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ORIGINAL PAPER

Biochar amendment enhanced soil nitrogen fractions and wheat yield after four to five years of aging in Loess Plateau, China

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Abstract

Soil fertility has become a major issue in the Loess Plateau, China. The present study explored the effects of maize straw biochar application on soil nitrogen (N) fractions, microbial biomass carbon (C), and wheat yields in a calcareous, sandy loam soil in the Loess Plateau region. Six maize straw biochar (BC) application rates were applied to the soil in July 2015, including control with no biochar (CK), BC1 (10 t ha^{-1}), BC2 (20 t ha^{-1}), BC3 (30 t ha^{-1}), BC4 (40 t ha^{-1}), and BC5 (50 t ha^{-1}). Wheat was cultivated in the amended soil for 5 years using routine mineral N and P fertilization practices. Four to 5 years after biochar application, the soil contents of total N, microbial biomass C, and amino acid N significantly increased by 9.0–30.9%, 55.1–81.4%, and 64.5–68.2% (in 2019) and 6.5–10.9%, 68.6–139.7%, and 66.9–77.2% (in 2020), respectively, as compared to CK. Moreover, the content of unknown-acidolyzable nitrogen decreased by 45.0–63.1% (in 2019) and 83.5–89.6% (in 2020) compared with CK, respectively. Application of BC3 increased the total acidolyzable nitrogen, acidolyzable ammonium nitrogen, and amino-acid nitrogen contents in 0 to 30-cm soil layer by 6.3–7.8%, 23.0–25.2%, and 62.2–0.9% (in 2019) and 14.7–18.0%, 23.5–29.0%, and 41.9–107.6% (in 2020), respectively, as compared with CK. However, after 4 and 5 years, nonacid hydrolyzed N was the highest in BC5 (50 t ha^{-1}) treatment, which increased by 27.0% and 44.8%, respectively, compared to the CK, while after 5 years, it was the lowest in BC3 (30 t ha^{-1}) treatment, decreased by 35.4%. After 5 years, all biochar treatments significantly improved wheat yields compared to CK. The highest wheat yield was obtained in the BC3 treatment, which was 21.6% and 24.8% higher than the CK in years 4 and 5, respectively. In conclusion, the application of biochar as a soil conditioner can significantly affect the soil total and organic N fractions and microbial biomass after aging for 4–5 years and has a positive effect on improving soil nutrient supply capacity.

Keywords Biochar · Soil nitrogen · Microbial biomass · Dryland farming

Introduction

Nitrogen is one of the main factors limiting soil fertility and crop growth (Li et al. 2018). Soil organic nitrogen is one of the major forms of soil nitrogen (Dempster et al. 2012). Its chemical cycle, i.e., nitrogen fixation, nitrification, assimilation, ammonification, and denitrification are important factors affecting soil nitrogen availability and play an important role in maintaining soil nitrogen supply (Song et al. 2020). Soil microbial biomass, as one of the important sources of nutrients needed for plant growth, is an important driving factor for the soil nutrient transformation cycle (Wang et al. 2017). Most Chinese soils have low organic matter content and fertility status, so incorporation of crop residues, i.e., straw to the field is a popular soil fertilization practice in the early stages of rural China (Hu et al. 2020). China has a lot of straw resources, and straw is considered nutrient-enriched

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agricultural residues. In 2014, the annual production of straw was around 700 million tons, and during the years 1995–2005, about 62% of all straw was burnt in the field (Ren et al. 2019). Direct incorporation of straw into the soil in agriculture systems, on the other hand, would enhance the content of soil organic carbon, foster microbial decomposition, and emit GHG, i.e., N_2O, CH_4, CO_2, and other GHG, resulting in soil nutrient depletion and significant global warming effects (Jing et al. 2020; Song et al. 2020).

Biochar is a highly aromatic carbon-containing solid substance produced by pyrolysis in the absence of oxygen or hypoxia, which has the characteristics of large specific surface area, adsorption, strong stability, and rich porosity (Haider et al. 2021a, b, c). In order to enhance the resource utilization of straw, crop straw can be processed into biochar (Hu et al. 2020). Straw biochar has been shown to enhance the content of soil organic matter while also maintaining soil quality, and straw-derived biochar has been shown to improve carbon sequestration in agricultural soils (El-Naggar et al. 2019; Jing et al. 2020). In recent years, the incorporation of biochar, as a new type of soil fertilizer improvement material, has become a trending research topic (Gul et al. 2015; Hu et al. 2020; Khan et al. 2021). Some studies suggest that the addition of biochar to agricultural soil can enhance the supply of nutrients in the soil for sustainable plant growth (Sarma et al. 2018; Haider et al. 2021b).

Nitrogen is needed for the metabolism of nucleic acid, enzymes, and nucleotides in plants, as well as for energy transfer (Manirakiza et al. 2019). The use of nitrogen is an essential process in the mineralization of organic carbon (Yang et al. 2019). Nitrogen can enhance the enzyme production and fertility status of soil, allowing carbon to be mineralized more effectively (Nieder et al. 2011). Nitrogen is the most limiting factor for microbial activity in semiarid and arid tropical zones of the world (Xu et al. 2003; Pokharel et al. 2020). Previous studies have indicated that adding biochar to agricultural soils may alter conditions that influence denitrification, nitrification, and other nitrogen transformation loss processes (Lehmann et al. 2011; Gul et al. 2015; Xu et al. 2016). Similarly, soil enzymes play an important role in the metabolic process that takes place in the soil, influencing the nutrient cycling, i.e., nitrogen and decomposition of soil carbon and nutrients (Oladele et al. 2019). Many factors influence the impact of biochar inclusion on soil enzyme production, including the form of biochar used, the relationship between enzymes and biochar, and the soil environment (Lehmann et al. 2011; Haider et al. 2021c).

Due to the prominent role of N nutrition in regulating crop production in semiarid and arid regions, the effect of biochar on soil nitrogen transformation processes are extremely important, as it must be replenished either as inorganic nitrogen fertilizer or cycled back to the soil in a complex organic form such as crop residues (Prommer et al. 2014). In the past few decades, research has focused on organic turnover mechanisms in the soil N cycle, such as organic matter depolymerization, which is currently thought to be the rate of limiting phase of dissolved organic N production (Hu et al. 2020). Biochar addition to soil may have a significant influence on the turnover of soil organic N fraction due to the properties stated above. Due to the numerous simultaneous soil N processes (Fig. 1), measuring and interpreting soil N transformation rates is complex.

The biochar application strongly impacts the activities of soil enzymes (Hu et al. 2020; Song et al. 2020). Although

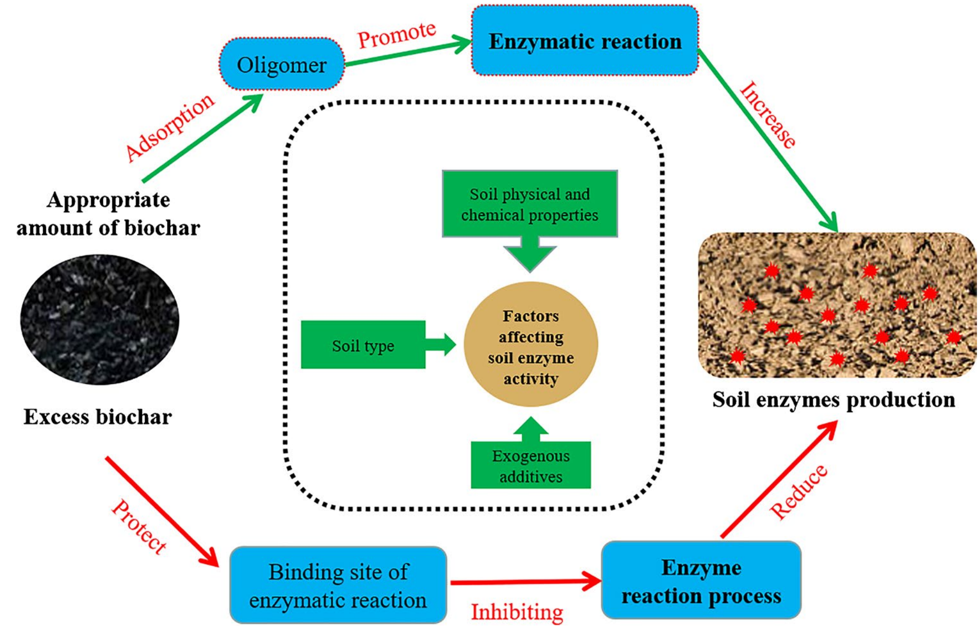

Fig. 1 Effects of biochar addition on soil enzyme production. Soil type, soil physical, chemical properties, and exogenous additives can affect soil enzyme activity. Adding an appropriate amount of biochar to the soil promotes enzymatic reaction by adsorbing oligomers in the soil, thereby increasing soil enzyme activity or enzyme production. However, excessive biochar addition inhibited the enzymatic reaction by protecting the binding sites of the enzymatic reaction, thereby reducing soil enzyme activity or enzyme production (modified from Prommer et al. 2014)

the studies on the impact of biochar application on soil under conventional fertilizer application are available (Xu et al. 2003; Li et al. 2004; Jia et al. 2017); however, the effect of biochar application on various soil organic nitrogen fractions and crop yield in the dry farmland in a semiarid region like Loess Plateau is not clear. As a result, it is critical to investigate the effects of biochar amendments on nitrogen transformation processes, particularly dry farmland in a semiarid region like Loess Plateau soils where nitrogen fertilizer use is excessive, to designate appropriate times for nitrogen addition. Therefore, this study was conducted to evaluate the effect of biochar incorporation on soil organic nitrogen fractions, activities of soil enzymes, and soil carbon mineralization in dry farmland on the Loess Plateau. It was hypothesized that the application of biochar may significantly improve soil total nitrogen content and soil microbial biomass due to biochar's alkaline nature and porous structure.

