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ORIGINAL ARTICLE

Persistent effects of biochar on soil organic carbon mineralization and resistant carbon pool in upland red soil, China

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Abstract

Biochar as a soil ameliorant has generated great interest for scientists in improving soil quality and carbon sequestration. The objective of this study was to investigate the persistent effects of biochar application on soil organic carbon (SOC) mineralization and soil-resistant carbon (C_r) in upland red soil. This experiment was conducted from September 2011 to May 2016. Biochar was applied only once in September 2011 at rates of 0, 2.5, 5, 10, 20, 30 and 40 t/ha in the field experiment, designated as treatments B_0, B_1, B_2, B_3, B_4, B_5 and B_6. The chemical properties, dynamics of SOC mineralization and soil-resistant carbon (C_r) were measured at the 1st and 6th year after biochar application. The results were as follows: biochar application at rates of 30 and 40 t/ha (B_5 and B_6 treatments) distinctly increased soil pH value and available P relative to B_0 in 2011. The pH value, available P, SOC, total N and C/N ratio in B_4, B_5 and B_6 treatments were significantly higher compared with the B_0 treatment, where the B_6 treatment increased the pH value by 0.80 and C/N ratio by 3.88 while increasing available P, SOC and total N by 24.18, 76.29 and 19.78%, respectively, compared with the B_0 treatment in 2016. The cumulative SOC mineralization (C_m) occupied around 4.62–6.91% of total organic carbon (C_t), which showed a declining trend in 2016 as compared to 2011. The C_m/C_t ratio also showed a declining trend with biochar amendment at both samplings. The C_r occupied around 26–46% of SOC and showed obviously increasing trends both in 2011 and 2016. We further found that C_m/C_t showed highly significant ($p < 0.01$) negative correlations with the rates of biochar application both in 2011 and 2016. The C_r, however, showed very significant ($p < 0.01$) positive correlations with the rates of biochar application both in 2011 and 2016. This study suggested that biochar application to upland red soil persistently improved soil properties and resistant carbon. Cumulative SOC mineralization was clearly restrained by biochar amendment. This study can provide scientific support for improving soil fertility and enhancing carbon sequestration by application of large amount of biochar (40 t/ha) in upland red soil.

Keywords Biochar · Red soil · Soil organic carbon mineralization · Resistant carbon

Introduction

The global soil organic carbon (SOC) pool, estimated at 1550 Pg C in the upper 100 cm, is an important component of the global carbon (C) cycle as it contains more C than the atmosphere (780 Pg C) and biosphere (620 Pg C) combined (Batjes 2014; Grace 2004). Thus, even minor changes would influence the atmospheric levels of carbon dioxide (CO_2) and the balance of global carbon. Consequently, there is strong interest in understanding SOC dynamics and the role of soils in mitigating CO_2 emission in solving the serious problem of global warming (Change 2007). The complex SOC pool is divided into an active SOC pool with a short turnover time (1–5 years), a slow SOC pool with an intermediate turnover time (20–40 years) and a resistant SOC pool with the longest turnover time (200–1500 years) (Parton et al. 1988). Fractions of SOC are important in maintaining soil fertility and are, therefore, more sensitive indicators of the emissions of greenhouse gases compared with the soil total organic carbon (Freixo et al. 2002; Lützow et al. 2002).

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Turnover time is directly determined by SOC mineralization which reflects the process of SOC converted into inorganic carbon.

Biochar, also termed black carbon, agrichar and charcoal, is in general the solid residue produced by thermal degradation of organic matter in the absence of oxygen (Scott and Jan 1984). During the process, 20–50% of biomass C is converted to the recalcitrant forms of C, dominantly made up of condensed aromatic C including small and fragmented graphene sheets that are highly resistant to microbial degradation (Glaser et al. 2002). Recently, biochar application to agricultural soils has attracted widespread attention as a soil amendment (Smith et al. 2010; Forbes et al. 2006; Fowles 2007). Additionally, because of its relative inertness, biochar applied to soil contributes to the refractory soil organic carbon and thus can reduce atmospheric CO_2 concentrations by sequestering carbon (Lehmann 2007; Mathews 2008).

