Dynamics of C and N in a clay loam soil amended with biochar and corn straw

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ABSTRACT
An incubation study was conducted to determine the influence of biochar and corn straw on CO₂-C emission, soil organic C, microbial biomass C and N, total N, and mineral N (NH₄⁺-N and NO₃⁻-N) in a clay loam soil. Six treatments viz., CK (Control); S (Soil + 1% straw); B1 (Soil + 0.5% biochar); B2 (Soil + 2% biochar); SB1 (Soil + 1% straw + 0.5% biochar); SB2 (Soil + 1% straw + 2% biochar) were tested with three replications. Results showed that straw addition to soil with or without biochar increased CO₂-C emission while sole-biochar addition (2%) reduced it. Straw and biochar also increased the soil microbial biomass C and N but greatest increase in microbial biomass N (111.9 µg g⁻¹) was recorded by biochar-straw combination. SOC and total N significantly increased following biochar and straw additions which suggest that organic amendments can improve soil chemical properties. Additionally, for soil mineral N, biochar reduced NH₄⁺-N and NO₃⁻-N concentrations while straw increased NH₄⁺-N concentration but greatly reduced that of NO₃⁻-N.

Key words: Biochar, Microbial biomass, Mineralization, Soil organic carbon, Straw, Total N.

INTRODUCTION
Biochar is the solid product of biomass obtained after pyrolysis which seems to be a very promising soil amendment. During the past decade, biochar has been adjudged to be a valuable product that gives room for soil improvement and carbon sequestration, in order to mitigate climate change (Peake et al., 2014). Incorporating biochar to soil as an amendment has been shown to influence physical, chemical, and biological properties of soil, as well as shows increase in soil pH, improvement in soil structure, soil aeration and water retention capacity (Mukherjee and Lal, 2013). Biochar is described as a heterogeneous material that varies widely in its chemical and physical properties. This variability depends on the parameters involved in pyrolysis and also on the materials used for biochar production (Atkinson et al., 2010). Considering that biochar is recalcitrant against microbial decomposition (Wang et al., 2015), positive (Fang et al., 2014), negative (Keith et al., 2011) and no effects (Kuzyakov et al., 2009) of biochar addition on mineralization of native soil organic matter (SOM) have been reported under laboratory conditions.

Addition of biochar to soil has been shown to change the soil nitrogen (N) dynamics by reducing the mineralization of biochar materials than the uncharred biomass (Song et al., 2013) and decreasing net N mineralization (Dempster et al., 2012). Clough and Condron (2010) reported that biochar can improve the quality of agricultural soils by increasing N retention and improving N use efficiency.

Straw, as an important organic fertilizer and renewable resource, contains C, N, P, K, and other nutrients in abundance and are readily available (Xu et al., 2010). Returning straw to the soil has been used to increase soil organic matter, improve soil physical properties, and increase crop productivity (Lou et al., 2011). Additionally, straw application has been used to improve the activity of soil microorganisms and promote soil nitrogen and carbon sequestration potential (Lu et al., 2009).

Many research works have been done to determine the influence of amendments on soil properties but few have looked into comparing the influence of biochar and straw on C and N dynamics. To understand clearly the change in soil C and N dynamics following biochar and straw additions, two biochar levels (0.5 and 2%) and a uniform rate of straw (1%) were applied to soil in a controlled environment. It is therefore expected that the quantity of added biochar would have a greater influence on selected soil properties. The objective of this study was to determine the influence of biochar and straw on C and N mineralization, soil organic C, microbial biomass and total N on a clay loam soil.

MATERIALS AND METHODS
Soil, biochar and straw: A high organic matter clay loam soil (Mollisol) was used for the experiment. The soil was collected from the Experimental and Practical Basement of Northeast Agricultural University, Harbin. Corn was planted on the soil in the previous cropping season. The soils were collected randomly at a depth of 0-20 cm, bulked to form a composite sample, then allowed to pass through < 2 mm
sieve and the basic properties determined (Table 1). Harvested corn residues (stems and leaves) were air-dried, crushed into tiny pieces with an electric grinder and allowed to pass through <2 mm sieve. Biochar was sourced from Jin and Fu Agriculture Company, China. It was produced from corn at a pyrolysis temperature of 450°C in an oxygen-restricted environment, and was crushed to pass through <2 mm sieve. The properties of biochar, straw and soil are listed in Table 1.

