



Combined biochar and nitrogen fertilization at appropriate rates could balance the leaching and availability of soil inorganic nitrogen

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ABSTRACT

Biochar has been proposed to ameliorate soil properties and plant growth. However, it remains unclear how the interaction between biochar and nitrogen (N) fertilizer impacts soil inorganic nitrogen (SIN) leaching and availability in dryland systems. Therefore, a two-year field experiment was carried out on the Loess Plateau in northern China to study the effects of biochar combined with N fertilizer on the leaching and availability of SIN. Biochar applied at 0, 20 and 40 t ha⁻¹ (B0, B1 and B2, respectively) interacted with three N fertilization levels (0, 120 and 240 kg N ha⁻¹; N0, N1 and N2, respectively). Winter wheat (*Triticum aestivum* L.) was cultivated in a winter wheat-summer fallow cropping system. We measured wheat aboveground biomass and residual SIN in the soil profile (0–60 cm at 10 cm intervals) using standard extraction methods (2 M KCL, shaking at 25 °C for 1 h). Additionally, to ascertain whether field-aged biochar captured SIN and to determine residual SIN availability, we also used a modified extraction method (2 M KCL, shaking at 60 °C for 2 h) and ion exchange membranes (IEMs) to extract SIN from plow layer soil (0–20 cm). Our results indicated that biochar application alone in the absence of N fertilization had no significant effect on wheat biomass or residual SIN in the soil profile. However, compared with the application of N fertilizer alone, the application of biochar at 20 t ha⁻¹ combined with N fertilizer not only increased wheat biomass by 12.2–13.8% but also significantly decreased residual NO₃⁻-N in the subsoil by 13.2–74.7%. Nevertheless, long-term N fertilization at 240 kg N ha⁻¹ led to large amounts of residual NO₃⁻-N without a significant increase in crop biomass, which inevitably increased the risk of leaching during the fallow period. Although the application of biochar at 40 t ha⁻¹ combined with N fertilizer more effectively decreased residual SIN in the subsoil, this approach was impractical because it decreased wheat biomass. Furthermore, the difference between NO₃⁻-N extracted via the modified method and via the standard method increased with biochar application under each N level. Thus, field-aged biochar absorbed a certain amount of NO₃⁻-N, thereby sequestering N in the soil after two years of N fertilization. Hence, biochar could reduce the residual NO₃⁻-N available for leaching during the fallow period. However, notably, overuse of biochar could reduce the amount of NO₃⁻-N available not only for leaching but also for crops. Ultimately, the application of biochar at 20 t ha⁻¹ combined with N fertilization at 120 kg N ha⁻¹ is a promising dual-win strategy for improving N availability while concurrently mitigating SIN leaching.

1. Introduction

Biochar is a carbonaceous product obtained by heat-treating biomass under conditions of limited or no oxygen (O) (i.e., pyrolysis) (Mukherjee and Zimmerman, 2013). Because of its interactions with plant-soil-microbial components, biochar has been used to improve soil properties and plant growth (Major et al., 2010; Zheng et al., 2013; Sika

and Hardie, 2014). Additionally, biochar adsorbs nutrients, increases the soil water-holding capacity and enhances soil microbial community structure, all of which contribute to increased nitrogen (N) retention (Zheng et al., 2013; Xu et al., 2016; Haider et al., 2017). However, reports concerning the leaching and availability of soil inorganic nitrogen (SIN) after biochar application are contradictory (Thi Thu Nhan et al., 2017).

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Increases in N availability in response to biochar addition are generally attributed to increased soil pH, reduced soil tensile strength and increased soil water-holding capacity (Gul et al., 2015). In addition, biochar usually increases plant biomass and N uptake in acidic soils but has little effect in neutral or alkaline soils (Liu et al., 2018). In contrast, biochar reduces N availability by increasing the available surface area, which leads to N adsorption, and by increasing the carbon (C):N ratio, which leads to N immobilization (Gul and Whalen, 2016). The main implications of this pattern may lead to decreases in both SIN availability and leaching (Thi Thu Nhan et al., 2017). However, the application of biochar combined with N fertilizer can offset N deficiencies caused by N immobilization (Prommer et al., 2014; Thi Thu Nhan et al., 2017). Studies have shown that the nutrient supply capacity of biochar in combination with N fertilizer is greater than that of biochar or N fertilizer alone (Schulz et al., 2013; Agegnehu et al., 2016). For example, Agegnehu et al. (2016) reported that, compared with the application of N fertilizer alone in an acidic Eutric Nitisol, the application of acacia biochar at 10 t ha⁻¹ combined with N fertilizer resulted in a slight increase in biomass, indicating increased N availability. In addition, the results of our previous simulation studies showed that apple branch biochar applications at less than 2% (w/w, approximately 40 t ha⁻¹) not only increased wheat yields (Li and Shangguan, 2018) but also significantly mitigated SIN leaching losses in a neutral silty clay soil (Li et al., 2018).

However, the available scientific evidence on this topic has been obtained mostly from short-term (< 6 months) incubation or soil column experiments, while results from long-term field studies focusing on the availability and leaching of SIN are lacking (Sorrenti and Toselli, 2016). Considering that the benefits of biochar vary with soil type, application rates of biochar and mineral fertilizer, and experimental conditions (Thi Thu Nhan et al., 2017; Liu et al., 2018), a better understanding of the long-term effects of combinations of biochar and N fertilization on the availability and leaching of SIN in natural dryland field conditions is necessary.

Fallow tillage methods play a major role in improving soil water storage in dryland agricultural systems (Sun et al., 2018). On the Loess Plateau of China, winter wheat is usually planted in early October and harvested in early June, while more than 60% of the annual rainfall occurs in the fallow season (Xiao et al., 2017). Therefore, most of the residual SIN in the soil is leached by this concentrated precipitation (Li et al., 2014; Dai et al., 2016). In addition, Dai et al. (2016) reported that N fertilization levels significantly affected NO₃⁻-N leaching, mainly from the topsoil. However, limited information is available about the effects of biochar on residual SIN and its availability for leaching under different N levels, which is important for N management in the field. SIN in the soil is typically quantified by extraction with 2 M KCl and shaking at 25 °C for 1 h (Keeney and Nelson, 1982). However, standard extraction methods may underestimate the NO₃⁻-N stocks of biochar-soil mixtures (Haider et al., 2016), and more than 50% of total NO₃⁻-N can potentially be released in subsequent extractions (Hagemann et al., 2017). Hence, a large amount of SIN may be captured by biochar and sequestered in the soil. The IEM method may provide a reliable measure of nutrient exchangeability over a range of soils and can accurately predict SIN availability (Johnson et al., 2005; Qian and Schoenau, 2007; Cambouris et al., 2014). Thus, a modified extraction method could be used to ascertain whether field-aged biochar captures SIN, and IEMs could be used to examine the availability of residual SIN.

