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Evaluating the impact of biochar on biomass and nitrogen use efficiency of sugarcane using ^{15}N tracer method

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N is an essential nutrient for sugarcane (Saccharum spp. Hybribds) growth. Excess chemical nitrogen fertilizer applied still a serious problem of China sugarcane plant. Biochar has shown promise in improving crop yield and N use efficiency (NEU).However its impact on sugarcane is not well-studied. To investigate how biochar impacts on sugarcane growth and nitrogen N use efficiency (NUE), a glasshouse pot experiment was conducted using the ^{15}N tracer method. Two cultivars, GT11 and B8, were chosen as test objects and were planted under low N(120 kg N hm^{-2}) and high N(600 kg N hm^{-2})condition, respectively. The effects of low and high biochar application rates (10 t hm^{-2} and 20 t hm^{-2}) on growth, nitrogen uptake, accumulation and distribution as well as NUE in GT11 and B8 were studied. Results showed that sugarcane biomass was not significantly affected by biochar application. N uptake by GT11 was significantly increased 23.91% - 45.42% by C20 and N120 condition at tillering stage and elongation stage. While N uptake by B8 showed a significant response to B10 and B20 with an increase of 27.27% and 30.40% at tillering stage,respectively. Biochar application led to 0.28% - 23.75% and 1.08% - 30.07% increase in NUE of GT11 and B8,respectively. The effect of biochar application of N from fertilizer(FF) was significant,however only C20 treatment shown remarkable response when under low N treatment. Our study suggest that the effects of biochar on sugarcane depend on varieties and the applied rate of biochar and N fertilizer.Biochar application with inorganic N could improve N uptake and N use of sugarcane.

KEYWORDS

biochar, nitrogen use efficiency, biomass accumulation, sugarcane, ^{15}N isotope tracer

Highlight

1. Biochar improve the biomass of sugarcane genotype with low NUE.
2. Biochar could increase the N uptake and N from fertilizer of sugarcane.
3. High biochar application improve sugarcane take more N from fertilizer.

Introduction

Biochar is a carbon-rich substance prepared under hypoxic or anaerobic, low temperature (<800 °C) conditions (Lehmann et al., 2005). Biochar has shown great application potential in soil improvement (Pan et al., 2021), crop yield increase (Omara et al., 2020), and environmental restoration (AliZahed et al., 2021; Başer et al., 2021). It has received extensive attention from domestic and abroad researchers. Nitrogen is the primary nutrient element for crop production. Numerous studies have focused on the effects of biochar on the N utilization of soil and crops. The raw material source and preparation temperature of biochar will affect its ability to absorb nitrogen N (Liao et al., 2018), as well as the growth and N use efficiency of crops (Shanta et al., 2016; Egamberdieva et al., 2019; Olszyk et al., 2020). Biochar can increase the yield of corn (Omara et al., 2020), wheat (Zee et al., 2017), rice (Huang et al., 2014; Ali et al., 2020), and other crops (Lou et al., 2016; Haque et al., 2019; Li et al., 2021), and increase the utilization rate of N fertilizer. Its short-term effect is more evident under the condition of soil nutrient deficiency; however, its effect on increasing the yield of fertile soil is far from significant or even invalid (Jeffery et al., 2017; Vijay et al., 2021). Huang et al. (2019) experiment with biochar application on rice for six consecutive seasons; the N utilization rate increased by 7%–11% only during the fifth and the sixth seasons, indicating that the application of biochar must be repeated for a long period of time to increase the internal N utilization and yield of rice. The impact of biochar on crop yield and N utilization varies with crop or biochar species, application amount, and time.

Guangxi, a major sugarcane planting province in China, accounts for more than 60% of the country's planting area and sugar production. The application of N fertilizer is an essential guarantee for increasing sugarcane yield and sugar content (Li et al., 2016). However, the current large-scale application of N fertilizer in sugarcane production has caused problems such as low fertilizer utilization, soil acidification, compaction, toxin accumulation, and reduced fertility (Zeng et al., 2020). Low N utilization efficiency of sugarcane is one of the main problems restricting the increase of sugarcane yield in China. Controlling or reducing the amount of N fertilizer application while continuously increasing sugarcane yield and minimizing the negative impact of excessive nitrogen fertilizer application has always been an important scientific issue for sugarcane-growing countries (Chandrasekaran et al., 2014; Li et al., 2016; Prasara et al., 2019). Previous studies have found that biochar can improve the root characteristics of the sugarcane seedlings and increase their root-shoot ratio (Liu et al., 2015). These effects may be related to the fact that biochar can increase soil pH, reduce N loss in the soil in the early and mid-term growth stages and promote the availability of nitrogen, phosphorus, and potassium in the soil (Liao et al., 2019a). However, these experiments are only the results of a single variety and a single nitrogen treatment and cannot fully reflect the effects of biochar on sugarcane growth and N utilization (Liao et al., 2019b). In this study, we selected two varieties with different nitrogen use efficiency and studied the effects of biochar on sugarcane growth, nitrogen absorption, cumulative distribution, and nitrogen utilization efficiency under different nitrogen treatment conditions using the ^{15}N tracer method to explore the effects of biochar on sugarcane growth and N utilization. The results provide theoretical and technical references for applying biochar in sugarcane production and reducing the dependence on nitrogen fertilizer.

