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REGULAR ARTICLE

Responses of crop nitrogen partitioning, translocation and soil nitrogen residue to biochar addition in a temperate dryland agricultural soil

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Abstract
Background and aims Low nitrogen (N) use efficiency endangers world crop production and ground water. Biochar has been well documented in sequestering C in agricultural soils and improving soil quality. The objective of this study was to assess the multiyear impacts of biochar addition on crop performance, with a specific emphasis on crop N uptake, partitioning, translocation and mineral N distribution in the soil profile.
Methods A 3-year field experiment was carried out on the semiarid Loess Plateau of northern China. Maize-straw biochar was added to a spring maize monoculture cropping system at rates of 0 (BC0), 10 (BC10), 20 (BC20) and 30 (BC30) t ha^{-1} and combined with NPK fertilization in April 2012.
Results Biochar addition increased the crop N uptake, grain N concentration and grain yield with an obvious increase in the kernel numbers per ear. In 2012, the highest rate of 30 t biochar ha^{-1} reduced leaf biomass and leaf N concentration at the V6 stage. Biochar addition had no effect on the pre-silking N accumulation but increased the post-silking N accumulation. Over the next two years, biochar addition increased the stem biomass and decreased the stem N concentration at the R1 and R6 stages. Biochar enhanced both the pre- and post-silking N accumulation; meanwhile, the N translocation efficiency in the biochar-added treatments was higher than in the control. The BC20 and BC30 treatments significantly improved the average NUE by 10.2% and 14.2%, respectively. Moreover, biochar addition decreased the soil NH_4^+-N concentration in the surface 20 cm soil layer in 2012, and the BC10 and BC20 treatments increased the soil NO_3^--N concentrations in the 10–20 and 0–20 cm soil layers, respectively, in 2013. Biochar addition reduced the total residual soil NO_3^--N and the extent of NO_3^--N leaching across the 0–200 cm soil profile.
Conclusions The application of biochar is an effective method to improve grain yield, N uptake and NUE while simultaneously avoiding substantial NO_3^--N leaching loss.

Keywords Biochar · N concentration · N uptake · N translocation · N use efficiency · Residual soil N

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Introduction

Nitrogen (N) is a critical macronutrient that is essential for crop growth and that contributes to an approximate 30 ~ 50% grain yield increase (Erisman et al. 2008; Bu et al. 2014; Jia et al. 2014). Application of N fertilizer can affect dry matter accumulation, N uptake and its partitioning to the crop's various organs (Dordas and Sioulas 2009), all of which are critical for determining final yield. Under field conditions, reducing soil mineral N to levels sub-optimal for crop demands can drastically restrict crop production (Salvagiotti et al. 2009). The top priority of crop production is to achieve high grain yield, and in order to ensure high yield, N fertilizer application to farmland soils is generally excessive and unlimited, primarily due to the difficulty in predicting accurate crop N requirements (Zhou et al. 2013). During the last decade, to support the largest population in the world, approximately 54% of the global increase in N fertilizer consumption for crop production occurred in China (Tian et al. 2012; Qian et al. 2014; Meng et al. 2016).

Increasing N fertilizer inputs in agriculture has caused a cascade of adverse environmental problems that go beyond limited crop productivity (Liu et al. 2003; Zhang et al. 2012a; Xia et al. 2016). The overuse of N fertilizer has led to N losses via ammonia (NH_3) volatilization, denitrification, and leaching losses (Ju et al. 2003; Zhao et al. 2006; Zhou et al. 2013). In return, N losses induce lower N utilization efficiency (NUE), especially in China, where the NUE is much lower than the global average level (Zhang et al. 2008; Tian et al. 2012). In the dryland soils of northern China, a large amount of N fertilizer accumulates in the soil profile in the form of nitrate-N (Zhou and Butterbach-Bahl 2014). Since rainfall coincides with maize growth in this region, the accumulated residual nitrate has the potential to leach into deep soil layers, resulting in a lower NUE and a higher risk of deteriorated ground water quality. Thus, it is crucial for agricultural development to find ways to simultaneously increase NUE and decrease N losses.

In recent years, one of the effectual approaches in improving crop productivity and mitigating global climate change has received increasing attention - biochar. Biochar is a carbon (C)-rich product with low density and high porosity, created by the thermal decomposition of organic materials at a preset temperature in the absence of oxygen (Lehmann and Joseph 2009; Lehmann et al. 2011). Numerous studies have documented that application of biochar can (a) increase the soil surface area (Chan et al. 2007), decrease soil bulk density (Xiao et al. 2016a) and improve soil aeration (Kolb et al. 2009), (b) increase the ion exchange capacity of soils and the nutrient retention capacity (Case et al. 2012; Zhang et al. 2013; Lone et al. 2015), and (c) change microbial community composition, enzyme activities and improve microbial biomass (Lehmann et al. 2011; Lone et al. 2015). Such changes could have beneficial effects on soil health (Atkinson et al. 2010) and indirectly impact plant growth (Jeffery et al. 2011). While due to a wide variety of biochar types, addition rates, soil properties and environmental conditions, positive, negative or non-relevant effects induced by biochar addition have been reported on crop biomass and grain yield (Kloss et al. 2014; Olmo et al. 2014; Rogovska et al. 2014). In addition, the application of biochar can also affect soil N dynamics, leading to an increase in inorganic N retention as a result of high adsorption ability of biochar, a reduction in NH_3 volatilization and nitrous oxide (N_2O) emissions due to increased nitrification and decreased denitrification, and an enhancement in biological immobilization of N because of microbial activity and labile organic compounds of biochar, all of which act synergistically to mitigate soil N losses potentially reducing the risk of air and water pollution (Clough et al. 2013; Lone et al. 2015), thereby improving plant assimilation and N use efficiency (Blackwell et al. 2010; Nguyen et al. 2017). However, some field studies found that biochar addition did not alter soil N dynamics (Sánchez-García et al. 2016) or even led to N immobilization or N gaseous loss, which reduced availability for plant roots (Schomberg et al. 2012; Nguyen et al. 2017). Hence, the influences of biochar addition need to be intensively investigated in soils of different regions.

Despite the extensive number of biochar studies examining plant yield and nutrient availability, information on how and if biochar addition changes plant N distribution and translocation is lacking. Post-silking N accumulation and N translocation from varying crop organs during the grain-filling period are deemed to be the two main sources of grain N content (Chen et al. 2015). The higher contribution of Post-N to grain indicated the stronger effect of soil available N on Post-N. The beneficial effect of biochar amendment on the soil mineral N retention and N availability may be the main reason for improving crop N uptake and partitioning. In addition, N accumulation was affected by available water (Dordas and Sioulas 2009). Previously we showed that biochar addition increased soil water holding capacity and plant available water content (Xiao et al. 2016b), both of which were conducive to improving soil nutrient availability. Biochar addition may also stimulate changes in plant root architecture that stimulate nutrient uptake through crop roots (Prendergast-Miller et al. 2014). Biochar has been demonstrated to increase crop productivity by approximately 10% (Jeffery et al. 2011; Liu et al. 2013), and the stronger grain demand induced higher N translocation (Ciampitti and Vyn 2012). Furthermore, the remobilization of N from vegetative organ to the grain is closely related to climatic conditions, management practice, soil nutrient and water availability, all of which are critical for determining the final grain yield (Dordas and Sioulas 2009). In dryland regions, uneven distribution of rainfall leads to recurrent drought and water deficit. Under stress conditions, the dry matter and N in vegetative organ can be used for grain filling, thereby enhancing the remobilization of N (Chen et al. 2015). To our knowledge, there is little information available on how the attainable grain yields induced by biochar additions are affected by crop N dynamics. Hence, exploring the correlation between the two sources is important for understanding the process of crop growth and crop N contribution to grain N, which in turn helps us analyze and interpret our experimental results.