Red soils, generally characterized by high risk of erosion and poor fertility, occupy approximately 2.04 million km^2 in southern China and are the most important soil resources in the tropical and subtropical regions of China (Xu et al. 2003). Furthermore, with the local population increasing and demand for food urgently increasing, efforts to restore the quality of red soil are imminent and biochar may play a key role in this endeavor. Many studies have shown that biochar application to agricultural soils has the potential for long-term sequestration of large amounts of C in the soil (Glaser et al. 2002; McHenry 2008; Steinbeiss et al. 2009; Tenenbaum 2009). It has also been reported that biochar amendment significantly increased SOC content and CO_2 emissions from the soils during a 500-day soil column incubation study (Rogovska et al. 2011). Other studies, however, observed no significant impact of biochar addition on CO_2 emission (Smith et al. 2010; Kuzyakov et al. 2009; Wang et al. 2011; Case et al. 2012). The differences in biochar impact on carbon sequestration may be attributed to variations in biochar raw feedstocks, pyrolysis conditions, biochar composition, application rates and soil types (Spokas and Reicosky 2009; Luo et al. 2011; Case et al. 2012). However, the majority of these studies reported the short-term impact of biochar on CO_2 emission in other soil. Few studies have focused on the long-term effects of biochar on SOC mineralization and resistant carbon content in upland red soil. The aim of this study was to investigate the persistent impacts of different application rates of biochar on the dynamics of SOC mineralization and soil-resistant carbon in upland red soil. The biochar was applied only once in the present study. The research results may provide a scientific basis for biochar's ecological effect and the balance of SOC in upland red soil of China.

Materials and methods

Site characteristics

The field experiment was conducted at the Institute of Red Soil, Jinxian County (116°20′24″N, 28°15′30″E), Jiangxi Province, China, from 2011 to 2016. The climate is characterized by cool dry winters and warm humid summers. The mean annual temperature and rainfall were 17.2 °C and 1549 mm, respectively. The soil is derived from Quaternary red clay. The soil had an initial pH of 4.54, SOC of 9.45 g/kg, total N of 1.06 g/kg, cation exchange capacity (CEC) of 15.2 cmol/kg and bulk density of 1.23 g/cm^3.

Seven treatments were established with biochar amendment at rates of 0, 2.5, 5, 10, 20, 30 and 40 t/ha, designated as B_0, B_1, B_2, B_3, B_4, B_5 and B_6, respectively. A randomized complete block design with three replications was laid out on the similar soil type and with consistent management. Each trial plot covered an area of 20 m^2 (4 m × 5 m). Biochar was spread on the surface of the soil and thoroughly mixed into a depth of 15 cm by manual plowing on August 18, 2011. No more biochar was supplemented in the subsequent years. We adopted the rapeseed–sweet potato rotation tillage system with rapeseed planted in October and harvested in mid-May, and the sweet potato planted in late May and harvested in late September. The chemical fertilizers used were urea for N, calcium superphosphate for P and potassium chloride for K. According to the method of local conventional fertilization, the rates of chemical fertilizer applied in each treatment were 90, 52.5 and 107 kg/ha for N, P_2O_5 and K_2O, respectively. All those fertilizers were applied to the soil surface and mixed homogeneously by manual plowing before rapeseed transplanting and sweet potato seeding. Additionally, a rate of 15 kg B/ha with borax was applied before seeding in the rapeseed season.

The biochar was produced from wheat straw pyrolyzed at approximately 500 °C in a vertical kiln made of refractory bricks in Sanli New Energy Company, Henan Province, China. Using this technology, 35% of the wheat straw dry matter would be expected to be converted to biochar (Pan et al. 2011). The original biochar was ground to pass through a 2-mm sieve to ensure uniform mixing into soil mass. The biochar amendment had an initial pH of 10.35, SOC of 467.2 g/kg, total N of 5.9 g/kg, total P of 14.43 g/kg, total K of 11.5 g/kg and cation exchange capacity (CEC) of 21.7 cmol/kg.

Soil sampling and analysis

Soil samples of seven treatments were collected at a depth of 0–15 cm after sweet potato harvesting on September

26, 2011, and rapeseed harvesting on May 5, 2016. The samples were sealed in plastic bags and transported to the laboratory within 2 days after sampling. Fine roots were removed manually, and the soil was air-dried and ground to pass 2- and 1-mm sieves successively. Each sample was divided into two parts: one part was used to determine basic soil properties, and the other was used for a laboratory incubation experiment. Basic soil properties were determined with the methods suggested by Bao (2000). The total SOC was measured via wet digestion using $K_2Cr_2O_7$ oxidation. Soil-resistant carbon was measured with acid hydrolysis consisting of refluxing soil in 6 M HCl (Zhang et al. 2017). Soil pH (H_2O) was determined with a glass electrode (soil/solution = 5 g:25 mL). Total N was measured with the Kjeldahl method. Soil available P was measured with a Mo–Sb colorimetry.

