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Effects of biochar on carbon pool, N mineralization, microbial biomass and microbial respiration from mollisol

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Biochar incorporation as a soil amendment has been shown to enhance soil quality. However, there has been conflicting reports on its short term effects on C and N mineralization and microbial biomass. An incubation experiment was conducted to determine the effects of three different levels (0.5, 1 and 2%) of biochar on carbon mineralization, soil organic carbon, nitrogen mineralization, microbial biomass and total nitrogen from mollisols of two different organic matter (high organic matter soil and low organic matter soil) levels. The experiment consisted of four treatments (Soil, Soil + 0.5% biochar, Soil + 1% biochar and Soil + 2% biochar) and each was replicated three times. Overall, soil respiration rate was reduced by biochar additions over a 100-day period. Two percent biochar application rate showed greatest CO₂-C reduction. Soil respiration in high organic matter soil was higher than low organic matter soil. NO₃⁻-N level was reduced by biochar addition in both high and low organic matter soils. Control (Soil) of the high organic matter soil showed the highest NO₃⁻-N (33.79 mg kg⁻¹) and NH₄⁺-N (7.23 mg kg⁻¹) values at 70 days. The total nitrogen was increased by biochar additions; 1 and 2% application rates showed the highest total nitrogen values. Biochar additions also increased soil microbial biomass carbon and soil microbial biomass nitrogen of both soils.

Key words: Biochar, C mineralization, N mineralization, microbial biomass.

INTRODUCTION

Biochar is the product of the thermal degradation of organic materials in the absence of air (pyrolysis) and is distinguished from charcoal by its use as soil amendment (Lehmann et al., 2011). It is considered as a carbon-rich organic matter with long residence time up to hundreds of years (Kuznyakov et al., 2009; Lehmann et al., 2015). Biochars made from diverse biomass species (feedstock)

are characterized by different morphological and chemical properties but also characteristically differ based on specific pyrolysis conditions (that is, final pyrolysis temperature or peak temperature, rate of charring or ramp rate, and duration of charring time) (Mukherjee and Zimmerman, 2013). Hence biochar properties and the effect on crop production depend

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on feedstock, pyrolysis conditions and soil type (Jeffery et al., 2011). The effect of biochar is said to be strongly connected to the soil properties and the climate but the correlations with crop yield are not completely clear (Cornelissen et al., 2018). Several authors (Agegnehu et al., 2016; Jeffery et al., 2017) have stated that the yield increases are related to an overall improvement of soil qualities. Biochar application is reported to stimulate (Wardle et al., 2008; Luo et al., 2011) or conversely, to suppress (Keith et al., 2011; Zimmerman et al., 2011) the mineralization of native soil organic carbon (SOC); these effects are termed positive and negative priming, respectively (Luo et al., 2011; Keith et al., 2011; Zimmerman et al., 2011). The differing results observed in previous studies may have been due to variations in the proportion of labile C in biochars (Luo et al., 2011), the presence or absence of plant-derived labile organic matter input in soil (Keith et al., 2011), and the degree of biochar ageing in soil (Zimmerman et al., 2011; Cross and Sohi, 2011; Liang et al., 2010).

Soil respiration is a product of several rhizospheric processes, which is root exudation, root respiration, and root turnover, as well as decomposition of litter and bulk soil organic matter from various pools with different characteristic turnover times (Luo et al., 2001). Release of CO₂ from soils due to the production of CO₂ by roots and soil organisms and, to a lesser extent, chemical oxidation of carbon compounds is commonly referred to as soil respiration. Soil surface CO₂ efflux, or soil respiration, is a major component of the biosphere's carbon cycle which is influenced by the environment change because it may constitute about three-quarters of total ecosystem respiration (Law et al., 2001). Application of biochar has been shown to have a variety of effects on the soil biota which may be associated with its impacts on C and N cycling. Biochar has the capacity to potentially sequester C, and also has agronomic benefits such as improving soil quality, nutrient availability and crop yield (Sohi et al., 2010; Spokas et al., 2012; Schulz and Glaser, 2012). Biochar amendments can alter soil N dynamics (Clough and Condon, 2010), increase soil pH, base saturation, available nutrient content, nutrient retention and CEC (Cation Exchange Capacity) (Tiessen et al., 1994; Glaser et al., 2002; Moreira et al., 2005; Mukherjee and Zimmerman, 2013), and decrease Al toxicity (Glaser et al., 2002).