In this study, we hypothesized that biochar addition would improve soil N availability and increase crop dry matter accumulation, as well as alter N partitioning and translocation, thereby improving N uptake and NUE. To investigate the dry matter and N accumulation process, we analyzed the medium-term effects of varying rates of biochar addition on crop growth in a temperate dryland field soil. The specific objectives of this study were (a) to quantify the effects of varying rates of biochar addition on maize dry matter accumulation, N content and N efficiency; (b) to identify the main source of grain N at maturity (i.e., pre-silking accumulated N versus post-silking accumulated N, and leaf translocated N versus stem translocated N) with different rates of biochar addition; and (c) to explore how the varying rates of biochar addition affected soil profile N dynamics. These results will help us determine the optimum biochar addition rate while considering both the positive and negative effects on crop growth and N uptake.

Materials and methods

Experiment site

This field experiment was carried out from 2012 to 2014 at the Changwu Agricultural and Ecological Experiment Station (35.28°N, 107.88°E; 1200 m elevation) on the Loess Plateau of China. The climate in the region is semi-arid with an average annual temperature of 9.1 °C and an average annual rainfall of 555 mm, 73% of which falls during the maize growth season. The mean annual evaporation from a free water surface is as high as 1565 mm. The rainfall was 481, 579 and 567 mm in 2012, 2013 and 2014, respectively, with 403, 421 and 375 mm falling during the maize growth season, respectively. Most precipitation occurred between July to September (approximately 60% of total rainfall). The distributions of the monthly precipitation and the air temperature during the observed three years are presented in Fig. 1. The region is rain-fed farmland, and the primary cropping system consists of one maize or wheat crop harvested per year. The soils at this site are Cumuli-Ustic Isohumosols (Gong et al. 2007).

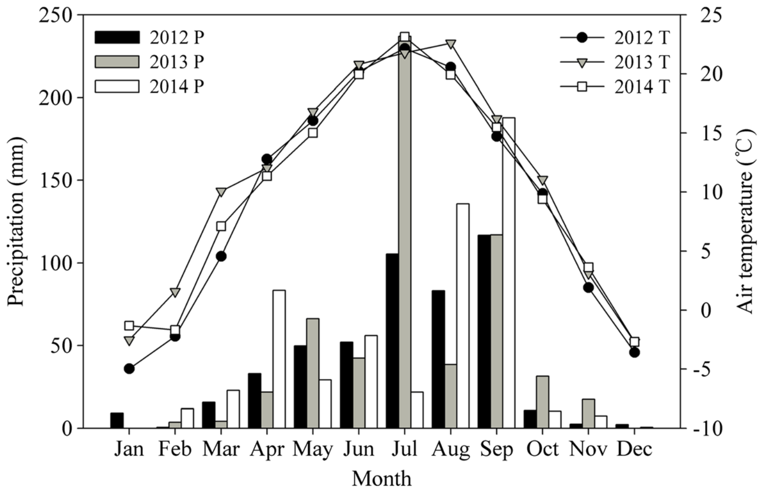

Fig. 1 Distribution of the monthly precipitation (vertical bars) and the air temperature (line) from 2012 to 2014 at the experimental site

Soil and biochar properties

Analyses of the basic physicochemical properties of the soil samples (0–20 cm) taken from the experimental field before sowing in 2012 indicated that the topsoil had a pH (1:2.5 H_2O) of 7.89; bulk density, 1.36 g cm^{-3}; total carbon, 19.9 g kg^{-1}; total N, 0.99 g kg^{-1}; available phosphorus (Olsen-P), 6.56 mg kg^{-1}; available potassium (NH_4OAc-K), 127.12 mg kg^{-1}; NO_3^--N, 8.79 mg kg^{-1}; and NH_4^+-N, 1.17 mg kg^{-1}. The biochar added to the study soils was manufactured from the pyrolysis of maize straw feedstock at 400 °C at the Sanli New Energy Company, Henan, China. The biochar was surface applied by hand in April 2012 before maize sowing and immediately incorporated into the soil to a depth of 0–20 cm with base fertilizers, utilizing both rotary and moldboard plow tillage. The biochar had a pH of 9.8; C, N and H contents of 59.16%, 0.98% and 1.69%, respectively; NO_3^--N, 1.6 mg kg^{-1}; NH_4^+-N, 1.11 mg kg^{-1}; cation exchange capacity (CEC), 37.33 cmol kg^{-1} and a specific surface area (Brunauer-Emmett-Teller) of 53.03 m^2 g^{-1}.

Experimental design and treatments

In this study, four biochar application rates (0, 10, 20 and 30 t ha^{-1}) were replicated three times providing 12 field plots; each plot was 56 m^2 (7 m × 8 m). The biochar treatments were labeled BC0, BC10, BC20 and BC30, respectively, based on the aforementioned rates. N fertilizer (225 kg ha^{-1}) was applied thrice in the form of urea: 40% was applied before sowing as a base fertilizer, 30% was top-dressed at the jointing stage, and 30% was top-dressed at the silking stage. Phosphorus (P) and potassium (K) were applied once before sowing as base fertilizers at a rate of 40 kg P ha^{-1} of calcium superphosphate and 80 kg K ha^{-1} of potassium sulfate. The rate and timing of N, P and K fertilization were the same in all plots. In each plot, maize seed (Pioneer 335) was sown 5 cm deep in April of each year at a density of 65,000 plants ha^{-1} using a hand-powered hole-drilling machine. The natural rainfall was the sole water supply for each plot.

Sampling and analyses

Plant sampling

The standard maize development stage system (Ritchie et al. 1992) was used to identify the main growth stages of the planted crop. In all 3 growing seasons, three adjacent plants per plot in a row were randomly selected at the 6-leaf (V6), silking (R1) and physiological maturity (R6) stages. Plants were cut at ground level and separated into the leaf laminae, the stem plus sheaths and the ears (i.e., husk + grain + cob). At the R6 stage, an area of 10 m^2 (four rows each 2.5 m long) in the middle of each plot were manually harvested to determine the grain yield and its components (ear numbers per 10 m^2, kernel numbers per ear, and 1000-kernel weight). The grain yield was calculated at 15.5% moisture content. All the fresh plant samples were first oven-dried at 105 °C for 30 min, then oven-dried at 80 °C until reaching a consistent weight, weighed and milled to a fine powder. The N concentration in each organ was determined by a modified Kjeldahl digestion method (Nelson and Somers 1973).

