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Research article

Animal carcass- and wood-derived biochars improved nutrient bioavailability, enzyme activity, and plant growth in metal-phthalic acid ester co-contaminated soils: A trial for reclamation and improvement of degraded soils

Hanbo Chen [a], Xing Yang [a,b], Hailong Wang [a,c,*], Binoy Sarkar [d], Sabry M. Shaheen [b,e,f], Gerty Gielen [g], Nanthi Bolan [h], Jia Guo [i], Lei Che [j], Huili Sun [k], Jörg Rinklebe [b,l]

[a] Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, Guangdong, 528000, China
[b] University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285, Wuppertal, Germany
[c] Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province, Zhejiang A&F University, Hangzhou, Zhejiang, 311300, China
[d] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK
[e] King Abdulaziz University, Faculty of Meteorology, Environment, and Arid Land Agriculture, Department of Arid Land Agriculture, 21589, Jeddah, Saudi Arabia
[f] University of Kafrelsheikh, Faculty of Agriculture, Department of Soil and Water Sciences, 33 516, Kafr El-Sheikh, Egypt
[g] Scion, Private Bag 3020, Rotorua, 3046, New Zealand
[h] Global Centre for Environmental Remediation, University of Newcastle, Newcastle, NSW, 2308, Australia
[i] Chengbang Eco-Environment Co. Ltd, Hangzhou, Zhejiang, 310008, China
[j] School of Engineering, Huzhou University, Huzhou, Zhejiang 313000, China
[k] Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, Guangdong, 510301, China
[l] University of Sejong, Department of Environment, Energy and Geoinformatics, 98 Gunja-Dong, Guangjin-Gu, Seoul, South Korea

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ABSTRACT

Reclamation of degraded soils such as those with low organic carbon content and soils co-contaminated with toxic elements and phthalic acid esters (PAEs) is of great concern. Little is known about the efficiency of plant- and animal-derived biochars for improving plant growth and physicochemical and biological properties of co-contaminated soils, particularly under low content of organic matter. Hence, a pot trial was carried out by growing pak choi (*Brassica chinensis* L.) to assess the influence of different doses (0, 0.5, 1, 2, and 4%) of animal (pig carcass) and wood (*Platanus orientalis*) derived biochars on soil properties, nutrient availabilities, plant growth, and soil enzyme activities in two soils containing low (LOC) and high (HOC) organic carbon contents and co-contaminated with di-(2-ethylhexyl) phthalic acid (DEHP) and cadmium (Cd). Biochar applications improved pH, salinity, carbon content, and cation exchange capacity of both soils. Addition of biochars significantly increased the bioavailability and uptake of phosphorus and potassium in the plants in both soils with greater effects from pig biochar than wood biochar. Biochar additions also significantly enhanced urease, sucrase, and catalase activities, but suppressed acid phosphatase activity in both soils. The impact of pig biochar was stronger on urease and acid phosphatase, while the wood biochar was more effective with sucrase and catalase activities. The biomass yield of pak choi was significantly increased after biochar addition to both soils, especially in 2% pig biochar treatment in the LOC soil. The positive response of soil enzymes activities and plant growth for biochar addition to the Cd and DEHP co-contaminated soils indicate that both biochars, particularly the pig biochar can mitigate the risk of these pollutants and prove to be eco-friendly and low-cost amendments for reclaiming these degraded soils.

* Corresponding author. Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, Guangdong, 528000, China.
E-mail address: hailong.wang@fosu.edu.cn (H. Wang).

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1. Introduction

Industrialization, urbanization, effluent irrigation, uncontrolled disposal of wastes, agricultural plastic mulch abuse, and other anthropogenic activities have resulted in unprecedented contamination of arable soils with heavy metal(loid)s (Qi et al., 2017; Bandara et al., 2020) and plasticizers, e.g., phthalic acid esters (PAEs) (Zhao et al., 2019). Di-(2-ethylhexyl) phthalic acid (DEHP) as a typical PAE, and cadmium (Cd) as a typical heavy metal, have posed alarming environmental and human health risks of these organic and metal contaminants globally (Antoniadis et al., 2017, 2019; He et al., 2018; Bandara et al., 2020). They can be taken up by plants, decreasing the yield and quality of crops, and can finally be accumulated in human body through the food web, damaging the functions of human organs including endocrine and reproductive systems (Qin et al., 2018; Chen et al., 2019). Simultaneously, poor organic matter content of soils has been identified as a major reason for the decline in soil quality and crop yield worldwide (Pulido-Fernández et al., 2013). Therefore, reclamation of degraded soils such as those co-contaminated with DEHP and heavy metal(loid)s, and soils with low organic matter content is of great importance. Achieving such reclamation via suitable low-cost amendments is an attractive option for soil restoration from both environmental quality and economic points of view (Lu et al., 2017; Palansooriya et al., 2019, 2020).

