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Short-term mesofauna responses to soil additions of corn stover biochar and the role of microbial biomass

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ABSTRACT

Biochar additions have been suggested to influence soil microbial communities that, through a cascade effect, may also impact soil fauna. In turn, any direct biochar effects on fauna can influence microbial communities through grazing, physical fragmentation of organic debris (and biochar) and modifying soil structure. If biochar creates a favorable environment for soil microorganisms, it is also plausible for fauna to be attracted to such microbially enriched habitats. However, how soil fauna respond to biochar addition to soil and what are the main factors that drive their behavior has rarely been experimentally addressed. Therefore, the behavior of two mesofauna species was assessed as a result of corn stover biochar (slow pyrolysis at 600 °C) additions to a loamy temperate soil, after preincubation for 2, 17, 31 and 61 d, and related to variations in microbial biomass and activity. Microbial biomass increased by 5–56% and activity by 6–156% with increasing biochar rates for the different preincubation times. Over the incubation time, microbial biomass did not change or increased at most 15% with the different biochar rates, while in turn microbial activity decreased steadily (around 70–80% at day 61). Enchytraeids generally did not show avoidance or preference to biochar when provided with an alternative unamended soil, while collembolans often showed avoidance responses. However, collembolan avoidance to biochar decreased or disappeared in biochar mixtures with higher microbial biomass and water extractable NH_4-N content, agreeing with the plausible role of microorganisms to potentially attract soil fauna after biochar applications. Avoidance response was mainly explained by environmental preferences of the test species and not by any toxic effect of the biochar in this study. However, avoidance after the application of biochar may still need to be considered due to the potential negative impacts of individuals' migration on soil ecosystem functioning.

1. Introduction

The addition of some biochars has been found to stimulate microbial abundance and activity (Pietikäinen et al., 2000; Steiner et al., 2004; Birk et al., 2009), which could then potentially influence nutrient cycles and crop productivity (Güereña et al., 2013). However, the literature about potential direct and indirect effects on soil fauna other than earthworms is scarce. This is surprising due to the key role of soil biota in some of the reported beneficial effects of some biochars on soil fertility (Lehmann et al., 2011) as well as the suspected effect of fauna on biochar persistence in soil, e.g., the reported capacity of earthworms to ingest and grind biochar particles, and to excrete biochar complexed with minerals (Topoliantz and Ponge, 2005; Ponge and Topoliantz, 2006).

Some positive effects on biota activity have been described in char-rich soil layers in burnt areas, observed as abundant fungal hyphae, as well as fresh and reprocessed fauna fecal pellets (Bunting and Lundberg, 1987; Phillips et al., 2000). Positive effects have also been predicted for bacterivore soil fauna gro ups in acid soils after the pH increase associated with biochar applications that favor bacteria (McCormack et al., 2013). Data from loamy temperate soils cropped to corn confirm enhanced faunal activity three years after the addition of increasing rates of biochar (3–30 Mg ha^{-1}), but only in combination with certain soil

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properties (Domene et al., 2014). Zhang et al. (2013) reported no variation in total nematode abundance after a wheat straw biochar addition of 2.4 Mg ha^{-1}, though higher diversity with biochar addition was observed as well as an increased abundance of fungivores. However, negative effects have also been occasionally observed, such as decreased faunal activity in an alkaline soil cropped to barley and amended at high addition rates (50 Mg ha^{-1}) with a gasification pine wood biochar (Marks, 2013).

The mechanisms underlying any of these effects have not been clearly demonstrated yet, although positive effects could at least partly be due to trophic effects resulting from biochar effects on soil microbial communities that certain fauna rely on. If microbial abundance is affected by biochar application, a cascade effect is expected on all soil fauna directly or indirectly relying on them (McCormack et al., 2013). In turn, effects on fauna could influence microorganisms themselves, due to the fauna regulation of organic matter decomposition by microbial grazing, but also by indirect effects resulting from litter fragmentation and soil structure modification carried out by some faunal groups (Bardgett, 2005).

Several explanations have been linked to the increased microbial abundance after biochar addition. Some authors have pointed out the refuge for microorganisms provided by biochar porosity (Lehmann et al., 2011; Ennis et al., 2012), while others have also suggested the increased nutrient and carbon availability and water retention around biochar, the sorption of noxious chemicals, and the increased pH in the specific case of acid soils (Lehmann et al., 2011). On the other hand, Pietikäinen et al. (2000) proposed the reduction of microbial cell leaching by direct retention in biochar as explanation.

