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Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China

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ABSTRACT

Although biochar amendment has been extensively evaluated as a promising strategy to improve soil quality, most evaluations have been conducted in the laboratory or under short-term field conditions, which restricted us to understand the long-term effects of biochar as a soil amendment. As the residence time of biochar in soils is expected to be hundreds to thousands of years, this study focused on revealing whether biochar addition influences soil physiochemical properties and fungal community composition in a black soil of northeast China over the long term. Biochar was added to the micro-plots at 0%, 2%, 4%, and 8% of the total mass of the top 20 cm of the soil in the spring of 2012, and soil samples were collected seasonally four times in 2014. The results indicate that soil pH, moisture, total C, total N, total P, NO_3^--N, available K and the C/N ratio significantly increased but soil bulk density and total K content decreased with biochar addition. The soil fungal abundance determined using quantitative real-time PCR showed that the number of fungal ITS gene copies increased with biochar addition. The soil fungal community composition determined using the Illumina MiSeq sequencing method showed that community diversity was not influenced by biochar addition but the community composition was influenced. The impact of biochar on changes in community composition was not reflected at the phylum level, but at the genus and operational taxonomic units (OTU) levels. The relative abundance of *Fusarium* decreased, but *Guehomyces* increased with biochar addition over the first three sampling dates. The relative abundances of several OTUs classified as potential crop pathogens decreased with biochar addition, suggesting that biochar amendment may be beneficial in terms of suppressing the occurrence of crop disease over the long term. In addition, canonical correspondence analysis indicated that fungal community composition was associated with soil parameters such as pH, soil moisture, total C, total N, total K and available K. The changes in these soil characteristics were highly correlated with the amounts of biochar addition, suggesting that the impacts of long-term biochar amendment on the soil fungal community occurred indirectly as a result of the alteration of soil physiochemical properties.

1. Introduction

Biochar is the solid product of thermal degradation of biomass under no oxygen or oxygen-deficient conditions (Lehmann and Joseph, 2009). The physiochemical properties of biochar vary with feedstocks and pyrolysis conditions (Gul et al., 2015). In general, biochar has a high pH, high surface area, large cation adsorption ability and high carbon/nitrogen ratio (Cantrell et al., 2012; Novak et al., 2013; Gul et al., 2015). Evidence has shown that biochar is highly resistant to decomposition when added to soil (Lehmann and Joseph, 2009; Graber et al., 2010). Therefore, biochar is considered as a good soil amendment for improving soil quality (Spokas and Reicosky, 2009; Sohi et al., 2010; Lehmann et al., 2011). To date, the reported beneficial effects of adding biochar to soil include increasing soil pH, strengthening soil water and nutrient retention, improving soil structure, increasing carbon

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sequestration and adsorbing pollutants, such as heavy metals and pesticides (Spokas and Reicosky, 2009; Sohi et al., 2010; Anderson et al., 2011; Cao et al., 2011; Gul and Whalen, 2016). However, applying biochar to soil also has some potential drawbacks. For example, the efficiency of agrochemicals, such as herbicides, insecticides and fungicides, may be reduced by biochar through absorption (Bonanomi et al., 2015). Biochar may also decrease soil N availability in N deficient soils due to the higher C:N ratio of the biochar or increase native soil organic matter loss (Muhammad et al., 2016). As a result of the beneficial characteristics of biochar, enhanced plant growth and increased crop yields were often observed in sub-boreal forest, paddy and vegetable soils following biochar addition (Graber et al., 2010; Uzoma et al., 2011; Robertson et al., 2012; Tong et al., 2014; Egamberdieva et al., 2016; Gul and Whalen, 2016). In contrast, several reports also indicated that adding biochar did not increase crop yield, especially in coarse-textured soils (Xie et al., 2013; Alburquerque et al., 2014; Reibe et al., 2015; Gul and Whalen, 2016). It should be noted that, most of these results were observed in short-term field trials or in pot experiments, the researches of targeting the effects of long-term biochar addition on agroecosystem are limited (Jones et al., 2012; Liang et al., 2014; Gul et al., 2015). Because the biochar ageing process is expected to result in the 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 (Major et al., 2010; Gul et al., 2015).

