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RESEARCH ARTICLE

Effects of biochar and maize straw on the short-term carbon and nitrogen dynamics in a cultivated silty loam in China

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Abstract Application of maize straw and biochar can potentially improve soil fertility and sequester carbon (C) in the soil, but little information is available about the effects of maize straw and biochar on the mineralization of soil C and nitrogen (N). We conducted a laboratory incubation experiment with five treatments of a cultivated silty loam, biochar produced from maize straw and/or maize straw: soil only (control), soil + 1 % maize straw (S), soil + 4 % biochar (B1), soil + 4 % biochar + 1 % maize straw (B1S), and soil + 8 % biochar + 1 % maize straw (B2S). CO_2 emissions, soil organic C, dissolved organic C, easily oxidized C, total N, mineral N, net N mineralization, and microbial biomass C and N of three replicates were measured periodically during the 60-day incubation using destructive sampling method. C mineralization was highest in treatment S, followed by B2S, B1S, the control, and B1. Total net CO_2 emissions suggested that negative or positive priming effect may occur between the biochar and straw according to the biochar addition rate, and biochar mineralization was minimal. By day 35, maize straw, irrespective of the rate of biochar addition, significantly increased microbial biomass C and N but decreased dissolved organic N. Biochar alone, however, had no significant effect on either microbial biomass C or N but decreased dissolved organic N. Mixing the soil with biochar and/or straw significantly increased soil organic C, easily oxidized C and total N contents, and decreased dissolved organic N content. Dissolved organic C contents showed mixed results. Notably, N was immobilized in soil mixed with straw and/or biochar, but the effect was stronger for soil mixed with straw, which may cause N deficiency for plant growth. The application of biochar and maize straw can thus affect soil C and N cycles, and the appropriate proportion of biochar and maize straw need further studies to increase C sequestration.

Keywords Biochar · Maize straw · Mineralization · Carbon fraction · Nitrogen fraction · Priming effect

Introduction

Soil organic matter (SOM) is an important source of nutrients for maintaining soil fertility for crop production. Optimum levels of soil organic carbon (SOC) are needed to retain water and nutrients, improve soil structure, and provide energy for soil microbes (Lal 2004). Various practices of soil management can maintain or increase SOC content, including the application of plant residues to soil (Bauer and Black 1994). Periodic inputs of crop residues as labile organic matter would directly increase C contribution, change SOC availability, and affect the turnover of the soil C sink, thereby influencing the fixation of soil C and the fluxes of greenhouse gases (Smith 2004). The increase in C contribution, however, would be minor and limited to the top 5 cm of soil in the first several years, and most of the C would be lost by leaching and in CO_2

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emissions (Novak et al. 2009). The increasing CO_2 released to the atmosphere is harmful to the environment (Ciais et al. 2013) and impedes the sustainable development of agriculture (Lichtfouse 2009). In addition, crop residues may temporarily delay the immobilization of soil nutrients and soil warming and increase the risk of disease (Procházková et al. 2003). The addition of biochar to soil may contribute to the reduction of these emission problems and promote soil fertility, due to its highly stable C and its inorganic nutrient content (Filiberto and Gaunt 2013; Lehmann et al. 2006; Sohi et al. 2010).

Biochar, the by-product of biomass pyrolysis, has received much attention for its ability to alter soil chemical, biological, and physical properties and for its effects on plant growth and climate change (Lehmann et al. 2011; Smider and Singh 2014; Steiner et al. 2007) due to its high pH, cation exchange capacity (CEC), and SOC content and recalcitrant nature (Jeffery et al. 2011; Kumar et al. 2013; Lehmann et al. 2011). Biochar is very stable in soil. Matovic (2011) calculated that biochar would continue to be a C sink for two centuries when added at a rate of 13.5 t ha^{-1} (3:100 biochar/soil) and that biochar could improve soil fertility and maintain chemical stable for a millennium. Lu et al. (2014) also suggested that biochar addition was an effective measure as a stable C sink. Biochar, however, is not indestructible in soil; some mineralizable biochar fractions can be rapidly incorporated into bacteria, leading to the loss of C (Hamer et al. 2004). Biochar can also have an impact on the decomposition of other forms of organic matter, such as native SOM. Wardle et al. (2008) found that biochar promoted native SOM decomposition, although the extent may have been affected by the quantity of biochar added. Prayogo et al. (2014) found that an addition rate of 0.5 % had no effect on C mineralization, but a rate of 2 % combined with litter decreased mineralization by 20 %. Zhang et al. (2012), however, reported that biochar addition to a rice paddy reduced greenhouse gas emissions, but the effects did not differ significantly between rates of addition of 10 and 40 t ha^{-1}. The rate of biochar addition may have a threshold for reducing the emission of greenhouse gases. A suitable rate of application to substantially decrease emissions of greenhouse gases remains unknown, however, due to the complex properties of soil and biochar.

Ogbonnaya et al. (2014) stated that SOM decomposition would likely be reduced by physical sorption in the pores or on the surface of biochar, but complex interactions appeared when labile organic matter was added. Both the stimulation and/or suppression of the decomposition of labile organic matter by biochar have been observed (Zavalloni et al. 2011; Zimmerman et al. 2011). Kuzyakov et al. (2009) showed that C mineralization increased with the addition of glucose. Liang et al. (2010) found that organic residues applied to soil amended with biochar decreased total C mineralization, because the residues were rapidly incorporated into aggregates. Keith et al. (2011) reported that biochar increased the retention of labile organic matter, but the labile organic matter increased biochar-C mineralization. The interactive effects of biochar on C mineralization are complex and may depend on the time since biochar addition, the temperature at which biochar is produced, origins of feedstock, soil textures, local environmental conditions, and management practices (Ennis et al. 2012; Luo et al. 2011; Woolf and Lehmann 2012). Zimmerman et al. (2011) observed both positive and negative interactive effects of biochar on mineralization in sandy soils.