Many research works (Spokas and Reicosky, 2009; Prayogo et al., 2014) have been done to determine how mineralization of C and N is affected by biochar application but few emphases have been made on testing the effects of different biochar application rates on microbial respiration, nitrogen mineralization, microbial biomass and soil organic carbon pool from mollisol of two different suborders with different organic matter levels. The rate of biochar application on the soil is expected to have serious influence on the impact of biochar on soil

processes, including microbial soil respiration and nitrogen mineralization. Therefore, a laboratory incubation method in which soil temperature and moisture regimes could be manipulated was employed. The objective of this study is to determine the effects of low and high biochar application rates on microbial soil respiration, soil organic carbon content, N mineralization as well as microbial biomass from mollisol of two different suborders with varying organic matter levels.

MATERIALS AND METHODS

Soil and biochar

Mollisol consisting of two levels of organic matter, high organic matter and low organic matter, was used for the experiment. The high organic matter soil which is of the Suborder Udolls (Dark colored) was obtained from the Experimental and Practical Basement of Northeast Agricultural University while the low organic matter soil which is of the Suborder Albolls (Light colored and high concentration of sand and silt) was obtained from Northeast Forestry University. The crop planted in the previous season on both soils was maize. The soils were collected randomly at a depth of 0-20 cm, after sieving to < 2 mm and the basic properties determined (Table 1). The biochar which was produced from corn at a pyrolysis temperature of 450°C in an oxygen-restricted environment in a batch system was provided by Jin and Fu Agriculture Co., China and was crushed to pass through 2 mm sieve. The properties of biochar and soils are listed in Table 1.

Incubation procedure and soil respiration

Soil sieved to 2 mm was amended with biochar at different rates, 0.5, 1 and 2% which are equivalent to 10, 20 and 40 t ha⁻¹ respectively. For incubation, dry weight soil equivalent to 25 g were placed in air-tight glass jars (0.3 L) in a completely randomized design for anaerobic incubation with 3 replicates. All treatments and control 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 (Tufekcioglu et al., 2001) during 100 days of incubation. The emitted CO₂ was trapped in 10 ml of NaOH which was titrated with HCl on days 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 after carbonate precipitation with BaCl₂.

Analytical methods

Soils from the jars were analyzed for selected chemical properties; soil organic carbon, microbial biomass C and N, Total N. Ammonium nitrogen and nitrate nitrogen contents were extracted with 2M KCl (1:10 w/v) after shaking for 2 h and determined colorimetrically, using the salicylate method as the variation of Berthelot-Phenate method. Microbial biomass C and N were determined 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

The statistical analyses were performed using SPSS 19.0 program.

Table 1. Physical and chemical properties of soils and biochar.

Parameter	Soil (High OM)	Soil (Low OM)	Biochar
pH	6.12	6.02	9.89
Total N (g kg ⁻¹)	0.7	0.34	6.89
Available N (mg kg ⁻¹)	Nd	Nd	*
Organic Carbon (g kg ⁻¹)	38.2	10.1	415.3
Available P (mg kg ⁻¹)	23.8	19.3	*
Total P (g kg ⁻¹)	Nd	Nd	10.26
Available K (mg kg ⁻¹)	185.6	180.1	25.9
Particle Size (g kg⁻¹)			
Sand	500	520	Nd
Silt	190	200	Nd
Clay	310	280	Nd
Textural Class (USDA)	Clay Loam	Clay Loam	Nd
Bulk Density (g cm ⁻³)	1.38	1.42	Nd

*<LOD of the technique, Nd = Not determined.

After testing of assumptions, One-Way analysis of variance (ANOVA) was performed followed by Duncan Multiple Range Test (DMRT). Results marked as significantly different are different at $P < 0.05$ unless specified in text. All reported values are means of three replicates.