In addition to the desirable soil physical and chemical properties caused by the application of biochar, biochar addition to soil undoubtedly shifts microbial community structure (Lehmann et al., 2011; Liao et al., 2016; Lucheta et al., 2016). Khodadad et al. (2011) found that the microbial communities in short-term and long-term biochar amended soils were separated into two distinct groups, and the overall bacterial diversity was decreased in short-term incubation with biochar. Moreover, lower bacterial diversity was also detected over the short-term biochar addition by Jin (2010), while the higher bacterial diversity was detected in biochar enriched soils with biochar addition over the long-term (Kim et al., 2007; O'Neill et al., 2009; Grossman et al., 2010). Generally, the labile substances in biochar, which can directly influence soil microbial communities, are quickly mineralized by microorganisms in short-term amendment (Steiner et al., 2008; Lehmann et al., 2011). However, the long-term effects of biochar on soil microbial communities may be achieved through indirect ways, such as the changes of soil physicochemical properties (*e.g.*, bulk density, pH and nutrient contents, *etc.*) (Ameloot et al., 2013; Gul et al., 2015). Nevertheless, compared with the short-term effects of biochar, the long-term effects of biochar on soil microbial communities have rarely been investigated.

Black soils, which are classified as dark Chernozems and also referred to as Mollisols, are one of the most important soil resources for crop production in China (Liu et al., 2010, 2015b). Original black soils are generally fertile and productive with high soil organic carbon contents (approximate 5%–8%), however, in order to feed the growing population, the arable black soils have been severely degraded over the past several decades because of long-term excessive and inappropriate agronomic practices (Yu and Zhang, 2004; Liu et al., 2010). In these cases, soil acidification and the loss of soil organic carbon have threated the sustainability of agricultural development in this region (Liu et al., 2003; Zhou et al., 2016). As the residence time of biochar in soils is confirmed to be hundreds to thousands of years (Kuzyakov et al., 2009; Lehmann and Joseph, 2009), the long-term residual effects of biochar on plant growth and on soil physicochemical and biological properties would be persisted, such as observed events in the biochar-enriched “Terra Preta” soils in the Amazon (Yin et al., 2000; Kim et al., 2007; O'Neill et al., 2009; Grossman et al., 2010). Although most studies using biochar to improve soil quality have been conducted on problem soils (*e.g.,* acid or saline soils, or soils with severe nutrient imbalances or low soil organic carbon) (Chen et al., 2013; Gul et al., 2015; Lu et al., 2015), the long-term effects of biochar amendment on fertile agricultural soils are also worthy of study (Jones et al., 2012).

The objectives of this study were to investigate the effects of biochar on soil physicochemical properties and microbial community structure in a black soil after a three-year addition. We hypothesized that the influences of biochar on soil physicochemical properties would continue to be presented over the long-term field application; therefore, the soil microbial community would also be changed with biochar addition. In this study, we focused on analysing the soil fungal community by evaluating the soil fungal abundance and community composition using quantitative real-time PCR (q-PCR) and Illumina MiSeq sequencing methods, respectively.

2. Materials and methods

2.1. Site description and experimental design

The long-term 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 (classified as Mollisol) with soil texture of clay loam. The biochar was a commercial powder product purchased from Liaoning Biochar Engineering Technology Research Center, China, and it was produced from corn stalks at a final temperature of 500–600 °C for 1 h. 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 addition rates of 0%, 2%, 4% and 8% were used, which were calculated as mass ratios of the top 20 cm of the soil (*i.e.,* 0, 50, 100 and 200 t ha^{-1}). These treatments are coded as C0, C2, C4 and C8, respectively. The biochar powder was spread on the soil surface and harrowed thoroughly to a depth of approximately 0–20 cm only one occasion on May 2012. Each treatment covered a total area of 7.7 m^2 (1.4 m × 5.5 m) with three replicates, and each micro-plot was separated by a plastic board inserted into the soil to a depth of approximately 40 cm. After adding biochar, the rotation system of soybean (2012) - maize (2013) - soybean (2014) 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} with urea, 90 kg P_2O_5 ha^{-1} with diammonium phosphate and 45 kg K_2O ha^{-1} with potassium sulphate; and for maize, the dosages were 180 kg N ha^{-1} with urea, 60 kg P_2O_5 ha^{-1} with diammonium phosphate and 60 kg K_2O ha^{-1} with potassium sulphate.

2.2. Soil sampling

Following the addition of biochar into the soil and subsequent cropping, soil samples were collected on 29 April (before seeding), 25 June (crop growing), 25 August (crop growing) and 18 October (after harvesting), 2014. The growing crop in this sampling year 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 to thoroughly homogenize and remove the visible roots, plant residues and stones. A portion of each soil sample was placed into a 50 mL centrifuge tube and stored