The various parameters referring to N uptake and N movement were calculated according to Dordas and Sioulas (2009) and Chen et al. (2015) as follows:

$$\text{Post-silking N content} = \text{Total N content at maturity} - \text{Total N content at silking} \tag{1}$$

$$\text{N translocation} = \text{Total N content at silking} - (\text{Leaf N content} + \text{Stem N content} + \text{Husk N content} + \text{Cob N content})\ \text{at maturity} \tag{2}$$

$$\text{N translocation efficiency}\ (\%) = (\text{N translocation}/\text{Total N content at silking}) \times 100 \tag{3}$$

$$\text{Leaf (Stem) translocated N} = \text{Leaf (Stem) N content at silking} - \text{Leaf (Stem) N content at maturity} \tag{4}$$

$$\text{Leaf (Stem) N translocation efficiency}\ (\%) = [\text{Leaf (Stem) translocated N}/\text{Leaf (Stem) N content at silking}] \times 100 \tag{5}$$

N harvest index (NHI, %) was calculated with the following formula (Huggins and Pan 1993):

$$\text{NHI} = (\text{Grain N content at maturity}/\text{Total aboveground N content at maturity}) \times 100 \quad (6)$$

The N use efficiency (NUE, kg kg^{-1}) was calculated with the following formula (Moll et al. 1982):

$$\text{NUE} = \text{Grain yield}/\text{N fertilizer application rate} \quad (7)$$

Soil sampling

Soil samples were taken from each plot at 10 cm intervals over the 0–100 cm soil depth and at 20 cm intervals over the 100–200 cm soil depth using a soil auger at harvest time (i.e., R6). The concentrations of nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) of the fresh soil samples were extracted with KCl solutions (1 mol L^{-1}, 50 ml for 5 g soil) for 1 h at 200 rev min^{-1} followed by filtration. Next, the extracts were analyzed using an automated flow injection analyzer (FLOWSYS, Italy). Residual soil NO_3^--N (kg ha^{-1}, Res-N) in the soil profile was considered to be the sum of the total storage in all of the sampled layers in the plot and was calculated using the following formula: Res-N = $h \times \rho_b \times p \times 10 / 100$, where h is the soil depth (cm), ρ_b is the soil bulk density (g cm^{-3}), and p is the soil concentration of NO_3^--N (mg kg^{-1}).

Data analyses

The reported results were the means of the three replicates. A one-way analysis of variance (ANOVA) was used to evaluate the measured parameters affected by the different rates of biochar addition. The differences between all the treatments were detected using least significant difference testing (LSD) at level of 0.05. Linear regression analysis was utilized to investigate the relationship between maize grain yield and dry matter accumulation during the pre- and post-silking periods. When considering the differences between years, a two-way analysis of variance was used with the different biochar treatments and the sampling years as two fixed factors. Statistical analyses and data plotting were performed using SPSS Statistics Software 19.0 and Sigma Plot 10.0, respectively.

Results

Crop organ N concentration and dry matter accumulation

The N concentrations of crop organs affected by varying rates of biochar addition are shown in Fig. 2. The BC10 and BC30 treatments significantly decreased the leaf N concentrations by 7.7% and 12.3%, respectively, compared with the control at the V6 stage in 2012, and no significant differences were observed in the other stages and years (Fig. 2a). Biochar addition did not have a statistically significant effect on the stem N concentration at the V6 stage but significantly reduced the stem N concentration at the R6 stage in each year. Additionally, the biochar-added treatments exhibited lower stem N concentrations at the R1 stage, but the differences between the biochar-added treatments and the control were not all significant. The ear N concentrations in the BC10 and BC30 treatments were lower than the control at the R1 stage in each year, while at the R6 stage, biochar additions increased the grain N concentrations (Fig. 2b). The husk and cob in the biochar-added treatments showed lower N concentrations than the control, and only significant in the third year.

The dry matter accumulation of the straw (stem, leaf and ear) and grain affected by varying rates of biochar addition are shown in Fig. 3. At the V6 stage in 2012, the BC10 treatment slightly increased the leaf and stem biomass ($P > 0.05$), whereas the leaf biomass in the BC30 treatment was significantly lower than the control by 21.4%; in 2013 and 2014, no significant differences were observed. At the R1 stage, biochar additions had no effects on the dry matter accumulations of leaf, stem

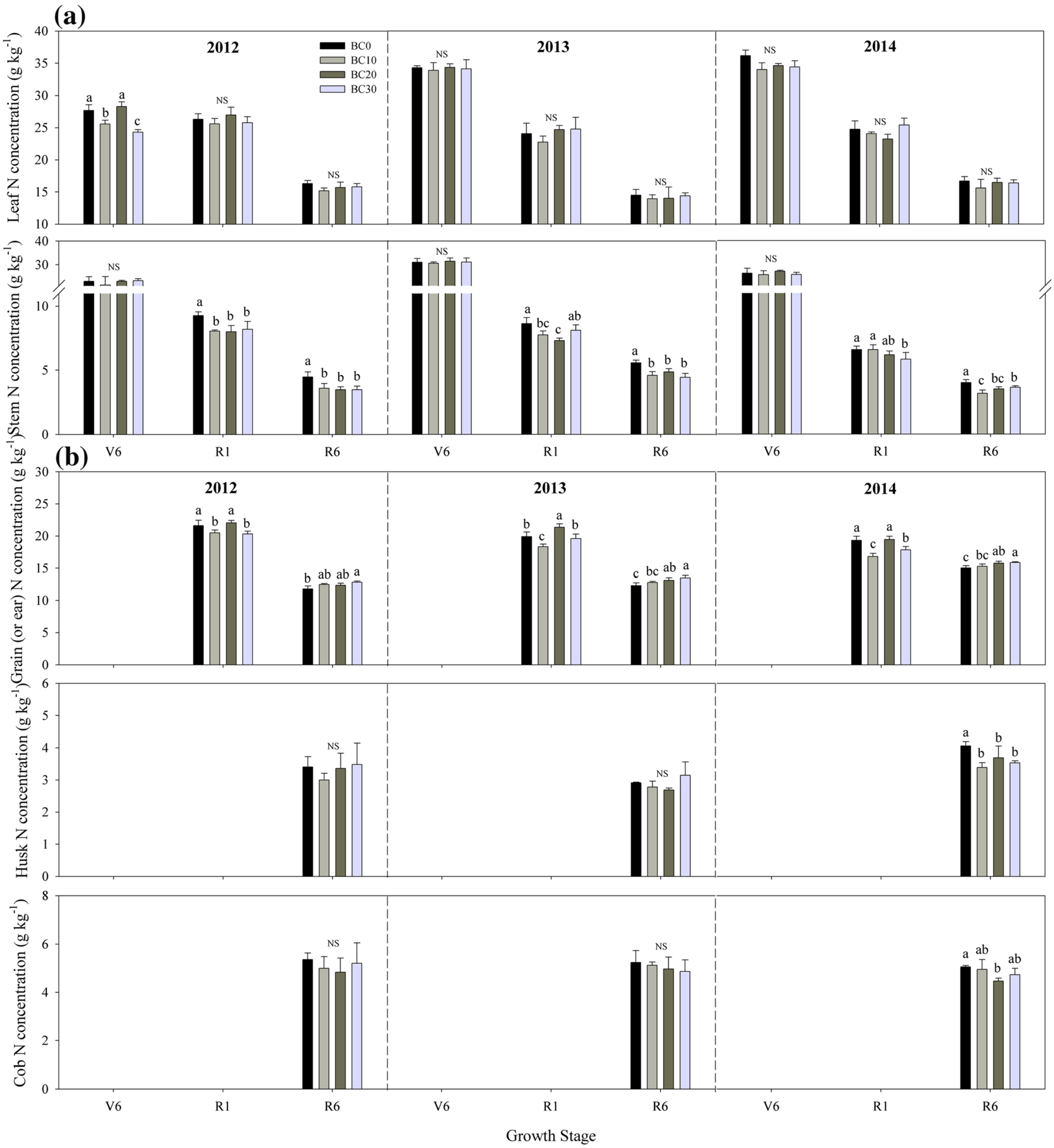

Fig. 2 The N concentrations of the leaf and stem (**a**) and the ear (i.e., grain + husk + cob; **b**) as affected by the different rates of maize-straw biochar at the V6, R1 and R6 stages of crop growth from 2012 to 2014. The bars represent the standard deviation of triplicates. Different letters indicate significant difference ($P < 0.05$) among treatment means. ns, Nonsignificant

and ear in 2012 but significantly increased them in 2013 with the exception of the ear biomass in the BC10 treatment; in 2014, the BC20 and BC30 treatments significantly increased the stem and ear biomass. At maturity, the BC20 and BC30 treatments significantly increased the grain biomass in each year. During the first two years, only the BC30 treatment significantly increased the straw biomass, while in the third year, all the biochar-added rates significantly increased the straw biomass. Positive linear relationships between the 3-

