



Interactions between biochar and litter priming: A three-source ^{14}C and $\delta^{13}\text{C}$ partitioning study



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ABSTRACT

Although it has been separately reported that biochar primes the decomposition of soil organic matter (SOM) or fresh organic matter, little is known about the simultaneous effects of biochar on SOM versus plant litter mineralization. We applied dual $^{13}\text{C}/^{14}\text{C}$ isotopic labels to partition soil CO_2 efflux and C pools into three sources: SOM, litter and biochar. Biochar made by slow pyrolysis (400 °C) of ^{14}C labeled residues of rice (*Oryza sativa*, C3) and maize (*Zea mays*, C4) litter were added separately or in combination to a silty Fluvisol with a C3 isotopic signature and incubated at 25 °C over a period of 6 months. Biochar decomposition was very slow, with a mean rate of 0.017% d^{-1} . Approximately 63% of biochar-derived CO_2 was produced in the first month. Mixing with litter reduced biochar mineralization by 14%. Addition of biochar alone to soil induced a cumulative positive priming effect (0.24 mg C g^{-1} soil) on SOM decomposition over 183 days, a much smaller effect than litter-induced priming (1.05 mg $\text{CO}_2\text{-C g}^{-1}$ soil). Compared to soils with only litter amended, biochar and litter added in combination decreased SOM mineralization by 19% while increasing litter mineralization by 6.9%, with no net changes in total CO_2 release. Increased litter- but not SOM-derived C in microbial biomass in the presence of biochar suggested that biochar caused preferential microbial utilization of litter over SOM. Given that immobilization of mineral N in the soil-litter mixture was markedly enhanced following the addition of biochar, we proposed that the biochar-induced preferential microbial utilization of litter over SOM was due primarily to alterations in N cycling. In conclusion, the priming effects of litter on SOM are changed by the presence of biochar.

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1. Introduction

Biochar, the product of low-temperature pyrolysis of organic matter, represents an inert form of C that is capable of persisting in soils or sediments for hundreds to thousands of years (Sohi et al., 2010; Kuzyakov et al., 2014). It has been suggested that converting biomass or plant residues to biochar represents an effective means of locking up otherwise easily decomposable C into a stable sink, thus reducing atmospheric CO_2 concentrations (Lehmann,

2007). Incorporation of biochar into soil has been shown to have many beneficial effects, such as increasing crop yield and mitigating soil nutrient losses (Sohi et al., 2010; Spokas et al., 2012; Clough et al., 2013). Therefore, interest in the use of biochar as a soil additive has been expanding because of its dual benefits of C sequestration and soil fertility improvement (Sohi et al., 2010).

However, biochar amendment may also induce changes in the decomposition of native soil organic matter (SOM), i.e. the “priming effect” (Kuzyakov, 2010), which must be carefully evaluated prior to the implementation of large-scale biochar application. Contradictory priming effects of biochar on SOM have been reported (Zimmerman et al., 2011; Maestrini et al., 2015; Wang et al., 2016). By synthesis of results from soil incubations with biochar, Maestrini et al. (2015) suggested that the addition of biochar resulted in a short-term

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positive priming effect of 0.32 mg C g⁻¹ soil on SOM (that is, mineralization of SOM was increased by 15%), whereas Wang et al. (2016) concluded that biochar addition had a mainly negative priming effect (-8.6% within 6 months) on SOM decomposition. The short-term priming of soil C mineralization as a result of biochar addition was generally related to the labile C fraction of biochar (Cross and Sohi, 2011; Maestrini et al., 2015; Wang et al., 2016), which would lead microbes to switch their food sources from SOM to the new C inputs, thereby resulting in a negative priming effect (Whitman et al., 2014). Alternatively, the labile C of biochar may benefit microbes capable of decomposing SOM and thus lead to positive priming effects (Singh and Cowie, 2014). The directions and magnitudes of the priming effects, caused by biochar addition, depend on a variety of factors, including feedstock and pyrolysis temperatures, the contents and quality of SOM, and soil nutrient availability, among many others (Blagodatskaya and Kuz'yakov, 2008; Fontaine et al., 2011; Maestrini et al., 2015; Wang et al., 2016).

As most research on biochar-induced priming effects treated the decomposition of bulk SOM as a whole (Maestrini et al., 2015; Wang et al., 2016), the heterogeneous soil C pools (e.g., litter and organic matter at various stages of decomposition) have rarely been differentiated in the priming. The dynamics of organic matter decomposition of different chemical characteristics might be affected to various extents by biochar (Farrell et al., 2015). According to Maestrini et al. (2015), short-term biochar addition had mainly positive priming effects on SOM (i.e. relatively old soil C) decomposition but negative priming effects on fresh organic matter like rhizodeposits or plant litter. However, responses of SOM and litter mineralization to biochar addition have not been studied simultaneously, despite the likelihood that complicated interactions exist between the decomposition processes of biochar, SOM, and litter. Although a few studies have reported that biochar addition to soil-plant systems might reduce the priming of SOM mineralization by the plant rhizosphere (Whitman et al., 2014; Keith et al., 2015; Weng et al., 2015), these studies failed to differentiate between the effects of biochar on C fluxes deriving from SOM and those from plant because of the difficulty of partitioning the three C sources (biochar, SOM, and plant); furthermore, they only included plant rhizosphere exudates but not the more recalcitrant plant residues. If biochar is widely used as a soil amendment, it will most likely be mixed with crop residues, microbial or animal necromass, and other types of organic residues. Given that different forms of C pools are of different relevance to soil CO₂ emissions, nutrient transformations, and microbial activities (von Lütow et al., 2008; Zeller and Dambrine, 2011; Chen et al., 2014), knowledge about the responses of various C fractions in soil to biochar is necessary for accurate assessments of the possible consequences of biochar application.

Our goal here was to explore the responses of SOM versus litter mineralization in response to biochar amendment in a soil mixed with maize straw over an incubation period of 6 months. We followed a three-source partitioning approach using dual isotopic labels (¹³C and ¹⁴C) developed by Blagodatskaya et al. (2011) to partition the decomposition of biochar, litter and SOM. The specific objectives were to (1) compare the priming effects of biochar versus maize straw on SOM decomposition when they were added separately, (2) differentiate the effects of biochar addition on SOM versus maize litter in soil-maize mixtures, and (3) provide insights into the possible consequences of biochar amendment on different soil organic pools.

2. Materials and methods

2.1. Soils and sampling

Soil used in this experiment was sampled from the plow layer

(Ap horizon, 0–10 cm) of a typical paddy rice field located in northern Jiangsu Province, China. This region receives 1000 mm of precipitation annually, and has an average temperature of 14 °C. The soil had a silty texture (silt: 87.93%; clay: 3.47%) and was tentatively classified as a Fluvisol. Soils collected from the field were passed through a 2-mm sieve, thoroughly homogenized, and handpicked to remove visible plant residues for incubation. Basic soil properties are listed in Table 1.

2.2. Production and chemical analyses of ¹⁴C labeled biochar

¹⁴C labeled biochar was produced from rice leaves uniformly labeled with ¹⁴C. Labeling procedures were the same as those used by Ge et al. (2012), in which young rice seedlings were grown in an air-tight glass chamber (80 cm × 250 cm, height 120 cm) for about 2 months. The ¹⁴CO₂ atmosphere inside the chamber was maintained via reactions between Na₂¹⁴CO₃ (1.6 × 10⁴ μg ml⁻¹ and 16.5 × 10³ Bq ml⁻¹) and HCl (2 M) in plastic beakers placed beside the rice seedlings. Atmospheric CO₂ concentration was maintained at ~350 ppm via repeated supplementation with Na₂¹⁴CO₃ every 2–3 d. Following completion of labeling, the shoots were cut off at the stem base of rice seedlings, oven-dried at 70 °C, and stored at 4 °C.

