The responses of soil organic carbon mineralization and microbial communities to fresh and aged biochar soil amendments

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Abstract

While biochar soil amendment has been widely proposed as a soil organic carbon (SOC) sequestration strategy to mitigate detrimental climate changes in global agriculture, the SOC sequestration was still not clearly understood for the different effects of fresh and aged biochar on SOC mineralization. In the present study of a two-factorial experiment, topsoil samples from a rice paddy were laboratory-incubated with and without fresh or aged biochar pyrolyzed of wheat residue and with and without crop residue-derived dissolved organic matter (CRM) for monitoring soil organic matter decomposition under controlled conditions. The six treatments included soil with no biochar, with fresh biochar and with aged biochar treated with CRM, respectively. For fresh biochar treatment, the topsoil of a same rice paddy was amended with wheat biochar directly from a pyrolysis wheat straw, the soil with aged biochar was collected from the same soil 6 years following a single amendment of same biochar. Total CO2 emission from the soil was monitored over a 64 day time span of laboratory incubation, while microbial biomass carbon and phospholipid fatty acid (PLFA) were determined at the end of incubation period. Without CRM, total organic carbon mineralization was significantly decreased by 38.8% with aged biochar but increased by 28.9% with fresh biochar, compared to no biochar. With CRM, however, the significantly highest net carbon mineralization occurred in the soil without biochar compared to the biochar-amended soil. Compared to aged biochar, fresh biochar addition significantly increased the total PLFA concentration by 20.3%–33.8% and altered the microbial community structure by increasing 17:1ω8c (Gram-negative bacteria) and i17:0 (Gram-positive bacteria) mole percentages and by decreasing the ratio of fungi/bacteria. Furthermore, biochar amendment significantly lowered the metabolic quotient of SOC decomposition, thereby becoming greater with aged biochar than with fresh biochar. The finding here suggests that biochar amendment could improve carbon utilization efficiency by soil microbial
INTRODUCTION

Biochar, a carbon (C)-rich solid produced from pyrolysis of biowastes in the absence of oxygen, is able to persist or remain deposited in soil for hundreds to thousands of years (Kuzyakov, Bogomolova, & Glaser, 2014; Lehmann et al., 2011). For this reason, biochar soil application has been widely accepted as an important strategy to increase soil organic carbon (SOC) sequestration (Sohi, 2012; Zheng, Han, et al., 2017). The impacts of biochar application on SOC dynamics have been reported in detail in the literatures (Ameloot et al., 2014; Jiang, Tan, Cheng, Haddix, & Cotrufo, 2019; Zheng et al., 2016). Previous studies showed that aging of biochar is a vital factor influencing SOC mineralization (Maestrini, Nannipieri, & Abiven, 2015; Wang, Xiong, & Kuzyakov, 2016). Lu et al. (2014) found that corn (C₄ plant) biochar addition had no effect on total soil CO₂ emissions but could significantly reduce CO₂ emissions by 64.9%–68.8% from native SOC in a cultivated sandy loam soil (C₃ soil) over an incubation period of 30 days. In another study, a 3.2 year incubation experiment conducted by Kuzyakov, Subbotina, Chen, Bogomolova, and Xu (2009) showed that fresh biochar addition had no effect on total SOC mineralization from two soil types within 2 months of incubation; however, a contrasting effect was found between the two soil types by biochar addition. CO₂ emission rates slightly increased to about 0.6 mg CO₂-C kg⁻¹ day⁻¹ in the loamy Haplic Luvisol, while they significantly decreased to 0.2–1.5 mg CO₂-C kg⁻¹ day⁻¹ in the loess. Recently, Zhao, Coles, and Wu (2015) compared the soil C mineralization rate between fresh and aged biochar amendments and observed a lower mineralization rate in aged biochar amendments. However, Spokas (2013) stated that CO₂ emission was enhanced two to eightfold under three weathered biochar additions in laboratory over a 100 day incubation. These results indicated that biochar addition in the short term (fresh biochar) and the long term (aged biochar) could influence the soil C mineralization intensity and direction with time through different mechanisms. Furthermore, these results suggest that aging of biochar in soil may alter SOC sequestration potential. To date, however, little information has been available on the response of organic C mineralization to biochar with aging in paddy soils.

