Maize Stover Biochar Accelerated Urea Hydrolysis and Short-term Nitrogen Turnover in Soil

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A soil incubation experiment was conducted to investigate the effect of biochar application (2% w/w) on urea hydrolysis and inorganic nitrogen accumulation. Fresh biochars were produced from maize stover that was pyrolyzed at 300 °C, 500 °C, and 700 °C. Then the matured biochars were obtained *via* a 50 days maturing process. Biochar prepared at 700 °C strongly accelerated the urea hydrolysis and increased soil pH. Fresh biochar, especially when pyrolyzed at low temperature, contained a relatively high concentration of labile carbon and 43% to 64% could be oxidized within 40 days of maturing incubation. The labile carbon in fresh biochars led to microbes thriving and resulted in an accelerated short-term nitrogen (N) turnover, *i.e.*, at an early stage of incubation, fresh biochar increased mineralization of soil organic N by 79 mg·kg^{-1} to 449 mg·kg^{-1}. However, a reduction of soil available N contents induced by microbial immobilization effect was observed at the end of incubation. The authors concluded that aged biochar was suitable for simultaneous soil amendment with urea rather than newly produced biochar. This is because aged biochar can avoid high soil available N accumulation; thus it can decrease the risk of inorganic N leaching loss.

INTRODUCTION

Burning of crop residues is commonly practiced after harvest in China as well as in other developing countries (Meng *et al*. 2011; Li and Wang 2013). Although this practice can reduce the amount of crop residuals directly, it results in significant pollution to adjacent regions from smoke and ash, resulting in many cities being covered by smog and haze. Moreover, residue combustion is a source of greenhouse gas emission, associated with global warming. Pyrolyzing stover biochar might be one optimal way for solving this problem without side effects (Meng *et al*. 2011). Biochar refers to carbon-rich materials produced from the pyrolysis of biomass under limited oxygen and relatively low temperatures. As a soil amendment, biochar has attracted considerable attention due to its ability to improve soil fertility and increase soil carbon sequestration. A biochar carbon sequestration effect mainly benefits from its recalcitrant characteristic formed during pyrolysis procedures (Lehmann 2003) and is beneficial due to its potential to protect soil organic matter from degradation (Whitman *et al*. 2015). Moreover, it was reported that biochar provides nutrient elements to plants and improves soil quality directly. Specifically, biochar may be enriched with elements including K, Mg, and Ca (Steiner *et al*. 2007; Cantrell and Martin 2011), and it is capable of improving soil water retention, cation exchange capacity, and nutrient retention capacity (Lehmann *et al*. 2003; Liang *et al*. 2006; Laird *et al*. 2010).

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It is generally accepted that the majority of biochar-C is chemically and biologically recalcitrant, but it still contains a minor fraction of bioavailable C (Major *et al.* 2010; Jones *et al.* 2011), and the concentration of labile carbon in biochar depends on the conditions during pyrolysis, as well as the composition of the feedstock biomass (Nelissen *et al.* 2012; Chen *et al.* 2016). There is also evidence that the labile carbon derived from biochar enhances soil microbial biomass growth and activity (Steiner *et al.* 2008). From this belief, there can be little doubt that biochar will play an important role on shifting microbial community structure and function involved in nitrogen dynamics.

Urea is an industrial product and the main form of nitrogen fertilizer used worldwide. The application of urea has been an important measurement for crop yield improvement and is a significant exogenous nitrogen source for soil. Thus, when biochar is amended into agricultural soil, it inevitably interacts with urea. Therefore, from an agronomic standpoint, the effect of biochar on the behaviours of urea in soil, as well as the combined effect of biochar and urea on soil's native nitrogen turnover, should be determined. However, only few studies have investigated the interaction between biochar and urea. Anderson *et al.* (2014) found that the relative proportion of nitrifiers and denitrifiers increases in biochar-amended soils subjected to large influxes of urine-derived N. Results from N-15 isotopic analyses by Reverchon *et al.* (2014) showed that biochar amendment accelerates the N dynamics and increases N losses, probably through ammonia volatilization or denitrification. Unfortunately, those studies lack the information about how biochar affect urea hydrolysis and the relationship with its labile carbon.

Because the time of urea hydrolysis in soil is usually shorter than 2 weeks, the authors conducted a short-term incubation experiment. The main objectives of this study were to 1) assess the labile carbon contents in biochar pyrolysis under different temperature and its stability; and 2) investigate the urea hydrolysis rate and inorganic N accumulation that was affected by biochar with and without labile carbon. It is hypothesized that 1) biochar can affect urea hydrolysis rate depending on its sorption ability or on improvement of soil quality, and 2) biochar labile carbon plays an important role on shifting soil organic N turnover.