For the production of ¹⁴C-enriched biochar, leaves of the ¹⁴C labeled rice were ball-milled, homogenized by passing through a 2-mm sieve, and then tightly filled into closed steel crucibles (iØ/ oØ = 46/50 mm × 40 mm high, wall thickness of 2 mm). The crucibles were then put into a muffle furnace, which was slowly heated at a rate of 4.3 °C min⁻¹ from 20 °C to 400 °C, kept at 400 °C for 10 h, and then switched off and left to cool to room temperature. After the charring process, the mass of the biochar product was only 31.7 ± 1.1% of the initial mass of the rice leaves. The biochar was milled and passed through a 0.5-mm sieve prior to incubation with soils.

The ¹⁴C specific activity of biochar was determined based on a protocol described by Wu and O'Donnell (1997). Briefly, about 0.04 g of biochar was digested at 165 °C for 8 min in a solution composed of 20 ml 0.2 M potassium dichromate mixed with 30 ml concentrated H₂SO₄ + H₃PO₄ (5:1, v:v). The ¹⁴CO₂ evolved during the digestion was trapped in 40 ml 0.4 M NaOH. To measure the ¹⁴C activity of trapped ¹⁴CO₂, a 1 ml aliquot of NaOH was mixed with 9 ml of RIA cocktail (Beckman, Germany) and counted for 5 min on a liquid scintillation counter (LS-6500, Beckman, Germany), following the process described by Ge et al. (2012).

The pH was measured using a pH electrode at a biochar:water ratio of 1:15 (following Luo et al., 2011) rather than the 1:5 ratio for soil, considering the generally high adsorption capacity of biochar (Sohi et al., 2010). For analyses of water-extractable components, biochar was extracted four times with deionized water at a biochar:water ratio of 1:12, and the extracts were combined. The concentration of NH₄⁺ in the water extracts was determined using the phenol-hypochlorite method (Lu, 1999), and NO₃⁻ was determined via reaction with VCl₃ using the method of Doane and Horwath (2003). Total dissolved organic C in biochar extracts was measured with a TOC analyzer (Multi N/C 2100, Analytik Jena, Germany), and total C and N contents of the biochar solid were measured using an elemental analyzer (FlashEA 1112 NC analyzer, Thermo, Italy). The δ¹³C value of biochar was determined by an isotope ratio mass spectrometer (IRMS; MAT 253, Thermo Finnigan, USA) with a precision of ±0.10‰, and expressed as following:

$$\delta^{13}\text{C}(\text{‰}) = \left[\left(R_{\text{sample}} / R_{\text{V-PDB}} \right) - 1 \right] \times 1000 \quad (1)$$

where R_{sample} and $R_{\text{V-PDB}}$ are the ¹³C/¹²C ratios of biochar and the

Table 1
Basic properties of biochar, soil, and maize straw.

	Biochar ^a	Soil ^b	Maize straw ^c
Total C (%)	40.6 ± 0.10	1.95 ± 0.03	45.4 ± 0.05
Total N (%)	5.38 ± 0.00	0.191 ± 0.001	1.41 ± 0.01
C:N ratio	7.56 ± 0.03	10.2 ± 0.01	32.3 ± 0.22
pH	8.04 ± 0.04	6.82 ± 0.10	ND
NH ₄ ⁺ content ^a (mg N g ⁻¹)	0.074 ± 0.004	0.003 ± 0.0003	ND
NO ₃ ⁻ content ^a (mg N g ⁻¹)	0.002 ± 0.0006	0.054 ± 0.004	ND
DOC ^b	2.43 ± 0.19	0.050 ± 0.004	ND
Specific ¹⁴ C activity (Bq mg ⁻¹)	10.2 ± 0.75	ND	ND
δ ¹³ C (‰)	-25.8 ± 0.22	-27.3 ± 0.21	-12.3 ± 0.33

^a NH₄⁺, NO₃⁻, and DOC of biochar were measured in water extracts.

^b DOC of soil was measured in 0.05 M K₂SO₄ extracts; NH₄⁺ and NO₃⁻ were extracted by 2 M KCl.

^c ND: not determined.

Vienna Pee Dee Belemnite (V-PDB) standard (Dawson et al., 2002), respectively.

2.3. Experimental layout and incubation conditions

To investigate the priming effects of biochar addition on the decomposition of SOM and litter, an incubation experiment was conducted over 183 days with four treatments (three replicates per treatment): (1) control, i.e. Soil with no addition, (2) Soil + Maize (in the form of maize straw), (3) Soil + Biochar, and (4) Soil + Maize + Biochar. Finely milled maize straw (leaves) or biochar prepared as above were thoroughly mixed at 2% with moist soils (35 g, oven-dry basis). The mixtures were adjusted to 60% water holding capacity (WHC) by addition of deionized water. To determine maximum WHC, soil mixtures were packed in the same way as during the incubation into a Büchner funnel, with the bottom of the funnel covered with filter paper to prevent the loss of soil or biochar/maize particles. Soil mixtures in the funnel were saturated with deionized water and left to drain gravimetrically for 24 h before being oven-dried to determine the water content (i.e. WHC). Additional water of 0.02 and 0.07 ml g⁻¹ mixture was added to treatments Soil + Biochar and Soil + Maize + Biochar, respectively, compared to Soil + Maize and Soil only. For incubation, each sample was placed in a 50-ml glass vial and incubated in a 1.2-l glass jar, with a vial containing 20 ml 1 M NaOH to trap the evolved CO₂ and 5 ml of water in the base of the jar to maintain soil moisture. Four blank jars containing only water and NaOH were also set up as above. The jars were sealed with a rubber bung and incubated at 25 °C for 183 days. The NaOH vials were periodically sampled and replaced with fresh vials on days 1, 3, 8, 15, 31, 62, 93, and 183.

2.4. Isotopic and chemical analyses

On each sampling day, the collected NaOH solution was treated in the following way to allow simultaneous determination of the ¹⁴C and ¹³C signatures of the evolved CO₂. First, a 5-ml aliquot was removed from the collected 20-ml NaOH solution and stored for later measurement of ¹⁴C activity on a scintillation counter (LS-6500, Beckman, Germany). The remaining 15 ml of the NaOH solution was then precipitated with excess 1 M SrCl₂ and titrated with 0.5 M HCl to determine the total CO₂ trapped by NaOH. The precipitate (SrCO₃) was washed with 50 ml deionized water and centrifuged at 9000 rpm, a process that was repeated three times to obtain clean SrCO₃ for ¹³C analysis. The SrCO₃ precipitate was dried at 50 °C prior to isotopic analysis using a MAT 253 IRMS equipped with Kiel IV Carbonate Device (Thermo Scientific, USA; precision: ±0.04‰). The ¹⁴C-CO₂ activity in the NaOH solution was measured following the procedures described by Ge et al. (2012) and

expressed in the unit of DPM (decay per minute).

Soil microbial biomass C (MBC) at the end of the incubation was determined using the chloroform fumigation–extraction method (Wu et al., 1990). The incubated soils were destructively sampled and thoroughly mixed, following which 10 g of moist soil from each sample was extracted with 40 ml 0.05 M K₂SO₄; another 10 g soil was first fumigated with chloroform for 24 h in a vacuum desiccator and then also extracted with 40 ml 0.05 M K₂SO₄. A 5-ml aliquot of the K₂SO₄ extracts was used for the analysis of total C concentrations using a TOC analyzer (Multi N/C 2100, Analytik Jena, Germany). Another 5-ml aliquot was frozen for later use in the analysis of ¹⁴C activity on a liquid scintillation counter (LS-6500, Beckman, Germany). The remaining 30 ml K₂SO₄ extracts were dried at 80 °C, with the resultant K₂SO₄ powder used to determine the ¹³C composition of MBC on an IRMS (MAT 253, Thermo Finnigan, USA).

