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# Response of microbial community structure and function to short-term biochar amendment in an intensively managed bamboo (*Phyllostachys praecox*) plantation soil: Effect of particle size and addition rate



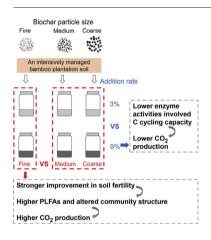
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#### HIGHLIGHTS

- The fine biochar increased microbial abundances and altered community structure.
- The fine biochar resulted in higher CO<sub>2</sub> emission than the other fractions.
- Higher addition rate generally reduced soil enzyme activities involving in C cycling
- Biochar effects on soil microbial community are particle size and rate dependent

#### GRAPHICAL ABSTRACT



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#### ABSTRACT

Biochar incorporated into soil has been known to affect soil nutrient availability and act as a habitat for microorganisms, both of which could be related to its particle size. However, little is known about the effect of particle size on soil microbial community structure and function. To investigate short-term soil microbial responses to biochar addition having varying particle sizes and addition rates, we established a laboratory incubation study. Biochar produced via pyrolysis of bamboo was ground into three particle sizes (diameter size < 0.05 mm (fine), 0.05–1.0 mm (medium) and 1.0–2.0 mm (coarse)) and amended at rates of 0% (control), 3% and 9% (w/w) in an intensively managed bamboo (*Phyllostachys praecox*) plantation soil. The results showed that the fine particle biochar resulted in significantly higher soil pH, electrical conductivity (EC), available potassium (K) concentrations than the medium and coarse particle sizes. The fine-sized biochar also induced significantly higher total microbial phospholipid fatty acids (PLFAs) concentrations by 60.28% and 88.94% than the medium and coarse particles regardless of addition rate, respectively. Redundancy analysis suggested that the microbial community structures were largely dependent of particle size, and that improved soil properties were key factors shaping them. The cumulative  $CO_2$  emissions from biochar-amended soils were 2–56% lower than the control and sharply decreased with increasing addition rates and particle sizes. Activities of  $\alpha$ -glucosidase,

β-xylosidase, N-acetyl-β-glucosaminidase, peroxidase and dehydrogenase decreased by ranging from 7% to 47% in biochar-amended soils over the control, indicating that biochar addition reduced enzyme activities involved carbon cycling capacity. Our results suggest that biochar addition can affect microbial population abundances, community structure and enzyme activities, that these effects are particle size and rate dependent. The fine particle biochar may additionally produce a better habitat for microorganisms compared to the other particle sizes.

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#### 1. Introduction

Biochar soil amendment has been well known as an attractive strategy to enhance soil carbon (C) storage, because it is largely resistant to biological degradation and can be stable in soil for hundreds of years (Kuzyakov et al., 2009). Application of biochar to soils can influence soil physical and chemical properties, nutrient availability and pollutant mobility (Joseph et al., 2010; Zheng et al., 2010), which may affect the biochar C sequestration potential and soil function. It has been shown that such interactions and changes in soil nutrient availability could depend on the particle sizes of both biochars and soils (Laird et al., 2009). Generally, larger particle biochar could exhibit low surface areas and are relatively ineffective at storing water and nutrients but increase aeration compared to the fine fractions. Comparatively, smaller-sized biochar particles could exhibit greater surface areas and water holding capacities and, in some cases, could probably absorb more nutrients (Taghizadeh-Toosi et al., 2012). Fine particles of biochar could be degraded by microbes more easily than coarse ones (Sigua et al., 2014), and could also remove heavy metal ions from aqueous solutions more efficiently (Zheng et al., 2010).

Theoretically, alterations in soil physicochemical properties induced by biochar-soil interactions with varying particle sizes would have differential effects on microbial communities and activities, because microorganisms are sensitive to environmental changes. A number of previous studies suggest that the addition of biochar can increase soil microbial biomass and activity, due to labile C contained in biochar which can serve as substrate for growth and thereby induce a positive priming effect (Farrell et al., 2013; Gomez et al., 2014; Khodadad et al., 2011; Luo et al., 2011). Biochar is also able to adsorb labile soil organic C, reducing nutrient availability to soil microorganisms and that of substances that inhibit microorganisms (Spokas et al., 2010), which resulted in a negative priming effect (Prayogo et al., 2014; Zimmerman et al., 2011). Besides, biochar could act as a suitable habitat for microbial growth and provide protection from predators (Pietikäinen et al., 2000; Quilliam et al., 2013). Obviously, such impacts of biochar on microbial community could be closely related to its particle size, although biochar type, soil type, and application rate could play certain roles (Chen et al., 2015; Gomez et al., 2014; Khodadad et al., 2011; Smith et al., 2010). However, little is known whether and how the particle size of biochar could affect the microbial population abundances and community structure and functionality as related to key soil processes such as C mineralization and soil enzyme activity.

