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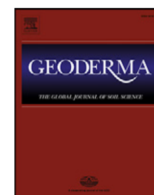

Changes in microbial community structure due to biochars generated from different feedstocks and their relationships with soil chemical properties

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Biochars produced from swine manure (SM), fruit peels (FP), *Phragmites australis* (PA) and *Brassica rapa* (BR) were applied at different rates to a sandy loam soil at 70% moisture. Phospholipid fatty acid (PLFA) measurements showed that feedstock type, biochar type and application rate significantly affected the soil microbial communities. PLFAs derived from bacteria, fungi, actinomycetes, G + ve and G − ve bacteria and sulfate reducers were higher with FP biochar at 3% and 1% weight: weight (wt:wt), respectively, followed by SM at 1% and PA biochar at 3%, than in the control soil. The control soil also contained higher concentrations of certain iso:anteiso PLFAs, which are indicative of environmental stress, than did biochar treated soils. Protozoa PLFAs only increased in PA 3% and BR 1% treatments. Redundancy analysis illustrated the relationships between microbial communities and chemical properties within biochar types and addition rates to soil. The analysis indicated that different biochars induced different chemical changes such as increased pH, dissolved organic carbon and total carbon and nitrogen in soil and changed the microbial community structure. These properties may be used as indicators of both soil improvement and C sequestration.

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1. Introduction

Increases in carbon fluxes between soils, plants and the atmosphere have been accepted as a major cause of global warming (Post et al., 1990). In recent years, there has been increased interest in soil carbon sequestration, using charcoal, biochar, or activated carbon, in agricultural soils as a strategy to help decrease the problem (Mathews, 2008). Biochar addition to soil influences soil pH, porosity, sorption capacity and cation exchange capacity. The porous structure provides an aerated, moist and protected habitat where aerobic microbial communities can develop. Biochars produced at higher temperatures are very resistant to microbial degradation but can change soil physico-chemical properties (Jindo et al., 2012). Under pyrolysis, cellulose, hemicelluloses, and lignin material become ash and alkali salts (Cao and Harris, 2010). Increased cation exchange capacity (CEC) promotes the adsorption of nutrients which, in turn, increase microbial growth, so enhancing the mineralization rate of organic compounds (Taghizadeh-Toosi et al., 2012). Biochars added to soils can decrease the nitrifying microbial community and inhibit nitrification (Elmer and Pignatellom, 2011; Wardle et al., 1998). The adsorption of N rich organic compounds onto the biochar surface contributed to the stimulation of ammonification and nitrification (Gundale and DeLuca, 2006). However, N immobilization also occurs on biochar surfaces, where substrates with high C:N ratios may be immobilized by microorganisms with low C:N ratios (Gundale and DeLuca, 2006; Jesus et al., 2010). Biochars also improve soil tilth (Glaser et al., 2002), crop yield (Graber et al., 2010; Silber et al., 2010), plant nutrient availability, (Lehmann et al., 2003; Steiner et al., 2008) and changes in microbial community structure (Kolton et al., 2011; Makoto et al., 2011; Pietikäinen et al., 2000).

However, biochar applications have some disadvantages in soils, as they may decrease soil N availability in N deficient soils due to the high C:N ratio of biochars and so can reduce crop yields. Other negative aspects of biochar on soil quality include decreased microbial activities, nutrient immobilization and acceleration of native soil organic matter loss (Jindo et al., 2012). With current technology, biochars cannot be removed from the soil once added. Jones et al. (2011) concluded that biochar application to soils will reduce the efficiency of applied herbicides. Biochar application to neutral soils increases soil pH and can stimulate the growth of neutrophilic plants and microbial communities. Biochar production also needs advanced technology to reduce its volatile components, recover nutrients and energy as well as requiring optimum conditions for production. Farmer training is also required to select specific biochar types and application rates for particular soils (Laird, 2008).

Previous studies have demonstrated that the structure and functioning of soil microbial communities are greatly influenced by soil properties (Bossio et al., 1998; Jesus et al., 2010; Lauber et al., 2008). Biochars have

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been applied to agriculture soils to improve soil quality, productivity and C sequestration (Glaser et al., 2002). Sandy soils generally contain low organic matter contents and are unable to retain moisture and nutrients, so are generally of low fertility, while some sandy soils contain high contents of base cations and micronutrients with less inorganic phosphorus (P) and nitrogen (N), which leaches out with heavy rain or irrigation (Weber et al., 2007). The addition of biochar to sandy soils can induce significant changes in microbial community structure and soil fertility (Ameloot et al., 2013). There is still incomplete understanding of the effects of biochars, generated from different feedstocks, on microbial community structure and the chemical properties of sandy soils.

Microbial phospholipid fatty acid (PLFA) analysis is used to determine the soil microbial community structure and responses to environmental stress and soil nutritional quality. It can also be used as an aid for monitoring constructive and sustainable ecosystem services (Kong et al., 2011; Steenwerth et al., 2002). Therefore, we hypothesized that biochar type and application rate changes certain chemical properties of soil with time, which can influence the microbial community structure. To test this, we used four biochars generated from different feedstocks for amendment of a sandy loam soil, representative of the Zhejiang province of China, to determine the influence of biochars on soil microbial community structure and chemical properties of a sandy loam soil.

