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ORIGINAL PAPER

Effects of three biochars on copper immobilization and soil microbial communities in a metal-contaminated soil using a metallophyte and two agricultural plants

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Abstract Biochar (BC) is a porous, carbonaceous material produced by slow pyrolysis of biomass under oxygen-limited conditions. BC production has been attracting research interest because it modifies soil physicochemical characteristics and improves the growth of plants in problem soils. These benefits may be best actualized for soils contaminated by metals, where remediation is hampered by metal toxicity to both plants and soil microbial communities. The objectives of this study were to evaluate the impact of the addition of chicken manure biochar (CMB), oat hull biochar (OHB), or pine bark biochar (PBB) on copper (Cu) bioavailability in a Cu-contaminated soil, the effectiveness of these BCs promoting plant growth, and its effects on soil microbial communities supporting these plants. A sandy soil (338 mg Cu kg^{-1}) was amended with CMB, OHB, and PBB, and the metallophyte *Oenothera picensis* or the agricultural species *Solanum lycopersicum* and *Lolium perenne* were grown for 3 months. The BCs produced an increase in soil pH, reduced the exchangeable Cu, and increased Cu bound to organic matter and residual fractions. All BCs enhanced the quality of contaminated soil and increased the plant biomass production, notably for *S. lycopersicum*, which grew until 12 times more than plants in non-amended soil. While BC addition reduced the concentration of Cu in soil pore water,

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the amendment did not reduce the concentrations of Cu in shoot tissues. BC additions also stimulated soil microorganisms, increasing basal respiration and DHA activity and modifying microbial communities, especially in soils supporting *L. perenne*. These results indicate that BCs represent an effective tool to remediate Cu-contaminated sandy soils.

Keywords Soil amendments · Metal-contaminated soils · Immobilization · Microorganisms

Introduction

Metal(loid) contamination is a common condition in soils surrounding smelters. Metals have high bioaccumulation potentials and are not biodegradable (Adriano et al. 2004). Some metals, such as copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), are essential nutrients and are involved in many biochemical reactions, but, at high concentrations, these metals may be toxic to soil biota, especially microorganisms (Giller et al. 1998). Soil microorganisms play important roles in developing soil structure and stability, carbon (C) and other nutrient cycling, and mediating plant–soil interactions (Frey-Klett et al. 2011; Seguel et al. 2013). The contamination of soils by metals, then, may impact ecosystem recovery and remediation efforts by specifically damaging the soil microbial community (Meier et al. 2015).

Biochar (BC) is the product derived from various feedstocks (ranging from lignocelluloses to manure) that are pyrolyzed at different temperatures (usually from 200 to 900 °C) (Shackley et al. 2012). The addition of BC improves soil structure and nutrition and may stimulate plant growth and invigorate microbial communities (Khodadad et al. 2011). In addition, the BC utilization may provide advantages to the remediation of metal-contaminated soils promoting plant growth and microbial activity, which may be attributed to (1) metal(loid) adsorption and decreased metal availability, (2) improvement in soil physiochemical properties such as soil pH and redox potential, (3) supplementation of recalcitrant C pools, (4) supply of limiting nutrients, and (5) improvement in microbial habitat by the BC structures that are suitable for microbial growth (Jones et al. 2011).

The incorporation of BC into Cu-contaminated soil reduced the pore water Cu, increased the soil microbial activity, and improved the growth of *Oenothera picensis*, a chilean native metallophyte (Meier et al. 2017a; Moore et al. 2017). Because of the diversity of soils that may be contaminated by metals, however, more information is required on the effects of BC on soil microbial communities and activities and the response of plants to these metals (Ahmad et al. 2016). Furthermore, there are few studies evaluating the behavior of native Cu metallophytes (slow growing, but adapted to high Cu concentrations) and traditional agricultural plants (fast growing and presumably sensitive to Cu) in response to BC and their effect on soil microbial communities. Such information is crucial for the development of effective approaches for phytoremediation of Cu-contaminated soils.

The objectives of this study were to assess: (1) the degree of immobilization of Cu by BC; (2) changes in other soil chemical properties associated with BC application; and (3) the diversity and the degree of activity of microbial communities in a Cu-contaminated soil amended with BC. We incorporated BCs produced from chicken manure (CMB), oat hull (OHB), and pine bark (PBB) into Cu-contaminated soil and evaluated the responses of plants and microbes in soils cropped with a native Chilean metallophyte, fragrant evening primrose (*O. picensis),* and two agricultural plants, tomato (*Solanum lycopersicum*) and perennial ryegrass (*Lolium perenne*).

Materials and methods

Soil collection and biochar production

A sedimentary Alfisol (Achreptic haploxeral) from Chilicauquén series was obtained from the Ventanas copper smelter situated in Puchuncaví Valley, Central Chile (32°46′30″S, 71°28′17″W). The information of the soil chemical analysis is available in Meier et al. (2017b). Briefly: pH (water) 5.29, organic matter 3%, cation exchange capacity (CEC) 4.63 cmol (+) kg^{-1}, Cu 338 mg kg^{-1}.

Three biochars were selected for this study, using as feedstocks chicken manure (CMB), oat hull (OHB), and pine bark (PBB), which were produced at the Center of Waste Management and Bioenergy, Universidad de La Frontera (Temuco, Chile). The pyrolyzer

conditions and physiochemical characterization of CMB and OHB are reported in Moore et al. (2017). Briefly, the temperature of pyrolysis reached was 500, 300, and 600 °C, for CMB, OHB, and PBB, respectively, and maintained for 2 h. Macro- and micronutrients were measured according to Sadzawka et al. (2006). Total C and N were measured in a CHNS/O analyzer (Fisons EA 1108 Analyzer, CE Instruments, Lancashire, UK). Cation exchange capacity (CEC) was determined according to McIntosh (1969), whereas carboxylic acidities were measured using methods proposed by Tan et al. (2005). Specific surface area (Brunauer–Emmett–Teller, BET) and pore volume were determined using a Quantachrome NOVA 1000e Analyzer (Quantachrome Instruments, Boynton Beach, FL, USA).

Plant growth experiment and copper fractionation on the soil

The soil was hand-mixed with CMB, OHB, or PBB at the rate of 0 (Control) or 3% w/w and watered at 60% water retention capacity for 2 weeks. Seeds of the metallophyte *O. picensis* and the agricultural plants *S. lycopersicum* and *L. perenne* were sterilized using 2% Chloramine-T solution for 5 min and then rinsed with distilled water. The seeds were initially sown in 500-mL pots in triplicate in a greenhouse (25/15 ± 3 °C day/night temperatures; 16/8 h light/dark photoperiod; 80–90% relative humidity). Seedlings were transplanted to 1-L pots containing BC-amended soils and allowed to grow for 90 days. A Rhizon sampler (Rhizosphere Research Products, Wageningen, the Netherlands) was connected to the pot to collect soil pore water samples (10–15 mL) on days 30, 60, and 90 during the plant growth experiment. The pH of soil pore water samples was measured immediately after collection. After that, the soil pore water sample was briefly refrigerated (5 °C) until the Cu analysis by atomic absorption spectrophotometry (AAS; UNICAM Solaar 969, Thermo Fisher Scientific, Waltham, MA, USA).

At harvest, shoots and roots were washed separately with Milli-Q water, oven-dried at 70 °C for 2 days, and weighed. Then, these samples were ground, ashed at 550 °C, and digested using concentrated HCl and HNO_3 and an acid solution composed by H_2O–HCl–HNO_3 mixture (8:1:1, v:v:v). Copper in the extract was analyzed by AAS.

