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Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil

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ABSTRACT

Biochar amendment in soil has been proposed as a carbon sequestration strategy which may also enhance soil physical and chemical properties such as nutrient and water holding capacity as well as soil fertility and plant productivity. However, biochar may also stimulate microbial activity which may lead to increased soil CO_2 respiration and accelerated soil organic matter (OM) degradation which could partially negate these intended benefits. To investigate short-term soil microbial responses to biochar addition, we conducted a 24 week laboratory incubation study. Biochar produced from the pyrolysis of sugar maple wood at 500 °C was amended at concentrations of 5, 10 and 20 t/ha in a phosphorus-limited forest soil which is under investigation as a site for biochar amendment. The cumulative soil CO_2 respired was higher for biochar-amended samples relative to controls. At 10 and 20 t/ha biochar application rates, the concentration of phospholipid fatty acids (PLFAs) specific to Gram-positive and Gram-negative bacteria as well as actinomycetes were lower than controls for the first 16 weeks, then increased between weeks 16–24, suggesting a gradual microbial adaptation to altered soil conditions. Increases in the ratio of bacteria/fungi and lower ratios of Gram-negative/Gram-positive bacteria suggest a microbial community shift in favour of Gram-positive bacteria. In addition, decreasing ratios of cy17:0/16:1ω7 PLFAs, a proxy used to examine bacterial substrate limitation, suggest that bacteria adapted to the new conditions in biochar-amended soil over time. Concentrations of water-extractable organic matter (WEOM) increased in all samples after 24 weeks and were higher than controls for two of the biochar application rates. Solution-state ^{1}H NMR analysis of WEOM revealed an increase in microbial-derived short-chain carboxylic acids, lower concentrations of labile carbohydrate and peptide components of soil OM and potential accumulation of more recalcitrant polymethylene carbon during the incubation. Our results collectively suggest that biochar amendment increases the activity of specific microorganisms in soil, leading to increased CO_2 fluxes and degradation of labile soil OM constituents.

1. Introduction

Biochar is the solid product resulting from the pyrolysis of organic material which has been proposed as a soil amendment to sequester carbon, enhance plant growth and ameliorate soil properties (Lehmann et al., 2011). Biochars generally have a high abundance of condensed aromatic structures and are expected to decompose more slowly in soil than their more labile precursor feedstocks, thus resulting in slower carbon turnover in the environment (Lehmann, 2007). Despite its chemically recalcitrant structure, previous studies suggest that the addition of biochar to soil may stimulate the activity of soil microorganisms such as bacteria and fungi on short timescales (Steinbeiss et al., 2009; Santos et al., 2012; Ameloot et al., 2013; Bamminger et al., 2014; Watzinger et al., 2014). This microbial stimulation may occur because of the potential utilization of biochar components as an energy source by microorganisms (Gomez et al., 2014; Watzinger et al., 2014), biochar serving as a habitat that affords protection to microorganisms from predators (Pietikäinen et al., 2000; Quilliam et al., 2013) as well as favourable improvements to soil properties such as water holding capacity, nutrient availability and pH buffering (Karhu et al., 2011; Wang et al., 2012; Basso et al., 2013; Ameloot et al., in press; Maestrini et al., 2014). However, enhanced microbial activity may have unintended consequences such as accelerated decomposition (priming) of soil organic matter (OM) and increased soil CO_2 emissions (Kuzyakov et al., 2000)

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which could partially negate the intended carbon sequestration benefits of biochar amendment in soil. As such, it is crucial to understand how soil microorganisms respond to the presence of biochar in soil before proceeding with large-scale application.

Biochar amendment has been studied extensively in agricultural systems (Kimetu and Lehmann, 2010; Cross and Sohi, 2011; Ameloot et al., 2013; Farrell et al., 2013; Domene et al., 2014; Gomez et al., 2014; Prayogo et al., 2014; Riedel et al., 2014; Watzinger et al., 2014) due to the potential benefits of biochar to plant productivity and crop yields whereas the application of biochar to forest ecosystems has received less attention (Steinbeiss et al., 2009; Khodadad et al., 2011; Santos et al., 2012; Maestrini et al., 2014; Ouyang et al., 2014). However, biochar amendment in forest soils warrants investigation as similar improvements to soil conditions may help increase forest productivity, especially if the soil is deficient in nutrients. For example, the Haliburton Forest and Wild Life Reserve Ltd. of southeastern Ontario is a privately managed, mixed hardwood temperate forest in which primary productivity is believed to be hindered by phosphorus-limited soil conditions resulting from high nitrogen deposition in this region (Gradowski and Thomas, 2006). This forest is currently under investigation as a potential site for biochar amendment as biochar may supplement soil phosphorus concentrations depending on the phosphorus content of the biochar feedstock (Wang et al., 2012). In addition, the alkaline nature of biochar may enhance the solubility of phosphate which may be bound to soil minerals such as iron and aluminium oxides at the acidic pH values that are often observed for forest soils (Cui et al., 2011). Moreover, the addition of biochar to soil has the potential to sequester carbon in a stable aromatic form to offset increasing atmospheric CO_2 emissions (Lehmann, 2007). However, it is important to investigate whether biochar addition substantially alters the soil microbial community and OM composition to better evaluate the feasibility of field-scale biochar application efforts.

