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Effects of biochar on earthworms in arable soil: avoidance test and field trial in boreal loamy sand

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ABSTRACT

Biochar is widely studied as a combined soil conditioner in agriculture and a potential carbon sink. The knowledge of the effects of field application of biochar on soil fauna remains limited. Earthworms are a globally common and important faunal group in arable soils and the purpose of our study was to determine the effects of biochar on earthworms under both laboratory and field conditions in a boreal loamy sand. An avoidance test using the earthworm *Aporrectodea caliginosa* Sav. was conducted for periods of 2 and 14 days with 16 g kg^{-1} spruce chip biochar. The same biochar was mixed into the top 10 cm of soil at 0 or 30 t ha^{-1} and its effect on earthworm density and biomass was studied over four and half months in a field experiment where wheat was grown with or without inorganic fertilizer application. In the avoidance test, biochar application did not affect the habitat choice of earthworms in the first 2 d, but after 14 d, they tended to avoid it. The avoidance was possibly the avoidance was possibly caused by a slight decline in soil water potential. Under field conditions the highest earthworm densities and biomasses were measured in biochar amended soils. None of the differences among the treatments studied were, however, statistically significant ($p > 0.05$). The time scale of the study was sufficient for reliably demonstrating the lack of strong toxic effects and immediate avoidance reactions caused by biochar application

1. Introduction

Biochar use as a soil amendment has been proposed as a greenhouse gas mitigation strategy due to the high stability of carbon (C) in it (Cheng et al., 2008; Lehmann et al., 2008; Woolf et al., 2010). The need for evaluating the suitability of biochar technology has increasingly promoted research in this area. Furthermore, biochar may also benefit soil functions (Glaser et al., 2002; Lehmann et al., 2008; Major et al., 2010; Vaccari et al., 2011) and, depending on its properties and those of the soil, it may increase soil pH (Vaccari et al., 2011), improve plant nutrition through the nutrients it contains (Major et al., 2010; Xu et al., 2013) and reduce nutrient leaching (Brockhoff et al., 2010; Güereña et al., 2012; Major et al., 2012). These effects may contribute to enhanced abundance and activity of soil organisms (Liang et al., 2010; Lehmann et al., 2011; Güereña et al., 2012), increased yields of agricultural crops (Major et al., 2010; Vaccari et al., 2011; Zhang et al., 2012) and improved environmental quality (Lehmann et al., 2011; Major et al., 2012).

The effects of biochar on the soil biological community have received little attention, with the exception of the rather well established increase in microbial biomass under most conditions (Liang et al., 2010; Lehmann et al., 2011; Güereña et al., 2012). In particular, the effects of biochar on soil fauna have so far been only sporadically studied (Lehmann et al., 2011). This is a clear shortcoming considering the high potential of soil animals, particularly earthworms, for ingesting, modifying and transporting biochar in the pedosphere (Topoliantz and Ponge, 2003, 2005; Eckmeier et al., 2007) and subsequently influencing the microbial activity (Lehmann et al., 2011). Earthworms are globally common members of soil communities and have favorable influences on soil physical structure, litter decomposition (Lavelle, 1988; Blouin et al., 2013) and soil nutrient availability for plants (Lavelle et al., 1998; Chaoui

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et al., 2003; Blouin et al., 2013). These functions make earthworms important organisms for assessing the effects of any substances added into soil, including biochar (Yeardley et al., 1996; Hund-Rinke and Wiechering, 2001; Busch et al., 2011; Li et al., 2011).

Favorable effects of biochar on earthworm behavior and activity have been attributed to decreased soil acidity (Topoliantz and Ponge, 2003, 2005; Van Zwieten et al., 2010; Busch et al., 2011) which could, when biochar particles are ingested and the pH in the gut of earthworms increased, assist earthworms in the assimilation of other resources (Weyers and Spokas, 2011). When earthworms ingest biochar particles containing high microbial biomass, it could contribute microbial enzymes to their digestive system (Topoliantz and Ponge, 2003). Negative responses to biochar by earthworms include avoidance and weight loss (Li et al., 2011) and decreased survival of *Eisenia fetida* Sav. (Liesch et al., 2010). These effects have been related to desiccation caused by high water retention of biochar (Li et al., 2011) or, in the case of poultry manure biochar, to the salinity, toxic effects of ammonia or to a rapid increase in soil pH (Liesch et al., 2010).

Previous experiments have not only given conflicting results on the effects of biochar on earthworms, but they have also been laboratory-based (Topoliantz and Ponge, 2003, 2005; Noguera et al., 2010; Van Zwieten et al., 2010; Busch et al., 2011; Li et al., 2011), and on earthworm species not common in agricultural soils (Van Zwieten et al., 2010; Busch et al., 2011; Gomez-Eyles et al., 2011; Li et al., 2011). Only two studies to our knowledge have been conducted under field conditions, neither of them comprised replicated treatments (Husk and Major, 2010; Weyers and Spokas, 2011).

Most field studies exploring the biochar-mediated changes in soil quality and plant growth have been conducted in (sub-) tropical (Major et al., 2010; Vaccari et al., 2011; Zhang et al., 2012) or temperate (Güereña et al., 2012; Jones et al., 2012) climates. Additional research is needed in colder climates, where soils are less affected by low contents of organic matter. For these reasons, this study explored the effects of spruce chip biochar on the earthworm species common in arable soils under both laboratory and boreal field conditions.