Materials and methods

Study site

The experiment was conducted at Lijiabu Town, Anding District, Dingxi City, Gansu Province, China (35°28′N, 104°44′E) (Fig. 2). It belongs to the hilly and gully region of the Loess Plateau and is also a typical dry farming area with an average altitude of 2000 m with average annual precipitation of 521 mm. The precipitation is mainly

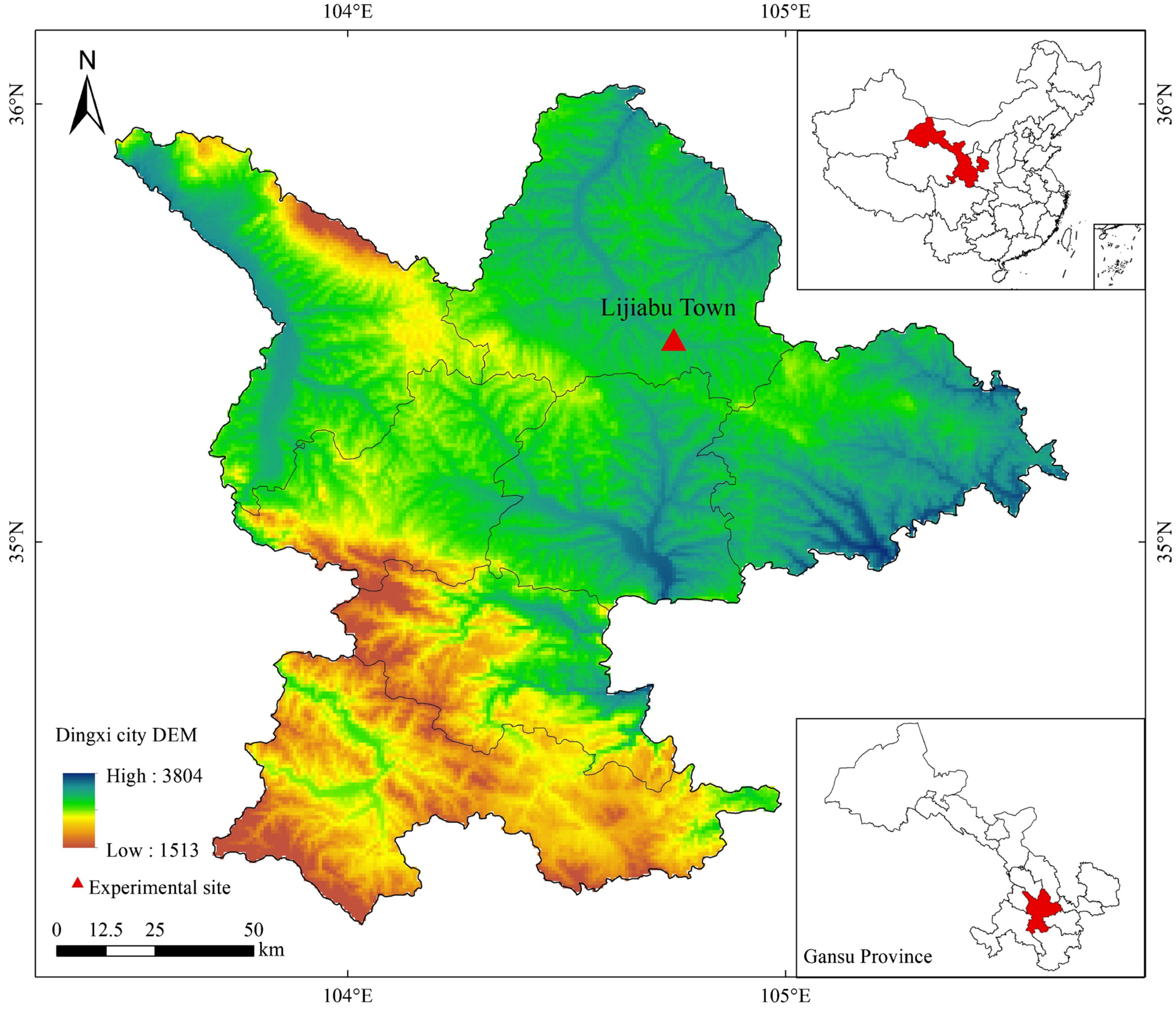

Fig. 2 Experimental site: Lijiabu Town, Anding District, Dingxi City, Gansu Province, China

concentrated in the months of July–September. The average annual temperature of this region was about 6.5 °C, and the average sunshine duration was about 2476.6 h each year. The experimental soil belonged to Loessial soil (Chinese Soil Taxonomy Cooperative Research Group 1995), which equates to Calcaric Cambisols in WRB soil classification (FAO 1990). The soil used was typically cultivated soils in the Loess Plateau of Northern China, i.e., calcareous, sandy loam soil with low organic matter and fertility. The experimental soil had soil pH 8.4, average bulk density 1.2 g cm^{-3}, wilting moisture 7.3%, saturated moisture 21.9%, organic matter content 12.0 g kg^{-1}, total nitrogen 0.8 g kg^{-1}, and total phosphorous (P_2O_5) 1.8 g kg^{-1}.

Experimental design and materials

The experiment was set up in 2015 as a single factor random block design comprising of 6 biochar application levels, i.e., CK (0 t ha^{-1}), BC1 (10 t ha^{-1}), BC2 (20 t ha^{-1}), BC3 (30 t ha^{-1}), BC4 (40 t ha^{-1}), and BC5 (50 t ha^{-1}). Each treatment was repeated 3 times in different test blocks, with a total of 18 plots, with an area of 16.8 m^2 (2.8 m × 6 m). The amount of biochar was calculated by natural air-dried weight. In March 2015, biochar was evenly spread into each plot according to the experimental design, and then it was incorporated into the plowing layer of soil with a rotary tiller (about 20 cm). From 2015 to 2020, the bread wheat variety "Dingxi 40" was planted in March and harvested in July. The wheat was seeded using a seeding rate of 188 kg ha^{-1} in a 20-cm spaced row set with a sowing depth of 7 cm. Before sowing in each year, urea 228 kg ha^{-1} (having 46% N) and superphosphate 750 kg ha^{-1} (having 14% P_2O_5), were applied accordingly to the conventional fertilizer rate, and the wheat was plowed according to the conventional farming method before sowing and after harvest (about 20 cm). At the end of July every year, the mechanical conventional method was used to harvest wheat, and the wheat in each plot is naturally air-dried and threshed, and the yield is recorded and measured. From 2015 to 2018, the experiment did not measure soil organic nitrogen fractions and microbial biomass and other related indicators.

The applied biochar was purchased from Liaoning Jinhefu Agricultural Science and Technology Cooperation Limited, which was prepared by pyrolysis of maize straw under 500 °C under anaerobic conditions, and 35% of the biomass could be converted into biochar. The basis properties of biochar were as follows; pH 9.2, cation exchange capacity 25.2 c mol kg^{-1}, specific surface area 11.3 m^2 g^{-1}, soluble organ carbon content 432.4 mg kg^{-1}, natural air-dried water content 5.1%, carbon content 53.3%, nitrogen content 1.0%, phosphorous content 0.3%, potassium content 0.5%, calcium content 0.8%, magnesium content 0.5%, and ash content 35.6%.

Collection and analysis of soil samples

After the harvesting of wheat in August 2020, soil samples were collected from 0 to 5 cm, 5 to 10 cm, and 10 to 30 cm depth by the 5-point method. After mixing, the samples were divided into two parts by quartile method, one of which was naturally dried through 0.25 mm and 0.15 mm and stored in a sealed bag, and other fresh samples were sieved through 2 mm and stored in an aseptic bag at 4 °C.

The air-dried soil samples (0.149 mm sieve) were weighed to determine the soil moisture contents. The soil sample was put into the bottom of the drying digestion tube, and a small amount of nonionic water (0.5–1.0 mL) was added to moisten the sample. The accelerant (2 g) and concentrated sulfuric acid (5 mL) were added and shaken well. The digestion tube was placed on the infrared digestion furnace for digestion. When the digestion liquid and soil particles were all turned gray and slightly green, they were continued to digest for 1 h. The cooled digestion tube was distilled directly into the Kjeldahl nitrogen analyzer, and the total nitrogen (TN) was determined by the Kjeldahl digestion method (Bremmer and Mulvaney 1982).