Soil pH was measured at a soil:water ratio of 1:5 using a pH electrode; NH₄⁺ and NO₃⁻ concentrations in the water extracts were determined as described in Section 2.2 (Lu, 1999; Doane and Horwath, 2003). Total soil C and N contents were measured with an elemental analyzer (FlashEA 1112 NC analyzer, Thermo, Italy).

2.5. Calculations and statistics

We followed the approach developed by Blagodatskaya et al. (2011) to partition CO₂ efflux or K₂SO₄-extractable dissolved organic C (DOC) in the Soil + Maize + Biochar treatment for the three sources (i.e. biochar, maize straw, and SOM). Briefly, we distinguished between C originating from biochar and C from maize/SOM based on ¹⁴C signals, and further partitioned maize- and SOM-derived C based on their δ¹³C signatures. Initially, the amount of biochar-derived C in a specific pool (CO₂ effluxes or DOC) was calculated based on the ¹⁴C activity in that pool:

$$C_{BC\text{-derived}} = \frac{(C_s - C_{bl}) \times 20}{^{14}C_{BC}/C_{BC}} \quad (2)$$

where $C_{BC\text{-derived}}$ (mg C) is the amount of biochar-derived C; C_s and C_{bl} (DPM) are the ¹⁴C activity in samples and the blank, respectively, in 1 ml NaOH solution; $^{14}C_{BC}$ (DPM g⁻¹ biochar) is the specific ¹⁴C activity of the biochar; and C_{BC} (mg C g⁻¹ biochar) is the C content of the biochar; 20 is the total volume (ml) of NaOH used to trap CO₂.

Next, the δ¹³C values (‰) of C originating from non-biochar sources (i.e. maize and SOM) were calculated based on the mass balance of stable isotopes:

$$\delta^{13}C_{\text{non-BC}} = \frac{\delta^{13}C_{\text{total}} \times C_{\text{total}} - \delta^{13}C_{BC} \times C_{BC\text{-derived}}}{C_{\text{total}} - C_{BC\text{-derived}}} \quad (3)$$

$$C_{\text{non-BC}} = C_{\text{total}} - C_{\text{BC-derived}} \quad (4)$$

Where $\delta^{13}\text{C}_{\text{total}}/C_{\text{total}}$ (mg C), $\delta^{13}\text{C}_{\text{non-BC}}/C_{\text{non-BC}}$ (mg C), and $\delta^{13}\text{C}_{\text{BC}}/C_{\text{BC-derived}}$ (mg C) are the $\delta^{13}\text{C}$ values/C contents of the total C pool (either NaOH-trapped CO_2 or DOC), C derived from non-biochar sources (maize and SOM), and biochar, respectively.

In the final step, maize- and SOM-derived C was calculated as follows:

$$C_{\text{MZ-derived}} = C_{\text{non-BC}} \times \frac{\delta^{13}\text{C}_{\text{non-BC}} - {}^{13}\text{C}_{\text{SOM}}}{\delta^{13}\text{C}_{\text{MZ}} - {}^{13}\text{C}_{\text{SOM}}} \quad (5)$$

$$C_{\text{SOM-derived}} = C_{\text{non-BC}} - C_{\text{MZ-derived}} \quad (6)$$

where $C_{\text{MZ-derived}}$ and $C_{\text{SOM-derived}}$ (mg C) refer to C derived from maize and SOM, respectively, and $\delta^{13}\text{C}_{\text{MZ}}$ and $\delta^{13}\text{C}_{\text{SOM}}$ are the $\delta^{13}\text{C}$ values of maize straw and SOM, respectively. Average $\delta^{13}\text{C}$ values of control soils at different sampling days were used as estimates of $\delta^{13}\text{C}_{\text{SOM}}$.

For treatments with only biochar or maize additions, C originating from SOM or biochar/maize was similarly calculated using Eqs. (2)–(6), except that some items for either biochar or maize were absent from the calculations. For K_2SO_4 -extractable DOC, no significant signals of ^{14}C activity could be detected and hence items related to biochar were not involved in the calculation. MBC derived from maize following incubation was calculated as follows:

$$\text{MBC}_{\text{MZ-derived}} = {}^f C_{\text{MZ-derived}} - {}^{\text{nf}} C_{\text{MZ-derived}} \quad (7)$$

$$\text{MBC}_{\text{SOM-derived}} = \text{MBC}_{\text{total}} - \text{MBC}_{\text{MZ-derived}} \quad (8)$$

where $\text{MBC}_{\text{total}}$, $\text{MBC}_{\text{MZ-derived}}$, and $\text{MBC}_{\text{SOM-derived}}$ ($\mu\text{g C g}^{-1}$ soil) represent the total MBC, MBC originating from maize, and SOM, respectively; ${}^f C_{\text{MZ-derived}}$ and ${}^{\text{nf}} C_{\text{MZ-derived}}$ ($\mu\text{g C g}^{-1}$ soil) are K_2SO_4 -extractable DOC deriving from maize in the fumigated and non-fumigated soils, respectively, which can be calculated using Eqs. (2)–(6).

The priming effect (PE) of biochar/maize-only addition on SOM decomposition was calculated as:

$$\text{PE} = \text{amended } C_{\text{SOM-derived}} - {}^{\text{CK}} C_{\text{SOM-derived}} \quad (9)$$

or expressed as relative PE (%):

$$\text{PE}\% = \frac{(\text{amended } C_{\text{SOM-derived}} - {}^{\text{CK}} C_{\text{SOM-derived}}) / {}^{\text{CK}} C_{\text{SOM-derived}}}{\times 100} \quad (10)$$

where $\text{amended } C_{\text{SOM-derived}}$ and ${}^{\text{CK}} C_{\text{SOM-derived}}$ are cumulative SOM-originating $\text{CO}_2\text{-C}$ (mg C g^{-1} soil) in soils amended with only biochar or maize and in control soils without additions, respectively.

PE of maize addition on biochar mineralization was calculated by:

$$\text{PE} = {}^{\text{MZ+BC}} C_{\text{BC-derived}} - {}^{\text{BC}} C_{\text{BC-derived}} \quad (11)$$

where ${}^{\text{MZ+BC}} C_{\text{BC-derived}}$ and ${}^{\text{BC}} C_{\text{BC-derived}}$ are cumulative biochar-originating $\text{CO}_2\text{-C}$ (% of total biochar-C input) in the Soil + Maize + Biochar and Soil + Biochar treatments, respectively.

For treatments Soil + Maize and Soil + Maize + Biochar, PE was calculated in order to examine the simultaneous effects of biochar addition on SOM versus maize straw mineralization:

$$\text{PE on maize} = {}^{\text{MZ+BC}} C_{\text{MZ-derived}} - {}^{\text{MZ}} C_{\text{MZ-derived}} \quad (12)$$

$$\text{PE on SOM} = {}^{\text{MZ+BC}} C_{\text{SOM-derived}} - {}^{\text{MZ}} C_{\text{SOM-derived}} \quad (13)$$

where ${}^{\text{MZ+BC}} C_{\text{MZ-derived}}$ / ${}^{\text{MZ+BC}} C_{\text{SOM-derived}}$ and ${}^{\text{MZ}} C_{\text{MZ-derived}}$ / ${}^{\text{MZ}} C_{\text{SOM-derived}}$ are cumulative maize-/SOM-derived $\text{CO}_2\text{-C}$ (mg C g^{-1} soil) in treatments Soil + Maize + Biochar and Soil + Maize, respectively.