RESULTS

Microbial soil respiration

Biochar application to soil had effects on CO₂-C release (Figure 1) with respect to soil alone. Difference was found for CO₂-C among the rates of application in the two soils. The mineralization of C was slightly depressed by 1% biochar addition and 2% biochar addition; however, stimulated by 0.5% biochar addition. The respiration rate of 0.5% biochar was higher at the beginning than the control until 20 days incubation (15 mg CO₂-C g⁻¹d⁻¹) for high organic matter soil (Figure 1a) and 15 days incubation (15.4 mg CO₂-C g⁻¹d⁻¹) for the low organic matter soil (Figure 1b). There was a sharp decline in all respiration rates during the first 25 days of incubation after which the respiration rate became steady. It was observed that the higher the rate of biochar applied, the lower the amount of CO₂-C released, which is an indication that there is decreased decomposition following biochar application to soils. High organic matter soil recorded higher soil respiration rate than low organic matter soil throughout the incubation period.

Soil organic carbon (SOC)

SOC increased with the increasing biochar application in all treatments for both high organic matter soil and low

organic matter soil at 50 and 100 days of incubation (Table 2). Soil + 1% biochar and Soil + 2% biochar were significantly ($P < 0.01$) higher than control (soil). The highest SOC value (58.8 g kg⁻¹) was shown by Soil + 2% biochar of the high organic matter soil at 100 days incubation.

N mineralization

The levels of NO₃⁻-N increased between 20 and 40 days for both soils (Table 3). Mineralization was highest in control (Soil), followed by Soil + 0.5% biochar. Biochar application significantly ($P < 0.05$) reduced mineralization at 20, 40, 60 and 90 days for the high organic matter soil; while significant ($P < 0.05$) reduction by biochar was observed at 40 and 100 days for the low organic matter soil. The highest NO₃⁻-N levels were recorded at 70 days for both soils. Between 20 and 40 days, NH₄⁺-N levels reduced in all soils (Table 4). Greatest NH₄⁺-N reduction was observed at 90 days, indicating net immobilization. Rates of immobilization were significantly ($P < 0.05$) highest in treatment receiving 2% biochar relative to other treatments. Control (Soil) was significantly ($P < 0.05$) higher than Soil + 2% biochar at 40 and 90 days for the high organic matter soil; while significant differences among treatments were observed at 20 and 90 days for the low organic matter soil.

Soil microbial biomass carbon (SMBC)

Variability of SMBC under different rates of biochar was large as shown in Figure 2. A sharp increase in SMBC was observed at 20 days for both soils with a further