On the other hand, soil samples from the plant growth experiment were air-dried and sequentially extracted for Cu according to Tessier et al. (1979) for easily exchangeable, carbonate-bound, Fe and Mn oxide-bound, organic, and residual-bound fractions. The reagents and operating conditions for the extraction steps are summarized in Park et al. (2011). Briefly, the extraction was carried out in 50-mL polyethylene centrifuge tubes. After each extraction step, the supernatant solution was separated from the solid phase by centrifugation (4000 rpm for 15 min). The supernatant solution was stored at 4 °C in polyethylene vessels for analysis, while the remaining soil was washed with 10 mL of Milli-Q water, and the washings were discarded after centrifugation. The residual Cu fraction was determined according to protocol EPA 3051A (USEPA 2007) in a microwave digester (PerkinElmer Titan MPS, Perkin Elmer Inc., Waltham, MA, USA). The supernatant solutions from all the steps were analyzed for Cu using EAAS.

Soil microbial activity

Dehydrogenase activity (DHA) and soil basal respiration were evaluated soon after harvesting according to Anderson and Domsch, (1990). For the DHA analysis, 3 g (dry weight basis) of fresh soil was weighed into 50-mL sterile centrifuge tubes with 3 mL of a 0.5% triphenyltetrazolium chloride (TTC) solution in 0.1 M tris buffer (pH 7.6–7.8) (Singh and Singh 2005) and incubated for 24 h at 37 °C. After that, 10 mL of methanol was added to each sample. The absorbance of the samples was measured at 485 nm against the blank (sample without soil) in a Thermo UV/Vis GENESYS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

For soil basal respiration, 18 g (dry weight basis) soil was weighed in a plastic tube which had its upper part perforated to allow gas exchange. The moisture of the soil was adjusted to 60% of the water retention capacity, and the plastic tube was sealed in a Schott bottle that contained 20 mL of NaOH solution (0.05 M). The soils were incubated for 24 h at 22 °C (Anderson and Domsch 1990). Plastic tubes were removed, and 2 mL of 0.5 M $BaCl_2$ solution was added to the solutions in the Schott bottles. After

precipitation of $BaCO_3$, phenolphthalein indicator was added followed by titration of the solution with 0.06 M HCl until the red color disappeared.

PCR-DGGE of the bacterial and fungal communities

The composition of bacterial and fungal communities was analyzed by PCR-DGGE according to the method described by Acuña et al. (2013) and modified by Moore et al. (2017). Total DNA in five soil samples from BC treatments and controls was extracted using FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's protocol. For bacterial community analysis, fragments of 16S rRNA were amplified by touchdown PCR using the specific primer set EUBf933-GC (Iwamoto et al. 2000).

Fungal ITS regions (18S rRNA) were amplified with the nested PCR using primer set ITS1F and ITS4 (for first round of PCR). One microliter from the first PCR was used as a template for the second PCR using primer set ITS1F and ITS2-GC; more details are reported in Moore et al. (2017).

The PCR products from each of the soil replicates from BC samples were visualized on an agarose gel stained with 1% (w/v) of ethidium bromide. Denaturing gradient gel electrophoresis (DGGE) analysis was performed using a DCode system (Bio-Rad Laboratories Inc., Hercules, CA, USA). PCR products (20 µL) were loaded onto a 6% (w/v) polyacrylamide gel with linear denaturing gradient (urea and formamide) ranging from 50 to 70% for 16S rRNA and from 35 to 65% for the 18S rRNA gene. The electrophoresis was run for 12 h at 100 V. The banding patterns were visualized by staining the gel 1:10,000 with (v/v) SYBR Gold (Molecular Probes, Invitrogen Co.) followed by image capture using a GelDoc-IT TS2 imaging system (Analytik Jena AG Company, Jena, Germany).

Finally, the similarities among the banding patterns of the DGGE were analyzed using clustering and dendrograms Phoretix 1D analysis software (Total Lab Ltd., Newcastle upon Tyne, UK). Based on the matrix obtained from Phoretix 1D analyses, the changes in the microbial community composition (presence or absence) and abundance of bacterial and fungal groups were visualized using nonlinear multidimensional scaling (NMDS) with PRIMER-v6 software (www.primer-e.com) and clustered using the Bray–Curtis similarity index.

Statistical analyses

All experiments were conducted with three replicates. The data collected were analyzed statistically using XLSTAT software. Tukey's multiple range tests were used to compare the means of the treatments. Variability in the data was expressed as the standard error. $p \leq 0.05$ was considered to be statistically significant.

Results

Biochar properties

CMB and PBB were strongly alkaline (9.1 and 9.2, respectively), whereas OHB was slightly alkaline (7.8) (Table 1). The CMB had higher macronutrient contents than OHB and PBB, but the least amount of carbon. The surface area of CMB was higher than that of OHB and PBB. All BCs had negative ζ potentials, highlighting PBB having the most negative potential ($-$ 40.5) (Table 1).

Effects of biochar on Cu immobilization in soil

The pH of soil pore water varied across treatments and collection period (Fig. 1a). In soils supporting *O. picensis*, the pH of pore water increased with the addition of OHB, PBB, and CMB (0.3, 0.4, and 1.0 units, respectively) compared to that of the Control over 90 days. A similar trend was observed for *S. lycopersicum*: The pH increased by 0.6, 0.7, and 1.2 units for OHB, PBB, and CMB, respectively. This pattern was also evident for soils from *L. perenne*, which had the largest difference between non-amended and BC-amended treatments (Fig. 1a).

The increase in pH due to BC addition was associated with reductions in Cu concentrations in the soil pore water by up to 11-fold for *O. picensis* amended with PBB compared to the Control on day 90 (Fig. 1b). For *S. lycopersicum*, the Cu concentration in soil pore water was reduced by 1.8-, 2.9-, and 3.4-fold by CMB, OHB, and PBB, respectively. For *L. perenne*, all BCs reduced pore water Cu by $\sim$ 5.5-fold compared to that of the Control (Fig. 1b).

All BCs reduced the easily exchangeable fraction of Cu in soils and increased the concentrations bounded to organic matter and residual fractions (Fig. 2). These

Table 1 General characteristics of the chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark (PBB) biochars

Parameter	CMB	OHB	PBB
Total C (%)	29.67	69.02	77.5
Total N (%)	2.13	1.06	0.69
C/N ratio (%)	13.92	65.11	112.31
P (g Kg^{-1})	19.4	2.1	2.21
K (g Kg^{-1})	17.2	11.3	0.372
Ca (g Kg^{-1})	54.0	1.0	75.8
Mg (g Kg^{-1})	5.1	1.3	30.6
S (g Kg^{-1})	4.6	–	15.5
pH (H_2O, 1:5)	9.1	7.81	9.2
Electrical conductivity (1:5, μS cm^{-1})	924	789	724
Total acidity (mmol g^{-1})	4.4	3.41	5.09
Carboxylic acid (mmol g^{-1})	0	0.28	0.30
Phenolic acid (mmol g^{-1})	4.4	3.13	4.79
BET (m^2 g^{-1})	11.51	0.16	1.30
Pore diameter (nm)	3.82	3.24	3.19
Pore volume (cm^3 g^{-1})	0.009	0.003	0.007
ζ potential (mv)	− 29.4	− 40.5	− 25.8

BET Brunauer–Emmett–Teller

effects were more pronounced for CMB, which decreased the Cu exchangeable fraction by a minimum of ∼ 71% in all treatments evaluated. OHB and PBB also decreased the exchangeable fraction of Cu by about 67% and increased the Cu bound to organic matter and residual fraction (mean of three plant species). The efficacy of CMB in sequestering Cu was greater than that of OHB and PBB (Fig. 2).