Materials and methods

Test materials

The sugarcane genotypes, Guitang 11 (GT11, N-inefficient) and B8 (N-efficient), were provided by the Germplasm Resource Garden of Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences. The preliminary test found that the nitrogen use efficiency of the two varieties differed by about 1.0 fold.

Experimental design and treatments combination

The pot experiment was conducted from February 2016 to February 2017 in the greenhouse at Sugarcane Research Institute, Guangxi Academy of Agricultural Science, Nanning, China. Plastic pots of 40 cm in diameter and 40 cm in height were used. Biochar at the rate of 0 g (C0, equivalent to 0 t hm^{-2}), 70 g (C10, equivalent to 10 t hm^{-2}), and 141 g (C20, equivalent to 20 t hm^{-2}) per pot was mixed with soil (30 kg) and incubated. We applied ^{15}N labeled urea at two rates: 1.8 g (N120, equivalent to 120 kg hm^{-2} nitrogen fertilizer) and 9.0 g per pot (N600, equivalent to 600 kg hm^{-2} nitrogen fertilizer). Each treatment had six replicates. Two sugarcane plants were planted in each pot. we applied P fertilizer 3.0 g (equivalent to 450 kg $P_2O_5{\cdot}hm^{-2}$) and potassium K fertilizer 2.0 g (equivalent to 225 kg K_2O hm^{-2})

to each pot, and all the fertilizers were applied only once as base fertilizer when sugarcane is planted. The biochar was mixed and incubated with the aired dry soil one day in advance. The germination, planting, and growth management of the seed stems were the same as the experiment by Liao et al. (2019).

The soil was collected from a depth of 0–20 cm from the sugarcane test field of the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences. It is classified as Fe-leachi-Stagnic Anthrosols (Cooperative Research Group on Chinese Soil Taxonomy, CRGCST 2001) with a pH of 6.25, electrical conductivity of 42.12 mV, total C content of 0.72 g kg^{-1}, organic matter content of 15.1 g kg^{-1}, alkali-hydrolyzable nitrogen (N) of 91.23 mg kg^{-1}, phosphorus (P) of 43.41 mg kg^{-1}, and potassium (K) of 152.03 mg·kg^{-1}.Soil was aired dry and was broken up less than 5 cm with a rubber mallet.

The test biochar was produced from cassava stems using pyrolysis conditions described by Liao et al. (2019). The total C, N, and H of biochar were 67.4%, 0.8%, and 2.18%, respectively. The content of total phosphorus, and potassium were3.53, and 13.40 g·kg^{-1}. We used ^{15}N-labeled urea (Shanghai Chemical Plant Ltd.) as the nitrogen fertilizer, with an abundance of 10.18% and a nitrogen content of 46%). Biochar was ground less than 2 mm before mixed and incubated with dry soil.

Plant yield contents

Samples of sugarcane were collected in three periods: the seedling period (four months after transplanting), the elongation period (seven months after transplanting), and the maturity period (12 months after transplanting). Three pots were collected for each treatment, and samples were classified according to roots, stems, dried leaves, and green leaves, and the fresh weight (FW) data were recorded simultaneously. Dried the collected materials at 60 °Ctill to constant weight, recorded the dry weights (DW, g/pot). The dried samples were ground to determine the total nitrogen content and ^{15}N abundance.

Determination of plant N content, total N uptake and nitrogen use efficiency

The abundance of ^{15}N was determined using an isotope mass spectrometer (Thermo Fisher, Waltham, MA, USA). Total N was determined by VAP50 Kjeldahl meter (Gerhardt, Königswinter, Germany). N uptake, Ndff (recovery percentage of plant-derived from ^{15}N-urea), N uptake from fertilizer, N uptake from the soil, and N use efficiency were calculated using the following equation (Omara et al., 2020):

$$\text{Total N uptake (TN uptake, g/pot)} = \text{total N concentration in plant} \times \text{total plant DW}$$

$$\%\text{Ndff} = \left({}^{15}\text{N abundance in plant - background}{}^{15}\text{N abundance}\right) / \left({}^{15}\text{N abundance in fertilizer- background}{}^{15}\text{N abundance}\right);$$
$$\text{where, the background}{}^{15}\text{N abundance} = 0.3663\ \%.$$

$$\text{Ndff (mg/pot)} = \text{Plant DW} \times \text{N content} \times \%\ \text{Ndff}$$

$$\text{N uptake from fertilizer (FF , \%)} = \text{Ndff (mg/pot)} \text{ x } 100 / \text{plant TN uptake}$$

$$\text{N uptake from soil (FS , \%)} = (\text{total N uptake - N uptake from fertilizer}) \times 100 / \text{plant TN uptake}$$

$$\text{Nitrogen use efficiency (NUE, \%)} = \text{N uptake} \times 100/\text{N applied}$$

Statistical analysis

All data were processed and analyzed using Excel 2007. SPSS19.0 statistical package program was used for the analysis of variance (SPSS Institute, USA). One-way snalysis of variance and the least significant difference test (LSD) were used to assess the statistical differences between the biochar treatments at N120 or N600 level. The level of significance was assessed by 0.05 probability level.