Numerous studies describing biochar as a suitable amendment for remediating organic pollutants (Zhang et al., 2013; Huang et al., 2018) and heavy metal(loid)s (Li et al., 2019a; Wu et al., 2017, 2019) in water (Li et al., 2019b; Mao et al., 2019; Shaheen et al., 2019) and soils (Yang et al., 2019; El-Naggar et al., 2020) have been published. For instance, Abbas et al. (2017) found that the Cd concentration in wheat was decreased after rice straw biochar amendment. They claimed that the probable reason could be the reduction of Cd concentration in soil pore water for immediate crop uptake after biochar addition, which is attributed to biochar-induced adsorption of Cd in soil, as also suggested by Rizwan et al. (2016). In addition, biochar is of benefit to the improvement of soil structure (i.e., aggregate formation) (Quan et al., 2020) and fertility (Li et al., 2019c; Wei et al., 2019), and thereby promoting crop growth (Dong et al., 2015; Li et al., 2018; Purakayastha et al., 2019).

China generates around 20 million pig carcasses yearly, and this number continues to climb every year (He et al., 2018). Additionally, urban green wastes such as tree branches have turned into a huge source of pollution and a hindrance to the benign development of ecological environment (Belyaeva and Haynes, 2010). Pyrolysis of pig carcasses and green wastes into biochar not only presents an efficient and environmentally friendly option for disposing these wastes (Yang et al., 2017) but also offers a tremendous scope for using the biochar for *in situ* remediation of soil contaminants while simultaneously improving soil productivity and crop yield.

In China, the area of vegetable cropping is second to grain production. Vegetables account for approximately 28.5% of the total diet in China, and pak choi (*Brassica chinensis* L.) is a typical widely-consumed leafy vegetable in daily life of the population (Yan et al., 2009). Wei et al. (2017) noted that the consumption of pak choi as a staple vegetable made a significant contribution to the estimated dietary intake of toxic metals such as Cd in Chinese population. As a consequence, it is of importance to reduce contaminant accumulation in pak choi and improve the crop yield and quality. It is well-accepted that soil enzymatic activity is sensitive to soil contaminants, accordingly considered to be a crucial parameter of soil health (He et al., 2019). Soil enzymes also have a critical influence on nutrient (e.g., Nitrogen (N), phosphorus (P) and potassium (K)) cycling and subsequent uptake by plants (Sarkar et al., 2016; Nie et al., 2018). Nutrient phytoavailability affects plant growth directly while contamination stress can inhibit plant growth by posing toxic effects.

Till date, little information is documented on the efficiency of plant- and animal-derived biochars for affecting nutrient bioavailabilities, enzyme activities, and plant growth in DEHP-metal co-contaminated soils. We hypothesize that co-contamination of soils with Cd and DEHP may affect the soil microbial activities, enzyme activities, nutrient bioavailability, and plant growth, and these effects may differ based on the soil organic carbon content. To verify this hypothesis, we conducted a pot-culture experiment using pak choi and two different soils treated with wood- and animal-derived biochars, i.e., pig carcasses and branches of *Platanus orientalis* Linn, to investigate the influence of these biochars on the bioavailability of soil nutrients, enzyme activity, and the pak choi growth under the combined pollution of Cd and DEHP in soils containing low and high organic carbon contents.

2. Materials and methods

2.1. Soil and biochar collection, preparation, and characterization

The studied soils were sampled from two near-by fields (0–20 cm of topsoil) located in the southwest of Hangzhou City (30°24′N, 119°71′E), China. The first soil is rich in its total organic carbon content (HOC: 3.08%) and was used as farmland to cultivate vegetables nearly for twenty years. The second soil was left fallow for the same period, and thus was poor in its organic carbon content (LOC: 0.75%). Both soils were air-dried, crushed, and sieved (3-mm mesh). In order to obtain co-contaminated soils, the two soils were spiked with DEHP at 50 mg kg^{-1} soil and Cd at 1.0 mg kg^{-1}. The concentration of Cd^{2+} was referred to the Level 3 of the Environmental Quality Standards for soils GB 15618–1995, and DEHP concentration was chosen according to a previous research (He et al., 2016). The Cd/DEHP-spiked soils were mixed homogenously, air dried, and used for the pot experiment.

Pig biochar (PB) was produced by the pyrolysis of whole pig carcasses, and wood biochar (WB) was prepared by pyrolysing shredded branches (3-mm mesh) of *Platanus orientalis* Linn., at 650 °C for 2 h. Both biochars were crushed and sieved (2-mm mesh) before mixing with the soils. The physicochemical properties of the studied biochars were determined using the methods described by Yang et al. (2016). The two biochars differed in many characteristics, such as ash content, cation exchange capacity (CEC), available P, surface alkalinity and specific surface area. More details about the experimental soil (Table S1) and biochar (Table S2) properties, soil spiking procedure with DEHP and Cd, and soil preparation and characterization are included in the Supporting Information (Appendix A) and published in Chen et al. (2019).

2.2. Pot trial

The pot trial was carried out in a greenhouse located in Zhejiang A&F University, Zhejiang Province, China, at temperature between 25 and 33 °C. Each ceramic pot (20 cm diameter, 19 cm height) was filled with either 3 kg of the Cd-DEHP contaminated LOC or HOC soil. Then the pig biochar and wood biochar were applied to the Cd-DEHP contaminated soils in the pots at five doses (i.e., 0 (control), 0.5, 1, 2 and 4%, w/w) and mixed well. In total, sixteen treatments plus two controls were set in this trial in four replicates (in total 72 pots).