Soil microbes play a key role in soil organic matter stabilization and nutrient cycling (Chen, Chen, et al., 2019; Watzinger et al., 2014). The effects of biochar on soil microbial community composition have been investigated using high-throughput sequencing or phospholipid fatty acid (PLFA) technology (Ameloot et al., 2014; Chen et al., 2017; Sheng & Zhu, 2018; Zhou et al., 2019). Xu et al. (2014) reported that high-throughput sequencing results in increased α-diversity significantly and the relative abundances of Flavmeovirgaceae and Chitinophagaceae related to C cycling after 45 days following biochar addition in a pot trial. Similarly, Watzinger et al. (2014) revealed that biochar addition significantly increased Gram-negative bacteria and actinomycetes from temperate soil in short-term incubation using ¹³C-PLFA. Chen et al. (2018) demonstrated that 3 year biochar soil amendment reduced the relative abundance of three dominant bacterial phyla related to C cycling, while increasing the abundance of Ascomycota, a key fungal community, and reducing the dependence of soil respiration on temperature. By contrast, a 6 year field experiment conducted by Tian et al. (2016) indicated that microbial metabolic activity strongly increased due to biochar amendment, but it did not change microbial community structure in paddy soil. These changes in soil would cause differences in exogenous organic C utilization (organic C sequestration) by microbial organisms. Although there were studies that reported that biochar amendment affected the soil microbial community composition in the short term or long term (Chen, Jiang, et al., 2019; Cheng, Hill, Bastami, & Jones, 2017; Sun, Meng, Xu, & Chen, 2016), the microbial usage of exogenous organic matter following the addition of fresh and aged biochar amendments is still unclear.

Paddy soil is one of the main soil types in agriculture systems in China, and it has a meaningful effect on soil C storage (Pan, Li, Wu, & Zhang, 2003). Crop residues are the main form of waste in agriculture production. In 2013, it was estimated that the total amount of crop residue production was approximately 3.8 × 10⁹ Mg/year in global agricultural ecosystems (Thangarajan, Bolan, Tian, Naidu, & Kunhikrishnan, 2013), and most crop residues are returned to the soil. Meanwhile, approximately 5%–15% of total residues enter into the environment in the form of soluble C (Cleveland, Neff, Townsend, & Hood, 2004), which is a very reactive fraction of organic C and easily utilized by soil microbes in terrestrial ecosystems (De Troyer, Amery, Moorleghem, Smolders, & Merckx, 2011). A study by Wang et al. (2017) showed that the dissolved organic carbon (DOC) content is generally higher in paddy soil with biochar amendment. However, a converse...
finding was reported by Zheng et al. (2016), who found that DOC content was significantly lower after 4 years of biochar amendment in paddy soil. It was reported that the biochar’s labile C may rapidly degrade within several days (Smith, Collins, & Bailey, 2010) or a few months (Kuzyakov et al., 2009) in soil. The biochar aging process develops a balance of chemical exchange and biological activity in the biochar-soil system, so the ‘fresh’ biochar effect on soil physicochemical and biological properties is likely different from the long-term effect of ‘aged’ biochar (Luo et al., 2017). Soil dissolved organic matter (DOM) could be adsorbed by the biochar due to its high porosity and large surface area (Smeebe et al., 2016). Pan, Zhou, Zhang, Qiu, and Chu (2006) stated that SOC sequestration could play a key role in stabilizing and increasing rice productivity and in sequestering C for mitigation of CO₂ emissions in paddy soils. Water management as a necessary agricultural practice is one of the crucial measures for crop production, especially in paddy soils. Straw residues that are returned to paddy soil produce much of the DOM due to seasonal flooding of agricultural practices. For C sequestration, it is essential to understand the organic C dynamics under the interactive effect of biochar and crop-derived DOM in paddy soil.

