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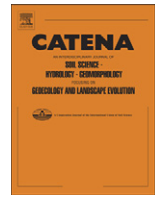

Effect of biochar on carbon fractions and enzyme activity of red soil

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ABSTRACT

To evaluate the changes in the pool of organic carbon fractions, aggregate stability and activity of enzymes, degraded red soil was amended with three different rates (0.5, 1.0 and 2%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$, $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$, $B_{2.0}$), with control as 0%. After 372 days, the incubated soils were analyzed for total organic C (TOC), potassium permanganate oxidizable C (POXC), light fraction organic C (LFOC), water soluble organic C (WSC), hot-water extractable C (HWC) and microbial biomass C (MBC), macroaggregates (>0.25 mm), dehydrogenase, β-glucosidase and urease. The highest macroaggregates, POXC, LFOC, HWC, MBC and enzyme activities were measured in the lowest rates ($W_{0.5}$ and $B_{0.5}$). MBC positively correlated with all labile organic C and macroaggregates, indicating that microbial activities result in mineralization of organic matter (OM) and contribute on bonding agent for macroaggregation. The C/N of the experimental soil negatively correlated with most of labile organic carbons and macroaggregates, which could be the effect of limited N availability on labile organic carbon fraction and aggregation. As compared to the control, lability index (LI) (changes in the lability of soil carbon) increased in $W_{0.5}$ and $B_{0.5}$ by 4 and 6%, respectively, whereas the carbon management index (CMI) (changes of the total carbon in the soil and its lability) increased by ranges of 50 to 286% in the treatments, and implies sequestration of organic C in soil. The high CMI is largely caused by high C sequestration and low lability differences between the treatments. Our results suggest that biochar application increases total organic carbon, stimulates microbial activities, in turn increases macroaggregation, and thus soil quality.
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1. Introduction

To meet ever increasing nutrition demands of the expanding human populations and make sustainable agriculture, restoration of degraded soil and improvement its quality is indisputable. The red soils of China are highly weathered and very susceptible to erosion (Zhang et al., 1996). Red soils occupy approximately 2.04 million km^2 in the southern China and are the most important soil resources in tropical and subtropical regions of China (Xu et al., 2003). These soils are typical of similar red soils that occur throughout tropical and sub-tropical South America, Africa and South East Asia; so that if the red soils of China could be successfully utilized then this would have wider implications for agriculture in other countries.

Red soils have high productivity potential. However, the long-term inappropriate utilization and management have caused severe degradation (Zhang and Xu, 2005). Depletion of soil organic matter, soil erosion and decline in soil structure are the major degradative processes. To sustain arable cropping systems there must be careful management on Chinese red soils. In other words, considerable attention has been focused on the restoration of soil organic matter and soil structure.

Zhang and Xu (2005) reported that degraded red soils could be greatly ameliorated through increasing the content of organic carbon (C) and improving soil aggregation. Similarly, Tisdall and Oades (1982) and Cerdà (1998) suggested that soil organic C is closely related to the formation and stability of soil aggregates. The increase in organic C and aggregate stability reduces soil losses (García-Orenes et al., 2012). Soil organic matter contributes to nutrient supply, improvement of soil physical properties, and protection from erosion and thus there would be a positive correlation between its content and soil quality (Janzen et al., 1997).

The soil organic carbon is a complex pool which is divided into labile pool with residence time of years to a few decades and recalcitrant pool with mean residence time of hundreds to thousands of years (Cheng et al., 2007). Fractions of soil organic carbon are more important in maintaining soil fertility and are, therefore, more sensitive indicators of the effects of management practices compared with the soil total organic carbon (Freixo et al., 2002; Lützow et al., 2000).

Due to their rapid response to environmental changes, the microbial biomass organic C (MBC) and light fraction of organic C (LFOC) are important fractions of soil organic matter, and play essential role in the short-term turnover of nutrients in soils (Alvarez et al., 1998). The

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ease of oxidation of the soil organic C by $KMnO_4$, permanganate oxidizable organic carbon (POXC), is a larger pool than that commonly measured as soil microbial biomass (Blair et al., 1995). Water-soluble carbon (WSC) is another labile fraction that can be used as an indicator of short-term changes in C status of soils (Haynes, 2000). The hot-water extractable carbon (HWC) being a component of the labile soil organic matter and also being closely related to soil microbial biomass and micro aggregation could therefore be used as one of the soil quality indicator (Ghani et al., 2003).

Many scientists showed interest in using biochar as soil amendment (Lehmann and Joseph, 2009; Sohi et al., 2010). Biochar is a highly stable compound created when biomass is heated to a temperature between 350 °C and 600 °C in the absence of oxygen. Biochar, as a soil amendment, can increase concentrations of soil organic matter, especially water-extractable organic carbon (Lin et al., 2012) and stimulate soil microbial activity (Lehmann et al., 2011). Microorganisms are largely responsible for decomposition of the organic matter via a variety of enzymes and hence, application of biochar is a method of replenishing degraded soil quality through improvement of the biological status of the soil, which usually implies an increase in enzyme activity (Albiach et al., 2000). We hypothesized that biochar increases organic matter in soils; therefore increases labile organic carbon, enzyme activity, and aggregate formation and, in turn improves soil quality.

To identify changes in soil organic carbon quality as influenced by biochar applications, measurements of the more active fractions of soil labile organic carbon are very important. To our knowledge, there is little information on the effect of biochar on organic carbon fractions and enzyme activity on red soil. Therefore, the objectives of this study were to evaluate the effect of biochar application on the amount of total organic C (TOC), labile soil organic C fractions (LFOC, POXC, WSC, HWC and MBC), enzyme activity, aggregate formation and the quality of the soil.