One-way ANOVA was used to compare soil properties and C fluxes among all treatments, followed by Duncan's post-hoc test. .

3. Results

3.1. Release of total CO_2

Over the course of the 183 days of incubation, the cumulative amount of CO_2 released was $1.94 \pm 0.09 \text{ mg C g}^{-1}$ in the control treatment (i.e. soil without addition) (Fig. 1). Amendment with biochar increased the cumulative CO_2 to $2.46 \pm 0.04 \text{ mg C g}^{-1}$ ($P < 0.01$). The increase in total CO_2 induced by biochar addition was higher between days 31–183 (0.35 mg C g^{-1}) than during the first 30 days (0.17 mg C g^{-1}).

The two treatments with maize straw incorporation showed much greater cumulative CO_2 efflux ($8.38 \pm 0.20 \text{ mg C g}^{-1}$) than the control soils or soils amended with biochar only. However, there was no difference in the cumulative CO_2 efflux between Soil + Maize and Soil + Maize + Biochar throughout the entire incubation period ($P > 0.05$).

3.2. Biochar decomposition with and without maize litter addition

Biochar decomposition was calculated based on the $^{14}\text{CO}_2$ efflux. Small percentages (2.77–3.86%) of biochar were decomposed over the 183 day incubation period (Fig. 2a), with a mean decomposition rate of $0.017\% \text{ d}^{-1}$. Decomposition rates of biochar were the highest during the first 3 days ($0.42 \pm 0.20\% \text{ d}^{-1}$), particularly in the Soil + Biochar treatment, then decreased by an order of magnitude ($0.035 \pm 0.019\% \text{ d}^{-1}$) during days 8–31 (Fig. 2b), and remained around $0.0075 \pm 0.002\% \text{ d}^{-1}$ for the next 5 months. More than 60% of the total CO_2 produced from biochar was mineralized in the first 31 days.

Mixing with litter consistently decreased the cumulative

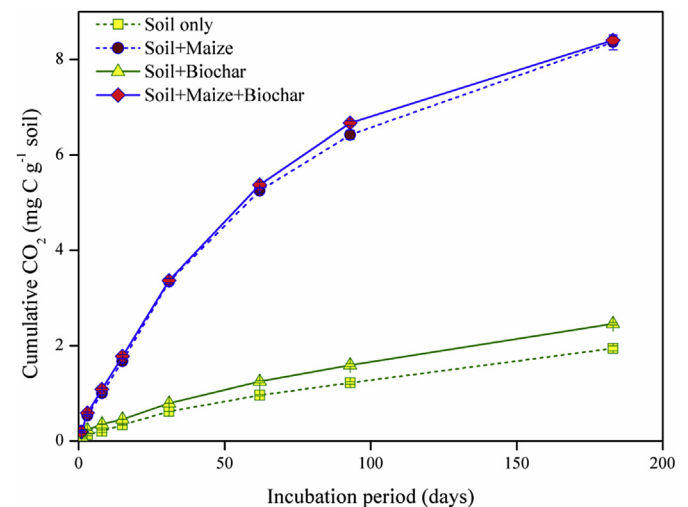


Fig. 1. Cumulative CO_2 respired throughout the incubation period (1–183 days). Error bars represent one SE ($n = 3$).

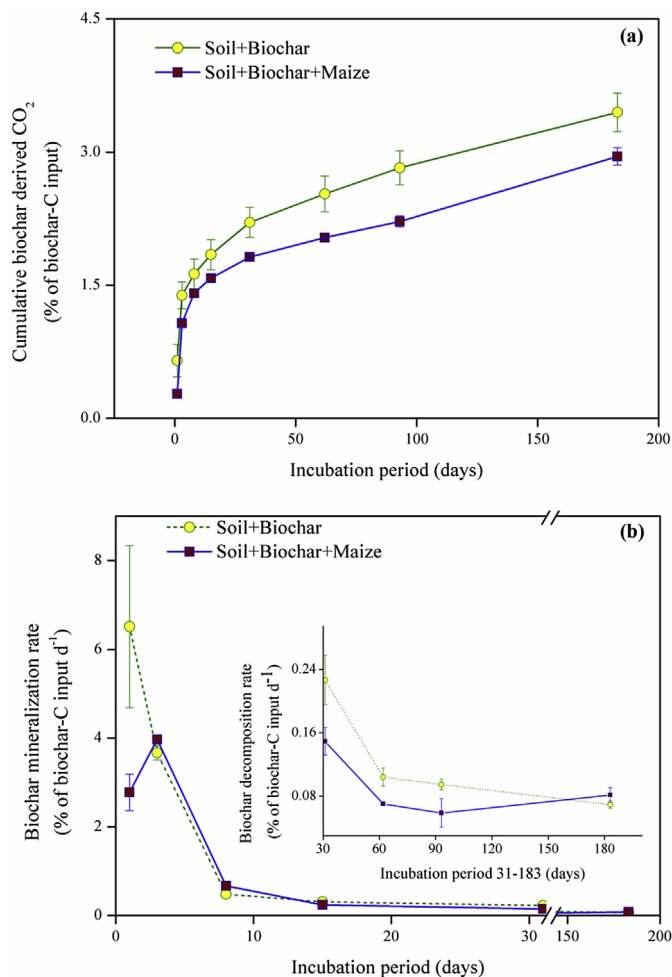


Fig. 2. Cumulative CO₂ derived from biochar (a) and mineralization rates of biochar-derived C (b). CO₂ is expressed as the percentage of total biochar C input. The inset in (b) shows biochar mineralization rates between days 31 and 183, with an expanded y-axis scale to improve clarity. Error bars represent one SE (n = 3).

decomposition of biochar (Fig. 2a); that is, litter addition resulted in a negative priming effect on biochar decomposition. At the end of the incubation period, biochar decomposition was 14% lower in the treatment containing litter than in the treatment without litter.

3.3. Decomposition of maize litter with and without biochar amendment

Litter contributed most of the CO₂ efflux from soils, accounting for up to $66 \pm 3.1\%$ of total CO₂ emissions. Over the entire incubation period, the cumulative CO₂ derived from litter was 5.34 ± 0.06 and 5.74 ± 0.01 mg C g⁻¹ soil in the Soil + Maize and Soil + Maize + Biochar treatments, respectively (Fig. 3a), indicating a cumulative relative priming effect of 6.86% on maize mineralization by biochar addition; however, the positive priming effect was mainly evident after the first month (Fig. 3a). Rates of litter decomposition were highest in the first 31 days (around 4.50 mg C g⁻¹ litter d⁻¹), slowly decreasing to 1.20 mg C g⁻¹ litter d⁻¹ over the next 62 days, and then to 0.38 mg C g⁻¹ litter d⁻¹ by the end of the incubation period (Fig. 3b).