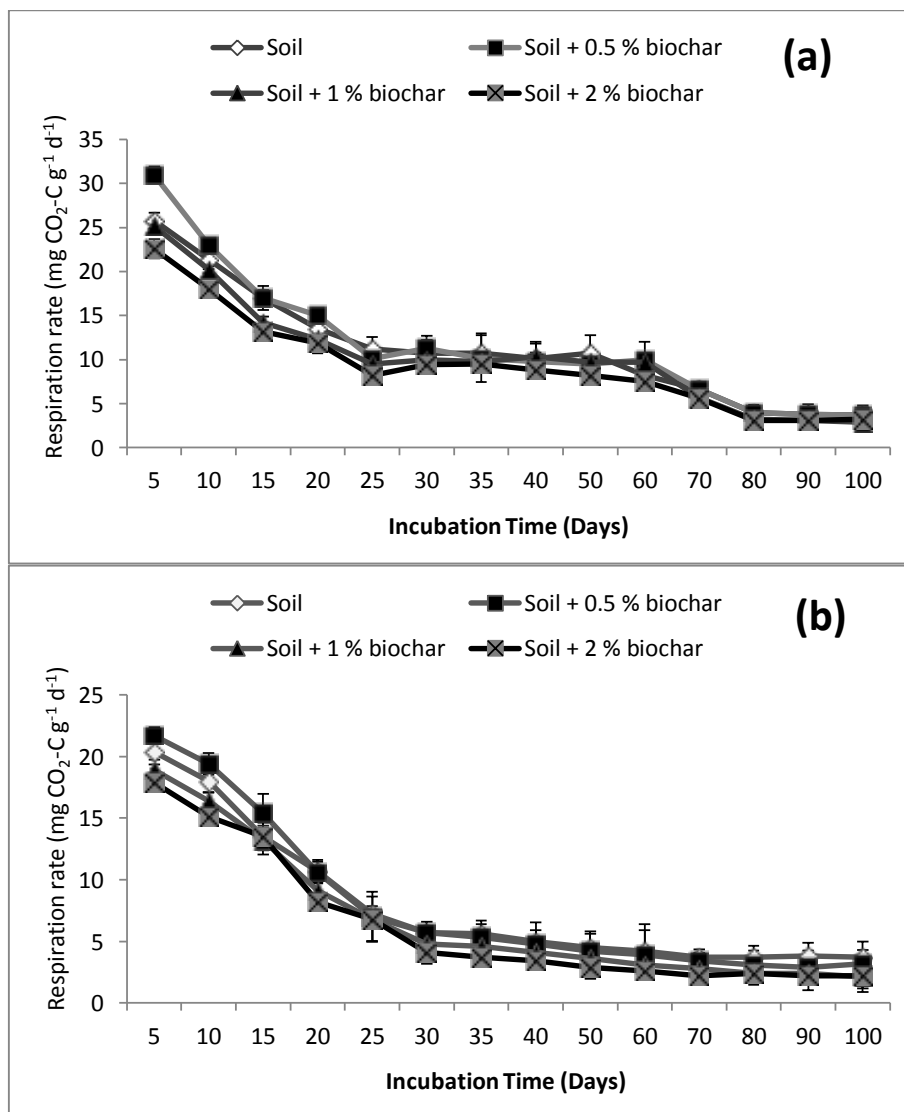


Figure 1. Respiration rates of high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Means ± SE, n = 3).

Table 2. SOC of high organic matter soil (a) and low organic matter soil (b) at 50 days and 100 days of incubation as affected by biochar additions (Mean ± SE, n = 3).

Soil code	Treatments	SOC (g kg ⁻¹)	
		50d	100d
(a)	Soil	37.3±1.12 ^d	37.6±1.79 ^c
	Soil + 0.5 % biochar	53.1±0.56 ^c	53.1±1.12 ^b
	Soil + 1 % biochar	56.4±1.12 ^b	55.8±1.96 ^b
	Soil + 2 % biochar	57.8±0.56 ^a	58.8±0.85 ^a
(b)	Soil	9.7±1.71 ^d	10.2±1.71 ^d
	Soil + 0.5 % biochar	21.8±1.12 ^c	22.5±1.71 ^c
	Soil + 1 % biochar	28.1±1.71 ^b	27.6±1.16 ^b
	Soil + 2 % biochar	36.7±1.41 ^a	37.1±1.71 ^a

Means followed by different letters are significantly different (P<0.01).