Plant growth and metal uptake

In general, the addition of BCs increased the plant dry weight and reduced the plant Cu uptake; these effects were dependent on the plant species and type of BC applied (Table 2). For *O. picensis*, OHB, PBB, and CMB increased the shoot biomass production by 24, 31, and 70%, respectively, compared to that of Controls. Similarly, PBB, OHB, and CMB increased the root biomass by 27, 31, and 54%, respectively. The BCs also increased the growth of *L. perenne* by ∼ 25% (mean of the three BCs) for shoots and roots. The most significant benefit of BCs was noted for the growth of *S. lycopersicum* because of its extreme sensitivity to Cu in non-amended soils. Shoot and root growth increased 14-fold and fourfold, respectively, with the addition of CMB, with substantial benefits also noted for the other BCs (Table 2).

The effects of BC on plant Cu concentration were dependent on the plant species and type of BC (Table 2). In *O. picensis*, CMB and OHB reduced the root Cu concentration by ∼ 50% and PBB by ∼ 30%, but no change was observed in shoots (Table 2). In *S. lycopersicum*, only CMB decreased the root Cu concentration (∼ 51% than that of the Control) and only PBB reduced the shoot Cu concentration. In *L. perenne*, no effect was evident either in the roots or shoots for any of the BCs (Table 2).

Soil microbial activity

The type of BC promoted, in most of the treatments, the microbial activity (i.e., basal respiration and DHA) compared to control soils, and these activities were influenced also by plant species (Table 3). In the case of *O. picensis*, the basal respiration increased by 20, 33, and 55% using OHB, CMB, and PBB treatments, respectively, compared to Control. A similar trend was observed in *S. lycopersicum*, with 2, 43, and 77% increases for OHB, CMB, and PBB treatments, respectively. This stimulation produced by BCs was even more pronounced in soils supporting *L. perenne*, with 186–228% increases in basal respiration compared to Controls (Table 3). The DHA activity was generally increased for all plants by BCs except for *O. picensis* grown in PBB (Table 3). In the case of *S.*

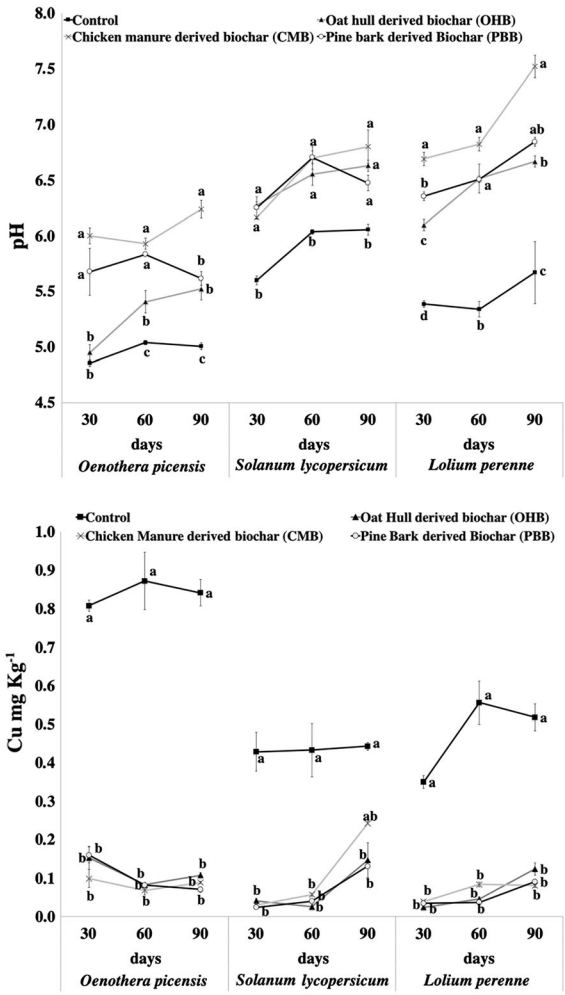
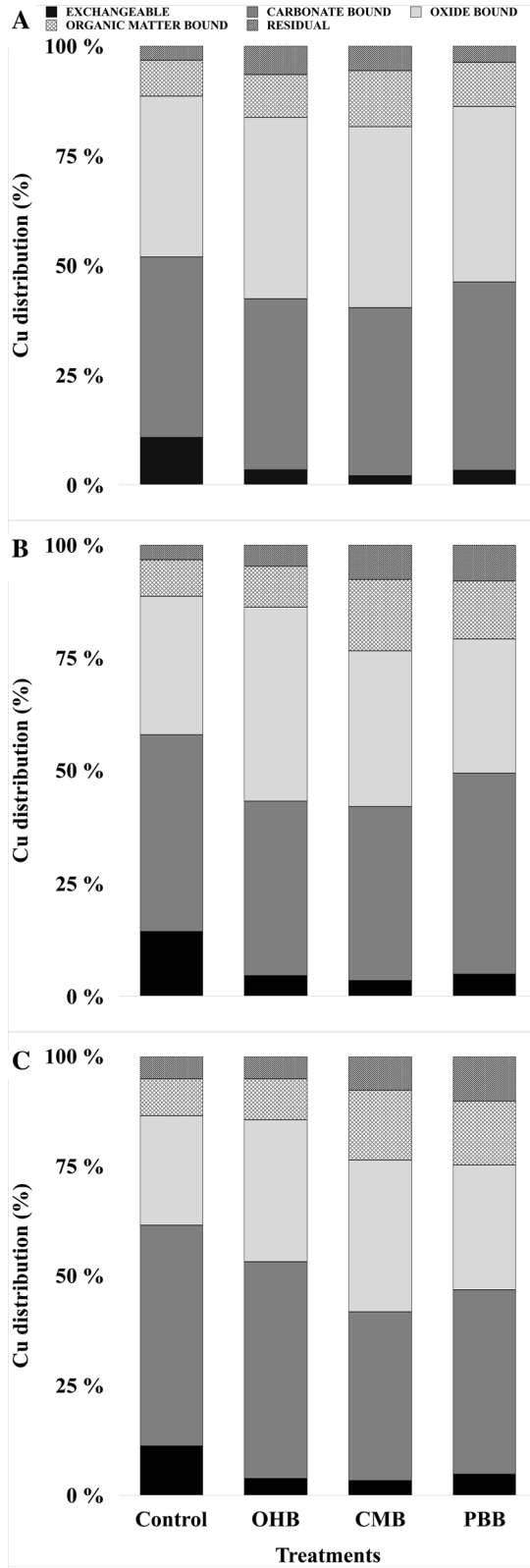


Fig. 1 pH (top) and Cu concentration (bottom) in soil pore water collected on days 30, 60, and 90 from Cu-contaminated soils amended with chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark biochar (PBB) at 0 or 3% (w/w). Each value represents the mean of three replicates ± standard error. Different letters within a column indicate a significant difference at $p \leq 0.05$ according to Tukey's multiple range tests

lycopersicum, the BCs increased the DHA by 80% (mean of results for three BCs), whereas PBB, OHB, and CMB increased it by 1.5-, 2.1-, and 2.4-fold for *L. perenne*, respectively.