3.4. CO₂ efflux from native SOM

Over the 183 days of incubation, decomposition of SOM decreased in the order Soil + Maize (cumulative over 183 days:

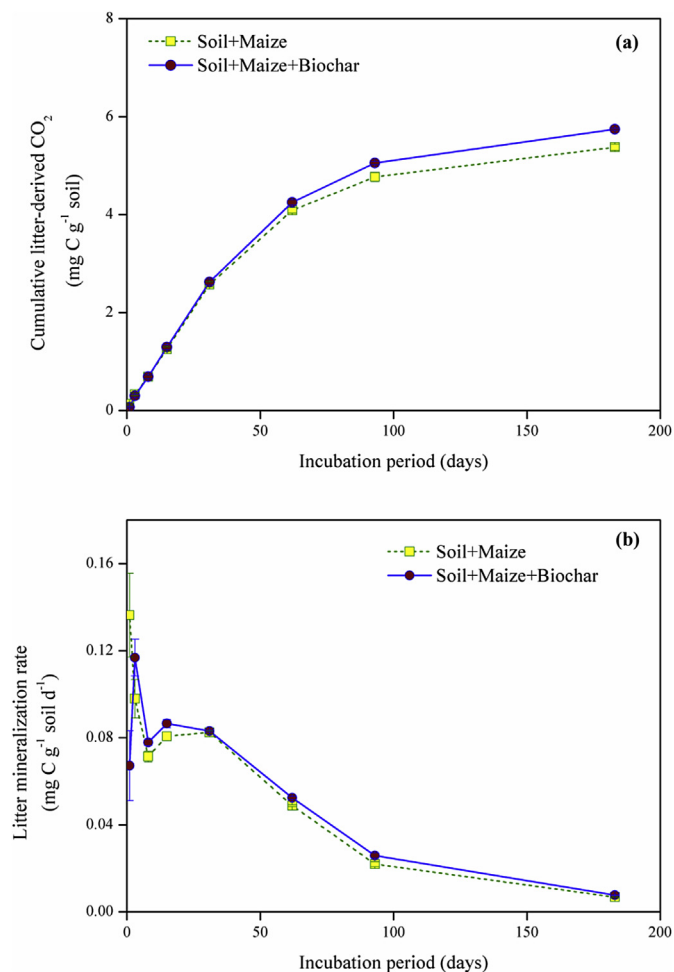


Fig. 3. Cumulative CO₂ (a) and mineralization rates of C (b) derived from litter (maize straw). Error bars represent one SE (n = 3).

2.99 ± 0.22 mg C g⁻¹ soil) > Soil + Maize + Biochar (2.42 ± 0.07 mg C g⁻¹ soil) > Soil + Biochar (2.18 ± 0.04 mg C g⁻¹ soil) > Soil only (1.94 ± 0.05 mg C g⁻¹ soil) (Fig. 4a). Therefore, compared with the control, all three amendments (maize, biochar, maize + biochar) induced net positive priming effects on SOM decomposition at the end of incubation.

The priming effects of various amendments on SOM differed in the order Soil + Maize (1.05 mg C g⁻¹ soil, 54.12%) > Soil + Maize + Biochar (0.48 mg C g⁻¹ soil, 24.74%) > Soil + Biochar (0.24 mg C g⁻¹ soil, 12.37%) (Fig. 4b). However, such priming effects were time-dependent. The cumulative priming effects induced by Soil + Biochar on SOM were largely negative or close to zero in the first 31 days but became positive over the next 5 months. In contrast, those by Soil + Maize were consistently positive throughout the incubation period and gradually increased over time. The dynamics of the priming effects induced by Soil + Maize + Biochar could be divided into three stages: initially positive in the first 8 days, declining to slightly negative until day 62 and thereafter positive once more.

3.5. Priming effects of biochar on SOM versus litter decomposition in soils containing maize straw

To assess the priming effects of biochar on SOM versus litter (i.e. maize straw) in soil–maize mixtures, the SOM- and litter-derived CO₂ effluxes from the Soil + Maize + Biochar treatment were

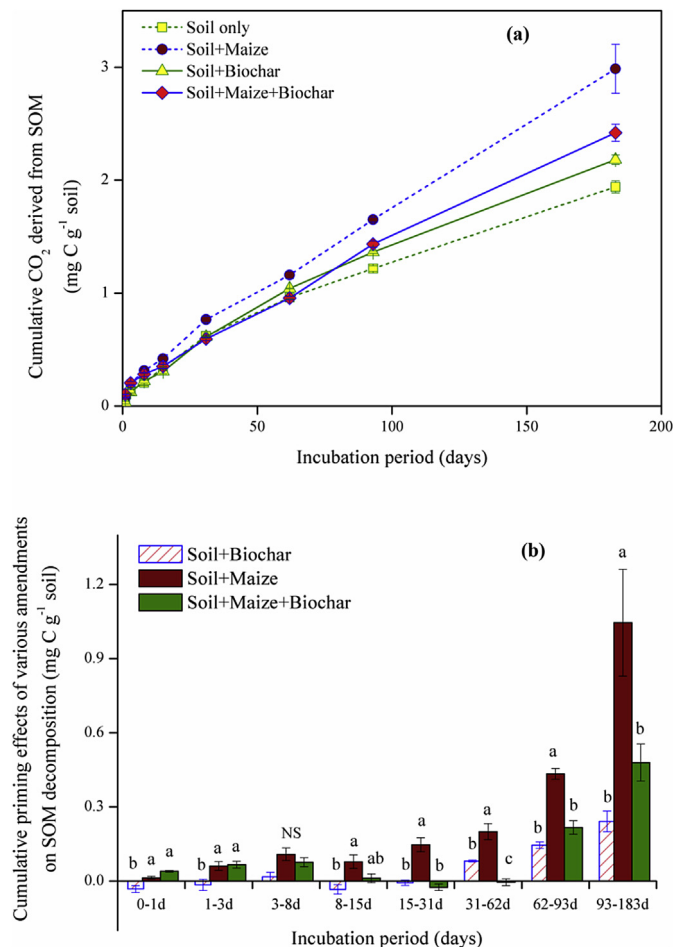


Fig. 4. Cumulative CO₂ derived from soil organic matter (SOM) (a) and the cumulative priming effects of different amendments on SOM decomposition with incubation time (b). The priming effects in (b) are based on comparisons to the control treatment (no additions). Letters in (b) for each sampling interval indicate significant differences between treatments. Error bars represent one SE (n = 3). NS: not significant (P > 0.05).

compared to those from the Soil + Maize treatment. The results revealed that biochar had opposing priming effects on SOM and litter mineralization (Fig. 5). During the first 1–3 days of incubation, biochar addition caused slight positive priming effects on SOM (0.004–0.026 mg C g⁻¹ soil), but thereafter the cumulative priming effects became negative and progressively decreased to -0.57 mg C g⁻¹ after 183 days (i.e. a relative priming effect of -19%). In contrast, the priming effects of biochar on litter mineralization were slightly negative in the initial 3 days, but then became positive and gradually reached 0.37 mg C g⁻¹ (a relative priming effect of 6.86%) after 183 days.

3.6. Microbial biomass C and its sources

At the end of the incubation period, MBC decreased in the order Soil + Maize + Biochar (321 ± 31 $\mu\text{g C g}^{-1}$ soil) \sim Soil + Maize (286 ± 33 $\mu\text{g C g}^{-1}$ soil) \sim Soil + Biochar (277 ± 30 $\mu\text{g C g}^{-1}$ soil) > Soil only (231 ± 19 $\mu\text{g C g}^{-1}$ soil). Partitioning of MBC into various sources (Fig. 6) revealed that following the 183 days of incubation, biochar-derived C was undetectable in the microbial biomass of the two biochar treatments (Soil + Biochar, Soil + Maize + Biochar). The SOM-derived MBC differed in the order Soil + Biochar (277 ± 17 $\mu\text{g C g}^{-1}$ soil) > Soil only (231 ± 19 $\mu\text{g C g}^{-1}$ soil) \sim Soil + Maize + Biochar (207 ± 22 $\mu\text{g C g}^{-1}$ soil) \sim Soil + Maize (197 ± 17 $\mu\text{g C g}^{-1}$ soil). After maize straw