All pots were fertilized with KH_2PO_4 and urea according to a basal dose of K_2O 0.2 g kg^{-1}, P_2O_5 0.32 g kg^{-1}, N 0.25 g kg^{-1} recommended for pak choi (He et al., 2016). The treatments were arranged in a complete randomized block design. The soil was maintained at 70% of the field water holding capacity for an initial period of 30 days to equilibrate the spiked Cd and DEHP into the soil. After the equilibration, ten pak choi seeds were sown at equal spacing in each pot, and after fifteen days, five strongest seedlings were kept after thinning out the rest. Watering with deionized water (2–3 times per week) was performed during the growth period to maintain the soil moisture status at the field capacity. After maturity (50 days), the matured pak choi shoots were harvested from the pots. The plants were rinsed with deionized water to get rid of the soil particles. The fresh plant shoots were oven-dried at 105 °C for

0.5 h and subsequently oven-dried at 65 °C until a constant weight was achieved. Dried plant shoots were crushed and sieved (0.25-mm mesh) before chemical analysis. After plant harvest, the soils in each pot were collected, homogenized and air-dried. Sampled soils were then ground to 2-mm and 0.25-mm for further chemical analysis.

2.3. Soil analysis

The dry and ground soils were analyzed for pH, electrical conductivity (EC), organic carbon content (OC), cation exchange capacity (CEC), and particle size distribution according to the methods described by Lu (2000). Soil available K was extracted using ammonium acetate, and analyzed by a flame photometer (FP640, Xinyi Instrument, China) (Lu, 2000). The concentration of available N was extracted using a micro-diffusion technique after alkaline-hydrolysis method (Lu, 2000). The available P was extracted using sodium bicarbonate ($NaHCO_3$) and measured by spectrophotometric method (UVA 132122, Thermo Electron Corporation, England) at 700 nm wavelength (Lu, 2000). The total Cd content of the soils was determined by digesting the soils with $HF–HClO_4–HNO_3$ (Carignan and Tessier, 1988). Potentially available Cd was extracted by diethylenetriaminepentaacetate acid (DTPA) (Lu, 2000). Cadmium was analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES Optima, 2000; PerkinElmer Co., USA). The DEHP was extracted and analyzed as per He et al. (2016). More details about the determination methods of Cd and DEHP concentrations in soil are provided in the Supporting Information (Appendix A).

2.4. Soil enzyme activities

The activities of urease, acid phosphatase, sucrase, and catalase were determined according to Dick et al. (1996). The urease activity was expressed as the mass of NH_3–N released per gram of dry soil after 24-h incubation with urea solution at 37 °C and determined by spectrophotometric method at 578 nm wavelength. The acid phosphatase activity was expressed as the mass of phenol released per gram of dry soil after 24-h incubation with a p-nitrophenyl phosphate substrate at 37 °C and determined by spectrophotometric method at 660 nm wavelength. The sucrase activity was expressed as mass of glucose released per gram of dry soil after 24-h incubation with glucose solution at 37 °C and determined by spectrophotometric method at 508 nm wavelength. The catalase activity was measured by titrating the residual hydrogen peroxide (H_2O_2) added after 20 min of soil exposure with 0.1 M potassium permanganate ($KMnO_4$). The catalase activity was expressed as the volume of 0.1 M $KMnO_4$ used per gram dry soil per minute (Dick et al., 1996).

2.5. Plant biomass and analysis of nutrients in plants

The dry weight of the plant shoots was recorded, and the samples were kept for further analysis. The N concentration was measured using an elemental analyzer (Flash EA1112, Thermo Finnigan, Italy). Plant shoots were digested with nitric acid (HNO_3), and the concentrations of P, K and Cd were determined. The P concentrations were quantified by spectrophotometric method (UVA 132122, Thermo Electron Corporation, England) at 700 nm (Lu, 2000). The concentrations of K were determined by a flame photometer (FP640, Xinyi Instrument, China). The concentrations of Cd were determined with ICP-OES (Optima 2000; PerkinElmer Co., USA) (Lu, 2000).

2.6. Data analysis

Data analysis was performed with the statistical package SPSS 17.0. Variability of the data was expressed in terms of standard deviation of four replicates. Analysis of variance (ANOVA) was used to assess differences between treatments, and $P < 0.05$ was supposed to be statistically significant. Pearson's correlation analysis with a significance level of $P < 0.01$ was performed to identify the correlation between variables.

3. Results and discussion

3.1. Biochar-induced changes on soil pH, salinity, CEC, and organic carbon

Soil pH significantly ($P < 0.05$) increased after application of the wood and pig biochars in both the LOC and HOC soils, and the impact of biochars was based on the applied dosage (Fig. 1A). The increase of soil pH can be explained by the high pH of biochars (9.5 for wood biochar and 10.0 for pig biochar; Table S2). We assume that when these alkaline biochars applied into the soil, the alkali salts (Fig. S1) might be released, thereby increasing the soil pH as indicated by Martinsen et al. (2015). Application of pig biochar made a greater impact on soil pH than wood biochar, which might be due to the higher pH, ash content, surface alkalinity, and alkali elements (e.g., Ca, Mg and K) of the pig biochar than wood biochar (Appendix A; Table S2). Biochar addition improved the status of the water-soluble salts, and thus increased the soil salinity, particularly in the HOC soil (Fig. 1 B), which might be due to the high mineral contents in the biochars (Fig. S1). However, the values of EC were still less than 0.3 dS m^{-1}, which means that the biochar treated soils would not suffer from high salinity.