Composition of bacterial and fungal communities

DGGE profiles and NMDS for the bacterial and fungal communities for all the treatments are shown in Figs. 3, 4, and 5. The DGGE profiles from BC-amended soils have different banding patterns



◀**Fig. 2** Copper fractions in soils following plant growth for 90 days on Cu-contaminated soil treated with chicken manure biochar (CMB), oat hull biochar (OHB), or pine bark-derived biochar (PBB) applied at 0 (Control) or 3% (w/w). Soils grown with: **a** *O. picensis*; **b** *S. lycopersicum*; and **c** *L. perenne*

compared to Controls, indicating significant changes in microbial communities in response to BC application (Figs. 3, 4, and 5). These changes are further modified by the plant species grown in the soils. For example, no clear difference was observed in bacterial and fungal communities for the metallophyte *O. picensis* for all the BC treatments (Fig. 3). However, changes in the bacterial communities in soils supporting *L. lycopersicum* indicated strong similarity among groups (70–80%) for OHB, CMC, and PBB treatments (Fig. 4); fungal communities were not so clearly separated. Differences were also evident in microbial and fungal communities in soils from *L. perenne* for all the BC treatments (Fig. 5).

The microbial structure and diversity from BC-amended soils were expressed by richness (S), Shannon–Wiener (H), and Simpson (D) indices (Figs. 6, 7, and 8). OHB, CMB, and PBB additions did not produce any change in the richness of bacterial and fungal communities in the case of *O. picensis* (Fig. 1a). The BCs promoted only bacterial diversity as indicated by Shannon–Wiener (Fig. 6b) and Simpson indices (Fig. 6c). For *S. lycopersicum*, BC additions increased bacterial, but not fungal, richness and diversity, whereas the opposite trend was observed in the case of *L. perenne* (Fig. 7), where all the BCs produced changes only in fungal community richness and diversity (Fig. 8).

Discussion

Biochar additions to a Cu-contaminated soil improved the chemical properties of the soil, stimulated the microbial activity, and increased the capacity of the soil to support plant growth, as has been reported or other soils and other metals (Lu et al. 2015; Park et al. 2011). The BC alkalinity was associated with base cations, including K^+, Ca^{2+}, and Mg^+, which increase the base saturation of soil and, therefore, increase the soil pH (Xu et al. 2012). Additionally, the presence of functional groups in the surface of BC such as

Table 2 Shoot and root biomass production and Cu concentration in plant tissues of *Oenothera picensis, Solanum lycopersicum*, and *Lolium perenne* grown in Cu-contaminated soil amended with chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark biochar (PBB) at doses of 0 or 3% (w/w)

	BC (%)	Biomass (g)		Cu concentration (μg g^{-1})	
		Shoot	Root	Shoot	Root
O. Picensis					
Control	0	3.72 ± 0.09^{c}	0.66 ± 0.05^{c}	24.21 ± 1.37^{a}	307 ± 15.6^{a}
OHB	3	4.63 ± 0.19^{bc}	0.87 ± 0.03^{ab}	24.22 ± 2.76^{a}	150 ± 18^{b}
CMB	3	6.32 ± 0.38^{a}	1.05 ± 0.05^{a}	20.98 ± 1.76^{a}	149 ± 25.1^{b}
PBB	3	4.89 ± 0.07^{b}	0.84 ± 0.05^{ab}	19.63 ± 1.78^{a}	202 ± 30.3^{b}
S. lycopersicum					
Control	0	0.81 ± 0.07^{c}	0.36 ± 0.03^{b}	38.72 ± 5.73^{a}	140 ± 7.7^{a}
OHB	3	9.37 ± 0.28^{b}	1.15 ± 0.01^{a}	24.92 ± 3.20^{ab}	118 ± 5.9^{a}
CMB	3	12.79 ± 0.96^{a}	1.57 ± 0.07^{a}	22.85 ± 3.39^{ab}	68.3 ± 3.9^{b}
PBB	3	8.82 ± 0.31^{b}	1.46 ± 0.18^{a}	21.11 ± 1.71^{b}	123 ± 13.6^{a}
L. perenne					
Control	0	6.40 ± 0.18^{b}	1.73 ± 0.17^{b}	48.05 ± 5.31^{a}	61.4 ± 3.5^{a}
OHB	3	7.73 ± 0.11^{a}	1.88 ± 0.12^{ab}	39.08 ± 6.28^{a}	61.1 ± 6.2^{a}
CMB	3	8.22 ± 0.42^{a}	2.29 ± 0.17^{ab}	34.44 ± 2.89^{a}	59.8 ± 4.5^{a}
PBB	3	8.71 ± 0.03^{a}	2.37 ± 0.07^{a}	37.18 ± 4.46^{a}	56.9 ± 2.0^{a}

Each value represents the mean of three replicates ± standard error. Different letters within a column indicate a significant difference at $p \leq 0.05$ according to Tukey's multiple range tests

Table 3 pH, microbial basal respiration, and dehydrogenase activity (DHA) in a Cu-contaminated soil amended with chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark biochar at doses of 0 or 3% (w/w)

	BC (%)	pH	Basal respiration (mg CO_2 kg^{-1} h^{-1})	DHA (mg TPF kg^{-1} 24 h^{-1})
O. Picensis				
Control	0	5.8 ± 0.03^{b}	12.5 ± 0.78^{c}	13.0 ± 0.46^{c}
OHB	3	6.1 ± 0.12^{b}	15.1 ± 1.20^{b}	21.0 ± 0.51^{a}
CMB	3	6.7 ± 0.15^{a}	16.6 ± 2.06^{b}	16.5 ± 0.31^{b}
PBB	3	6.2 ± 0.01^{b}	19.4 ± 3.19^{a}	9.6 ± 0.12^{d}
S. lycopersicum				
Control	0	5.5 ± 0.09^{c}	13.4 ± 0.78^{c}	11.5 ± 0.2^{b}
OHB	3	6.1 ± 0.01^{b}	13.6 ± 0.37^{c}	21.2 ± 0.06^{a}
CMB	3	6.7 ± 0.08^{a}	19.2 ± 2.80^{b}	20.6 ± 0.03^{a}
PBB	3	6.2 ± 0.06^{b}	23.7 ± 1.88^{a}	20.6 ± 0.38^{a}
L. perenne				
Control	0	6.2 ± 0.08^{c}	12.3 ± 0.65^{b}	8.8 ± 0.21^{d}
OHB	1	6.8 ± 0.03^{b}	23.1 ± 2.16^{a}	19.2 ± 0.22^{b}
CMB	1	7.4 ± 0.10^{b}	28.0 ± 2.54^{a}	21.2 ± 0.28^{a}
PBB	5	6.9 ± 0.12^{a}	22.9 ± 2.49^{a}	13.7 ± 0.48^{c}

Each value represents the mean of three replicates ± standard error. Different letters within a column and season indicate a significant difference at $p \leq 0.05$ according to Tukey's multiple range tests