On the basis of the above information, we conducted a two-year biochar field experiment on the Loess Plateau of northern China. We hypothesized that combining biochar and N fertilizer might increase N availability and mitigate leaching. In addition, biochar could capture SIN, especially in high-biochar-application-rate treatments, thus reducing residual SIN exchangeability. The specific objectives of this research were to (1) study the long-term effects of combining biochar with N fertilizer on SIN leaching and availability in the field; (2) determine whether field-aged biochar retains SIN; and (3) assess the

Table 1
Initial characteristics of the soil.

pH	CEC (cmol kg ⁻¹)	Clay	Silt	Sand	SOC	Total N
7.99	21.01	250.3	679.3	70.3	13.15	0.73
Total P	Total K	NO ₃ ⁻ -N	NH ₄ ⁺ -N	Available P	Available K	
0.82	16.55	3.02	2.57	13.92	206.2	

Note: CEC is the cation exchange capacity. Clay < 0.002 mm, silt 0.002–0.05 mm, sand 0.05–2 mm. SOC is the soil organic carbon. The units of clay, silt, sand, SOC, total N, total P and total K are grams per kilogram. The units of NO₃⁻-N, NH₄⁺-N, available P and available K are milligrams per kilogram.

effects of biochar on the availability of residual SIN under different N levels.

2. Materials and methods

2.1. Experimental site, soil and biochar

This field experiment was conducted from October 2015 to July 2017 in Yangling, Shaanxi, China (34°18'15" N, 108°02'30" E; 530 m elevation). The experimental site is located in the southern region of the Loess Plateau. The climate of the area is characterized as semihumid, with a mean annual precipitation of 599 mm (2016–2017), of which more than 70% occurs between June and October (Fig. S1).

The soil (0–20 cm layer) physical and chemical properties determined before the experiment are shown in Table 1. The soil type is a Lou soil (Eum-Orthic Anthrosol) and is considered silty clay according to the USDA system. The soil pH was measured with a pH meter at a soil to water ratio of 1:2.5 (w/v). The cation exchange capacity (CEC) was measured via the ammonium acetate compulsory displacement method (Gaskin et al., 2008). The soil organic C content was assayed via dichromate oxidation (Nelson et al., 1982), and the total N content of the soil was assayed using the Kjeldahl method (Bremner and Mulvaney, 1982). NO₃⁻-N and NH₄⁺-N were extracted by shaking for 1 h with a 2 M KCl solution and analyzed via flow injection analysis (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany). The total phosphorus (P) content of the soil was determined using the molybdenum blue method after digestion with H₂SO₄-HClO₄ at 300 °C for 2 h (Qian et al., 2013). Available P was extracted with 0.5 M sodium (Na) bicarbonate and quantified using the molybdenum blue method (Jin et al., 2016). Soil total potassium (K) was quantified by digesting the soil samples in a mixture of HF and HClO₄, and soil available K was measured via the neutral ammonium acetate extraction method (Bolland et al., 2002). The concentration of K in the digest or leachate was then measured by atomic absorption spectrophotometry (ICE 3000, Thermo, USA).

Apple (*Malus pumila* Mill.) branches constituted the feedstock for biochar production. The furnace temperature was increased from ambient room temperature to 450 °C at a rate of 30 °C min⁻¹, after which the temperature was maintained at 450 °C for approximately 8 h. The biochar was ground and subsequently passed through a 5 mm sieve for use. The physicochemical properties of the biochar are shown in Table 2, and the applied measurement methods have been described previously (Li et al., 2017). Briefly, the pH was measured with a pH meter at a biochar to water ratio of 1:2.5 (w/v). The specific surface area of the biochar was assessed using the Brunauer-Emmett-Teller (BET) method (Micro ASAP2460, Micromeritics, USA), and the electrical conductivity (EC) was determined in a 1:5 (w/v; g cm⁻³) biochar:water mixture. The concentrations of elemental C, N, hydrogen (H) and O in the biochar were determined using an elemental analyzer (Flash 2000, Thermo Fisher, USA). After the biochar was digested by aqua regia (produced by mixing nitric acid and hydrochloric acid together at a volumetric ratio of 1:3), the total K, P, Na, calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and lead (Pb) contents were measured using an inductively coupled

Table 2
Physical and chemical characteristics of the biochar used in this study.

Surface area (m ² g ⁻¹)	pH	CEC (cmol kg ⁻¹)	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C:N	H (g kg ⁻¹)	O (g kg ⁻¹)	Total P	Total K
14.22	9.67	34.65	670.15	5.70	117.57	21.71	71.79	1802.1	6003.4
NO ₃ ⁻ -N	NH ₄ ⁺ -N	Available P	Na	Ca	Mg	Fe	Cu	Mn	Zn
0.52	1.86	23.68	639.2	24185.1	3196.5	5745.8	9.9	91.5	37.3

Note: CEC is the cation exchange capacity. The units of total P, total K, NO₃⁻-N, NH₄⁺-N, available P, Na, Ca, Mg, Fe, Cu, Mn and Zn are milligrams per kilogram.

plasma (ICP) optical spectrometer (Vista Axial, Varian Medical Systems, USA). Available P was extracted with 0.5 M Na bicarbonate and quantified using the molybdenum blue method (Jin et al., 2016). Additionally, scanning electron microscopy (SEM) images (JSM-6360 LV, JEOL, Japan) were used to examine cross-sections of the biochar samples, which exhibited an definitive tubular pore structure (Fig. S2). The variability in functional groups of the biochar was investigated via Fourier transform infrared (FTIR) spectroscopy (Vertex 70 FTIR, Bruker Corporation, Germany) (Fig. S3).

2.2. Experimental design

This study used a randomized block design that included two experimental factors. Biochar was applied at 0, 20 and 40 t ha⁻¹ (B0, B1 and B2, respectively, hereafter); N (in the form of urea) was applied at 0, 120 and 240 kg N ha⁻¹ (N0, N1 and N2, respectively, hereafter). Only winter wheat (*Triticum aestivum* L. cv. Xiaoyan No. 22) was cultivated, and each treatment was replicated three times. Each plot had an area of 16 m² (i.e., 4 × 4 m), and the plots were separated by pathways that were 0.5 m wide to avoid cross-contamination and treatment effects. Biochar was manually added to the plow layer soil (0–20 cm) once using a shovel until mixing occurred before sowing in October 2015. Basal applications of urea to the plow layer soil were performed once a year before sowing (i.e., in mid-October 2015 and 2016). No tillage occurred during the growth stage, although weeds were regularly removed by hand. Wheat was harvested manually at maturity on 28 May 2016 and 2 June 2017 by cutting the aboveground biomass at the soil level, and the four central rows of plants were then oven dried to a constant weight at 65 °C to determine the total aboveground biomass. During the experiment, natural rainfall was the sole water supply, and the fallow period lasted from June to mid-October. To represent the availability of NO₃⁻-N and NH₄⁺-N accurately at the end of the cropping cycle, three pairs of cation and anion IEMs were randomly inserted into the 0–20 cm layer of soil in each plot in October 2016 and then removed in June 2017. An increasing extraction time generally leads to increasing ion accumulation on these membranes (Johnson et al., 2005), and a long period of extraction (greater than three months) may predict equilibrium with the N concentration in the soil (Drohan et al., 2005). The cation and anion IEMs (20 × 2 cm, HeCEM Grion 7321 and HeAEM Grion 7171, Hangzhou Grion Environmental Technology Co., Ltd, China) were imbedded with plastic stakes prior to the experiments; the counterions on the resin membranes were Na⁺ and HCO₃⁻ (Johnson et al., 2005).