Applications of 4% pig and wood biochars were more effective in increasing the CEC in both soils than the lower doses (Fig. 1C). The increase of soil CEC after addition of biochars might be explained by the high surface alkalinity and ash content of the biochars as indicated in Table S2 (Appendix A). Wood biochar addition was more efficient in increasing the organic carbon content of soil than pig biochar (Fig. 1D), which can be explained by its higher content of carbon than pig biochar (Table S2). For instance, the highest soil organic carbon contents were noticed at 4% wood biochar treatments, which increased by 5.4 folds in the LOC soil and 0.7 folds in the HOC soil, as compared to the control.

3.2. Impact of pig and wood biochars on the bioavailability and uptake of N, P, and K

The pig biochar addition caused a more profound impact than wood biochar on increasing the bioavailability and uptake of K and P in the soils (Fig. 2A and B). The maximum values of available K were corresponded to 4% pig biochar treatment, with up to 4.1-fold increase in the LOC soil and up to 2.1-fold increase in the HOC soil. In addition, the concentrations of available P in pig biochar-amended LOC soil increased by 1.0–3.5 folds, and increased by 0.4–0.8 folds in the HOC soil. Simultaneously, the concentrations of K and P in the plant tissues also significantly ($P < 0.05$) increased as the pig biochar application dosages increased (Fig. 2D and E). As compared to the controls, significant increases of available K in soils were also noticed after wood biochar addition, which increased by 0.7–1.8 and 0.3–0.5 folds in the LOC and HOC soils, respectively. However, wood biochar amendment showed a non-significant ($P > 0.05$) effect on the bioavailability of P neither in LOC nor HOC soil.

The impact of the pig biochar on the availability and uptake of P and K was significantly stronger than wood biochar. Increasing the bioavailability and uptake of K and P in the pig biochar-treated soils than the wood biochar-treated soils is likely due to its obviously higher content of P and K than the wood biochar (Table S2; Appendix A). The energy dispersive X-ray spectrometry (EDS) data of Fig. S1B (Appendix A) indicate that pig biochar contains much higher P, and alkali elements (e.g., Ca, Mg and K) than wood biochar. These results indicate that pig biochar is a much better source of P and K to soils than wood biochar. In addition, we assume that the biochar-induced improvement in soil pH and CEC could be another reason for improving the status and availability of P and K in the pig biochar-treated soil, as mentioned by DeLuca et al. (2009) and Haefele et al. (2011). We also assume that the

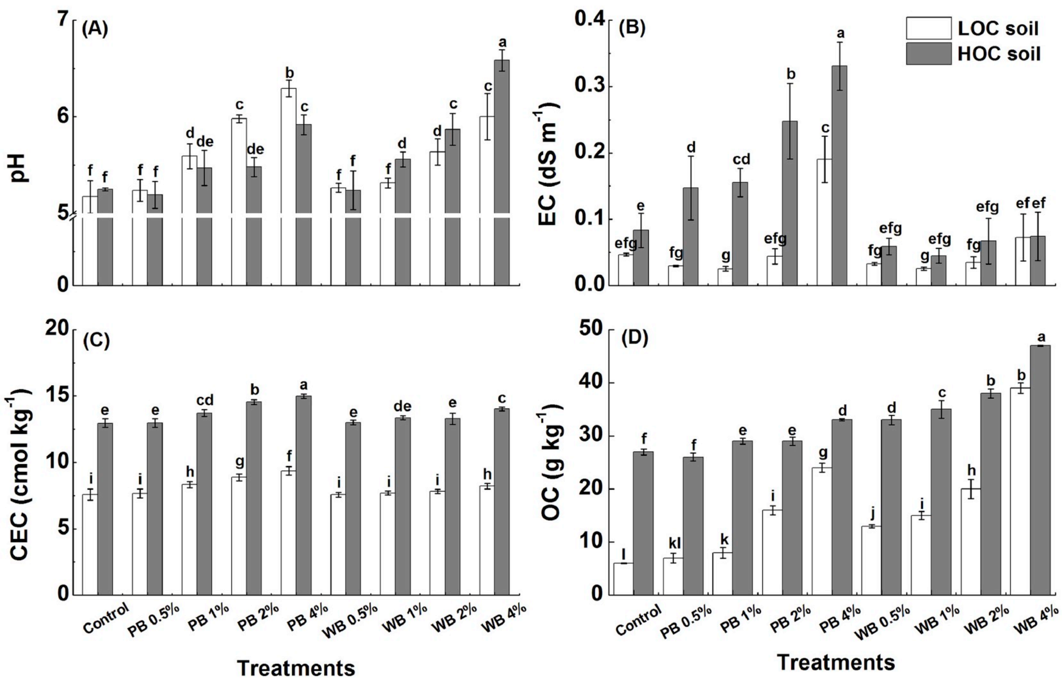

Fig. 1. Effect of biochar treatments on the pH (A), electrical conductivity (EC) (B), cation exchange capacity (CEC) (C), and organic carbon (OC) (D) in the low organic carbon (LOC) soil and high organic carbon (HOC) soil. Control: untreated soil contaminated with Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n = 4). Different lower-case letters above the columns indicate significant difference between treatments ($P < 0.05$).