2. Material and methods

2.1. Soil collection and characterization

A sandy loam soil was collected from the Zhejiang province of China. The soil particle composition data was given in our previous study (Dai et al., 2013b). The soil has been classified as a “Psammaquent” soil according to the USDA classification system and in the Chinese classification system it is categorized as a ‘Red Earth’ (Wu et al., 2009b). The soil was collected from the surface horizon (0–10 cm depth) as a composite of individual soil cores as described by Wu et al. (2009b). Obvious vegetation, roots and stones were removed then the soil was air dried and passed through <2 mm mesh. Subsamples were taken for physical and chemical analyses (Agricultural Chemistry Committee of China, 1983). Briefly, soil pH was measured at a soil to water ratio of 1:2.5 (w/v). Clay, loam and sand contents were determined by the hydrometer method. Water holding capacity (WHC) was determined by saturating soil samples with water, draining for 4 h and determining the water held gravimetrically at 105 °C by weighing to constant weight. The C and N concentrations of biochar and soils were determined by an elemental analyzer (Flash EA 1112, Thermo Finnigan). For total K, the soil samples were digested with H_2O_2 and HNO_3 at 120 °C for 4 h and K determined by atomic absorption spectrometry (NovAA300, Analytik Jena, Germany).

2.2. Biochar production and characterization

Four feedstocks, swine manure (SM), fruit peels (FP), *Phragmites australis* (PA) and *Brassica rapa* (BR) were collected from around Hangzhou. The plants were air-dried, chopped into small pieces (less than 1 mm) approximately and pyrolyzed at 500 °C in a muffle furnace (Shanghai Yi Zhong Electricity Furnace Inc., Shanghai, China) under oxygen limited conditions. The pyrolysis temperature was raised at a rate of 26 °C min^{-1} and then held at 500 °C for two hours (Dai et al., 2013b; Muhammad et al., 2013). The resulting biochar (BC) was cooled then ground (<0.2 mm) prior to use.

Biochars were subjected to the following physicochemical analysis: ash content, pH, carbon (C), hydrogen (H), nitrogen (N) and oxygen (O). To estimate ash content, 1.0 g of the ground biochars was heated at 500 °C for 8 h in a muffle furnace. Ash content was calculated from: ash content (%) = (mass of ash / mass of biochar) × 100. The pH of biochars was measured in deionized water at the ratio of 1:10 wt/v, by an Orion 720 pH meter with combined electrodes. The elemental (C, H, and N) contents of biochars were measured by elemental analyzer (Flash EA 1112, Thermo Finnigan), and the O content was calculated by difference. Volatile organic matter (VOC) was measured as the weight loss after heating the biochars in a covered Ni crucible at 950 °C for 7 min.

2.3. Experimental design

Prior to application of the four biochars, soil moisture was adjusted to 70% of WHC and the soil was incubated at 25 °C for one week to activate the existing microbial communities. The soil was amended with 1% and 3% (w:w) of the different biochars. Each biochar, in triplicate (n = 3), at both rates, was thoroughly mixed with soil to ensure homogeneous distribution. The biochar amended soils were transferred to plastic bags and moisture maintained at 70% of WHC throughout the incubation experiment. Soil subsamples were collected after 90 days from each treatment, freeze dried and stored at −70 °C for PLFA analysis and at −20 °C for physico-chemical analysis.

2.4. Chemical analysis of control and amended soil after incubation

The pH was measured at a 1:2.5 (soil:water) ratio. The dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were measured in 2 g of fresh moist soil, following shaking with 20 ml of distilled water in 50 ml polypropylene bottles on a reciprocating shaker at 200 rev min^{-1} for one hour. Soil extracts were centrifuged at 4000 *g* for 10 min. DOC and DON were analyzed in the supernatants after passing through <0.45 μm filter papers. The concentrations of DOC and total dissolved N (TDN) were determined with a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). The soil NH_4^+ and NO_3^- were extracted by 1.0 M KCl at a 1:10 soil to solution ratio, and then determined by a continuous flow analytical system (Skalar SAN++ System, Netherlands). The total carbon (Ct) and N were determined with an automatic C/N analyzer (NA1500, Carlo ERBA). The CO_2 was measured in separate soil incubations with identical treatments to the main experiment (i.e. soils amended with the same biochar types, rates and moisture contents and unamended soils). Empty glass flasks served as blanks. The flasks also contained 15 ml 1 M NaOH to trap CO_2 evolved from soil at 24 h. The NaOH was titrated with HCl in the presence of $BaCl_2$ and the CO_2 evolved from the soils was then determined. The 70% WHC was maintained with deionized water throughout. All measurements are the means of 3 replicate determinations and are expressed on an oven-dried soil basis (105 °C, 24 h).

2.5. PLFA extraction

The microbial biomass in biochar treated soil after 90 days incubation was estimated from total PLFA concentrations and microbial community composition determined by PLFA analysis. The PLFAs from freeze-dried soil (3.0 g) samples were extracted, fractionated and methylated (Bardgett et al., 1996; Bligh and Dyer, 1959; Frostegård et al., 1993), using a slightly modified method of Wu et al. (2009a). In brief, extractable lipids were dissolved in chloroform then fractionated on silica bonded phase columns (SPE-Si, Supelco, Poole, UK). Neutral lipids, glycolipids and phospholipids were eluted with chloroform, acetone and methanol, respectively. In each sample, fatty acid 19:0 was added as an internal standard before methylation. Methyl esters of PLFAs were obtained by subjecting the phospholipid fraction to mild alkaline hydrolysis followed by its recovery in chloroform. The solvents were removed by evaporation under N_2 and the resulting fatty acid methyl esters identified by GC (N6890, Agilent) fitted with an MIDI SHERLOCKS microbial identification system (version 4.5, MIDI). The individual fatty acid methyl esters were expressed as nmol g^{-1} soil.

The nomenclature used for fatty acids was: The C=C in long chain fatty acids and position of the double bond were represented as (0) from the methyl end of the molecule. Cis and Trans geometry were indicated by c and t suffixes. The a and i prefixes indicated the anteiso and iso branching in the molecule, while the methyl group on the

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