Soil microbial biochar (MBC) and microbial biomass nitrogen (MBN) were determined using fumigation-extraction methods based on the difference between C or N extracted with 0.5 mol·L^{-1} K_2SO_4 through chloroform-fumigation and unfumigated soil samples, using K_{EC} (0.45) and K_{EN} (0.54) factors, respectively (Varma and Oelmüller 2007).

Soil organic nitrogen fractions were determined by the Bremner method (Bremner 1965). For the preparation of soil acid hydrolysate, the sample soil of about 10.00 g was allowed to be sieved by passing through a 100-mesh sieve. The soil was put into a triangular flask with a grinding joint, n-octanol (2 drops) and 6 M HCI (20 mL) were added to the flask. The bottle was shaken to mix the soil with acid. The bottle was placed into a far-infrared digestion furnace with a temperature control function, installed in a condensing tube with glass grinding joint at the bottle mouth, and was connected to condensed water. After hydrolysis, the sample was filtered while hot with medium speed blue ribbon filter paper, and the filtrate was collected in a 100 mL beaker, and the remaining residue was washed with deionized water to make the volume of filtrate 60 mL. The beaker containing the filtrate was incubated in an ice bath; 5 M NaOH was added drop by drop into the solution and stirred to reach pH 5. The pH was neutralized with 0.5 M NaOH to the pH of 6.5 ± 0.1.

For the determination of total nitrogen acidolysis (TAN), acidolysis solution (5 mL) was sucked with a pipette with a wide end and was put into the digestion tube, nitrogen-fixing mixed catalyst (0.5 g) and concentrated sulfuric acid (2 mL) were added, and the mixture was heated at 380 °C until the digestion solution was clear. After cooling, the digestion tube was connected with the Kjeldahl nitrogen apparatus to estimate the TAN.

For the determination of acid hydrolyzed ammonia nitrogen (AMN), neutralized acidolysis solution (10 mL) was taken and put into the digestion tube, 3.5% MgO suspension (2.5 mL) was added, and AMN was determined with the Kjeldahl nitrogen determinator.

For the determination of acid-hydrolyzed ammonia nitrogen, neutralized acid hydrolysate (10 mL) was taken into the digestion tube; phosphoric acid borax buffer (pH 11.2; 10 mL) was added. The acid-hydrolyzed ammonia nitrogen was determined with the Kjeldahl nitrogen determinator. Amino-sugar nitrogen was calculated by subtracting acid-hydrolyzed ammonia nitrogen from the determination result.

For the estimation of amino acid nitrogen (AAN), neutralized acid hydrolysate (5 mL) was taken into a 50-mL small beaker, and 0.5 M NaOH solution (1 mL) was added. The sample was heated in 100 °C boiling water until the solution was reduced to 2 mL. After cooling at room temperature, citric acid (1 g) and hydrated ninhydrin (0.2 g) were added. The beaker was incubated at 100 °C in a water bath for 10 min. The mixture solution (10 mL) was taken, phosphoric acid borax buffer (20 mL), and 5 M NaOH (2 mL); the solution mixture was distilled for 4 min and was then titrated.

The unknown nitrogen (UAN) was calculated using the following formula:

$$Unknownnitrogen(UAN) = totalnitrogenacidolysis(TAN) - (acidhydrolyzedammonianitrogen(AMN) + amino - sugarnitrogen(ASN) + aminoacidnitrogen(AAN))$$

The nonacid nitrogen was calculated by subtracting the total nitrogen acidolysis from the total nitrogen (TN).

Statistical analysis

The observed parameters were sorted by Excel 2016, the figures were developed out by Sigma-Plot 12.5, and the data were analyzed statistically by SPSS 19.0 software. Duncan's new multiple range test was used for mean separation at $p \leq 0.05$ level. The correlation attributes were calculated by the Pearson method.

Results

Total soil nitrogen

Biochar treatments had a significant effect on soil nitrogen content, which was significantly higher in the 0 to 5-cm soil layer as compared to the 10 to 30-cm soil layer (Table 1; Fig. 3). The total nitrogen contents of BC4 and BC5 treatments in the 0 to 5-cm soil layer in 2019 were significantly improved by 10.7% and 11.4%, respectively as compared to control treatment ($p < 0.05$, Fig. 3a). The total nitrogen content of BC4 and BC5 treatments increased by 9.8% and 10.9%, respectively, as compared to control in 2020 ($p < 0.05$, Fig. 3b). In the 5–10 cm layer, the total nitrogen content of BC3, BC4, and BC5 treatments in 2019 increased by 26.4%, 28.8%, and 30.9%, respectively, higher than control ($p < 0.05$, Fig. 3a), and the total nitrogen content of BC3, BC4, and BC5 treatments increased by 6.55%, 8.75%, and 10.25%, respectively, higher than control in 2020 ($p < 0.05$, Fig. 3b). In the 10 to 30-cm soil layer, the total nitrogen content of BC2, BC3, BC4, and BC5 treatments in 2019 were increased by 7.7%, 7.9%, 8.3%, and 9.0%, respectively, higher than control ($p < 0.05$, Fig. 3a), the total nitrogen content of BC2, BC3, BC4, and BC5 treatments were increased by 3.6%, 6.6%, 8.8%, and 10.3%, respectively, higher than control in 2020 ($p < 0.05$, Fig. 3b). However, there was no significant difference in total nitrogen content between the 2 years under BC1 treatment ($p < 0.05$, Fig. 3a; $p < 0.05$, Fig. 3b).

Soil organic nitrogen fractions

Total acidolyzable nitrogen

The effects of biochar on the grouping of total acidolyzable nitrogen are given in Figs. 4 and 5. In 2019 and 2020, total acidolyzable nitrogen in the 0 to 30-cm soil layer increased firstly and then decreased with an increase of biochar addition and showed a decreasing trend within the deepening of the soil layer (Fig. 4a). The total acidolyzable nitrogen in the 0 to 30-cm soil layer ranged from 0.7 to 0.8 g kg^{-1} in 2019 and from 0.6 to 0.8 g kg^{-1} in 2020, respectively, accounting for a 69.8 to 88.2% increase in 2019 and a 66.1% to 86.9% increase in 2020, respectively, of total acidolyzable nitrogen in soils. The addition of biochar was greater than or equal to BC2, the total acidolyzable nitrogen content was significantly increased ($p < 0.05$). In 2019 and 2020, BC3 treatment increased the total acidolyzable nitrogen content in the 0 to 30-cm soil layer by 6.3–7.8% and 14.7% and 18.0% compared with control ($p < 0.05$), followed by BC4 and BC3 treatment in 2019, BC3 and BC4 treatment in 2020, and BC5 increased the last.

Amino acid nitrogen

The amino acid nitrogen contents in soil increased with the increase of biochar supplemental level, and the highest content was observed in BC5 as compared to other treatments

Table 1 Analysis of variance (*F* value) for soil organic nitrogen fractions, microbial biomass carbon, nitrogen, and crop yield

Years	Soil layer	Sources	d.f	TN	AAN	AMN	UAN	ASN	AIN	MBC	MBN	Wheat yield
2019	0–5 cm	Replication	2	1.515 ns	10.559**	3.050 ns	11.568**	0.292 ns	0.963 ns	0.178 ns	0.214 ns	0.315 ns
		Treatment	5	2.560 ns	91.788**	41.867**	48.481**	7.619**	1.076 ns	67.942**	42.243**	1.747 ns
		Error	10									
		Total	17									
	5–10 cm	Replication	2	1.613 ns	6.894*	1.060 ns	3.100 ns	0.359 ns	1.046 ns	0.056 ns	0.227 ns	
		Treatment	5	5.195*	77.447**	36.728**	18.398**	5.013*	2.662 ns	66.655**	28.221**	
		Error	10									
		Total	17									
	10–30 cm	Replication	2	3.804 ns	2.144 ns	1.480 ns	0.846 ns	3.829 ns	3.693 ns	1.930 ns	0.595 ns	
		Treatment	5	12.591**	67.352**	50.481**	12.737**	7.772**	3.028 ns	75.951**	31.922**	
		Error	10									
		Total	17									
2020	0–5 cm	Replication	2	0.809 ns	1.074 ns	0.358 ns	0.849 ns	0.525 ns	1.155 ns	0.707 ns	2.140 ns	115.169**
		Treatment	5	6.418**	71.790**	27.031**	9.116**	7.526**	2.798 ns	16.455**	2.827 ns	48.536**
		Error	10									
		Total	17									
	5–10 cm	Replication	2	0.160 ns	0.242 ns	0.012 ns	3.375 ns	0.096 ns	1.545 ns	0.605 ns	3.822 ns	
		Treatment	5	4.875*	65.069**	16.059**	12.447**	6.780**	1.787 ns	19.418**	1.718 ns	
		Error	10									
		Total	17									
	10–30 cm	Replication	2	0.164 ns	1.737 ns	0.994 ns	4.232*	1.057 ns	1.442 ns	0.066 ns	4.546*	
		Treatment	5	6.063**	50.908**	17.763**	15.746**	13.403**	9.510**	16.634**	3.997*	
		Error	10									
		Total	17									

d.f. = degree of freedom; *significant at 0.05 probability; **significant at 0.01 probability; *ns*, no significance; *TN*, total nitrogen; *AAN*, amino acid nitrogen; *AMN*, ammonium nitrogen; *UAN*, unknown-acidolyzable nitrogen; *ASN*, amino-sugar nitrogen; *AIN*, non-acidolyzable nitrogen; *MBC*, microbial biomass carbon; *MBN*, microbial biomass nitrogen

