Soil carbon mineralization following biochar addition associated with external nitrogen

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Biochar has been attracting increasing attention for its potentials of C sequestration and soil amendment. This study aimed to understand the effects of combining biochar with additional external N on soil C mineralization. A typical red soil (Plinthudults) was treated with two biochars made from two types of plantation-tree trunks (soil-biochar treatments), and was also treated with external N (soil-biochar-N treatments). All treatments were incubated for 42 d. The CO₂-C released from the treatments was detected periodically. After the incubation, soil properties such as pH, microbial biomass C (MBC), and microbial biomass N (MBN) were measured. The addition of biochar with external N increased the soil pH (4.31-4.33) compared to the soil treated with external N only (4.21). This was not observed in the comparison of soil-biochar treatments (4.75-4.80) to soil only (4.74). Biochar additions (whether or not they were associated with external N) increased soil MBC and MBN, but decreased CO₂-C value per unit total C (added biochar C + soil C) according to the model fitting. The total CO₂-C released in soil-biochar treatments were enhanced compared to soil only (i.e., 3.15 vs. 2.57 mg and 3.23 vs. 2.45 mg), which was attributed to the labile C fractions in the biochars and through soil microorganism enhancement. However, there were few changes in soil C mineralization in soil-biochar-N treatments. Additionally, the potentially available C per unit total C in soil-biochar-N treatments was lower than that observed in the soil-biochar treatments. Therefore, we believe in the short term, that C mineralization in the soil can be enhanced by biochar addition, but not by adding external N concomitantly.

Key words: Biochar, carbon sequestration, nitrogen, soil amendment.

INTRODUCTION

Biochar incorporation into soils can potentially sequester C and amend soils (Yu et al., 2010; Mulcahy et al., 2013; Spokas, 2013; Zhang et al., 2014). However, soil C mineralization can be altered by biochar within a short time, and the mechanism underlying this process warrants further investigation (Verheijen et al., 2014). A small fraction of labile C in biochar can be mineralized within a short period (Kuzyakov et al., 2009) and can stimulate soil microorganism growth (Quilliam et al., 2013). Biochar can provide a substrate for soil microorganisms, thereby enhancing microorganism activity (Gomez et al., 2014). This microbial growth induces soil C mineralization or degradation (Smith et al., 2010; Luo et al., 2011). However, other studies indicate that biochar alters the soil microbial community structure rather than biomass (Anders et al., 2013) and suppresses soil C mineralization through its adsorptive and biochemical effects (Jones et al., 2011). This variation in behavior and activity requires further clarification. An additional benefit of biochar is the potential to ameliorate soil acidification through the breakdown of carbonates contained in biochar (Bruun et al., 2014).

Nitrogen fertilizer application associated with biochar improves N utilization efficiency through mineral retention and biological fixation, and has the potential to increase N uptake by crops (Borchard et al., 2012). Thus, C sequestration and soil amendment can be simultaneously achieved by using biochar with N fertilizer. However, few studies have described soil C mineralization kinetics following biochar addition with external N, which is important for biochar C sequestration. External N addition can enhance soil microorganism growth, which may promote organic C degradation in soils (Gundale and DeLuca, 2007). Abiotic C mineralization dominates C mineralization process in a relatively short time (e.g., weeks to months) after biochar addition to soils (Cheng et al., 2006). However, this process may be modified when external N is combined with biochar as the additional N can affect soil microorganisms (Kolb et al., 2009), improving the soil microbial biomass C when compared to biochar alone (Chan et al., 2008). In another study, this...
association results in a decrease in soil pH over a period of 55 d (Clough et al., 2010), which may cause a decrease in soil microorganism activities (Aciego Pietri and Brookes, 2008). Therefore, combination of biochar with external N does not always promote soil C mineralization, as evidenced by the limited effect of biochar when combined with dairy manure on soil CO₂ release, relative to biochar alone (Sarkhot et al., 2012). Elucidation of soil C mineralization after biochar addition with external N is necessary not only for understanding soil organic C kinetics and soil amendment performance, but also for determining the effectiveness of C sequestration.