In this study, we hypothesized that (a) lower organic C mineralization would occur in the soil with aged biochar amendment compared to the soil with fresh biochar addition due to labile C depletion, and (b) soil microbial community composition would be modified differently by the fresh and aged biochar soil amendment and would utilize exogenous organic C differently. The main aims of this study were to (a) determine the variation of organic C mineralization dynamics under fresh (short-term) and aged (long-term) biochar amendment in a paddy soil, and (b) investigate response of soil microbial community and organic C mineralization to fresh and aged biochar soil amendments followed by exogenous C addition.

2 | MATERIALS AND METHODS

2.1 | Field experiment

2.1.1 | Site description

The field experiment site was located in Jing-tang Village (31°24′N, 119°41′E) in Yixing Municipality, Jiangsu Province, China. The local climate is a subtropical humid monsoon climate with a mean annual temperature of 15.7°C and a mean annual precipitation of 1,177 mm. The soil type is a typical paddy soil and is classified as a Hydroagric Stagnic Anthrosol (Gong, 1999). The basic soil properties before biochar amendment were as follows: bulk density of 1.01 g/cm³, pH(H₂O) of 5.36, SOC of 23.5 g/kg, total N(TN) of 2.37 g/kg, cation exchange capacity of 18.1 cmol (+)/kg, and clay content of 390 g/kg. The local traditional farming system is a typical rice–wheat rotation in this region.

2.1.2 | Field experiment treatment

The field experiment was set up in May 2009 after wheat harvest and included two treatments: without and with biochar soil amendment. To exaggerate the biochar’s effect on soil properties, the biochar was applied only once to soil at a rate of 40 t/ha for biochar treatment. The biochar was spread on the soil surface and mixed homogeneously with soil before rice planting. Each plot size was 20 m² (4 m × 5 m) in area with three replicates and laid out in a randomized block design. Urea, calcium biphosphate, and KCl were used as N, P, and K fertilizers in the field following local agronomic practices at the rates of 300 kg N/ha, 89 kg P/ha, and 169 kg K/ha, respectively.

2.2 | Incubation experiment

2.2.1 | Soil sampling and pretreatment

Soil samples from without and with biochar soil amendments for laboratory incubation were sampled randomly from the surface layer (0–15 cm) of each plot by using an Eijkelkamp soil core sampler (5 cm in diameter) after the rice harvest in 2015 and shipped to the laboratory. Then, the samples were air-dried after removing the roots from the soil. Prior to the incubation experiment, the soil samples were passed through a 2 mm sieve and homogenized. The topsoil basic properties are listed in Table 1.

To compare the response of C mineralization and soil microbial communities to aged and fresh biochar soil amendments, the 6 year soil with biochar amendment in the field was used for the aged biochar-amended treatment, and the soil with the fresh biochar from the same pyrolysis procedure was used for fresh biochar-amended treatment. The mixture

| Sample                | Bulk density (g/cm³) | pH (H₂O)   | Organic C (g/kg) | TN (g/kg) |
|-----------------------|----------------------|------------|------------------|-----------|
| Soil without biochar  | 0.99 ± 0.05          | 5.59 ± 0.09| 23.1 ± 0.14      | 2.48 ± 0.08|
| Soil amended with biochar | 0.90 ± 0.02          | 6.05 ± 0.11| 31.3 ± 0.24      | 2.50 ± 0.02|

Table 1 Basic properties of topsoils (0-15 cm) from the field soil samples
rate of the fresh biochar was calculated according to the difference in C content between the field soil samples without and with biochar amendment. The biochar used in this study was produced commercially by Sanli New Energy Company in Henan Province, China. Wheat straw was pyrolyzed at a mid-temperature and slow carbonization in a vertical kiln at a temperature range of 350–550°C varying from the start to the endpoint. Basic properties of biochar were: organic C: 467.2 g/kg, total N: 5.90 g/kg, pH (H2O): 10.4, DOC: 530.3 g/kg, surface area: 8.92 m2/g, and ash content: 20.8%.