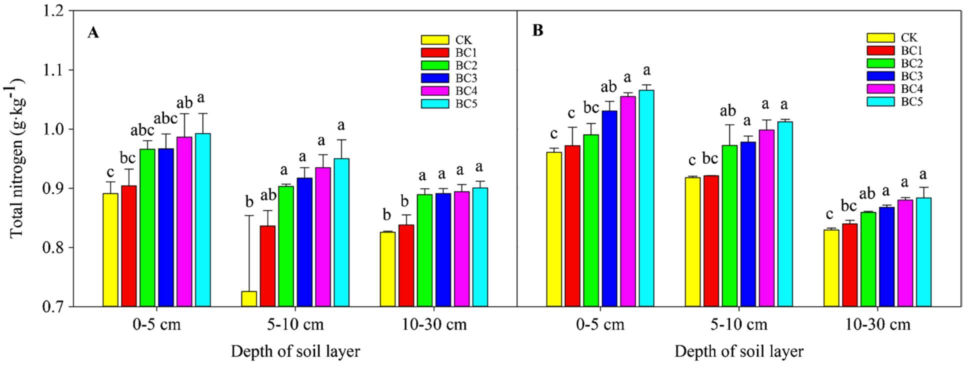

Fig. 3 Effects of biochar addition on soil total nitrogen in different soil layers during **a** 2019 and **b** 2020. The results are the averages and standard deviations of three replicates. Bars sharing the same case letters, for interaction and main effects for a parameter, do not differ significantly at $p \leq 0.05$. CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; and BC5 = 50 t ha^{-1}

(Fig. 4). In 2019 and 2020, the variation range of amino acid nitrogen contents in the 0- to 30-cm soil layer was 0.2 to 0.5 g kg^{-1} and 0.2 to 0.5 g kg^{-}, respectively, accounting for 28.6–43.9% and 27.5–45.1% of total soil nitrogen, respectively (Tables 1 and 2). Compared with control treatment, in the 0 to 5-cm soil layer, when biochar supplemental level was greater than BC1, amino acid nitrogen contents in soil significantly increased by 14.8–68.2%, respectively, in

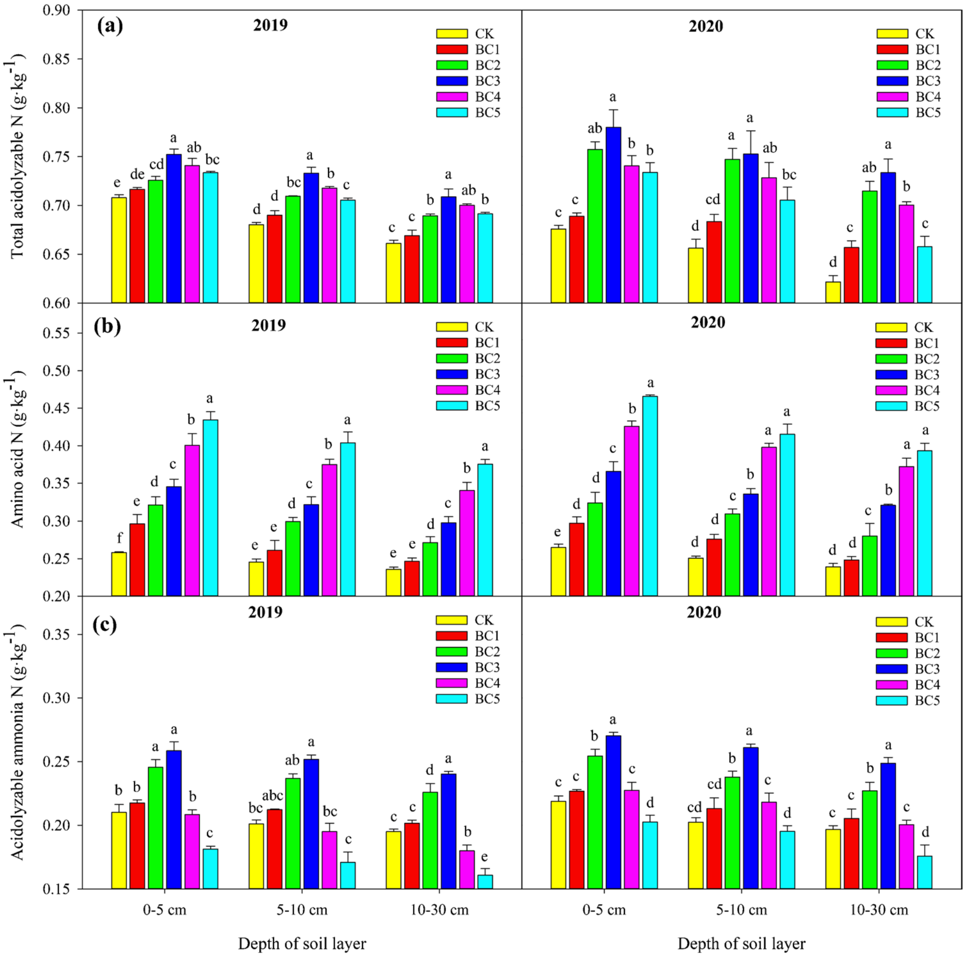

Fig. 4 Effects of biochar addition on soil total acidolyzable N (**a**), amino acid N (**b**), and acidolyzable ammonia N (**c**) in different soil layers during years 2019 and 2020. The results are the averages and standard deviations of three replicates. Bars sharing the same case letters, for interaction and main effects for a parameter, do not differ significantly at $p \leq 0.05$. CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; and BC5 = 50 t ha^{-1}

2019 and 12.4–77.2%, respectively, in 2020 ($p < 0.05$); in the 5 to 30-cm soil layer, when biochar supplemental level was significantly greater than or equal to BC2, amino acid nitrogen contents in soil significantly increased by 15.1–64.5%, respectively, in 2019 and 17.6–66.9%, respectively, in 2020 ($p < 0.05$). In addition, the amino acid nitrogen contents in the 0 to 30-cm soil layer were increased by 4.4–6.7% in 2020 as compared with that in 2019.

Acidolyzable ammonia nitrogen

The acidolyzable ammonia nitrogen contents in soil were firstly increased and then decreased with the increase of biochar supplemental level (Fig. 4; 2019c; 2020c). In 2019 and 2020, the variation range of acidolyzable ammonia nitrogen contents in the 0 to 30-cm soil layer was 0.2 to 0.3 g kg^{-1} and 0.2 to 0.3 g kg^{-1}, respectively, accounting for 17.9 to 27.5% and 19.0 to 28.7% of total soil nitrogen (Tables 1 and 2). Compared with control, BC3 treatment enhanced the acidolyzable ammonia nitrogen contents in soil by 23.0% and 23.2% in 2019 and 23.5% and 26.4% in 2020 in the 0 to 5-cm and 10 to 30-cm soil layers, respectively ($p < 0.05$), followed by BC2 treatment, which increased the acidolyzable ammonia nitrogen contents by 16.9% and 15.9% in 2019 and 16.3% and 15.5% in 2020 in the 0 to 5-cm and 10 to 30-cm soil layers, respectively ($p < 0.05$). In the 5 to 10-cm soil layer, compared with control, the acidolyzable ammonia nitrogen contents in the BC3 treatment increased by 25.2% in 2019 and 29.0% in 2020, respectively. However, under BC5 treatment, soil acidolyzable ammonia nitrogen contents were significantly decreased by 13.7 to 17.5%, respectively, in 2019 and 7.5 to 10.6%, respectively, in 2020. There was no significant difference in the soil acidolyzable ammonia nitrogen contents between BC1 and BC4 treatments.