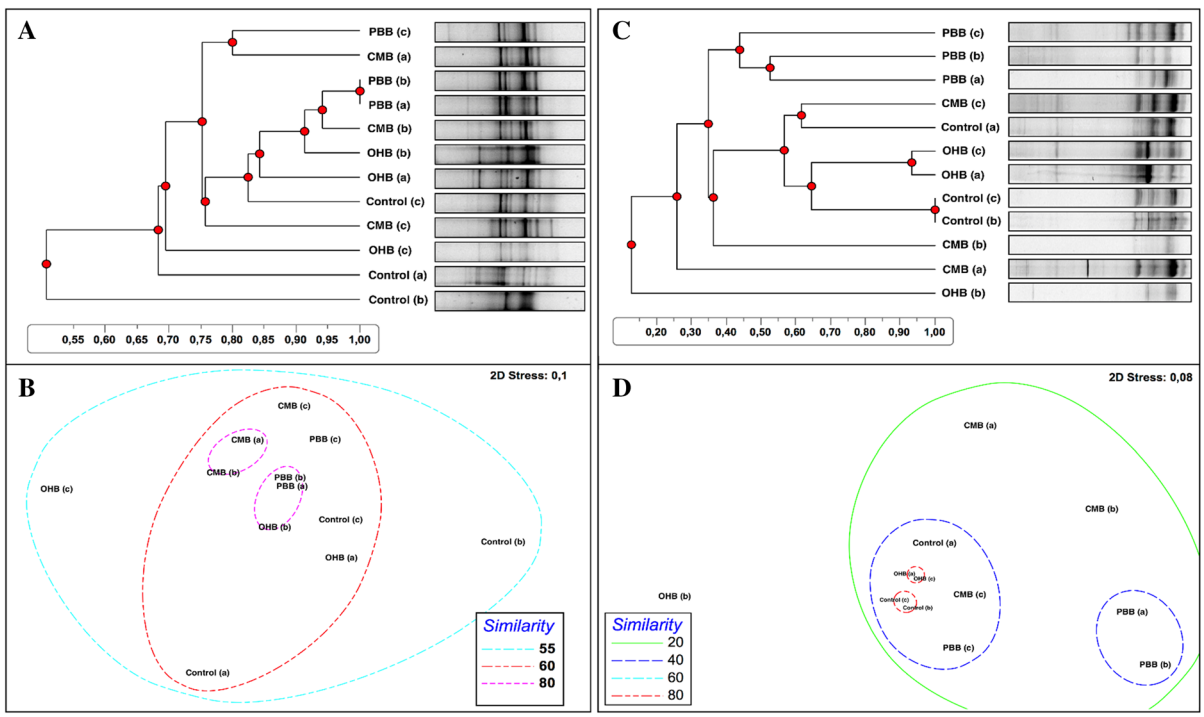


Fig. 3 Dendrogram of denaturing gradient gel electrophoresis (DGGE) profiles (**a**, **c**). Non-metric multidimensional scaling (NMDS) (**b**, **d**) of microbial communities from *Oenothera picensis* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at doses of 0 or 3% (w/w). **a**, **b** Correspond to bacterial communities, whereas **c**, **d** correspond to fungal communities

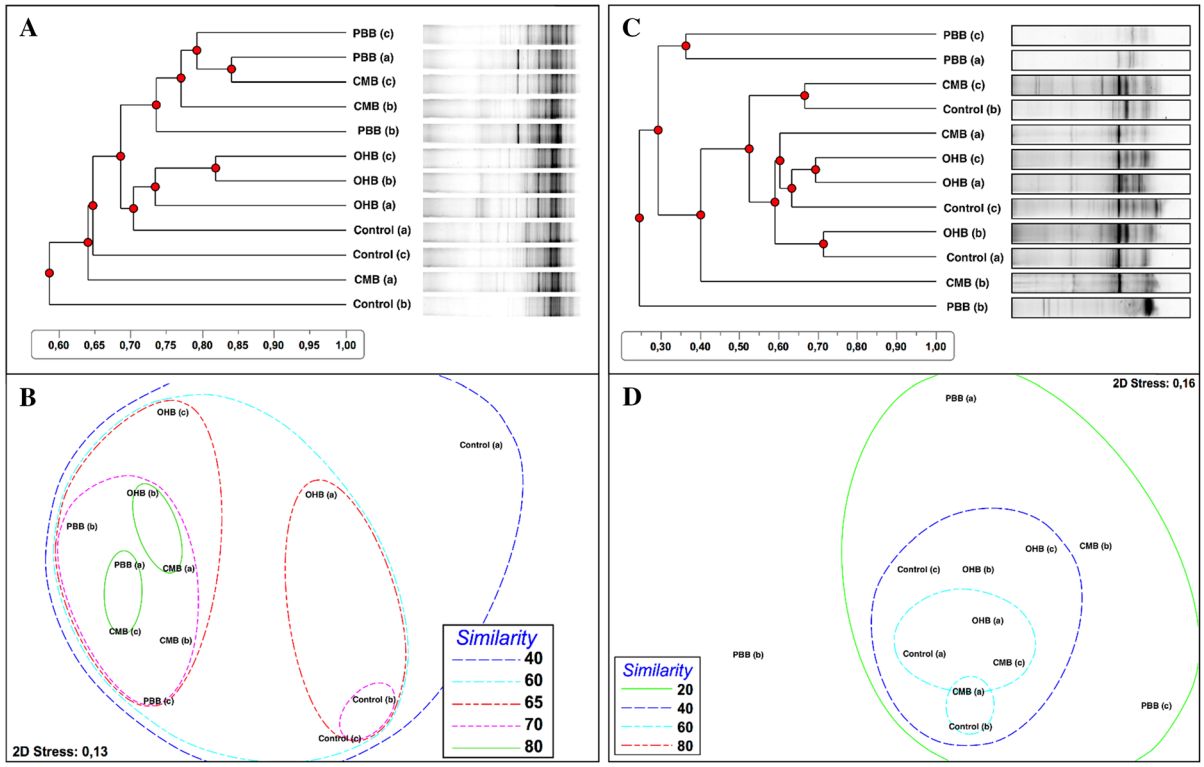


Fig. 4 Dendrogram of denaturing gradient gel electrophoresis (DGGE) profiles (**a**, **c**). Non-metric multidimensional scaling (NMDS) (**b**, **d**) of microbial communities from *Solanum lycopersicum* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at doses of 0 and 3% (w/w). **a**, **b** Correspond to bacterial communities, whereas **c**, **d** correspond to fungal communities

carboxyl, phenolic, and hydroxyl (–COOH, C–OH, –OH) may contribute to the formation of stable compounds with Cu, reducing its bioavailability and toxicity (Yuan et al. 2011).

Influence of biochar on Cu distribution and plant uptake

Bioavailability is critical to the toxicity of metals to soil microorganisms and plants growing in metal-contaminated soils (Giller et al. 1998; Cornejo et al. 2008; Meier et al. 2017a). The major factors influencing the speciation distribution of heavy metals in soil include intrinsic properties of metals, physicochemical soil characteristics (pH, organic matter, and oxide content), and environmental factors (temperature and moisture). BCs can modify some chemical properties of the soil, such as soil pH and CEC, indirectly promoting metal immobilization. Moreover, the immobilization and stability of metals occur by the complexation of metals with functional groups on BCs. For the Cu ion, the specific and the negatively charged BC immobilizes the positive Cu cations via the electrostatic attractions (Zhang et al. 2013). Although the BCs used in the current study differed in their physicochemical characteristics (Table 1), they generally were all beneficial in remediating the Cu-contaminated soil. OHB and PBB had a much lower surface area (0.16 and 1.30 $m^2\ g^{-1}$, respectively) than CMB (11.51 $m^2\ g^{-1}$), but other properties such as the pH, pore structure and volume, and a negative ζ potential suggest that Cu immobilization by OHB and PBB was also of high magnitude (Table 1, Figs. 1 and 2), and BCs were effective at sequestering Cu from available pools in the soil (Zhao et al. 2013). Our results show that BCs decreased available Cu by reducing the exchangeable Cu fraction and transferring Cu to soil organic matter and residual fraction pools (Fig. 2), which have been previously reported by Moore et al. (2017). These patterns indicated that Cu is being sequestered in BC particles, which would play an important role in remediation of Cu-contaminated soils.