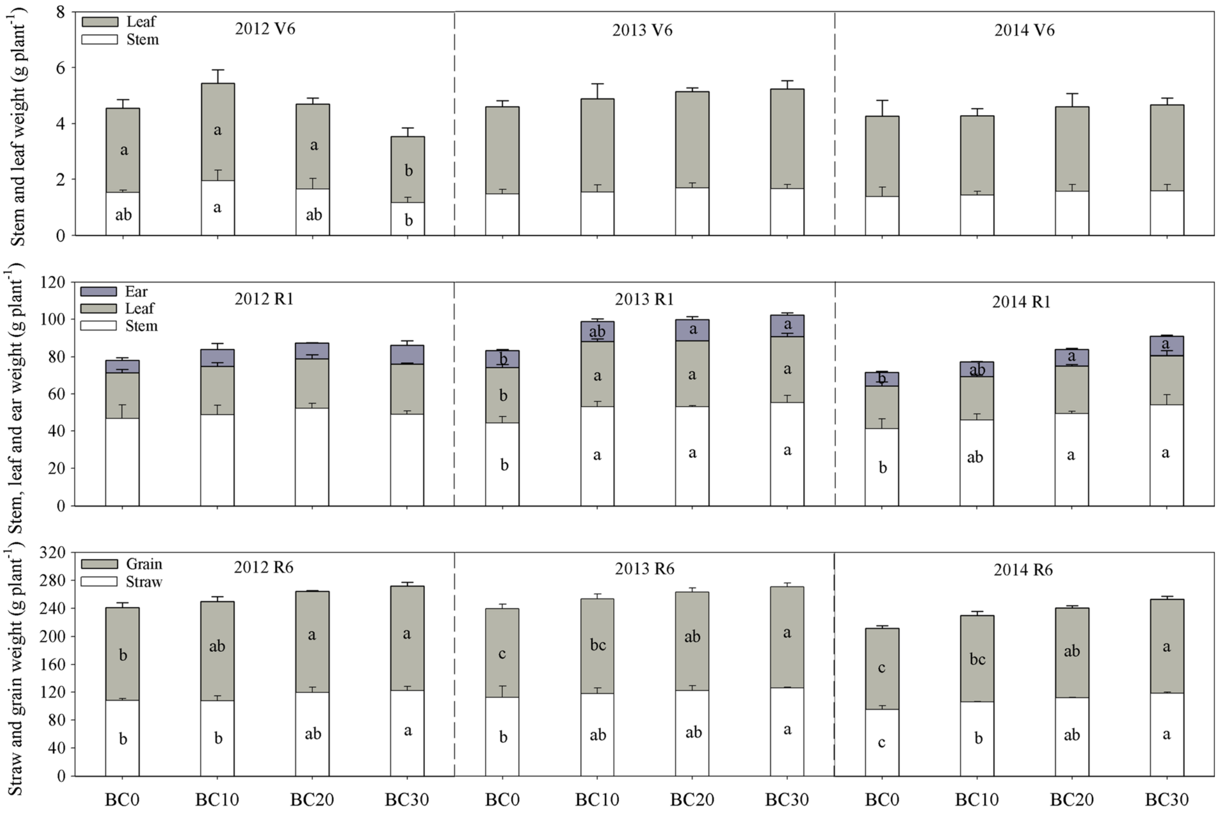

Fig. 3 The dry matter accumulation of the straw (stem, leaf and ear) and the grain as affected by the different rates of maize-straw biochar at the V6, R1 and R6 stages from 2012 to 2014. The bars represent the standard deviation of triplicates. Different letters indicate significant difference ($P < 0.05$) among treatment means

year maize grain yields and the dry matter accumulations during the pre- and post-silking periods were observed in Fig. 4. The determination coefficient (R^2) and the significance during the post-silking period were higher than that during the pre-silking period. The ratios of dry matter accumulation during the post-silking period to the total aboveground dry matter amount at maturity were 61% ~ 68.4% in all treatments.

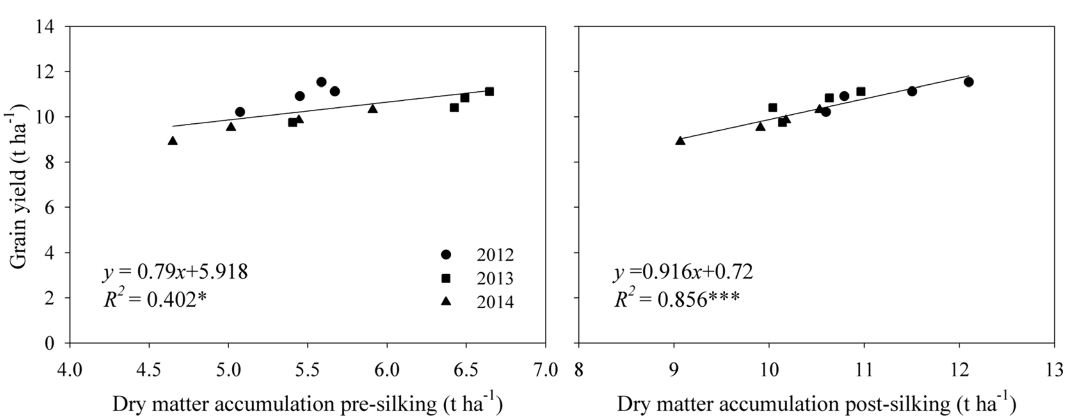

Fig. 4 The maize grain yield response to dry matter accumulation during pre- and post-silking periods. Relationships were fitted to three years of data. * Significant at $P < 0.05$; *** Significant at $P < 0.001$

N accumulation and translocation

The biochar rate and sampling year had significant effects on the aboveground N accumulations during the pre- and post-silking periods, but the interaction was not statistically significant (Table 1). In 2012, no significant differences in the aboveground N accumulations were observed during the pre-silking period; biochar additions increased N accumulations during the post-silking period, but only significant in the BC30 treatment, at 41.7% more than the control. Meanwhile, the biochar-added treatments showed a higher ratio of Post-N/Total-N, and the BC30 treatment significantly increased the ratio at 16.5% more than the control. Over the next two years, biochar addition increased both Pre-N and Post-N accumulations, especially in the BC20 and BC30 treatments, and the ratio of Post-N/Total-N showed a tendency to decrease with biochar applications.