Amino-sugar nitrogen

The application of biochar caused a significant increase in the amino-sugar nitrogen content (Fig. 5; 2019a; 2020a). In 2019 and 2020, the variation of amino-sugar nitrogen content in the 0 to 30-cm soil layer was 0.02–0.07 g kg^{-1} and 0.03–0.09 g kg^{-1}, respectively, accounting for 2.5 to 7.8% in 2019 and 2.8 to 8.9% in 2020 of the total nitrogen contents in soil (Tables 1 and 2). BC3 treatment had the highest content of amino-sugar nitrogen in the 0 to 30-cm soil layer, which

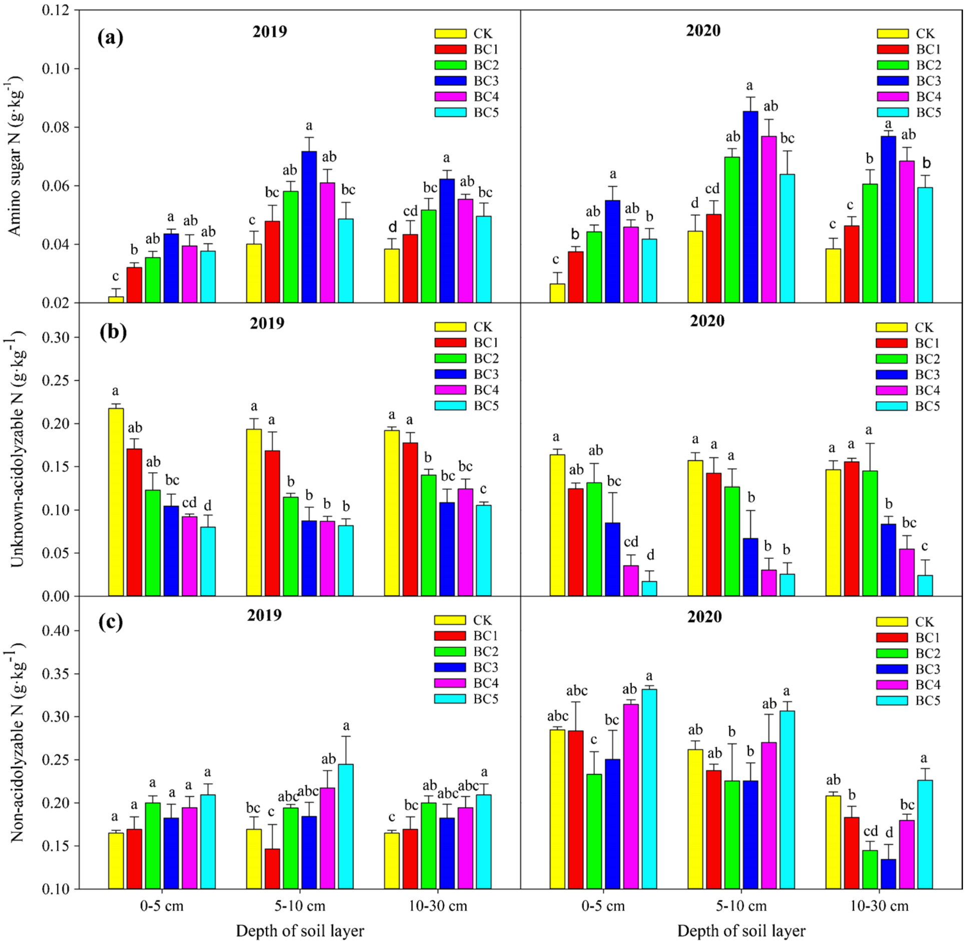

Fig. 5 Effects of biochar addition on soil amino-sugar N (**a**), unknown-acidolyzable N (**b**), and non-acidolyzable N (**c**), in different soil layers during years 2019 and 2020. The results are the averages and standard deviations of three replicates. Bars sharing the same case letters, for interaction and main effects for a parameter, do not differ significantly at $p \leq 0.05$. CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; and BC5 = 50 t ha^{-1}

increased by 62.2 to 97.3% in 2019 and 91.9 to 107.6% in 2020 ($p < 0.05$), followed by BC4 treatment, that increased by 44.3–78.7%, respectively, in 2019 and 72.8–77.7%, respectively, in 2020 ($p < 0.05$). The amino-sugar nitrogen contents in BC2 treatment increased by 34.6–60.6%, respectively, in 2019 and 56.9–67.2%, respectively, in 2020, while in the BC1 treatment, only the 0 to 5-cm soil layer amino-sugar nitrogen content significantly increased by 45.3% in 2019 and 41.5% in 2020 ($p < 0.05$). The amino-sugar nitrogen contents under BC5 treatment, in the 0 to 30-cm soil layer increased by 29.2–70.6%, respectively, in 2019 and 43.6–57.7%, respectively, in 2020. However, the difference was nonsignificant compared with that of control in the 5–10 cm layer in 2019.

Unknown-acidolyzable nitrogen

Unknown-acidolyzable nitrogen contents showed a decreasing trend with the increase of biochar supplemental level (Fig. 5). In 2019 and 2020, the variation range of unknown-acidolyzable nitrogen contents in the 0 to 30-cm soil layer was 0.08–0.22 g kg^{-1} and 0.02–0.16 g kg^{-1}, respectively, accounting for 8.0–24.5% and 1.6–18.5% of total nitrogen in the soil (Tables 1 and 2). Compared with control, unknown-acidolyzable nitrogen contents in BC3, BC4, and BC5 treatments was significantly decreased, among which BC5 treatment had the largest decreases of 45.0–63.1% in 2019 and 83.5–80.7% in 2020 ($p < 0.05$), followed by BC4 treatment by 35.2–57.6% in 2019 and 62.6–80.7% in 2020; in BC3 treatment, the reduction rate was 43.4–54.8% in 2019 and 42.0–48.1% in 2020. Similarly, in BC2 treatment, the unknown-acidolyzable nitrogen contents of 5–30 cm soil were significantly reduced by 26.9–40.5%. The effect of BC1 treatment on unknown-acidolyzable nitrogen contents in soil from the year 2019 to 2020 remained nonsignificant.

Non-acidolyzable nitrogen

In 2019 and 2020, the variation range of non-acidolyzable nitrogen contents in the 0 to 30-cm soil layer was 0.15–0.24 g kg^{-1} and 0.13–0.33 g kg^{-1}, respectively, accounting for 17.3–25.9% and 15.5–31.1% of the total nitrogen content in soil (Tables 1 and 2). With the increase of the biochar addition, the non-acidolyzable nitrogen contents in the 0 to 30-cm soil layer firstly decreased and then increased and showed a downward trend with the deepening of the soil layer (Fig. 4; 2019c; 2020c). In

Table 2 Proportion of soil organic nitrogen fractions to total soil nitrogen

Year	Depth of soil layer (cm)	Treatment	AAN (g kg^{-1})	AMN (g kg^{-1})	UAN (g kg^{-1})	ASN (g kg^{-1})	AIN (g kg^{-1})
2019	0–5	CK	29.01 ± 0.98c	23.59 ± 1.23bc	24.46 ± 1.89a	2.49 ± 0.61b	20.46 ± 3.62a
		BC1	32.83 ± 2.82bc	24.12 ± 1.66b	18.89 ± 2.33b	3.57 ± 0.43a	20.60 ± 3.94a
		BC2	33.31 ± 2.38bc	25.45 ± 1.70ab	12.72 ± 3.41c	3.68 ± 0.47a	24.83 ± 2.18a
		BC3	35.74 ± 0.22b	26.78 ± 2.02a	10.88 ± 2.81 cd	4.52 ± 0.49a	22.08 ± 4.09a
		BC4	40.84 ± 5.55a	21.20 ± 1.65c	9.36 ± 0.11d	3.99 ± 0.44a	24.62 ± 6.65a
		BC5	43.89 ± 3.91a	18.32 ± 1.47d	8.04 ± 2.24d	3.81 ± 0.62a	25.93 ± 4.52a
	5–10	CK	28.92 ± 0.91c	23.69 ± 0.55b	22.80 ± 2.84a	4.72 ± 0.90c	19.87 ± 2.48ab
		BC1	31.32 ± 3.60bc	25.43 ± 1.30ab	20.19 ± 4.59a	5.73 ± 1.04abc	17.33 ± 5.06b
		BC2	33.15 ± 1.25bc	26.21 ± 0.51a	12.73 ± 0.93b	6.43 ± 0.60abc	21.47 ± 0.63ab
		BC3	35.12 ± 2.01b	27.47 ± 1.10a	9.53 ± 2.88b	7.84 ± 1.13a	20.03 ± 2.51ab
		BC4	40.11 ± 0.32a	20.91 ± 2.03c	9.32 ± 1.39b	6.51 ± 0.60ab	23.15 ± 2.89ab
		BC5	42.66 ± 4.77a	17.97 ± 0.47d	8.66 ± 1.54b	5.12 ± 0.95bc	25.60 ± 4.45a
	10–30	CK	28.57 ± 0.62d	23.61 ± 0.34c	23.23 ± 0.87a	4.65 ± 0.74c	19.95 ± 0.68b
		BC1	29.41 ± 0.16d	24.07 ± 0.65c	21.20 ± 2.71a	5.19 ± 1.01bc	20.14 ± 2.34b
		BC2	30.53 ± 1.79d	25.39 ± 0.90b	15.79 ± 1.63b	5.81 ± 0.68b	22.48 ± 1.19ab
		BC3	33.38 ± 1.09c	26.97 ± 0.82a	12.22 ± 3.24bc	6.99 ± 0.52a	20.44 ± 2.78ab
		BC4	38.07 ± 2.10b	20.13 ± 1.36d	13.91 ± 2.19bc	6.19 ± 0.34ab	21.70 ± 2.03ab
		BC5	41.70 ± 1.02a	17.87 ± 1.16e	11.72 ± 0.79c	5.52 ± 0.94bc	23.20 ± 1.97a
2020	0–5	CK	27.77 ± 1.09d	22.79 ± 0.95bc	17.06 ± 0.97a	2.76 ± 0.67c	29.63 ± 0.26ab
		BC1	30.95 ± 3.13 cd	23.37 ± 1.11b	12.81 ± 0.52ab	3.88 ± 0.48b	28.99 ± 4.27abc
		BC2	33.03 ± 1.35bc	25.71 ± 1.41a	13.33 ± 4.29ab	4.47 ± 0.39ab	23.45 ± 3.76c
		BC3	35.89 ± 1.95b	26.23 ± 0.37a	8.35 ± 6.08bc	5.32 ± 0.65a	24.20 ± 4.98bc
		BC4	40.93 ± 1.32a	21.57 ± 1.01c	3.36 ± 2.00 cd	4.35 ± 0.43ab	29.80 ± 1.09ab
		BC5	44.34 ± 0.71a	19.00 ± 0.76d	1.59 ± 1.98d	3.92 ± 0.59b	31.14 ± 0.84a
	5–10	CK	27.46 ± 0.62d	22.05 ± 0.57bc	17.12 ± 1.83a	4.85 ± 1.03d	28.51 ± 1.85ab
		BC1	30.18 ± 1.26 cd	23.13 ± 1.59b	15.44 ± 3.40a	5.45 ± 0.89 cd	25.79 ± 1.38ab
		BC2	32.20 ± 1.92bc	24.55 ± 2.17ab	13.16 ± 4.10a	7.18 ± 0.08abc	22.91 ± 6.49b
		BC3	34.72 ± 1.55b	26.68 ± 0.11a	6.82 ± 5.77b	8.72 ± 0.80a	23.06 ± 3.81b
		BC4	40.38 ± 1.43a	21.88 ± 1.85bc	3.05 ± 2.46b	7.73 ± 1.2ab	26.95 ± 4.87ab
		BC5	41.56 ± 2.09a	19.29 ± 0.88c	2.51 ± 2.25b	6.32 ± 1.42bcd	30.31 ± 1.94a
	10–30	CK	28.92 ± 0.81d	23.71 ± 0.68c	17.64 ± 2.14a	4.64 ± 0.79d	25.08 ± 1.05ab
		BC1	29.70 ± 1.08 cd	24.47 ± 1.80bc	18.54 ± 0.69a	5.52 ± 0.71 cd	21.77 ± 2.41bc
		BC2	32.82 ± 3.42c	26.41 ± 1.28ab	16.87 ± 6.45a	7.06 ± 0.99b	16.83 ± 2.13de
		BC3	37.36 ± 0.46b	28.66 ± 1.08a	9.65 ± 1.85b	8.86 ± 0.42a	15.47 ± 3.35e
		BC4	42.78 ± 1.93a	22.78 ± 0.55c	6.24 ± 3.12bc	7.78 ± 0.96ab	20.41 ± 1.25 cd
		BC5	45.13 ± 3.06a	19.92 ± 1.92d	2.66 ± 3.42c	6.74 ± 0.93bc	25.55 ± 2.09a

