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Copper immobilization by biochar and microbial community abundance in metal-contaminated soils

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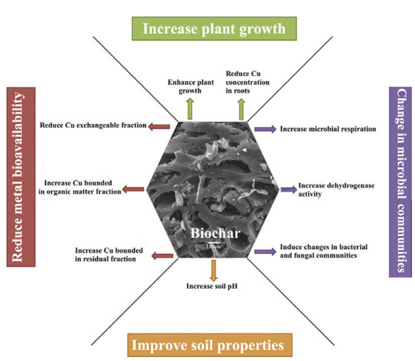
HIGHLIGHTS

- Biochar reduced bioavailable Cu fraction up to 10 times.
- Biochar increased microbial activities in soil.
- Biochar produced changes in fungal and bacterial communities.
- Biochar play a positive role promoting better plant growth.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar (BC) is gaining attention as a soil amendment that can remediate metal polluted soils. The simultaneous effects of BC on copper (Cu) mobility, microbial activities in soil using metallophytes have scarcely been addressed. The objective of this study was to evaluate the effects of biochar BCs on Cu immobilization and over soil microbial communities in a Cu-contaminated soil evaluated over a two-year trial.
A Cu-contaminated soil (338 mg kg^{-1}) was incubated with chicken manure biochar (CMB) or oat hull biochar (OHB) at rates of 1 and 5% w/w. Metallophyte *Oenothera picensis* was grown over one season (six months). The above process was repeated for 3 more consecutive seasons using the same soils.
The BCs increased the soil pH and decreased the Cu exchangeable fraction Cu by 5 and 10 times (for OHB and CMB, respectively) by increasing the Cu bound in organic matter and residual fractions, and its effects were consistent across all seasons evaluated. BCs provided favorable habitat for microorganisms that was evident in increased microbial activity. The DHA activity was increased in all BC treatments, reaching a maximum of 7 and

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Immobilization
Microorganism

6 times higher than control soils in CMB and OHB. Similar results were observed in microbial respiration, which increased 53% in OHB and 61% in CMB with respect to control. The BCs produced changes in microbial communities in all seasons evaluated. The fungal and bacterial richness were increased by CMB and OHB treatments; however, no clear effects were observed in the microbial diversity estimators.
The physiochemical and microbiological effects produced by BC result in an increase of plant biomass production, which was on average 3 times higher than control treatments. However, despite being a metallophyte, *O. picensis* did not uptake Cu efficiently. Root and shoot Cu concentrations decreased or changed insignificantly in most BC treatments.

1. Introduction

Anthropogenic activities such as metal mining, smelting and refining contaminate soils with metals (Choppala et al., 2012). Several methods for the remediation of metal-polluted soils have been developed, including land excavation, soil washing and stabilization to reduce metal bioavailability (Hakeem et al., 2015; Meier et al., 2012; Pilon-Smits, 2005). Nonetheless, effective low-cost practices are needed to solve this problem. Over the past decade, a concept of in situ immobilization of metals in contaminated soils has become popular for its effectiveness at remediation, and plants are emerging as low-cost phytoremediation methods (McGrath and Zhao, 2003). Nevertheless, the success of phytoremediation depends on the ability of plants to produce biomass, which is in fact difficult in a soil with metals at phytotoxic levels (Ginocchio et al., 2004). Therefore, certain soil amendments may be used to remediate metal contaminated soils, thereby making them suitable for plant establishment (Meier et al., 2011). Among those amendments the biochar emerges as an interesting option to promote the phytoremediation of metal polluted soils (Lu et al., 2015; Meier et al., 2017a).

Biochar (BC) is "a porous carbonaceous solid produced by the thermochemical conversion of organic materials in an oxygen depleted atmosphere" (Shackley et al., 2012). There have been attempts to apply BC to remediate metal-contaminated soils (Choppala et al., 2012; Lu et al., 2015; Meier et al., 2017a). A number of studies have demonstrated that BC could sorb metals from soils, such as Cu, thereby reducing metal availability (Igalavithana et al., 2017; Meier et al., 2017a; Park et al., 2011). Recently, the use of biochar has been suggested as an amendment for the in situ stabilization of metals in Cu-contaminated soils (Buss et al., 2012; Meier et al., 2017b; Park et al., 2011). However, most of these experiments did not study the concomitant effects of BC on plant growth, soil microbial activity and communities measured over the long term, which is very important to assess the Cu bioavailability in order to implement phytoremediation programs (Lu et al., 2015).