Table 1 The aboveground N accumulation during the pre-silking (Pre-N) and post-silking periods (Post-N), and the ratio of N accumulation during the post-silking period to the total aboveground N amount at maturity (Post-N/Total-N) under the different biochar-added treatments in 2012, 2013 and 2014

Year	Treatment	Pre-N (kg ha^{-1})	Post-N (kg ha^{-1})	Post-N/Total-N (%)
2012	BC0	79.7a†	65.8b	45.0b
	BC10	80.7a	78.4ab	49.2ab
	BC20	86.2a	79.0ab	47.8ab
	BC30	84.4a	93.2a	52.5a
2013	BC0	82.9c	70.3b	45.9a
	BC10	91.4bc	70.4b	43.5a
	BC20	97.7ab	74.6ab	43.4a
	BC30	101.0a	78.2a	43.7a
2014	BC0	59.6b	96.7b	61.9a
	BC10	64.5b	100.3ab	60.9a
	BC20	69.4ab	109.6a	61.2a
	BC30	78.8a	109.4a	58.1a
Source of variation				
Biochar (BC)		***	***	ns
Year (Y)		***	***	***
BC × Y		ns	ns	ns

† Means followed by different letters within a column in the same year are significantly different at $P < 0.05$ according to the LSD test. *** Significant at $P < 0.001$; ns, Nonsignificant

To explore the impacts of biochar addition on N translocation in the nutritional organs of the crop, we analyzed the results of the leaf, stem and total straw N translocation amount and efficiency in Table 2. The analysis of variance indicated that the biochar rate, sampling year and the interaction had significant effects on the leaf, stem and total straw N translocation amounts and efficiency. Biochar addition had no effect on the leaf and total straw N translocation amounts and efficiency in 2012, but significantly increased them in 2013. In addition, the BC30 treatment significantly decreased the stem N translocation amount and efficiency in 2012, but on the contrary, significantly increased them in 2013. In 2014, the BC10 treatment significantly increased the stem N translocation amount and the leaf and stem N translocation efficiency; the BC20 treatment significantly increased the stem N translocation amount; the BC30 treatment significantly increased the leaf, stem and total straw N translocation amounts and efficiency, with the exception of the stem N translocation efficiency.

Crop N uptake, NHI and NUE

At maturity, the biochar rate and sampling year showed a significant influence on the N uptake, NHI and NUE, but the interaction was not statistically significant (Table 3). In comparison with the control, the straw N uptakes of the BC20 and BC30 treatments were higher in each year, but only significant in 2012 by 12.3% and 17.1%, respectively; while no any obvious effect was observed in the BC10 treatment. The grain N uptakes of all the biochar-added treatments were significantly higher than that of the control by 10 ~ 26 kg ha^{-1} and increased with increasing rates of the biochar application. The NHIs in the biochar-added treatments were higher than that in the control, but only significant in 2013. The higher rates of biochar addition improved the maize N use efficiency (NUE). The BC20 and BC30 treatments significantly increased the NUE by 8.9% and 12.9% in 2012, 11.2% and 14.1% in 2013, and 10.7% and 15.9% in 2014, respectively.

Grain yield and component

Analysis of variance indicated that the biochar rate and sampling year exhibited statistically significant influences on the maize grain yield and kernel numbers per ear ($P < 0.001$, Table 4). As biochar addition increased,

Table 2 The leaf translocated N (Leaf Tra-N), stem translocated N (Stem Tra-N), leaf (stem) N translocation efficiency, total straw N translocation (Tra-N) and N translocation efficiency under the different biochar treatments in 2012, 2013 and 2014

Year	Treatment	Leaf Tra-N (kg ha^{-1})	Leaf N translocation efficiency (%)	Stem Tra-N (kg ha^{-1})	Stem N translocation efficiency (%)	Tra-N (kg ha^{-1})	N translocation efficiency (%)
2012	BC0	19.7a†	46.7a	17.0a	60.1a	36.1a	45.2ab
	BC10	20.2a	46.5a	14.3ab	55.7ab	36.9a	45.6a
	BC20	20.3a	43.3a	15.6ab	57.1ab	37.3a	43.2ab
	BC30	18.7a	41.0a	14.1b	53.9b	33.4a	39.6b
2013	BC0	22.6b	48.5b	8.1b	32.4b	31.2b	37.6b
	BC10	28.7a	55.5a	12.0ab	44.4ab	42.3a	46.1a
	BC20	31.7a	55.9a	9.7b	37.8b	45.6a	46.6a
	BC30	31.7a	55.4a	15.7a	54.0a	48.7a	48.2a
2014	BC0	12.3b	35.0b	5.0b	32.2b	16.4b	27.4b
	BC10	14.6ab	40.1a	10.4a	52.8a	22.7ab	35.2ab
	BC20	12.5b	32.4b	8.6a	43.3ab	21.8ab	31.4ab
	BC30	17.4a	39.9a	10.6a	45.8ab	29.2a	36.7a
Source of variation							
Biochar (BC)		**	ns	**	**	**	*
Year (Y)		***	***	***	***	***	***
BC × Y		*	*	**	**	*	*

† Means followed by different letters within a column in the same year are significantly different at $P < 0.05$ according to the LSD test. * Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns, Nonsignificant

Table 3 The maize straw and grain N uptake, N harvest index (NHI) and N use efficiency (NUE) at maturity under the different biochar treatments in 2012, 2013 and 2014

Year	Treatment	N uptake (kg ha^{-1})		NHI (%)	NUE (kg kg^{-1})
		Straw	Grain		
2012	BC0	43.5b†	101.9b	70.0b	45.4b
	BC10	43.8b	115.3a	72.5a	48.5ab
	BC20	48.9a	116.3a	70.4b	49.5a
	BC30	51.0a	126.6a	71.3ab	51.3a
2013	BC0	51.6a	101.6c	66.3b	43.3b
	BC10	49.1a	112.7b	69.7a	46.3ab
	BC20	52.1a	120.2ab	69.8a	48.2a
	BC30	52.3a	127.0a	70.8a	49.4a
2014	BC0	43.2ab	113.1c	72.4a	39.5c
	BC10	41.7b	123.0b	74.6a	42.3bc
	BC20	47.6ab	131.3ab	73.4a	43.8ab
	BC30	49.7a	138.5a	73.6a	45.8a
Source of variation					
Biochar (BC)		***	***	***	***
Year (Y)		***	***	***	***
BC × Y		ns	ns	ns	ns

† Means followed by different letters within a column in the same year are significantly different at $P < 0.05$ according to the LSD test. *** Significant at $P < 0.001$; ns, Nonsignificant

Table 4 The maize grain yield, ear numbers per 10 m^2, kernel numbers per ear and 1000-kernel weight at harvest under the different biochar treatments in 2012, 2013 and 2014

Treatment	Grain yield (t ha^{-1})			Ear numbers per 10 m^2			Kernel numbers per ear			1000-kernel weight (g)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
BC0	10.2b†	9.8c	8.9c	62a	63a	61a	460b	442c	428c	290a	293a	288a
BC10	10.9ab	10.4bc	9.5bc	61a	62a	60a	468ab	451bc	437bc	293a	296a	290a
BC20	11.1a	10.8ab	9.9ab	62a	64a	60a	486a	470ab	458ab	301a	296a	293a
BC30	11.5a	11.1a	10.3a	64a	65a	62a	493a	481a	472a	307a	305a	295a
Source of variation												
Biochar (BC)		***			ns			***			ns	
Year (Y)		***			**			***			ns	
BC × Y		ns			ns			ns			ns	

† Means followed by different letters within a column in the same year are significantly different at $P < 0.05$ according to the LSD test. ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns, Nonsignificant

the maize grain yield and kernel numbers per ear showed an increasing trend. The BC20 and BC30 treatments significantly increased the grain yields and kernel numbers per ear by 8.9% ~ 15.9% and 5.7% ~ 10.2%, respectively, compared with the control across the three years. The BC30 treatment exhibited a trend of increasing the ear numbers per 10 m^2 and the 1000-kernel weight presented an increased trend under the biochar-added treatments, but no significant effects were observed.