2. Materials and methods

2.1. Soil sampling and analysis

A laboratory experiment was conducted to study the influence of biochar on total organic carbon, labile soil organic carbon and enzyme activity. The experimental red soil for this study was sampled at Meijiawu, suburban of Hangzhou. The soil is highly weathered (Plinthic Hapli Udic Ferrosols in Chinese Soil Taxonomic Classification System; and Typic plinthustults in USAD Soil Taxonomy), derived from quaternary red clay parent material and characterized by low pH. The present land-use is tea garden.

Particle size distribution was determined by a pipette method. Soil pH was determined through a suspension sample with a soil (air-dried) to water (w/v) ratio of 1:2.5 and measured with a pH meter (Pansu and Gautheyrou, 2006). Soil organic carbon was determined by dichromate oxidation (Nelson and Sommers, 1982). Total nitrogen (TN) in soil was measured using the Kjeldahl method after H_2SO_4 digestion in the presence of K_2SO_4–$CuSO_4$–Se catalyst (Bremner, 1996).

2.2. Biochar analysis

The oak wood (*Quercus phillyraeoides*) and bamboo (*Phyllostachy edulis*) biochar used for this study, was pyrolyzed at 600 °C for 2 h (was purchased from Linan Yaoshi charcoal production Limited company located in Hangzhou City). The pH was determined in deionized water at the ratio of 1:10 wt/v (Gaskin et al., 2008) by Orion 720 pH meter. The carbon, hydrogen, and nitrogen contents of the biochars were determined using a CHN elemental analyzer (Flash EA 1112, Thermo Finnigan). The oxygen content was estimated by mass difference (100% − C, H, N and ash %). The ash content was determined according to ASTM D-1762-84 (2007) by combusting the biochar at 750 °C for 6 h in open crucibles on a dry weight basis. The BET (Brunauer–Emmet–Teller) surface areas was measured via N_2 adsorption multilayer theory using a Nova 2200e surface area analyzer (Quantachrome, Boynton Beach, FL) (Chen et al., 2008).

2.3. Incubation experiment

The soil sample was passed through 5 mm sieve whereas the biochars through 0.25 mm sieve. Based on the mass of the soil (total 2 kg), the biochars were added at a rates of (0.5, 1.0 and 2%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$, $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$, $B_{2.0}$), with control as 0%. After mixing the soil and the biochars thoroughly, they were wetted with deionized water to about 30% water holding capacity of the experimental soil. All pots were covered with plastic film and then a small hole was made to allow gaseous exchange. The pots were incubated at a constant temperature of 25 °C. Based on evaporation loss, the soil moisture was kept constant by regular weighing of the pots. For each treatment, triplicate samples were prepared. The samples were arranged in complete randomized design. After 372 days, the incubated soils were used to analyze water-stable aggregate, TOC, TN, LFOC, POXC, WSC, HWC, MBC and dehydrogenase, β-glucosidase and urease.

2.4. Aggregate stability

The fraction of aggregates was measured using a wet sieving method (Kemper, 1986). Air-dried soil samples of 50 g were placed at the top of a stack of sieves (2, 1, 0.5 and 0.25 mm). The screen was lowered to the water surface to allow the dry soils to become moist via capillary action, and then soils were sieved for 10 min with a stoke length of 10 cm and a frequency of 35 strokes per minute. Soils that remained in each sieve were transferred to a container, dried (60 °C), and weighed.

2.5. Labile organic carbon fractions

The light soil organic carbon fraction (LFOC) was determined using the density fractionation method as described by Gregorich and Janzen (1996). Briefly, 10 g of air-dried soil (<2 mm) was placed in a 100 ml centrifuge tube with 50 ml of NaI solution (1.70 g cm^{-3}). After shaking about 10 times by hand, the tube was sonicated using an ultrasonic disintegrator for 15 min at 400 W, and then centrifuged at 1200 g for 10 min. The supernatant with floating particles was decanted into a vacuum filter unit with a glass-fiber filter (Whatman GF/C) and the NI solution was collected for reuse. The process repeated twice without sonication. The light fraction collected on the filter was washed three times with 0.01 mol/l $CaCl_2$ to remove excess NaI, and then washed three times with deionized water. The three subfractions were combined, oven-dried at 60 °C for 48 h, weighed and stored for analysis. Water soluble carbon (WSC) and hot-water extractable carbon (HWC) were measured as described by Ghani et al. (2003). Soil samples (equivalent to 3 g oven dry weight) were weighed into 50 ml polypropylene centrifuge tubes. These were extracted with 30 ml of distilled water for 30 min on an end-over-end shaker at 30 rpm and at 20 °C, centrifuged for 20 min at 3500 rpm and all the supernatant was filtered through 0.45 μm cellulose nitrate membrane filter into separate vials for carbon analysis. This fraction of the soil organic carbon was classified as water soluble C (WSC). A further 30 ml of distilled water was added to the sediments in the same tubes. These tubes were shaken on a vortex shaker for 10 s to suspend the soil in the water. The tubes were capped and left for 16 h in a hot-water bath at 80 °C. At the end of the extraction period, each tube was shaken for 10 s on a vortex shaker to ensure that HWC released from the SOM was fully suspended in the extraction medium. These tubes were then centrifuged for 20 min at 3500 rpm. The supernatants were filtered through 0.45 μm cellulose nitrate membrane filters. The extract was analyzed for carbon with a multi N/C analyzer. Permanganate oxidizable C was measured as described by Blair et al. (1995). Finely ground air-dried soil samples were reacted with 333 mM $KMnO_4$ by shaking at 60 rpm for 1 h. The suspension

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