2.2.2 | Crop-derived dissolved organic matter preparation and addition rate

Wheat straw harvested from the field was air-dried and chopped prior to use. The crop residue was extracted for 24 hr by distilled water (1:40 by wt:vol). The supernatant solution was passed through a 0.45 μm filter membrane and immediately added to the soil. The amount of crop residue-derived dissolved organic matter (CRM) was added at the rate of 400 ml solution/kg soil (equivalent to 320 mg C/kg soil) for the treatments with CRM addition.

2.2.3 | Incubation experiment

The incubation experiment was designed as a two-factorial of biochar and CRM, including six treatments with three replications: (a) soil without biochar (Control), (b) control with fresh biochar addition (BCF), (c) soil with a 6 year biochar (aged) amendment in the field (BCA), (d) Control with CRM addition (C-control), (e) BCF with CRM addition (C-BCF), and (f) BCA with CRM addition (C-BCA). One hundred grams of treated soil (dry weight equivalent) was put into a 500 ml jar, and then, the prepared CRM solution was added into the designated jars at the rate mentioned above. Each treatment contained 24 jars, three of which were used to monitor the C mineralization, and the remaining ones were used to determine the DOC, microbial biomass C (MBC), and PLFA. All treatment samples were incubated at constant moisture (60% water holding capacity) and temperature (25 ± 1.0°C) in the incubator. To maintain the soil moisture, deionized water was added to the jars by weight during the incubation. The respired CO2 was sampled after 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 23, 26, 29, 34, 40, 50, and 64 days of incubation. At the end of incubation, MBC and PLFA were analyzed. The metabolic quotient (qCO2) was calculated as the ratio of basal respiratory C (mg CO2-C kg−1 hr−1) to MBC (mg/kg).

2.3 | Determination of soil properties, DOC, MBC, and PLFA

Soil bulk density was measured by a 100 cm3 cylinder at the rice harvest. SOC and TN were determined following the protocol described by Lu (2000). The soil pH was measured using a 1:2.5 soil-to-water ratio. DOC was extracted by distilled water according to Zheng, Chen, et al. (2017). Soil MBC was extracted by 0.5 mol/L K2SO4 using the chloroform-fumigation method (Vance, Brookes, & Jenkinson, 1987). The DOC and MBC contents were analyzed by a Total Organic C Analyzer (multi N/C 2100; Analytik Jena AG).

The composition of the soil microbial community was estimated by PLFA, which was determined with a slightly modified method according to Frostegard and Baath (1996). Briefly, 3.0 g of freeze-dried soil was extracted twice using 15.0 and 10.0 ml single-phase chloroform/ methanol/citrate buffer mixture (v/v/v = 1/2/0.8; pH = 4.0). The phospholipids were separated from neutral lipids and glycolipids using silica acid columns (Supelco, Inc.), and then phospholipids were methylated to provide fatty acid methyl esters using mild alkaline methanolysis at 37°C in a water bath. The PLFA methyl esters were separated and identified using a gas chromatograph (N6890; Agilent) fitted with a MIDI Sherlock microbial identification system (Version 4.5; MIDI). Methyl nonadecanoate fatty acid (19:0) was added as an internal standard before methylation. The concentration of PLFA was expressed as nmol/g soil on a soil dry weight base. PLFA biomarkers of fungi (18:2ω6,9c and 18:1ω9c), Gram-positive bacteria (G+‐bacteria, i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (G‐ bacteria, 16:1ω7c, 16:1ω9c, 17:1ω8c, cy17:0, 18:1ω7c, and cy19:0), and actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0) were used to represent different microbial groups (Frostegard & Baath, 1996; Luo et al., 2017). Two ratios were also calculated: G+‐bacteria to G‐bacteria, 15:00 and 10.0 ml single-phase chloroform/ methanol/citrate buffer mixture (v/v/v = 1/2/0.8; pH = 4.0). The phospholipids were separated from neutral lipids and glycolipids using silica acid columns (Supelco, Inc.), and then phospholipids were methylated to provide fatty acid methyl esters using mild alkaline methanolysis at 37°C in a water bath. The PLFA methyl esters were separated and identified using a gas chromatograph (N6890; Agilent) fitted with a MIDI Sherlock microbial identification system (Version 4.5; MIDI). Methyl nonadecanoate fatty acid (19:0) was added as an internal standard before methylation. The concentration of PLFA was expressed as nmol/g soil on a soil dry weight base. PLFA biomarkers of fungi (18:2ω6,9c and 18:1ω9c), Gram-positive bacteria (G+‐bacteria, i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (G‐ bacteria, 16:1ω7c, 16:1ω9c, 17:1ω8c, cy17:0, 18:1ω7c, and cy19:0), and actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0) were used to represent different microbial groups (Frostegard & Baath, 1996; Luo et al., 2017). Two ratios were also calculated: G+‐bacteria to G‐bacteria, 15:00 and 17:00). The PLFA data were standardized to the mole percentages (mol%) of individual PLFA and principal component analysis was carried out for the examination of the variation microbial community structure in the soil.