**Incubation procedure and C mineralization:** The sieved soil was amended with three levels of biochar (0, 0.5 and 2%) which are equivalent to 0, 10 and 40 Mg ha⁻¹ respectively. For anaerobic incubation, equivalent to 25 g dry weight soils with 3 replications were placed in air-tight glass jars (0.3 L) in a completely randomized design. The soil-biochar combinations were mixed thoroughly in order to achieve a uniform distribution. In the treatments receiving straw (S, SB1 and SB2), the straw was added at a uniform rate of 1% (20 Mg ha⁻¹) and was thoroughly mixed. There were six treatments in total; CK (unamended soil); S (soil + 1% straw), B1 (soil + 0.5% biochar); B2 (soil + 2% biochar); SB1 (soil + 1% straw + 0.5% biochar); SB2 (soil + 1% straw + 2% biochar). All treatments were moistened to 60% of their water holding capacity and incubated for 100 days at 25°C in the dark. Water content was regularly checked gravimetrically and adjusted with de-ionized water. Carbon mineralization was measured as CO₂-C using alkaline trap (Tufekcigil et al., 2001) during incubation. Emitted CO₂ was trapped in 10 ml of NaOH and was titrated with HCl using destructive sampling method on days 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 after carbonate precipitation with BaCl₂.

**N mineralization:** The treatments were destructively sampled at 20, 40, 60, 80 and 100 days of incubation for determination of mineral N in the forms of ammonium nitrogen and nitrate nitrogen. Soil NH₄⁺-N and NO₃⁻-N contents were extracted with 50 ml of 2 M KCl (1:10 w/v) after shaking for 2 h and the extracts were analyzed using an automatic flow analyzer (FIStar 5000 Injection Analyzer, Warrington, USA).

**Microbial biomass, SOC and Total N:** Microbial biomass C and N were determined from fresh soil samples by fumigation and extraction technique as described by Vance et al., (1987). SOC was measured using wet oxidation with K₂Cr₂O₇ while total N was measured using Kjeldahl method as described by Gupta (2006).

**Statistical analysis:** Statistical analysis of the data sets was performed using SPSS 19.0 program (SPSS Inc., Chicago, USA). After testing of assumptions, the data sets were analyzed using one-way Analysis of Variance (ANOVA) which was followed by Duncan Multiple Range Test (DMRT) at P<0.05 significance level for means comparison.

**RESULTS AND DISCUSSION**

**C mineralization:** The soil C mineralization was influenced by biochar and straw additions (Fig 1). A steep decline in C mineralization rate was observed in first 25 days of incubation (Fig 1a). Straw additions significantly (P<0.01) increased C mineralization rate throughout the 100-day incubation period in comparison with the unamended soil. Treatments receiving biochar and straw combinations also recorded higher CO₂-C emission than those receiving sole biochar and control. The low biochar concentration (B1) did not have any effect on C mineralization when compared with the unamended soil. Greatest CO₂-C release was recorded by treatments S and SB1, and they were significantly (P<0.01) higher than other treatments. However, B2 (2% biochar) recorded the lowest C mineralization rate. It significantly (P<0.01) reduced CO₂-C evolved in comparison with the control. Significant differences (P<0.01) were also observed for cumulative C mineralization among the treatments (Fig 1b). Differences in cumulative CO₂-C evolved followed the trend, S > SB1 > SB2 > B1 > CK > B2, with values ranging between 22.6 and 202.8 mg CO₂-C g⁻¹. Our result is consistent with the works of Zhu et al. (2017) and Oduguhenro et al. (2018). Increase in microbial growth and activity following biochar addition to soil have been reported by Lu et al. (2015) as the labile C fraction is beneficial to microbes, but this improvement did not lead to increase in CO₂-C efflux. Reason adduced to this could be as a result of low labile organic carbon content of the biochar used for our experiment. Conversely, straw addition with or without biochar significantly (P<0.01) increased C mineralization rate and cumulative C mineralization. The sudden increase in microbial activities accompanying straw additions which contains sufficient labile organic C could be responsible for the increase in CO₂-C efflux during incubation, and similar results have been reported by Li et al. (2018).