The values in the table are mean ± SD ($n = 3$), means sharing the lowercase letters, for a parameter do not differ significantly at $p \leq 0.05$; *AAN*, amino acid nitrogen; *AMN*, ammonium nitrogen; *UAN*, unknown-acidolyzable nitrogen; *ASN*, amino-sugar nitrogen; *AIN*, non-acidolyzable nitrogen; *CK*, without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; BC5 = 50 t ha^{-1}

the 0 to 10-cm soil layer compared with the control treatment, the non-acidolyzable nitrogen contents in the 5 to 10-cm soil layer of BC5 treatment increased by 44.8% in 2019, and there was a nonsignificant difference in the non-acidolyzable nitrogen contents in the other biochar treatments ($p < 0.05$). Correspondingly, in the 10 to 30-cm soil layer, compared with the control treatment, the non-acidolyzable nitrogen contents in BC2 and BC5 treatment increased by 21.4% and 26.9%, respectively, in 2019, and the non-acidolyzable nitrogen contents in BC2 and BC3 treatments decreased by 30.4% and 35.4%, respectively, in 2020 ($p < 0.05$).

Proportion of soil organic nitrogen fractions to soil total nitrogen

In 2019 and 2020, the proportion of organic nitrogen fractions in soil treated by biochar to soil total nitrogen was in descending order as follows; amino acid nitrogen > non-acidolyzable nitrogen > acidolyzable ammonium nitrogen > unknown-acidolyzable nitrogen > amino-sugar nitrogen (Table 2). In the 0 to 30-cm soil layer, the ratio of soil amino acid nitrogen to soil total nitrogen was increased gradually with the increase of biochar supplemental level. Compared with control, the ratio of soil acidolyzable ammonium nitrogen content to soil total nitrogen in BC3, BC4, and BC5 treatments increased significantly, among which BC5 treatment increased by 14.1–16.6% in 2019 and 14.1–16.6%, respectively, in 2020 ($p < 0.05$). The proportion of acidolyzable ammonium nitrogen content to soil total nitrogen showed a trend of increase and then decrease. Compared with control, BC3 treatment showed the highest increase, increasing by 13.5–16.0%, respectively, in 2019 and 15.5–21.0%, respectively, in 2020 ($p < 0.05$). BC5 treatment showed the highest decrease, decreasing by 22.3–24.3%, respectively, in 2019 and 12.5–16.6%, respectively in 2020 ($p < 0.05$). The proportion of unknown-acidolyzable nitrogen content in soil total nitrogen showed a decreasing trend. In 2019, the proportion of unknown-acidolyzable nitrogen content in soil total nitrogen of other biochar treatments decreased significantly except BC1 treatment, which showed a nonsignificant difference compared with the control. In 2020, the proportion of unknown-acidolyzable nitrogen content to soil total nitrogen in BC3, BC4, and BC5 treatments significantly decreased, and BC5 treatments showed the highest decrease by 49.6–67.1% in 2019 and 84.9–90.7% in 2020, respectively. The proportion of amino-sugar nitrogen to total nitrogen in soil showed a trend of first increasing and then decreasing, with the highest increase in BC3 treatment, 50.3–81.5% in 2019 and 79.8–93.1% in 2020, respectively, and the highest increase in the 0 to 5-cm soil layer was observed. The ratio from non-acidolyzable nitrogen to total nitrogen in soil layers increased by 16.3% in 2019 in BC5 treatment, decreased by 20.9% and 32.9% in BC2 treatment, and decreased by 38.2% and 18.6% in BC3 and BC4 treatments, respectively.

Soil microbial biomass carbon and nitrogen

Compared with control, the addition of biochar significantly increased the soil microbial biomass carbon in the 0 to 30-cm soil layer in 2019 (Fig. 6a and b). The maximum increase of microbial biomass carbon in BC5 treatment was 55.1–81.4% ($p < 0.05$). However, in the 0 to 30-cm soil layer, the soil microbial biomass carbon content decreased in 2020 compared with that in 2019. In the 0 to 5-cm soil layer, the soil microbial biomass carbon content decreased in 2020 compared with that in 2019. In the 0 to 5-cm soil layer, the soil microbial biomass content of BC1 treatment did not differ significantly compared with control. In the 5 to 30-cm soil layer, the soil microbial biomass content of BC4 treatment had a nonsignificant difference compared with control. In addition to the above conditions, other biochar treatments could increase the soil microbial biomass carbon content in 0 to 30-cm soil layer, and the increase of microbial biomass carbon content in BC5 treatment was 68.6–139.7%. Compared with control in 2019, soil microbial biomass nitrogen content in biochar treatment improved significantly

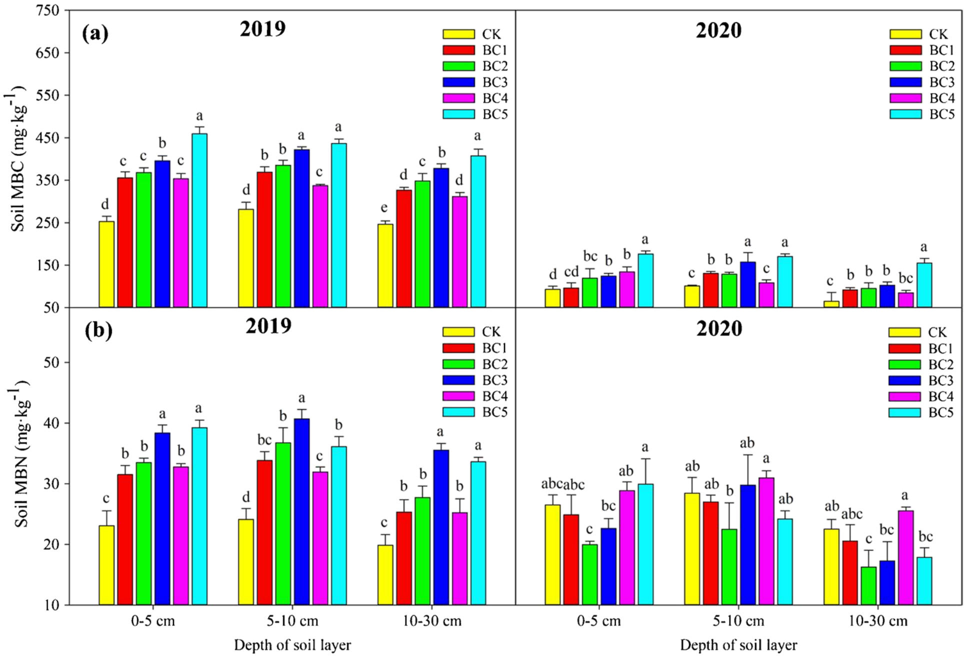

Fig. 6 Effects of biochar addition on soil microbial biomass carbon (**a**) and soil microbial nitrogen (**b**) in different soil layers during years 2019 and 2020. The results are the averages and standard deviations of three replicates. Bars sharing the same case letters, for interaction and main effects for a parameter, do not differ significantly at $p \leq 0.05$. CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; BC5 = 50 t ha^{-1}. *MBC*, microbial biomass carbon; *MBN*, microbial biomass nitrogen