EXPERIMENTAL

Materials

Biochar production

Maize stover biochar was used in this study. After being dried to a constant weight at 65 °C and milled to 1 cm^3 to 2 cm^3, the maize stover was mixed thoroughly and placed in crucibles covered with lids then pyrolyzed in a programmed muffle furnace (Beijing Zhongxing Laboratory Equipment Inc., Beijing, China) at a heating rate of 10 °C/min from room temperature to 300 °C/500 °C/700 °C and maintained for 30 min. The obtained biochar was marked as FBC300/500/700.

To produce matured biochar that was termed as MBC300/500/700, a 50-day incubation was conducted. The main aim of the maturing process was to remove the labile carbon from biochar. First, the microbial inoculums were mixed with each fresh biochar at a rate of 1:10 (w:w), and then incubated at 35 °C for 50 days. During the incubation process, the same volume of microbial inoculum was added when the mixtures

were dried. Subsequently, biochar was air-dried for a future study. The microbial inoculums added were freshly obtained from the extracts of grassland soil, which exhibited high biological activity. Specifically, 100 g of topsoil (0 cm to 10 cm, incubated under 60% water holding capacity (WHC) at 30 °C for 2 d) was mixed with 200 mL of distilled water and shaken for 30 min at 200 rpm. After standing for 12 h the supernatant obtained was microbial inoculums.

Methods

Measurement of biochar properties

For elemental analyses, sieved biochar were milled to achieve a homogenous fine powder. C, N, and O contents were measured by using an elemental analyzer (Elementar Macro Cube, Langenselbold, Germany). The H content was calculated by the difference method (Yue *et al*. 2016). The biochar specific surface area and pore characteristic were determined by using a surface area and pore analyzer (V-sorb 4800, Jinaipu, China). The ash content and volatile matter were determined based on a method described by the IBI Biochar Standards Version 2.1. The biochar pH value was measured at a biochar:water ratio of 1:25.

Biochar's labile carbon fraction was determined by assessing the CO_2 that evolved from biochar over time (40 days) through the method described by Nelissen *et al*. (2014) and Cheng *et al*. (2008b). Biochar was thoroughly mixed with sand in a ratio of 1 g of biochar to 20 g of sand and placed into a 450-mL Mason jar. Distilled water, microbial inoculum, and micronutrient solution were added to the jar to maintain biological activity. Approximately 2 mL of microbial inoculum was added to each jar, which was the same as described above to produce matured biochar; then 2 mL micronutrient solution that contained 4 mM NH_3NO_3, 4 mM $CaCl_2$, 2 mM KH_2PO_4, 1 mM K_2SO_4, 1 mM $MgSO_4$, 25 mM $MnSO_4$, 2 mM $ZnSO_4$, 0.5 mM $CuSO_4$, and 0.5 mM Na_2MoO_4 was also added to each jar. The experiments were initiated immediately after wetting. The evolved CO_2 was trapped in 10 mL of 0.1 N NaOH, which was placed in a small vessel inside the sealed incubation jars. The NaOH vessels were removed and replaced with a new vessel on day 1, 3, 5, 7, 11, 15, 23, 31, and 40. The trapped CO_2 was precipitated by an excess of 1 M of $BaCl_2$ solution and back-titrated with standard 0.5 M of HCl to quantify the evolved CO_2.

Soil incubation experiment

The incubation experiment included the following 8 treatments: (1) no amendment (control); (2) urea amendment (U); (3, 4, and 5) soil amendment with urea and FBC300, FBC500, FBC700, marked as F300, F500, and F700, respectively; and (6, 7, and 8) soil amendment with matured biochar, which was marked as M300, M500, and M700. Each treatment included 3 replications. The soil used was typical brown earth from northeast China, which was collected from a local farmland near Shenyang Agricultural University (41°83′N, 123°58′E). The top 15 cm of soil was collected following a maize harvest. Afterward, the soil was air-dried and sieved by using a 2-mm sieve. The basic properties of the soil were as follows: pH of 7.14 (soil:water = 1:2.5); bulk density of 1.29 g·cm^{-3}; total N content of 1.69 g·kg^{-1}; available N of 41.5 mg·kg^{-1}, and SOC of 10.60 g·kg^{-1}.

In each replication, 100 g of soil was thoroughly mixed with 0 g (control and U treatment) or 2 g of biochar (equal to 55 t·ha^{-1}) and placed into a 450-mL mason jar. Then

10 mL urea solution (6 g·L^{-1}) was applied to the soil (equal to 300 kgN·ha^{-1}, except for the control soil). Distilled water was added to bring the moisture content to 60% of WHC, and the samples were incubated at 27 °C for 3 weeks. The remaining urea in the soil was determined after 12 h, 24 h, 36 h, and 48 h using the method proposed by Douglas and Bremner (1970). The indophenol blue colorimetric method was used to assay the urease activity on day 14. Available nitrogen was measured after 2 days and end of incubation by an alkaline hydrolysis method. Moreover, the microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were extracted with 0.5 M K_2SO_4 and determined by a C/N analyzer (Analytik Jena 3100, Jena, Germany) at the end of incubation.

