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Changes of bacterial community compositions after three years of biochar application in a black soil of northeast China

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ABSTRACT

Although biochar has been widely evaluated as a soil-amendment, the response of soil bacterial community to biochar addition, especially after several years' addition, has not yet been fully understood. Here, we studied the effect of a single addition of biochar on bacterial community compositions in a black soil of northeast China. The biochar was added with dosages of 0%, 2%, 4% and 8% of the total mass of the top 20 cm soils in the spring of 2012, and soil samples were collected seasonally four times in 2014. The abundance and composition of bacterial community were determined using quantitative real-time PCR and Illumina MiSeq sequencing methods, respectively. The results showed that soil pH, moisture, total C, total N, total P, NO_3^--N, available K and the C/N ratio significantly increased with biochar addition, but that soil bulk density and total K content decreased. The bacterial abundance increased with biochar addition, especially at a higher dosage. The biochar addition increased the alpha-diversity of soil bacterial community and changed the bacterial community compositions. Taxonomic analyses showed that *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi* and *Bacteroidetes* were the dominant phyla in this study, and the relative abundances of *Acidobacteria* decreased but *Chloroflexi* increased with biochar addition. Additionally, biochar addition increased the relative abundances of *Bacillus* and *Pedomicrobium*, but decreased the relative abundance of *Bradyrhizobium*. Canonical correspondence analysis indicated that bacterial community compositions were closely associated with soil parameters such as pH, total C, total N and total K. Given the changes of these soil parameters were highly correlated with the amounts of biochar addition, which suggested that the impacts of long-term biochar amendment on the soil bacterial community were linked to the alteration of soil physiochemical properties.

1. Introduction

Biochar is a solid material produced by high temperature pyrolysis of biomass (such as manure, organic waste, bioenergy crops and crop residues) under limited or no oxygen conditions (Lehmann and Joseph, 2009). Biochar has some excellent physiochemical properties, such as high pH, high surface area, porosity, large cation adsorption ability and high carbon/nitrogen ratio (Cantrell et al., 2012; Gul et al., 2015; Novak et al., 2013), and the biochar characteristics vary with different feedstocks and pyrolysis conditions (Gul et al., 2015; Gunes et al., 2015). Generally, biochar produced at high temperature is very resistant to microbial degradation (Graber et al., 2010; Jindo et al., 2012; Lehmann and Joseph, 2009) and application of biochar to soils is proposed as a good strategy for improving soil quality (*e.g.*, soil pH, soil water and nutrient retention), increasing soil carbon content, improving soil structure and adsorbing pollutants, such as heavy metals and pesticides (Anderson et al., 2011; Bruun et al., 2014; Lehmann, 2007; Liu et al., 2014b; Wang et al., 2015).

Except of soil physicochemical properties changed with biochar amendment, soil microorganisms are also sensitive to biochar addition (Kim et al., 2007; Lehmann et al., 2011; Xu et al., 2016). Hu et al. (2014) reported that the addition of the forest litter-derived biochar to a forest soil for 96 days changed the soil bacterial community, increased the bacterial diversity and enriched the main bacterial taxa, such as the phylum *Actinobacteria*. In another experiment, Khodadad et al. (2011) reported

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that biochar amendment decreased the bacterial diversity in tropical forest soils after 188 days incubation but increased the relative abundance of bacterial phyla *Actinobacteria* and *Gemmatimonadetes*. It should be noted that, most of the researches on the investigation of the impact of biochar on soil microbial communities were conducted in short-term experiments (*i.e.* within one year) (Ameloot et al., 2013; Gomez et al., 2014; Jeffery et al., 2011). Only a few papers reported the influences of biochar on soil microbial communities after adding several years or even longer times (Jones et al., 2012; Kim et al., 2007; Qin et al., 2016), which restricted us to fully understand the long-term effects of biochar on soil microorganisms. For example, in ancient pyrogenic carbon-enriched soils, higher bacterial populations and greater diversity were detected in Amazonian Dark Earths (Terra Preta) than in adjacent pristine forest soils (O'Neill et al., 2009). In an acidic rice paddy, Zheng et al. (2016) also observed that biochar addition increased the bacterial diversity and shifted bacterial community composition after four years of single application of biochar. Because biochar is very stable when it added to soils (Wang et al., 2016) and the biochar ageing process is expected to develop an equilibrium for chemical exchange and biological activity in the biochar-soil system, the long-term effects of "aged" biochar on soil physicochemical and biological properties are likely differed from those of "fresh" biochar (Gul et al., 2015; Major et al., 2010). Therefore, the long-term field experiments of testing the impact that biochar on soil ecological environments are needed (Jones et al., 2012). However, up to date, the responses of soil bacterial community compositions and soil parameters to long-term biochar application have not yet been well examined in the fertile agricultural soils, particularly in the black soils of northeast China.

