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Biochar application influences microbial assemblage complexity and composition due to soil and bioenergy crop type interactions

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ABSTRACT

Enhancement of soil fertility and mitigation of atmospheric greenhouse gases are potential benefits of biochar amendments with proximate links to microbiological processes, yet the impact of biochar on the soil microbial community is poorly resolved. Here, we assessed changes in bacterial community composition and microbial assemblage patterns with biochar amendment in two soils with contrasting fertility under two cropping systems; perennial grass and annual sweet corn rotation at two time periods. Overall, soil type exerted the greatest effect on the soil microbial community, followed by sampling time and cropping system which exerted a greater effect on the microbial community than biochar treatment. The influence of biochar on community composition was most pronounced in the highly weathered, low fertility Oxisol, than the high fertility Mollisol. We further investigated microbial assemblage architecture using random-matrix theory based molecular ecological network analyses. Microbial assemblage complexity increased with biochar amendment, with the largest impact associated with the Oxisol, accompanied by more negative OTU co-variations, indicating enhanced competition and/or niche partitioning in concert with an increase in the number of putative "keystone species". Network analysis suggests that biochar addition, especially in the highly weathered, low fertility Oxisol, confers higher-level organization, competition, and complexity to the soil microbiome that may result in higher resistance to change due to environmental perturbation and thereby increase system sustainability.

1. Introduction

The global conversion of natural ecosystems to intensive, continuous agricultural land use has led to the widespread depletion of soil organic carbon (SOC) stocks (Post and Kwon, 2000) that negatively impacts soil fertility and ultimately may reduce biomass production over time (Lal, 2015). Carbon (C) loss through decomposition in response to deforestation and warmer conditions results in carbon dioxide (CO_2) and methane (CH_4) emissions that can contribute to global atmospheric concentrations of greenhouse gases (GHG) (Lal, 2012, 2004). Thus, developing sustainable agricultural practices and supporting "climate smart soils" that enhance SOC sequestration and potentially offset agricultural sources of greenhouse gas emissions are of critical importance to addressing global food, fuel and fiber needs (Campbell et al., 2014; Paustian et al., 2016).

One potential component of sustainable management is the application of biochar, a C-rich product of biomass pyrolysis, as a soil amendment. First described within the highly weathered, infertile soils of central Amazonia, patches of persistent, anthropogenic dark-colored soil (*terra preta*), characterized by large reserves of charred materials, have maintained their fertility for several thousand years (Glaser, 2007; Glaser and Birk, 2012). Compared to the surrounding soils, *terra preta* is less acidic, contains higher nutrient concentrations (P, Ca, N, Mg) and remains high in soil organic matter, despite intensive cultivation (Barrow, 2012; Glaser and Birk, 2012). Biochar within the *terra preta* is thought to be key to the observed changes in soil physical and chemical properties, leading to nutrient retention, improved crop yields and thus can potentially address decreases in soil fertility as a potential C sink (Lehmann et al., 2006).

Modeled on the C-rich *terra preta*, biochar amendments were proposed as an approach to ameliorate soil quality (Laird, 2008; Lehmann et al., 2011, 2006). Biochar contains a large portion of aromatic compounds recalcitrant to microbial degradation and thus may enhance long-term C sequestration in terrestrial systems (Laird, 2008; Lehmann

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et al., 2006; Noyce et al., 2015). However, the sorption and residence time of biochar in soil is dependent on its physical and chemical properties (Keiluweit et al., 2010) and is a result of a combination of feedstock and pyrolysis temperature (Bourke et al., 2007; Deenik et al., 2011). Observed alterations of soil chemical and physical properties with biochar application may result in a shift in the composition of the native soil microbial community, but not necessarily total microbial biomass (Anders et al., 2013; Anderson et al., 2011; Harter et al., 2014; Steinbeiss et al., 2009). By influencing the activity of microbial functional groups, subsequent changes in soil physiochemical properties induced by biochar addition may suppress GHG emissions and further increase the climate change mitigation potential of the system (Lehmann et al., 2011; Liu et al., 2012; Wang et al., 2012).

As the proximate driver of soil processes underlying C and N cycling, the response of microbial functional groups to biochar addition is critical to understand and anticipate. For example, in one study biochar increased potential nitrogen (N) fixation and enhanced the activity of nitrous oxide (N_2O) reducing bacteria in water-saturated soil microcosms (Harter et al., 2014). In another system, biochar shifted microbial community composition to favor Gram-negative Proteobacteria (Anderson et al., 2011; Orr and Ralebitso-Senior, 2014), thus providing a mechanistic explanation for improved N-cycling through complete denitrification (Jones et al., 2013; Mills et al., 2008; Orr and Ralebitso-Senior, 2014). Changes in soil pH and nutrient availability associated with biochar amendment also may select for a subset of the microbial community (Su et al., 2017; Ventura et al., 2007). For example, biochar addition promoted the abundance of Actinomycetes with no significant changes in total microbial biomass in temperate forest soils (Anders et al., 2013).