2.3. Soil sampling and analyses

After harvest in 2017, the soil was sampled at five locations that were randomly selected within each plot at depths of 0 to 60 cm at 10 cm intervals using a soil drilling sampler whose inner diameter was 4 cm. All samples were sieved through a 2 mm screen, after which the roots and other debris were removed. Every five samples from each plot were subsequently mixed together to form a single sample; these samples were immediately stored in cooled boxes that were then transported to the laboratory for the measurements of NO₃⁻-N and NH₄⁺-N concentrations.

NO₃⁻-N and NH₄⁺-N concentrations in the soil profile (0–60 cm grouped by 10 cm intervals) were determined using the standard extraction method. Briefly, field-fresh soil (10 g) was placed in 40 mL of 2 M KCl, after which it was shaken at 200 rpm at 25 °C for 1 h and then filtered (round filter ø 90 mm) (Keeney and Nelson, 1982; Haider et al., 2016). An automated flow injection analyzer (Autoanalyser 3, Bran + Luebbe, Germany) was used to measure the NO₃⁻-N and NH₄⁺-N concentrations in the extracts. The SIN concentration was determined as the sum of the NO₃⁻-N and NH₄⁺-N concentrations. Additionally, we used the concentration data to calculate the stocks of NO₃⁻-N, NH₄⁺-N and SIN in the soil profile using the following equation:

$$S_N = C_N \times D \times H \times 10^{-1}$$

where S_N is the stock of the different N forms (kg ha⁻¹); C_N is the concentration of NO₃⁻-N, NH₄⁺-N or SIN (mg kg⁻¹); D is the bulk density (g cm⁻³, Table S1); and H is the thickness of the soil layer (cm).

Soil samples from the plow layer (0–20 cm) were also extracted via modified extraction methods. Briefly, field-fresh soil (10 g) was placed in 40 mL of 2 M KCl, after which it was shaken at 200 rpm at 60 °C for 2 h and then filtered (round filter ø 90 mm). Generally, an extraction temperature greater than 50 °C facilitates SIN captured by biochar release, likely shortening equilibration times (Haider et al., 2016; Hagemann et al., 2017), because the 2-dimensional surface water flow in biochar particles changes to a 3-dimensional inner-pore water flow and becomes much faster when the temperature surpasses 50 °C (Conte et al., 2014). The IEMs were washed with distilled water to remove adherent soil and then extracted by shaking in 50 mL of 2 M KCl at 200 rpm at 25 °C for 1 h (Cambouris et al., 2014). The SIN concentrations of the extracts were measured with an automated flow injection analyzer.

2.4. Statistical analysis

The reported results are the means of the three replicates. Two-way ANOVA (at a $p < 0.05$ significance level) was performed to assess significant differences, and multiple comparisons were adjusted for Duncan's multiple range test at a probability level of 0.05. Correlation analysis was performed using Pearson's correlation (for normally distributed data) or Spearman's correlation (for abnormally distributed data). All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, USA).

3. Results

3.1. Effects of biochar on wheat aboveground biomass under different N levels

The aboveground biomass of wheat was significantly influenced by the biochar, N fertilizer and their interactions (Fig. 1). Biochar application alone did not significantly increase the aboveground biomass but had a slightly positive effect. However, compared with the B0 treatment, the B1 treatment under N1 and N2 significantly increased the aboveground biomass by 13.8% and 12.2%, respectively. In contrast, the B2 treatment under N1 and N2 negatively affected the aboveground biomass (Fig. 1). Compared with B0N1, B2N1 significantly reduced the

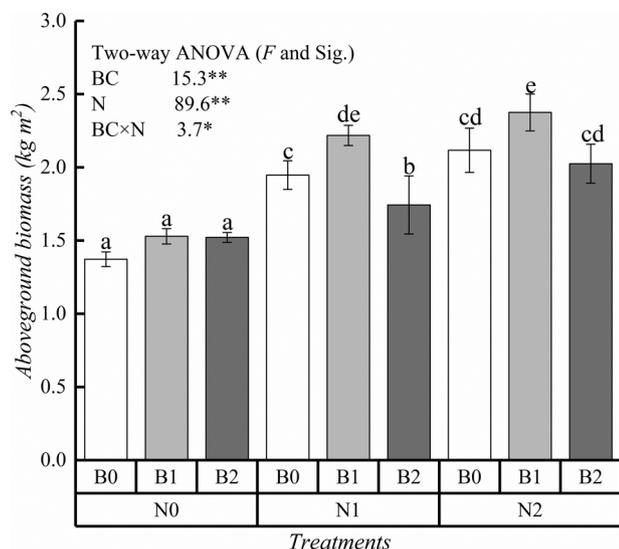


Fig. 1. Total wheat aboveground biomass under different treatments. B, N and B × N represent biochar and nitrogen fertilizer application rates and their interaction, respectively. ** $p < 0.001$; * $p < 0.05$. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha⁻¹ and 240 kg N ha⁻¹, respectively. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha⁻¹ and 40 t ha⁻¹, respectively. The different letters on the bars indicate significant differences ($p < 0.05$).

aboveground biomass by 10.6%.

3.2. Residual NO₃⁻-N, NH₄⁺-N and SIN in the soil profile

Overall, the biochar, N fertilizer and their interactions significantly influenced the concentrations and stocks of NO₃⁻-N, NH₄⁺-N and SIN in the soil profile, all of which were negatively correlated with biochar application rate (Table 3). Additionally, the SIN was more strongly correlated with NO₃⁻-N than with NH₄⁺-N.

3.2.1. Residual NO₃⁻-N

Regardless of the biochar application rate, residual NO₃⁻-N increased with increasing N fertilization level (Fig. 2 and Table 3). Furthermore, the application of biochar under N0 increased the NO₃⁻-N stock in the plow layer soil but did not significantly affect the NO₃⁻-N stock in the subsoil (20–60 cm) (Fig. 2d). However, the NO₃⁻-N concentrations and stocks decreased with biochar application under N1 and N2 (Fig. 2). Compared with the application of N fertilizer alone under N1 and N2, the B1 treatment reduced the NO₃⁻-N stocks in the subsoil by 13.2% and 74.7%, respectively. Nevertheless, the NO₃⁻-N stock in the subsoil of B1N2 was approximately tenfold greater than that of

Table 3

Results of two-way ANOVA and correlation analyses of variables in the soil profile.

