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Long-term biochar application influences soil microbial community and its potential roles in semiarid farmland

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ABSTRACT

Biochar addition to soil can change soil physicochemical properties, resulting in a shift of the soil microbial community. However, it is uncertain how long-term biochar application affects the soil microbial community and diversity in drylands. To determine the underlying mechanism, a 3.5-year spring maize (*Zea mays* L.) field experiment with biochar applications was conducted to elucidate the effect of biochar on soil microbial abundance and community composition as well as its potential applications in drylands of the Loess Plateau in Northwest China. Soil samples from a 0–20 cm depth for four biochar treatments, including 0 (C0, as the control), 10 (C10), 30 (C30) and 50 (C50) t ha^{-1}, were examined using phospholipid fatty acid (PLFA) analysis. It was found that the proportion of arbuscular mycorrhizal fungi (AMF) and the ratio of AMF/saprotrophic fungi (SF) correlated with the biochar levels, for example, the C30 treatment significantly decreased the absolute SF but increased the ratio of AMF/SF. Especially, both the AMF/SF and Fungi/Bacteria ratios were significantly increased in the C50 treatment, suggesting that high amounts of biochar could increase fungal rather than bacterial diversity. In addition, soil organic C (SOC) ($P < 0.01$), $KMnO_4$-oxidizable C ($KMnO_4$-C) ($P < 0.01$), and the C management index (CMI) ($P < 0.01$) were confirmed to play significant roles in shaping the soil microbial community composition. SOC and total N were significantly increased by biochar application, and total P was increased in the C30 treatment. However, compared with the C0 treatment, the C50 treatment significantly decreased $KMnO_4$-C and the CMI, suggesting the proper level of biochar addition to soil should be considered for the improvement of soil organic materials. Accordingly, biochar application at 30 t ha^{-1}, which was connected with a decreased absolute SF and an increased AMF/SF ratio, could be a choice for improving soil quality and nutrient availability in semiarid farmland.

1. Introduction

The carbon-rich by-product produced by thermal degradation of organic materials, such as agricultural crop residues and wood waste, in an oxygen-depleted environment is termed 'biochar' (Lehmann et al., 2011). Biochar represents an emerging technology that is increasingly being recognized for its potential role in carbon sequestration, reducing greenhouse gas emissions, waste management, renewable energy, soil improvement, crop productivity enhancement and environmental remediation (Kuppusamy et al., 2016). Although the agronomic and environmental benefits of biochar application have been recently supported by a number of studies (Atkinson et al., 2010; Lehmann et al., 2011), the possible effects on soil organisms such as microbes are not yet fully elucidated.

Do biochar micropores provide a favorable or unfavorable environment for microorganisms living? Kuppusamy et al. (2016) reported that changes in the microbial biomass induced by biochar were not equally spread across different functional groups because the altered soil environment might cause some microorganisms to become competitively dominant by inhibiting other microbial groups, resulting in changes in the community composition and structure. Biochar can change soil microbial communities and abundance (Grossman et al., 2010; Jindo et al., 2012), perhaps altering the activity of beneficial soil microorganisms and nutrient cycles (Bruun et al., 2014). Lehmann et al. (2011) revealed that the microbial activity was enhanced by the addition of biochar, whereas Graber et al. (2010) discovered that the microbial reproduction rate was greatly reduced by the application of biochar. Warnock et al. (2010) and George et al. (2012) reported that

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arbuscular mycorrhizal fungi (AMF) abundance decreased with the addition of biochar. AMF play critical roles in soil C and N sequestration as well as in soil aggregation, explaining the vast majority of variability in soil aggregation in the ecosystem (Wilson et al., 2009). Moreover, Chan et al. (2008) and Durenkamp et al. (2010) found that biochar had no effect on the microbial biomass and indicated that the activation of biochar rendered a habitat less suitable for microbial survival. Recently, Chen et al. (2013) reported that the bacterial and fungal diversity were increased and decreased, respectively, by biochar in paddy soil. More recently, Zheng et al. (2016) also found that biochar addition increased the bacterial diversity and shifted the bacterial community composition, whereas Tian et al. (2016) found that biochar did not alter the microbial community structure in paddy soil. However, until now, few reports have been available on the changes in the soil microbial community with biochar addition in drylands.

The possible connections between the characteristics of biochar and soil microorganisms and the implications of biochar on soil quality have not yet been studied systematically (Kuppusamy et al., 2016). Fungi play key roles in the degradation of soil quality (Lenhart et al., 2012). Saprotrophic fungi (SF) mainly act on litter decomposition and nutrition cycling (Koukol et al., 2006), while AMF contribute greatly to soil aggregation (Wilson et al., 2009). Additionally, Leake et al. (2004) revealed that the extraradical mycelium of AMF consisted of 20–30% of the soil microbial biomass in addition to being the C storage pool, taking almost 15% of soil organic C (SOC). Moreover, biochar positively improved the formation of soil macroaggregates (Herath et al., 2013), which might be induced by biochar interactions with mineral soils (Joseph et al., 2010). Although biochar application can enhance SOC (Lehmann et al., 2011; Kuppusamy et al., 2016), it is uncertain whether the microbial community is correlated with SOC during the addition of biochar. Therefore, it is postulated that fungal abundance, especially AMF abundance increased by biochar application, correlates with the increase of SOC. Although the effects of biochar application on soil microbial communities have been extensively evaluated, most evaluations were conducted in the laboratory or under short-term field conditions (i.e., within one year) (Ameloot et al., 2013; Gomez et al., 2014). Only a few studies reported the impacts of biochar application on soil microbial communities after treatment for several years or even longer times (Qin et al., 2016; Yao et al., 2017a,b), restricting our full understanding of the long-term effects of biochar application. Because the biochar aging 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 different from those of “fresh” biochar (Gul et al., 2015; Yao et al., 2017a). However, until now, there have been few reports on the changes in the soil microbial community resulting from biochar application in long-term field experiments.