Soil N concentration and residue

The soil NO_3^--N and NH_4^+-N concentrations in the 0–200 cm soil profile at harvest are shown in Fig. 5. In 2012, the BC10 treatment had a similar tendency as the control, but the peak of NO_3^--N concentration occurred in the 50–60 cm layer, which was deeper than the control (40–50 cm layer). The NO_3^--N concentrations with the BC20 and BC30 treatments across the 20–70 cm soil profile were lower than the control. No obvious differences among all the treatments were observed across the 70–200 cm soil profile. In 2013, the BC10 and BC20 treatments significantly increased the NO_3^--N concentrations in the 10–20 and 0–20 cm soil layers, respectively. Additionally, biochar addition reduced the NO_3^--N concentration across the 20–80 cm soil profile, and no obvious difference was observed across the 80–200 cm soil profile. In 2014, there were significant differences in the distribution of the soil NO_3^--N between the biochar-added treatments and the control. The peaks of NO_3^--N concentrations with the biochar-added treatments occurred in the 30–40 or 40–50 cm soil layers, while the control with two peaks of NO_3^--N concentrations occurred in the 20–30 and 60–70 cm soil layers. The NO_3^--N concentration of the control was higher than the biochar-added treatments across the 10–30 and 70–160 cm soil profiles. In 2012, the NH_4^+-N concentrations in the 0–20 cm layer were BC0 > BC10 > BC20 > BC30, and these differences were statistically significant. No significant differences in the distribution of NH_4^+-N among all the treatments were observed in 2013 and 2014.

Analysis of variance indicated that the biochar rate, sampling year and the interaction had significant effects on the residual NO_3^--N in the soil profile except across the 0–200 cm soil layer (Table 5). Biochar addition led to lower residual soil NO_3^--N, which was mainly located in the 0–100 cm layers in the first two years and in the 60–200 cm layers in the third year. In 2012, biochar additions significantly decreased the soil NO_3^--N residues in the 0–60 cm layer and the BC20 treatment also significantly decreased it in the 60–100 cm layer. The BC20 and BC30 treatments significantly decreased the soil NO_3^--N residues in the 60–100 and 0–100 cm layers, respectively, in 2013 and in the 0–200 and 60–200 cm layers, respectively, in 2014. Overall, biochar additions decreased the soil NO_3^--N residues across the 0–200 cm profile by 23.3 ~ 59.4 kg ha^{-1} in 2012, 19.6 ~ 70.1 kg ha^{-1} in 2013, 25.8 ~ 97.1 kg ha^{-1} in 2014, respectively.

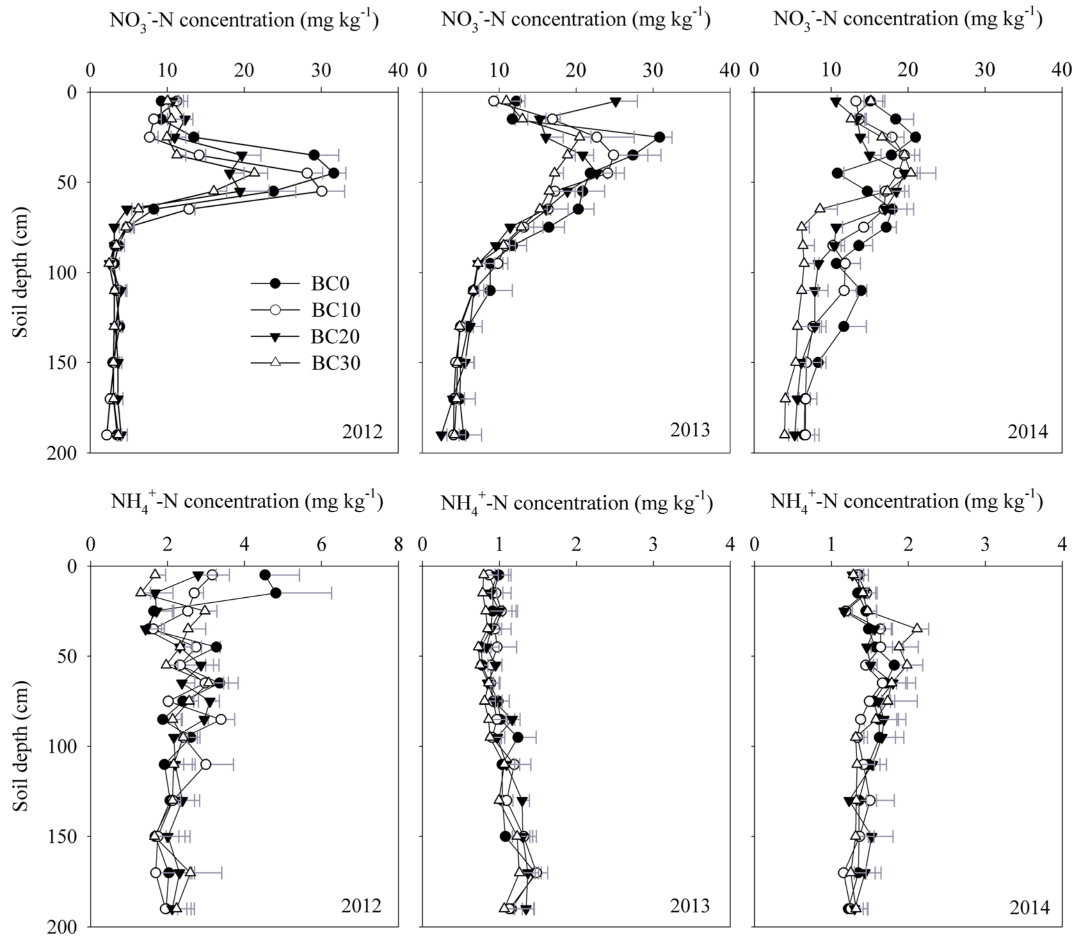

Fig. 5 The soil NO_3^--N and NH_4^+-N concentration in the 0–200 cm soil profile at harvest under the different biochar treatments in 2012, 2013 and 2014. The bars represent the standard deviation of triplicates

Discussion

Biochar effects on N concentration and dry matter accumulation in maize

Biochar additions affected crop N concentrations in different plant tissues, and the influences were different at varying crop growth stages and varying croping years (Fig. 2). At the V6 stage in 2012, as evident from the lower leaf N concentration and the higher leaf biomass with the BC10 treatment, we speculated that more robust maize growth could likely dilute leaf N concentration, which was consistent with Rogovska et al. (2014). The reduction in the leaf N concentration was most visible at the highest application rate (30 t biochar ha^{-1}), which was consistent with our previous conclusion that the incorporation of biochar at the rate of 30 t ha^{-1} inhibited maize development during the early maize growth period in the first year (Xiao et al. 2016a). The remarkable reduction in the leaf N concentration and N uptake with the BC30 treatment may be due to the creation of a limited N environment caused by the high C/N ratio of biochar which led to inorganic N immobilization by microorganisms (Parton et al. 2007; Clough et al. 2013) or to the characteristics of the specific biochar produced at low temperatures such as in our study, which enhanced biochar adsorption surface and thereby increased initial N immobilization (Nguyen et al. 2017) or to the stimulated volatilization of urea-N influenced by the elevated pH of biochar (Schomberg et al. 2012; Huang et al. 2017). Similarly, lower leaf N concentrations and N uptake, induced by biochar addition, were observed in some temperate soils cropped to corn and ryegrass as reported by Rajkovich et al. (2012) and O'Toole et al. (2013), respectively. Whereas the inhibition was limited and temporary, no