Concentration variable	Two-way ANOVA			Spearman correlation		Pearson correlation		
	B	N	B × N	B	N	NO ₃ ⁻ -N	NH ₄ ⁺ -N	SIN
NO ₃ ⁻ -N	**	**	**	-0.165**	0.840**	1	0.147	0.992**
NH ₄ ⁺ -N	*	*	*	-0.199**	-0.041		1	0.273**
SIN	**	**	**	-0.253*	0.793**			1
Stock variable	Two-way ANOVA			Spearman correlation		Pearson correlation		
	B	N	B × N	B	N	NO ₃ ⁻ -N	NH ₄ ⁺ -N	SIN
NO ₃ ⁻ -N	**	**	**	-0.173*	0.839**	1	0.266**	0.988**
NH ₄ ⁺ -N	*	*	*	-0.194*	-0.058		1	0.413*
SIN	**	**	**	-0.332*	0.729**			1

Note: All analytical data are the values of variables in the soil profile (0–60 cm, at 10 cm intervals), and the results show the overall situation. B, N and B × N represent biochar and nitrogen fertilizer and their interactions, respectively. SIN is the sum of NO₃⁻-N and NH₄⁺-N. ** $p < 0.001$; * $p < 0.05$.

B1N1 (Fig. 2e and f).

3.2.2. Residual NH₄⁺-N

The concentrations of NH₄⁺-N under all treatments fluctuated along the soil profile (Fig. 3a, b and c). In particular, B1N1 increased the NH₄⁺-N concentrations and stocks throughout the soil profile (Fig. 3b and e). The B2 treatment generally had a decreasing effect on the concentration of NH₄⁺-N. The stock of NH₄⁺-N in the subsoil of B2N0 was significantly lower (10.9% lower) than that of B0N0 (Fig. 3d). In addition, compared with B0N2, B2N2 significantly reduced the NH₄⁺-N stock in the plow layer soil by 24.4% (Fig. 3f).

3.2.3. Residual SIN

Residual SIN increased with N fertilization level, which was attributed mainly to residual NO₃⁻-N (Fig. 4 and Table 3). Biochar did not significantly affect the concentrations and stocks of SIN in the soil profile under N0 (Fig. 4a and d). However, SIN in the subsoil decreased with biochar application under N1 and N2 (Fig. 4).

3.3. NO₃⁻-N, NH₄⁺-N and SIN in plow layer soil extracted by the modified method and IEMs

3.3.1. NO₃⁻-N extracted via the modified method and IEMs

The concentration of NO₃⁻-N extracted via the modified extraction method (NO₃⁻-N_m) was consistently 2.0–109.7% greater than that extracted via the standard extraction method (NO₃⁻-N_s) (Fig. 5). Biochar application increased the difference between NO₃⁻-N_m and NO₃⁻-N_s (NO₃⁻-N_{m-s}), which was influenced by the interaction of biochar and N fertilizer (Fig. 5 and Table 4). In particular, B1N2 presented greater NO₃⁻-N_s, NO₃⁻-N_m and NO₃⁻-N_{m-s} concentrations than did the other treatments. The concentration of NO₃⁻-N extracted from IEMs (NO₃⁻-N_{IEM}) ranged from 14.22 to 173.46 mg m⁻² and increased in response to N fertilization; however, the same concentration generally decreased with biochar application, with the exception of that in B1N1 (Fig. 5 and Table 4).

3.3.2. NH₄⁺-N extracted via the modified method and IEMs

The concentration of NH₄⁺-N extracted via the modified extraction method (NH₄⁺-N_m) was -2.0–24.4% greater than that extracted via the standard extraction method (NH₄⁺-N_s) (Fig. 6). The difference between NH₄⁺-N_m and NH₄⁺-N_s (NH₄⁺-N_{m-s}) was affected only by N fertilization (Table 4). In addition, the concentration of NH₄⁺-N extracted from IEMs (NH₄⁺-N_{IEM}) generally tended to increase with increasing biochar application under each N level (Fig. 6).

3.3.3. SIN extracted via the modified method and IEMs

Overall, the concentration of SIN extracted via the modified method (SIN_m) was 5.0–45.3% greater than that of SIN extracted via the standard method (SIN_s) (Fig. 7). SIN_s, SIN_m and the difference between them (SIN_{m-s}) were significantly affected by biochar, N fertilizer and

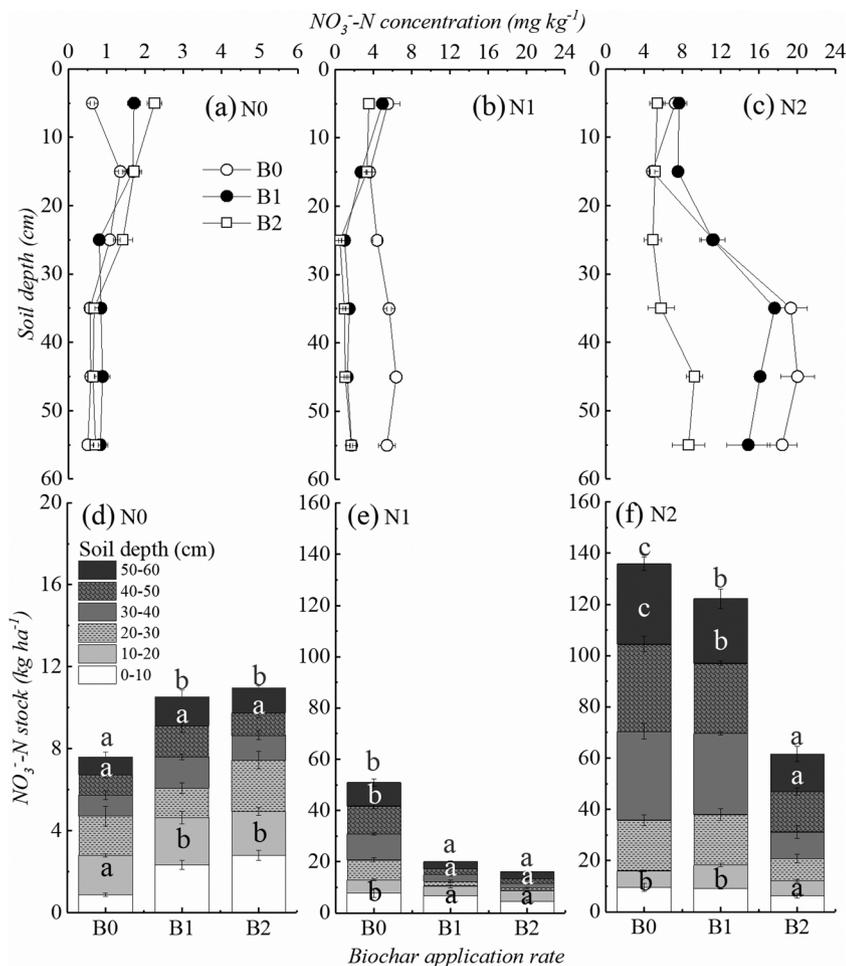


Fig. 2. NO_3^- -N concentrations and stocks in the soil profile. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha^{-1} and 240 kg N ha^{-1} , respectively. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha^{-1} and 40 t ha^{-1} , respectively. The different lowercase letters in subgraphs d, e and f indicate significant differences ($p < 0.05$) between treatment means: the black letters in the 10–20 cm section refer to the plow layer soil (0–20 cm), the white letters in the 50–60 cm section refer to the subsoil (20–60 cm), and the dark gray letters above the bar refer to the total soil profile (0–60 cm).

their interactions (Table 4). As shown in Fig. 7, biochar application increased the SIN_s , SIN_m and SIN_{m-s} under N0. However, under N1 and N2, SIN_s decreased with biochar application, whereas SIN_m was not influenced, with the exception of that in B1N2. Additionally, the concentration of SIN extracted from IEMs (SIN_{IEM}) under N1 and N2 generally decreased with biochar application, which was consistent with the results for NO_3^- - N_{IEM} (Figs. 5 and 7).

