Maize stover biochar increases urea (^{15}N isotope) retention in soils but does not promote its acquisition by plants during a 4-year pot experiment

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ABSTRACT

Biochar as a soil amendment has been shown to improve soil quality and crop growth. However, biochar's effect on urea-N use efficiency in long term is not well elucidated. Here we studied urea-N (^{15}N isotope) allocation in plants and soil in the presence of maize (*Zea mays* L.) stover biochar (equivalent to 46 t ha^{-1}) during a 4-yr pot trial. Results showed that biochar only increased maize biomass (about 9%) with high amount of urea addition, which indicates the increased maize dry weight by biochar application could be attributed to synergistic effects between biochar and urea. Soil total N contents and fertilizer N retention were increased by 20% and 10.47% to 94.52%, respectively, indicating that biochar was more capable for fertilizer N retention than promote plant adsorption. Moreover, inorganic N content in biochar treatment was greatly increased, which implies the increased N mineralization. In total, we concluded that biochar application was a potential urea enhancer during plant production.

Key words: Biochar, ^{15}N isotope, N retention, N uptakes, *Zea mays*.

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INTRODUCTION

Nitrogen is an essential element for plant growth. The application of inorganic N fertilizer to agricultural soil is shown as an effective practice for crop yield improvement, but its overuse or even at a normal level could all result in N losses (Zhu and Chen, 2002; Smolders et al., 2010). The N leachate is considered as one of the primary pollutants (Power et al., 2001), contributing to eutrophication for aqueous systems and the global warming by the emission of gaseous N. One of the great concerns within agricultural context lies in methods for effectively using N fertilizers.

Many methods have been proposed to improve fertilizer-N use efficiencies. Recommended N fertilization is introduced into practice, because of the varied N acquisition rates in terms of crop varieties, and physiological stages. A slow-release N fertilizer is used to satisfy crop demand with less N losses to the environment (Chalk et al., 2015). Nitrification and urease inhibitors are applied along with N fertilizer, which can enhance NH_4^+-N retention by reducing its conversion to mobile NO_3^--N in the soil that finally turns into gaseous N (Xu et al., 2000). Lehmann (2007) firstly proposed the potential of using biochar to improve N retention and decrease N leaching, which was inspired by the research conducted on 'terra preta' in Amazon. Biochar is the solid remains of thermally decomposed organic matter with limited oxygen supply at temperatures < 700 °C (Lehmann and Joseph, 2009; Novak et al., 2009). Mechanisms underpin biochar's effects on N retention in soil are not well understood, but some potential processes have been proposed. The positive effect of biochar on N retention was attributed to adsorption of NH_4^+-N, and the intrinsic properties of biochar such as the high cation-exchange capacity (CEC) and water holding capacity. These effects further result in reduction in NO_3^--N leaching, N_2O emissions, and NH_3 volatilization (Yanai et al., 2007; Singh et al., 2010; Taghizadeh-Toosi et al., 2011; Schimmelpfennig et al., 2014). Biochar is also suggested to increase N bioavailability and use efficiency by promoting plant growth (Zheng et al., 2013; Partey et al., 2015). For instance, Huang et al. (2013) reported a tight relationship between rice yield and the agronomic N use efficiency in the presence of biochar amendment. Likewise, N loss occurred when biochar induced decrease in wheat biomass (Reverchon et al., 2014). Labile organic C in fresh biochar was found to decrease N availability by provoking microbial proliferation (Lehmann et al., 2003; Ippolito et al., 2012). Biochar charged with nutrients or microbial inoculants, or amended with

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nutrients are suggested beneficial for plant production. However, because of these positive and negative effects, biochar can induce neutral effects on N cycling under some instances (Jones et al., 2012).