2.4 | Data process

The cumulative amount of mineralized C (C(t); mg CO2-C/ kg soil) was plotted against time (t), and a combined...
first-plus-zero-order kinetic model was fitted to the data using the Levenberg–Marquardt algorithm:

\[ C(t) = C_e \left(1 - \exp\left(-k_e \times t\right)\right) + k_s \times t, \]

where this model assumes an initial size of easily mineralizable C pool \( (C_e) \), which is diminished according to first-order kinetics (Stanford & Smith, 1972), and the more resistant fraction is mineralized by zero-order kinetics (Sleutel, Neve, Roibas, & Hofman, 2005). In Equation (1), \( k_e \) is a rate constant for the easily mineralizable C pool (day\(^{-1}\)), and \( k_s \) is the slow C pool mineralization rate (mg CO\(_2\)-C kg\(^{-1}\) soil day\(^{-1}\)).

Net C mineralization (\( \Delta C_{min} \)) of CRM addition was calculated as:

\[ \Delta C_{min} = C_{min \, CRM} - C_{min \, non-CRM}, \]

where \( C_{min \, CRM} \) is the total C mineralization of C-control, C-BC\(_{F}\), and C-BC\(_{A}\) treatments, respectively; \( C_{min \, non-CRM} \) is the total C mineralization of Control, BC\(_{F}\), and BC\(_{A}\) treatments, respectively.

All data were expressed as the means of three replicates with 1 SD. One-way analysis of variance (ANOVA) was used to analyze the effect of the biochar. Two-way ANOVA was used to test the interaction effect of biochar and CRM. All statistical analyses were carried out using the JMP11.0 software (SAS Institute). The significant differences between the means were tested using Tukey’s honestly significant difference at \( p < .05 \). All figures were plotted using Origin 8.0 software.

3 | RESULTS

3.1 Soil CO\(_2\) release dynamics and variation in the mineralizable C pool under different treatments

Overall, the CO\(_2\) release dynamics showed a similar trend under different treatments (Figure 1a,b). CO\(_2\) release sharply declined in the first 6 days, and then gradually stabilized with time during the whole incubation period for all treatments. The fresh biochar addition significantly increased CO\(_2\) efflux. The mean CO\(_2\) release rate increased by 20.9%–53.9% in the BC\(_F\) than in the Control within 1–8 days (\( p < .05 \)). In contrast, the consistently lower CO\(_2\) release rate in aged biochar-amended soil was observed across the whole incubation period. With CRM addition, the CO\(_2\) release rate increased by 26.8%–79.5%, 16.4%–43.2%, and 20.3%–86.3% in the C-control, C-BC\(_{F}\), and C-BC\(_{A}\) treatments, respectively, within 1–8 days compared with the corresponding biochar treatment without CRM addition. The cumulative C mineralization was extreme significantly affected by the biochar amendment and CRM addition individually (\( p < .01 \)), but no interaction effect (biochar \( \times \) CRM) was found (\( p > .05 \); Figure 2; Table S1).