4. Discussion

4.1. Effects of combined biochar and N fertilizer applications on the availability and leaching of SIN

Our results indicated that the application of biochar alone had no significant effect on N availability or SIN leaching, but the effects of biochar were most evident when combined with N fertilization (Figs. 1 and 3), which is in agreement with the findings of several other authors (Van Zwieten et al., 2010; Antonio Albuquerque et al., 2013; Li and Shanguan, 2018). Additionally, biochar application alone had a slightly positive effect on crop biomass and significantly increased the residual NO_3^- -N in the plow layer soil (Figs. 1 and 2d). These positive effects were likely not due to the nutrients present in the biochar and were instead attributed mostly to biochar-stimulating microorganisms that mineralize soil native organic N, thereby eliminating N starvation (Ameloot et al., 2015; Gul and Whalen, 2016), because the biochar contained lower concentrations of NO_3^- -N (0.52 mg kg^{-1}) than did the initial soil (3.02 mg kg^{-1}) (Tables 1 and 2).

Compared with N fertilization alone, the B1 treatment significantly increased aboveground biomass, but the opposite pattern was observed in response to the B2 treatment. This finding suggested that N

availability was sensitive to the interactions of biochar and N fertilizer and thus affected plant assimilation (Thi Thu Nhan et al., 2017), and this sensitivity strongly depended on the biochar application rate. Baiga and Rao (2017) reported that coapplication of N with biochar at 10 t ha^{-1} could improve dry matter production and N uptake because of improved soil N mineralization parameters such as mineralization potential and the coefficient of the mineralization rate. In our work, B1 combined with N fertilization not only increased N availability but also mitigated NO_3^- -N leaching. Hence, the increase in crop biomass observed under B1N1 and B1N2 may partly account for the significant decrease in residual SIN in the subsoil (Figs. 1 and 4). Similarly, Xiao et al. (2017) reported that the application of biochar at $20\text{--}30 \text{ t ha}^{-1}$ combined with N fertilizer at 225 kg ha^{-1} significantly increased maize N uptake and reduced both total residual soil NO_3^- -N and the extent of NO_3^- -N leaching. A surprising finding of our work was that there was no significant difference between the wheat biomass in B1N1 and B1N2 (Fig. 1); however, the residual SIN content mainly in NO_3^- -N was much greater in the subsoil of B1N2 than in that of B1N1 (Fig. 4). Thus, long-term N fertilizer applications at 240 kg N ha^{-1} exceeded the needs of the crops, which inevitably led to large amounts of residual NO_3^- -N and increased the risk of leaching during the fallow period, which was accompanied by concentrated rainfall (Dai et al., 2016; Xiao et al., 2017). Compared with the other fertilization treatments, the B2 treatment more effectively protected SIN against leaching; however, this level of application is impractical because of reduced crop production (Figs. 1 and 4). Therefore, the B1N1 combination was the best strategy for achieving the dual goals of improving N availability and mitigating SIN leaching.

In line with the results of the B2 treatment under fertilization, other studies have also indicated that biochar amendment does not increase

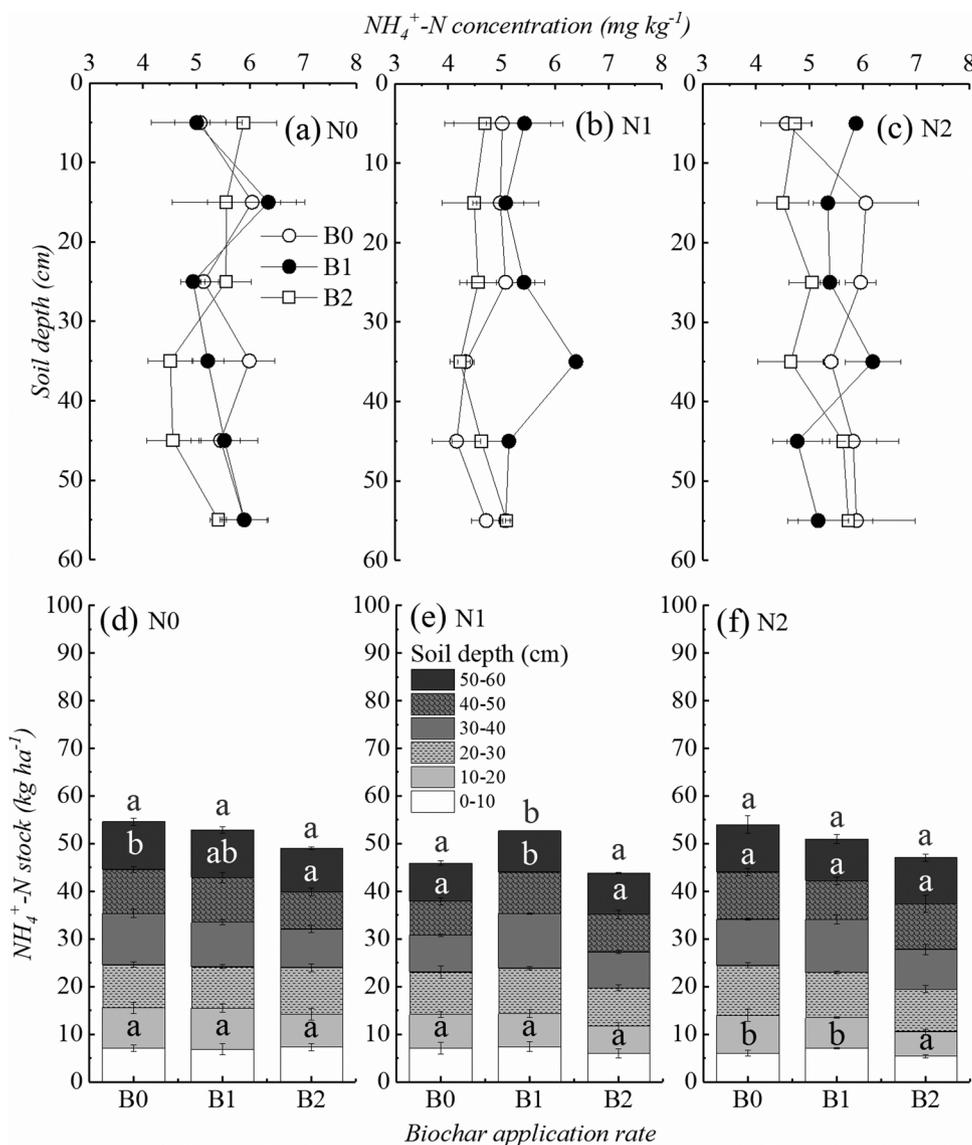


Fig. 3. $\text{NH}_4^+\text{-N}$ concentrations and stocks in the soil profile. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha^{-1} and 240 kg N ha^{-1} , respectively. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha^{-1} and 40 t ha^{-1} , respectively. The different lowercase letters in subgraphs d, e and f indicate significant differences ($p < 0.05$) between treatment means: the black letters in the 10–20 cm section refer to the plow layer soil (0–20 cm), the white letters in the 50–60 cm section refer to the subsoil (20–60 cm), and the dark gray letters above the bar refer to the total soil profile (0–60 cm).