biochar-induced enhancement of soil microbial and enzyme activities (Section 3.3) could be a reason for increasing the bioavailability of P and K in the wood biochar-treated soils. In this respect, Wardle et al. (2008) and Gul et al. (2015) indicated that biochar application might promote the growth and activity of soil microorganisms via facilitating the soil structure (e.g., soil temperature, moisture and aeration), and therefore enhance P and K mineralization. Additionally, we hypothesize that the mitigated biotoxicity of Cd and DEHP could enhance P and K uptake. Previous studies (Sun et al., 2018; Chen et al., 2019) found that the existence of Cd and DEHP would damage the cell membranes in plants, and the destruction of cell membranes seriously affected the absorption of nutrient elements by blocking the transmembrane transport. Therefore, application of biochars might indirectly promote the absorption of P and K by plants via alleviating the stress of contaminants in soil.

The biochars impact on soil available N content was stronger in the LOC soil than the HOC soil (Fig. 2C). The available N concentration in the LOC soil significantly ($P < 0.05$) decreased with the addition of both biochars. However, the shoot N concentrations increased after both biochars addition (Fig. 2F). We hypothesize that the decrease of N availability in the LOC soil after the addition of both biochars could be ascribed to their specific properties (i.e., high porosity, specific surface area and CEC; Table S2), which might increase the sorption of NO_3^- (pore filling) and NH_4^+ (cation exchange), as also reported by other studies (e.g., Olmo et al., 2016; Purakayastha et al., 2019). In addition, the C-rich biochars used in the current study would increase the C/N ratio of biochar-amended soil, which might inhibit the mineralization rate of soil organic N by reducing the activities of microorganisms, and thereby decrease the N availability, as the similar interpretations were previously reported by Haefele et al. (2011). The increase of N concentration in plants might not be due to the extra N provided by biochar, because most of N would be non-bioavailable in biochar pyrolyzed at a temperature higher than 500 °C (650 °C in this study) (Zheng et al., 2013; Lu et al., 2014; Feng et al., 2020). The increase of shoot N concentrations after biochar application could be attributed to the improvement of N utilization efficiency after biochar application into the soil, according to the results reported by Zheng et al. (2013) and Purakayastha et al. (2019).

Additionally, the influence of biochar application on the availability of K, P and N of the LOC soil was more noticeable than that of the HOC soil, which suggested that the soil organic carbon content had a strong association with the effectiveness of biochar application on impacting the soil fertility. Yang et al. (2016) demonstrated that the higher organic carbon content increased the soil buffering capacity. Therefore, in our present study, it is interpretable that biochar application had more advantages in improving the physicochemical properties and nutrient availability of the LOC soil than that of HOC soil.

3.3. Impact of pig and wood biochars on enzyme activities

Soil enzyme activities, as biological/biochemical indicators of soil quality, are closely related to the behavior of soil microorganisms, and could be affected by soil contamination (He et al., 2019; Bandara et al., 2020). As shown in Fig. 3, application of biochars had positive effect on the activities of urease, sucrase, and catalase, and the effectiveness differed based on biochar type and dose, and soil type. Compared to the untreated soils, the urease activity of the LOC and HOC soils treated with all doses of pig biochar increased by 19.0–133.6% and 58.3–213.0%, respectively (Fig. 3A). In the case of wood biochar treatments, only the 4% dose led to a significant ($P < 0.05$) increase in urease activity in LOC soil; however, in the HOC soil, the urease activity was significantly increased for all biochar treatments as compared to the control (Fig. 3A). However, application of 4% wood biochar had a greater impact on enhancing the activities of sucrase and catalase than urease. The maximum values of sucrase activity were noticed at 4% wood biochar treatment, with up to 12.5-fold increase in the LOC soil, and 4.6-fold increase in the HOC soil, as compared to the control soil (Fig. 3C). The wood biochar was more effective (increased by 85.8–150.8% in LOC soils, and 27.2–65.2% in HOC soils) than pig biochar application (increased by 35.0–140.5% in LOC soils, and 19.0–60.5% in HOC soils) on increasing the catalase activity in the soils (Fig. 3D).