Results

The effect of biochar and N fertilizer treatment on biomass of sugarcane

A considerable difference in the effect of biochar co-treatment with N fertilizer on the biomass of the two varieties was observed, as shown in Table 1. Two varieties were significantly different in the DW of root (p = 0.005), stem (p< 0.001), leaves (p< 0.001), and total DW (p< 0.001). The accumulation of sugarcane biomass was more affected by nitrogen application rate (p< 0.001), with an exception as the root DW (p = 0.208). Biochar could increase the DW of GT11 under low nitrogen conditions (N120). Total DW was 73.05, 195.21, and 347.49 g/pot at tillering stage, elongation stage, and maturation stage without biochar application (C0), respectively. Compared with control C0, total DW increased by 1.38%–16.28%, 4.98%–17.41% (p< 0.05) and 0.25%–6.61% after C10 and C20 treatment, respectively. These were mainly due to DW increases in the stem, green leaf, and root. Biochar application, under N600 treatment, did not show any significant effect of increasing DW of GT11.

TABLE 1 Effects of biochar and N fertilizer treatment on dry weight(DW) of two sugarcane varieties.

Variety	N treatment	Growth stages	Biochar treatment	Root (g/pot)	Shoot (g/pot)	Green Leaves (g/pot)	Senescence leaves (g/pot)	Whole plant (g/pot)
GT11	N120	Tillering stage	C0	16.34a	27.08a	23.36a	6.27a	73.05a
			C10	15.23a	26.77a	24.55a	7.51a	74.06a
			C20	17.85a	31.71a	28.03a	7.34a	84.94a
		Elongated stage	C0	37.60a	112.51a	17.03a	28.07a	195.21b
			C10	44.67a	117.79a	17.67a	24.80a	204.93ab
			C20	43.83a	140.60a	20.30a	24.47a	229.20a
		Mature stage	C0	38.79a	235.46a	22.93a	50.30a	347.49a
			C10	25.16b	241.41a	22.68a	59.12a	348.37a
			C20	32.36ab	253.97a	20.19a	63.92a	370.45a
	N600	Tillering stage	C0	11.93a	55.72a	63.60a	3.00a	134.26a
			C10	9.53a	52.70a	60.24a	4.74a	127.21a
			C20	15.63a	44.54a	54.90a	3.95a	119.02a
		Elongated stage	C0	38.67a	216.27a	41.37a	42.97a	339.27a
			C10	43.13a	179.30a	35.73a	37.60a	295.77a
			C20	40.30a	216.99a	36.60a	33.20a	327.09a
		Mature stage	C0	35.05a	417.05a	47.77a	68.84a	568.71a
			C10	42.89a	347.51a	52.16a	110.37a	552.94a
			C20	47.93a	307.89a	27.38a	85.37a	468.57a
B8	N120	Tillering stage	C0	12.42a	29.58a	29.03a	7.90a	78.93a
			C10	12.85a	18.23b	36.09a	8.12a	75.29a
			C20	13.26a	22.69ab	29.81a	8.40a	74.16a
		Elongated stage	C0	67.07a	134.36a	25.33a	32.83a	259.59a
			C10	48.47b	132.79a	23.87a	52.20a	257.33a
			C20	44.20b	122.77a	23.67a	25.13a	215.77a
		Mature stage	C0	50.65a	191.77a	21.30a	63.52a	327.24a
			C10	43.48ab	172.81a	20.71a	62.33a	299.33a
			C20	39.21b	207.76a	24.61a	78.28a	349.86a
	N600	Tillering stage	C0	9.36a	38.92a	58.81a	3.46a	110.55a
			C10	7.92a	41.60a	54.71a	6.06a	110.29a
			C20	8.15a	40.33a	58.60a	4.16a	111.24a
		Elongated stage	C0	52.33a	181.08b	40.07b	48.63a	322.11a
			C10	48.50a	183.96b	40.63b	24.87b	297.96a
			C20	50.70a	217.39a	47.83a	45.40a	361.32a
		Mature stage	C0	34.54ab	278.75a	46.08a	115.52a	474.89a
			C10	45.99a	267.49a	59.29a	117.38a	490.15a
			C20	32.75b	319.98a	47.96a	108.78a	509.47a
F-values and significance level								
Variety				.005	<0.001	.054	<0.001	<0.001
C treatment				0.527	0.551	0.635	0.331	0.718
N treatment				0.208	<0.001	<0.001	<0.001	<0.001
C and N interaction(C × N)				0.013	0.105	0.485	0.783	0.189

C0, 0 t hm^{-2} biochar; C10, 10 t hm^{-2} biochar; C20, 20 t hm^{-2} biochar; N120,120 t hm^{-2} N fertilizer; N600,600 t hm^{-2} N fertilizer; Different letters within the columns show a significant difference between the treatments at P=0.05 according to the LSD test.

