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Microbial community structure and predicted bacterial metabolic functions in biochar pellets aged in soil after 34 months

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ABSTRACT

Biochar has been shown to induce changes in soil microbial communities, which is initially attributed to the labile organic carbons stimulating degraders over short time periods. However, little is currently understood about microbial communities as well as their metabolic traits on biochar pellets (BC) aged in soil for prolonged periods of time. It was hypothesized that microbial communities affected by biochar may result in changes in the metabolic traits. The activities of bacteria and fungi were compared by measuring their biomarkers including muramic acid (MurN) and glucosamine (GluN). The structure of soil microbial communities on BC aged in soil after 34 months was evaluated using 454 pyrosequencing. The metabolic functions of the bacterial community were predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). Pyrolysates in BC were analyzed using pyrolysis-gas chromatography/mass spectroscopy. The statistically significant decrease of GluN/MurN in BC in comparison with the adjacent soil (ADJ) and the control indicates higher accumulated bacterial activity than that of fungi. The structure of bacterial communities greatly shifted in biochar pellets in comparison with their fungal counterparts. *Proteobacteria* and *Actinobacteria* of bacterial community were more abundant in BC than in ADJ and control soils. At class level there were greater bacterial OTUs in BC than ADJ and control soils. The changes of pyrolysates in BC may indicate the deconstruction of biochar. The PICRUSt indicated the bacterial community in BC shifted from preferring metabolizing carbohydrates to xenobiotics. This was supported by the high concentration of water soluble carbon present in BC. The current study suggests that biochar may preserve soil organic matter through mediating bacterial metabolic capacities in carbon sources.

1. Introduction

Biochar materials are often produced by intentional, low-temperature pyrolysis (<700 °C) of organic matter in zero or low oxygen environments (Lehmann, 2007). Due to the diverse properties of raw materials and varying pyrolysis highest temperatures and the retention time, biochar products have highly variable characteristics such as pH, cation exchange capacity (CEC), content of inorganic nutrients and porous structure. As a result, the amendment of biochar into soil caused inconsistent effects on the physico-chemical proprieties of soil, soil microbial activities and plant growth (Biederman and Harpole, 2013).

Abiotic degradation was reported to occur at the surface of biochar particles (Moreno et al., 2000), but biotic effects, in particular soil microbial activities, are pivotal to shaping biochar surface interactions once introduced into soils (Lehmann et al., 2011). Biochar encompasses a variety of carbon moieties ranging from insufficiently charred organic matter, char and graphite to soot (Masiello, 2004), or their mixtures. These organic carbons can be categorized into labile organic carbons (LOCs) (e.g., low molecular weight aliphatics and aromatic compounds) (Spokas et al., 2011; Hale et al., 2012), intermediate labile organic carbon (ILOCs) (e.g., high molecular weight aliphatics and aromatic compounds) (McBeath et al., 2011; Keiluweit et al., 2010), and inert organic carbon (IOCs) (e.g., graphite) (Bernal, 1924). Each component may have different recalcitrant capacity or carbon sequestration potential when subjected to microbial activities. In natural ecosystems, the organic substances in biochar may have different heat tolerances during pyrolysis (Vancampenhout et al.,

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2009). In fact, a range of volatile organic carbons in fresh biochar have been detected by using pyrolysis-gas chromatography/mass spectroscopy (Py-GC–MS) (Spokas et al., 2011).

A growing body of evidence suggests that biochar induces changes in the structure of soil microbial communities (Chen et al., 2013; Rutigliano et al., 2014; Sun et al., 2013) over short time periods. This was suggested to correlate with the LOCs in fresh biochar (Sun et al., 2015). However, the long term effect of biochar on soil microbial communities should not be solely attributed to the LOCs in biochar provided their short life span (O'Neill et al., 2009; Kim et al., 2007). A further assumption is that the ILOCs in biochar may provide available organic carbon sources for microorganisms when subjected to soil aging. However, the response of microbial communities on biochar pellets after prolonged aging in soil has not been well addressed.

The present study was conducted to examine the soil microbiome on biochar pellets as compared to the adjacent soil and non-biochar amended soil after 34 months of soil aging using 454 pyrosequencing. Muramic acid (MurN) and glucosamine (GluN), reflecting the survival history of bacteria and fungi were measured to compare their activities during biochar aging. It was hypothesized that microbial communities affected by biochar may result in changes in the metabolic traits. Functions of bacterial communities were further predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013). PICRUSt is a bioinformatics software package designed to predict metagenome functional content and in turn ecosystem functionality, from reference full genome and marker genes such as 16S rRNA. To verify the PICRUSt results related to metabolism of carbohydrates and xenobiotics, water-soluble carbon contents in biochar pellets, adjacent soil and non-biochar amended soil were also determined.