The inconsistent feedback in respect to biochar's impact on soil N could come from the varied biochar types, occurrence of irrigation or precipitation, N fertilizer amount and forms. Also, most of the available data are collected from the short-term experiment. These short-term effects could not be extrapolated to the long-term one. In the soil-water-air interfaces, N loss and conversion occur at multiple pathways, which make it difficult to trace the original N amended in soil. Isotopic analysis has already been used to unravel the mechanisms governing N cycling in plant soil systems. The use of N isotope provides opportunities for monitoring the fate of N-fertilizer in soils (Taghizadeh-Toosi et al., 2012), but it has rarely been applied on biochar studies.

Recently, biochar technology was introduced into the bio-wastes management regime (Meng et al., 2011). Traditional practice such as burning on field after grain harvest brings about severe debate, because of air pollution. And crops residues are difficult to be decomposed when ploughed back to soils, which usually results in the reduced germination rates for crops. However, in order to avoid negative effect occurred, the interaction between fertilizer and biochar should be studied prior to its field application.

To address these knowledge gaps mentioned above, we conducted a multi-year pot trial using maize stover biochar and urea (^{15}N labeled) as a tracer which was the most commonly used from 2012 to 2015. The aim of this study was to: i) Evaluate the effect of biochar on maize growth with presence of N fertilizer, ii) investigate the biochar effect on N adsorption and fertilizer N allocation in plant, and iii) examine the effect of biochar on fertilizer N retention in soil and its bioavailability in long term. We hypothesize that biochar would be an optimal product for stovers resource utilization and soil fertility improvement.

MATERIALS AND METHODS

The pot trial was established in 2012 in Shenyang Agricultural University, Shenyang, China. Two treatments were employed: Soil amended with 0 t ha^{-1} biochar, but urea (NF); and soil amended with 40 t ha^{-1} biochar and urea (BF). Each treatment was triplicated. Top soil (0-15 cm) was collected from a local fallow land adjacent to Shenyang Agricultural University (41°83' N, 123°58' E). The soil was classified as a Hapli-Udic Cambisol according to Food and Agriculture Organization (FAO). To determine the basic soil properties (Table 1), the soil was air-dried and sieved through a 2-mm sieve. Soil total C and N contents were measured using an elemental analyzer (Elementar Macro Cube, Langenselbold, Germany). Mo-Sb-Vc-method and flame photometry method were used to determine soil total P and K contents after soil sample was melted by sodium hydroxide.

The maize stover biochar used in this study was produced using a commercial pyrolysis equipment under "oxygen-limited" conditions at 400-500 °C (Jinhefu Agricultural Development Company, Liaoning, China). The basic properties of the biochar were shown in Table 1.

To increase the hydraulic conductivity, 1.2 kg quartz sand was firstly added in 20 L pots (height of 33 cm, and average diameter of 14 cm). In the next step, 17 kg soil was mixed with 0.246 kg biochar, before transferring to each pot, to achieve a rate equivalent 46 t ha^{-1} in field. The control was just 17.246 kg soil. Two maize seeds (*Zea mays* L. 'Zhengdan 958') were planted in the center of each pot at approximately 4 cm depth (1 to 10 May depending on the weather conditions in each year). After germination, one seedling was kept. During the four growing seasons, 0.89 g P_2O_5 and 0.74 g K_2O were applied to one pot for each season. The pots were employed under natural environment, but an awning was used when severe rainfall occurred. The soil moisture was kept at around 50%-60% of the water holding capacity.

Nitrogen fertilizer application

^{15}N labelled urea (10.12% atom abundance) was used as an N fertilizer (Shanghai Research Institute of chemical industry). In reference of the traditional N application amount used by farmers in Northern China, full recommended fertilization of 9 g urea per pot (equal to 284 kg N ha^{-1}) in 2012 and 2013, and 45% recommended fertilization of 4 g urea per pot (equal to 126 kg N ha^{-1}) in 2014 and 2015 was applied, respectively. Five grams urea were applied as planting and 4 g urea dissolved by water were applied into the soils 3 wk after germination in 2012 and 2013. In 2014 and 2015, only 4 g urea were applied about 3 wk after germination.