We hypothesize that the enhancement of urease, sucrase, and catalase activities of the biochar-treated soils might be due to the high mineral and nutrient contents, porosity and surface area of the added biochars (Table S2; Fig. S1), which provided a habitat for

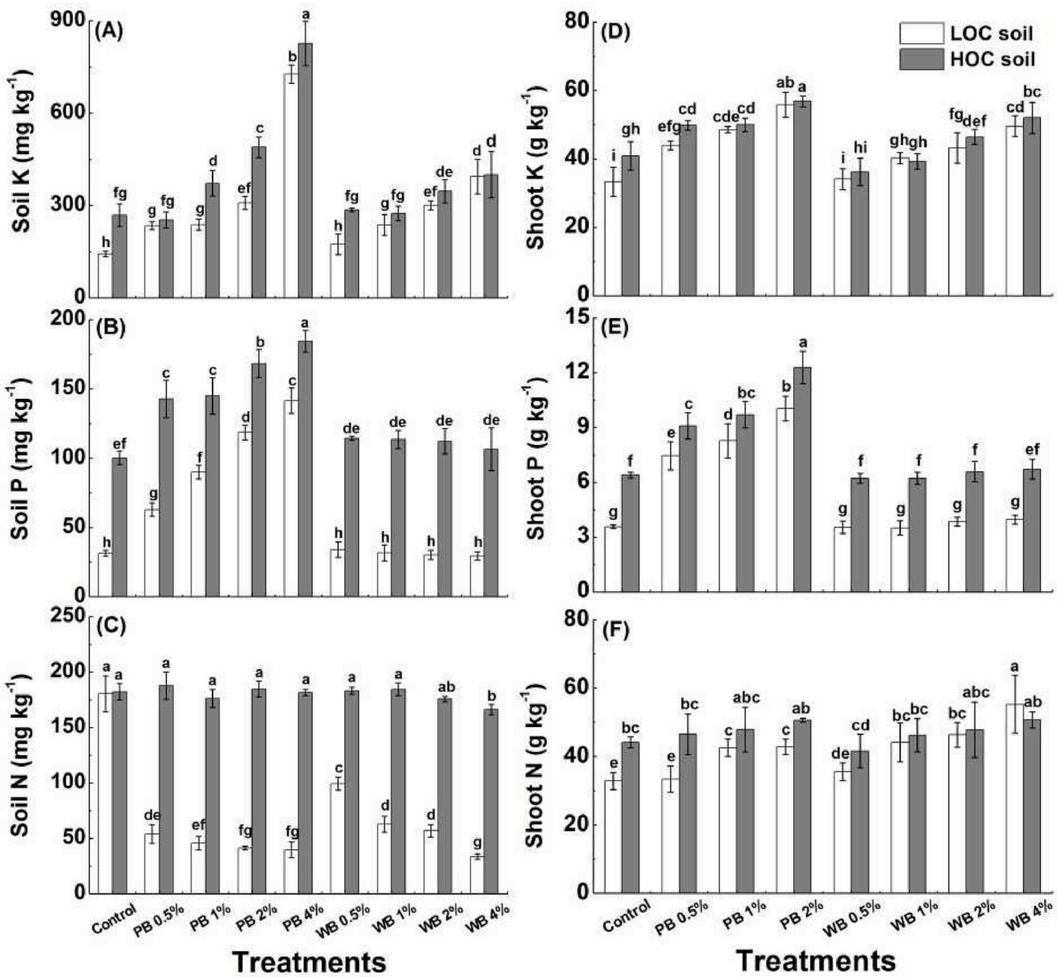

Fig. 2. Effect of biochar treatments on the available K (A), P (B) and N (C) in the low organic carbon (LOC) soil and high organic carbon (HOC) soil, and the uptake of K (D), P (E) and N (F) in plant shoots. Control: untreated soil contaminated with Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n = 4). Different lower-case letters above the columns indicate significant difference between treatments ($P < 0.05$).

microorganisms with ample aeration, water, and nutrients, which might be a reason for improving the growth and reproduction of soil microorganisms, as reported by Gul et al. (2015) and Bandara et al. (2020), and thereby promoting soil enzymatic activities.

Toxic metals, such as Cd ions, might deactivate the enzyme proteins, and thus inhibit soil enzymatic activities (Tan et al., 2018). Also, DEHP might affect the production of enzymes by causing dysfunction in the structure of cell membrane (Chen et al., 2019). Improving the activities of urease, sucrase, and catalase in the biochar treated soils as compared to the untreated soils indicated that both biochars mitigated the negative impact of Cd and DEHP on these enzyme activities in these contaminated soils. In our previous study (Chen et al., 2019), both the wood and pig biochars, particularly pig biochar, were able to reduce the bioavailability of Cd and DEHP in both soils. Therefore, we assume that the biochar-induced reduction of Cd and DEHP toxicity in biochar-amended soils might promote the soil enzyme activities in these soils as compared to the untreated ones. Pearson's correlation analysis in the present study provided a proof that the urease activity negatively correlated to the concentration of extractable Cd ($r = -0.489$, $P < 0.01$, $n = 72$), and the catalase activity negatively correlated to the DEHP concentration in soil ($r = -0.527$, $P < 0.01$, $n = 72$). These results indicated that biochar application was able to reduce the Cd and DEHP bio-toxicity through adsorption/immobilization of those contaminants onto biochar (Qin et al., 2018), as suggested by the improvement of most of the enzymatic activities examined in this study.