Bamboo forests play a significant role in sequestering atmospheric CO<sub>2</sub> for long-term storage in biomass due to its wide distribution, rapid growth and high yields (Zhou et al., 2011). Lei bamboo (*Phyllostachys praecox*) is one of the major cash crops commonly grown in eastern China for high-value edible bamboo shoots. To obtain a high shoot yield, intensive management typically including large inputs of mineral fertilizers and application of winter mulch was widely performed. The addition of mulch and long-term fertilization (as high as 720 kg N ha<sup>-1</sup> year<sup>-1</sup>), however, has been shown to lower fertilizer use efficiency and increase degradation of the environment due to soil acidification, increased nutrient leaching and loss of biodiversity (Qin et al., 2014; Zhang et al., 2013). Therefore, it is important to explore alternative management practices such as amendments to improve soil quality and increase C stocks simultaneously. Biochar could be one of the candidates as it can increase soil C storage efficiently and provide

multiple environmental benefits such as improved soil fertility and reduced greenhouse gas emissions (Harter et al., 2014; Lehmann et al., 2011). A few studies have reported that biochar derived from bamboo and bamboo leaf feedstock can decrease CO<sub>2</sub> emissions and increase C sequestration in paddy soil and Chinese chestnut plantations (Liu et al., 2011; Wang et al., 2014). However, the application of biochar to bamboo forest ecosystems has received less attention compared to other agricultural systems. Additionally, it is important to understand how soil microorganisms and soil enzymes respond to the application of biochar, particularly as affected by particle size, before proceeding with large-scale operations.

Taking into consideration the abovementioned issues, the purpose of this study was to examine whether biochar amendments differing in particle sizes and application rates alter soil microbial population abundances and community structure as well associated CO<sub>2</sub> emissions and enzyme activities in an intensively managed bamboo plantation soil. We hypothesized that: (1) bamboo biochar addition could ameliorate soil acidification and enhance soil C and nutrient retention in the intensively managed soil, (2) biochar addition could influence microbial community abundance and structure but this effect may be particle size and rate dependent, and (3) response of soil CO<sub>2</sub> emissions and enzyme activities to biochar addition may also depend on particle size and rate possibly due to changes in the microbial communities.

#### 2. Materials and methods

#### 2.1. Soil and biochar characterization

Soil was sampled from a Lei bamboo stand located in Jincheng Township, Lin'an County, Zhejiang Province, China (30°15′ N, 119°43′ E). The region has a monsoonal subtropical climate with four distinct seasons. The mean annual precipitation and temperature of the sampling site are 1420 mm and 15.8 °C, respectively. The bamboo plantations were managed according to local management practices which consisted of application of mineral fertilizers three times a year in mid-May, mid-September and before mulching in the winter. The total amounts of the mineral fertilizers can range as high as 1.1 t ha<sup>-1</sup> of urea and 2.2 t ha<sup>-1</sup> of compound fertilizer (N:  $P_2O_5$ :  $K_2O = 15$ : 15: 15). Mulch consisting of mixed bamboo leaves and rice chaff was applied to the soil surface of the bamboo stands in the winter and removed in the following spring. The intensive management regime of bamboo stands is further described by Qin et al. (2014). The soil is classified as Ferrisols in the FAO soil classification system with a sandy loam texture (clay: 11.6%, silt: 39.2% and sand: 49.2%). Samples of topsoil (0–20 cm) were collected and homogenized to form a composite soil in November 2013. After air drying, the soil was passed through a 2 mm sieve for use.

The biochar used in this experiment was made from bamboo sticks via pyrolysis at a temperature of 700 °C for 4 h in a closed container under oxygen-limited conditions. After pyrolysis, the biochar was crushed to three particle sizes: diameter size of <0.05 mm (fine), 0.05-1.0 mm (medium) and 1.0-2.0 mm (coarse) before application.

#### 2.2. Incubation experiments

The effect of biochar addition rates of 0% (control), 3% and 9% (w/w) were examined. Together with the three particle sizes, 7 treatments were prepared as follows: 0% biochar (CK), 3% fine biochar (3%F), 3%

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