Previously, most studies of the effect of biochar on soil microbial communities focused on biomass and composition change, e.g., species richness and abundance. The recent emergence of random matrix theory-based molecular ecological network analysis revealed robust associations among taxa within the soil microbial community (Barberán et al., 2012; Shi et al., 2016; Zhou et al., 2010). The generation of large environmental sequencing datasets offer an opportunity to identify co-occurrence patterns and interdependent relationships among taxa (e.g. OTUs) within the microbial community (Faust and Raes, 2012; Hallam and McCutcheon, 2015) by analyzing the topology of the nodes and characteristics of microbial network assemblages. In this study, we determined the effect of biochar amendment on bacterial community composition and assemblage patterns in two contrasting soil types in Hawaii under two cropping systems using a highly replicated targeted sequencing approach. We emphasize the impact of soil type and biochar amendment on bacterial community network architecture and, by doing so, reveal relationships between specific network modules and environmental factors and identify large changes in assemblage composition in response to amendment and cultivation.

2. Materials and methods

2.1. Study sites and experimental design

Field trials were conducted on the island of Oahu, Hawaii, United States at the Waimanalo (21°20′15″N; 157°43′30″W) and Poamoho (21°32′30″N; 158°05′15″W) agricultural experimental research stations of the College of Tropical Agriculture and Human Resources, University of Hawaii Manoa. Waimanalo has a mean annual precipitation and mean annual temperature of 95 cm and 23 °C (Soil Survey staff, accessed 7/25/2013). The soil, of the Waialua series, is a fertile Mollisol with 55% clay, strong shrink-swell properties, is slightly acidic (pH 6.2) and has a moderately high cation exchange capacity (CEC) (Soil Survey staff, accessed 7/25/2013). Poamoho has a mean annual precipitation and mean annual temperature of 127 cm and 22.5 °C (Soil Survey staff, accessed 7/25/2013). The soil, of the Wahiawa series, is an acidic (pH 5.2) Oxisol with 44% clay rich in kaolinite and iron oxides with a low CEC (Soil Survey staff, accessed 7/25/2013).

The biochar, supplied by Diacarbon Energy, Inc. (Burnaby, BC Canada), was produced at 600 °C in a continuous flow reactor, composed of 80% woodchip (spruce, pine and fir) and 20% anaerobic digester residue. Biochar was applied to the field at a 1% rate by volume (45.36 kg/plot). All plots were amended with 10.89 kg/plot of fish bone meal (9.07%N; 2.38%P; 0.63%K; 1.49%Ca; 0.13%Mg). Lime was applied to all plots at Poamoho (13.61 kg/plot) to improve soil pH of the acidic Oxisol for cultivation.

The field experiment consisted of biochar application and corresponding control plots in two cropping systems in two contrasting soil types. Each site had two crops, napiergrass (*Pennisetum perpereum* var. green bana, cultivated as a potential biofuel feedstock) and sweet corn (*Zea mays*, var. Hawaiian Supersweet #9, a regionally important food crop) with eight plots of each crop and two bare plots (Fig. S1). Napiergrass and sweet corn plots were planted in December 2013 and February 2014, respectively, planted plots were 4.57 m by 6.10 m and bare plots were 2.29 m by 3.05 m. At each site, four napiergrass plots, four corn plots and one bare plot were randomly chosen for biochar amendment. Napiergrass was planted December 2013 at Waimanalo and Poamoho, approximately 10 cuttings were planted per row with 121.92 cm row spacing. Sweet corn seeds were planted February 2014 and April 2014 at Waimanalo and Poamoho, respectively, with 76 cm spacing between rows. Napiergrass was harvested by ratoon, i.e., a form of zero-tillage management by cutting the grass near the surface leaving the soil and root system intact for vegetative regeneration, every 6 months and corn was conventionally harvested approximately 72 days after planting.

Soils were collected at two sampling times for microbial community analyses. Samples from napiergrass plots and bare plots at both sites were collected December 2013, 5–13 d after planting, ~1 month after biochar amendment. Poamoho corn plot soils were collected April 2014, 14 days after biochar amendment and 7 days after planting. Waimanalo corn plots were collected March 2014, 14 days after biochar amendment and planting. All soils from the initial sampling are referred to as “pre-plant” henceforth. The second set of samples was obtained from all plots after the second crop rotation several days before harvesting, approximately one year later. Soils from corn plots were collected October 2014 from Poamoho and November 2014 from Waimanalo. Soils from napiergrass plots and bare plots from both sites were collected December 2014. Soils from the second collection are here on referred to as “pre-harvest”. For all soil sampling, each plot was split in half and three half-plot 0–10-cm depth cores (8-cm diameter) were taken randomly and mixed for each sample to create a composite sample. Four of these composite samples were taken per half-plot, for a total of 8 replicates per plot and were transported on dry ice and stored at −80 °C until DNA extraction.

2.2. Soil chemical analyses

Base cations were determined using a 1M ammonium acetate ($NH_4C_2H_3O_2$) (pH 7) extraction with a soil to $NH_4C_2H_3O_2$ ratio of 1:20 (Sparks et al., 1996), shaken for 30 min and filtered through a Whatman 42 filter paper and frozen until analysis for calcium (Ca), sodium (Na), magnesium (Mg) and potassium (K) content (QuikChem 8500 Series Automated Ion Analyzer, Lechat Instruments, Loveland, Colorado). Soil pH was measured (Accumet Research AR20, Fisher Scientific, Waltham, MA, USA) and total soil C and N was analyzed using oxidative combustion (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA) (Table S1).

2.3. DNA extraction and Illumina MiSeq sequencing

Genomic DNA was extracted from 0.25 g soil using MoBio Powerlyzer PowerSoil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA, USA). To improve lysis and desorption of DNA from the