(Fig. 6). BC3 treatment had the highest increase in soil microbial biomass nitrogen contents, increasing by 66.3–78.9%, respectively, while BC4 treatment had a small increase in soil microbial biomass nitrogen content increasing by 27.0–42.1%, respectively, in 2019–2020. In 2020, the 0 to 5-cm soil layer showed a trend of the first decrease and then increase, and the 5 to 10-cm and 10 to 30-cm soil layers showed a trend of the first decrease, then increase and then again decrease trends, and the minimum values were all presented in BC2 treatment. BC2 treatment in the 10 to 30-cm soil layer significantly reduced 27.7%, compared with control, and a nonsignificant difference was found in other treatments compared with control (Fig. 6). The soil microbial biomass nitrogen content in the 0 to 30 cm soil layer decreased compared with that in 2019. In 2019 and 2020, the variation range of B_C/B_N in the 0 to 30-cm soil layer was 11.0–12.4% in 2019 and 2.9–3.6%, respectively, in 2020 as compared to control (Table 3). Compared with control, the addition of biochar in 2020 increased the B_C/B_N in the 0 to 30-cm soil layer (Table 3).

Wheat yield

The addition of biochar caused a significant increase in wheat yield to varying degrees (Table 1; Fig. 7). In 2019 and 2020, the yield of wheat under the treatment of biomass charcoal was higher than that of CK. However, in 2019, only under the BC3 treatment, the wheat yield significantly increased by 21.6% compared with CK; and in 2020, the biomass charcoal treatment significantly increased the wheat yield compared with CK. The BC1, BC2, BC3, BC4, and BC5 treatments caused a 5.2%, 17.9%, 24.8%, 14.6%, and 10.3% increase in wheat grain yield, respectively, compared with control.

Correlation of soil organic nitrogen fractions with soil total nitrogen and microbial biomass carbon and nitrogen

In 2019, soil MBC content had a significantly positive correlation with soil AAN, AIN, and ASN content ($p < 0.01$), and a significant negative correlation with soil UAN content

Table 3 Effects of biochar treatment on soil microbial biomass carbon/microbial biomass nitrogen ratio under different soil layers

Year	Depth of soil layer (cm)	Microbial biomass carbon/microbial biomass nitrogen					
		CK	BC1	BC2	BC3	BC4	BC5
2019	0–5	10.96	11.28	10.98	10.31	10.78	11.69
	5–10	11.66	10.90	10.48	10.36	10.56	12.08
	10–30	12.38	12.87	12.54	10.63	12.35	12.11
2020	0–5	3.49	3.86	5.96	5.47	4.64	5.88
	5–10	3.55	4.82	5.72	5.27	3.50	7.04
	10–30	2.87	4.44	5.83	5.93	3.31	8.68

CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; BC5 = 50 t ha^{-1}

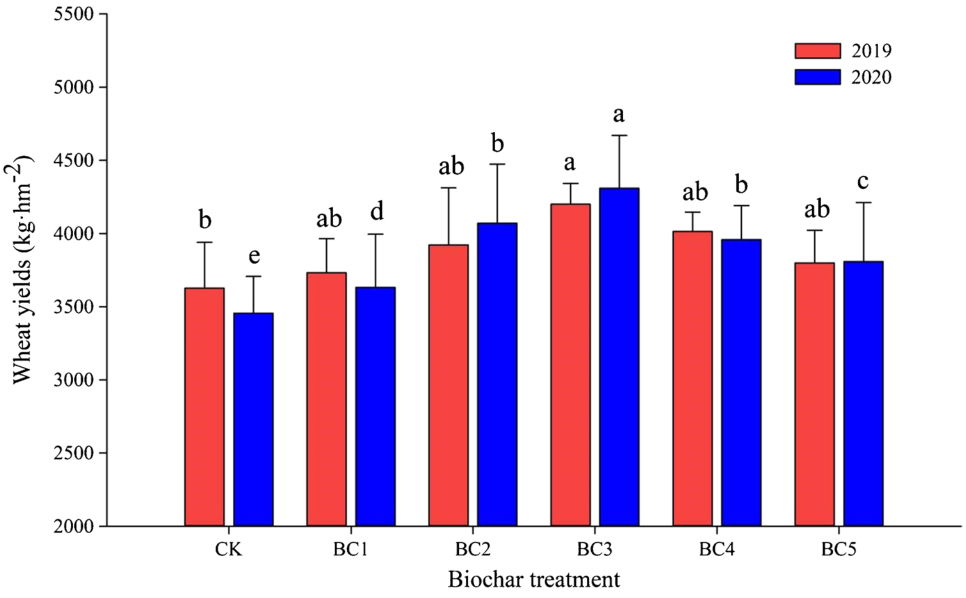

Fig. 7 Effects of biochar addition on wheat yield during years 2019 and 2020. The results are the averages and standard deviations of three replicates. Bars sharing the same case letters, for interaction and main effects for a parameter, do not differ significantly at $p \leq 0.05$. CK = without biochar; BC1 = 10 t ha^{-1}; BC2 = 20 t ha^{-1}; BC3 = 30 t ha^{-1}; BC4 = 40 t ha^{-1}; BC5 = 50 t ha^{-1}

($p < 0.01$; Table 4). However, correlations between soil MBC content and soil AMN and ASA in 2020 were nonsignificant. In 2019, soil MBN content was positively correlated with AAN, AIN, and ASN contents ($p < 0.01$), and with AMN content ($p < 0.05$), and were negatively correlated with the UAN content ($p < 0.01$). In 2020, there was a positive correlation between MBN content and AIN content ($p < 0.01$). There was a significant positive correlation between TN content and AAN and AIN content in 2 years ($p < 0.01$), and a significant negative correlation between TN content and UAN content in 2 years ($p < 0.01$).

Discussion

After 5 years, maize straw biochar amendment significantly affected soil total N, organic N fractions, microbial biomass C, and wheat yields. Soil total N increased with increasing biochar application rate, with the highest content in the BC5 treatment (Fig. 3). Biochar has a large specific surface area (11.3 m^2 g^{-1}), which can enhance the soil nitrogen uptake (Long et al. 2019) and reduce the loss caused by nitrogen leaching (Li et al. 2017), thus significantly increasing the total nitrogen content in the soil. The effect of biochar on microbial biomass carbon and nitrogen is not consistent due to differences in soil and biochar type in previous studies (Walelign and Mingkui 2015; Oladele et al. 2019). In the current study, the content of soil MBC increased with the increase of biochar content; on the other hand, the application of biochar increased the soil pH (Haider et al. 2021a), enhanced the soil water holding capacity (Pokharel et al. 2020) and porosity (Haider et al. 2021b), reduced soil bulk density (Rumpel 2011), and provided optimum living conditions for soil microorganisms survival (Gul et al. 2015), thus increasing the soil microbial biomass. Due to the presence of the higher carbon content and rich nutrient elements of biochar, the application of biochar can directly provide optimum nutrients for microbial growth (Chan and Xu 2009), and the strong adsorption of nutrients, i.e., nitrogen and phosphorous enhances the ability of soil to supply nutrients (Lehmann et al. 2011). The content of MBN decreased at first and then increased and then again decreased with the increase in the concentration of biochar, but the difference among treatments was not significant, and the content of BC1 (10 t ha^{-1}) was the lowest in each soil layer. This might be due to the high pH value of biochar used in this experiment. With the increase of biochar concentration in soil, the soil pH value increases, which leads to a decrease in the fungal biomass in the soil. Consistently, Yuan et al. (2019) found that the soil MBN content was the highest after the addition of (30 t ha^{-1}) wheat straw biochar for 1 year and decreased gradually with the increase of application rate, which may be due to the difference of soil acidity, biochar properties, pyrolysis temperature for biochar processing, feedstock used for biochar, climatic condition variations, and may be due to the different sampling years after adding biochar (Yang et al. 2019; Haider et al. 2021b). The ratio of B_C/B_N is an important index to measure the availability of soil microbial carbon and nitrogen and the change of microbial composition. The ratio of B_C/B_N showed a trend of increasing first and then decreasing and then increasing with the increase of biochar concentration in soil. Under the condition of conventional fertilizer, the addition of excessive biochar increased the C/N ratio of soil, resulting in the decrease of the proportion of active nitrogen that could be directly utilized by microorganisms in the soil (Yang et al. 2019), and the sequestration of nitrogen (Oladele et al. 2019), which was more like a carbon pool than a nitrogen source (Zhang et al. 2016) to soil microorganisms.

