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Effects of bamboo biochar on soybean root nodulation in multi-elements contaminated soils

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ABSTRACT

Improvements in plant physiological performance by means of biochar application in soils contaminated by multi-elements are determinants of agroecosystem functioning. This study analyzed the effects of bamboo-derived biochar on root nodulation and plant growth in a moderately acidic Andosol (pH = 5.56) contaminated with multi-elements during a 70-day investigation of soybean growth.

Bamboo biochar that had been pyrolyzed at a temperature below 500 °C was applied to soils at three different and moderately high rates (5%, 10%, and 15%, w/w). Biochar amendment beyond 5% stimulated root nodulation as well as soybean growth. The nodule weight per root system was significantly enhanced by 186% and 243% over the control at the 10% and 15% addition rates, respectively. The primary explanation for these stimulatory effects was attributed to an increase in the K and Mo supplies for plant uptake that was induced by the biochar application, whereas the increased availability of P contributed to a lesser extent. Leaf CO_2 assimilation rate was slightly enhanced at the highest application rate, but this enhancement was not associated with an increase in biomass. The incorporation of biochar into the soil reduced extractable-NH_4NO_3 Cd, Cu, Mn, Ni, and Zn, but not Pb, regardless of the application dose. This change was accompanied by a significant ($P < 0.05$) suppression of the uptake od trace elements in soybean shoots at the optimum application rate (10%); the degree of reduction followed this order: Pb > Mn > Cd > Zn > Cu > Ni. The increase in soil pH and the diffusion/adsorption of trace elements onto the biochar may have contributed to the lowering of the concentration of trace elements in the soil as well as in soybean shoots.

1. Introduction

Anthropogenic activities are the main cause of trace element pollution in the environment, and soil constitutes a major natural sink for trace elements. Humans discharge trace elements from a variety of sources and contaminate large areas of land annually. Trace elements take various physico-chemical forms in soils. Depending on the soil physico-chemical structure as well as the quantity and quality of the trace elements released into the soil environment, extensive remedial approaches have currently been developed for soil remediation. *In situ* stabilization of contaminants using numerous amendments rich in organic matter, such as biosolids, compost, and manures, have been tested, and successful results have been achieved toward reducing the bioavailability of trace elements in soils contaminated with multi-metals (Bolan et al., 2003; Adriano et al., 2004; Walker et al., 2004; Clemente et al., 2006; Ok et al., 2011). In recent years, attention has focused on biochar as a unique soil amendment. Biochar is a recalcitrant form of organic carbon (C) compounds that are produced by the thermal decomposition of biomass under temperatures usually between 300 °C and 1000 °C and under low oxygen concentrations.

Biochar has potential application in various fields including soil conditioning and decontamination (Cao et al., 2009; Karami et al., 2011; Buss et al., 2012; Ippolito et al., 2012; Uchimiya et al., 2012; Lucchini et al., 2014a, 2014b), offsetting of greenhouse gas emissions (Liu et al., 2011; Feng et al., 2012; Singla and Inubushi, 2014; Martin et al., 2015), enhancement of microbial proliferation (Smith et al., 2010; Jones et al., 2011, 2012; Ducey et al., 2013; Bamminger et al., 2014; Prayogo et al., 2014), and plant growth stimulation (Lehmann et al., 2003; Rondon et al., 2007; Steiner et al., 2007; Graber et al., 2010).

Many studies have demonstrated that biochar can significantly immobilize certain trace elements, such as Cd, Cu, Pb, and Zn, in the soil by increasing the soil pH (Beesley et al., 2010; Houben et al., 2013; Lu et al., 2016), creating secondary mineral precipitation (Cao et al., 2011), as well as by adsorbing trace elements onto the microporous structures of biochar (Beesley and Marmiroli, 2011) and onto its active

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organic functional groups (Uchimiya et al., 2011a).

Biochar-induced trace element immobilization in the soil limits the influx of trace elements into the plant roots and shoots, thereby counteracting the retardation of plant growth by trace elements and/or by stimulating growth. Several hypotheses suggest how biochar stimulates plant growth. These include increased nutrient availability due to increased microbial activity, such as that of arbuscular mycorrhizal fungi (AMF; Warnock et al., 2007), increased N retention (Guerena et al., 2013) caused by reduced leaching of NO_3^- due to its sorption onto biochar, microbial immobilization (Zheng et al., 2013), and increased plant uptake (Steiner et al., 2007). In leguminous species, biochar amendment enhanced growth and yield through increased biological nitrogen fixation (BNF; Rondon et al., 2007; Mia et al., 2014) as well as by the enhanced availability of nutrients, such as P (Nishio and Okano, 1991; Rondon et al., 2007; Tagoe et al., 2008), K (Lehmann et al., 2003; Rondon et al., 2007; Mia et al., 2014), and increases in the micronutrients B and Mo (Rondon et al., 2007). While a substantial body of data on the effects of pyrolyzed biochar on BNF and root nodulation exists, insufficient information is available concerning the effects of biochar on root nodule formation in soils contaminated with multi-metals. Here, we assess whether biochar application may have ameliorating effects on the physiological performance of soybean during trace element suppression in a soil contaminated with multi-metals. Soybean, as a leguminous plant, was chosen in order to determine whether the application of biochar in soil contaminated with multi-metals might enhance the nitrogen fixation process by stimulating the formation of root nodules. We hypothesized that biochar from bamboo (as a fast-growing timber crop) would confer positive effects on the abundance of root symbionts, primarily reflected by the formation of root nodules, even in soil contaminated with multi-metals. Notably, we chose to use a fertile soil, which is practically and economically representative of the fertile agricultural areas where the biochar would normally be applied.