After biochar was applied, the total DW of B8 was slightly higher than that of the control (C0), mainly under high nitrogen (N600) and high carbon (C20) conditions. Under this condition, the total DW of B8 was 111.24 g/pot, 361.32 g/pot, and 509.47 g/pot at tillering stage, elongation stage, and maturation stage, respectively, which were 0.63%, 12.17%, and 7.28% higher than that of C0 treatment, but did not reach a significant level of difference. These increases are mainly attributable to an enhancement in the stem and leaves DW.

Effects of biochar and N fertilizer treatment on nitrogen accumulation and distribution in sugarcane

As shown in Table 2, the N accumulation in stems, green leaves, and senescence leaves of the two varieties was significantly different after treatment ($p< 0.001$). Both carbon and N treatments significantly affected N accumulation in sugarcane stems, senescence leaves, green leaves, and whole plants ($p< 0.001$), but there was no significant interaction between carbon and N treatments. Biochar treatment promoted the increase of nitrogen accumulation in stem, and leaves of both varieties at different growth stages.

The nitrogen accumulation of GT11 in the whole plant was between 665–4854 mg/pot under low nitrogen conditions. Compared with C0 treatment, the N accumulation of GT11 increased by 7.97%–45.42% after biochar application, while N accumulation of roots, stem, and leaves also increased 2.05%–50.68% in each reproductive period. In particular, C20 treatment significantly increased the nitrogen accumulation of the whole plant in the tillering and elongation stages, which were 23.91% ($p< 0.05$) and 45.42% ($p< 0.05$) higher than the control (665 mg/pot and 3338 mg/pot), respectively. Green leaf nitrogen accumulation also increased by 33.74% ($p< 0.05$) and 43.85% ($p< 0.05$) compared with the control of 368.81 mg/pot and 453.99 mg/pot, respectively, while 50.68% ($p< 0.05$) increase in stem and 40.70% ($p< 0.05$) increase in roots were obtained mainly during the elongation stage.

Under high nitrogen condition, biochar application could boost the N accumulation in the whole plant. In GT11, N accumulation increased by 0.33%–10.43% at the tillering and elongation stages but did not reach a significant difference level. These increases were mainly due to the growth of nitrogen accumulation in roots, stem, and leaves, especially under the C20 treatment in the elongation period. The nitrogen accumulation significantly increased by 25.26%, 10.10%, and 8.25% compared to the respective controls of roots (708.38 mg/pot), stem (4536.27 mg/pot), and leaves (1148.21 mg/pot).

The biochar effect on B8 showed a similar change trend as GT11. The accumulation of whole plant nitrogen of B8 increased by 2.43%–45.23% at each growth stage after biochar was applied, irrespective of N treatment application. At the tilling stage, the nitrogen accumulation after biochar application significantly increased by 27.27%–30.40%, compared with the control (704 mg/pot) at N120 conditions; however, it significantly increased by 36.47% after C20 treatment at N600 condition. Biochar treatment promoted the green leaf nitrogen accumulation of B8 by 8.62%–58.15% at each growth stage. After C10 and C20 treatments, the green leaf nitrogen accumulation reached 634.14–659.57 mg/pot and 841.42–885.62 mg/pot in the tillering and elongation stages, which were significantly higher than C0 by 52.05%–58.15% and 32.45%–39.41%, respectively.

Biochar effect on N from soil and N from fertilizer

As shown in Table 3A, under high and low nitrogen condition without biochar application, the nitrogen in GT11 roots, stem, and leaves in each growth period, was chiefly came from the soil, accounting for 64.62%–82.02%. While the nitrogen from fertilizer (FF) accounted for 17.98%–35.38%. Under low nitrogen conditions, FF of GT11 was between 21.51%–35.03%,which was lower than C0 treatment by 0.02% - 6.32%.The FF of GT11 after C20 treatment was between 43.60%–69.34%,which was higher than C0 by 19.87% - 37.70%.Under high nitrogen conditions, biochar treatment significantly increased FF of GT11 roots, stem, and green leaves with the value between 53.44.22% - 68.87%, which was more than CO by 30.76-40.68%. Further, the value of FF with C10 treatment was higher than that with C20 treatment by 0.03% - 4.31%.

Data from Table 3B show that the performance trend of B8, after biochar and nitrogen co-treatment, was similar to that of GT11. In the case of no nitrogen application (C0), the FF of B8 roots, stem, and leaves was between 18.94% to 31.23%, and FS was between 68.77%–81.06%.Under N120 condition, C10 treatment could slightly increase the FF of B8 roots,stem and leaves by 0.16% - 2.07%. With C20 treatment, FF of B8 was increased up to 55.95-72.02% which was higher that C0 by 16.21% - 40.79%.FF of B8 increased corresponding to N fertilizer applied rates. When N fertilizer applied rate reached 600 kg hm^{-2}, FF of B8 was between 53.17% - 72.03%,that was more 10.00% - 38.43% than C0 treatment. And also observed that FF of B8 with C10 treatment was higher than that with C20 treatment by 0.93% - 15.76%.