Statistical analyses

A one way ANOVA was performed using SPSS version 19 (IBM Corporation, New York, USA) to analyze the data. Significant differences among means were assessed by the least significant difference (LSD) test at 0.05 probability level. Figures shown in this article were made by using Graphpad Prism 5 (GraphPad Software, Inc., La Jolla, USA).

RESULTS AND DISSCUSSION

Characteristics of Biochar

The pyrolysis temperature significantly influenced the basic characteristics of the biochar (Tables 1 and 2). When the pyrolyzing temperatures were increased from 300 °C to 700 °C, the biochar carbon contents increased by 8.4%, while oxygen and hydrogen contents decreased by 9.32% and 4.3%. Accordingly, the ratios of O/C and H/C decreased from 0.39 and 0.12 to 0.2 and 0.4. This observation indicates that the degree of carbonization was accelerated with increasing pyrolysis temperature. During the 50 days of maturing processes, 1.6%, 2.0%, and 0.56% of the total carbon in biochar was lost from FBC300, FBC500, and FBC700, respectively. The biochar total N content exhibited a small change in the maturing processes, only MBC700 increased 0.34% compared with FBC700. The O contents in matured biochar were higher than fresh biochar, one promising reason was some oxygen-containing function groups were formed during maturing incubation (Cheng *et al*. 2008a).

Table 1. Elemental Composition of the Fresh and Matured Biochar

Biochar	C (%)	N (%)	O (%)	H (%)	O/C	H/C
FBC300	58.61 ± 0.65 d	1.67 ± 0.02 a	22.95 ± 0.32 b	7.23 ± 0.12 a	0.39	0.12
FBC500	63.80 ± 0.25 b	0.84 ± 0.01 d	14.70 ± 0.35 d	7.21 ± 0.04 a	0.23	0.11
FBC700	67.02 ± 0.52 a	1.06 ± 0.04 c	13.63 ± 0.14 e	2.93 ± 0.17 c	0.20	0.04
MBC300	57.02 ± 0.28 e	1.61 ± 0.02 a	24.48 ± 0.41 a	6.72 ± 0.21 b	0.43	0.12
MBC500	61.80 ± 0.17 c	0.91 ± 0.02 d	20.29 ± 0.35 c	7.01 ± 0.17 ab	0.33	0.11
MBC700	66.46 ± 0.43 a	1.40 ± 0.02 b	14.09 ± 0.29 d	3.05 ± 0.04 c	0.21	0.05
Value represents means; ± represents standard errors; Different lower case letters represent significant difference (Least significant difference (LSD) test, p < 0.05)						

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Table 2. Properties of the Fresh and Matured Biochar

Biochar	Labile Carbon (mg·g^{-1})	Volatile Matter (%)	Ash (%)	Fixed Carbon (%)	pH	Surface area (m^2·g^{-1})	Pore diameter (nm)	Pore volume (cm^3·g^{-1})
FBC300	14.55 ± 0.95 a	41.42 ± 0.45 a	8.02 ± 0.01 f	50.56 ± 0.46 c	7.03 ± 0.01 f	24.50 ± 0.87 c	49.71 ± 11.36 a	0.20 ± 0.09 c
FBC500	11.93 ± 0.46 b	23.08 ± 0.19 b	13.12 ± 0.23 d	63.79 ± 0.42 b	9.39 ± 0.02 c	26.81 ± 1.07 c	41.80 ± 4.37 ab	0.33 ± 0.03 b
FBC700	9.80 ± 0.27 c	15.29 ± 0.48 c	13.66 ± 0.08 c	71.05 ± 0.78 a	10.35 ± 0.01 a	73.69 ± 1.89 a	27.92 ± 10.56 b	0.49 ± 0.08 a
MBC300	5.25 ± 0.12 d	42.08 ± 0.88 a	9.18 ± 0.10 e	48.74 ± 0.57 c	7.29 ± 0.01 e	20.43 ± 3.12 c	50.02 ± 7.21 a	0.22 ± 0.03 c
MBC500	5.06 ± 0.48 d	22.34 ± 0.37 b	14.80 ± 0.05 b	62.86 ± 0.42 b	8.95 ± 0.01 d	19.12 ± 4.03 d	39.87 ± 5.55 ab	0.25 ± 0.05 c
MBC700	5.68 ± 0.80 d	14.16 ± 0.59 c	15.65 ± 0.10 a	70.18 ± 0.49 a	9.84 ± 0.02 b	56.21 ± 5.56 b	24.80 ± 4.43 b	0.33 ±0.02 b