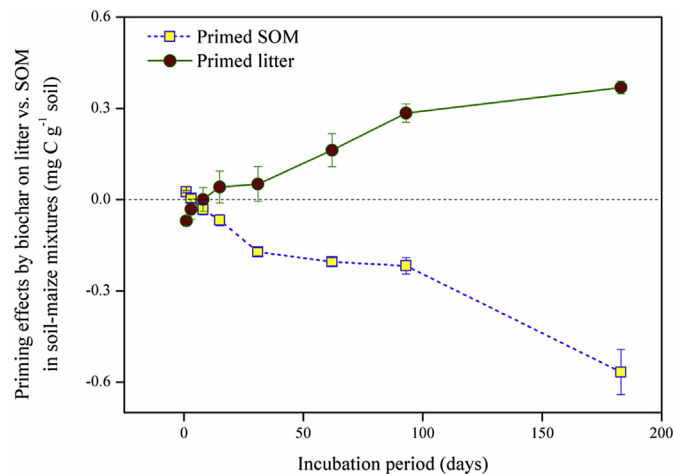


Fig. 5. Priming effects of biochar on the mineralization of litter (maize straw) versus soil organic matter (SOM) in soil-litter mixtures. The priming effects are differences in SOM mineralization between the Soil + Maize + Biochar and Soil + Maize treatments. Error bars represent one SE (n = 3).

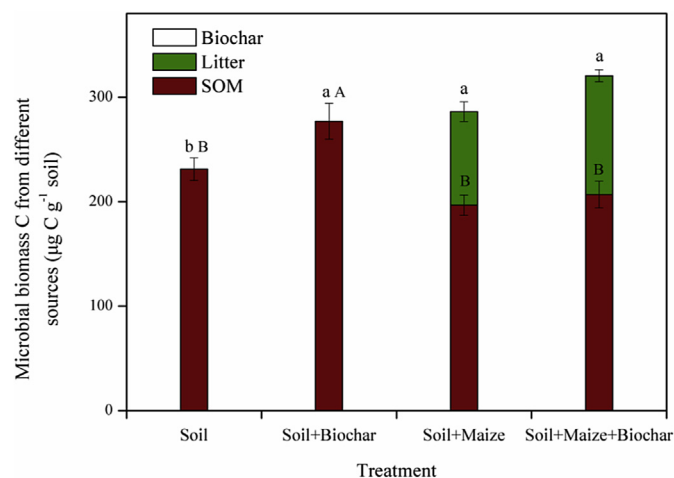


Fig. 6. Microbial biomass C (MBC) originating from three sources following incubation. Lowercase letters indicate significant differences in total MBC; uppercase letters indicate significant differences in MBC deriving from soil organic matter (SOM) between treatments. Error bars represent one SE (n = 3).

addition, a significant proportion (29–37%) of MBC was found to originate from the maize straw. Maize-derived MBC under Soil + Maize and Soil + Maize + Biochar treatments were 89 ± 16 $\mu\text{g C g}^{-1}$ and 114 ± 10 $\mu\text{g C g}^{-1}$, respectively, an indication that biochar enhanced the incorporation of maize-derived C into the soil microbial biomass.

4. Discussion

4.1. Negative priming effects of litter addition on biochar decomposition

Biochar labeled with ¹⁴C allowed for effective quantification of the slow rates of decomposition, because ¹⁴C analysis is more sensitive than that of stable C isotopes (Kuzyakov et al., 2009, 2014; Gocke et al., 2011). By this approach we found that only 2.77–3.86% of added biochar was mineralized at the end of the incubation period, when its decomposition rate was as low as 0.0075 d⁻¹. Also using ¹⁴C-labeled biochar, Kuzyakov et al. (2009) reported that 1.84–2.10% of total biochar input was decomposed after a 2-month

incubation period, which is very close to our results (Fig. 2a). The mean decomposition rate of our biochar over the whole incubation period ($0.015\text{--}0.021\text{ d}^{-1}$) was quite close to the average biochar decomposition rate (0.023 d^{-1}) in incubation studies lasting < 0.5 years in a synthesis study by Wang et al. (2016). However, relatively smaller percentages (1.8–1.9%) of ^{14}C -labeled biochar were mineralized in a study by Bruun and EL-Zehery (2012) over 451 days, which might be due to the fact that our soils were wetter (water content: 37%) than those (15%) used by Bruun and EL-Zehery (2012) and hence favored biochar decomposition. Decomposition rates of our biochar in the first month were one to two orders of magnitude faster than over the next 5 months (Fig. 2b), most likely due to the initially rapid mineralization of the labile biochar components (Bruun et al., 2008; Hilscher et al., 2009; Cross and Sohi, 2011). Biochar used in this study had a high DOC content (Table 1), an indicator of the presence of labile C.

Our observation of litter-induced negative priming effects on biochar mineralization is consistent with the result of Bruun and EL-Zehery (2012) in that straw addition decreased the mineralization of ^{14}C -labeled biochar, but contrasts with the findings of Hamer et al. (2004) and Kuzyakov et al. (2009) where glucose addition increased biochar mineralization. Litter added here (maize straw) and in Bruun and EL-Zehery (2012) was obviously more recalcitrant than glucose and thus would have a less stimulatory effect on microorganisms. Adding labile organics (e.g. glucose, sucrose) into soil tended to stimulate the fast-growing microbes (r-strategists) that primarily utilize easily available substrates (Blagodatskaya et al., 2009; Chen et al., 2014), which would trigger the co-metabolism of labile C in biochar (Hamer et al., 2004; Kuzyakov et al., 2009). In contrast, the more recalcitrant litter should favor the slow-growing K-strategists (Fontaine et al., 2011; Chen et al., 2014), which primarily mineralize the stable organic compounds in soil. In addition, adding litter might have caused a “dilution effect”, that is, maize straw diluted the available biochar compounds and thus reduced the relative uptake of biochar by microorganisms. Furthermore, we speculate that organic substances released from litter decomposition may cover the surfaces and clog the pores of biochar, thereby slowing biochar mineralization (Keith et al., 2011). Consistent with these two mechanisms, biochar mineralization was reduced immediately following litter addition (Fig. 2b). Although slightly more water (0.05 ml g^{-1} mixture) was added to the Soil + Maize + Biochar treatment than to Soil + Biochar to maintain 60% WHC, this alone is unlikely to have decreased the mineralization of biochar or the diffusion of CO_2 out of the soil. However, some incubation studies have indicated that plant residues have positive priming effects on biochar decomposition (Keith et al., 2011; Luo et al., 2011). This inconsistency with our results is most likely due to the differences in biochar and soil properties, as well as the quality of plant residues.

4.2. Priming effects of biochar on native SOM

Adding biochar alone to soil increased total CO_2 emissions and induced a net positive priming effect on SOM decomposition (Figs. 1 and 4a). The magnitude of the priming effect (0.24 mg C g^{-1} soil over 6 months, 12.4% of control) was comparable to the 1-year average (0.32 mg C g^{-1} soil, 15% of control) reported by Maestrini et al. (2015). Two major mechanisms have been proposed to explain the short-term priming effects of biochar on SOM decomposition, both concerning the labile components of biochar. First, the co-metabolism, i.e. the labile C in biochar activated soil microorganisms decomposing SOM (Hamer et al., 2004; Luo et al., 2011; Zimmerman et al., 2011; Maestrini et al., 2015). The labile components of biochar, which make up about 3% of total biochar-C with a mean residence time of 108 days as estimated by Wang et al.