crop growth but does significantly reduce SIN stocks in the subsoil (Güereña and Riha, 2013; Hagemann et al., 2017). A meta-analysis revealed that excess applications of biochar ($\geq 80 \text{ t ha}^{-1}$) significantly inhibit plant biomass and N uptake (Liu et al., 2018). In addition, a previous pot-based experiment conducted by our group indicated that high rates of biochar applications (approximately 80 t ha^{-1}) led to significant reductions in wheat yields, mainly due to $\text{NO}_3^- \text{-N}$ capture by biochar (Li and Shangguan, 2018). However, the threshold of the beneficial biochar application rate in the field was lower than that in incubation and pot-based experiments, likely due to the limited water resources in the natural dryland field. Although biochar may increase the total soil water-holding capacity, much of this is below the permanent wilting point, so plant available water may actually decrease under dry conditions (Haider et al., 2017), exerting concomitant effects on the availability of SIN, particularly in the form of soluble $\text{NO}_3^- \text{-N}$ (Xu et al., 2016; Li et al., 2018). Additionally, large amounts of biochar may adsorb large amounts of $\text{NO}_3^- \text{-N}$ for an extended period, thus leaving less $\text{NO}_3^- \text{-N}$ available for both plant uptake and leaching (Haider et al., 2016; Sun et al., 2017; Thi Thu Nhan et al., 2017), which will be discussed more in the next chapter. Such findings help to elucidate why biochar treatments under fertilization significantly reduce residual SIN, although the B2 treatment did not increase crop biomass.

Many studies have indicated that less $\text{NH}_4^+ \text{-N}$ is leached from

biochar-treated soil columns than from untreated soil columns (Zheng et al., 2013; Sika and Hardie, 2014; Pratiwi et al., 2016). However, in our work, the amount of residual $\text{NH}_4^+ \text{-N}$ in the subsoil slightly decreased in response to only B2 (Fig. 3). This finding was obtained because $\text{NH}_4^+ \text{-N}$ may be readily adsorbed onto negatively charged clay minerals in silty clay soil (Li et al., 2018). In addition, woody biochar usually presents a lower CEC, fewer acidic functional groups, and lower labile C compound contents than does crop-derived and herbaceous biochar (Harvey et al., 2012; Wang et al., 2016; Thi Thu Nhan et al., 2017). Additionally, biochar applied at a rate greater than 40 t ha^{-1} generally induces a significant increase in soil NH_3 volatilization, although woody biochar tends to decrease soil NH_3 volatilization because of an adsorption effect (Liu et al., 2018). However, verification of whether the B2 treatment led to an increase in NH_3 volatilization and thus reduced $\text{NH}_4^+ \text{-N}$ content requires further investigation. Moreover, B1N1 increased the stock of $\text{NH}_4^+ \text{-N}$ in the soil profile to a particularly high level (Fig. 5b), which was likely not due to pronounced $\text{NH}_4^+ \text{-N}$ adsorption (Haider et al., 2016). Hence, the relatively higher $\text{NH}_4^+ \text{-N}$ and lower $\text{NO}_3^- \text{-N}$ concentrations observed in B1N1 may be due to relatively lower nitrification rates, as suggested by Sun et al. (2017), and reduced NH_3 volatilization, as reported by Clough et al. (2013).

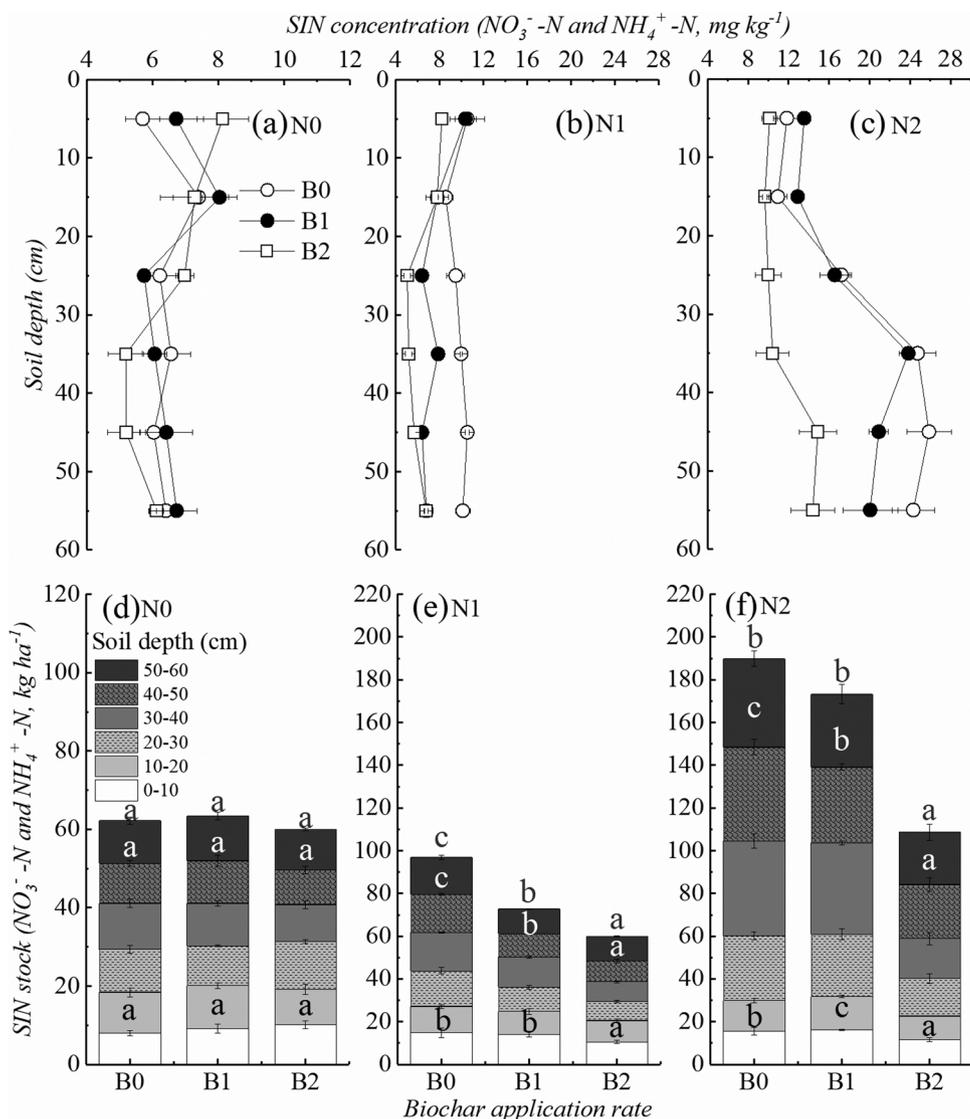


Fig. 4. SIN (NO_3^- -N and NH_4^+ -N) concentrations and stocks in the soil profile. N0, N1 and N2 refer to the non-urea conditions and conditions in which urea was added at 120 kg N ha⁻¹ and 240 kg N ha⁻¹, respectively. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha⁻¹ and 40 t ha⁻¹, respectively. The different lowercase letters in subgraphs d, e and f indicate significant differences ($p < 0.05$) between treatment means: the black letters in the 10–20 cm section refer to the plow layer soil (0–20 cm), the white letters in the 50–60 cm section refer to the subsoil (20–60 cm), and the dark gray letters above the bar refer to the total soil profile (0–60 cm).