Biochar addition can also alter soil processes involving nitrogen (N), because soil C and N availability and N turnover rates are strongly linked (Cayuela et al. 2014; Prayogo et al. 2014). N dynamics following biochar application, however, was variable due to the characteristics and quantity of biochar applied, soil conditions, and timescales (Prayogo et al. 2014; Wu et al. 2012). Song et al. (2013) found that biochar addition decreased soil inorganic N content more than uncharred biomass due to its highly porous surface by decreasing N mineralization (Knoblauch et al. 2011) or by the sorption of nitrogenous compounds on the biochar (Castaldi et al. 2011; Dempster et al. 2011). Castaldi et al. (2011), however, reported that biochar addition increased net N mineralization, although a 3-year field trial showed that biochar addition had no long-term effects on N mineralization (Jones et al. 2012). Mineralizable N fractions did not increase in biochar treatments after 127 days of incubation (Schomberg et al. 2012). Biochar, though, can increase N retention and improve N use efficiency by improving the quality of agricultural soil (Clough and Condron 2010). Further study is still needed to investigate the effects of biochar addition on N dynamics and the underlying mechanisms.

Applying biochar to agricultural soils can clearly be beneficial to soil fertility and agronomic gains because of its impacts on soil physicochemical properties and supply of nutrients available to plants (Novotny et al. 2009). Biochar addition, however, would increase the interactions with periodic inputs of crop residues, either retained on or added to fields after harvest. Any stimulation of the decomposition of native SOC or crop residues would decrease C sequestration induced by the biochar. The impact of biochar on soil C and N cycling thus requires more study before large-scale application is adopted. Little data are yet available on the effects of biochar on the decomposition of native SOC or crop residues. Our current understanding of the effects of applying biochar or exogenous labile organic matter to soil on C and N cycling is poor. We also do not know whether and how biochar interacts with labile organic matter and if biochar has any priming effect on C or N dynamics.

We hypothesized that biochar would be fairly stable for short-term applications to silty loam soil and that the mineralization of labile organic matter may vary with the quantity of biochar added. The main aims of this study were to (1) determine the effect of biochar and/or maize straw on C and N mineralization in cultivated silty loam soil, (2) evaluate the interactive effects induced by applications of maize straw with a high and low rate of biochar addition, and (3) quantify the changes in soil C and N fractions following the addition of biochar with or without maize straw.

Materials and methods

Characterization of the soil, biochar, and maize straw

Soil was sampled from study sites at the Changwu Agricultural and Ecological Experimental Station (35.28° N, 107.88° E; 1200 m a.s.l.) on the Loess Plateau in northwestern China. Removing the top soil of 5 cm, the soils were collected using a small front-ender loader in each plot without crops and then thoroughly mixed. The samples were passed through a 2-mm sieve to remove plant material and large aggregates and then air-dried. The soil was classified as a Cumuli-Ustic Isohumosol of the USDA classification system (Gong et al. 2007) and had a silty-loam texture. Soil C and N contents at 0–20 cm were 13.34 and 0.99 g kg^{-1}, respectively, with a C/N ratio of 20.02, and the pH was 7.89 (1:2.5 soil/water).

Maize straw harvested from the study sites was air-dried and crushed prior to use. The straw had total contents of C and N of 584.7 and 6.8 g kg^{-1} (85.99 C/N), respectively, and a pH of 6.72.

The biochar used in this experiment was produced from maize straw at a temperature of 450 °C. The biochar was not aged before use and was oven-dried at 105 °C for 24 h and then sieved to 2 mm prior to use and analysis. The biochar had the following characteristics: C content of 447.3 g kg^{-1}, N content of 9.8 g kg^{-1} (45.64 C/N), bulk density of 0.3 g cm^{-3}, and a pH of 9.8 (1:2.5 biochar/water).

Experimental design

We conducted an incubation experiment with five treatments of soil and different rates of biochar and maize straw: (1) soil only (control), (2) soil + 1 % maize straw (g straw g^{-1} soil, S), (3) soil + 4 % biochar (g biochar g^{-1} soil, B1), (4) soil + 1 % maize straw + 4 % biochar (B1S), and (5) soil + 1 % maize straw + 8 % biochar (B2S). The treatments were incubated in 108 plastic jars, 18 jars (10 g dry weight, three jars without soil, blank) for determining soil CO_2 emissions, and 90 jars (100 g dry weight) for determining soil C and N dynamics during the incubation. The biochar and straw were ground and sieved at 2 mm to increase the surface area/volume ratio and maximize the interactions with soil particles and microbes. The biochar and/or straw were then homogeneously mixed to an equivalent of 100 g of dry soil for each jar. The mixtures were wetted immediately before experiment initiation. The soil-water content was adjusted to 20 % (*w*/*w*) during incubation by weighing and adding distilled water. The jars were arranged in a random block design and incubated in the dark at 25 °C.

C mineralization

This C mineralization incubation included two parts, one part for soil CO_2 emission (nine samples each time) and the other part for C fractions determination (three samples each sampling time, six sampling times). One hundred grams of air-dried soil was placed in 250-mL plastic jars; three replicate subsamples were moistened to 20 % soil-water content. The CO_2 released was trapped in vials containing 10 mL of 1.0 mol L^{-1} NaOH placed inside the jars. Another vial containing 10 mL of water was also placed in each jar to maintain humidity. The vials containing NaOH were replaced with new vials on days 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 37, 45, and 52 of the incubation and were removed on day 60. The trapped CO_2 was precipitated with 1.0 mol L^{-1} $BaCl_2$ and then titrated with standard 0.1 mol L^{-1} HCl to quantify the released CO_2.