Laboratory incubation

The air-dried samples from every treatment (equivalent to 100 g) were sieved to 4 mm and adjusted to 60% of water holding capacity and then incubated in 1 L hermetically sealed canning jars at 25 °C in the dark. A vial with NaOH (20 mL, 0.5 M) was placed in each jar to capture the CO_2 emitted. The CO_2 traps were replaced at various intervals (2nd, 4th, 8th, 13th, 18th, 25th, 34th, 39th, 46th, 53rd, 62nd, 68th, 79th and 90th day after incubation). Three replicate samples of each treatment were conducted and control jars contained no soil. The emission of CO_2 for each interval was measured by titration of residual NaOH to pH 7.0 with 0.4 M HCl, after prior precipitation (with 20 mL of 1 M $BaCl_2$) of the carbonates formed (Ameloot et al. 2013).

Statistical analysis

One-way analysis of variance (ANOVA) was performed by SPSS 20.0. The differences in the group means were examined using the Duncan significant difference test with a significance level of $p < 0.05$. Pearson's correlation coefficients were calculated to determine how the biochar application rates and SOC mineralization and resistant carbon are related.

Results

Soil physicochemical properties

A distinct improvement of soil basic properties was observed in 2011 and 2016 with biochar amendment. For the 1st year after biochar application to upland red soil, an increasing trend of pH value and available P was found in the B_5 and B_6 treatments which were amended with 30 and 40 t/ha biochar, relative to the B_0 treatment (Table 1a). For the 6th year after biochar addition, when the amounts of biochar were 20, 30 and 40 t/ha (B_4, B_5 and B_6 treatments), the pH, available P, SOC, total N and *C*/*N* ratio were significantly higher compared to B_0 treatment. The B_6 treatment increased the pH value by 0.80 and *C*/*N* ratio by 3.88 while increasing available P, SOC and total N by 24.18, 76.29 and 19.78%, respectively, compared with

Table 1 Soil chemical properties in (a) 2011 and (b) 2016

Treatments	pH	Available P (mg/kg)	SOC (g/kg)	Total N (g/kg)	*C*/*N*
(a) 2011					
B_0	4.61 ± 0.01c	36.88 ± 0.69f	8.46 ± 0.25bc	1.00 ± 0.03ab	8.50 ± 0.24ab
B_1	4.59 ± 0.01bc	38.57 ± 0.79e	8.37 ± 0.15c	0.99 ± 0.03ab	8.46 ± 0.26ab
B_2	4.70 ± 0.23bc	39.07 ± 0.43de	8.29 ± 0.29c	0.87 ± 0.00b	9.52 ± 0.00ab
B_3	4.95 ± 0.25ab	39.98 ± 0.43d	8.36 ± 0.05c	1.00 ± 0.09a	8.41 ± 0.78b
B_4	4.94 ± 0.19abc	42.13 ± 0.22c	8.78 ± 0.29b	0.90 ± 0.02ab	9.76 ± 0.19a
B_5	5.09 ± 0.12a	43.28 ± 0.27b	9.70 ± 0.19a	1.00 ± 0.07ab	9.76 ± 0.68a
B_6	5.07 ± 0.28a	47.81 ± 0.89a	8.80 ± 0.02b	0.94 ± 0.13ab	9.52 ± 1.40ab
(b) 2016					
B_0	4.54 ± 0.04e	38.62 ± 0.87e	7.55 ± 0.04e	0.91 ± 0.02d	8.32 ± 0.10c
B_1	4.46 ± 0.05e	38.87 ± 0.45e	7.44 ± 0.19e	0.90 ± 0.02d	8.22 ± 0.28c
B_2	4.66 ± 0.04d	39.28 ± 0.66de	8.47 ± 0.39d	0.95 ± 0.02cd	8.90 ± 0.57c
B_3	5.02 ± 0.05c	40.24 ± 0.27d	8.71 ± 0.07d	0.98 ± 0.01bc	8.88 ± 0.11c
B_4	5.10 ± 0.09c	42.87 ± 0.75c	10.48 ± 0.37c	1.03 ± 0.08ab	10.26 ± 1.10b
B_5	5.20 ± 0.04b	45.36 ± 0.58b	11.36 ± 0.36b	1.07 ± 0.01a	10.64 ± 0.34b
B_6	5.34 ± 0.02a	47.96 ± 1.09a	13.31 ± 0.11a	1.09 ± 0.04a	12.20 ± 0.57a