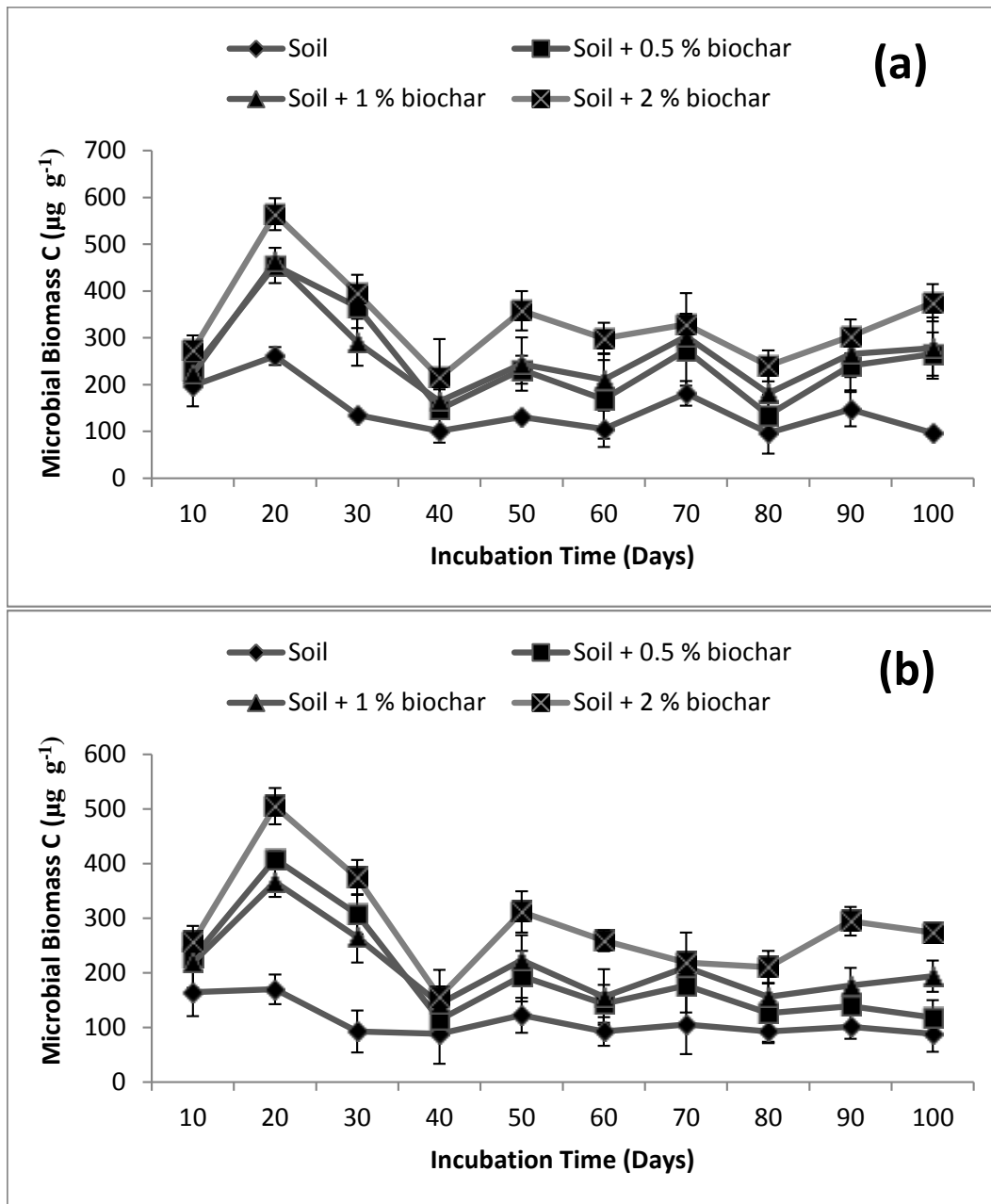


Figure 2. Soil MBC for high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Mean \pm SE, n = 3).

decline at 40 days. SMBC was highest at 20 days for both high organic matter soil and low organic matter soil (Figure 2a and b). The results of the soil microbial biomass analysis also indicate that the high organic matter soil contains the greatest microbial biomass C. Treatment receiving 2% biochar was significantly ($P < 0.05$) higher than other treatments throughout the incubation period. The variations in the microbial biomass C among the different soil types is an indication of the differences in their microbial activities.

Soil microbial biomass nitrogen

At day 50 of the high organic matter soil (Table 5), treatment receiving 2% biochar showed the highest SMBN ($111.9 \mu\text{g g}^{-1}$) and it was significantly ($P < 0.001$) higher than other treatments; while for the low organic matter soil, treatment receiving 2% biochar ($80.8 \mu\text{g g}^{-1}$) was also significantly ($P < 0.001$) higher than other treatments. At day 100 of the high organic matter soil (Table 5), the treatment receiving 2% biochar showed the

Table 3. NO₃⁻-N levels in biochar-amended high organic matter soil (a) and low organic matter soil (b) during incubation.