(2016), are presumably water soluble C (Luo et al., 2011) and largely composed of nonaromatic substances (Singh et al., 2012). Generally, this labile C was most intensively mineralized in the initial few days to weeks following amendment to soil (Kuzyakov et al., 2009; Keith et al., 2011; Singh et al., 2012; Singh and Cowie, 2014), leading to the strongest priming effects in the first 20 days (Maestrini et al., 2015). Second, microbes may have switched their C sources from the recalcitrant SOM to the easily available C in biochar, thereby resulting in negative priming effects on SOM mineralization (Wang et al., 2016). In addition, a few studies have found that carbonates contained in biochar may contribute to the initial CO_2 flush following biochar addition (Jones et al., 2011; Bruun et al., 2014). This abiotic mechanism, although not a major pathway of CO_2 release from low-temperature biochar, as suggested by Bruun et al. (2014), might still lead to an overestimation of biochar decomposition and should be considered when evaluating biochar-induced priming of SOM decomposition.

During the first month of incubation, when the rate of biochar decomposition was highest (Fig. 2a and b), the priming effects were largely negative or slightly above zero (Fig. 4b). This was most likely due to the preferential microbial utilization of labile C in biochar rather than co-metabolism, given that strong mineralization of biochar occurred during this period. Consistent with this are the results of the meta-analysis performed by Maestrini et al. (2015), which showed that many studies reported negative priming effects on SOM by biochar in the first 20 days of incubation. An initial preferential use of exogenous labile C inputs by microbes was also noted by Kuzyakov and Bol (2006). However, it is noteworthy that carbonates in biochar may have contributed to the biochar-derived CO_2 but not to the priming; given that such carbonates may be generated during pyrolysis or originate from the feedstocks of biochar (Tchomgui-Kamga et al., 2010; Jones et al., 2011; Bruun et al., 2014), it is likely that they are isotopically (^{14}C) undistinguishable from biochar. This can lead to overestimates of biotically mediated CO_2 release from biochar (Bruun et al., 2014) and therefore biochar-induced priming effects. To what extent carbonate-derived CO_2 contributes to overestimation of biochar decomposition has not yet been quantified, however, and requires further investigation.

Over the course of the 5 months that followed, however, biochar addition resulted in consistently positive priming effects on SOM decomposition (Fig. 4b). This is not well explained by co-metabolism alone, as biochar decomposition proceeded at a rather slow rate ($0.00066\text{ mg biochar-C g}^{-1}\text{ soil d}^{-1}$) during this period, compared to the magnitudes of SOM-derived CO_2 effluxes ($0.010\text{ mg C g}^{-1}\text{ soil d}^{-1}$) and priming effects ($0.0031\text{ mg C g}^{-1}\text{ soil d}^{-1}$). Alternatively, biochar amendment must have induced some lasting changes in soil, such as improved aeration and higher water holding capacity (Herath et al., 2013), more nutrients (Prendergast-Miller et al., 2014), and alterations to enzymatic activities or microbial biomass (Lehmann et al., 2011). Biochar addition alone significantly increased microbial biomass (Fig. 6) and enhanced soil mineral N contents by 274% at the end of the incubation period (Table 2). The increased mineral N might partly originate from biochar itself (Taghizadeh-Toosi et al., 2012; Maestrini et al., 2014), but should be mainly released when soil microbes mined SOM for C/nutrients as primed by the labile C inputs from biochar (Nelissen et al., 2012; Maestrini et al., 2014). The latter mechanism was facilitated by the feedstock of our biochar (straw), since biochar made from woody biomass tended to suppress soil N mineralization compared to straw-made biochar (Hansen et al., 2016). Moreover, previous studies reported that biochar increased activities of soil enzymes involved in N cycling, such as aminopeptidase and N-acetylglucosaminidase (Lehmann et al., 2011). Application of the ^{15}N tracing technique is needed to disentangle the various N-fluxes in soil N cycling affected by biochar amendment (Nelissen

Table 2
Soil properties following incubation.^a

Treatment	pH (H ₂ O)	Total C (%)	Total N (%)	Total C:N	DOC ^a (mg C g ⁻¹)	NH ₄ ⁺ (mg N Kg ⁻¹)	NO ₃ ⁻ (mg N Kg ⁻¹)
Soil only	7.08 ± 0.08 a	1.83 ± 0.07 d	0.19 ± 0.01 d	9.85 ± 0.18 a	10.9 ± 0.22 a	10.2 ± 0.40 a	37.2 ± 2.32 c
Soil + Maize	6.99 ± 0.73 a	2.16 ± 0.12 c	0.22 ± 0.01 c	9.61 ± 0.59 a	13.8 ± 1.58 a	9.01 ± 0.26 b	188 ± 1.27 a
Soil + Biochar	6.65 ± 0.24 a	2.49 ± 0.07 b	0.28 ± 0.01 b	8.87 ± 0.02 b	11.0 ± 1.38 a	7.03 ± 0.86 c	123 ± 39.0 b
Soil + Maize + Biochar	6.79 ± 0.10 a	2.78 ± 0.09 a	0.31 ± 0.01 a	9.01 ± 0.11 b	15.3 ± 3.11 a	5.70 ± 0.30 d	130 ± 0.06 b

Different letters in the same columns indicate significant differences ($P < 0.05$). Duncan's test was used for the comparison.

^a Measured in 0.05 M K₂SO₄ extracts.

et al., 2012).

Biochar-induced changes in soil microbiological and nutrient properties would further profoundly affect SOM mineralization (Chen et al., 2014; Qiu et al., 2016). Therefore, mechanisms of commonly observed positive priming effects of biochar on SOM decomposition might differ from that resulting from the addition of glucose or other simple organic substances to soil. Biochar-induced priming is likely based on more lasting alterations in soil fertility and microbial activity, and thus may remain stable even after depletion of labile biochar compounds (Zimmerman et al., 2011; Maestrini et al., 2015). In summary, the supplementation of soils with biochar to promote C sequestration must take into account the long-term impacts of biochar on soil nutrient status, enzymatic activity, and microbial composition (Lehmann et al., 2011).

4.3. Differential impacts of biochar on SOM and litter decomposition

Our successful partitioning of C fluxes from biochar, SOM, and litter suggested that the dual-isotope labeling approach (Ruf et al., 2006; Blagodatskaya et al., 2011, 2014) was a viable means to simultaneously examine the interactive priming of the three C sources. The results indicated that biochar addition to soil-litter mixtures increased litter decomposition while reducing that of SOM. To our knowledge, partitioning of the complex interactions of three C sources in biochar-induced priming has not been previously reported (Maestrini et al., 2015).

The accelerated decomposition of maize litter following the addition of biochar was consistent with the results of Novak et al. (2010), who found that the presence of biochar increased the mineralization of switchgrass litter. In contrast, however, Bruun and EL-Zehery (2012) reported that biochar addition did not significantly affect ¹⁴C-labeled straw decomposition, and several other studies reported reduced mineralization of plant residues in soils containing biochar (Liang et al., 2010; Keith et al., 2011; Jones et al., 2012). The discrepancy might be partially attributed to differences in experimental conditions or biochar/soil properties; for example, the biochar investigated by Liang et al. (2010) and Jones et al. (2012) had been present in soils for several or thousands of years, and thus the initial effects were long finished.

Whereas biochar addition promoted the decomposition of maize straw, it reduced that of SOM (Fig. 5), suggesting preferential microbial utilization of litter over SOM in the presence of biochar. Indeed, significantly more microbial biomass C was derived from maize straw when biochar was present as compared to soils with only maize added (Fig. 6). Similarly, Novak et al. (2010) found that biochar addition to switchgrass-soil mixtures resulted in accelerated decomposition of switchgrass litter compared to soils without biochar, leading the authors to suggest that the presence of biochar caused a shift in microbial decomposition from SOM to switchgrass residues. In a temperate forest soil, Singh et al. (2014) also found that the amount of free light fraction of soil C, a relatively fresh C fraction mainly composed of decomposed plant residues (von Lütow et al., 2008), decreased more than stabilized SOM in response to biochar amendment.