Sample analysis

For each growing period, maize grains and stovers were collected and separated on 15-20 October. Plant samples were dried in oven at 65 °C until receiving a constant weight. The dry weight of grains and stovers were measured. Plant samples were then finely grinded before measuring

Table 1. Basic properties of the tested soil and biochar.

	Total C	Total N	Total P	Total K	Available N	pH	WHC	Bulk density	Surface area
	%		g kg^{-1}		mg kg^{-1}		%	g cm^{-3}	m^2 g^{-1}
Soil	1.12	0.15	0.22	2.05	69.33	7.30	49.99	1.29	NA
Biochar	64.50	1.14	0.38	3.23	14.15	9.11	NA	0.35	1.33

For pH determination, soil:water 1:2.5; biochar:water 1:25.
WHC: Water-holding capacity, NA: not available.

the total N content by elemental analyzer (Elementar Macro Cube, Langenselbold, Germany). ^{15}N abundance was determined using isotope ratio mass spectrometry (Thermo Fisher, Waltham, Massachusetts, USA).

After harvest, 50 g bulk soils were collected from each pot, air dried and passed through a 100-mesh sieve for determining the total N content using elemental analyzer. In 2014 and 2015, inorganic N contents in soils were determined: 10 g fresh soil samples were collected (0-20 cm) on day 1, 3, 5, 10, 17, 26, 31 and 60 immediately after urea was applied. The exchangeable NO_3^--N and NH_4^+-N were extracted using 2 M KCl, and determined by continuous flow analyzer (SEAL AA3, Jean, Germany). During the 4-yr experiment, sub-samples of the sieved soils were finely grinded for determination of the total ^{15}N abundance. The natural ^{15}N abundance values of soil and plants were determined by samples from control treatment where unlabelled urea was added.

The N derived from fertilizer (Ndff, %) and N use efficiency (NUE, %) were calculated by the following equations (Wu et al., 2011; Güereña et al., 2013):

$$Ndff = {}^{15}N_s \,/\, {}^{15}N_u \qquad [1]$$

$$NUE = Ndff \times N_p \,/\, N_i \qquad [2]$$

where $^{15}N_s$ is the ^{15}N enrichment in soil or plant sample (%), $^{15}N_u$ is the ^{15}N enrichment in urea (%), N_p refers to the total N in plant (g), and N_i for the total N input (g).

Statistical analysis

All data were analyzed by independent *t*-test at a significance level of 0.05 using SPSS version 19 (IBM Corporation, Armonk, New York, USA). Figures were generated by GraphPad Prism 5 (GraphPad Software, San Diego, California, USA).

RESULTS

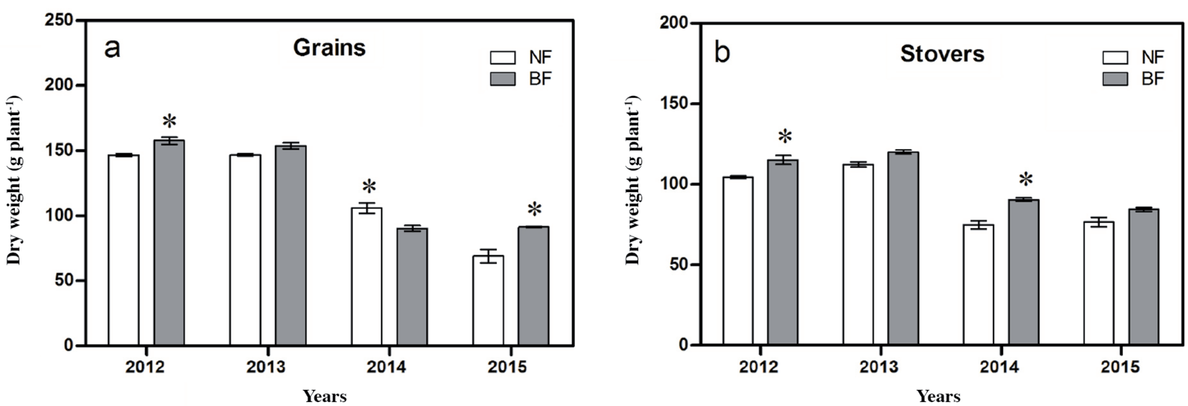
Effect of biochar on maize biomass and N uptake