Laboratory incubation with standardized conditions is an effective method to examine C mineralization (Lefèvre et al., 2014). First-order models can be used to describe C mineralization kinetics after the addition of external materials (e.g., biochar) (Zhou et al., 2012; Quilliam et al., 2013). Plantation wastes of *Pinus massoniana* Lamb. and *Cunninghamia lanceolata* (Lamb.) Hook. are widely distributed in southern China; such wastes could be pyrolyzed to promote C sequestration and soil amendment. This study investigated C mineralization in a typical acidic soil of southern China, after adding biochar and external N. We hypothesized that plantation-waste biochar when combined with external N could amend acidic soils and enhance on soil C mineralization more effectively than biochar alone.

**MATERIALS AND METHODS**

**Sample collection**
Topsoil samples (0-30 cm) from a typical quaternary red soil (Plinthudults) were collected in Zhejiang Province (27°02′-31°11′ N, 118°01′-123°25′ E). The average annual temperature range is 15-19 °C and the mean annual precipitation is 1300 mm. The pH (1:2.5 w/v), organic C content and total N content of the soil sample were 4.84, 4.20 g kg⁻¹, and 0.28 g kg⁻¹, respectively. The proportions of sand (2.00-0.05 mm), silt (0.05-0.002 mm), and clay (< 0.002 mm) in soil samples were 21.67%, 37.00%, and 41.33%, respectively. Freshly cut *P. massoniana* and *C. lanceolata* trunks were collected as biochar feedstock.

**Biochar preparation**
The trunk samples were air-dried and shattered to provide 1-2 cm³ pieces, which were then placed in covered crucibles. The samples were pyrolyzed in a programmed muffle furnace (Shanghai Jinghong Laboratory Equipment Inc., Shanghai, China). A maximum temperature of 450 °C was selected as it was close to the temperature of natural forest fire (Wolf et al., 2013). The temperature raised at 20 °C min⁻¹ and the maximum temperature were maintained for 1 h. Biochars made from *P. massoniana* and *C. lanceolata* trunks were ready (labeled as PB and CB, respectively) when the furnace cooled to room temperature without disturbance. After being passed through a 1 mm sieve, the biochar samples were sealed in brown jars and stored in the dark.

**Biochar characteristic analysis**
Each biochar sample was mixed with deionized water (1:5 w/v) and then stirred with an electromagnetic stirrer for 2 min. After equilibrating for 1 h, the pH was determined with a digital pH meter (Sanxin-MP521, Shanghai Youyi Co., Shanghai, China). Volatile matter content was determined by measuring the difference in weight loss with the combustion of the biochar sample in a ceramic crucible at 950 °C for 6 min, and the ash content was determined by placing the sample at 750 °C for 6 h in a programmed muffle furnace (ASTM, 2007). The concentrations of elemental C, H, and N in the biochar sample were analyzed with an elements analyzer (Flash EA 1112, Thermo Finnigan, Milan, Italy). The O content was calculated using the weight difference, assuming that the biochars consisted of C, H, N, and O only. The results were determined using the ash-free dry weight. The carbonate content was measured using a volumetric analysis method as described in the literature (Yuan et al., 2011).

The Brunauer-Emmet-Teller (BET) surface area was measured using the N gas adsorption-desorption method on an Autosorb-1-C analyzer (Quantachrome Instruments, Boynton Beach, Florida, USA). Each biochar sample was analyzed for approximately 5 h after being degassed at 300 °C, with the total time dependent on the time taken to reach stabilization. The surface area was calculated with a multipoint plot over a P/P₀ range of 0.05-0.35. Fourier-transform infra red (FTIR) methodology was employed to detect any surface functional groups on a Nicolet 5700 (Nicolet Instrument, Thermo Electron Corporation, Madison, Wisconsin, USA). The biochar sample was initially mixed with potassium bromide (KBr) at the ratio of approximate 1%, and then this mixture was milled using an agate mortar and pressed into a pellet. The FTIR spectrum was normalized to the highest peaks in the fingerprint (4000-400 cm⁻¹) with a resolution of 4.0 cm⁻¹.