The analysis of phospholipid fatty acids (PLFAs) is an established technique for monitoring rapid changes in living soil microbial populations in response to environmental change and provides information about the abundance of several classes of microorganisms such as Gram-positive and Gram-negative bacteria, actinomycetes and fungi (Zelles, 1999). In addition, ratios of PLFA concentrations can be used to examine microbial community shifts and may indicate whether specific microorganisms are favoured as soil conditions change (Guckert et al., 1986; Bossio and Scow, 1998; Feng and Simpson, 2009). Several studies have applied PLFA analysis to monitor changes in soil microbial activity following biochar application, including the incorporation of biochar into microbial PLFAs (Steinbeiss et al., 2009; Santos et al., 2012; Farrell et al., 2013; Ameloot et al., in press; Gomez et al., 2014; Prayogo et al., 2014; Watzinger et al., 2014). These studies reported increased activity of different types of microorganisms such as Gram-positive bacteria (Ameloot et al., 2013), Gram-negative bacteria (Ameloot et al., 2013; Gomez et al., 2014; Prayogo et al., 2014; Watzinger et al., 2014), actinomycetes (Prayogo et al., 2014; Watzinger et al., 2014) and fungi (Steinbeiss et al., 2009) after the addition of biochar to different soil types that vary in mineralogy and OM content. As such, PLFA extraction and quantification provides a reliable method for analyzing the active microbial contribution to soil OM. Solution-state ^{1}H nuclear magnetic resonance (NMR) spectroscopy is an emerging technique for the analysis of soil OM and can provide detailed structural information about water-soluble soil OM components, including microbial-derived compounds such as peptides, amino acids and carbohydrates (Feng and Simpson, 2011). This information can be used in conjunction with PLFA extractions to further examine soil microbial responses to changing environmental conditions. However, to date, solution-state NMR techniques have not been used to study compositional changes in soil OM following biochar amendment.

We conducted a laboratory soil incubation study in which sugar maple wood biochar was amended at three concentrations (5, 10 and 20 t/ha) in a forest soil for 24 weeks. We used soil PLFA extractions, CO_2 respiration measurements and solution-state ^{1}H NMR analysis of water-extractable organic matter (WEOM) to monitor changes in microbial activity throughout the incubation. We hypothesize that biochar amendment will alter soil conditions and stimulate microbial activity, resulting in higher concentrations of PLFAs, higher soil CO_2 respiration and increased microbial solution-state ^{1}H NMR signatures in WEOM. We also hypothesize that these increases will occur to a greater extent at higher biochar concentrations.

2. Materials and methods

2.1. Description of soil and sampling site

Soil was sampled at a site in the Haliburton Forest and Wild Life Reserve Ltd. of southeastern Ontario, Canada (45.29° N, 78.64° W) in June 2013. The forest contains a mixture of hardwood trees and is dominated by sugar maple (*Acer saccharum* Marshall) with contributions from red maple (*Acer rubrum* L.), eastern hemlock (*Tsuga canadensis* L. Carrière), yellow birch (*Betula alleghaniensis* Britton) and American beech (*Fagus grandifolia* Ehrh.). The mean annual precipitation and temperature of the sampling site are 1074 mm and 5 °C, respectively (Environment Canada, 2014; Sackett et al., in press).

The forest floor litter consisting primarily of decomposing leaves was carefully scraped away using a hand spade prior to sampling of the mineral soil. Mineral soil samples (0–20 cm) were collected using a shovel. The soil is classified as a Brunisol under the Canadian System of Soil Classification, an Inceptisol under the USDA Soil Taxonomy system and a Cambisol under the FAO World Reference Base soil classification system (Soil Classification Working Group, 1998). The soil is derived from granite bedrock of the pre-Cambrian Canadian Shield and has a sandy to sandy loam texture (Sackett et al., in press). This soil type is not highly weathered and did not have any visible horizon development i.e., no major changes in the colour of the soil with depth to 20 cm and no obvious organic-mineral horizon transition. As such, the entire 0–20 cm of soil was homogenized to form a composite soil which was used for the incubation study. The soil was passed through a 2 mm sieve after collection to remove rocks and residual plant material.

Soil bulk density was measured by collecting three replicate soil cores from within 1 m of the soil sampling site. The volume of the soil samples was equated to the volume of the corer. The soils were placed in pre-weighed, sealed plastic bags and were weighed by difference immediately after returning to the laboratory, approximately 3 h after soil collection. The density was calculated by dividing the mass of soil by the volume, and the average of the three measurements was taken to be the bulk soil density, which was found to be 1.04 ± 0.10 g/cm^3.

2.2. Biochar feedstock and production conditions

Sugar maple wood was selected as the biochar feedstock due to its prevalence in the Haliburton Forest (Gradowski and Thomas, 2006). To prepare biochar, approximately 25 g of dry, untreated sugar maple wood chips (Montana Grilling Gear, Brantford, ON, Canada) were placed inside capped stainless steel mesh canisters and loaded into an insulated tube furnace (model GSL-1100X, MTI Corporation, Richmond, CA, USA) under a continuous flow of nitrogen gas (0.01 MPa). The furnace temperature was ramped from