Biochar plus urea (BF) generally increased maize biomass in comparison with the urea addition (NF) (Figure 1). Higher dose of urea promoted the growth of maize in 2012. When urea addition was decreased, BF improved maize stover biomass by 20.95%, but decreased maize grain weight by 18.78% compared with NF in 2014. With the same amount of urea, BF improved the biomass of maize for both the grain and stoves in comparison with NF in 2015.

The addition of urea and the initial soil contributed 48%-61% and, 39%-52% of the total N acquired by plants (Table 2). The addition of biochar did not show significant impact on the ratio of N sources from urea and soil during 2012 to 2014, but biochar addition (BF) increased the total N uptake by plants from fertilizer N to 46.62% than NF in 2015.

The recovery of urea-N in grains and stovers were calculated and the results were shown in Table 3. Most

Figure 1. Maize grain (a) and stover (b) dry weight of NF and BF treatments.



NF treatment: Soil amended with urea only; BF treatment: soil amended with urea and 40 t ha^{-1} biochar.
*Significant differences between NF and BF treatments in same year at $p < 0.05$ level. Error bars represent standard error.

Table 2. Maize N adsorbed from urea and soil.

	Treatments	Total maize N	N derived from urea		N derived from soil	
			Amount	Ratio	Amount	Ratio
		g plant^{-1}	g plant^{-1}	%	g plant^{-1}	%
2012	NF	2.68 ± 0.09	1.30 ± 0.22	48.33 ± 3.37	1.38 ± 0.11	51.67 ± 3.37
	BF	2.79 ± 0.13	1.47 ± 0.10	52.83 ± 1.84	1.31 ± 0.06	47.16 ± 1.84
2013	NF	2.38 ± 0.03	1.47 ± 0.05	61.65 ± 2.33	0.91 ± 0.06	38.36 ± 2.33
	BF	2.56 ± 0.07	1.51 ± 0.07	58.98 ± 1.22	1.05 ± 0.01	41.02 ± 1.22
2014	NF	1.48 ± 0.06	0.85 ± 0.04	57.47 ± 1.41	0.63 ± 0.03	42.54 ± 1.41
	BF	1.56 ± 0.07	0.89 ± 0.43	57.22 ± 0.07	0.67 ± 0.03	42.79 ± 0.07
2015	NF	**1.0 ± 0.13**	**0.56 ± 0.06**	56.68 ± 1.46	0.46 ± 0.07	43.35 ± 1.46
	BF	**1.33 ± 0.07**	**0.79 ± 0.04**	59.17 ± 0.15	0.54 ± 0.03	40.84 ± 0.15

NF: Soil amended with urea only; BF: soil amended with urea and 40 t ha^{-1} biochar.
Values represent means ± standard errors (n = 3).
Data in bold indicate significant differences between the NF and BF treatments ($p < 0.05$).

Table 3. Recovery of labeled fertilizer N in grain and stover of maize.