Statistical analysis showed that biochar and nitrogen treatment significantly affected the FF and FS of roots, stem, and leaves of the two varieties. There were significant differences between different treatment concentrations, and a significant interaction effect existed between biochar and nitrogen treatments.

Biochar effects on nitrogen use efficiency

As shown in Table 4, the NUE of sugarcane was significantly influenced by biochar and nitrogen treatment ($p< 0.001$). Variety mainly affected the NUE of green leaves ($p = 0.002$) and senescence leaves ($p< 0.001$).All in all, Biochar and N fertilizer treatment could increase the whole plant NUE of GT11 and B8 in all growth stages and organs (roots, stem, and leaves).The NUE of GT11 was 0.17%-26.60% higher than that of CK at different growth stages and different organs, B8 also show 0.05%-30.07% higher than CK. Yet two varieties show different response to biochar and N fertilizer applied rate.

TABLE 2 Effects of biochar and N fertilizer application on N uptake of two sugarcane varieties.

Variety	N treatment	Growth stages	Biohar treatment	Root (mg/g)	Shoot (mg/g)	Green Leaves (mg/g)	Senescence leaves (mg/g)	Whole plant (mg/g)
GT11	N120	Tillering stage	C0	87.10a	173.31a	368.81b	36.46b	665b
			C10	85.25a	161.20a	427.63ab	44.74a	718ab
			C20	92.63a	191.73a	493.25a	46.87a	824a
		Elongated stage	C0	617.73b	2092b	453.99b	174.13a	3338b
			C10	742.97ab	2381ab	505.46ab	177.70a	3807b
			C20	869.16a	3152a	653.05a	179.52a	4854a
		Mature stage	C0	279.95a	1505a	227.19a	231.80b	2244a
			C10	172.44b	1522a	216.55a	306.96ab	2217a
			C20	229.03ab	1626a	199.14a	356.23a	2410a
	N600	Tillering stage	C0	73.44a	354.98a	1064a	16.43a	1509a
			C10	56.34a	334.62a	1108a	26.89a	1526a
			C20	101.98a	302.01a	1081a	28.89a	1514a
		Elongated stage	C0	708.38a	4536ab	1148ab	287.89a	6680ab
			C10	827.04a	3766b	1061b	288.83a	5943b
			C20	887.30a	4994a	1242a	252.69a	7377a
		Mature stage	C0	261.32a	2783a	467.74a	328.59a	3841a
			C10	322.54a	2306a	561.01a	565.02a	3755a
			C20	364.10a	2083a	226.28a	499.12a	3173a
B8	N120	Tillering stage	C0	77.24a	156.92a	417.06b	53.27a	704b
			C10	80.69a	101.50b	659.57a	54.88a	896a
			C20	92.06a	128.31ab	634.14a	64.28a	918a
		Elongated stage	C0	997.03a	2207a	635.27a	276.13b	4116a
			C10	782.05b	2423a	841.42a	443.02a	4489a
			C20	769.04b	2319a	885.62a	242.11b	4216a
		Mature stage	C0	302.10a	1061a	207.25a	370.64a	1941ab
			C10	280.89a	1019a	225.12a	368.78a	1893b
			C20	274.39a	1253a	260.86a	520.17a	2308a
	N600	Tillering stage	C0	64.90a	237.55a	949.47b	23.92a	1275b
			C10	54.69a	246.86a	991.72b	43.93a	1337ab
			C20	60.51a	288.60a	1359a	31.05a	1740a
		Elongated stage	C0	901.78a	3676a	1301b	399.59a	6279a
			C10	863.03a	3578a	1452b	225.41b	6119a
			C20	959.32a	5003a	2052a	430.46a	8445a
		Mature stage	C0	241.88a	1802a	501.00a	709.44a	3255a
			C10	309.03a	1676a	652.63a	764.84a	3402a
			C20	232.96a	2194a	576.46a	690.45a	3694a
F-values and significance level								
Variety				.054	.001	<0.001	<0.001	.685
C treatment				0.257	0.002	<0.001	0.042	<0.001
N treatment				0.116	<0.001	<0.001	<0.001	<0.001
C and N interaction (C × N)				0.162	0.148	0.521	0.821	0.159

C0,0 t hm^{-2} biochar;C10, 10 t hm^{-2} biochar; C20, 20 t hm^{-2} biochar;N120,120 t hm^{-2} N fertilizer; N600,600 t hm^{-2} N fertilizer; Different letters within the columns show a significant difference between the treatments at P=0.05 according to the LSD test.