Value represents means; ± represents standard errors; Different lower case letters represent significant difference (Least significant difference (LSD) test, $p < 0.05$)

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As shown in Table 2, the ash and fixed carbon contents increased from 8.02% and 50.56% (FBC300) to 13.66% and 71.05% (FBC700), respectively. In contrast, biochar's labile carbon fraction and biochar volatile matter decreased by 18.34% and 4.7 mg·g^{-1}, respectively. After 50 days maturing incubation, the ash contents in matured biochar were 9.18%, 14.8%, and 15.65%, which was 1.16%, 1.7%, and 2% higher than fresh biochar, respectively. Nevertheless, the volatile matter showed no change, thereby the fixed carbon decreased 1.82%, 0.93%%, and 0.87% in MBC300, MBC500, and MBC700, respectively.

Pyrolysis process created small pores and damaged the large pores (Yue *et al*. 2016), resulting in pore diameter decreased from 49.7 to 27.9 nm when pyrolyzing temperatures were increased from 300 °C to 700 °C. It also can be concluded that more pores were created by increasing pyrolyzing temperature. The total pore volume in FBC700 was 1.69 times as FBC300; consequently, the biochar surface area increase from 24.50 m^2·g^{-1} to 73.68 m^2·g^{-1}. A reduction of surface area and pore volumes were detected upon MBC700, possible reasons are that pore destruction or increase in oxygen-containing surface functional groups during the incubation (Ghaffar *et al*. 2015).

The biochar pH values increased from 7.03 to 10.35 with the increase of pyrolyzing temperature from 300 °C to 700 °C. Furthermore, the maturing processing minimized the range of pH values, which indicated that biochar alkalinity was neutralized by the maturing processes. As shown in Table 2, MBC300 presented a pH of 7.29, and was slightly higher than that of FBC300. However, the pH values for FBC500 and FBC700, which showed strong alkalinity, decreased 0.44 and 0.51 with mature incubation, respectively.

The results showed (Fig. 1) that within fresh biochar, the CO_2 evolution occurred during the entire 40 days of incubation and in first 25 days for matured biochar. The biochar pyrolyzed at low temperatures (300 °C and 500 °C) usually contained a considerable amount of carbon that was easily mineralized (Table 2). For instance, the total evolved biochar of CO_2-C from FBC300 was 14.6 mg/g of biochar, which was significantly ($p < 0.5$) higher than FBC500 (11.9 mg·g^{-1}) and FBC700 (9.8 mg·g^{-1}).
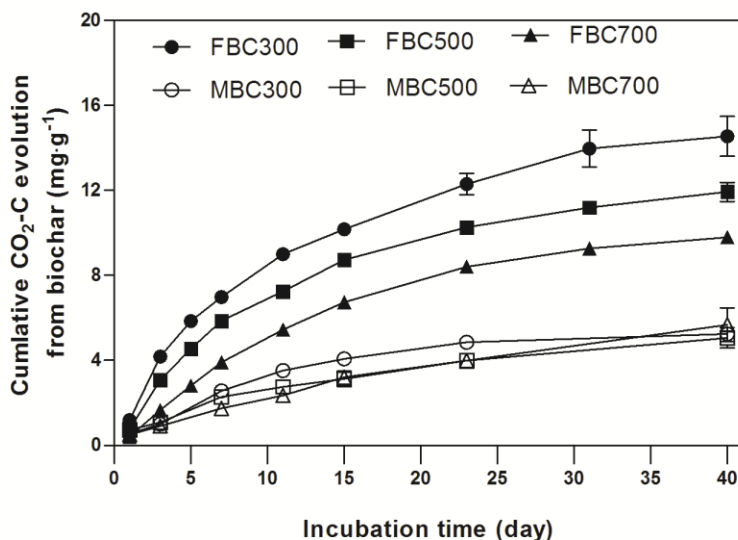

Fig. 1. Cumulative carbon mineralized from fresh and matured biochar during 40 days of incubation, (mean ± SE; n = 3)

Smaller amounts of CO_2-C evolved from matured biochar (< 5.67 mg·g^{-1}) as its labile carbon was mineralized during maturing processes. No significant differences were observed among MBC300, MBC500, and MBC700.