Scarce evidence exists about how fauna interact with biochar particles or soils to which biochar was added. Data are mostly obtained with earthworms in avoidance tests, by introducing individuals in a vessel containing soil and a soil-biochar mixture and assessing their distribution after a period of time. Avoidance tests with soil fauna are based on the ability of organisms to escape from unsuitable environments due to pollution (Amorim et al., 2005; Loureiro et al., 2005; Lukkari and Haimi, 2005; Natal-da-Luz et al., 2008a) or due to unsuitable environmental conditions outside their ecological preferences (Natal-da-Luz et al., 2008b; Chelinho et al., 2011; Domene et al., 2011). Avoidance tests are based on the chemoreception capacity of most soil animals and have a high ecological relevance (Natal-da-Luz et al., 2009) since avoidance responses under field conditions are equivalent to mortality in terms of ecosystem composition and function. This is why earthworm avoidance tests, together with plant germination tests, have been proposed as quick screening tests for the ecotoxicological characterization of biochars before their use in the field (Major, 2009). Conversely, avoidance test methods, initially designed for pollutant testing, allow the detection of preference behavior for practices enhancing the soil function as habitat such as the addition of biochars.

The type of feedstock, the pyrolysis procedure used, and the rate of addition are the most plausible factors that could explain faunal responses to biochar, but also the type of soil and changes in soil properties caused by biochar addition. As an example, Van Zwieten et al. (2010), comparing the response of earthworms (*Eisenia andrei*) in soil mixtures of two slow pyrolysis papermill waste biochars in an acid and an alkaline soil (10 Mg ha^{-1}, equivalent to a 2 and 1.5% addition, respectively), demonstrated preference for soil-biochar mixtures in the acid soil but not in the basic soil. This behavior was associated with a $CaCl_2$ pH increase (from 4.2 to 5.1–5.9) and higher microbial activity after the addition of biochar to the acid soil, and not observed in the basic soil. Preference response was assumed to be the result of a more suitable environment for this species and pointed out the importance of the type of soil for evaluating the impact of biochar addition on soil biota. Excessive liming has also been linked to toxic effects of e.g., a poultry biochar (Liesch et al., 2010), which might have been detected with avoidance responses. Li et al. (2011) reported that the avoidance by the earthworm *Eisenia fetida* for an apple wood sawdust biochar was entirely explained by water content in soil-biochar mixtures, since avoidance disappeared when moisture was adjusted to field capacity.

The appeal of soil fauna for biochar has been inferred from field observations in biochar-enriched soils after wildfires (Topoliantz and Ponge, 2005; Ponge and Topoliantz, 2006), and plausibly explained by microbial abundance, although this has been rarely addressed experimentally in the available literature, with a few studies based only on earthworm species. A variety of responses have been reported in them, ranging from preference (Van Zwieten et al., 2010; Busch et al., 2012; Hale et al., 2013; Chan et al., 2008), to no effect or avoidance (Chan et al., 2008; Liesch et al., 2010; Li et al., 2011; Van Zwieten et al., 2010; Tammeorg et al., 2014), although these studies have not checked or had failed to empirically find a correlation with microbial abundance.

The main aim of this study was to assess the main drivers for the behavior of two soil mesofauna species exposed to soil-biochar mixtures, which may be representative for the potential short-term responses of soil fauna under field conditions. We hypothesized that a relationship of such a response exists with microbial biomass and activity.

2. Methods

2.1. Mesofauna species, soil and biochar

The test species of this study were considered to be representative of soil mesofauna due to their contrasting feeding and life habits and exposure routes. *Folsomia candida* is a predominantly fungivorous species living in soil pores (Fountain and Hopkin, 2005), while *Enchytraeus crypticus* lives in close contact with soil pore water (Römbke, 2003) as most enchytraeids do and mostly feeds on bacteria and plant debris (Didden and Römbke, 2001).

An agricultural soil cropped to corn was collected at the Cornell Musgrave Research Farm (Aurora, New York, USA) in early spring 2008. Soil had a 42% sand, 31% silt and 27% clay, total C content of 16.2 g kg^{-1}, total N of 1.6 g kg^{-1}, and a 1 N KCl pH around 7.3 (see Rajkovich et al., 2012 more details). Soil was collected after snowmelt and before any fertilizer or pesticide was applied, and then air-dried, homogenized, and sieved to 5 mm. Soil was defaunated by long-term storage (two years), and by carrying out two freezing–thawing cycles (24 h at −20 °C, 24 h at 20 °C) before the beginning of the experiment. The corn stover biochar in this study was produced by slow pyrolysis (30 min, 600 °C) at BEST Energies Inc. (Somersby, Australia). Biochar had a high alkalinity (KCl pH = 10) and intermediate volatile matter content (26%) (see Güereña et al., 2013 for this and additional details on biochar composition).

2.2. Avoidance test setup

Avoidance tests in both species were carried out in accordance with ISO (2011), a test initially designed for *F. candida*. The only modification for collembolans was that individuals were aged 24–32 days instead of the 10–12 days proposed in the test to maximize their recovery at the end of the test. *E. crypticus* was continuously cultured so the availability of clitellated adults was ensured during the experiment.

Soil-biochar mixtures (0, 0.2, 0.5, 2, 7 and 14%, w/w) were prepared and moistened to 50% of the maximum water holding capacity. Moistening was carried out with deionised water containing 5% (v/v) of an inoculant solution to reintroduce the