Means ± SE followed by the same letter within a column are not significantly different at $P < 0.05$ (the same as for Tables 2 and 3) the B_0 treatment in 2016 (Table 1b). Additionally, the pH value, available P, SOC, total N and *C*/*N* ratio in B_4, B_5 and B_6 treatments were higher in 2016 as compared to the respective treatments in 2011.

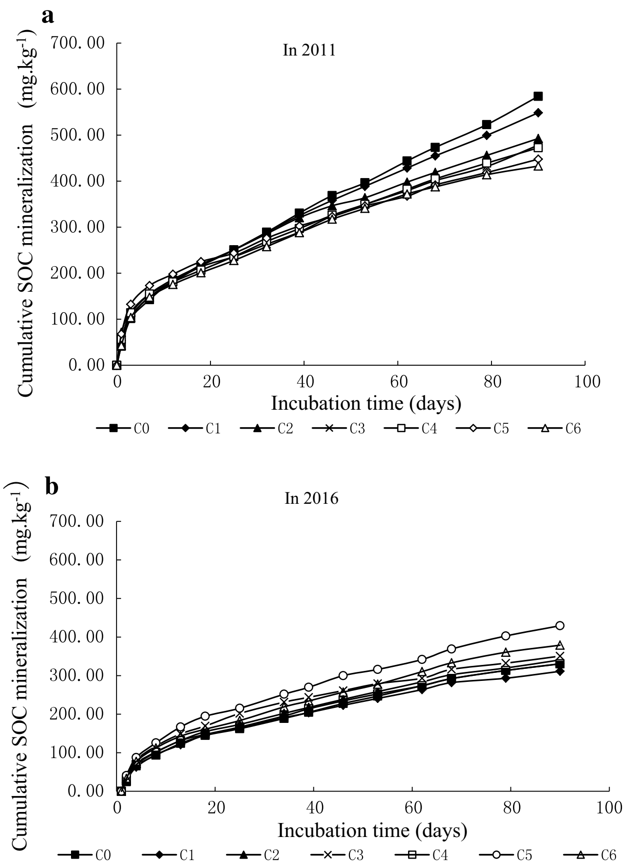

Fig. 1 The dynamics of cumulative SOC mineralization in upland red soil under different biochar applications in **a** 2011 and **b** 2016

The characteristics of SOC mineralization in upland red soil

The curve slopes varied markedly with incubation times, which exhibited rapid SOC mineralization rates at the beginning of the measurement period and slower thereafter, in all treatments. The mineralization rates initially differed strongly between all treatments, but substantially decreased within 7 days of incubation and were constant after 30 days (Fig. 1a, b). In the 1st year after biochar addition into the upland red soil, the highest initial mineralization rate was measured in B_5 treatment with 30 t/ha biochar application (Fig. 1a). With the extension of incubation time until the 39th day, the B_0, B_1 and B_2 treatments had higher mineralization rates than the other treatments (Fig. 1a).

In the 6th year after biochar application to upland red soil, the mineralization rates in the B_5 and B_6 treatments were significantly higher than in the other treatments (Fig. 1b). The data showed that mineralization rates in 2016 significantly decreased as compared to rates in 2011.

The cumulative SOC mineralization (C_m) means the sum of daily amounts of SOC release during the whole incubation time, which tended to increase with incubation time. 432.78–584.28 mg/kg SOC release was measured for individual soil samples for the 1st year after biochar application to upland red soil, representing 4.62–6.91% of total organic carbon (Table 2). The treatments with biochar application had significantly lower C_m than the B_0 treatment ($p < 0.05$, Table 2). In addition, the C_m decreased with increasing rate of biochar application in red soil, where the B_6 treatment decreased strongly by 25.93% compared with the respective control (B_0 treatment) (Table 2). For the 6th year after biochar application to upland red soil, the C_m varied from 311.20 to 428.71 mg/kg over the incubation time, which was 2.85–4.38% of total organic carbon (Table 2). The fluctuation among these treatments was not significantly different compared with the B_0 treatment, aside from the B_5 and B_6 treatments. The C_m in all treatments showed decreasing trends in 2016 as compared to that in 2011.