Effect of Biochar on Urea Hydrolysis rate and Available Nitrogen contents

Urea hydrolysis rate

The urea hydrolysis rate was accelerated significantly by biochar addition in 48 h (Fig. 2), particularly for fresh biochar. The residual urea-N in biochar-amended soils was lower than that in the urea addition only soil and decreased rapidly at the start of incubation. After 24 h of incubation, 185.6 mg urea-N·kg^{-1}, 119.8 mg urea-N·kg^{-1}, and 238.8 mg urea-N·kg^{-1} was hydrolyzed in FBC300-, FBC500-, and FBC700-amended soil, respectively. This was equal to 66.3%, 42.8%, and 85.3% of the total added urea-N, respectively. However, only 28.7% urea-N was hydrolyzed in the control soil (280 mg urea-N·kg^{-1} soil was added initially). After 48 h of incubation, urea-N in fresh biochar-amended soil was almost completely hydrolyzed, but the U treatment still contained about 50 mg urea-N·kg^{-1} soil. This result indicated biochar advanced at least one day or longer of the urea completely hydrolysis time than control.

The matured biochars showed a weak effect on accelerating urea hydrolysis, especially for biochar pyrolysis at 700 °C. The remaining urea-N in M700 treatment was 26.3%, 14.6%, and 13.5% higher than that of F700 treatment at 24 h, 36 h, and 48 h, respectively.
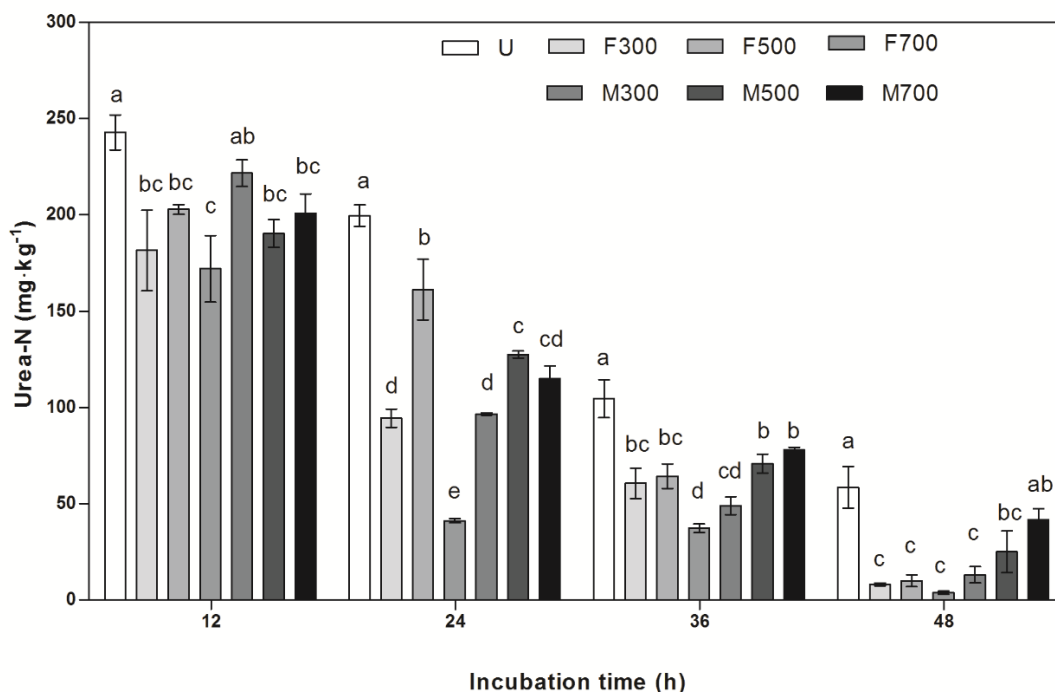

Fig. 2. Soil residual urea-N contents of different treatments within 48 h, different letters on the columns of the same incubation time represent significant differences at p < 0.05 level by LSD test; Error bars represent standard errors of the means (n = 3)

Available nitrogen contents

As shown in Fig. 3, at the early incubation stage, the addition of urea could significantly increase the soil available N *via* a "priming effect", as only 80.3 mg urea-$N \cdot kg^{-1}$ was hydrolyzed during one day of incubation (Fig. 2), and the control soil contained available N of approximately 40 $mg \cdot kg^{-1}$, which indicated there were approximately 221 $mg \cdot kg^{-1}$ [341.7 (total available N) – 40 (from soil) – 80.3 (from urea) = 221.4] soil N mineralized by urea-N addition. Moreover, biochar addition increased the soil N that was mineralized at the early incubation stage. Approximately 671 $mg \cdot kg^{-1}$, 620 $mg \cdot kg^{-1}$, and 299 $mg \cdot kg^{-1}$ N was mineralized from F300, F500, and F700 treatments, respectively. Matured biochar showed a significantly weak mineralization effect on soil N; Approximately 273 $mg \cdot kg^{-1}$, 251 $mg \cdot kg^{-1}$, and 282 $mg \cdot kg^{-1}$ N was mineralized by MBC300, MBC500, and MBC700 addition, respectively.
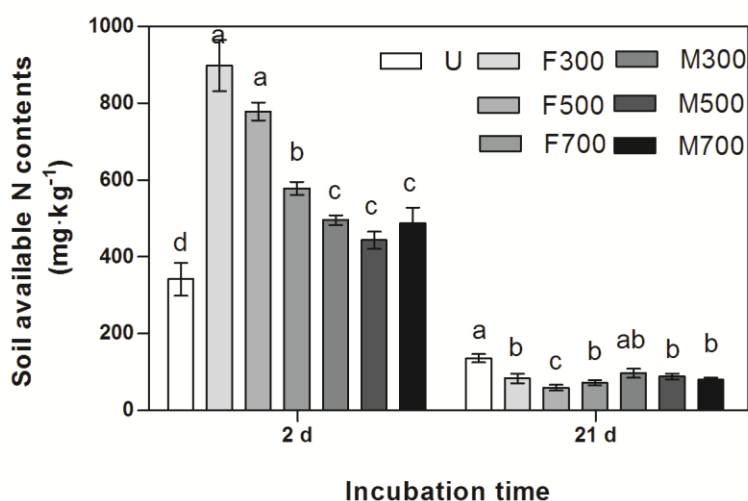