We hypothesized that the effects of long-term biochar application on soil physicochemical properties would continue to be effective in field conditions; therefore, the soil microbial community would also be affected by biochar application. Accordingly, in this experiment we assessed the effects of biochar application on soil quality and microbial community through a 3.5-year biochar amendment field trial.

2. Materials and methods

2.1. Site description

The study was conducted in 2012–2015 at the Changwu Agricultural and Ecological Experimental Station (35.28°N, 107.88°E, 1200 m altitude) located in a semiarid area on the Loess Plateau of China. The annual mean air temperature is 9.7 °C, and the average annual precipitation is 580 mm (previous 50 yrs) with 73% of rainfall during the maize-growth season. The annual mean air temperature and precipitation amount averaged 10.2 °C (range 9.4–11.0 °C) and 547 mm (range 481–579 mm), respectively, in 2012–2015. The soil at the study site originated from loess with a silt loam texture, as qualified by the USDA texture classification system. In April 2012, prior to the start of the experiment, the soil properties in the top 20 cm were as follows: bulk density at 1.36 g cm^{-3}; pH at 7.89; organic C at 8.25 g kg^{-1}; total N at 0.99 g kg^{-1}; available phosphorus (Olsen-P) at 6.56 mg kg^{-1}; available potassium (NH_4OAc-K) at 127.12 mg kg^{-1}; and mineral N (NO_3^--N and NH_4^+-N) at 9.96 mg kg^{-1}.

2.2. Experimental design and field management

Four biochar treatments were initiated at rates of 0 (C0, as the control), 10 (C10), 30 (C30) and 50 (C50) t ha^{-1}, and the biochar (< 1 mm diameter) was produced from maize straw at 400 °C by the Sanli New Energy Company in Henan, China. The biochar had a bulk density of 0.4 g cm^{-3}, a pH of 9.8, and a specific surface area (Brunauer-Emmett-Teller) of 53.0 m^2 g^{-1}, as well as C, N and H content of 59.16%, 0.98% and 1.69%, respectively. The treatments were applied to 56 m^2 (8 m × 7 m) plots arranged in a random block design with three replicates. Biochar was spread over the soil by hand and immediately plowed into 0–20 cm soil depth in April 2012 before maize sowing. Chemical fertilizers were yearly spread over the soil at rates of 90 kg N ha^{-1} in the form of urea (containing 46% N), 40 kg P ha^{-1} in the form of calcium super phosphate (12% P_2O_5) and 80 kg K ha^{-1} in the form of potassium sulfate (45% K_2O); the soil was then plowed to mix the fertilizer into the subsoil. Maize was planted 5 cm deep with a density of 65,000 plants ha^{-1} using a hole-sowing machine at the end of April and was harvested at the end of September. All plots were top-dressed twice with 67.5 kg N ha^{-1} (urea, 46% N) during the jointing and silking stages using the same handheld device that is used for sowing. Weeds were periodically removed by hand during the maize-growing season.

2.3. Soil sampling and measurements

Soil samples were collected from a 0–20 cm soil depth on 27 September 2015 after harvesting. In each plot, soil cores were randomly drilled using a T sampler (4 cm in diameter) with five replicates combined as one composite sample. The samples were then stored in airtight polypropylene bags, placed in a cooler box at approximately 4 °C and transported to the laboratory. After the removal of visible roots and fresh litter material, the composite samples were homogenized and passed through a 2-mm sieve. The fresh sub-samples used for phospholipid fatty acid (PLFA) analysis were freeze-dried and kept in a desiccator before extraction. The other sub-samples were air-dried and measured for pH. Air-dried subsamples of < 2 mm soil particles were ground to pass through a 0.15 mm sieve for the detection of organic C (SOC), total N (TN), total P (TP), and $KMnO_4$-oxidizable C ($KMnO_4$-C). The pH was measured with a standard combination electrode in a 1:2.5 (w/v) suspension in deionized water (Zheng et al., 2013). The TP content was determined by digestion with $HClO_4$-H_2SO_4 and measured using spectrophotometry (UV2300, Shimadzu, Japan). SOC, TN, and $KMnO_4$-C were measured according to Luo et al. (2016). The C management index (CMI) was calculated according to Luo et al. (2016), and the control was used as a reference soil in this study.

The PLFA extraction method used in this study was described by Zhang et al. (2014). We used nonadecanoic acid methyl ester (19:0, Sigma) as the internal standard. Thirty-three individual PLFAs (C14–C20) consistently present in the samples were used to indicate the total microbial biomass. The sum of i14:0, a15:0, i15:0, i16:0, a17:0 and i17:0 was used to indicate gram-positive bacteria (Gm +) (Yu et al., 2016), and the sum of 16:12OH, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c and cy19:0 was used to indicate gram-negative bacteria (Gm–) (Yu et al., 2016). The sum of 18:2ω6,9c and 18:1w9c was used to indicate saprotrophic fungi (SF) (Zhang et al., 2014), and 16:1w5c was used to indicate AMF (Zhang et al., 2014; Liu et al., 2015). The sum of