Table 2 Cumulative SOC mineralization values measured at different treatments in 2011 and 2016

Treatments	2011			2016		
	C_m (mg/kg)	C_t (g/kg)	C_m/C_t (%)	C_m (mg/kg)	C_t (g/kg)	C_m/C_t (%)
B_0	584.28 ± 0.99a	8.46 ± 0.25bc	6.91 ± 0.21a	330.67 ± 22.61cd	7.55 ± 0.04e	4.38 ± 0.33a
B_1	548.66 ± 8.52b	8.37 ± 0.15c	6.56 ± 0.19b	311.20 ± 13.88d	7.44 ± 0.19e	4.18 ± 0.08a
B_2	491.41 ± 1.25c	8.29 ± 0.29c	5.94 ± 0.21c	339.98 ± 15.09cd	8.47 ± 0.39d	4.03 ± 0.01ab
B_3	478.36 ± 5.88d	8.36 ± 0.05c	5.72 ± 0.06c	346.94 ± 13.42c	8.71 ± 0.07d	4.02 ± 0.18ab
B_4	472.52 ± 4.32d	8.78 ± 0.29b	5.39 ± 0.16d	328.1 ± 21.03cd	10.48 ± 0.37c	3.16 ± 0.31c
B_5	447.76 ± 3.61e	9.70 ± 0.19a	4.92 ± 0.13e	428.71 ± 18.70a	11.36 ± 0.36b	3.78 ± 0.04b
B_6	432.78 ± 4.64f	8.80 ± 0.02b	4.62 ± 0.04f	376.80 ± 24.55b	13.31 ± 0.11a	2.85 ± 0.21c

C_m the cumulative SOC mineralization, C_t the total organic carbon

The ratio of cumulative SOC mineralization to total organic carbon (C_m/C_t) reflected the capacity of soil mineralization and could mask the variations in total organic carbon changes in different conditions (Chen et al. 2008a, b). It showed a significant decreasing trend in treatments with biochar application relative to the B_0 treatment both in 2011 and 2016 (Table 2). However, there existed a widely decreased C_m/C_t ratio for all treatments in 2016 as compared to that in 2011 (Table 2).

Persistent effect of biochar application on soil-resistant carbon in upland red soil

For the 1st year after biochar application to upland red soil, the soil-resistant carbon (C_r) varied from 26.24 to 37.74% of total organic carbon, where significant increases were observed in the B_3, B_4, B_5 and B_6 treatments compared with the B_0 treatment (Table 3). Moreover, there was a general increasing trend for the content of C_r with increasing rates of biochar application. For the 6th year after biochar application to upland red soil, the ratio of C_r:C_t varied from 36.18 to 53.13% and the B_5 treatment had the highest value. The content of C_r in treatments with biochar application, which increased with increasing biochar application, was significantly higher than in the control (B_0) treatment (Table 3). As compared to 2011, there were different enhancements of the C_r for all seven treatments in 2016, where the B_5 and B_6 treatments strongly increased by 55.13 and 67.77%, respectively (Table 3).

Relationships between cumulative SOC mineralization, resistant carbon and biochar

A correlation analysis was carried out to verify the persistent effect of biochar on cumulative SOC mineralization (C_m) and soil-resistant carbon (C_r) in upland red soil (Table 4). There was a significant positive correlation between the biochar application rate and C_r content (the correlation coefficients were 0.898 for 2011 and 0.617 for 2016). However, there was a highly significant negative correlation between the biochar application rate and the ratio of C_m/C_t in 2011 and 2016 (the correlation coefficients were − 0.892 for 2011 and − 0.908 for 2016). Also, the relationships between C_m content, the ratio of C_r/C_t and biochar were very significant ($r_1 = -0.859$, $r_2 = 0.848$) in 2011 but not significant in 2016 (Table 4).