Fig. 3. Soil available N contents in urea applied soil at early and end of incubation, different letters represent significant differences at p < 0.05 level by LSD test; Error bars represent standard errors of the means (n = 3)

After 21 days of incubation, soil available N contents in biochar treatments (except for F700) were significantly ($p < 0.05$) lower than that in the U treatment. Among the biochar amended soil, the F500 treatment showed a minimal available N concentration of 59.6 $mg \cdot kg^{-1}$ among all treatments.

Effect of Biochar on Soil Urease Activity

The urease activity was determined on week 2 of the incubation. As shown in Fig. 4, compared with the control soil, the addition of urea alone inhibited urease activity by 35.5%. This phenomenon may have been caused by the substantial increase in soil pH value during urea hydrolysis, which could lead to further stability of soil organic–inorganic colloid, urease, and urea, and result in urease inactivation (Ran *et al.* 2000). Biochar potentially increased the urease activity. Among the amendments of biochar, together with urea-N, the urease activity in F500, F700, and M300 was 0.324 mg NH_4^+–$N \cdot kg^{-1}$, 0.314 mg NH_4^+–$N \cdot kg^{-1}$, and 0.309 mg NH_4^+–$N \cdot kg^{-1}$ soil per day, respectively, which was significantly ($p < 0.05$) higher than that in U treatment (0.191 mg NH_4^+–$N \cdot kg^{-1}$ soil per day). No significant differences were detected amongst the fresh and matured biochar.
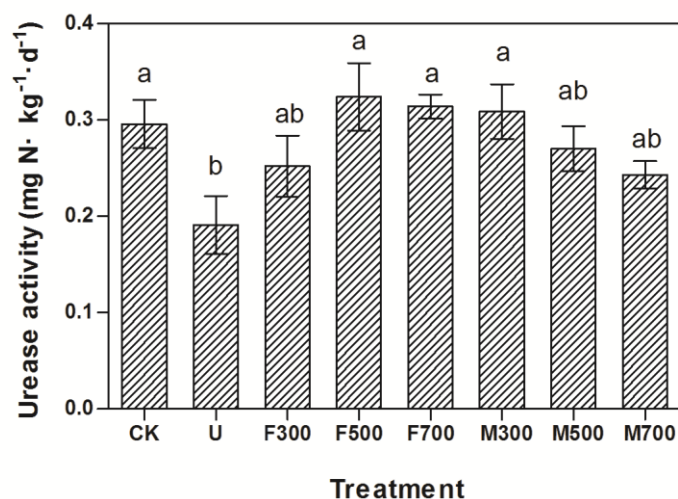
Fig. 4. Urease activity in control and biochar-amended soil; Different letters represent significant differences at p < 0.05 level by LSD test; Error bars represent standard errors of the means (n = 3)

Effect of Biochar on Soil Microbial Biomass

As shown in Fig. 5a, the addition of FBC300, FBC500, and FBC700 increased soil MBC by 176.7 mg·kg^{-1}, 140.85 mg·kg^{-1}, and 64.11 mg·kg^{-1}, respectively, when compared to U treatment. The MBC contents increased with decreased biochar pyrolysis temperature and a statistical analysis showed a positive correlation ($R = 0.96$) between fresh biochar's labile carbon content and soil MBC contents. In matured biochar amended treatments, compared with the U treatment, the MBC contents only increased in MBC300- and MBC500-amended soil by 74% and 57.1%, respectively. The soil MBN contents were also increased by biochar addition (Fig. 5b); nevertheless, the significant difference was only found in FBC500-amended soil, which was 50% higher than the U treatment.