Soil code	Treatments	NO ₃ ⁻ -N (mg kg ⁻¹)					
		20 d	40 d	60 d	70 d	90 d	100 d
(a)	Soil	15.32±0.27 ^a	23.37±0.90 ^a	19.17±1.11 ^a	33.79±1.27 ^a	26.84±1.08 ^a	27.13±0.55 ^a
	Soil + 0.5 % BC	15.19±0.24 ^a	21.47±1.59 ^b	17.34±0.10 ^b	33.44±2.65 ^a	25.10±1.24 ^{ab}	24.94±0.05 ^a
	Soil + 1 % BC	14.15±0.47 ^b	21.06±0.62 ^b	16.10±1.07 ^b	31.74±1.83 ^a	24.22±0.57 ^b	24.38±3.35 ^a
	Soil + 2 % BC	13.93±0.49 ^b	21.28±0.36 ^b	16.77±0.34 ^b	32.94±0.06 ^a	23.30±1.06 ^b	26.43±1.74 ^a
(b)	Soil	6.52±0.47 ^a	7.99±0.76 ^{ab}	9.73±0.43 ^b	26.68±2.04 ^a	12.60±3.26 ^a	17.56±1.76 ^a
	Soil + 0.5 % BC	6.44±0.33 ^a	9.44±1.42 ^a	9.35±1.10 ^a	25.55±2.72 ^a	11.94±0.83 ^a	15.67±0.56 ^{ab}
	Soil + 1 % BC	6.51±0.22 ^a	6.99±1.47 ^b	8.56±0.57 ^a	24.35±1.06 ^a	9.81±0.17 ^a	14.72±0.81 ^b
	Soil + 2 % BC	5.97±0.68 ^a	8.81±0.25 ^{ab}	8.46±0.83 ^a	23.68±2.05 ^a	9.54±0.97 ^a	14.21±1.90 ^b

Means followed by different letters are significantly different (P<0.05), BC = Biochar.

Table 4. NH₄⁺-N levels in biochar-amended high organic matter soil (a) and low organic matter soil (b) during incubation.

Soil code	Treatments	NH ₄ ⁺ -N (mg kg ⁻¹)					
		20 d	40 d	60 d	70 d	90 d	100 d
(a)	Soil	3.28±2.19 ^a	0.70±0.05 ^a	2.42±0.59 ^a	7.23±0.81 ^a	0.52±0.08 ^a	0.73±0.10 ^a
	Soil + 0.5 % BC	2.85±0.98 ^a	0.58±0.08 ^a	3.37±1.05 ^a	5.27±2.50 ^a	0.470±0.03 ^a	0.68±0.13 ^a
	Soil + 1 % BC	3.26±1.14 ^a	0.60±0.13 ^a	2.60±0.33 ^a	2.27±1.56 ^a	0.30±0.05 ^b	0.67±0.08 ^a
	Soil + 2 % BC	2.60±0.35 ^a	0.38±0.18 ^b	2.00±1.28 ^a	2.00±0.35 ^a	0.28±0.03 ^b	0.57±0.19 ^a
(b)	Soil	4.42±2.05 ^a	0.68±0.06 ^a	3.15±0.33 ^a	2.92±2.05 ^a	0.45±0.06 ^a	0.72±0.10 ^a
	Soil + 0.5 % BC	1.97±0.86 ^b	0.67±0.08 ^a	2.70±0.61 ^a	2.62±0.72 ^a	0.43±0.01 ^a	0.72±0.10 ^a
	Soil + 1 % BC	2.62±1.17 ^{ab}	0.78±0.03 ^a	2.33±0.31 ^a	2.32±0.89 ^a	0.32±0.05 ^b	0.72±0.14 ^a
	Soil + 2 % BC	1.75±0.08 ^b	0.67±0.10 ^a	2.00±1.22 ^a	2.90±1.33 ^a	0.30±0.03 ^b	0.73±0.08 ^a

Means followed by different letters are significantly different (P<0.05), BC = Biochar.

highest SMBN (76.67 µg g⁻¹) and it was significantly (P<0.001) different from other treatments. Treatments receiving 0.5 and 1% biochar were not significantly different from each other. For the low organic matter soil, treatment receiving 2% biochar (74.6 µg g⁻¹) was significantly (P<0.001) higher than other treatments.