Three replicates of each treatment were prepared in another 90 jars (five treatments three replicates, six sampling times) in total. Microbial biomass C (MBC), SOC, easily oxidized C (EOC), and dissolved organic C (DOC) contents were measured during incubation. MBC content was determined with the fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Fresh soil samples fumigated with chloroform and un-fumigated samples (12.5 g) were extracted with 50 mL of 0.5 mol L^{-1} K_2SO_4, and the extracts were analyzed with a Phoenix 8000 TOC analyzer (Teledyne Tekmar, Mason, USA). MBC was calculated as the difference in extractable organic C between the fumigated and unfumigated soil using a conversion of 0.45 (Wu et al. 1990). DOC content was measured with distilled water: an air-dried subsample (10.0 g) was extracted with 50 mL of distilled water, and the extracts were analyzed as for the DOC. EOC content was measured with 333 mmol L^{-1} $KMnO_4$, based on the difference between subsamples and the blank (only $KMnO_4$) to calculate the EOC content (Lefroy et al. 1993).

N mineralization

Three replicate jars were destructively sampled for mineral N analysis on days 1, 2, 6, 14, 21, and 35. Soil NO_3^--N and NH_4^+-N were extracted with 50 mL of 1 mol L^{-1} KCl for each subsample (equal to 5.0 g of air-dried soil, 10:1 KCl solution/soil). The samples were shaken for 1 h (200 rpm) at room temperature and filtered through Φ12.5-mm filter paper. The extracts were analyzed with an automatic flow analyzer (FIAstar 5000 Flow Injection Analyzer, Warrington, UK).

Soil dissolved organic N (DON) was measured colorimetrically (Krom 1980) using the DOC extracts and Kjeldahl digestion (Brookes et al. 1985). Microbial biomass N (MBN) was extracted from the MBC extracts using Kjeldahl digestion and was measured colorimetrically (Krom 1980). Total N (TN) content was quantified using an automatic Kjeltec 2300 analyzer unit (FOSS, Hoeganaes, Sweden).

Data analyses

Calculations

Net C mineralization ($C_{min\ net}$) was calculated as

$$C_{min\ net} = (C_{min\ treatment} - C_{min\ control}) \Big/ (C_{soil} + C_{treatment}) \quad (1)$$

where $C_{min\ treatment}$ is the total amount of CO_2 emission from soil treated with biochar and/or maize straw, $C_{min\ control}$ is the total amount of CO_2 emission from the control, and C_{soil} and $C_{treatment}$ are the SOC content and the C content of the corresponding maize straw/biochar treatment, respectively (Ameloot et al. 2013).

Net N mineralization ($N_{min\ net}$) was calculated as

$$N_{min\ net} = (N_{min\ treatment} - N_{min\ control}) \Big/ (N_{soil} + N_{treatment}) \quad (2)$$

where $N_{min\ treatment}$ and $N_{min\ control}$ are the amounts of mineral N at the end of incubation for a straw/biochar treatment and the control, respectively, and N_{soil} and $N_{treatment}$ are the N contents of the soil and the corresponding straw/biochar treatment, respectively (Ameloot et al. 2013).

Statistical analyses

Data obtained from the incubation experiment were expressed as means of three replicate incubations ± SE. All data were tested for homogeneity of variance and normality of distribution. One-way analysis of variance (ANOVA) was used to evaluate the effects of biochar and maize straw on all the measured parameters at each sampling period, followed by a Tukey's post hoc test at $P < 0.05$ using IBM SPSS Statistics 19 (SPSS Inc., Chicago, USA).

Results

C mineralization

The rate of CO_2 emission and cumulative CO_2 emission for all five treatments had similar trends during the 60 days of incubation (Fig. 1a, b). The emission rate was rapid at the beginning of incubation and decreased with incubation time (Fig. 1a). The CO_2 emission rates, highest on the first day of incubation, were 193.68, 874.70, 182.09, 842.09, and 734.05 mg C kg^{-1} soil day^{-1} for control, S, B1, B1S, and B2S, respectively. A rate of 4 % biochar without straw had no significant effect on the emission rate relative to the control ($P < 0.05$). The addition of straw to biochar-treated or biochar-untreated soil produced similar large increases in emission rate. The CO_2 emission rate was significantly higher in S than in B1S and B2S during the first several days but did not differ among the three treatments by day 60.

The cumulative CO_2 emission did not differ significantly between the control and B1 during the incubation (Fig. 1a, Table 1), suggesting that biochar mineralization by itself was probably minimal, and a rate of 4 % biochar had no priming effect on CO_2 emission in the biochar-amended system. The cumulative CO_2 emission, however, was significantly lower in B1 than in S, B1S, and B2S (Fig. 1b, Table 1; $P < 0.05$). The corrected cumulative emission was higher in biochar-untreated soil (S minus control) than in biochar-treated soil (B1S minus B1), suggesting that appropriate biochar addition suppresses the decomposition of straw. The cumulative emission was significantly lower in B2S than in S in the first 10 days of incubation, and the difference decreased thereafter. The cumulative emission was higher in B2S than in S after 60 days of incubation. S, however, still had the highest net amount of mineralized C ($C_{min\ net}$), followed by B2S, B1S, and B1 by 60 days of incubation (Table 1). The total net emitted CO_2 was significantly lower in B1S than in S and B1 combined, indicating a possible negative priming effect on C mineralization between maize straw and biochar added at appropriate rates (Table 1).