Some specific elements in pyrolytic materials are disproportionately lost to the atmosphere, fixed into recalcitrant forms, or liberated as soluble oxides during the heating process, which affects the chemical composition of the biochar surface (Chan and Xu 2009). Consequently, the elemental composition of biochar varied with different pyrolyzing temperatures. Consistent with previous research, the current study showed that biochar's total C contents increase significantly with increasing pyrolyzing temperatures (Bruun *et al*. 2011; Enders *et al*. 2012), while O and H contents, O/C and H/C significantly decreased, indicating that the degree of carbonization and aromaticity was accelerated (Kim *et al*. 2012). In addition, low-temperature-pyrolyzed biochar contained a relatively large proportion of active compounds such as violate matters and labile carbon, but 700 °C biochar contained more fixed carbon. This result can be explained by the fact that during pyrolysis, a series of cleavage and polymerization reactions occur, thereby resulting in thermally stable fixed C structures (Spokas *et al*. 2012), which are directly related to increased biochar C content. Increasing pyrolyzing temperatures also typically leads to a loss of easily decomposable substances, volatile compounds, and elements (*e.g.*, O, H, N, and S) and thus concentrates other nutrients present in biochar, including C, Ca, Mg, and K (Kim *et al*. 2012).
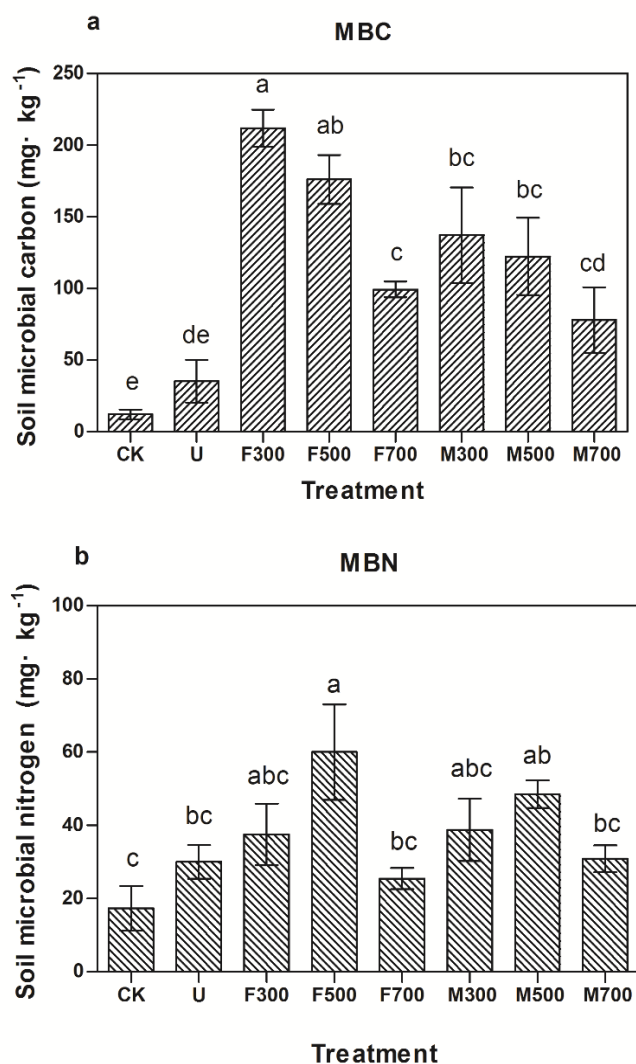
Fig. 5. Soil microbial biomass content of a) soil microbial carbon, and b) soil microbial nitrogen; Different letters represent significant differences at p < 0.05 level by LSD test; Error bars represent standard errors of the means (n = 3)

Nitrogen transformation is the most complex process during pyrolysis (Enders *et al.* 2012); in general, the total N content reaches a maximum between 300 °C and 399 °C and decreases at higher temperatures (Ippolito *et al.* 2015). This literature result is consistent with the authors' result that biochar at 300 °C possessed maximum total N contents. However, the minimum total N contents were observed in biochar at 500 °C, which was caused by the increased hydrolysis temperature that led to violated N. Nevertheless, the total N was not concentrated, similar to that in biochar at 700 °C.

Biochar surface area and pore characteristic reflect its adsorption capability, which will affect nutrition cycling and microbial thrive in soil after biochar was applied (Sun *et al.* 2016). In present study, the author found that biochar pore characteristic was well developed under high pyrolysis temperature. So urea-N adsorbed by biochar may be one potential reason for the low urea-N contents observed in biochar amended soil.