Microbes (bacteria and fungi) are a ubiquitous and integral part of soils. Microbes play significant roles in the recycling of soil C, N, P, S, and micronutrients, thereby making them available to plants (Lehmann et al., 2011). Heavy metals that are in bioavailable forms adversely affect soil microbes by reducing their populations and changing the community structure and diversity (Carrasco et al., 2006). The adverse impacts result in decreased soil enzyme activity (Karami et al., 2011) and interference in plant-soil-metals associations (Meier et al., 2012). BC can significantly alter the microbial population; this may explain the positive effects of BC on soil enzymatic activity, plant growth, and nutrient cycling (Meier et al., 2017b; Scheer et al., 2011). Therefore, the interactions between BC and soil microbes should be determined to gain a better understanding of agro-ecosystems and to develop effective soil systems. Although there are some studies that show the impact of BC on microbial variations in soil (Buss et al., 2012; Domene et al., 2014; Lu et al., 2015), there are few reports evaluating the concomitant effects of BC on Cu-endemic metallophytes, soil microbial activity and the richness of bacterial and fungi communities measured over the long term.

We proposed a study evaluating the impacts of BCs originating from different feedstocks and produced at different pyrolysis temperatures on Cu mobility, microbial activity, fungal and bacterial communities and diversity and their effects on the growth of the Cu metallophyte *Oenothera picensis*, a metallophyte plant that naturally grows in Cu polluted environments from Central Chile.

2. Materials and methods

2.1. Soil collection and properties

Soil was obtained from the vicinity of the Ventanas Cu smelter, a division of National Copper Corporation of Chile (CODELCO), situated in the Puchuncaví Valley of Central Chile (32°46′30″ S, 71°28′17″ W). The soil is a sedimentary Alfisol (Achreptic haploxeral) from the Chilicauquén soil series and had fine sandy loam texture (clay 13%, sand 65%, silt 22% - González et al., 2008). Some of the soil properties are as follows: pH 5.29, OM 3%, CEC 4.63 cmol (+) kg^{-1}, Cu 338 mg kg^{-1}, N 6 mg kg^{-1}, P 11 mg kg^{-1}, K 21 mg kg^{-1} and Zn 17 mg kg^{-1} (all the nutrients are the available fraction); more information on soil properties is available in Meier et al. (2017a).

2.2. Biochar production and analysis

Chicken manure biochar (CMB) and oat hull biochar (OHB) were produced at 500 and 300 °C, respectively, at the Center of Waste Management and Bioenergy, Universidad de La Frontera, Temuco Chile (Park et al., 2011; Meier et al., 2017b). The pyrolyzer was fed at full load (5 kg) and then purged with nitrogen gas to displace air before starting the process. The temperature during the pyrolysis process was increased at a rate of 3.6 °C min^{-1} until the desired temperature of 500 or 300 °C was reached; the temperature was maintained at this level for 2 h.

The physicochemical properties of CMB and OHB are presented in Table 1. The pH (1:5) of BC was measured electrochemically with a pH meter (Thermo Scientific Orion 3 Star pH Benchtop). Total C and N were measured using a CHNS/O analyzer (Fisons EA 1108 Analyzer). Major and trace available elements and electrical conductivity (EC) were determined according to Sadzawka et al. (2006). Total and carboxylic acidities were determined using the $Ba(OH)_2$ and $Ca(C_2H_3O_2)_2$ methods, respectively, according to Tan (2005). The specific surface area (Brunauer–Emmett–Teller, BET) and pore volume were determined using a Quantachrome NOVA 1000e Analyzer by adsorbing/desorbing nitrogen at 77 K on/from samples previously dried and outgassed at 160 °C for 16 h. The surface charge of the sample was determined by measuring the zeta potential (ζ potential) of colloidal BC according to Tan (2005) using a Zetasizer Nano ZS (Malvern Instruments Ltd.); Smoluchowski's formula was used to convert the electric mobility into the ζ potential.

2.3. Plant growth experiment

The soil was mixed with CMB or OHB (0, 1 and 5% w/w) by hand until homogeneity was achieved. Accordingly, the BC treatments were