Total N

The Total N values following biochar addition are shown in Figure 3. The high organic matter soil was higher in soil Total N (Figure 3a). Higher biochar application rate increased soil Total N throughout the incubation period in relation to control. Treatment receiving 2% biochar showed the highest Total N value and it was significantly (P<0.05) higher at 70 and 100 days. Slight increase in Total N was shown by Soil + 2% biochar of the low organic matter soil in the first 20 days (Figure 3b). Soil + 2% biochar was significantly (P<0.05) higher at 10, 20, 60 and 80 days with respect to other treatments. The control soil showed the lowest soil Total N value throughout the 100 days incubation.

DISCUSSION

Many research works conducted in China have shown that biochar application to agricultural soils has little or no effect on carbon mineralization (CO₂-C efflux). There is decreased decomposition or negative priming following biochar application to soils which inadvertently leads to a reduction in soil respiration. Findings from this research showed that biochar treatment with highest application rate 2%, which is equivalent to 40 t ha⁻¹ recorded the lowest mineralization, and it is in agreement with other findings (Liu et al., 2011; Case et al., 2014; Schimmelpennig et al., 2014). Soil + 2% biochar reduced CO₂-C mineralization by 15.8 % in the high organic matter soil and 16.1 % in the low organic matter soil at 10 days incubation. Even though improvement in the growth of soil microorganisms following biochar application has been reported (Chen et al., 2015; Lu et al., 2015), it did not amount to an increase in soil respiration. The reason that can be adduced to this could be that the fine particles in biochar might have taken up the evolved CO₂-C to an extent; thereby limiting its release to the atmosphere.