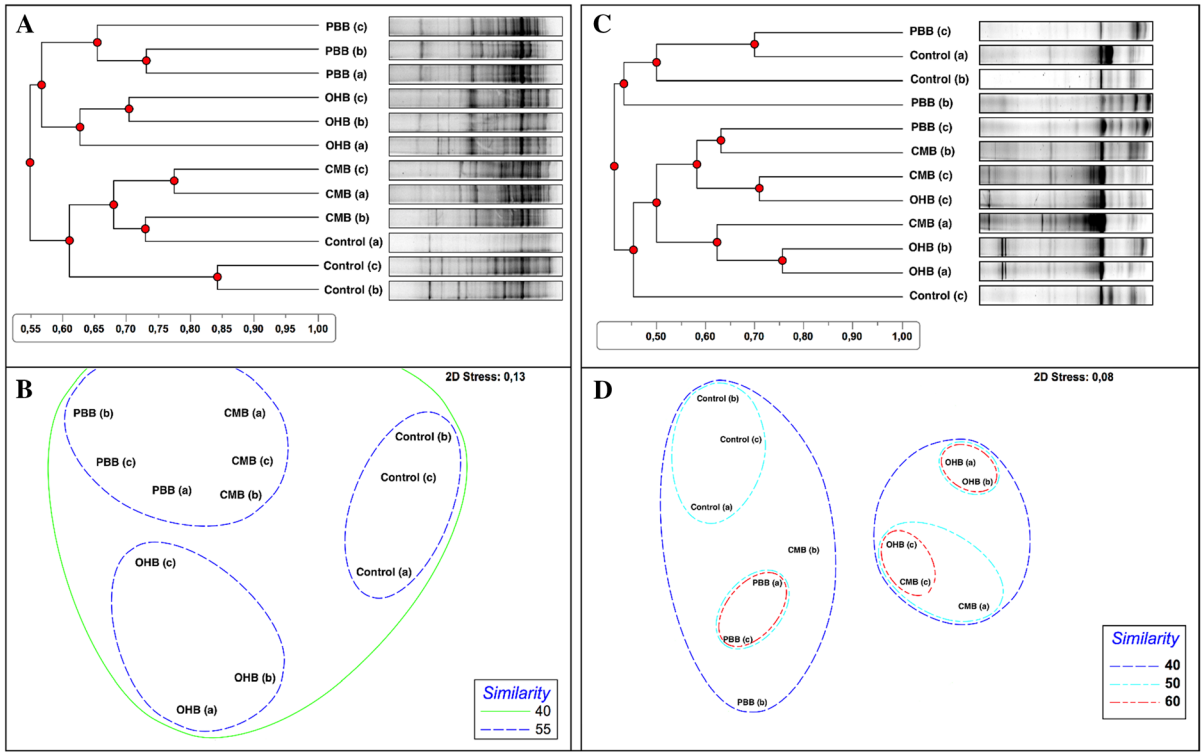


Fig. 5 Dendrogram of denaturing gradient gel electrophoresis (DGGE) profiles (**a**, **c**). Non-metric multidimensional scaling (NMDS) (**b**, **d**) of microbial communities from *Lolium perenne* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at doses of 0 and 3% (w/w). **a**, **b** Correspond to bacterial communities, whereas **c**, **d** correspond to fungal communities

The BC applications were effective in increasing the pH of soil pore water (Fig. 1a). Soil pH has strong effects on the solubility and mobility of Cu; thus, an increase in pH reduced the Cu concentration in soil pore water when compared to the Control treatments (Fig. 1b). This is supported by several studies indicating that the application of BC increases the pH of acid soils, thereby enhancing the adsorption and complexation of Cu cations on both BC and native soil clays and (oxy)hydroxides and reducing Cu bioavailability (Ahmad et al. 2012; Cornejo et al. 2008; Moore et al. 2017). This benefit can be attributed to the "liming effect" of BC alkaline chemical due to the presence of oxides, hydroxides, and carbonates (Beesley et al. 2011). The increase in soil pH causes precipitation of metal (oxy)hydroxides, which reduces, in the current study, Cu mobility (Figs. 1 and 2) (Park et al. 2011; Lucchini et al. 2014).

Copper ion immobilization by organo-metal complex formation with BC components may also play a role in reduced plant toxicity and, thus, increases in shoot and root biomass production in Cu-contaminated soil (Table 1) (Park et al. 2011; Oustriere et al. 2016). This effect is evident from the increased Cu in the organic fraction of soils amended with BCs (Fig. 2). Although we observed substantial reductions in pore water Cu and exchangeable Cu as well as increased Cu in organic and residual pools, the influence on plant Cu concentrations was relatively modest in spite of the growth benefits (Table 2). Indeed, the largest reductions in tissue Cu were noted for the roots of *O. picensis*, a native metalliferous plant. This suggests that, while BCs reduced Cu in pore water and its uptake by this metallophyte, the impacts on Cu accumulation in the agricultural plants may be less pronounced because these species limit Cu uptake in general. Thus, while the reduction in pore water Cu is not strikingly evident as a chemical signature in *L. perenne* or *S. lycopersicum*, the reduction in free Cu in the rhizosphere has significant benefits to the plant. Increases in plant growth (and microbial activity) may also be attributed to BC-derived nutrients (Lu et al. 2015),