Discussion

Persistent effect of biochar on SOC mineralization in upland red soil

Ameloot et al. (2013) reported that SOC mineralization in all treatments showed an initial flush, after which the CO_2 flux continued at much slower rates, similar to our results (Fig. 1). This is because the labile or volatile components in soil are rapidly degraded, followed by slow to negligible degradation of the stable components (Ameloot et al. 2013; Zimmerman et al. 2011). Also, soil's rewetting has been shown to induce bursts in microbial activity and increasing C mineralization (Bengtsson et al. 2003; Borken and Matzner 2009), which would have resulted in high SOC decomposition. Indicators such as biochars' properties, types of soils and interactions between biochars and soils also play important roles in SOC mineralization (Zimmerman et al. 2011). After biochar application to upland red soil in 2011, the initial mineralization rate in the B_5 treatment was the highest among all treatments,

Table 4 Relationships between cumulative SOC mineralization, resistant carbon and biochar in upland red soil

	Indicators	C_m	C_r	C_m/C_t	C_r/C_t
Biochar	2011	− 0.859**	0.898**	− 0.892**	0.848**
	2016	0.209	0.617**	− 0.908**	0.099

**Correlation is significant at 0.01 level

Table 3 Soil-resistant carbon measured at different treatments in 2011 and 2016

Treatments	2011			2016		
	C_r (g/kg)	C_t (g/kg)	C_r/C_t (%)	C_r (g/kg)	C_t (g/kg)	C_r/C_t (%)
B_0	2.22 ± 0.02c	8.46 ± 0.25bc	26.24 ± 0.01d	2.73 ± 0.31d	7.55 ± 0.04e	36.18 ± 0.04b
B_1	2.24 ± 0.10c	8.37 ± 0.15c	26.80 ± 0.03d	3.02 ± 0.13bc	7.44 ± 0.19e	40.60 ± 0.01ab
B_2	2.42 ± 0.08bc	8.29 ± 0.29c	29.23 ± 0.01cd	3.12 ± 0.51bc	8.47 ± 0.39d	37.09 ± 0.08b
B_3	2.64 ± 0.21b	8.36 ± 0.05c	31.57 ± 0.02bc	3.34 ± 0.17bc	8.71 ± 0.07d	46.4 7 ± 0.02b
B_4	3.11 ± 0.40a	8.78 ± 0.29b	35.50 ± 0.05ab	3.70 ± 0.11b	10.48 ± 0.37c	38.38 ± 0.01b
B_5	3.41 ± 0.11a	9.70 ± 0.19a	35.08 ± 0.01ab	5.29 ± 0.73a	11.36 ± 0.36b	53.13 ± 0.05a
B_6	3.32 ± 0.05a	8.80 ± 0.02b	37.74 ± 0.01a	5.75 ± 0.62a	13.31 ± 0.11a	43.22 ± 0.05ab

C_r the soil-resistant carbon, C_t the total organic carbon which was consistent with total organic carbon content. This may be related to the plant residues and nutrient transport in soil. Previous study had also found that the growth of rapeseed and sweet potato was significantly higher in treatments with large amount of biochar amendment (≥ 30 t/ha) than that in no biochar treatment (Liu et al. 2014). So there would be plenty of plant residues providing nutrient and labile organic carbon for the process of SOC mineralization (Xiao et al. 2015). Besides, Chen et al. (2008a, b) had reported that the total organic carbon pool had significant positive correlation with mineralization rate. Furthermore, Luo et al. (2011) had reported that biochar application can affect the abundance and activity of soil microorganisms, which are critical to soil function and ecosystem services, in turn affecting soil structure and stability, nutrient cycling, aeration, water use efficiency and C storage capacity (Lehmann et al. 2006; Chen et al. 2015). After 39 days of incubation, the mineralization rates in B_4, B_5 and B_6 treatments were lower than B_0 treatment. This may be explained that biochar surfaces can adsorb many SOC (Kuzyakov et al. 2009) and therefore reduce its availability. Biochar in this study produced at 500 °C contain a large proportion of condensed aromatic structures (Yuan et al. 2011), which contribute to its greater recalcitrance (Li et al. 2013), thus decreasing the rate of SOC mineralization in later period of incubation. In consequence, SOC mineralization rates decreased sharply in soils with high rates of biochar application (B_4, B_5 and B_6 treatments).