On other hand, the acid phosphatase activity decreased significantly ($P < 0.05$) in the biochar treated soil as compared to the control (Fig. 3 B). Pig biochar addition decreased (14.1–39.8% in LOC soil and 18.2–44.7% in HOC soil) the acid phosphatase activity more than wood biochar application (15.7–31.3% in LOC soil and 13.5–32.1% in HOC soil), in comparison to untreated soils (Fig. 3B). We hypothesize that the reduction of acid phosphatase activity in the biochar treated soils could be interpreted by the associated increase of soil pH (Fig. 1), as also indicated by Chen et al. (2013) and Yang et al. (2016). Wang et al. (2018) reported that the acid phosphatase activity depended on soil microbial activities and soil pH. The optimum pH of acid phosphate activity is 4.0–5.0 as mentioned by Wang et al. (2018); however, our soil pH increased to 6.5 after biochar addition, which might cause an inhibitory effect on the acid phosphatase activity. A significant negative correlation between acid phosphatase activity and soil pH was observed in this study ($r = -0.434$, $P < 0.01$, $n = 72$), which also presented an evidence for our hypothesis.

The acid phosphatase activity relates to P transformation and cycling in the soil, and the urease is a crucial factor in soil N mineralization (Yang et al., 2016; Wang et al., 2018). As mentioned above, the pig biochar had a more profound influence on soil N and P availability than wood biochar (Fig. 2). Therefore, pig biochar amendment had greater effect on the urease and acid phosphatase activities in soil, and the reason might be the higher N and P contents, CEC, surface alkalinity of pig biochar than wood biochar (Table S2). Sucrase and catalase activities depended on soil organic carbon content, and therefore, the higher C content in wood biochar than pig biochar might enhance sucrase and

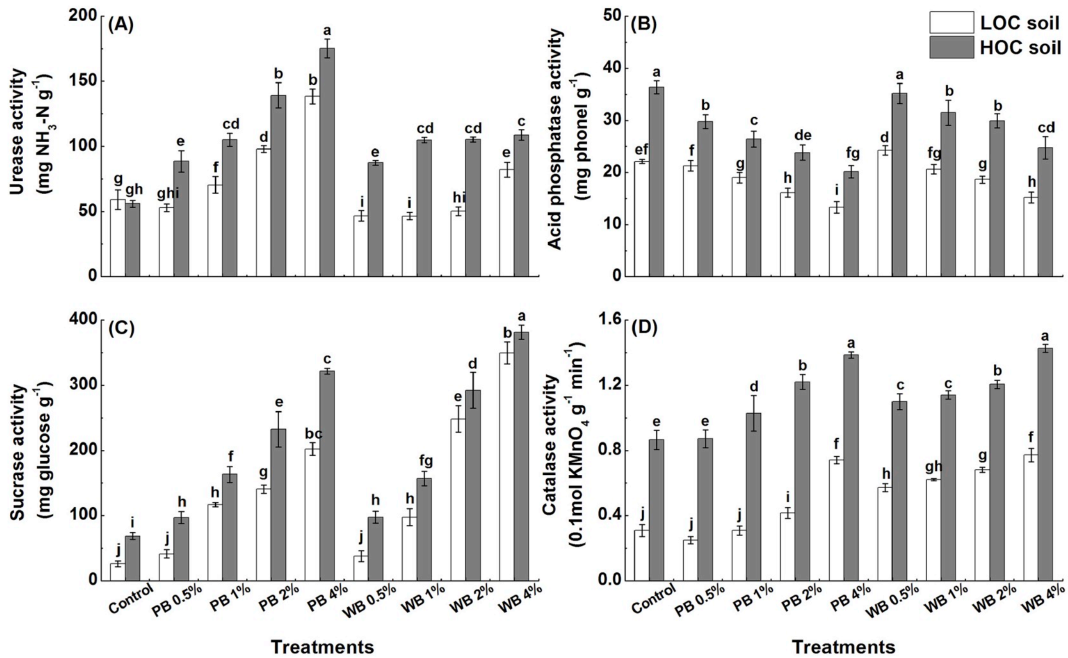

Fig. 3. Effect of biochar treatments on the activities of urease (A), acid phosphatase (B), sucrase (C) and catalase (D) in the low organic carbon (LOC) soil and high organic carbon (HOC) soil. Control: untreated soil contaminated with Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n = 4). Different lower-case letters above the columns indicate significant difference between treatments ($P < 0.05$).

catalase activities in the biochar-amended soils. Further research should be performed to determine the reasons for different response of biochar-amended soil enzyme activities to the LOC and HOC soils.

3.4. Impact of pig and wood biochars on plant growth

Given that the seeds of pak choi in 4% pig biochar treatments did not germinate normally, we eliminated the plant data from that treatment in the statistical analysis. The fact that pak choi did not germinate well in the 4% pig biochar treatments in the pot trial suggested that high pig biochar dosage (4%) inhibited plant growth, as observed also by Schmidt et al. (2014) and Khan et al. (2015). Schmidt et al. (2014) reported that high biochar dosage might cause nutrient immobilization in soils, particularly the mineral N, which consequently would restrict plant growth. Another possible reason was that biochar application increased the available NH_4^+-N concentration in the soil to a level which led to a stress condition for the plant (Khan et al., 2015).