N mineralization

The mineral N contents of the soils treated or untreated with biochar and maize straw are shown in Fig. 2. Mineral N decreased significantly and rapidly after the addition of biochar or/and straw, perhaps due to N adsorption and retention (Prayogo et al. 2014). Mineral N did not change significantly with incubation time in the control and B1. Mineral N in soils with straw had the same trend. Mineral N decreased significantly due to the presence of straw in soils treated or untreated with biochar ($P < 0.05$) and decreased to approximately 10 % of the initial mineral N content in S, B1S, and B2S. These results suggested that the straw had a greater effect than biochar on mineral N and therefore produced a significantly lower $N_{min\ net}$. The addition of biochar increased mineral N in soils mixed with straw after 35 days of incubation, with no significant difference of $N_{min\ net}$ between B1S and B2S, suggesting that adding more biochar did not correspondingly stimulate higher N mineralization in the soils with straw (Table 1).

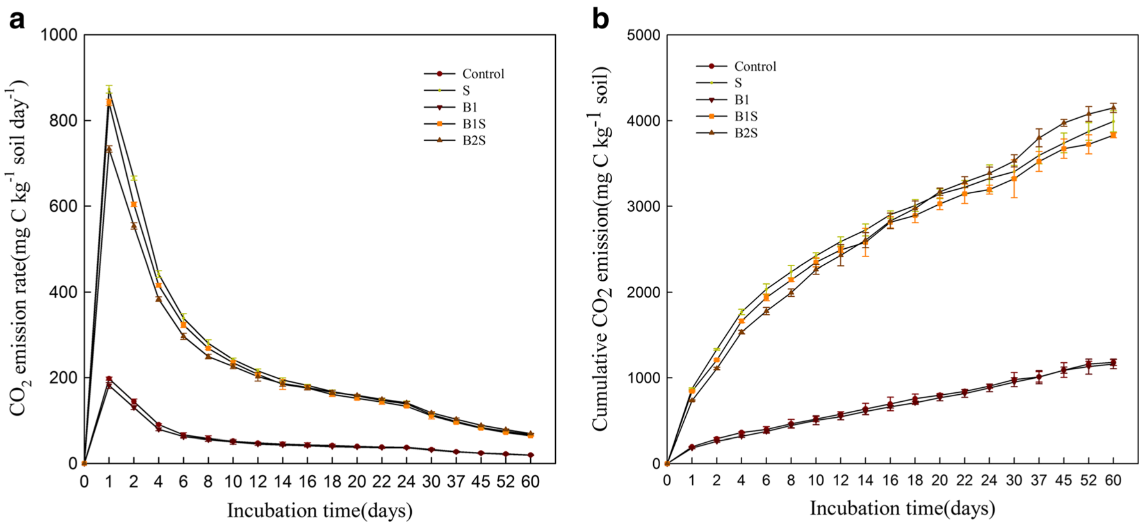

Fig. 1 CO_2 emission rate (**a**) and dynamics of cumulative CO_2 emission (**b**) during incubation under different treatments (n = 3; *control*, soil only; *S*, maize straw; *B1,* biochar; *B1S*, 1 % maize straw + 4 % biochar; *B2S*, 1 % maize straw + 8 % biochar)

MBC and MBN contents

MBC was highest on day 1 of incubation in all treatments and decreased significantly thereafter (Fig. 3). A rate of addition of 4 % biochar without straw had no significant effect on MBC, except on day 6 of the incubation. The addition of straw, however, significantly increased MBC with and without biochar on day 1 and thereafter. Soil treated only with straw tended to have the highest MBC (115.41–140.14 mg C kg^{-1} soil), followed by B1S (86.54–142.80 mg C kg^{-1} soil) and B2S (75.49–141.86 mg C kg^{-1} soil) beginning on day 6. Biochar addition at a rate of 8 % with straw contributed less to MBC than did 4 % biochar with straw.

Similar to MBC, MBN was significantly higher on day 1 than on day 2. MBN in soil mixed with biochar did not differ significantly from the control only at the beginning of incubation. The addition of straw to soils with and without biochar increased MBN significantly compared with the control over the first 35-day period ($P < 0.05$). The addition of biochar at a rate of 4 % with straw did not increase MBN.

SOC, DOC, and EOC contents

Additions of biochar and/or maize straw significantly increased SOC but with no significant change over a 35-day period (Fig. 4). SOC increased most in soil containing 8 % biochar and 1 % straw; the mean SOC content was nearly fivefold higher than in the control, followed by B1S, B1, and S ($P < 0.05$). DOC was also affected by the biochar and/or straw ($P < 0.05$, Fig. 4b), although the contents differed with incubation time. DOC in B1 decreased significantly on day 1 and did not vary significantly thereafter but remained significantly lower than in the control, suggesting a negative effect of biochar without straw on the decomposition of SOM. DOC was highest in S by day 35 and was significantly higher than in B1S and B2S. The straw had a significantly greater effect on the increase in DOC; adding biochar at either rate decreased the effect. Similar to SOC and DOC, EOC was significantly affected by straw and/or biochar, despite the variability ($P < 0.05$). The treatments with straw with or without biochar had significantly higher EOC contents by day 35 compared to the control.