Soil acid hydrolyzed organic nitrogen is an active part of the soil nitrogen pool, which is easily distributed by human activities (Xu et al. 2016). The transformation rate of nonacid hydrolyzed nitrogen in the soil is slow, so it is a stable and difficult mineralization component in the soil nitrogen pool. In the current study, the addition of biochar significantly increased the content of total nitrogen in the soil acid hydrolysis but had no significant effect on the content of nonacid nitrogen. The overall performance of soil organic nitrogen was as follows amino acid nitrogen > non-acidolyzable nitrogen > acidolyzable ammonium

Table 4 Pearson correlation analysis of soil organic nitrogen fractions, soil total nitrogen (TN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN)

Year	Index	AAN	AIN	AMN	UAN	ASN
2019	MBC	0.666**	0.392**	ns	0.740**	0.357**
	MBN	0.598**	0.353**	0.322*	0.732**	0.404**
	TN	0.605**	0.725**	ns	0.582**	ns
2020	MBC	0.674**	0.472**	ns	0.603**	ns
	MBN	ns	0.573**	ns	ns	ns
	TN	0.639**	0.801**	0.299*	0.437**	ns

*Significant at 0.05 probability; **significant at 0.01 probability; *ns*, no significance; *TN*, total nitrogen; *AAN*, amino acid nitrogen; *AMN*, ammonium nitrogen; *UAN*, unknown-acidolyzable nitrogen; *ASN*, amino-sugar nitrogen; *AIN*, non-acidolyzable nitrogen; *MBC*, microbial biomass carbon; *MBN*, microbial biomass nitrogen

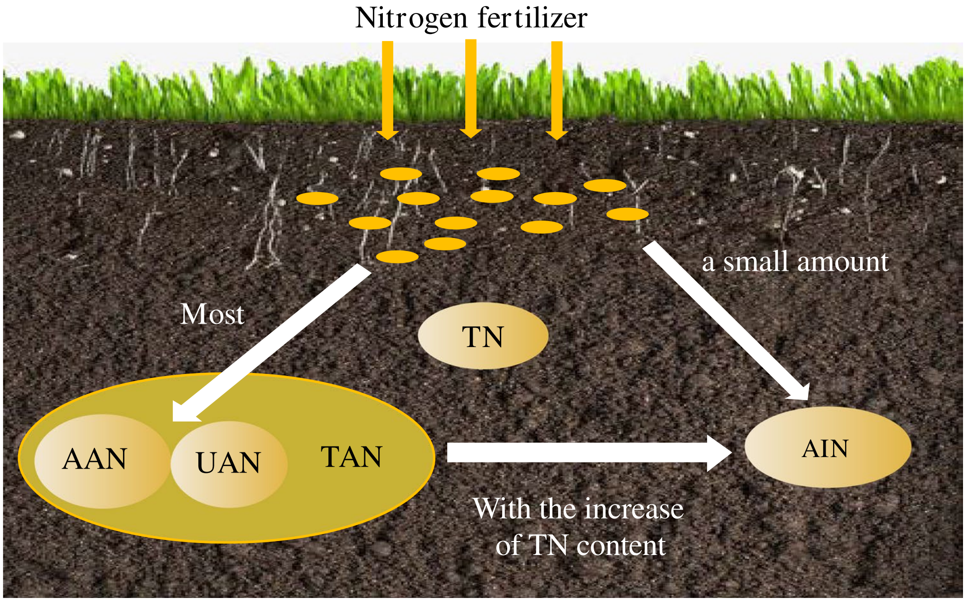

Fig. 8 Effect of nitrogen application on soil organic nitrogen dynamics. After the application of nitrogen fertilizer in the soil, most of them would be transformed into soil amino acid nitrogen (AAN) and acid-hydrolyzed unknown nitrogen (UAN), and only a small part would be transformed into nonacid hydrolyzed nitrogen (AIN). However, with the increase of soil total nitrogen (TN) content, soil total acid-hydrolyzed organic nitrogen (TAN) would be transformed into nonacid hydrolyzed nitrogen (AIN)

nitrogen > unknown-acidolyzable nitrogen > amino-acidolyzable nitrogen, which was similar to many research results (Zhang et al. 2016). The results showed that with the increase in the biochar concentration, the changing trend of acid hydrolyzed total nitrogen and nonacid hydrolyzed nitrogen was opposite, and the nonacidified nitrogen decreased at first and then increased. Some studies (such as Lu et al. 2009; Li et al. 2013) reported that when nitrogen fertilizer was applied in soil, most of them were transformed into amino acid nitrogen and acid-hydrolyzed unknown nitrogen. With the increase of soil total nitrogen content, acid-hydrolyzed organic nitrogen begins to transform to nonacid hydrolyzed nitrogen (Fig. 8). The soil total nitrogen content increases with the increase of biochar concentration, which is consistent with the results of the above researchers. Acid ammonium nitrogen and acid amino-acid nitrogen are important sources of plant absorption and utilization of mineralization nitrogen, as well as the source and pool of soil mineralization organic nitrogen (Zhang et al. 2016), which is closely related to soil nitrogen supply capacity (Xiang et al. 2013). Amino acid nitrogen may come from the deamination of amino sugar, amide, purine, and pyrimidine, the decomposition of hydroxyl and other amino acids, and the release of fixed ammonium in the soil (Hu et al. 2020).

The proportion of acid ammonium nitrogen and acid amino acid nitrogen in organic nitrogen fractions was higher, which was the same as that of Dang et al. (2011) in the study of typical soil organic nitrogen fractions in the Loess Plateau. Correlation analysis showed that amino acid nitrogen was positively correlated with soil total nitrogen and MBC, and acid hydrolyzed ammonium nitrogen was positively correlated with soil total nitrogen, which indicated that the application of biochar promoted the degradation of macromolecular complex organic matter by soil microorganisms on the basis of increasing soil total nitrogen and MBC content and increased inorganic nitrogen source and low molecular organic nitrogen in the soil (Li et al. 2017). However, the content of both decreased with the deepening of the soil layer, which was mainly due to the application of chemical fertilizers according to the conventional amount of fertilizers before sowing, which led to the higher nitrogen content in the surface layer of soil than in the lower layer. The mineralization rate of unknown nitrogen in the soil is slow, and it is easy to accumulate (Li et al. 2013; Fawzy et al. 2020). It is mainly composed of non-α-amino acid nitrogen, aliphatic amine, and aromatic amine (Xu et al. 2003; Nieder et al. 2011).

With the increase of biochar concentration, the content of unknown nitrogen in each soil layer showed a reducing trend. The correlation analysis showed that its content was negatively correlated with soil total nitrogen, amino acid nitrogen, and MBC content, which indicated that the addition of biochar promoted the decomposition and transformation of acid hydrolyzed unknown nitrogen in the soil. The proportion of amino-acid nitrogen in soil total nitrogen and acid hydrolyzed organic nitrogen fractions was relatively low (Nieder et al. 2011; Song et al. 2020), which was mainly due to the residue of the microbial cell wall (Jing et al. 2020). The content mainly reflected the accumulation degree of dead microorganisms in the soil (Hu et al. 2020). The results showed that the content of amino-acid nitrogen increased at first and then decreased with the increase of biochar concentration and deepening of the soil layer, and the content of biochar (30 t ha^{-1}) was the highest, which was similar to the existing results (Yuan et al. 2019). The addition of optimum an amount of biochar improved the soil environment and provided a good environment for microbial survival (Haider et al. 2021a). Similarly, with the increasing concentration of biochar, the water-holding capacity of soil increased and the growth and reproduction of microorganisms were inhibited. In crux, the contents of total soil nitrogen, acid-hydrolyzed ammonium nitrogen, and amino-acid nitrogen were the highest under BC3 treatment, while those of unhydrolyzed nitrogen was the lowest. Under BC5 treatment, the contents of amino-acid nitrogen and acid-hydrolyzed unknown nitrogen were the highest and lowest, respectively, which indicated that the active part of soil organic nitrogen was under BC3 and BC5 treatments. Therefore, in the dry farming area of the Loess Plateau, the application of biochar is of great significance to improve the soil nitrogen supply capacity and crop yield.

Conclusions

The current study demonstrated that the soil total N, amino acid N, microbial biomass C, and wheat yield increased, while unknown nonacid hydrolyzable N decreased after 4–5 years of addition of maize straw biochar (10–50 t ha^{-1}). However, variations were apparent among different levels of biochar application. The BC3 treatment (30 t ha^{-1}) had the highest wheat yield among different treatments, which was mainly attributed to the beneficial effect of this treatment on soil N fractions, soil microbial biomass N, acid-hydrolyzed ammonium nitrogen, and amino-acid nitrogen content. Overall, the application of biochar at an optimum level can help improve soil nutrient supply capacity, soil microbial activities, and productivity of field crops in semiarid and arid regions.

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Author contribution P.Z. contributed to data collection and write up of the manuscript; F.U.H. contributed to writing, reviewing, and editing; S.H., M.F., and C.X. participated in coordination and helped to draft the manuscript; C.L. contributed to supervision and funding acquisition. All authors have read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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