Cheng *et al*. (2006) and Zimmerman (2010) reported the short-term oxidation of labile carbon fraction from biochar. According to Bruun *et al*. (2011), 90% of biochar-C losses occurred within the first 20 days of incubation. In the present study, the total CO_2-C that evolved from FBC300, FBC500, and FBC700 was 14.5 mg·g^{-1}, 11.9 mg·g^{-1}, and 9.8 mg·g^{-1} during 40 days of incubation, which was equal to 2.5%, 1.87%, and 1.46% of total biochar carbon losses, respectively. These values matched the range of 0.3% to 3% biochar C losses reported by Zimmerman (2010) and Hamer *et al*. (2004). The 50 days of maturing processes resulted in 42.9% to 63.9% of biochar's labile carbon mineralized into CO_2, which was equal to 0.6% of total biochar-C in FBC700 and 1.6% of total biochar-C in FBC300.

Only few studies have examined the effect of biochar on available nitrogen concentrations associated with urea-N addition (Gao and Cai 2015; Hangs *et al*. 2015), and no researchers have detected how biochar affected the urea-N transformation in soil. In the present study, the authors found that biochar significantly accelerated urea hydrolysis and increased the soil available nitrogen content greatly at the beginning of incubation. The accelerated urea hydrolysis can be explained by the following two reasons: i) Increased urease activity. The enhanced urease activity in the presence of biochar is presumably caused by a combination of different mechanisms: a) biochar provides energy-rich organic compounds (Luo *et al.* 2013; Singh and Cowie 2014) and microbial habitat that promote the growth of microorganisms resulting in the production of microbial urease (Zantua and Bremner 1977); and b) urease activity is optimum under slightly alkaline conditions (Dharmakeerthi and Thenabadu 2013), and biochar is a known liming agent; ii) Soil pH corresponds well to urea hydrolysis rate and nitrification processes (Gao and Cai 2015). This is consistent with the authors' result that the urea hydrolysis rate increased with enhanced biochar pH value (Table 3).

Table 3. pH Values in Soil Amended with or without Urea and Biochar in the End of Incubation (Soil:Water = 1:2.5)

	CK	U	F300	F500	F700	M300	M500	M700
pH	7.23 ± 0.05	6.89 ± 0.02	6.91 ± 0.02	6.85 ± 0.07	7.27 ± 0.02	6.93 ± 0.08	6.72 ± 0.05	6.72 ± 0.10
Value represent means, ± represents standard errors; n = 2								

Unlike biochar derived from agricultural wastes, sewage sludge, or manure-based feedstock, crop stover or straw biochar typically exhibit relatively low bioavailable N to plants ($< 2\%$) (Xie *et al*. 2013). Thus, as mentioned by previous findings that biochar can potentially mineralize N (Anderson *et al*. 2011; Nelissen *et al*. 2012), the authors concluded that the large amounts of available N in biochar-amended soil (Fig. 4) originated from the net mineralization of soil N. In detail, the labile carbon in biochar provides carbon sources that promote microbes' growth and reproduction, and urea-N provided an inorganic N source. As a result, the soil showed a net mineralization effect by biochar and urea addition, especially for biochar that contained a relatively high concentration of labile carbon.

However, at the end of incubation, the available nitrogen in biochar amended soils was lower than in the urea-alone treatment. The authors concluded that the reduction of available nitrogen in biochar treatments was a result from a net immobilization effect that caused the thriving of soil microbes (Fig. 5). As former researchers illustrated, the microbial demand for inorganic N increases with biochar addition, thereby immobilizing

N biotically (Novak *et al*. 2010; Nelissen *et al*. 2012, 2014). Nelissen *et al*. (2012) demonstrated that low-temperature biochar containing high concentrations of bioavailable C would probably induce net nitrogen immobilization. However, in this study, no differences in inorganic N contents were observed among the fresh and matured biochar treatments. In addition, it was reported that the reduction of inorganic nitrogen may also result from the adsorption by biochar (Ding *et al*. 2010; Singh *et al*. 2010; Spokas *et al*. 2012), but related works to prove this observation were not performed in the present study.

CONCLUSIONS

1. As pyrolysis temperature increased from 300°C to 700°C, biochar carbonization degree was accelerated and more pores were created, resulting in an increase of biochar surface area from 24.50 $m^2 \cdot g^{-1}$ to 73.68 $m^2 \cdot g^{-1}$.

2. Biochar, especially when newly produced, accelerated the rate of urea hydrolysis, and biochar enhanced soil urease activity and soil pH values were the main reasons for this effect.

3. Biochar contained a fraction of labile carbon, which corresponded to 1.5% to 2.5% of biochar total carbon, and this fraction of carbon promoted microbial to thrive, during which lead to soil nitrogen mineralized.

4. The highest concentration available of N in fresh biochar amended soil was from urea transformation and soil nitrogen mineralization. Therefore, the authors concluded that aged biochar was suitable for simultaneous soil amendment with urea rather than newly produced biochar, as it can promote available N accumulation in a short time; thus, an increase in the risk of inorganic nitrogen leaching loss.

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