Table 1 The emitted carbon content by CO_2 (C_{min}) and N content (N_{min}) and the net C mineralization ($C_{min\ net}$, Eq. (1)) and net N mineralization ($N_{min\ net}$, Eq. (2)) at the end of the mineralization experiment

Treatment	C_{min} (mg kg^{-1})	$C_{min\ net}$ (%)	N_{min} (mg kg^{-1})	$N_{min\ net}$ (%)
Control	1190.99 ± 5.25d	–	39.56 ± 0.41a	–
S	4043.72 ± 20.81b	20.49 ± 1.01a	3.86 ± 0.02d	−3.58 ± 0.29b
B1	1164.40 ± 14.94d	−0.18 ± 0.02c	34.75 ± 0.37b	−0.47 ± 0.04a
B1S	3824.43 ± 12.16c	16.76 ± 0.52b	5.18 ± 0.06c	−3.32 ± 0.35b
B2S	4140.49 ± 23.04a	16.85 ± 0.74b	4.86 ± 0.07c	−3.22 ± 0.40b

Different lowercase letters in the same column indicate treatment means significantly different at $P < 0.05$. Means ± SE (n = 3)

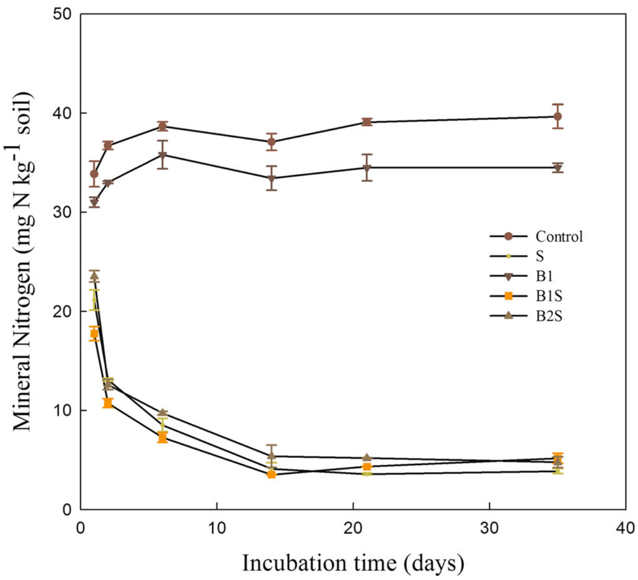

Fig. 2 Dynamics of mineral nitrogen during incubation in all treatments (n = 3; *control*, soil only; *S*, maize straw; *B1*, biochar; *B1S*, 1 % maize straw + 4 % biochar; *B2S*, 1 % maize straw + 8 % biochar)

TN and dissolved organic N contents

The addition of biochar and/or maize straw significantly increased TN by 0.05–0.57 g kg^{-1} and significantly decreased DON (Fig. 5, $P < 0.05$). TN content in the treatments was in the order B2S > B1S > B1 > S > control, independent of incubation time. Adding biochar without straw produced a much higher increase in TN and a smaller decrease in DON than adding straw without biochar by day 35, perhaps partly due to the high N content and minimal mineralization of biochar. Similar to mineral N, DON content did not differ significantly among the straw treatments with or without biochar after 35 days of incubation.

Discussion

CO_2 emissions

Biochar or organic residue can strongly affect soil CO_2 emissions (Badía et al. 2013; Wu et al. 2012), but in our study, the application of biochar had no effect on cumulative CO_2 emissions during incubation (Fig. 1), which was consistent with the results showed by Wang et al. (2014). Increased cumulative CO_2 emission may be contributed to increased pools of labile organic C (Uchida et al. 2012), so the low amount of labile organic C in our experiment might be the most likely scenario of decreased cumulative CO_2 emissions in the biochar-alone treatment compared to the other treatments. Cross and Sohi (2011) found that biochar addition produced from sugarcane bagasse at 350 and 450 °C decreased SOM decomposition in grassland with high soil C content (3.64 %). Kuzyakov et al. (2009) similarly reported that biochar produced from *Lolium* shoot litter at 400 °C could decrease the decomposition of native SOM from an initial rate of 0.05 to 0.0013 % day^{-1} after 2 or 3 months. These studies suggested that the decreased decomposition of SOC might be attributed to the high porosity and large surface area/volume ratio of biochar which would help to adsorb SOM on the biochar surface or absorb it in the pores, which could decrease the availability of the SOM and isolate it from microbes, thereby decreasing C mineralization (Brewer et al. 2009; Zimmerman et al. 2011). Biochar would increase soil organic C stability due to increases in C–C/C–H and COOH groups during the incubation, indicating that organic C was bonded to the biochar (Darby et al. 2016).

Cumulative CO_2 emissions in treatments added with maize straw in the presence or absence of biochar were higher (Fig. 1). This finding was consistent with that reported by Wu et al. (2012), who found a significant increase in soil