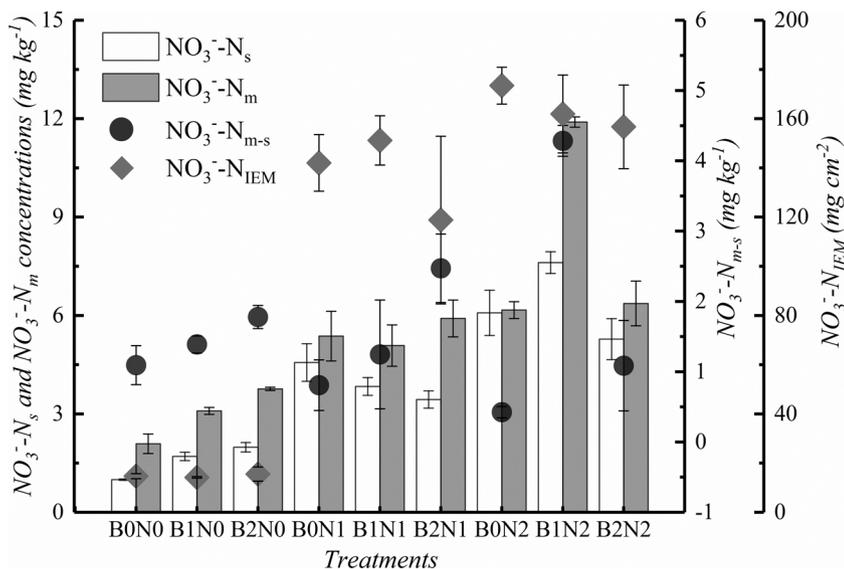


Fig. 5. NO_3^- -N concentrations extracted via different methods from plow layer soil. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha⁻¹ and 40 t ha⁻¹, respectively. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha⁻¹ and 240 kg N ha⁻¹, respectively. NO_3^- -N_s is the NO_3^- -N extracted via the standard method (2 M KCl, shaking for 1 h at 25 °C). NO_3^- -N_m is the NO_3^- -N extracted via the modified method (2 M KCl, shaking for 2 h at 60 °C). NO_3^- -N_{m-s} is the difference in NO_3^- -N extracted via the modified method and via the standard method. NO_3^- -N_{IEM} is the NO_3^- -N extracted from IEMs.

Table 4
Relationships among N forms extracted via different methods and the results of two-way ANOVA.

Parameter	Method	Correlations			Results of two-way ANOVA			
		Standard	Modified	IEMs	B	N	B × N	R ²
NO ₃ ⁻ -N	Standard	1	0.903**	0.857**	**	**	**	0.963
	Modified		1	0.694**	**	**	**	0.971
	IEMs			1	NS	**	NS	0.952
NH ₄ ⁺ -N	Standard	1	-0.246	-0.395*	NS	*	NS	0.265
	Modified		1	0.158	NS	NS	NS	0.238
	IEMs			1	**	*	NS	0.551
NO ₃ ⁻ + NH ₄ ⁺ (SIN)	Standard	1	0.883**	0.782**	*	**	**	0.920
	Modified		1	0.695**	**	**	**	0.947
	IEMs			1	NS	**	NS	0.952
NO ₃ ⁻ -N _{m-s}	-	-	-	-	**	NS	**	0.863
NH ₄ ⁺ -N _{m-s}	-	-	-	-	NS	*	NS	0.388
SIN _{m-s}	-	-	-	-	*	*	**	0.650

Note: The correlations are between different extraction methods, and the ANOVA refers to the comparisons among treatments. B, N and B × N represent biochar and nitrogen fertilizer application rates and their interaction, respectively. ** $p < 0.001$; * $p < 0.05$; NS: not significant; R²: proportion of the explained variance. NO₃⁻-N_{m-s}, NH₄⁺-N_{m-s} and SIN_{m-s} are the differences in NO₃⁻-N, NH₄⁺-N and SIN extracted via the modified method and the standard method.

4.2. Biochar capture of SIN and availability of residual SIN

To determine whether the reduced SIN content observed under the biochar treatments was partly due to sequestration by biochar and the availability of residual SIN, we used a modified extraction method and IEMs to extract SIN from plow layer soil. The results indicated that shaking the soil samples at a high temperature for a long time could facilitate the release of SIN in biochar-amended soil, especially for NO₃⁻-N. In addition, the NO₃⁻-N_{m-s} and SIN_{m-s} generally increased with biochar application under each N level (Figs. 5 and 7). This finding explains the diminishing of NO₃⁻-N, which was captured by field-aged biochar and is usually underestimated by the standard extraction method (Haider et al., 2016). Moreover, the modified extraction method may still be insufficiently powerful to extract all biochar-captured NO₃⁻-N, which is sometimes retrieved via KCl extraction and depends on the biochar pore size distribution and the capture mechanism involved (Haider et al., 2016; Hagemann et al., 2017). These characteristics suggest that biochar exhibits a certain potential to retain N in the soil and that underestimating NO₃⁻-N retention in biochar-amended soils is possible, despite the use of NO₃⁻-N_m.

NO₃⁻-N_{IEM} decreased with biochar application after two years of N fertilization (Fig. 5), which suggests that biochar reduces the residual NO₃⁻-N available for both leaching and crop uptake. Similarly, Antonio

Albuquerque et al. (2013) reported that biochar applications at 2.5% (w/w) resulted in decreased SIN_{IEM} and aboveground N concentrations in wheat, regardless of the mineral fertilization level. Sika and Hardie (2014) reported that leached pine wood biochar-amended soils contained only low concentrations of exchangeable NO₃⁻-N (5.8–8.0 mg kg⁻¹) and NH₄⁺-N (0–7.3 mg kg⁻¹), despite the observed strong decrease in SIN leaching. Additionally, some studies have detected improved mineral N accumulation using a standard extraction method in biochar-amended soils but without a concurrent increase in yield (Borchard et al., 2012; Güereña and Riha, 2013), because some of the NO₃⁻-N_s present in biochar-amended soils may already be unavailable (Borchard et al., 2012; Haider et al., 2016). However, the NO₃⁻-N retained in the biochar-amended soil was clearly resistant to leaching, which could explain why B1N2 increased NO₃⁻-N_s in the plow layer soil but reduced both NO₃⁻-N_{IEM} and NO₃⁻-N_s in the subsoil (Figs. 2 and 5). Therefore, although there were significant positive correlations between NO₃⁻-N_{IEM} and NO₃⁻-N_s or NO₃⁻-N_m (Table 3), NO₃⁻-N_m provides only an index of the relative “true” stock in biochar-amended soils in most cases and may be inadequate as a basis for precise soil management recommendations. Additionally, the quantification of how much biochar-captured NO₃⁻-N can be regarded as belonging to the available N pool for crop uptake while avoiding leaching requires further research.