2. Materials and methods

2.1. Biochar

The biochar was produced by pyrolising chips of debarked spruce (*Picea abies* (L.) H. Karst.) in a continuously pressurized carboniser (Preseco Oy, Lempäälä, Finland) at 550–600 °C for 10–15 min. The biochar was cooled overnight in an airtight silo and then ground. The particle size distribution was determined by dry sieving. The ash content and the total elemental composition of the biochar were determined by dry ashing after Miller (1998). A 1.5 g sample was dry-ashed in a laboratory muffle furnace (Nabertherm Program Controller C19, Nabertherm, Lilienthal, Germany) by raising the temperature to 500 °C within 2 h and holding it there for 3 h. The ash was further transferred into an Erlenmeyer flask with 100 mL 0.2 M HCl, boiled for 30 min, transferred quantitatively into a 100-ml measurement flask, adjusted to the volume with deionized water, and finally filtered through a quantitative, ashless filter paper (Whatman, Grade 589/3, blue ribbon, pore size 2 μm, GE Healthcare, UK). Dilutions were carried out with 0.2 M HCl, if needed. The elemental concentrations of extracts were analyzed by an inductively coupled plasma optical emission spectroscopy (ICP-OES; Thermo-Fisher iCAP3600 MFC Duo, Thermo Fisher Scientific, Cambridge, UK). The total C and N contents of the biochar were determined by Dumas dry combustion with a VarioMax CN analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The content of hydrogen (H) was determined by combustion with a CHN-1000 elemental analyser (LECO Corp. St. Joseph, MI, USA).

The volatile matter (VM) of the biochar was determined according to ASTM D3175-02 (2002), by heating the biochar in covered crucibles at 910 ± 30 °C for 7 min in the muffle furnace and determining the VM content as the weight lost from the sample. The pH of the biochar was measured in a 1:5 (w/w) suspension of biochar in deionized water with a combination pH electrode. The $CaCO_3$ liming equivalence of the biochar was determined by a method adapted after Rowell (1994). A 2.5 g sample of biochar was treated with 50 mL of 1 M HCl, for 2 h (including mild boiling for 45 min). Thereafter the solutions were cooled and titrated with 0.1 M NaOH.

The content of carbonate–C in the biochar was determined based on the gas chromatographic analysis of the carbon dioxide (CO_2) released by hydrochloric acid (HCl) as follows. First 1 g of biochar was accurately weighed and transferred into a 1205-mL glass bottle, and wetted with 5 mL of deionized water. Next, instantly after adding 20 mL of 1 M HCl into the bottle, the bottle was closed air-tight with a chlorobutyl septum. The bottles were kept for 3 d at 22 °C and gently shaken several times. A gas sample was taken from each bottle through the septum and the CO_2 evolved was determined by a gas chromatographic (HP 5890 Series II, Hewlett Packard, Palo Alto, CA, USA) method described in more detail by Penttilä et al. (2013). The amount of CO_2 evolved was calculated by multiplying the CO_2 concentration difference between samples and blanks with the volume of bottle, and converting it to the carbonate–C content assuming the validity of the ideal gas equation. The method was validated by measurements of known amounts of $CaCO_3$. Organic C (C_{org}) was calculated by subtracting carbonate–C from total C.

The concentration of total polycyclic aromatic hydrocarbons (PAH) was determined according to a protocol combining the methods of Hale et al. (2012) and Hilber et al. (2012). The Soxhlet extractions (0.5 g of biochar, 90 mL of toluene, 6 h, 160 °C) were spiked with 1,1-binaphthyl as an internal standard before extraction. The toluene was reduced to 1 mL and PAH content was analyzed by gas chromatography mass spectroscopy analysis.

The Brunauer–Emmett–Teller specific surface area (BET SSA) was determined by the N_2 adsorption technique with a single point (at 0.30 partial pressure) method using samples ground and sieved to pass 0.063 mm mesh and pre-heated at 300 °C for 30 min before analysis. The BET SSA was measured at −196.15 °C with a Micromeritics Flowsorb 2300 gas adsorption analyser (Micromeritics Co., Norcross, USA).

The scanning electron microscopy (SEM) images of 0.2 mm sieved biochar samples were obtained after scattering the samples onto double-sided tape fixed to an aluminum sample holder and sputter coating with 5 nm of platinum (Quorum Q150TS, Quorum Technologies Ltd., East Grinstead, UK). SEM images were taken with a FEI Quanta 250 Field Emission Gun Scanning Electron Microscope (FEI Co., Philips, Eindhoven, Netherlands) using primary electron beam energy of 10 keV.

2.2. Field experiment

2.2.1. Site and soil

A field experiment was started in May 2011 at the Viikki Research and Experimental Farm, University of Helsinki, Finland (60°13′42″ N 25°2′34″ E). The field had been cropped with wheat (*Triticum aestivum* L. emend Thell.) and barley (*Hordeum vulgare* L.) with conventional moldboard plowing for the preceding six years.

The experimental soil was an Endogleyic Umbrisol (WRB, 2007) with a loamy sand texture (Soil Survey Division Staff, 1993). Particle size analysis of samples taken from the uppermost 30 cm soil layer was conducted by the pipette method (Elonen, 1971). The soil comprised 83% sand, 15% silt and 2% clay. Soil chemical