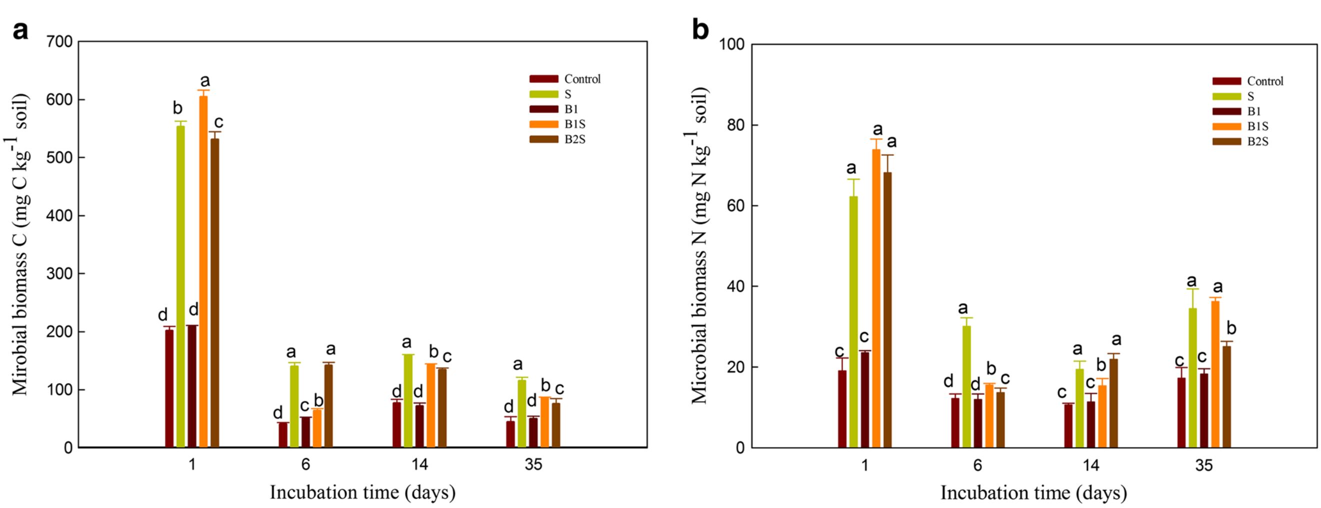

Fig. 3 Microbial biomass carbon (**a**) and microbial biomass nitrogen (**b**) in different treatments on different sampling dates during the incubation. Means ± SE of three replicates. Treatments: *control*; *S*, 1 % maize straw; *B1*, 4 % biochar; *B1S*, 1 % maize straw + 4 % biochar; *B2S*, 1 % maize straw + 8 % biochar. *Different letters* for each incubation period indicate significantly different treatment means at $P < 0.05$

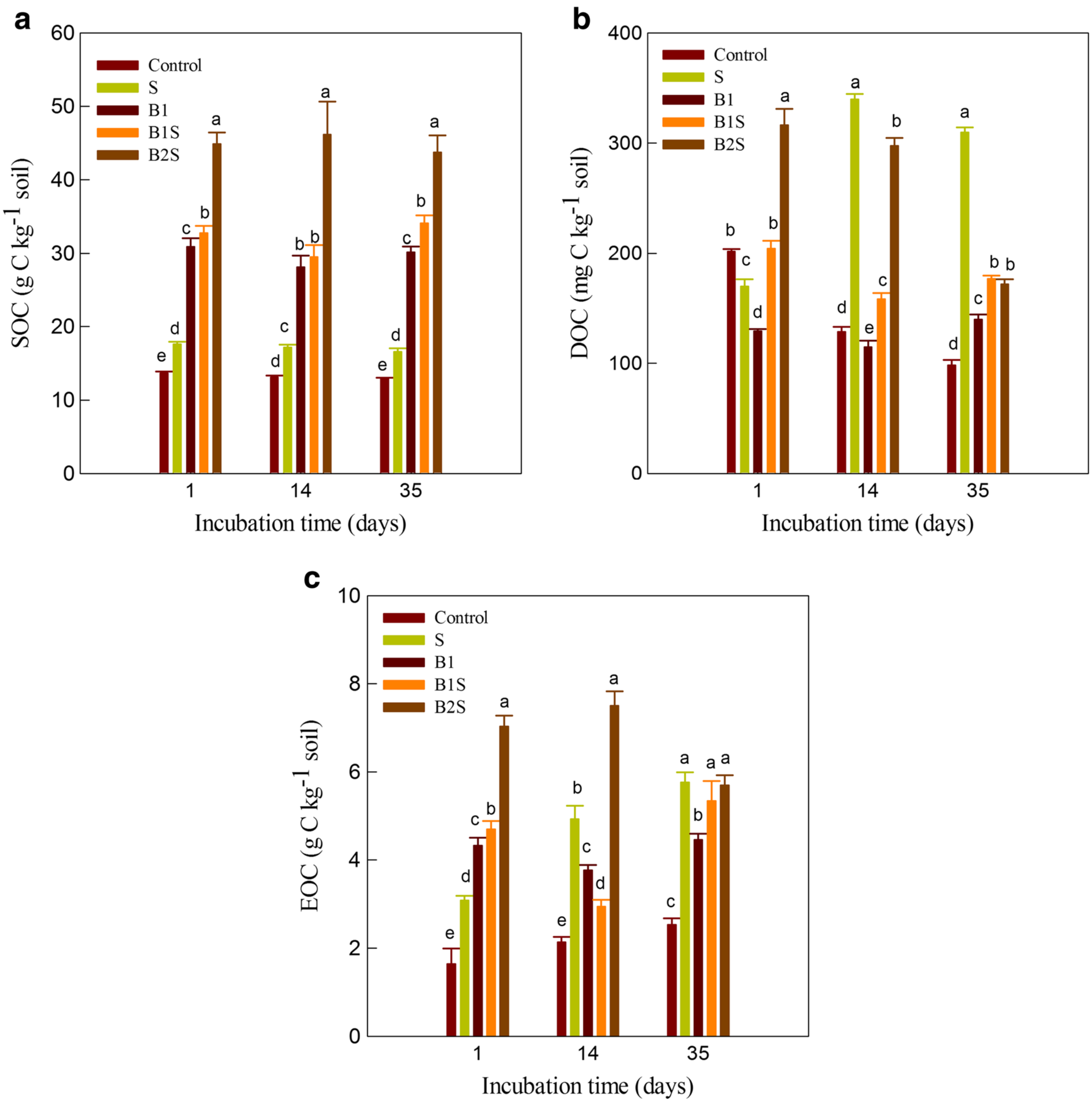

Fig. 4 Soil organic carbon (SOC), dissolved organic carbon (DOC), and easily oxidized carbon (EOC) on different sampling dates during incubation. *Different letters* for each incubation period indicate significantly different treatment means at $P < 0.05$ ($n = 3$)