There was a general trend of biochar application to soil leading to decreased ratio of C_m/C_t (Zimmerman et al. 2011). This effect in 2011 was more significant relative to 2016. The basic lower organic carbon (8.46 g/kg) in the present study led to a low active organic carbon content for soil mineralization and, as a consequence, a negative priming effect on the C_m. An interaction of microbial biomass carbon and SOC decomposition being restrained by biochar may be another explanation for such decreases in C_m/C_t in treatments with biochar application (Dempster et al. 2012; Kasozi et al. 2010). Liu et al. (2016) had reported that a high ratio of C/N with low microbial N availability in soils simultaneously suppressed soil C mineralization. Moreover, in soils amended with high rates of biochar, lower soil density and good aeration would play a crucial role in enhancing aerobic microbial activity and respiration rate after easily decomposed components (sugars, starches and amino acids) were quickly depleted. However, the differences in C_m/C_t between 2011 and 2016 in this study would be relative to the persistent effects of biochar in upland red soil. Biochar was incorporated into soil in the first year and no more was supplemented in subsequent years. Hence, plenty of labile organic substances were decomposed for soil respiration and microbial activity in the 1st year after biochar application to red soil. In the following years, available nutrients may be the main limiting factors for SOC mineralization. On the other hand, changes in the microbial community composition or enzyme activities may be responsible for lower mineralization of SOC in 2016 compared with 2011. Also, in the present study, the mechanism of biochar's long-term negative effect on SOC mineralization in upland red soil could not be elucidated (Freddo et al. 2012).

Persistent effect of biochar on resistant carbon in upland red soil

The content of C_r could reflect the stability of SOC (Yan et al. 2012), which was around 26–46% of total SOC in 2011 and 2016. This was due to the great deal of stable SOC via organo-mineral interactions (Keith et al. 2011; Lin et al. 2012; Singh and Cowie 2014). There was an obvious increasing trend for C_r and the ratio of C_r/C_t with increasing rate of biochar application in 2011 and 2016. It was primarily explained that a large amount of promoted plant residue with abundant difficult-to-decompose humus retained in red soil (Jeffery et al. 2011; Liu et al. 2013). The factors affecting SOC mineralization also are relevant to C_r. For instance, the structure of biochar surfaces, the microbial biomass carbon and the ratio of C/N in soil would all affect C_r content. In particular, the smaller pore size but higher micropore volume of the biochar surface had potential to adsorb SOC and coalesce into soil aggregates (Liang et al. 2010). In addition, the higher levels of C/N in soil amended with biochar (Table 1) displayed a limiting effect on microbial activities and benefit to carbon sequestration.

Relationships between cumulative SOC mineralization, resistant carbon and biochar in upland red soil

The biochar-derived C storage has been considered possible for hundreds of years in Terra Preta from the Amazon Basin (Lehmann et al. 2006; Woolf and Lehmann 2012), which would suggest a longtime effect on crop growth and soil physicochemical properties even after a single amendment in field experiments (Major et al. 2010; Haefele et al. 2011; Lentz and Ippolito 2012; Zhang et al. 2012). The significant positive correlation between the rate of biochar application and C_r both in 2011 and 2016 would be related to increased adsorption of SOC on biochar pore surfaces and to improved soil aggregation as well (Oguntunde et al. 2004; Gaskin et al. 2010; Van Zwieten et al. 2010). Furthermore, a significantly higher content of SOC after biochar amendment would reduce the ratio of O:C that is suitable for C sequestration in soil (Luo et al. 2016). The rate of biochar application and C_m/C_t had a highly significant negative correlation both in 2011 and 2016. This was primarily responsible for the persistent improvement of red soil properties when amended with biochar.

Conclusions

Incorporation of biochar can persistently improve basic properties of upland red soil, where this effect in 2016 was better than that in 2011. The soil pH and available P in the B_5 and B_6 treatments were distinctly higher than in the B_0 treatment in 2011, and the pH, available P, total N and *C*/*N* ratio in B_4, B_5 and B_6 treatments were significantly higher in comparison with B0 treatment in 2016.

The C_m content was significantly decreased by biochar amendment, and the ratio of C_m/C_t showed declining trends with the rates of biochar application increasing in upland red soil during 2011 and 2016. There were decreasing trends for C_m and C_m/C_t in 2016 as compared to 2011. Also, the C_r occupied around 26–46% of total SOC and showed a clearly increasing trend with increasing rate of biochar application in 2011 and 2016. Significantly higher C_r in 2016 was observed as compared to that in 2011. Consequently, amendment with large amount of biochar (40 t/ha) may be considered as a good strategy for persistently enhancing soil carbon sequestration and reducing CO_2 emission in upland red soil. Furthermore, this effect was stronger in 2011 than in 2016.

Overall, the C_m/C_t showed highly significant negative correlations with the rates of biochar application both in 2011 and 2016. However, the C_r showed significant positive correlations with the rates of biochar application both in 2011 and 2016.

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