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Compared to the Control, cumulative C mineralization amount significantly increased by 28.9% in BC F, whereas a significantly low cumulative C mineralization was observed in the BCA. Similarly, for the CRM addition treatments, the highest cumulative C mineralization occurred in C‐BC F, followed by Control and C‐BC A. The cumulative C mineralization amount increased by 24.3%, 15.2%, and 16.3% in the C‐control, C‐BC F, and C‐BC A treatments, respectively, due to addition of CRM, compared with the corresponding biochar treatment without CRM addition (Figure 2).

The easily mineralizable C pool ($C_e$), mineralization rate of the slow C pool ($k_s$), and the $k_e$ were extremely and significantly affected by the biochar amendment and CRM addition ($p < .01$; Table 2; Table S2). The size of $C_e$ significantly increased by 45.9%–79.6% under addition of CRM compared to the treatments without CRM addition. For the treatments without CRM addition, the $C_e$ sizes were calculated in the following order: BCF (238.8 mg C/kg) > Control (169.8 mg C/kg) > BCA (125.6 mg C/kg). Similarly, the $k_s$ significantly increased by 27.9% in the BC F compared to the Control. By contrast, the $k_s$ significantly decreased by 40.1% in the BC A compared to the Control.

### 3.2 Dynamics of DOC under different treatments

The dynamics of DOC content in different treatments exhibited a similar trend as the CO$_2$ release rates during the whole incubation period (Figure 3a,b). There was a significant decrease in the DOC content, from 282.0 mg/kg to 39.7 mg/kg and from 346.9 mg/kg to 43.8 mg/kg in the treatment without and with CRM addition, respectively, in the first 8 days. A slow decrease was observed in the later incubation period. The lowest DOC occurred in aged biochar soil during the whole incubation period. The DOC was significantly higher in the BC F than in the Control within the first 8 days, but there was no significant difference between the BC F and the Control from 14 to 64 days. Meanwhile, the DOC of all treatments increased by the CRM addition, especially in the early incubation period (i.e., <8 days). However, there were no significant differences in DOC between the C‐control and the C‐BC F.

### 3.3 The variation in MBC and microbial $q_{CO2}$ under different treatments

Biochar amendment significantly affected the MBC content, while the CRM addition had no effect on the content of MBC (Table 3; Table S3). Compared to the Control, soil MBC content significantly increased by 63.1% and 67.9% under the BC F and BC A, respectively. However, there was no significant difference ($p > .05$) in MBC content between the BC F and BC A. For the treatments with CRM addition, compared to C‐control, the MBC content significantly increased by 35.5% and 41.7% for the C‐BC F and C‐BC A, respectively. As shown, $q_{CO2}$ decreased by 21.5% and 63.9% in the BC F and BC A, respectively, compared to the Control (Table 3). In addition, $q_{CO2}$ decreased by 11.8% and 58.9% in the C‐BC F and C‐BC A, respectively, compared to the C‐control. However, there were no significant differences ($p > .05$) in $q_{CO2}$ between the Control and C‐control, BC F and C‐BC F, or BC A and C‐BC A.

### 3.4 PLFA analysis

Regardless of CRM addition, compared to soil without the biochar-amended treatment, the total PLFA concentration...
and individual PLFA markers of fungi, G⁺-bacteria, and G⁻-bacteria significantly increased by 19.0%–40.2% in BCF, and 19.5%–29.7% in C-BCF (p < .05; Figure 4a). However, there was no significant difference in the total PLFA concentration among the aged biochar-amended soil and without biochar-amended soil (p > .05). With the CRM addition, the total PLFA concentration was significantly increased by 20.4% in the C-control than in the Control. On the contrary, there was no difference (p > .05) in the total PLFA concentration between the treatments with and without CRM addition under fresh or aged biochar soil amendments, indicating a weak response of PLFA to CRM addition. The ratio of G⁺-bacteria/G⁻-bacteria and fungi/bacteria was significantly affected by biochar and CRM, and there was a synergistic effect between biochar and CRM on the G⁺-bacteria/G⁻-bacteria ratio (Figure 4b,c; Table S4). The aged biochar soil amendment significantly increased the ratio of G⁻-bacteria/G⁺-bacteria and fungi/bacteria by 32.2%–55.0% and 13.1%–36.5%, respectively, compared to the without and with fresh biochar addition under the same CRM addition treatments.