2. Materials and methods

2.1. Setup of pot microcosms and sampling procedure

Biochar pellets (diameter 2–3 mm, height 3–5 mm) were generated using corncob biochar added with 5% clay as an adhesion agent (Fig. S1). The characteristics of biochar pellets were total C 40.1%, total N 10 ($g\,kg^{-1}$), total P 0.3 ($g\,kg^{-1}$), total K 43.2 ($g\,kg^{-1}$) and pH 7.7. Biochar pellets were blended with soil at a rate of 5% (w/w, excluding water and clay) (Sun et al., 2013). Ten kilograms of soil or soil amended with biochar pellets was transferred into 12.5 L pots, and 3 replicates were employed for biochar amended soil and non-biochar amended soil. Non-biochar amended soil was used as a control. Control and biochar-amended soils were subjected to soybean cultivation for 4 months, no tillage for 9 months, soybean cultivation for 4 months and then no tillage for 17 months. After 17 months, 27 months and 34 months, biochar pellets were collected, air dried and well mixed prior to analysis using Py-GC–MS. Whole soil genome DNA and microbial biomarkers extractions and water-soluble carbon analysis were conducted using non-biochar amendment soil (control), biochar pellets (BC) and adjacent soil (ADJ) after 34 months. When water content was over 60%, BC and ADJ soil were separated using sterile tweezers, and samples were stored at −80 °C prior to analysis. Triplicates were conducted for each assay.

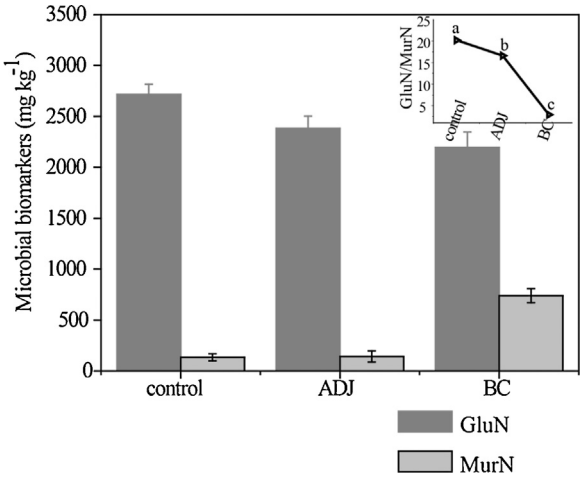

Fig. 1. Glucosamine (GluN) and muramic acid (MurN) concentrations and their ratio in non-biochar amendment (control), adjacent soil (ADJ) and biochar pellet (BC). Statistically significant differences by Tukey's *t*-test ($P < 0.05$) are denoted by different lowercase letters.

Table 1
Estimates of Chao1, ACE and Shannon index in non-biochar amendment (control), adjacent soil (ADJ) and biochar pellet (BC).

Treatment	16S (V1-V3)			18S (ITS)		
	Chao1	ACE	Shannon	Chao1	ACE	Shannon
Control	2680 b	3339 c	6.6 a	381 b	364 b	3.9 a
ADJ	3330 a	3994 b	6.4 a	416 a	409 a	4.1 a
BC	3482 a	4216 a	6.5 a	354 c	356 b	3.7 a

Statistically significant differences by Tukey's *t*-test ($P < 0.05$) are denoted by different lowercase letters

2.2. Determination of amino sugars

Amino sugars were determined according to Zhang and Amelung (1996). In brief, sample corresponding to a about 4 mg organic carbon was combined with 100 μg myo-inositol as an internal standard, and hydrolyzed with 10 mL of 6 M HCl and incubated at 105 °C for 8 h. The released amino sugars were separated from impurities by neutralization with 0.4 M KOH. Prior to derivatization, 100 mg of methylglucamine was added as a recovery standard. Aldononitrile derivatives of the amino sugars were prepared by heating the samples in 0.3 mL of a derivatization reagent consisting of (32 mg hydroxylamine hydrochloride mL^{-1} and 40 mg 4-(dimethylamino) pyridine mL^{-1} in pyridine–methanol 4:1(v/v)) at 75 °C for 30 min. After acetylation with 1 mL of acetic anhydride at 75–80 °C for 20 min, dichloromethane was added, and excess derivatization reagents were removed by washing with 1 mL of 1 mol L^{-1} HCl and 1 mL of water two times each. The remaining organic phase was dried under an air stream at 45 °C and dissolved in 0.3 mL ethyl acetate-hexane (1:1, v/v). The amino sugar derivatives were separated on a HP 6890 GC equipped with a HP-5 fused silica column (30 × 0.25 mm ID with 0.33 mm film) and a flame ionization detector. Amino sugars were quantified using inositol as the internal standard and methylglucamine as the recovery standard.

2.3. DNA extraction, PCR amplification and pyrosequencing

Genomic DNA in samples was extracted using a Fast DNA® Spin Kit (Omega Biotek, America) according to the manufacturer's instructions. PCR was performed using 454 sequencing adaptor linked primers flanking the hyper variable V1–V3 region of the 16S rDNA gene: F (5′-AGAGTTTGATCCTGGCTCAG-3′) and R (5′-TTACCGCGGCTGCTGGCAC-3′). The PCR thermal regime consisted of an initial denaturation of 4 min at 94 °C, followed by 27 cycles of 30 s at 94 °C, 45 s at 55 °C, 1 min at 72 °C and a final cycle of 7 min at 72 °C. PCR was also conducted using 454 sequencing adaptor linked primers for ITS region of the 18 S rDNA gene: F (5′-