Under low nitrogen conditions (N120), the whole plant NUE of GT11 remarkably improved by C20 treatment at tillering and elongated stages. Compared with the C0 treatment, the whole plant NUE of GT11 increased from 21.54% and 47.40% to 45.29% and 74.00% at tillering and elongated stages after C20 treatment, respectively. Under N600 conditions, biochar just significantly improved the whole plant NUE at tillering stages. The whole plant NUE with C10 and C20 treatment was up to 20.60% and 19.98%, that was more than C0 treatment by 12.14% and 11.52%, respectively.

TABLE 3A Effects of biochar and N fertilizer treatment on FF and FS of GT11.

Variety	N treatment	Growth stages	Biochar treatment	FF (%)				FS (%)			
				Root	Shoot	Green Leaves	Senescence leaves	Root	Shoot	Green Leaves	Senescence leaves
GT11	N120	Tillering stage	C0	30.85b	33.58b	29.18b	23.73b	69.15a	66.42a	70.82a	76.27a
			C10	24.53c	30.72c	26.44b	22.88b	75.47a	69.28a	73.56a	77.12a
			C20	57.53a	66.34a	59.54a	43.60a	42.47b	33.66b	40.46b	56.40b
		Elongated stage	C0	27.79b	34.93b	29.70b	32.94b	72.21a	65.07a	70.30a	67.06a
			C10	26.65b	35.03b	28.48b	30.51b	73.35a	64.97a	71.52a	69.49a
			C20	59.43a	69.34a	63.13a	58.05a	40.57b	30.66b	36.87b	41.95b
		Mature stage	C0	27.49b	28.84b	22.05b	26.69b	72.51a	71.16a	77.95a	73.31a
			C10	25.91b	28.82b	21.51b	24.19c	74.09a	71.18a	78.49a	75.81a
			C20	60.82a	66.14a	53.96a	52.45a	39.18b	33.86b	46.04b	47.55b
	N600	Tillering stage	C0	26.13c	31.15b	25.37b	19.89b	73.87a	68.85a	74.63a	80.11a
			C10	61.23a	66.81a	57.23a	33.22a	38.77b	33.19b	42.77a	66.78b
			C20	56.92b	65.07a	56.13a	34.81a	43.08b	34.93b	43.87b	65.19b
		Elongated stage	C0	28.50b	35.38b	29.34c	31.14c	71.50a	64.62a	70.66a	68.86a
			C10	60.33a	68.87a	63.88a	60.01a	39.67b	31.13b	36.12b	39.99b
			C20	59.37a	66.65a	61.32b	57.09b	40.63b	33.35b	38.68b	42.91b
		Mature stage	C0	23.70b	25.36b	17.98b	21.84b	76.30a	74.64a	82.02a	78.16a
			C10	59.68a	65.93a	53.64a	53.44a	40.32b	34.07b	46.36b	46.56b
			C20	59.65a	66.04a	53.55a	50.32a	40.35b	33.96b	46.45b	49.68b
Variety				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C treatment				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N treatment				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C and N interaction (C × N)				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

C0,0 t hm^{-2} biochar;C10, 10 t hm^{-2} biochar; C20, 20 t hm^{-2} biochar;N120,120 t hm^{-2} N fertilizer; N600,600 t hm^{-2} N fertilizer; FF,N from fertilizer; FS, N from soil; Different letters within the columns show a significant difference between the treatments at P=0.05 according to the LSD test.

Data from Table 4 shows that the whole plant NUE of B8 was observably increased after biochar applied under N600 conditions. At tillering stage, the whole plant NUE was up to 17.97% and 23.13% from 6.76% by C10 and C20, respectively, and was up to 57.70% and 61.90% from 42.87% at elongated stage, and was up to 48.99% and 46.48% from 18.92% at mature stage. While under N120 conditions, biochar mainly significantly improved the whole plant NUE at tillering stage with C20 treatment.

Discussion

Effects of biochar on sugarcane biomass

In accordance with previous report (Liao, 2019), biochar have little effect on the biomass accumulation of sugarcane. This is in agreement with some studies reported a decline in crop yield with biochar application (Zhu et al., 2014; Jay et al., 2015; Olszyk et al., 2020).Experiment on rice conducted by Xie et al. (2011) showed that grain yield decreased by 2% at first year after wheat straw biochar application. Previous researches seldom concern about different varieties yield response to the biochar impact. In this study, we found that the sugarcane variety GT11 with low NUE could significantly increase the biomass in the elongation period under low nitrogen treatment (N120), this is consistent with many studies which have shown that the effect of biochar on crop yield is not substantial in fertile soil; however, it becomes effective in poor soil (Xie et al., 2013; Omara et al., 2020; Haider et al., 2022). While the variety B8 with slightly higher NUE did not show a significant increase. We speculate that this difference arises from the differences in the response of different sugarcane genotypes to biochar. And this indicated that the impact of biochar on sugarcane yield is very complex.

Effects of biochar on nitrogen accumulation in sugarcane

The results from this study demonstrate that biochar could improve the nitrogen accumulation of the two sugarcane varieties under high and low nitrogen conditions, especially in the tillering and elongation stages, where the nitrogen accumulation of the whole sugarcane plant and green leaves

TABLE 3B Effects of biochar and N fertilizer treatment on FF and FS of B8.