2. Materials and methods

2.1. Preparation of soil for the experimental set up

The soil used in the current study was an Andosol that was contaminated with multi-elements including elevated concentrations of Cd, Cu, and Ni (pseudo-total concentrations: 30, 138.5, and 57.6 mg/kg, respectively) all beyond the maximum permissible concentration (MPC) for soil (Crommentuijn et al., 2000). The concentration of the remaining metals including Cr, Mn, and Zn, were below the MPC (65.2, 764.4, and 99.8 mg/kg, respectively). The physico-chemical characteristics of the soil prior to the start of the experiments are shown in Table 1. The soils used in the experiment were sieved through a 5-mm mesh and fertilized with potassium chloride (70 mg/kg), ammonium sulfate (160 mg/kg), and ammonium dihydrogen phosphate (50 mg/kg) solutions and placed in propylene containers. The source of nitrogen (N) applied to the soil samples was therefore limited to the ammonium-containing compounds. The conversion of ammonium to nitrate via the nitrification process will thus maintain a gradual supply of nitrate to the soil system. To correct for trace element readings, 2-g soil samples were used to measure the initial soil moisture content. Biochar produced by the pyrolysis of bamboo wood in steel ring furnaces was mixed thoroughly with the soil at concentrations of 5%, 10%, and 15% (w/w) before potting. The general properties of the biochar used in this study are as follows; Total C content: 83.6%, Volatile matter content (%): 7.4, pH (H_2O; 1:10 w/v): 8.5, EC: 19.1 (mS/m), ash: 5% and bulk density: 0.62 (g/cm^3). We chose 15% as the maximum concentration used for amendment because in our preliminary experiment, 20% was the highest concentration used, at which the results were unsatisfactory. A control soil (1100 g oven-dried weight) as well as biochar-amended bulk soils (1100 g oven-dried weight) were placed in plastic pots (internal diameter and depth, 159 mm; height, 200 mm).

Table 1
Chemical composition of the soil sample.

Properties	Value
pH (H_2O)	5.56
EC (mS m^{-1})	22.5
CEC (cmolc kg^{-1})	29.0
Total C (g kg^{-1})	65.2
Total N (g kg^{-1})	3.0
Texture (loam)	
Sand %	58
Silt %	35.15
Clay %	6.85
Pseudo-total metals (mg kg^{-1})	
Cd	30.0
Cu	138.5
Ni	57.6
Mn	764.4
Zn	99.8
Pb	4.7
Al	20580
Fe	32298
Ca	168.1
Mg	486.4
Na	500.0
K	256.1
B	75.0
Si	19.5
Mo	0.1

Five soybean seeds were planted in each pot, and were watered with distilled water to 62% of the maximum water-holding capacity. After one week of germination, the seedlings were thinned to one vigorous plant. The pot trials were maintained in a greenhouse under natural light conditions for 70 d following thinning of the seedlings; each experiment was conducted in triplicate. The pots were periodically weighed and watered as required throughout the experiment. At harvest, roots were also examined for the number and fresh weight of nodules using dissecting forceps and a hand held magnifier.

2.2. Gas exchange measurements

The CO_2 assimilation rate (An) of soybean leaves was determined 30 d following seed germination using an Li-6400 Portable Photosynthesis Analysis System (Li-COR Inc., Lincoln, NE, USA). The An of each sample was measured under an external CO_2 concentration of 380 μmol/mol supplied from a CO_2 steel gas cylinder and at a light intensity of approximately 1000 μmol/m^{2}s provided by an light-emitting diode red/blue light source. Leaf chlorophyll (Chl) was monitored non-destructively on the same day with a SPAD-502 apparatus (Minolta, CO., Ltd., Osaka, Japan). To account for leaf heterogeneity, SPAD measurements were taken at different locations on the leaves (n = 30).

2.3. Plant and soil analysis

For chemical analysis, dried plant tissues were finely ground using an electric mill. Samples of approximately 0.5 g of plant materials were placed in 75 mL digestion tubes followed by the addition of 1 mL distilled water and 4 mL concentrated H2SO4; then, the digestion tubes were left to stand overnight.

The following day, the tubes were preheated to 60 °C and the temperature was gradually raised to 100 °C for 4 h with the addition of 2 mL H2O2 every 2 h. The digested samples were cooled and adjusted to a volume of 50 mL using distilled water. The filtrates were then stored at 4 °C prior to analysis. Plant height was measured from the soil surface to the tip of the stem. Estimations of the mobile fraction (soluble + exchangeable) of trace elements in the soil were performed using 1 M NH_4NO_3 as the standard (DIN, 1995). The amounts of pseudo-total trace elements and background elements were also determined using