearson correlation results (Table S1). This suggests that soil OC was a major explanatory variable and thus played a major role in the microbial decomposer community and enzyme activity compared with metal immobilization.

Nonetheless, the positive impacts on the measured microbial and biochemical properties were greater with low than high-temperature biochar in accordance with greater volatile matter content in the former (Table 1). This is further supported by higher C turnover rate of low-temperature biochar (4.2%) than that of high-temperature biochar (3.5%) observed after 120 days of incubation. The higher atomic O/C ratio and lower content of fixed C in low-temperature biochar (Table 1) also imply an organic amendment with less recalcitrance and low aromaticity. Further evidence is the lower qM value in the B600-amended (1.8%) than the B400-amended (2.3%). This indicates that the biochar produced at a high pyrolysis temperature is more resistance to microbial decomposition, in agreement with previous studies (Khadem and Raiesi 2017). Hence, a low-temperature bagasse biochar can support a larger microbial community with more microbial biomass and metabolic activity in the Cd/Pb contaminated soils. The strong relationships between soil microbial properties (Table S1) suggest that the higher microbial activity and biomass with the biochar produced at low temperature were accompanied by higher enzyme activities.

In this experiment, the soil microbial population was exposed to metal toxicity resulting from both Cd and Pb, which may act interactively due to competitive sorption processes (Huang et al. 2017; Xu et al. 2018; Ni et al. 2019; Jiang et al. 2020). In unamended treatments, the co-contamination of soil with Cd and Pb reduced microbial and biochemical properties compared with single Cd or Pb contamination, which was due to the greater availability of one metal in the presence of the other metal. This suggests that soil co-contamination by Cd and Pb might have a greater

harmful impact on the soil microorganisms. Similarly, earlier reports showed microbial biomass and respiration, and enzyme activity were inhibited more in soil co-spiked with Cd and Pb compared with soils spiked with Cd or Pb alone (Khan et al. 2010; Huang et al. 2017; Zhan et al. 2018; Xu et al. 2018). Indeed, the great reductions in microbial and biochemical functions under the co-contamination of metals might suggest a synergistic inhibitory effect of Cd and Pb when both metals coexist in the soil (Huang et al. 2017). Interestingly, biochar application improved microbial properties and processes in soils co-contaminated with Cd and Pb, but this positive impact of biochar was often less pronounced in the co-contaminated soils than in single-contaminated soils. This could be attributed to the greater reduction in Cd availability than in Pb availability in co-spiked soils amended with biochar under competitive adsorption conditions (Fig. 1). These observations would also confirm the results of metal availability described previously: (1) soil Cd might impose a synergistic effect on Pb availability with biochar in the co-presence of heavy metals and (2) the lower efficiency of biochar to reduce the availability of heavy metals (here Pb) when Pb and Cd coexisted in the soil.

5 Summary and conclusions

In support of our first hypothesis, sugarcane bagasse biochar alleviated the negative effect of metal toxicity on microbial and biochemical properties of the soils spiked either separately or jointly with Cd and Pb. However, the beneficial impacts of biochar were lower in co-contaminated soils than in single-contaminated soils, confirming our second hypothesis. In addition, the positive effect of low-temperature biochar (400 °C) on microbial and biochemical functions was more pronounced than that of high-temperature biochar (600 °C). Biochar addition could lessen the detrimental effect of metals on microbial properties of metal co-contaminated through the supply of the easily available substrates and essential elements as well as metal immobilization. Therefore, it can be concluded that the addition of biochar prepared from sugarcane wastes is a cost-effective remediation method, and promising strategy to restore the co-contaminated soils by reducing metal availability, and improving microbiological and biochemical conditions. However, this study was conducted using an incubation experiment with artificially spiked soils, and large-scale and long-term field experiments using naturally contaminated soils are needed to verify these results.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42773-021-00123-0.

Declarations

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