Variety	N treatment	Growth stages	Biochar treatment	FF (%)				FS(%)			
				Root	Shoot	Green Leaves	Dried leaves	Root	Shoot	Green Leaves	Dried leaves
B8	N120	Tillering stage	C0	24.12b	27.02b	25.00b	18.94b	75.88a	72.98a	75.00a	81.06a
			C10	24.09b	28.86b	25.16b	19.36b	75.91a	71.14a	74.84a	80.64a
			C20	56.16a	66.49a	58.62a	35.15a	43.84b	33.51b	41.38b	64.85b
		Elongated stage	C0	27.34b	31.23b	27.58b	25.82b	72.66a	68.77a	72.42a	74.18a
			C10	25.84b	31.66b	29.65b	27.56b	74.16a	68.34a	70.35a	72.44a
			C20	55.95a	72.02a	65.31a	60.50a	44.05b	27.98b	34.69b	39.50b
		Mature stage	C0	27.97b	29.98b	20.22b	25.26b	72.03a	70.02a	79.78a	74.74a
			C10	26.13b	29.13b	20.70b	24.02c	73.87a	70.87a	79.30a	75.98a
			C20	63.68a	66.87a	57.38a	57.55a	36.32b	33.13b	42.62b	42.45b
	N600	Tillering stage	C0	23.57c	28.40b	25.01b	18.28b	76.43a	71.60a	74.99a	81.72a
			C10	59.54a	66.15a	58.53a	29.70a	40.46b	33.85b	41.47b	70.30a
			C20	53.17b	66.16a	57.70a	28.28a	46.83b	33.84b	42.30b	71.72a
		Elongated stage	C0	26.74b	33.60c	30.69c	28.45c	73.26a	66.40a	69.31a	71.55a
			C10	64.46a	72.03a	65.90a	62.20a	35.54b	27.97b	34.10b	37.80b
			C20	58.77a	66.27b	62.01b	55.11b	41.23b	33.73b	37.99b	44.89b
		Mature stage	C0	26.48b	29.69b	22.48c	26.00b	73.52a	70.31a	77.52a	74.00a
			C10	63.94a	66.39a	57.88a	58.27a	36.06b	33.61b	42.12b	41.73b
			C20	60.73a	66.90a	54.42b	55.81a	39.27b	33.10b	45.58b	44.19b
F-values and significance level											
Variety				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C treatment				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N treatment				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C and N interaction (C × N)				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

C0,0 t hm^{-2} biochar;C10, 10 t hm^{-2} biochar; C20, 20 t hm^{-2} biochar;N120,120 t hm^{-2} N fertilizer; N600,600 t hm^{-2} N fertilizer; FF,N from fertilizer; FS, N from soil; Different letters within the columns show a significant difference between the treatments at P=0.05 according to the LSD test.

significantly increased. Both biochar and nitrogen treatments greatly affected nitrogen accumulation in stem and leaves of the two varieties, but there was no significant interaction between them. These results are consistent with the effect of biochar on nitrogen accumulation in other crops. Huang et al. (2014) found that biochar promoted rice fertilizer nitrogen uptake by about 23%–27%, thus increasing rice grain yield by 6%–8%. Khan et al. (2021) also found that applying biochar under nitrogen reduction conditions can increase the nitrogen absorption of rice by 13%, thus increasing rice grain yield and NUE by 36% and 35%, respectively. Biochar can significantly increase nitrogen accumulation and the proportion of nitrogen obtained by crops from fertilizers or soil. This is probable due to biochar application can effectively improve soil structure, increase soil pH value, and facilitate the release of soil-available nitrogen and other nutrient availability (Frimpon et al., 2021). Our previous studies have also observed (Liao et al., 2018, 2019) that biochar can increase the nitrogen content in the soil and augment the soil nitrogen retention in the early growth stage of sugarcane, which could explain how biochar is capable of promoting the increase of nitrogen accumulation in the early growth stages of sugarcane.

The effect of biochar on sugarcane nitrogen use efficiency

Our experiment found that biochar can indeed improve the nitrogen utilization rate of sugarcane, where the NUE of roots, stem, and leaves increased to a certain extent. Under high and low nitrogen conditions, the nitrogen use efficiency of the whole plant, green leaves, and roots of GT11 was significantly improved, yet B8 got a huge boost under high nitrogen conditions. The positive influence of biochar on NUE is consistent with several reports. Omara et al. (2020) demonstrated that *Zea mays* L. grain yield, N uptake, and NUE increased by 25%, 28%, and 46%, respectively, with fertilizer N-biochar-combinations treatment compared to N

TABLE 4 Effects of biochar and N fertilizer treatment on N use efficiency of two sugarcane varieties.