Table 1: Basic physical and chemical properties of soil, straw and biochar.

| Parameter                  | Soil (g kg⁻¹) | Straw (g kg⁻¹) | Biochar (g kg⁻¹) |
|----------------------------|---------------|---------------|-----------------|
| pH                         | 6.12          | 7.02          | 9.89            |
| Total N                    | 0.7           | 5.0           | 6.89            |
| Available N (mg kg⁻¹)      | 24.4          | -             | *               |
| Organic Carbon (g kg⁻¹)    | 38.2          | 400.2         | 415.3           |
| Available P (mg kg⁻¹)      | 23.8          | 2.3           | *               |
| Total P (mg kg⁻¹)          | 0.48          | -             | *               |
| Available K (mg kg⁻¹)      | 185.6         | 36.2          | 25.9            |
| Particle Size (g kg⁻¹)     | Sand          | 500           | -               |
|                            | Silt          | 190           | -               |
|                            | Clay          | 310           | -               |
| Textural Class (USDA)      | Clay Loam     | -             | -               |
| Bulk Density (g cm⁻³)      | 1.38          | -             | -               |

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(2015) as the labile C fraction is
Soil organic carbon (SOC): Biochar and straw additions had influence on SOC as expected as a result of their high C content (Table 2). SOC increased with biochar application rate; treatment receiving a combination of straw and 2% biochar application (SB2) showed the highest SOC and was significantly (P<0.01) higher than unamended soil (CK), straw (S), 0.5% biochar (B1) and straw + 0.5% biochar (SB1). Moreover, treatment S was also significantly (P<0.01) higher than CK. The high level of SOC in biochar-treated soils may be as a result of the potential of biochar to increase soil recalcitrant C and this will remain in the soil much longer than carbon supplied in the form of residues and biogenic soil organic matter (Jefrey et al., 2009). Even though treatment effect was high, no significant change in SOC was observed over the 100-day incubation, which is an indication of reasonable resistance to microbial mineralization as reported by Novak et al. (2010).

Soil microbial biomass carbon and nitrogen (SMBC and SMBN): The change in SMBC following addition of biochar and straw is shown in Fig 2. Treatments effect on SMBC was large, with B2 showing the highest value (564.2 µg g⁻¹) at 20 days and CK showing the lowest value (96.8 µg g⁻¹) at 80 days. Treatment S was also significantly (P<0.05) higher than CK throughout the incubation period which is a sign that biochar and straw can act as a source of carbon for microorganisms and also improve their growth and activity in the soil solum. Our finding is in contrast to the work of Zhu et al. (2017) who reported that straw addition significantly increased microbial biomass while biochar addition without crop residues had no effect on SMBC. However, Zhang et al. (2014) reported an increase in SMBC following biochar addition to a sandy loam.

![Figure 1: C mineralization rate (A) and cumulative C mineralization (B) of soil amended with biochar and straw during incubation (Mean ± SE, n=3).](image1)

![Figure 2: Microbial biomass C as affected by biochar and straw additions during incubation (Mean ± SE, n = 3).](image2)

Table 2: SOC as affected by biochar and straw additions during incubation (Mean ± SE, n = 3).

| Treatments | SOC (g kg⁻¹) |
|------------|--------------|
|            | 20 d         | 40 d         | 60 d         | 80 d         | 100 d        |
| CK         | 39.1±2.01d   | 38.4±1.96d   | 38.4±0.85d   | 39.0±2.33d   | 38.2±3.41c   |
| S          | 51.2±1.71c   | 52.1±1.16c   | 51.9±2.56c   | 51.8±1.71c   | 51.2±2.52d   |
| B1         | 53.1±1.12c   | 53.1±0.56c   | 52.3±0.65c   | 52.0±2.96c   | 53.1±1.12cd  |
| B2         | 59.2±0.56a   | 59.0±0.65ab  | 60.0±0.85ab  | 59.0±0.85ab  | 58.8±0.85ab  |
| SB1        | 56.6±0.85b   | 56.8±1.79b   | 56.6±1.41b   | 56.8±1.79b   | 55.7±0.85bc  |
| SB2        | 61.3±0.32a   | 61.4±1.17a   | 61.8±0.85a   | 60.1±0.65a   | 61.4±0.56a   |