CO_2 flux after application of bamboo leaves. Similar results were also reported by Zavalloni et al. (2011) and Kuzyakov et al. (2009). The labile organic C derived from the maize straw may have created favorable conditions for soil microorganisms, which subsequently increased soil respiration and cumulative CO_2 emissions (El-Naggar et al. 2015; Usman et al. 2013). The increase in MBC, indicating the growth of soil microorganisms after application of maize straw, might also contribute to the increase in cumulative CO_2 emission (Zavalloni et al. 2011). Adding 4 % biochar to straw-treated soil, however, decreased CO_2 emission, suggesting the immobilization of soil organic C and decrease in microbial activity due to the sorption of organic nutrients to the biochar (Darby et al. 2016; Zimmerman et al. 2011). This proposal was questioned by Steiner et al. (2004), who reported that application of biochar and easily decomposable matter would not affect the rate of CO_2 emission but could immobilize SOM by increasing microbial biomass.

Adding more biochar in the presence of maize straw altered the dynamics of the priming effect. Cumulative CO_2 emission was higher in B2S than in S on day 60 after being significantly lower in the first 10 days of incubation (Fig. 1b). Similarly, change was also observed in the study of adding glucose to biochar-treated soil. Adding glucose would increase biochar mineralization several fold within a few weeks and then not differ from the control thereafter (Hamer et al. 2004; Kuzyakov et al. 2009). It suggested that the effect may have temporarily contributed to co-metabolic priming after glucose addition and would decrease during exposure (Naisse et al. 2015), along with the priming effect, and microbial biomass

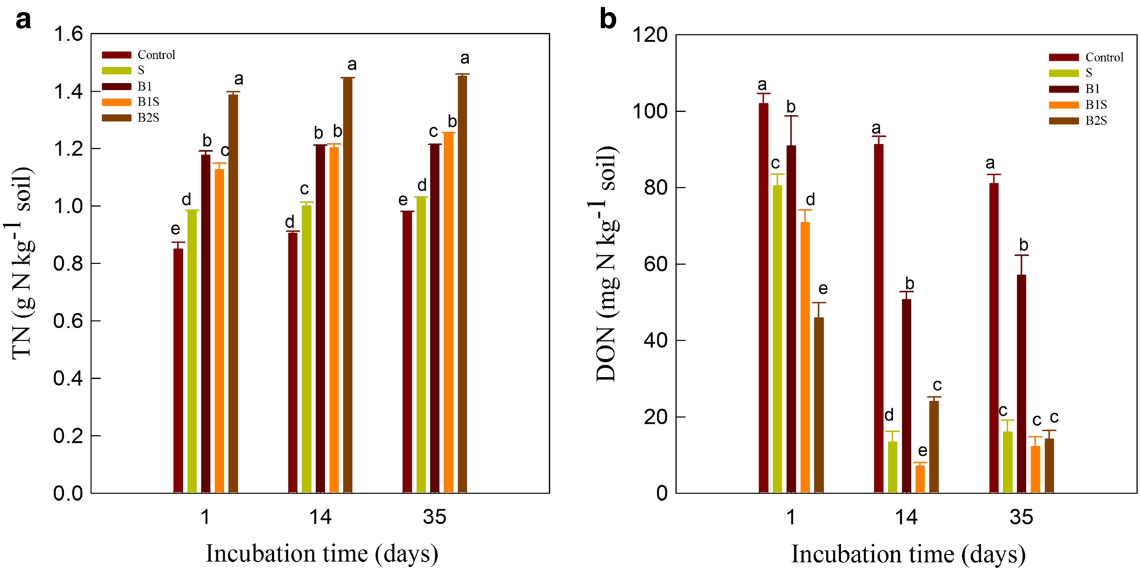

Fig. 5 Soil total nitrogen (TN) (**a**) and dissolved organic nitrogen (DON) (**b**) under different treatments on different sampling dates during incubation. *Different letters* for each incubation period indicate significantly different treatment means at $P < 0.05$ ($n = 3$)

and soluble C contents increased (Hamer et al. 2004). This shifting effect has been previously reported (Zimmerman et al. 2011), suggesting that the direction of the priming effect on C mineralization may change with the dominant mechanism in the long term. The responses of mineralization to the addition of biochar with or without labile organic matter are thus more chemically complex and require further investigation, especially in an agro-ecosystem where fresh residues are periodically added.

N mineralization

The effect of biochar and crop residues on soil N mineralization has received less attention than the effect on C mineralization. Our results showed that biochar addition in the presence or absence of maize straw decreased the accumulation of mineral N (Fig. 2, Table 1), consistent with previous studies (Novak et al. 2010; Prayogo et al. 2014; Zavalloni et al. 2011), and this effect can be extended to decreasing mineral N by leaching (Singh et al. 2010). Interestingly, our results showed that biochar addition alone significantly decreased mineral N concentrations but had no effect on C mineralization (Table 1). Previous studies showed that a C/N ratio of approximately 20 was a threshold for N mineralization and immobilization in SOM (Calderon et al. 2015). The C/N ratios of biochar and straw in this study were 45.64 and 85.99, respectively, suggesting that N immobilization would occur when biochar and maize straw were applied into the soil. Wu et al. (2012) also showed that a high rate of added straw would immobilize inorganic N. Mineral N decreased quickly in the treatments containing straw within 14 days, which may be contributed to the net N immobilization induced by the microbial immobilization (Figs. 2 and 3b), resulting in the added available C which could not be matched by a sufficient amount of added available N (Deenik et al. 2010). Yao et al. (2012) and Zheng et al. (2013) also reported that biochar could decrease the amount of N available to plants. The effects of biochar on N depend on the conditions of soil quality and biochar property. Fast-pyrolysis biochar applied to soil immobilized mineral N, but slow-pyrolysis biochar produced a net mineralization of N over a 65-day period (Bruun et al. 2012). Maize straw can temporarily immobilize N in soil (Recous et al. 1995), so crop residues may provide more available C to microbes and increase the microbial demand for N. In addition, $N_{min\ net}$ did not differ among S, B1S, and B2S treatments containing straw (Table 1), suggesting that soil mineral N would not be affected by the rate of biochar addition in soil amended with straw. Consistent with the decrease in mineral N, DON decreased in soil amended with crop residues (Fig. 5b, Prayogo et al. 2014), resulting in a large increase in the soil soluble C/N ratio (data not shown), indicating the increase of immobilization of soil available N.