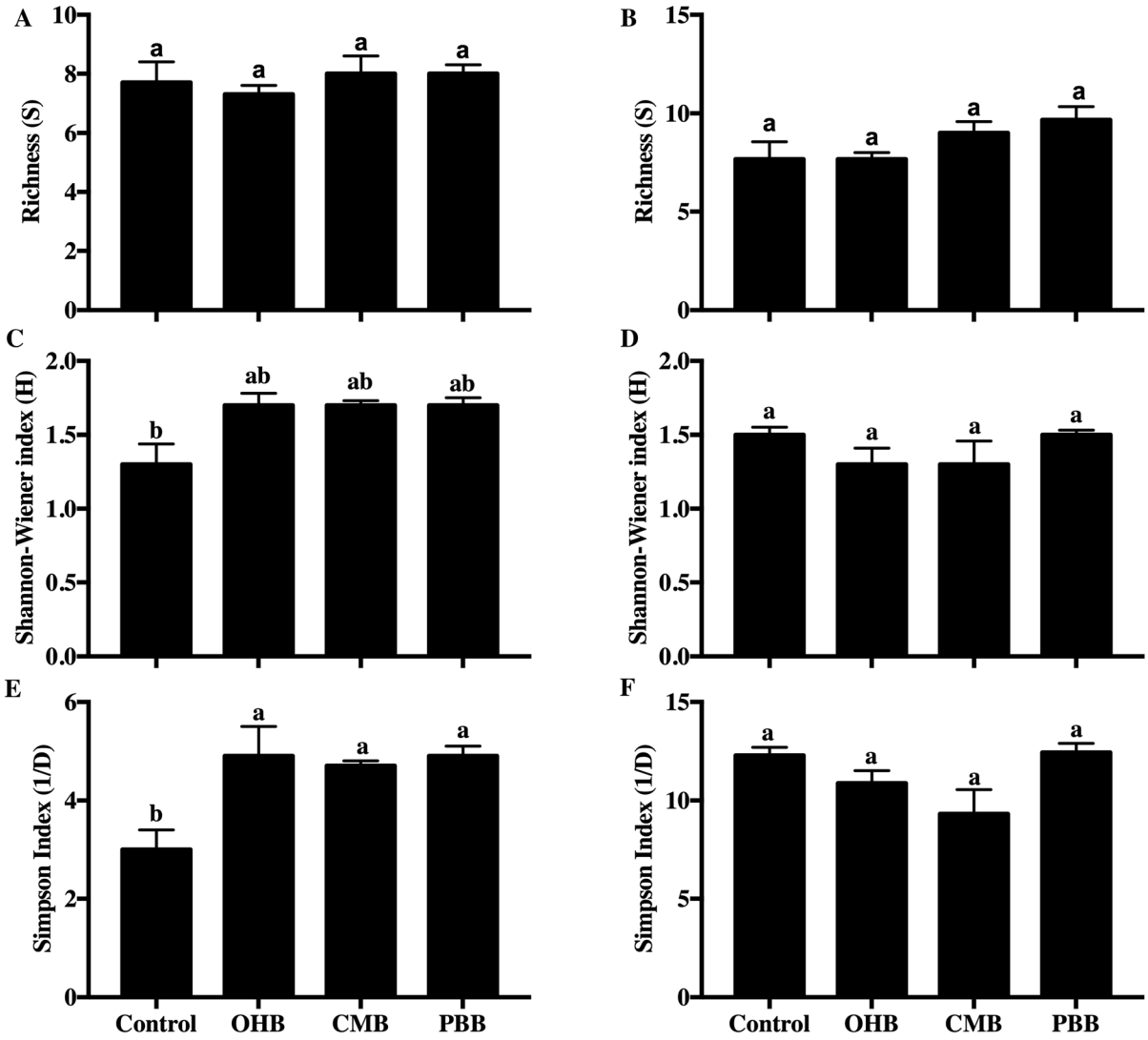


Fig. 6 Genetic richness (**a**, **b**), Shannon–Wiener index (**c**, **d**) and Simpson index (**e**, **f**) of bacterial and fungal communities supported by *Oenothera picensis* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at 0 (control) or 3% (w/w). **a**, **c**, **e** correspond to bacterial communities, whereas **b**, **d**, **e** correspond to fungal communities

which explains the better performance of CMB than OMB and PBB, respectively.

Effect of biochar on soil microbial properties

Soil basal respiration and dehydrogenase activity

Microbial activity plays a central role in the sequestration and processing of C and nutrients in soils. Metal-mediated changes in microbial communities or their activity would be expected to limit nutrient cycling and the capacity of contaminated soils to support plant growth. In the current study, an increase in the basal respiration, reflecting higher microbial activity, was noted for soils amended with BCs (Table 3). This is consistent with previous reports in which BC addition increased the microbial activity and CO_2-C liberation from soils (Jones et al. 2011; Meier et al. 2017a, b; Moore et al. 2017). To further assess the impacts of Cu on microbial activity, we measured the biological oxidation of organic compounds in soil by dehydrogenases, which act as proton

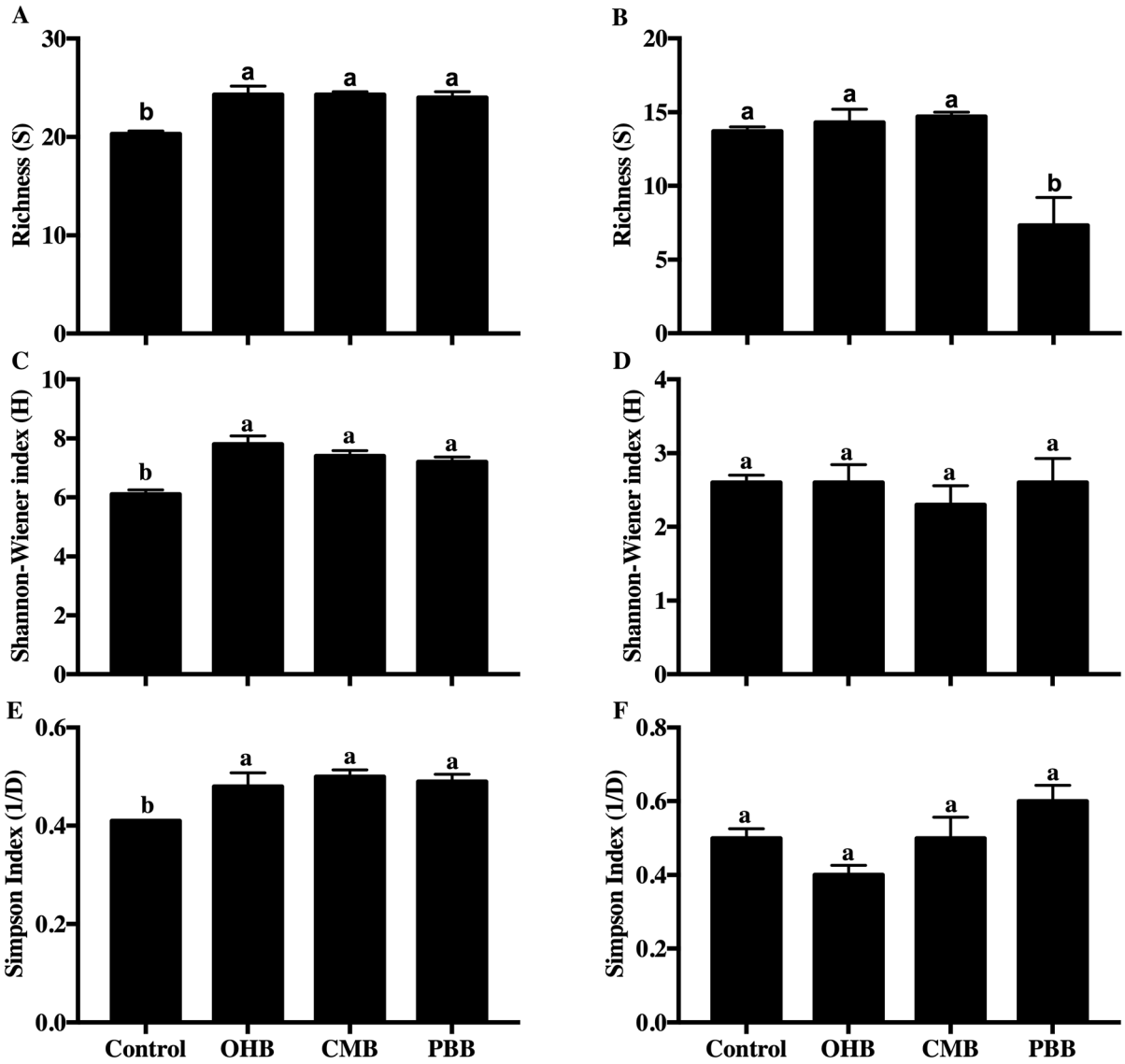


Fig. 7 Genetic richness (**a**, **b**), Shannon–Wiener index (**c**, **d**) and Simpson index (**e**, **f**) of bacterial and fungal communities supported by *Solanum lycopersicum* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at 0 (Control) or 3% (w/w). **a**, **c**, **e** Correspond to bacterial communities, whereas **b**, **d**, **e** correspond to fungal communities

acceptors in C oxidation reactions (Burns et al. 2013), being indicative of the overall microbial activity (Gu et al. 2009; Salazar et al. 2011). The addition of BCs to Cu-contaminated soil increased the DHA activity compared to Control treatments (except in *O. picensis* amended with PBB), and OHB was, in general, more effective than CMB and PBB (Table 3). This is in accordance with Igalavithana et al. (2017), who observed clear patterns of variation of DHA activity in heavy metal-contaminated soils after the application of BCs due to the immobilization of metals. Together, these studies suggest that BCs may stimulate microbial activity by first reducing the availability of free Cu in the soil solution and second by providing available C sources and nutrients to soil microbes. Both of these processes would increase microbial proliferation, thus increasing DHA activity (Lehmann et al. 2011), and also promote further Cu immobilization in microbial biomass (Meier et al. 2017a).