Means followed by different letters are significantly different (P<0.01).
Fig 3: Microbial biomass N as affected by biochar and straw additions during incubation (Mean ± SE, n = 3). Means followed by different letters are significantly different (P<0.05).

The change in SMBN among treatments was also large as shown in Fig 3. Highest SMBN (111.9 µg g⁻¹) was recorded by SB1 at 20 days and it was significantly (P<0.001) higher than other treatments. Treatments S, SB1 and SB2 were significantly (P<0.001) higher than B1 and B2. The large changes observed in SMBC and SMBN at different incubation days indicate differences in microbial activity and also suggest that the presence of organic matter and nutrients in both biochar and straw could provide energy for soil microorganisms and also provide a favourable environment for them to thrive.

Total N: The influence of biochar and straw on soil total N is shown in Fig 4. It is the sum of nitrate (NO₃⁻), nitrite (NO₂⁻), organic nitrogen and ammonia. Straw and biochar treatments significantly increased the total N content in soil in comparison with the unamended soil. SB1 and SB2 recorded the highest total N content (1.03 g kg⁻¹) at 90 days followed by B2 (0.99 g kg⁻¹) at 70 days while the lowest total N content (0.65 g kg⁻¹) was recorded by CK at 10 days. The increase in total N following biochar and straw additions is an indication that biochar and straw are beneficial to the accumulation of soil total N and consequently improve soil total N stocks. Similar results have been reported by (Zhang et al., 2015; Agegnehu et al., 2016).

N mineralization: Differences in NO₃⁻-N levels among treatments is shown in Fig 5a. Highest NO₃⁻-N mineralization (33.79 mg kg⁻¹) was shown by CK at 80 days. Biochar and straw additions both significantly (P<0.01) reduced soil NO₃⁻-N levels when compared with CK but straw (S) and biochar-straw combinations (SB1 and SB2) showed the lowest NO₃⁻-N levels. In contrast to NO₃⁻-N, treatment S significantly (P<0.01) increased NH₄⁺-N mineralization when compared with other treatments (Fig 5b). Biochar addition reduced soil NH₄⁺-N levels, with B2 showing the greatest reduction.
throughout incubation. Straw-biochar combinations also reduced soil NH$_4^+$-N levels. A decrease in NH$_4^+$-N levels was observed between days 20 and 100, which is an indication of net immobilization. Greatest NH$_4^+$-N value (5.27 mg kg$^{-1}$) was shown by treatment S at 80 days and it was significantly (P<0.01) higher than other treatments while the lowest value (0.38 mg kg$^{-1}$) was shown by B2 at 40 days, with no significant difference (P>0.05). Similar results have been reported by (Prayogo et al., 2014; Zhu et al., 2017). Our findings also showed that even though the lesser rate of biochar application reduced NO$_3^-$-N level; it had no effect on cumulative C mineralization. Reason for the reduction could be due to the high C/N ratio of biochar and greater potential for immobilization as well as the adsorption of both NH$_4^+$ and NH$_3$ by their particles from soil solution thus reducing solution mineral N at least temporarily, but maybe concentrating it for microbial use and activity (Lehmann et al., 2006).

**CONCLUSION**

The results from this incubation study showed that addition of biochar and corn straw influenced C and N mineralization. Biochar incorporation reduced C mineralization rate while straw significantly increased C mineralization when compared with the unamended soil. Biochar and straw both increased soil MBC and MBN which is an indication that the organic amendments provided a more suitable environment for microorganisms in the short term. The SOC and total N content of the soil were also improved by biochar and/or straw additions. However for soil mineral N, both straw and biochar reduced NO$_3^-$-N concentration while straw addition increased NH$_4^+$-N.

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