Treatments		N uptake from fertilizer					
		Total	N in grain	N in stover	Grain N/Total N	Stover N/total N	NUE
		g plant^{-1}			%		
2012	NF	1.30 ± 0.14	1.01 ± 0.10	0.29 ± 0.20	77.55 ± 0.53	22.45	30.88 ± 3.00
	BF	1.47 ± 0.10	1.14 ± 0.09	0.33 ± 0.01	77.37 ± 0.71	22.63	35.09 ± 2.46
2013	NF	1.47 ± 0.53	1.19 ± 0.03	0.28 ± 0.02	81.02 ± 0.81	18.98	34.92 ± 1.27
	BF	1.51 ± 0.69	1.25 ± 0.05	0.26 ± 0.02	82.59 ± 0.67	17.41	35.94 ± 1.65
2014	NF	0.85 ± 0.04	0.68 ± 0.05	0.17 ± 0.01	79.92 ± 0.16	20.08	45.56 ± 2.14
	BF	0.89 ± 0.43	0.69 ± 0.03	0.20 ± 0.02	77.86 ± 2.04	22.14	47.75 ± 2.28
2015	NF	**0.56 ± 0.06**	0.41 ± 0.06	0.15 ± 0.03	72.84 ± 2.39	27.16	**30.13 ± 2.03**
	BF	**0.79 ± 0.04**	0.59 ± 0.05	0.20 ± 0.00	74.60 ± 1.75	25.40	**42.10 ± 2.32**

NF: Soil amended with urea only; BF: soil amended with urea and 40 t ha^{-1} biochar.
Values represent means ± standard errors (n = 3).
Data in bold indicate significant differences between the NF and BF treatments ($p < 0.05$).

urea-N was accumulated in maize grains, only 17.4% to 27.2% of urea-N accumulated in stovers. Biochar addition did not induce significant differences in N recovery by maize during 2012-2014, but a significant increase by 39.86% was obtained in 2015.

Effect of biochar on the total N, and ^{15}N retention in soils

Biochar amendment (BF) significantly ($p < 0.05$) increased soil total N contents by 0.14 and 0.24 g kg^{-1} compared with NF treatment in 2012 and 2013 (Figure 2a). The atom ^{15}N value was enhanced by 0.32% by biochar addition in 2013, as a result, the amount of N from urea increased by 0.09 and 0.68 g pot^{-1}, and the residual rate increased by 2.28% and 8.3% (Table 4). However, biochar addition showed nonsignificant impact on soil total N content (Figure 2a) when urea addition was reduced in 2014 and 2015. Although the atom ^{15}N value decreased by 0.18% compared with NF treatment in 2015 (Figure 2b), the total amount of fertilizer N in soil and the residual rate showed nonsignificant reduction (Table 4).

Effect of biochar addition on NUE and loss rate

While the total N derived from urea adsorbed by maize were not affected by biochar addition during 2012 and 2013, but the amount of fertilizer N retained in soils were

Table 4. Total soil N (TN) and urea-N retained in soils during 2012-2015.

	Treatments	TN in soil	Urea-N in soil	
			Amount	Residual rate
		g pot^{-1}		%
2012	NF	**13.86 ± 0.20**	**0.54 ± 0.02**	**12.82 ± 0.45**
	BF	**15.75 ± 0.45**	**0.63 ± 0.05**	**15.10 ± 1.24**
2013	NF	**13.14 ± 0.27**	**0.74 ± 0.04**	**8.79 ± 0.46**
	BF	**16.38 ± 0.46**	**1.43 ± 0.10**	**17.09 ± 1.23**
2014	NF	13.23 ± 0.28	1.03 ± 0.06	10.01 ± 0.61
	BF	14.04 ± 0.59	0.91 ± 0.07	8.84 ± 0.65
2015	NF	13.05 ± 0.45	1.07 ± 0.04	8.84 ± 0.36
	BF	14.40 ± 0.45	1.01 ± 0.02	8.30 ± 0.13

