



## Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China



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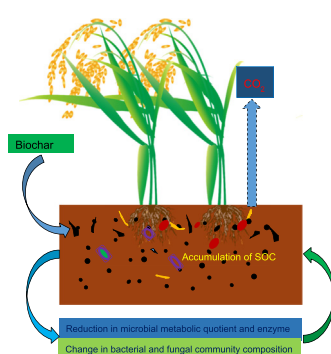
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### HIGHLIGHTS

- Biochar changed soil properties in a rice paddy four years after incorporation.
- Biochar induced a lower microbial metabolic quotient and enzyme activity.
- Biochar altered both bacterial and fungal community structures.
- Fungal rather than bacterial community composition was more affected by biochar.

### GRAPHICAL ABSTRACT



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### ABSTRACT

While numerous studies both in laboratory and field have showed short term impacts of biochar on soil microbial community, there have been comparatively few reports addressing its long term impacts particular in field condition. This study investigated the changes of microbial community activity and composition in a rice paddy four years after a single incorporation of biochar at 20 and 40 t/ha. The results indicated that biochar amendment after four years increased soil pH, soil organic C (SOC), total N and C/N ratio and decreased bulk density, particularly for the 40 t/ha treatment compared to the control (0 t/ha). Though no significant difference was observed in soil basal respiration, biochar amendment increased soil microbial biomass C and resulted in a significantly lower metabolic quotient. Besides, dehydrogenase and  $\beta$ -glucosidase activities were significantly decreased under biochar amendment relative to the control. The results of Illumina Miseq sequencing showed that biochar increased  $\alpha$ -diversity of bacteria but decreased that of fungi and changed both bacterial and fungal community structures significantly. Biochar did not change the relative abundances of majority of bacteria at phylum level with the exception of a significant reduction of Actinobacteria, but significantly changed most of bacterial groups at genus level, particularly at 40 t/ha. In contrast, biochar significantly decreased the relative abundances of Ascomycota and Basidiomycota by 11% and 66% and increased the relative abundances of Zygomycota by 147% at 40 t/ha compared to the non-amended soil. Redundancy analysis (RDA) indicated that biochar induced changes in soil chemical properties, such as pH, SOC and C/N, were important factors driving community composition shifts. This

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study suggested that biochar amendment may increase microbial C use efficiency and reduce some microorganisms that are capable of decomposing more recalcitrant soil C, which may help stabilization of soil organic matter in paddy soil in long term.

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## 1. Introduction

Produced by pyrolysis of biomass under limited oxygen condition, biochar is dominantly composed of aromatic compounds that are largely resistant to biological degradation together with a few labile and leachable compounds (Khodadad et al., 2011; Lehmann et al., 2011). Owing to its abundance of recalcitrant C and millennium scale mean residence time, conversion of plant residues to biochar has been well known as an attractive strategy to enhance C storage and for mitigation of atmospheric carbon dioxide (CO<sub>2</sub>) emission (Lehmann, 2007). Recently, the addition of biochar to soil has received increasing attention due to its positive role in improving soil quality, remediating of contaminated soils and reducing non-CO<sub>2</sub> greenhouse gases (GHGs) emission (Cayuela et al., 2013; Khan et al., 2013; Zhang et al., 2010). However, compared with its effect on soil chemical properties, biochar's effect on soil biological properties has received much less attention.