The dissolved organic C (DOC), total soluble N (TSN), nitrate N, and ammonium N in the biochar were analyzed according to Gaskin et al. (2008). One gram biochar sample (0.3 mm sieve) was mixed with 20 mL of deionized water in a disposable cellulose nitrile filter (0.45 μm) flask. The flask was shaken for 5 min (180 rpm) and then vacuum filtered. The leachate was collected after this process which was replicated five times to measure DOC and TSN using a multi N/C 3100 analyzer (Analytik Jena AG, Jena, Germany), and to measure nitrate N and ammonium N using a flow injection analyzer (SA-4000, Skalar Co., Breda, The Netherlands).

**Incubation experiment**
Twenty gram soil samples (dw; 2 mm sieve) were treated with N (NH₄NO₃) and the biochar samples. The
N addition rate of 0.15 N g kg\(^{-1}\) was selected based on local agricultural practices (Ye et al., 2012). Soil-biochar treatments consisted of soil + 1% PB (SP1), soil + 1% CB (SC1), soil + 2% PB (SP2), and soil + 2% CB (SC2). Soil-biochar-N treatments consisted of soil + 1% PB + N (SP1N), soil + 1% CB + N (SC1N), soil + 2% PB + N (SP2N), and soil + 2% CB + N (SC2N). Both the PB and CB samples were mixed with quartz sand (at 2% based on SP2 or SC2) which had been washed with 1 M HCl and then distilled water to a neutral pH (i.e., sand-biochar treatments; labeled as QP2 and QC2, respectively). Treatments of soil + N (SN) and soil only (CK) were set as controls. All treatments were replicated three times.

Soil treatments were adjusted to 40% water-holding capacity (WHC), and all treatments were placed into 1 L Erlenmeyer flasks. A tube containing 5 mL NaOH (0.10 M) solution was placed into each flask to trap CO\(_2\). The flask was sealed with a rubber stopper and incubated at 25 °C in the dark. The CO\(_2\) captured by the NaOH solution was titrated with 0.1 M HCl solution at 1, 2, 4, 8, 12, 15, 22, 32, and 42 d incubation periods. The results were calculated as C contents in CO\(_2\) (CO\(_2\)-C). At each titration, each flask was ventilated for 2 min and a new tube containing fresh NaOH solution replaced the old one. During the incubation, the moisture in the soil treatments was maintained at 40% WHC by weekly weighing and adding distilled water.

### Soil properties analysis after incubation

Each soil sample was air-dried and mixed with deionized water (1:2.5 by w/v). The mixture was stirred with a magnetic stirrer for 1-2 min, and equilibrated for 30 min. The pH was measured with a digital pH meter. Five grams of moist soil sample were mixed with 25 mL ultra-pure water and shaken for 90 min (180 rpm). After being centrifuged for 15 min (4000 rpm), the mixture was filtered through a 0.45 µm millipore filter. The extract was measured for DOC and DON on a multi N/C 3100 analyzer. The soil microbial biomass C (MBC) was estimated by the difference (EC) between the soluble total N extracted from fumigated and unfumigated samples according to Equation [1] (Vance et al., 1987).

\[
\text{MBC} = \frac{\text{EN}}{2.64}
\]

where, 2.64 and 1.85 in the equations are dimensionless constants.

### Statistics

The data among different treatments was compared using the one-way ANOVA with the Tukey’s significant (P < 0.05) difference as a post hoc test using SPSS v18.0 (SPSS Inc., Chicago, Illinois, USA). A bivariate correlation (Pearson, two-tailed) was applied to determine the correlation between pH and total cumulative CO\(_2\)-C. A first-order kinetic model was employed to fit cumulative CO\(_2\)-C evolution using Origin 8.5 (OriginLab Corp., Northampton, Massachusetts, USA). The first-order model is given in Equation [3]:

\[
\text{C}_t = \text{C}_0 (1 - e^{-kt})
\]

where, \(\text{C}_0\) is mineralized C at time t; \(\text{C}_0\) is the potentially available C (labile C potential); \(k\) is apparent rate constant; and \(t\) is time of incubation (Zhou et al., 2012).