Fig. 4 showed that all treatments (except in the 0.5% wood biochar treatment) significantly ($P < 0.05$) increased the dry weight of plant shoots, as compared to the control. The highest dry weight of plant shoots was observed in the 2% pig biochar treatment, which was 10.1 and 0.5 folds higher than the control in the LOC and HOC soil, respectively. In both soils, pig biochar amendment was more effective in enhancing the shoot dry weight than wood biochar amendment. In addition, the impact of biochar on the plant dry weight in the LOC soil was stronger than that in the HOC soil. Increasing the dry weight biomass of the plants in the biochar treated soils can be explained by the associated increase of soil nutrient availabilities (Fig. 2) and improved soil physical and chemical properties (Fig. 1), as discussed in the previous sections and reported by other studies (e.g., Haefele et al., 2011; El-Naggar et al., 2018; Purakayastha et al., 2019). The pig biochar had higher nutrient contents than wood biochar (Table S2), and thus pig biochar showed a greater effect on the plant growth than wood biochar.

Cadmium and DEHP can negatively affect the plant growth in contaminated soils (Chen et al., 2019). In our studied soils, the relationships between plant growth and Cd and DEHP concentrations in the plant were negative (Fig. S2). Improvement of plant biomass in the biochar treated soils as compared to the untreated soils indicated that both biochars, particularly 2% of pig biochar can mitigate the negative impact of Cd and DEHP on the plant growth in these contaminated soils. This positive impact is in agreement with Lu et al. (2014), Salam et al. (2019) who demonstrated that the addition of biochars increased the plant biomass in metals contaminated soils through improving the soil pH. In our experimental soils, the increased soil alkalinity (Fig. 1A) could be a reasonable factor for immobilizing Cd and DEHP in the soil and reducing their uptake by the plants (Chen et al., 2019), and thus promoting the crop productivity after biochar application to the acidic soil. In our previous study (Chen et al., 2019), we found that biochar application decreased Cd and DEHP bioaccumulation in pak choi shoot, and the pig biochar application was more efficient in comparison with wood biochar. The effect of biochars on the shoot dry weight was more

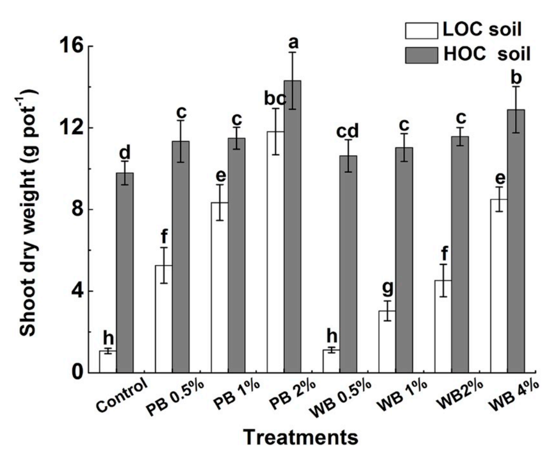

Fig. 4. Effect of biochar treatments on plant shoots dry weight. LOC: low organic carbon content; HOC: high organic carbon content; Control: untreated soil contaminated with Cd-DEHP; PB: pig biochar; WB: wood biochar. Error bars are standard deviation of the means (n = 4). Different lower-case letters above the columns indicate significant difference between treatments ($P < 0.05$).

prominent in the LOC soil than in the HOC soil, which agrees with reports by Haefele et al. (2011) and Zhang et al. (2012) who concluded that biochar produced from crop straw increased rice yield more significantly in barren soils than fertile soils.

4. Conclusions

Our study provided promising information of using animal carcass- and wood-derived biochars for reclamation of degraded soils such as Cd-DEHP co-contaminated soils and soils with low organic matter content. Both biochar treatments improved soil properties (e.g., pH, carbon content, and CEC), increased the bioavailability of P and K in the soils and the uptake of P, K and N by pak choi, and improved the activities of urease, sucrase, and catalase activities. Both biochars, particularly 2% pig biochar, increased the plant biomass, especially in the LOC soil. The positive response of soil enzyme activities and plant growth due to biochar addition in the Cd-DEHP co-contaminated soils indicated that these two biochars could mitigate the risk of Cd and DEHP in soils and improve the soil quality. Pig biochar had higher pH, ash content, surface alkalinity, CEC, and nutrient content than wood biochar; therefore, the former showed more potential to improve soil properties, nutrient availability, and urease activities, and thereby enhanced the crop yield more than wood biochar. This study offers a preliminary understanding of employing pig biochar as an emerging eco-friendly biosorbent for improving soil fertility and crop quality in heavy metal-PAE co-contaminated soils, as well as a cost-effective and applicable fertilizer in degraded soils.

CRediT authorship contribution statement

Hanbo Chen: Investigation, Writing - original draft, Writing - review & editing. **Xing Yang:** Writing - original draft, Investigation. **Hailong Wang:** Funding acquisition, Conceptualization, Methodology, Supervision, Writing - review & editing. **Binoy Sarkar:** Writing - review & editing. **Sabry M. Shaheen:** Writing - review & editing. **Gerty Gielen:** Writing - review & editing. **Nanthi Bolan:** Conceptualization, Writing - review & editing. **Jia Guo:** Investigation. **Lei Che:** Investigation. **Huili Sun:** Investigation. **Jörg Rinklebe:** Conceptualization, Writing - review & editing.

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Appendix A. Supplementary data

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