Microbial community structure was altered by biochar addition (Figure 5). The first two principal components correlated meaningfully with the individual PLFA (loading plot in Figure 6). High positive loadings on the PC1 axis, such as in the BCA and C-BCA, correlated with higher mole percentages of 16:1ω7c and 18:1ω7c (G⁻-bacteria) and 18:2ω6,9c (fungi), with negative loadings from i16:0 and a17:0 (G⁺-bacteria) mole percentages. In contrast, PC2 had mainly positive loadings from 17:1ω8c (G⁻-bacteria) and i17:0 (G⁺-bacteria) mole percentages, with negative loadings from i14:0 and a15:0 (G⁺-bacteria) mole percentages.

### Table 3

| Treatment | MBC (mg C/kg soil) | Soil respiration rate (mg C kg⁻¹ soil hr⁻¹) | qCO₂ (mg C g⁻¹ MBC hr⁻¹) |
|-----------|------------------|---------------------------------|-------------------|
| Control   | 210.7 ± 26.8 b    | 0.87 ± 0.04 c                   | 4.17 ± 0.64 a     |
| BCF       | 343.7 ± 29.4 a    | 1.12 ± 0.06 b                   | 3.27 ± 0.34 b     |
| BCA       | 353.8 ± 17.5 a    | 0.53 ± 0.04 d                   | 1.51 ± 0.19 c     |
| C-control | 260.2 ± 9.5 b     | 1.08 ± 0.05 b                   | 4.15 ± 0.28 a     |
| C-BCF     | 352.7 ± 18.9 a    | 1.29 ± 0.07 a                   | 3.67 ± 0.05 ab    |
| C-BCA     | 368.7 ± 43.6 a    | 0.62 ± 0.05 d                   | 1.70 ± 0.34 c     |

Two-way ANOVA

|                         | ** | ** | ** |
|-------------------------|----|----|----|
| Biochar                 | ns | ns | ns |
| CRM                     |    | **| ns |
| Biochar × CRM           | ns | ns | ns |

Note: Different letters in a single column indicate significant difference between the treatments at p < .05. ‘**’ and ‘ns’ indicate significant levels at p < .01, and not significant (p > .05), respectively. Abbreviations: BCA, the soil with biochar applied in the field; BCF, Control with fresh biochar addition; C-BCA, BCA with CRM addition; C-BCF, BCF with CRM addition; C-control, Control with CRM addition; Control, soil without biochar.
DISCUSSION

4.1 Decomposition of C under fresh/aged biochar soil amendments and its response to crop-derived DOM addition

Some laboratory incubation studies showed an immediate or short-term increase in total CO₂ emissions following the addition of fresh biochar (Yousaf et al., 2017; Zhao et al., 2015). In agreement with these results, our present study showed that fresh biochar significantly increased the total CO₂ release, especially in the initial days of incubation (Figure 1). The primary reason was that relatively small portion of labile C in
Fresh biochar was decomposed by microorganisms during the early stages of incubation. More labile C fractions (containing higher H/C) existed in the fresh biochar, especially for biochar that was made at lower pyrolysis temperatures (Bian et al., 2016; Liu et al., 2019; Mukherjee, Zimmerman, & Harris, 2011). They can be utilized more easily by soil microorganisms over short periods (Chen et al., 2017). Consequently, a significant increase in CO2 release was found over short time, because an abundance of nutrients contained in the biochar boosted microbial growth and reproduction (Figure 4a) and accelerated organic matter decomposition (Sheng & Zhu, 2018; Singh, Cowie, & Smernik, 2012). In comparison, most of the degradable fractions in aged biochar that had been incorporated in the soil for several years were preferentially consumed by the soil microorganisms. A large amount of aromatic C remaining in the aged biochar increased C stability (Chen, Chen, et al., 2019). We found that aged biochar soil amendment suppressed the total C mineralization (Figure 2). Zimmerman, Gao, and Ahn (2011) reported that addition of biochar derived from grasses increased the C mineralization during the early period of incubation (first 90 days), whereas the addition of biochar derived from grasses at high temperatures (525 and 650°C) decreased to a lower CO2 release during the later incubation period (250–500 days), compared to the soil without biochar treatment. In the present study, the size of easily mineralizable C pool and CO2 release rate were significantly lower in the aged biochar-amended soil than in the fresh biochar-amended soil, suggesting that biochar amendment could modify C cycling and increase water availability for microbial growth and metabolism in soil. For one thing, biochar has higher porosity and large surface area/volume ratio (Zimmerman et al., 2011), which could increase its absorption ability and fix organic compounds to prevent biological decomposition (Smeybe et al., 2016). Owing to the adsorption of dissolved organic compounds to the biochar surface or micropores, the bioavailability of labile organic compounds could be limited and reduce the exogenous C mineralization under biochar addition. In addition, the effect of microbial community change on CRM decomposition under biochar amendment will be discussed in the below section.