Table 5 Residual soil nitrate-N (kg ha^{-1}) across the soil profile after harvest under the different biochar treatments in 2012, 2013 and 2014

Year	Treatment	0–60 cm	60–100 cm	100–200 cm	0-200 cm
2012	BC0	159.3a†	26.2ab	43.2a	228.7a
	BC10	134.9b	31.2a	39.3a	205.4b
	BC20	123.2b	17.8c	47.8a	188.8bc
	BC30	105.9c	22.0bc	41.3a	169.2c
2013	BC0	171.7a	75.6a	78.5a	325.8a
	BC10	170.8a	72.1a	63.2a	306.1ab
	BC20	164.0a	60.4b	65.2a	289.6b
	BC30	130.7b	60.6b	64.4a	255.7c
2014	BC0	134.3a	78.4a	122.6a	335.3a
	BC10	136.3a	70.2ab	103.0ab	309.5ab
	BC20	122.5b	61.2b	85.3bc	269.0bc
	BC30	136.1a	36.3c	65.7c	238.1c
Source of variation					
Biochar (BC)		***	***	***	***
Year (Y)		***	***	***	***
BC × Y		***	***	**	ns

† Means followed by different letters within a column in the same year are significantly different at $P < 0.05$ according to the LSD test. ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ns, Nonsignificant

suppressed effects were observed at other stages, probably due to the decrease in labile organic compounds and the surface adsorption capacity of biochar. Similarly, in a greenhouse pot experiment, Kloss et al. (2014) observed a short-term growth inhibition induced by biochar application despite additional mineral fertilization. Hence, in order to optimize crop response in the short term, adequate N fertilization is needed for the initial growing stage with biochar incorporation at a high rate. Additionally, the lower leaf N concentrations were not observed in the other stages, although sometimes leaf biomasses were higher in the biochar-added treatments, which were speculated to be due to an increase in leaf photosynthetic efficiency with biochar addition by influencing the activities of specific enzymes or the uptake of Mg, both of which affected chlorophyll synthesis (Olmo et al. 2014; Wang et al. 2014). At the V6 stage, presumably both due to the lower stem biomass (1.2 ~ 2.0 g) in all plots and no significant differences induced by biochar addition in stem biomass, biochar addition had no influence on stem N concentration in any year. Although biochar addition had no negative effects on the stem and ear biomass at the R1 stage in 2012, a marked decrease in the stem and ear N concentrations was observed and was likely due to lower inorganic N availability. At the R1 and R6 stages in 2013 and 2014, the lower stem N concentrations in the biochar-added treatments were mostly due to an increase in stem dry matter accumulation, causing a "dilution" effect. This result was similar to the results of a pot trial reported by Kammann et al. (2011), who observed a reduction in leaf N concentration with a relatively nutrient-rich peanut hull biochar, and the reduction was attributed to elevated N use efficiency caused by the 60% increase in biomass at the same accumulated amount of N.

Grains are formed after crop silking, and the dry matter accumulation during the post-silking period is important for the grain formation and kernel filling. The contributions of the dry matter accumulation during the post-silking period were much higher than the dry matter accumulation during the pre-silking period (Fig. 4), which is consistent with a previous study (Ciampitti and Vyn 2012) which found that the increase in maize grain yield depended on the consistent improvement of dry matter accumulation, especially during the post-silking period. At maturity, a small but significant increase in the grain N concentration was observed in the higher biochar-added rates treatments, which synchronously increased the grain biomass, thereby improving the maize grain yields and grain N uptake. The ear numbers per 10 m^2 and 1000-kernel weight presented slightly beneficial effects on the grain biomass production in the biochar-added treatments, and the higher grain biomass most likely corresponded to an obvious increase in the kernel numbers per ear (Table 4). For cereal crops, numerous field studies in the northern latitudes have reported positive biochar impacts on grain yields (Gathorne-Hardy et al. 2009; Vaccari et al. 2011; Zhang et al. 2012b). In this study, the differences between the biochar-added treatments and the control gradually increased with increasing biochar application rates. Overall, biochar addition did not significantly increase biomass in the early stages, but as the accumulation of dry matter increased, the biomass improvements, in contrast to the control, became clear. At maturity, the BC30 treatment had marked increases in both the grain and straw biomass.

Biochar effects on N accumulation and translocation

Grain N comes from either new N being taken up during the grain-filling period or N translocated from the vegetative organs (Mueller and Vyn 2016). Under N stress conditions, higher Post-N accumulation may be a better indicator of the final grain yield than the Pre-N accumulation (Akintoye et al. 1999; Mueller and Vyn 2016). The excessive post-silking N accumulations in the biochar-added treatments were used to form grain, which was consistent with the above conclusion that the grain N concentrations in the biochar-added treatments were higher than the control (Fig. 2). Additionally, the higher N accumulation during the post-silking period in the biochar-added treatments could also reduce N remobilization from leaf to grain, maintaining functional stay-green leaf that accumulated more dry matter, thereby increasing the grain yield (Lee and Tollenaar 2007), which could explain the lower leaf and stem N translocation in the BC30 treatment. When the post-silking accumulation of N is less than the grain N requirements, translocated N will be accelerated (Chen et al. 2015). Over the next two years, biochar addition increased both pre- and post-silking N accumulation, but no differences in the ratio of N accumulation at post-silking to the total aboveground N amount induced by biochar addition were observed, which indicated that the pre-silking N accumulation was likely dominant for grain N accumulation. Our results give further evidence that biochar addition increased pre-silking N accumulation and N translocation (Tables 1 and 2). Simultaneously, an obvious increase in leaf and stem N translocation was observed with biochar addition, and the higher contribution of translocated N may be responsible for the higher grain biomass in the biochar-added treatments (Fig. 3). The N translocation efficiency indicates the ability of the crop to remove N from vegetative organs (Dordas and Sioulas 2009). In the final two years, larger amounts of pre-silking N accumulation from the leaf and stem were translocated to the maize grains during the grain-filling period in the biochar-added treatments, as evidenced by the higher leaf and stem translocation efficiency (Table 2), which presumably led to the dramatic increase in the grain N concentrations at maturity. In addition, the leaf had a much higher fraction of translocated N into the grain than the stem due to the high leaf N concentration at the R1 stage and the leaf is the main organ for photosynthesis (Fig. 2, Chen et al. 2015). The NHI represents a measure of the crop's ability to utilize the acquired N for grain production (Fageria and Baligar 2005). Similar to the conclusion of a higher NHI induced by a biochar-fertilizer composite reported by Joseph et al. (2013), a small but significant increase in the NHI in 2013 was observed in the biochar-added treatments (Table 3), indicating that more translocated N could occur in the grain-filling stages despite the higher post-silking N accumulation already achieved in the biochar treatments (Tables 1 and 2). This is because of the higher N demand resulting from the greater grain dry matter accumulation in the biochar-added treatments (Fig. 3 and Table 4). At maturity, our results showed that the incorporation of biochar clearly increased the grain N accumulation but only had a slight influence on straw N accumulation, consistent with the above conclusion that biochar addition accelerated the translocation of more N for grain production from the straw N. In this paper, averaging the values of the three years, the BC20 and BC30 treatments significantly improved the NUE by 10.2% and 14.2%, respectively. Biochar addition increased the crop N uptake and improved N utilization, which eventually contributed to an improvement in the NUE. These results agree with findings of previous studies that showed that wheat straw biochar combined with NPK chemical fertilizer increased rice yield and the agronomic N use efficiency in the field experiments of paddy soils (Joseph et al. 2013; Qian et al. 2014). Additionally, in a field trial with SOC-poor calcareous soil, Zhang et al. (2012b) found that maize yield and N use efficiency under N fertilization treatments were significantly increased with biochar addition at rates of 20 and 40 t ha^{-1}.