The black soils are classified as Mollisols, according to the USDA Soil Taxonomy system (Soil Survey Staff, 2014), and also named as dark Chernozems, according to the Canadian system of soil classification (Soil Classification Working Group, 1998). The black soil mainly distributed in Northeast China, is regarded as one of the most important soil resources for crop growth and plays a crucial role in ensuring national food security (Liu et al., 2015; Wang et al., 2008). An original black soil has high fertility, superior physicochemical characteristics with high soil organic carbon contents (approximate 5%–8%) (Liu et al., 2010; Wang et al., 2008; Yu and Zhang, 2004). However, in order to feed the growing population, a large proportion of agricultural black soils underwent fertility deterioration and soil erosion over the past several decades because of long-term intensive cultivation (Liu et al., 2003). Several aspects of soil deterioration were pronounced in the arable black soils, such as soil acidification (Zhou et al., 2016), decrease of soil organic carbon (Liu et al., 2003), and soil compaction (Zhang et al., 2006), etc. In view of the physicochemical properties of biochar (Gul et al., 2015), we hypothesized that applying biochar was an effective practice to counter the deterioration of arable black soils. Considering the residence time of biochar in soils is hundreds to thousands of years (Kuzyakov et al., 2009; Lehmann and Joseph, 2009), the long-term residual effects of biochar on black soil ecosystem should be persisted, such as observed in biochar-enriched "Terra Preta" soils in the Amazon (Grossman et al., 2010; Kim et al., 2007; O'Neill et al., 2009; Yin et al., 2000). Therefore, in this study, we aimed to explore the potential influences of biochar on bacterial community composition and to reveal the relationship between community composition and physicochemical properties of black soil from a long-term view. We hypothesized that the soil bacterial community structure would be changed with long-term biochar addition after the soil physicochemical properties were influenced.

2. Materials and methods

2.1. Site description and experimental design

The biochar experimental plot was set up in the experimental garden of the Northeast Institute of Geography and Agroecology (45°41′48″N, 126°38′12″E), Harbin, Heilongjiang Province, China, in the spring of 2012. The soil is a typical black soil. The biochar was a commercial powder product purchased from Liaoning Biochar Engineering Technology Research Center, China, which was produced from corn stalks. The total carbon, nitrogen and potassium contents of the biochar were 715 g kg^{-1}, 6.9 g kg^{-1} and 16.14 g kg^{-1}, respectively, and the pH of the biochar was 8.87 (weight ratio of biochar/water was 1/10).

Biochar was supplied only once at the beginning of the experiment. Adding rate was 0%, 2%, 4% and 8% as calculated with mass ration of top 20 cm soils (*i.e.,* 0, 5, 10 and 20 kg m^{-2}), which encoded as C0, C2, C4 and C8, respectively. The biochar material was evenly spread on the soil surface and harrowed thoroughly to a depth of approximately 20 cm in May 2012. Each treatment plot was separated by the plastic board inserted into the soil to a depth of approximately 40 cm. Each treatment covered a total area of 7.7 m^2 (1.4 m × 5.5 m) with three replicates. After adding biochar, the annual cropping rotation of soybean–maize–soybean was conducted alternately. An equal amount of chemical fertilizers was applied as base fertilizer when sowing seeds. For soybean, the application dosages were 45 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1} and 45 kg K_2O ha^{-1}; and for maize, the dosages were 180 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1}.

2.2. Soil sampling and soil property determination

The soil samples were collected on 29 April (before seeding), 25 June (crop growing), 25 August (crop growing) and 18 October (after harvesting) in 2014 when the growth crop was soybean. Each sample was a mixture of more than 5 individual soil cores collected at a depth of 0–15 cm. The soil samples were sieved through a 2-mm mesh and the visible roots, plant residues and stones were manually removed. A portion of each soil sample was placed into a 50 ml centrifuge tube and stored at −80 °C for soil DNA extraction, and the remaining soil was air-dried at room temperature to measure the soil physicochemical properties.

Soil physicochemical properties were analysed based on the methods described in Lu (1999). Soil pH was determined from a soil water suspension (1:2.5 w/v) by using a pH metre. Soil moisture content and bulk density were measured gravimetrically. Soil total carbon (TC) and total nitrogen (TN) contents were measured using an Elemental analyser (VarioEL III, Germany). Soil total phosphorus (TP), available phosphorus (AP), ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were digested or extracted with H_2SO_4—$HClO_4$, 0.5 M $NaHCO_3$ and 2.0 M KCl respectively, and then assayed using a continuous flow analytical system (SKALAR SAN^{++}, The Netherlands). Soil total potassium (TK) and available potassium (AK) were digested or extracted with HNO_3—$HClO_4$—HF and 1.0 M NH_4Ac respectively, and then quantified using inductively coupled plasma-atomic emission spectrometry (ICPS-7500, Shimadzu, Japan).

2.3. Soil DNA extraction

The total DNA was extracted from the frozen soil samples (0.5 g wet weight) by using a Fast DNA® Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions, and its quality was estimated using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). Extracted DNA was diluted in