### RESULTS

The PB and CB had similar elemental concentrations (C, H, N, and O), DOC, nitrate N, pH, volatile matter, and ash (Table 1). Biochar CB had higher amounts of TSN and ammonium N than PB, while PB had larger surface areas. Carbonates were not detected in either of the biochar samples. Similar functional groups were observed on both PB and CB (Figure 1). Peaks at around 3385 cm\(^{-1}\) were assigned to aromatic structures. Peaks at 1705 and 1600 cm\(^{-1}\) were attributed to carbonyl and carboxyl structures, respectively.

Biochar additions increased cumulative CO\(_2\)-C release (Figure 2 and Table 2). Soil-biochar treatments had higher levels of cumulative CO\(_2\)-C evolution than soil-biochar-N treatments; labeled as QP2 and QC2, respectively.

### Table 1. Characteristics of biochars made from Pinus massoniana trunk (PB) and made from Cunninghamia lanceolata trunk (CB).

| Items                  | Biochars |
|------------------------|----------|
|                       | PB       | CB       |
| Element C, mg kg\(^{-1}\) | 704.60   | 712.10   |
| Element H, mg kg\(^{-1}\) | 23.90    | 24.70    |
| Element N, mg kg\(^{-1}\) | 1.30     | 3.20     |
| Element O, mg kg\(^{-1}\) | 270.20   | 260.00   |
| O/C                   | 0.38     | 0.37     |
| H/C (x10\(^{2}\))     | 3.39     | 3.47     |
| DOC, g kg\(^{-1}\)     | 2.54     | 1.88     |
| TSN, mg kg\(^{-1}\)    | 27.23    | 45.92    |
| Nitrate N, mg kg\(^{-1}\)| 3.54     | 2.60     |
| Ammonium N, mg kg\(^{-1}\) | 10.76   | 22.18   |
| pH                    | 5.01     | 4.88     |
| Volatile matter, mg kg\(^{-1}\) | 10.10 | 9.70   |
| Ash, mg kg\(^{-1}\)   | 14.00    | 10.30    |
| Carbonsates, mg kg\(^{-1}\) | ND      | ND      |
| BET surface area, m\(^2\) g\(^{-1}\) | 224.30 | 145.50 |

DOC: Dissolved organic C; TSN: total soluble N; BET: Brunauer-Emmet-Teller; ND: not detected.
The measured cumulative CO₂-C values were normalized to total C (added biochar C + native soil C), which were then fitted using first-order kinetic models (Figure 3). The kinetic model adequately described the degree of conformity between the experimentation data and the model-predication as indicated by the $R^2$ coefficients (Table 2). Whether or not associated with external N, the biochar additions did decrease the cumulative CO₂-C evolution per unit total C (Figure 3 and Table 2). According to the first-order model, the biochar additions, and in particular the 2%-additions decreased the labile C potential per unit total C ($C_0$). The rate constant ($k$) values were higher in the soil-biochar treatments than in the other treatments. Soil-biochar and soil-biochar-N mixtures had lower mineralizable C potential per unit total C ($C_1 + C_2$) than CK and SN.

Small changes in soil DOC and DON after biochar addition alone were observed (Table 3). Soil-biochar-N treatments demonstrated higher levels of nitrate N and ammonium N than SN, but the differences were not observed in the comparison of soil-biochar treatments to CK. The soil amended with biochar and external N improved the soil MBC and MBN contents. Soil pH values could be ranked as: soil-biochar treatments = CK > soil-biochar-N treatments > SN.