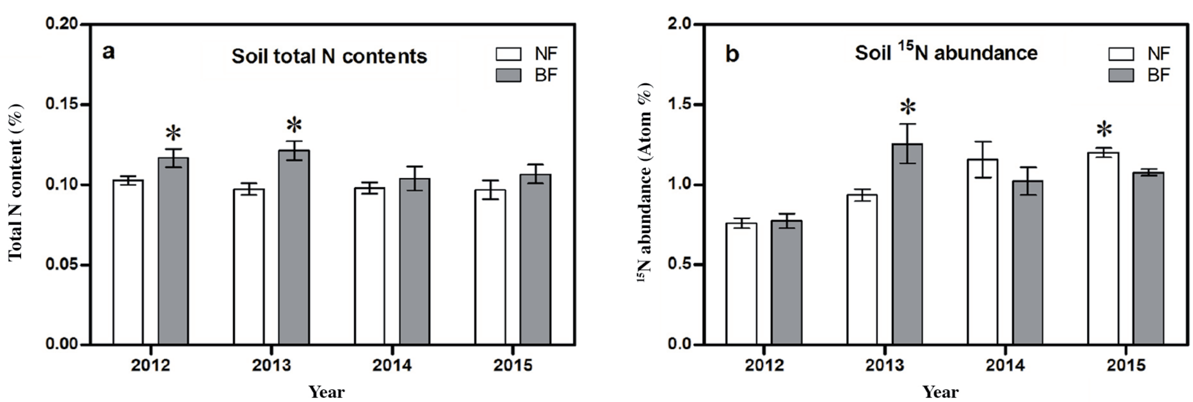
NF: Soil amended with urea only; BF: soil amended with urea and 40 t ha^{-1} biochar.
Values represent means ± standard errors (n = 3).
Data in bold indicate significant differences between the NF and BF treatments ($p < 0.05$).

greatly enhanced (Table 5). However, after reduction of N usage, the fertilizer N retained in soils in 2015 showed nonsignificant differences between NF and BF treatments. But the NUE increased about 2.90% and the N loss rate decreased 2.75% in biochar treatment *vs*. non-biochar treatment.

Effect of biochar on soil inorganic N contents

The NH_4^+-N and NO_3^--N contents were measured immediately after urea application in 2014 and 2015.

Figure 2. Soil total N content (a) and ^{15}N abundance (b) in BF and NF treatments.



NF treatment: Soil amended with urea only; BF treatment: soil amended with urea and 40 t ha^{-1} biochar.
The asterisk indicates significant differences between the NF and BF in same year at $p < 0.05$ level. Error bars represent standard error.

Table 5. Recovery of N-urea in maize and soil, and loss rate during 2012-2013 and 2012-2015.

Treatments		N-urea uptake by maize		Residual N-urea in soil		N loss
		Amount	NUE	Amount	Residual rate	Loss rate
		g pot^{-1}	%	g pot^{-1}	%	%
2012-2013	NF	2.76 ± 0.07	32.94 ± 0.87	**0.74 ± 0.04**	**8.79 ± 0.46**	**58.27 ± 1.25**
	BF	2.98 ± 0.16	35.56 ± 1.89	**1.43 ± 0.10**	**17.09 ± 1.23**	**47.35 ± 2.83**
2012-2015	NF	**4.08 ± 0.03**	**33.64 ± 0.27**	1.07 ± 0.04	8.83 ± 0.36	**57.40 ± 0.05**
	BF	**4.41 ± 0.07**	**36.33 ± 0.60**	1.01 ± 0.02	8.30 ± 0.13	**54.65 ± 1.05**