Soil NO_3^--N and NH_4^+-N

Peer-reviewed reports have clarified the potential role of biochar addition with regard to the retention of NO_3^--N in the soil (Clough et al. 2013). In the first and third year, our study demonstrated that biochar addition had no influence on the NO_3^--N concentration in the mixed soil layer (0–20 cm) at harvest, which was similar with a 4-month field experiment in a calcareous loamy soil by Zhang et al. (2012b), who reported that no changes were observed in soil mineral N concentrations caused by wheat-straw biochar additions (20 and 40 t ha^{-1}) but eventually resulted in significant increases in maize yield and agronomic N use efficiency. In the second year, the BC10 and BC20 treatments increased the soil NO_3^--N concentrations in the 10–20 and 0–20 cm soil layers, respectively, attributed to the enhancement in soil NO_3^--N retention or the promotion of soil mineralization. Although no experimental data directly implied that the BC30 treatment improved NO_3^--N content, the high N uptake ability by the crop in the BC30 treatment indirectly demonstrated that biochar can enhance the adsorption capacity of soil, making NO_3^--N available to the crop. An improvement in nitrate retention induced by biochar addition can be explained by the existence of base functional groups on biochar surface or chemisorption through H-bonding (Chintala et al. 2013; Clough et al. 2013; Nguyen et al. 2017). Biochar addition decreased soil bulk density and increased soil porosity, thereby altering soil oxygen content and improving water holding capacity as shown by our previous study (Xiao et al. 2016b), which were beneficial to soil mineralization and N availability.

Nitrate is the dominant form of soil residual N. In northern China, although rainfall is relatively sparse, the high intensity of precipitation in a short period plays an important role in the accumulation of soil NO_3^--N and its subsequent leaching across the soil profile (Fan et al. 2010). Due to occasional heavy rainfalls occurred during the grain-filling stage in each year, percolating water transported the surface NO_3^--N to the deep soil layer (Ju and Christie 2011; Zhou and Butterbach-Bahl 2014), and the depth of the NO_3^--N leaching increased year by year. In our study, we observed that biochar addition reduced the extent of NO_3^--N leaching into the soil profile, thereby reducing the risk of NO_3^--N leaching loss. In addition, biochar addition decreased the total amount of NO_3^--N accumulation, especially at rates of 20 and 30 t biochar ha^{-1}. The enhanced microbial activity induced by biochar addition may be one of the reasons for reduced soil nitrate accumulation, due to an elevated microbial immobilization of N (Steiner et al. 2008; Lehmann et al. 2011; Zavalloni et al. 2011). Moreover, the intense rainfalls probably improved conditions for denitrification and N losses (NO, N_2O and N_2), which has also been found to be affected by biochar addition (Clough et al. 2013; Saarnio et al. 2013; Cayuela et al. 2014). Previous studies have shown that light rainfalls were beneficial to urea hydrolysis and improved the concentration of NH_3 in soil solution which promoted NH_3 volatilization; while heavy rainfalls hindered NH_3 volatilization by transporting fertilizer-N into the deep soil with percolating water (Kissel et al. 2004; Sanz-Cobena et al. 2011). The improved soil water holding capacity induced by biochar additions (data not shown) in our study might stimulate urea hydrolysis and thereby increased NH_3 volatilization.

Under dryland farming, the high concentration of NH_4^+-N only appeared for a short period after fertilization in the surface soil and the NH_4^+-N concentration across the soil profile was sustained at a low and constant level < 4 mg kg^{-1} (Ju et al. 2003; Zhao et al. 2006). In our study, the highest concentration of NH_4^+-N was 4.8 mg kg^{-1} in the control in 2012. Our results also showed that biochar addition clearly decreased the soil NH_4^+-N concentration only in the surface 20 cm layer in 2012 presumably through two mechanisms: biochar either stimulated NH_3 volatilization or promoted nitrification converting the NH_4^+-N to NO_3^--N or both. In our prior study, an obvious increase in soil pH (0.12 ~ 0.16 units) induced by biochar addition was observed only in 2012, and the elevated soil pH was considered suitable for NH_3 volatilization (Schomberg et al. 2012). However, Mandal et al. (2016) observed that biochar reduced NH_3 volatilization by increasing the NH_3 adsorption capacity of biochar, where higher pH or alkaline biochar increases NH_3 volatilization. Additionally, in acidic and subtropical cropland soils, Zhao et al. (2014) reported that biochar stimulated the nitrification process, attributed to the greater increase of soil pH caused by biochar addition. However, we did not find evidence that biochar addition significantly increased the NO_3^--N concentration at harvest in 2012. There were no effects of biochar addition on the NH_4^+-N concentration at harvest in 2013 and 2014. In addition, little difference was observed in the deeper soil layers (20–200 cm) among all the treatments after harvest in each year. This finding may be related to the fact that the soil NH_4^+-N only increased quickly in the surface soil layer after fertilization due to the rapid hydrolysis of the urea, and then declined to its original level due to nitrification, which resulted in minimal leaching of the NH_4^+-N deep to the soil profile (Liu et al. 2003).

Biochar reduced N leaching loss and improved mineral N, thereby increasing the soil available N. Our previous studies have proved that the application of biochar improved soil aeration via altering soil bulk density and porosity (Xiao et al. 2016a), and increased soil available water (Xiao et al. 2016b), which beneficially changed soil N cycle leading to greater soil mineralization and inorganic-N availability. Moreover, the favourable environment induced by biochar addition also promoted maize roots growth (Xiao et al. 2016a), thereby improving N uptake through maize roots. All of these acted synergistically to increase maize dry matter accumulation, and improve N uptake and NUE.

Conclusion

The results demonstrated that during the early growth period in the first year, the highest biochar addition rate reduced leaf biomass and leaf N concentration at the V6 stage potentially due to limited inorganic N. In addition, no effect of biochar addition in the pre-silking N accumulation was observed in 2012. In other words, the post-silking N and dry matter accumulations were dominant for the grain N accumulation and grain formation due to N stress during the early growth period. While over the next two years, biochar addition enhanced both pre- and post-silking N accumulation, and N translocation efficiency in the biochar-added treatments was also higher, attributed to the higher contribution of translocated N induced by biochar addition. Although biochar addition decreased the stem N concentration, a small but significant increase in the grain N concentration was observed in the higher biochar addition rates, which synchronously increased the grain biomass. Biochar significantly increased the NHI in 2013 and improved the NUE in each year. The incorporation of biochar significantly decreased the soil NH_4^+-N concentration in the biochar-mixed soil layer in the first year. In addition, the BC10 and BC20 treatments obviously increased the soil NO_3^--N concentrations in the 10–20 and 0–20 cm soil layers, respectively, in the second year. Biochar addition reduced the total amount of soil NO_3^--N accumulation and mitigated the extent of NO_3^--N leaching across the soil profile. Overall, the application of biochar has great potential in improving spring maize grain yield and N use efficiency while simultaneously reducing the risk of NO_3^--N leaching loss. However, further investigations are still required to clarify the fate of N and to uncover the mechanisms underlying these processes with biochar addition in long-term field trials.

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