**DISCUSSION**

Effects of biochar addition on soil C mineralization

No change was observed in the soil DOC after biochar additions, which is similar to previous findings (Jones et al., 2012). The CO₂-C evolution distribution and higher levels of total CO₂-C released in SP2 vs. QP2 and SC2 vs. QC2 + CK (Table 4) suggest that soil C mineralization can be enhanced by the biochar additions, which is consistent with the previous report.
Table 3. Soil properties after incubation.

| Treatments | DOC | DON | MBC | MBN | Nitrate N | Ammonium N | pH |
|------------|-----|-----|-----|-----|-----------|------------|----|
| SP1        | 177.61 ± 15.25a | 4.56 ± 0.67a | 40.05 ± 1.97b | 8.20 ± 0.82b | 6.56 ± 0.90a | 5.08 ± 0.77a | 4.80 ± 0.02c |
| SC1        | 197.83 ± 21.45a | 4.79 ± 0.11a | 38.80 ± 7.25b | 7.80 ± 2.72ab | 6.37 ± 1.14a | 5.95 ± 0.64a | 4.77 ± 0.01c |
| SP2        | 182.34 ± 24.35ab | 4.00 ± 0.47a | 44.15 ± 10.14b | 8.01 ± 2.21ab | 5.62 ± 0.72a | 4.53 ± 0.79a | 4.79 ± 0.01c |
| SC2        | 219.36 ± 25.35ab | 4.21 ± 0.28a | 39.06 ± 5.70b | 8.83 ± 1.55b | 6.18 ± 0.83a | 4.70 ± 0.82a | 4.75 ± 0.01c |
| SP1N       | 201.52 ± 16.35ab | 191.34 ± 3.63b | 38.63 ± 7.92b | 8.59 ± 2.26ab | 86.99 ± 1.11c | 82.34 ± 0.57c | 4.33 ± 0.03b |
| SC1N       | 213.51 ± 14.71a | 193.57 ± 8.71b | 42.41 ± 8.74b | 7.32 ± 3.44ab | 84.23 ± 0.18c | 82.55 ± 0.81c | 4.31 ± 0.03b |
| SP2N       | 220.83 ± 23.42ab | 186.30 ± 10.82b | 38.61 ± 6.77b | 8.75 ± 1.75b | 88.63 ± 1.20c | 80.51 ± 1.74c | 4.31 ± 0.03b |
| SC2N       | 195.98 ± 26.82ab | 181.89 ± 16.5b | 42.25 ± 7.81b | 9.17 ± 1.22b | 89.83 ± 1.57c | 81.73 ± 1.39c | 4.33 ± 0.03b |
| CK         | 236.58 ± 18.93b | 4.11 ± 0.45a | 21.48 ± 2.83b | 4.31 ± 0.03b | 4.77 ± 0.01c | 4.31 ± 0.03b | 4.31 ± 0.03b |
| SN         | 203.19 ± 4.82ab | 186.23 ± 14.51b | 19.32 ± 3.10a | 4.21 ± 0.03b | 60.51 ± 1.74c | 51.08 ± 2.02b | 4.21 ± 0.03a |

Table 4. Total amounts of released CO₂-C during the incubation.

| Treatments | Amounts of CO₂-C (mg) |
|------------|-----------------------|
| SP1        | 2.66 ± 0.13cd        |
| SC1        | 2.94 ± 0.26de        |
| SP2        | 3.15 ± 0.31e         |
| SC2        | 3.23 ± 0.38e         |
| SP1N       | 2.14 ± 0.30bc        |
| SC1N       | 2.02 ± 0.11bc        |
| SP2N       | 2.41 ± 0.17cd        |
| SC2N       | 2.25 ± 0.36d         |
| QP2        | 0.92 ± 0.05a         |
| QC2        | 0.81 ± 0.24a         |
| CK         | 1.65 ± 0.26b         |
| QP2 + CK   | 2.57 ± 0.17cd        |
| QC2 + CK   | 2.45 ± 0.16cd        |
| SN         | 1.46 ± 0.07b         |
| QP2 + SN   | 2.38 ± 0.02cd        |
| QC2 + SN   | 2.72 ± 0.02d         |

QQ2 + CK, QC2 + CK, QP2 + SN, and QC2 + SN represent the sum of CO₂-C released from the sand-biochar treatments and from the controls (CK or SN).