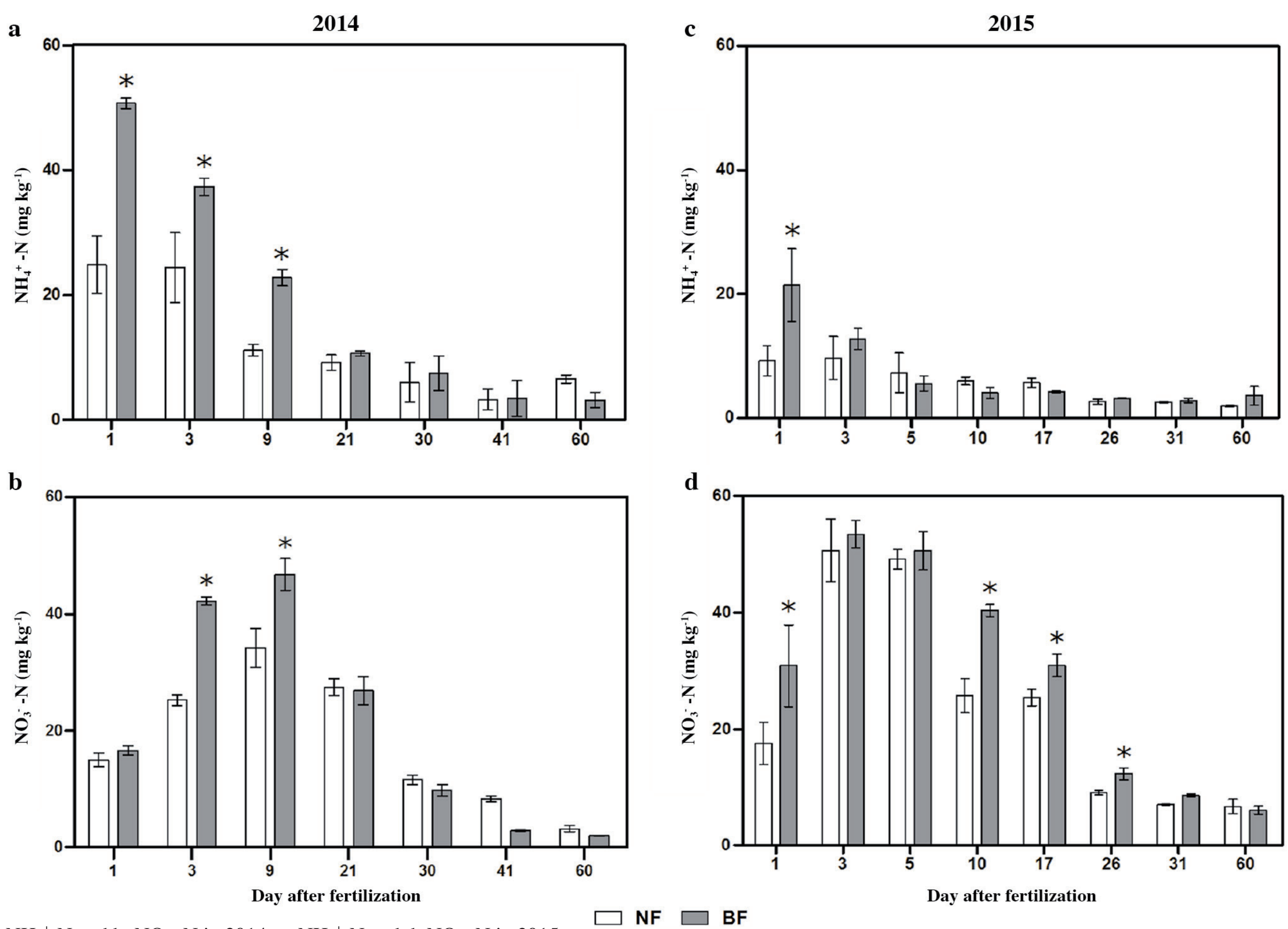
NF: Soil amended with urea only; BF: soil amended urea with and 40 t ha^{-1} biochar.
NUE: Nitrogen use efficiency.
Values represent means ± standard errors (n = 3).
Data in bold indicate significant differences between NF and BF treatments ($p < 0.05$).

Results showed that biochar increased both soil NH_4^+-N and NO_3^--N in the short period after urea addition (Figure 3). Ten days after the fertilization in 2014, NH_4^+-N and NO_3^--N concentrations in BF were greater than that in NF. The concentration of NH_4^+-N in BF reached the peak concentration of 50.7 and 21 mg kg^{-1} the first day after urea addition in 2014 in 2015, respectively, which was 2.0 and 2.26 times of the values in NF (Figures 3a and 3b). Biochar treatment showed higher NO_3^--N concentration at sampling time (day 3 and 9 in 2014; day 1, 10 and 17 in 2015) (Figures 3b and 3d).