The exact mechanisms underlying the differential effects of biochar on litter versus SOM decomposition are unclear at present. Although extra water (0.07 ml g⁻¹) was added to maintain 60% WHC following the amendment of biochar to soil + litter mixtures, this could not explain the differential responses of litter versus SOM to biochar since water should have changed mineralization of both litter and SOM in the same direction. Here we propose a conceptual framework that explains the priming of SOM in responses to litter-only and litter + biochar additions (Fig. 7). Increased SOM mineralization in the presence of exogenous C-rich substrates could be due to microbes mining SOM to meet their nutrient requirements (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010). Therefore, maize straw (with high C:N ratios) would induce significant and lasting positive priming effects on SOM decomposition (Fig. 4) because microbes mined N from SOM (Fontaine et al., 2011; Qiu et al., 2016). On the other hand, providing available N along with C-rich litter would lead to preferential litter utilization by microorganisms, lessening their N acquisition and hence decreasing SOM decomposition (Fontaine et al., 2011; Qiu et al., 2016). We assumed this to be a plausible cause of the decreased SOM but increased maize mineralization when biochar was present. The NH₄⁺ contained in biochar itself increased soil NH₄⁺ contents by nearly 50% (Table 1, 0.7 g biochar added to 35 g d.w. soil), which partially met the N requirements of microbes utilizing maize straw. Moreover, NH₄⁺ and NO₃⁻ in the soil-litter-biochar mixtures were significantly lower than in soils amended with only litter, suggesting that biochar promoted the immobilization of mineral N in the presence of litter (Table 2). This implies enhanced N uptake and/or retention by microbes in soil-litter mixtures due to biochar addition, which benefits litter decomposition and reduced the necessity of microbes to mine N from the recalcitrant SOM. The enhancement of N immobilization in soils containing crop residues as a result of biochar addition has also been reported by other researchers (Novak et al., 2010; Prayogo et al., 2014), but the mechanisms remain unclear. We hypothesize that (1) the labile C component of biochar may have provided additional C sources for microbial N immobilization, as suggested by Deenik et al. (2010); and (2) the adsorptive capacity of biochar facilitates the co-location of C, nutrients, and microbes (Lehmann et al., 2011), resulting in greater microbial N uptake efficiency and N immobilization.

Consequently, more attention should be paid to the impacts of biochar on N processes, rather than solely to the labile C fraction of biochar, when explaining its priming effects. Biochar is a significant source of N for organisms (de la Rosa and Knicker, 2011; Taghizadeh-Toosi et al., 2012) and influences C dynamics by altering soil N processes (Bruun et al., 2012; Nelissen et al., 2012). The nutrients in biochar may also lead to preferential growth of plant roots toward biochar particles (Prendergast-Miller et al., 2014), which would further influence C processes via rhizosphere effects (Kuzyakov, 2002).

4.4. Implications of biochar-induced priming effects for C sequestration in croplands

Converting tree, grass, and crop residue biomass to biochar for

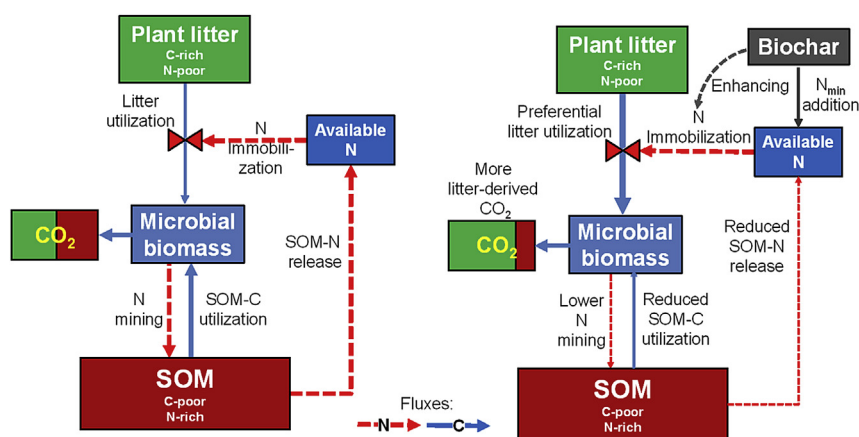


Fig. 7. A Conceptual diagram explaining the mechanisms underlying the differential priming of soil organic matter (SOM) versus litter by biochar. The left part presents a generally assumed mechanism of priming effects on SOM decomposition by exogenous C inputs (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010), that is, microbes mine N from the N-rich SOM to meet their N requirements for utilization of the C-rich litter. The right part explains the preferential microbial utilization of litter over the more recalcitrant (C-poor) SOM caused by biochar addition, due to the addition of mineral N (N_{min}) from biochar and/or the enhanced microbial N uptake and retention via the promotive effects of biochar on N immobilization. The preferential decomposition of litter versus SOM results in higher proportions of litter-derived CO₂ emissions.

use as a soil additive has been considered as a promising approach to lock up the otherwise easily decomposable C in soil and thus reduce atmospheric CO₂ (Lehmann, 2007). However, complete pyrolysis of crop residues in agroecosystems would mean depriving soils of C inputs from straw incorporation, a traditional form of crop-residue management that extends a variety of beneficial agronomic effects, such as improving soil aggregation, enzymatic activities, nutrient contents, and crop yields (Liu et al., 2014; Zhao et al., 2016). On the other hand, the effects of biochar on SOM dynamics, crop yields, and other agronomic aspects remain uncertain, and must be critically evaluated prior to the wide-scale application of biochar to agroecosystems (Spokas et al., 2012; Singh et al., 2015).

Our results suggested that pyrolysis of only part of the crop residues might be a more feasible approach than complete pyrolysis of the litter, at least in terms of SOM retention in agroecosystems. Incorporation of maize straw alone greatly stimulated SOM decomposition (a positive priming effect of 53.9%), which was markedly mitigated (24.7%) by adding biochar together with maize straw (Fig. 4b). Previous studies have reported that amendments of biochar together with rice straw reduced CH₄ emissions from paddy rice fields (Liu et al., 2011). Based on these results, we suggest that pyrolyzing part of the crop residues may be a viable option for achieving the beneficial effects of biochar, such as soil C sequestration and greenhouse-gas mitigation, while avoiding the potential risks of complete crop residue pyrolysis and leaving the soil microorganisms with little easily available C.

5. Conclusions

Using three-source partitioning of C fluxes by combination of ¹³C natural abundance and ¹⁴C labeling, we found that the incorporation of biochar into soils containing maize litter accelerated the decomposition of litter but decreased the decomposition of SOM; as a consequence of these opposing effects, there were no significant changes in total CO₂ efflux.

The preferential microbial utilization of litter was further confirmed by the increased microbial biomass C derived from maize straw but not from SOM in response to biochar addition. Co-metabolism alone – the triggering of microbial activities by the labile components of biochar – was not sufficient to account for these effects, as the opposing impacts of biochar on litter and SOM were more evident at later incubation stages (after 1 month), when the labile C from biochar had been largely depleted. The

preferential microbial utilization of litter over SOM in the presence of biochar was most likely related to the altered soil N processes following biochar addition, either due to the increased mineral N from biochar or greater microbial N uptake and/or retention via enhanced N immobilization. Given that biochar incorporation alone, or when combined with litter, induced smaller priming effects on SOM and consequently CO₂ losses than litter addition alone, we suggest that pyrolysis of only a part of crop residues may represent a feasible option for promoting C sequestration and greenhouse-gas mitigation in croplands compared to the complete pyrolysis of all crop residues.

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