Effects of biochar combined with external N on soil C mineralization

Increased levels of CO₂-C in the soil-biochar-N vs. soil-biochar treatments (Figure 2) and relatively few differences in the total cumulative CO₂-C values between soil-biochar-N treatments and SN (Table 2) suggest that when biochar is added with external N, it cannot facilitate soil C mineralization in the short term. This conclusion is supported by the non-significant differences (P > 0.05) in the total amount of CO₂-C released in SP2N vs. QP2 + SN and SC2N vs. QC2 + SN (Table 4), and by the lower levels of potentially available C (Cₐ) per unit total C in soil-biochar-N vs. soil-biochar treatments (Table 2). Decreased soil pH and few changes in microbial biomass between soil-biochar-N and soil-biochar treatments (Table 3) suggest that the restrictive effects of biochar additions associated with external N on microorganism activity contribute to the mineralization suppression since soil acidity is critical to soil microbial functionality (Aciego Pietri and Brookes, 2008). Further evidence is provided by the correlation between pH and the total cumulative CO₂-C values in the N-present treatments (r = 0.625, P < 0.05, n = 15). The suppression effects observed in this study is different from previous findings in the literature (Gundale and DeLuca, 2007). This may be attributed to the different type of external N (i.e., glycine in the literature) because biochar additions associated with urea (another external N) has also lowered soil pH after more than 50 d (Clough et al., 2010). The higher levels of mineral N and increased pH in soil-biochar treatments vs. SN confirm that biochar can retain external added mineral N (Güereña et al., 2013), and suggest that the biochars can amend acidic soils due to the oxygen-containing functional groups (Figure 1) (Xu et al., 2012). Simultaneously, this association can maintain organic C in the soil in the short term. Thus, the biochars associated with mineral N fertilizer can amend acidic soils more effectively than the biochars alone.

(Brun et al., 2011). Labile C fractions in both PB and CB is a main source of C mineralization in the short term (Cheng et al., 2006), as shown by the labile C fraction contents (Table 1) and released CO₂-C in QP2 and QC2 (Table 2 and Figure 2), which may incur soil C mineralization (Luo et al., 2011). The increased soil microbial biomass (Table 3) suggests that soil microorganism growth has been promoted by the biochar additions as the labile C fractions benefit microorganisms (Calvelo Pereira et al., 2011). Thus, both the C mineralization of abiotic process in the biochars and the microorganism process in the soil, contributes to the CO₂-C enhancement following the addition of biochar. However, the habitat-availability effects of biochar on soil microorganisms are relatively minor in the short term (Quilliam et al., 2013), which accounts for the non-significant differences in both the released CO₂ and microbial biomass between the soil-PB and soil-CB treatments; even though there are notable differences in the surface areas between PB and CB (Table 1).
Carbon mineralization kinetics according to model fitting

According to the first-order kinetic model applied in this study, the turnover rate of available C increases following the addition of biochar but slows when external N is added simultaneously, as demonstrated by the distribution of k (Zhou et al., 2012), and responds to the decline of C mineralization in soil-biochar-N vs. soil-biochar treatments. However, the amount of C mineralization is minor compared to the total C (Figure 3 and Table 2), suggesting that the biochar additions (whether or not they are associated with external N) can improve C storage in the soil. This is supported by the lower values of the potential labile C per unit total C in the soil-biochar-N and soil-biochar treatments compared to the controls (Table 2). This result is similar to previous findings in that the short-term C loss should no compromise the ability of biochar to store C in soils (Smith et al., 2010; Jones et al., 2011).

CONCLUSIONS

This study has demonstrated that C mineralization in the soil can be enhanced by the biochars through stimulating soil microorganisms in the short term, and that this process is not sensitive to simultaneous additions of external N. Carbon storage in the soil is improved after the addition of biochar but slows when external N is added simultaneously, as demonstrated by the distribution of k (Zhou et al., 2012), and responds to the decline of C mineralization in soil-biochar-N vs. soil-biochar treatments. However, the amount of C mineralization is minor compared to the total C (Figure 3 and Table 2), suggesting that the biochar additions (whether or not they are associated with external N) can improve C storage in the soil. This is supported by the lower values of the potential labile C per unit total C in the soil-biochar-N and soil-biochar treatments compared to the controls (Table 2). This result is similar to previous findings in that the short-term C loss should no compromise the ability of biochar to store C in soils (Smith et al., 2010; Jones et al., 2011).

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