Soil C and N fractions

Only application of biochar would not affect soil microbial biomass significantly, though the amount of MBC and MBN was different (Fig. 3). An analysis of phospholipid fatty acids indicated that biochar addition in the absence of crop residues had no effect on microbial biomass (Prayogo et al. 2014). Biochar likely had no significant effect on soil microbial activity, or any effect may have been negated by increased aggregation or microbial toxicity when added to the cultivated silty loam soil (Hamer et al. 2004; Verheijen et al. 2010). Previous studies showed that stimulated by microbial growth, treatments with crop residues in the presence or absence of biochar had higher contents of microbial biomass and soluble C and N, suggesting that the addition of decomposable organic matter to soil would increase the availability of C to microbes more than biochar (Bruun et al. 2008; Prayogo et al. 2014). ^{14}C-biochar was not detected in the MBC after 20 days of incubation (Bruun et al. 2008), and only 2.5 % of the biochar C was assimilated by microbes after a longer incubation of 624 days (Kuzyakov et al. 2009). The rate of biochar addition would also not contribute to microbial activity in the presence of maize straw (Fig. 3). Zavalloni et al. (2011) found that the addition of biochar at a rate of 0.5 % had no significant effect on MBN in an incubation experiment, and a high C/N ratio of the biochar suggested that the biochar would not act as a N source for microbial organisms (Zhang et al. 2014). Our results were also in agreement with those of Durenkamp et al. (2010), who reported that biochar changed the amount of MBC and MBN extracted with 0.5 M K_2SO_4 and determined by the fumigation-extraction method. Moreover, fumigation-extraction methods may underestimate microbial biomass due to absorption in the biochar micropores (Liang et al. 2010). An initial increase in microbial activity, however, would gradually decrease with incubation time, and the depletion of the easily available compounds over time would decrease microbial biomass by the end of the incubation (Fig. 3). MBC content, consistent with the CO_2 flux, decreased gradually with incubation time along with a higher consumption of labile organic C, indicating a positive correlation between MBC and the rate of CO_2 emission ($R^2 = 0.99$, $P < 0.01$).

It is widely accepted that organic materials applied into soil would alter soil labile organic C fractions (Singh et al. 2007; Emmerling et al. 2002). Our results showed that concentrations of soil DOC and EOC were significantly higher in the treatments containing straw in the presence or absence of biochar than in the control (Fig. 4), indicating that crop residues have a higher amount of easily decomposable substrates, partially due to the decomposition of biochar, as also reported in an incubation study of 84 days (Wengel 2006). Similarly, wheat straw combined with biochar significantly increased soil labile organic carbon, while biochar or wheat straw alone increased it less than the combination (Zavalloni et al. 2011). EOC, increased after maize straw and biochar addition, might be stimulated by increase in soil microorganism activity and quantity. In our study, DOC content was highest in treatment-added maize straw (S), except at the first day, and DOC was significantly lower in the soils treated with biochar (Fig. 4), indicating that biochar may encapsulate DOC in the pores or adsorb it on the surface (Pietikäinen et al. 2000). An increase in labile organic carbon by maize straw was exceeded by a decrease in biochar encapsulation (Zimmerman et al. 2011), in agreement with a study where biochar did not increase microbial biomass (Zavalloni et al. 2011).

The SOC and TN contents in this silty loam after treatment with biochar increased significantly and did not decrease significantly after 35 days of incubation (Figs. 4a and 5a), suggesting some recalcitrance to microbial mineralization. Other studies have also reported a similar resistance to biochar in soil (Liang et al. 2006; Novak et al. 2010). SOC content in our study was not correlated with short-term cumulative CO_2 emission ($R^2 = 0.46$, $P = 0.09$) indicating that the relationship between SOC and cumulative CO_2 emission was complex. The addition of biochar alone did not stimulate cumulative CO_2 production, but straw addition significantly increased cumulative CO_2 emission (Fig. 1a, $P<0.05$). Easily decomposable substrates in the maize straw may initially be mineralized by microbes (Novak et al. 2010).

Conclusions

The incubation experiment found that the addition of maize straw and biochar affected soil C and N cycling. The addition of biochar alone significantly decreased cumulative CO_2 emission and increased SOC content, but the addition of straw alone increased net C mineralization compared to the control. The addition of straw combined with different rates of biochar showed variable effects on cumulative CO_2 emissions. Biochar alone significantly decreased mineral N while the addition of maize straw increased N immobilization, thus decreasing the tendency of N leaching and interfering with the use of N by plants. This possibility requires further investigation and mechanistic understanding. Further studies are also required for understanding the appropriate proportion of biochar and straw to reduce cumulative CO_2 emissions and increase C sequestration in silty loam soil before it can be incorporated into the soil as a long-term C sink.

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