NH₄⁺-N sorption or retention is usually attributed to an increase in

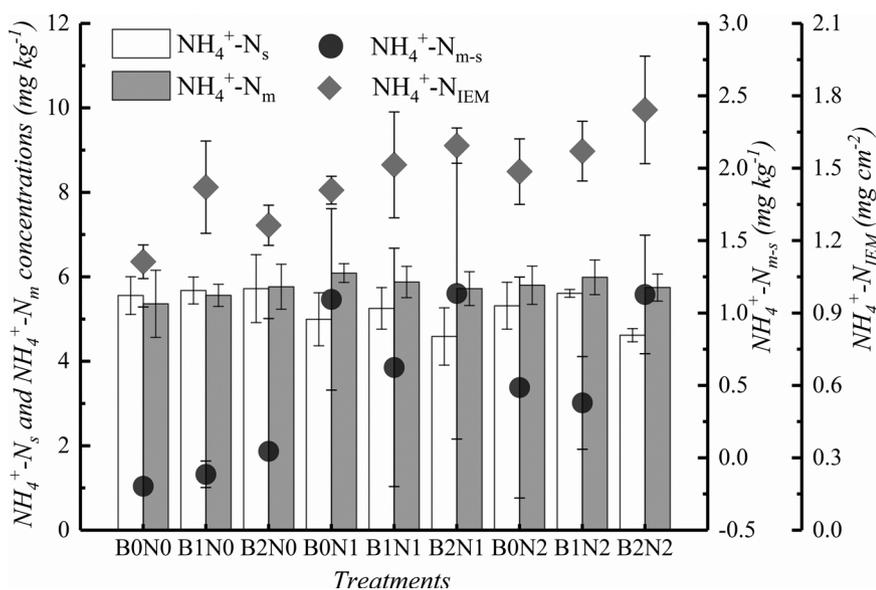


Fig. 6. NH₄⁺-N concentrations extracted via different methods in the plow layer soil. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha⁻¹ and 40 t ha⁻¹, respectively. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha⁻¹ and 240 kg N ha⁻¹, respectively. NH₄⁺-N_s is the NH₄⁺-N extracted via the standard method (2 M KCl, shaking for 1 h at 25 °C). NH₄⁺-N_m is the NH₄⁺-N extracted via the modified method (2 M KCl, shaking for 2 h at 60 °C). NH₄⁺-N_{m-s} is the difference in NH₄⁺-N extracted via the modified method and via the standard method. NH₄⁺-N_{IEM} is the NH₄⁺-N extracted from IEMs.

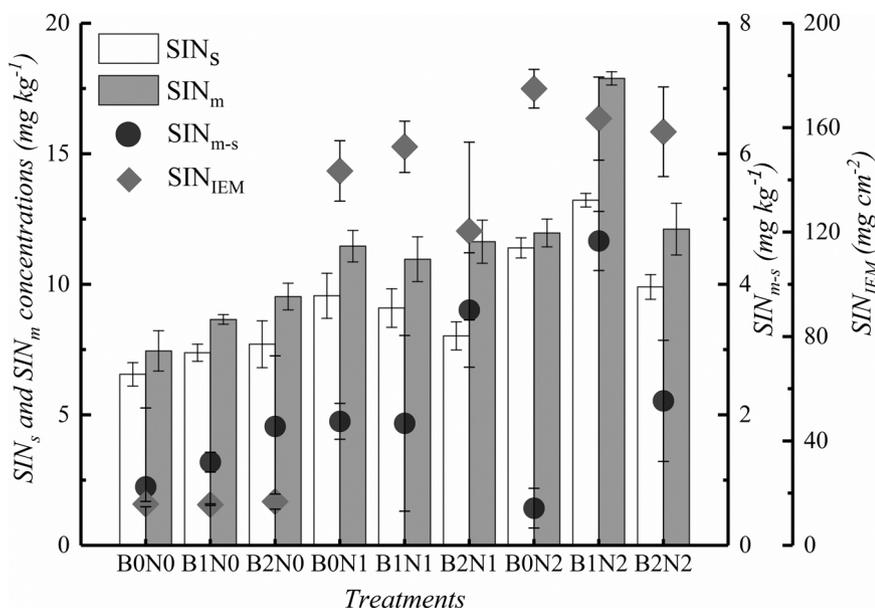


Fig. 7. SIN (NO_3^- -N and NH_4^+ -N) concentrations extracted via different methods from plow layer soil. B0, B1 and B2 refer to the application of no biochar and biochar applications in the plow layer soil at 20 t ha^{-1} and 40 t ha^{-1} , respectively. N0, N1 and N2 refer to non-urea conditions and conditions in which urea was added at 120 kg N ha^{-1} and 240 kg N ha^{-1} , respectively. SIN_s is the SIN extracted via the standard method (2 M KCl, shaking for 1 h at 25°C). SIN_m is the SIN extracted via the modified method (2 M KCl, shaking for 2 h at 60°C). SIN_{m-s} is the difference in SIN extracted via the modified method and via the standard method. SIN_{IEM} is the SIN extracted from IEMs.

CEC induced by biochar (Haider et al., 2017), because the carboxylic groups and fewer acidic groups, such as phenols and carbonyls, on the surface of biochar have a negative charge and can adsorb NH_4^+ -N through electrostatic attraction (Haider et al., 2016; Liu et al., 2018). However, NH_4^+ -N adsorbed onto negatively charged functional groups may be easily extracted via the standard method (Clough et al., 2013). Therefore, only the B2 treatment slightly increased NH_4^+ - N_{m-s} (Fig. 6). Additionally, the N compounds adsorbed by biochar may be desorbed over time and become available (Taghizadeh-Toosi et al., 2012), which contributes to the increased NH_4^+ -N exchangeability represented by NH_4^+ - N_{IEM} (Fig. 6).

5. Conclusions

Biochar application alone had no significant effects on wheat biomass or SIN leaching. However, the B1N1 treatment represents a promising strategy for achieving the dual goals of increasing N availability and mitigating the leaching of SIN (mainly in NO_3^- -N) in dryland systems. Compared with all the treatments, the B2 treatment more effectively mitigated SIN leaching; however, this treatment also significantly limited N availability. Hence, the overuse of biochar will make less SIN available not only for leaching but also for crop uptake. Biochar can adsorb NO_3^- -N after two years of field aging; as such, biochar application generally reduces the amount of NO_3^- -N available for leaching during the fallow period.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2019.02.013>.

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