4.2 | The effect of fresh/aged biochar soil amendments on microbial community composition and its implications for C sequestration

A lower $qCO_2$ was observed in biochar-amended soil compared to the no biochar-amended soil (Table 3). It suggested that biochar amendment could enhance the C utilization efficiency of microorganisms (Chen et al., 2018). This finding was also supported by Zheng et al. (2016), who reported that biochar decreased the microbial metabolic quotient 4 years after a single incorporation in a slightly acidic rice paddy. Similarly, Zhou et al. (2017) found an overall decrease of 13% in $qCO_2$ due to biochar addition by meta-analysis. Lehmann et al. (2011) demonstrated that biochar particles may generate microhabitats in the soil. The nutrients, substrates, and microorganisms were co-localized on biochar surfaces, thus mediating higher microbial biomass but lower C mineralization. The decreases in $qCO_2$, especially in aged biochar-amended soils, may be attributed to the improved microbe-suitable habitats because biochar could provide moisture and nutrients and increase water availability for microbial growth and

![Figure 7](image-url)
such as biochar C (Jiang et al., 2016), and the G+‐bacteria thus, they could benefit from the addition of aromatic C, toward G−‐bacteria over other types of microorganisms (2014) reported that the soil microbial community shifts (Figure 4a). Gomez, Denef, Stewart, Zheng, and Cotrufo were significantly more by the addition of fresh biochar (Ameloot et al., 2013). Lower microbial reproduction rate due to the liming potential (Luo et al., 2017). Shifts in microbial community composition could be suppressed due to biochar‐amendment. Moreover, it is different for survival strategies of bacteria and fungi. With fast high growth rates but few protective and/or structural compounds, soil bacteria typically act as r‐strategists. By comparison, soil fungi usually behave as k‐strategists, with slow growth rates but high C use efficiency (Six et al., 2006). The increase of bacteria/fungi ratio was limited due to CRM addition in the biochar soil amendments. Therefore, the biochar‐amendment could increase the efficiency of microbial utilization for exogenous C and reduce net C mineralization, especially in the aged biochar‐amended soil.

Long‐term (aged) biochar amendment significantly decreased total C mineralization in soil, while it was enhanced under the presence of short‐term (fresh) biochar. Furthermore, with the addition of exogenous C, its decomposition could be suppressed due to biochar‐amendment, especially in the aged biochar soil amendment. Fresh biochar addition can increase the microbial biomass, while the aged biochar‐amendment had no effect on the total PLFA concentration. Microbial community composition was altered mainly by an increase in the proportion of bacteria (17:1ω8c and i17:0) in fresh biochar amendment and fungi (18:2ω6, 9c) in aged biochar amendment. Moreover, biochar amendment, particularly aged, significantly decreased the microbial metabolic quotient, suggesting that biochar amendment could enhance the microbial use efficiency of C and the C sequestration potential of paddy soils.

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