Soil microorganisms play a key role in nutrient cycling, mineralization and immobilization of soil organic C (SOC) (Six et al., 2006). Present short-term scale studies both in the laboratory and in the field have showed that after incorporation into soil, biochar could induce significant changes in soil microbial activity and community composition, which in turn could affect plant performance and the mineralization of SOC (Ameloot et al., 2013; Farrell et al., 2013; Gomez et al., 2014; Graber et al., 2010; Luo et al., 2013). For example, Kolton et al. (2011) reported an improved growth of sweet pepper with biochar addition as a result of an augment of a few plant growth promoting bacteria in the rhizosphere. In another pot experiment, Khodadad et al. (2011) showed that addition of oak and grass-derived biochar to a forest soil for 188 days stimulated the relative abundances of some taxa-specific microbial communities, such as the phyla Actinobacteria and Gemmatimonadetes. Enhanced microbial biomass C (up to 20% of total microbial biomass) due to biochar addition was reported to increase native SOC mineralization (known as positive priming effect) after 90 days of incubation (Luo et al., 2013). Similarly, Ameloot et al. (2013) also reported that biochar treatment specifically stimulated a few Gram-positive and Gram-negative bacteria by analysis of phospholipid fatty acids (PLFAs) and resulted in a higher CO<sub>2</sub> emission compared to the non-amended treatment. It is generally accepted that some labile C contained in biochar could serve as substrates for growth of some taxa-specific microbial communities and stimulate their activities (Farrell et al., 2013; Gomez et al., 2014; Khodadad et al., 2011). In addition, biochar could act as a suitable habitat for microbial growth and protection from predators (Pietikäinen et al., 2000). Biochar induced improvements in the soil physical and chemical properties, such as increased pH, aeration, water holding capacity and cation exchange capacity, were also believed to help enhance microbial activities and functions (Lehmann et al., 2011). In fact, such changes in soil property induced by biochar were demonstrated to be obvious and strong, particularly in a first short period (Ameloot et al., 2013). So far, it is unclear whether such changes in soil property still remain several years after a single incorporation and their impacts on microbial community due to limited long-term field studies.

It is demonstrated that the labile C in biochar could rapidly be degraded within a few days (Smith et al., 2010) or several months (Kuzayakov et al., 2009), which indicated that the role of fresh biochar as direct substrates for microbial growth may disappear several

months later. As reported by Kuzayakov et al. (2009), the biochar-derived C in the microbial biomass declined to 1.5% to 2.6% after 1.7 years of incubation. Besides, biochar could be aged both biotically and abiotically and its adsorption capacity of toxic compounds such as heavy metals, polycyclic aromatic hydrocarbons and organic pesticides may decrease over time (Hale et al., 2011). Unlike the laboratory incubation, field condition is characterized by changing temperature, frequency tillage, cycling of wetting and drying, which may decrease the impacts of biochar on both soil chemical and biological properties. However, recent studies showed that biochar induced changes, such as the liming effect, sustainable high concentration of SOC as well as improved soil aggregation could be found in the fields several years after addition (Jones et al., 2012; Liu et al., 2014a; Liu et al., 2014b). The aging process after years of incorporation may enhance interactions of biochar with native organic matter and clay minerals and retain more nutrients in the soil (Major et al., 2010). Two years after biochar incorporation, Ameloot et al. (2014) observed a lowered soil microbial activity and abundance and shifted community composition in biochar amended soil with 49 t/ha rates compared to the non-amended soil, which may also indicate a microbiological mechanism of stabilization of soil organic matter under long term biochar addition. Therefore, several years of incorporation, biochar may still have an impact on microbial community, which may not be similar to that of the short term. However, up to date changes in microbial community composition under biochar amendment are still poorly addressed, particularly in the field conditions.

Rice paddy soil covers approximately 20% of the world total irrigated croplands (Frolking et al., 2002), and plays an important role in grain production, SOC storage and GHGs mitigation (Pan et al., 2003). It was characterized by low C turnover with a dominance of anaerobic microbial communities under mostly anoxic conditions (Conrad and Klose, 1999). Recently, biochar was tentatively incorporated into paddy soils, and was demonstrated to be effective in improving soil quality and reducing N<sub>2</sub>O emissions in field experiments across south China (Liu et al., 2012; Zhang et al., 2010). Biochar amendment also caused a reduction in CH<sub>4</sub> emission by increasing the activity and abundance of methanotrophic proteobacteria (Feng et al., 2012). Our previous investigations also suggested that biochar incorporation after one or two rice seasons could significantly enhance bacterial abundance, community diversity and modify the community compositions, particularly of the bacteria involved in N cycling (Chen et al., 2013, 2015). Since the key role of microbial community in regulating dynamics of soil organic matter and GHGs emissions, it is crucial to establish the long-term effect of biochar on soil microbial community activity and composition under field condition before scaling up large application in agriculture.

We hypothesized that the biochar remained in the soil and its induced changes in soil properties several years after a single incorporation could have an impact on microbial community composition and activity and change SOC decomposition in turn. The effects of biochar on physical, chemical and microbial parameters were examined in a rice paddy soil amended with biochar in May 2010 located in southwest China. Illumina Miseq sequencing technique was used to assess the composition of both bacterial and fungal communities. Soil respiration (a measurement of the total activity of the soil microbial community) was monitored during a 28-day incubation.

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