DISCUSSION

A positive response to crop growth by biochar application was observed in our study, this is consistent with previous findings that biochar and N fertilizer increased crops production (Van Zwieten et al., 2010a; Zheng et al., 2013; Huang et al., 2014). However, only high amount of urea application received the positive effect. This indicates the increased maize dry weight by biochar application could be attributed to the indirect effect, such as synergistic effects between biochar and urea, rather than direct nutrient supply

Figure 3. Inorganic nitrogen content in soils after urea application in 2014 and 2015.



a: NH_4^+-N and b: NO_3^--N in 2014; c: NH_4^+-N and d: NO_3^--N in 2015.
NF treatment: Soil amended with urea only; BF treatment: Soil amended with urea and 40 t ha^{-1} biochar.
The asterisk indicates significant differences between the NF and BF in same year at $p < 0.05$ level. Error bars represent standard error.

from biochar. As our result showed (Table 1) the biochar available N content only was 14.15 mg kg^{-1}. Research from Xie et al. (2013) also found that crop stover or strew biochar usually exhibit relatively low bioavailable N to plants (< 2%), even it contained considerable amount of N. This was likely caused by the formation of recalcitrant heterocyclic N-containing compounds during the pyrolysis process (Cantrell et al., 2012).

Despite the slight influence on N use efficiency by plants, biochar addition increased soil's capacity on N retention from urea and the original soil with high urea addition (2012 and 2013). However, the N retention in soil was greatly reduced when urea application rate decreased. Possible mechanisms for biochar's effect on N retention have been summarized as processes: i) NH_4^+-N absorbed by biochar due to CEC (Liang et al., 2006) or surface acid function groups (Clough and Condron, 2010); ii) biochar's labile C fractions increased soil microbial N immobilization (DeLuca et al., 2006; Chan et al., 2007; Steiner et al., 2008; Van Zwieten et al., 2010b); iii) increased nutrient retention because of the promotion in water-retention capacity and deduction of soil bulk density in the presence of biochar. In our study, however, inorganic N content in BF was greatly increased. This implies the increased N mineralization by biochar addition. The enhanced N mineralization could stimulate N retention and increase N acquisition and crop yield.

To date, however, there are inconsistent conclusions on whether biochar stimulates net N mineralization or immobilization. Many studies showed that biochar accelerated soil N cycling with increased gross N mineralization (Nelissen et al., 2012; 2014). Anderson et al. (2011) found the increased abundance and activity of microorganisms involved in soil N cycle by biochar. Luo et al. (2011) attributed it to the labile organic C remaining in the fresh biochar, which stimulates microbial activities on N mineralization. However, Kuzyakov et al. (2009) and Dempster et al. (2012) showed mineralization of organic N was not changed by biochar addition.

After the 4-yr consecutive growing experiment, the average NUE in BF treatment was 3% greater than NF treatment, but fertilizer N retained in soil showed nonsignificant difference, as a result, the total N loss rate was reduced about 2.8% by biochar addition. We concluded that the main way for biochar reduce N losses in present study were denitrification, because in our experiment, water leaching from pots was greatly reduced, so that N run-off could be ignored. Also, ammonia (NH_3) volatilization only takes a small fraction of the total N from urea by previous study in our lab (Liu et al., 2015). Biochar is shown to reduce N_2O fluxes and potential mechanisms have been summarized by Clough et al. (2013): i) biochar inhibits denitrification and N_2O production by improving soil aeration (Yanai et al., 2007); ii) labile C in biochar promotes dinitrogen (N_2) formation; iii) enhanced N_2O reductase activity for promoting N_2 formation and reducing N_2O production (Firestone et al., 1980).

CONCLUSIONS

Our results showed that biochar addition did not directly increase N acquisition from urea by plants. However, biochar decreased the N loss and enhanced N retention in soil. The author concluded that biochar is a potential urea enhancer to achieve a desirable plant yield with low risk of N leaching during agricultural production.

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