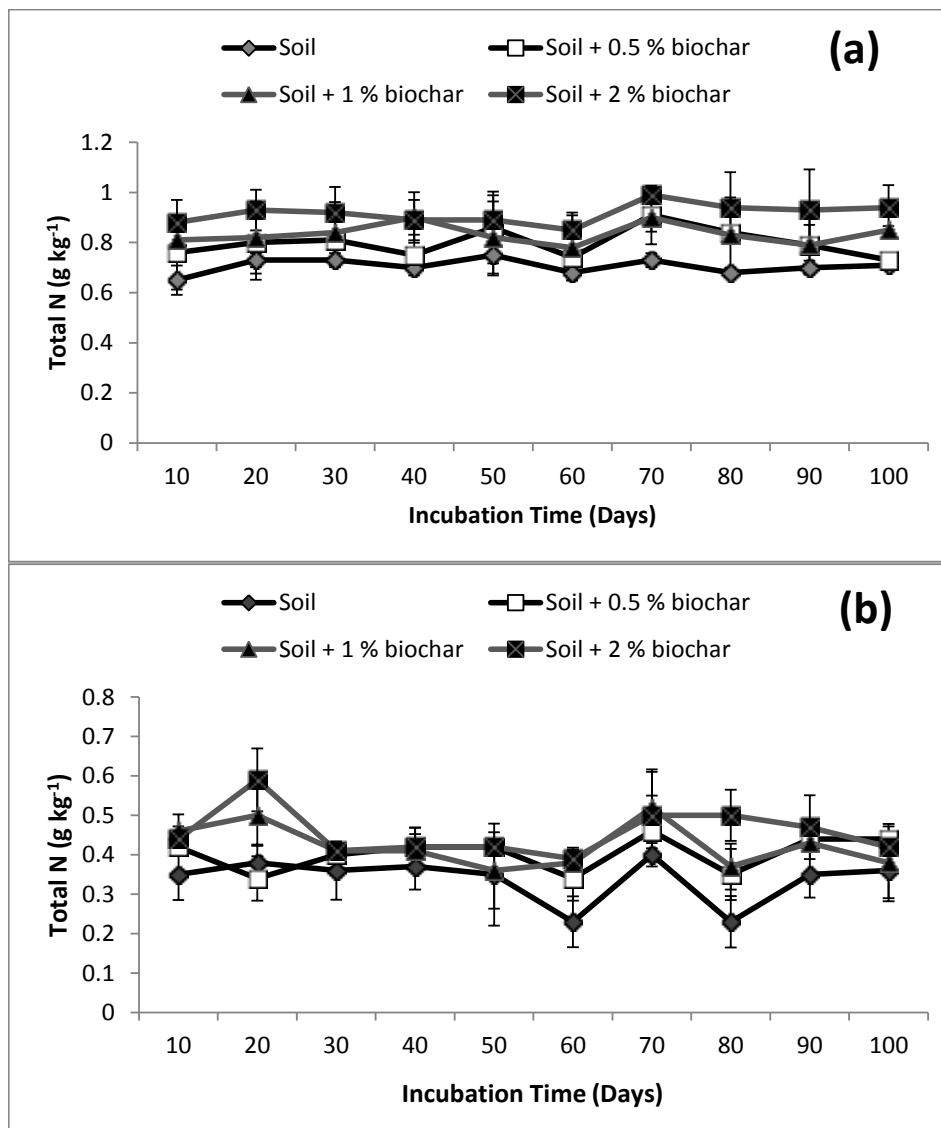


Figure 3. Soil Total N for high organic matter soil (a) and low organic matter soil (b) amended with biochar at 0.5, 1 and 2% levels (Mean \pm SE, n = 3).

Biochar additions largely increased SOC and contributed to carbon storage. This finding is in agreement with the work of Biederman and Harpole (2012). The observed increases in SOC following biochar addition were expected, considering the high carbon content (81%) and recalcitrant carbon of biochar.

Mineral nitrogen content ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) in the rhizosphere soil is an important indicator of threats to soil by nitrogen saturation (Fingerman et al. 2011). The N mineral forms are the forms in which agricultural crops take in nitrogen from soil. The findings from this research showed that biochar when added to soil reduced N mineralization and accumulation of $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ which is in consonance with other works (Novak et al., 2010; Taghizadeh-Toosi et al., 2011; Zavalloni et al.,

2011). High biochar concentration significantly reduced $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ concentrations. The reason for this could be as a result of a change in soil pH that follows biochar addition which inadvertently affects the structure of soil microbial organisms.

The changes showed in SMBC among treatments points out the activities of microorganisms and breakdown of organic matter. The results from this study established that SMBC increased with biochar application relative to control, which is an indication that biochar addition to soils can accelerate the growth of microbes (mostly bacteria and fungi). Same results have been reported by several authors (Anderson and Domsch, 1993; Tian et al., 2008; Kolb et al., 2008; Aciego and Brookes, 2009; Liang et al., 2010; Lehmann et al., 2011).

The effect of biochar on soil microbial biomass and activity depends mainly on biochar type (feedstock and pyrolysis temperature). For SMBN, biochar had positive effects on soil microbial biomass nitrogen. The addition of biochar increased the microbial composition of both soils and enhanced the release of biomass N. Biochar concentration also affected SMBN release from soils following the death of these microbes. This result is in consonance with the work of Zhang et al. (2014).

Total N is the sum of total kjeldahl nitrogen (ammonia, organic and reduced nitrogen) and nitrate-nitrate. Biochar additions increased soil Total N, which is consistent with the work of Zhang et al. (2012b) who recorded a significant maize increase, accompanied by increased soil Total N content following application of different levels of a nutrient rich wheat-straw biochar (20 and 40 t ha⁻¹); but this is in contrast to the findings of Jones et al. (2012). The tendency of biochar amendment to increase soil Total N depends on the feedstock used in biochar production as well as the pyrolysis temperature. It also depends on soil properties as well as the prevailing weather and climatic conditions. Biochar properties such as large surface area and high porosity could also be responsible for the changes in Total N when added to the soil.

Conclusion

In conclusions, this study shows that the addition of biochar to Mollisols reduced soil respiration rate throughout the 100-days incubation period. Higher biochar concentration caused a further decline in soil respiration rates. Incorporation of biochar to soils increased SOC and soil Total N content; hence high biochar rate can be applied to soils as amendment to boost SOC and soil Total N. A positive effect on soil microbial biomass carbon SMBC and soil microbial biomass nitrogen SMBN was also shown by biochar. However, biochar addition reduced mineral soil nitrogen in the forms of NO₃⁻-N and NH₄⁺-N.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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