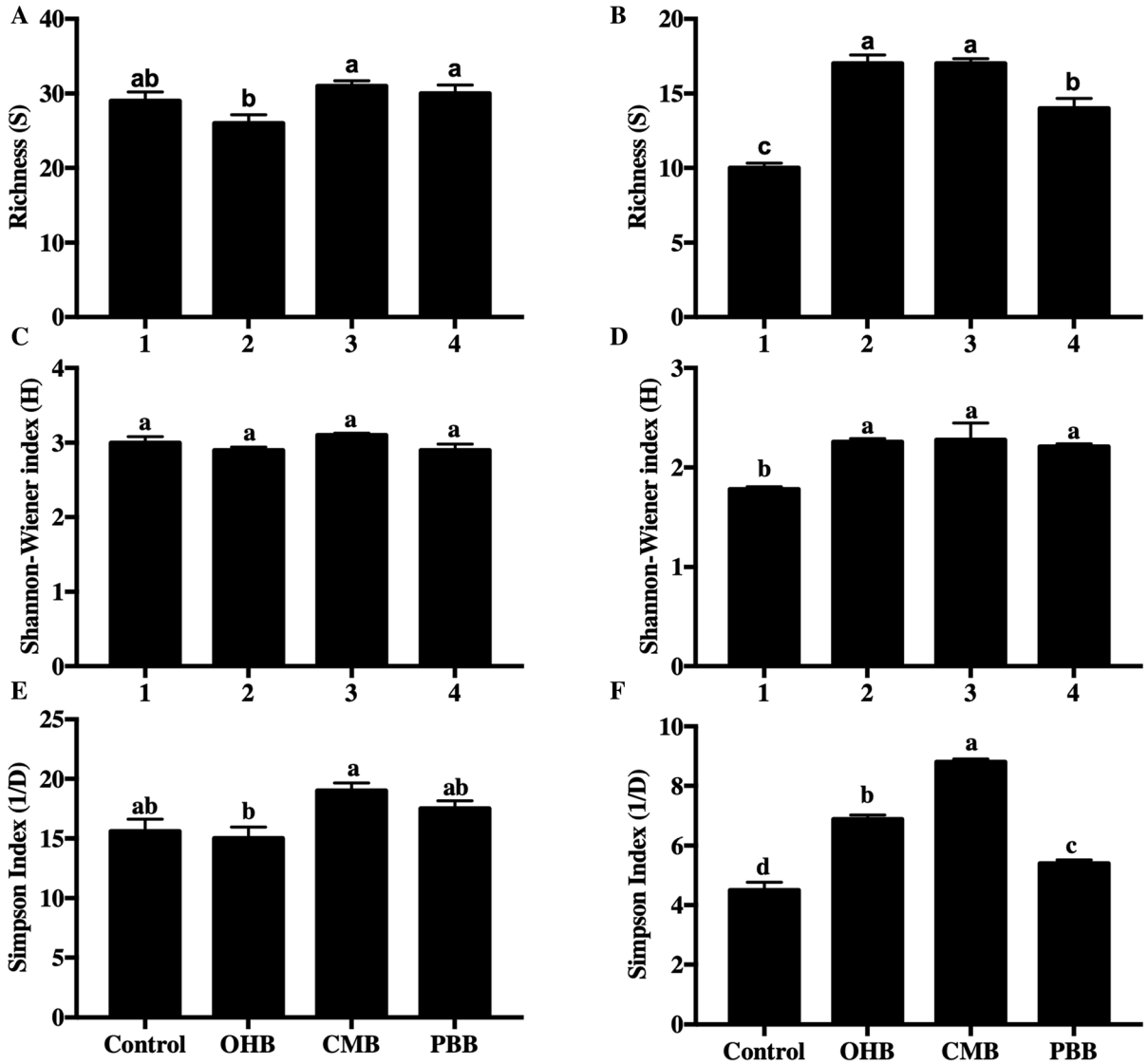


Fig. 8 Genetic richness (**a**, **b**), Shannon–Wiener index (**c**, **d**) and Simpson index (**e**, **f**) of bacterial and fungal communities supported by *Lolium perenne* growing in a Cu-contaminated soil with the addition of chicken manure biochar (CMB), oat hull biochar (OHB), and pine bark-derived biochar (PBB) applied at 0 (Control) or 3% (w/w). **a**, **c**, **e** Correspond to bacterial communities, whereas **b**, **d**, **e** correspond to fungal communities

Soil microbial communities

The structure and function of microbial communities within soils are complex, and the presence and variable abundance of these groups, mainly bacteria and fungi, have a profound effect on soil function and health, as does the application of organic matter and BC (Atkinson et al. 2010). Past studies have reported that the application of BCs to soils affects microbial functions rather than microbial community structure in soils supporting agricultural plants, such as wheat (Rutigliano et al. 2014), and in soils supporting native Cu metallophytes, such as *O. picensis* (Moore et al. 2017). In contrast to these studies, we observed changes in the microbial community structure under the influence of BCs and these changes were functions of the plant species and type of BC used (Figs. 3, 4, and 5). BC additions influenced bacterial and fungal richness and diversity of Cu-contaminated soils in comparison with non-amended soils, and these

changes were more evident using CMB probably due to the higher nutrient content of the feedstock used (Hass et al. 2012: Meier et al. 2017a, b). However, the changes observed were highly variable, which is in accordance with soil heterogeneity in general. Domene et al. (2014) also observed high variability among microbial communities in field plot replicates, which might have prevented significant BC effects from being detected. A similar trend was observed by Moore et al. (2017), who found changes in soil bacterial and fungal populations evaluating *O. picensis* in a 2-year experiment. In contrast to these studies, changes were detected in the agricultural plants in the current work (Figs. 3 and 4). The literature indicates that BC application benefits microbial communities through changes in soil physical and chemical characteristics (Lehmann and Joseph 2009; Atkinson et al. 2010), providing protection to microorganism residing into its porous structure (Pietikäinen and Kiikkilä 2000), supplying C sources and macronutrients (Smith et al. 2010; Thies and Rillig 2009), and/or immobilizing pollutants (Moore et al. 2017). In the current study, the increases in bacterial diversity after BC amendment of soil supporting agricultural plants may be attributed to reductions in Cu in pore water (Fig. 1) and the alkalinity of biochar (liming effect) and to the contribution of some nutrient by the amendment (Fig. 1, Table 1). That these differ among BC sources may contribute to differences in microbial and plant response to BC addition, and such variation should be considered in soil remediation activities.

Conclusions

The present study demonstrated that BCs increased the soil pH, decreased the Cu bioavailability, helped support the growth of both metalliferous and agricultural plants, and promoted the microbial activity and community diversity. However, these BC effects were dependent on the type of biochar used and the plant species grown in the Cu-contaminated soil. BC incorporation into the Cu-contaminated soil reduced the Cu availability by ~ 70%, decreasing Cu in the exchangeable fraction while increasing Cu in organic matter and residual fractions. The physicochemical changes in the soil produced by BC application stimulated the growth of *O. picensis* (up to 68%), *S. lycopersicum* (up to 12.3-fold), and *L. perenne* (up to 36%). These improvements in growth were not associated with reductions in Cu concentrations in plant roots or shoots, indicating a complex dynamic between Cu bioavailability, uptake, and toxicity. Soil microbial activity (respiration and DHA) also increased with BC application, which may be attributed to changes in the diversity and richness of bacterial and fungal communities in BC-amended soil. Through their influences on the soil physicochemical characteristics, benefits to plant growth, and stimulation of the soil microbial community, BC application represents an effective tool that can be used to enhance remediation of Cu-contaminated soils.

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