Variety	N treatment	Growth stages	Biochar treatment	Root (%)	Shoot (%)	Green Leaves (%)	Senescence leaves (%)	Whole plant (%)
GT11	N120	Tillering stage	C0	3.20b	6.84b	12.62b	1.01b	21.54b
			C10	2.51b	5.84b	13.31b	1.21b	20.45b
			C20	6.31a	12.47a	24.17a	2.40a	45.29a
		Elongated stage	C0	2.52c	25.80a	15.85b	2.03c	47.40b
			C10	12.11b	23.68a	16.94b	6.38b	53.16b
			C20	18.17a	25.66a	34.82a	12.25a	74.00a
		Mature stage	C0	9.05a	25.53a	7.28b	5.92b	51.65b
			C10	5.26b	25.70a	8.78ab	5.48b	63.48a
			C20	9.84a	31.61a	10.99ab	8.54a	51.92b
	N600	Tillering stage	C0	0.45b	2.60b	6.34b	0.08a	8.46b
			C10	0.81ab	5.26a	14.92a	0.21a	20.60a
			C20	1.37a	4.64ab	14.34a	0.24a	19.98a
		Elongated stage	C0	4.74b	20.79b	7.92b	2.11b	48.47a
			C10	11.73a	18.27ab	15.95a	4.07a	54.44a
			C20	12.40a	23.51a	17.93a	2.96ab	54.80a
		Mature stage	C0	2.58a	22.84a	6.56ab	3.31a	39.52b
			C10	4.56a	25.02a	9.35a	7.09b	53.12a
			C20	5.09a	25.91a	2.85b	6.81b	45.11ab
B8	N120	Tillering stage	C0	2.20a	4.97 b	12.25b	1.18b	18.18b
			C10	2.26a	3.47b	19.48a	1.25b	23.36b
			C20	1.83a	10.05a	21.80a	2.62a	36.33a
		Elongated stage	C0	9.65b	24.26a	20.52a	8.27a	64.22a
			C10	9.52b	31.62a	29.31a	8.61a	71.65a
			C20	12.65a	25.61a	27.33a	3.44b	65.30a
		Mature stage	C0	9.93a	26.21b	11.03b	4.93a	56.87ab
			C10	8.65a	34.80a	10.35b	5.47a	52.88b
			C20	10.25a	29.62ab	17.61a	7.04a	64.16a
	N600	Tillering stage	C0	0.36a	1.59b	5.58c	0.11b	6.76b
			C10	0.77a	3.81a	13.63b	0.41 a	17.97a
			C20	0.75a	4.47a	18.37a	0.22ab	23.13a
		Elongated stage	C0	5.59b	22.56a	9.41b	2.67b	42.87b
			C10	13.10a	21.73a	22.50a	3.30b	58.70ab
			C20	13.26a	23.37a	20.97a	5.61a	61.90a
		Mature stage	C0	1.52b	12.57b	4.33b	2.66b	18.92b
			C10	4.66a	26.17a	10.48a	8.88a	48.99a
			C20	3.33a	24.63a	9.09a	7.30a	46.48a
F-values and significance level								
Variety				0.575	0.965	0.002	<0.001	0.757
C treatment				<0.001	<0.001	<0.001	<0.001	<0.001
N treatment				<0.001	<0.001	<0.001	<0.001	<0.001
C and N interaction (C × N)				<0.001	0.75	0.43	<0.001	<0.001

C0,0 t hm^{-2} biochar;C10, 10 t hm^{-2} biochar; C20, 20 t hm^{-2} biochar;N120,120 t hm^{-2} N fertilizer; N600,600 t hm^{-2} N fertilizer; Different letters within the columns show a significant difference between the treatments at P=0.05 according to the LSD test.

fertilizer single treatment in sandy loam soil. Ye et al. (2020) conducted a three-year fixed-point experiment in stratospheric soil in Northeast China to study the effects of biochar and controlled-release of nitrogen fertilizers on rice yield, nitrogen use efficiency, residual nitrogen, and nitrogen balance in soil-crop systems. Their study found that yield and nitrogen use efficiency increased by 10.2% and 16.5%, respectively, after adding biochar. On the other hand, the experimental results showed that nitrogen accumulation, nitrogen use efficiency, and biomass in leaves were greatly improved, but biochar did not show a significant promoting effect on stem as harvested organs, suggesting that there is a complex transformation relationship in sugarcane 'source–sink,' thereby affecting the accumulation of stem biomass, which requires further research in later experiments.

Conclusion

With consistent biochar treatments and growing conditions, two sugarcane varieties varied in response to biochar but with some general patterns. Results showed a positive effect of application of biochar in genotype with low NUE and low N condition. High biochar applied rate could effectively reduce the stress of high N level on the growth of sugarcane. However, this experiment is mainly a barrel planting experiment under greenhouse conditions, and many years of multi-pilot field experiments are still needed in the follow-up tests. Nonetheless, the effect of biochar treatment on the physiological and biochemical indicators of sugarcane will be carried out in the future, and the relationship between the nitrogen balance of the biochar–sugarcane–soil system will be studied to verify and evaluate the effect of biochar on the nitrogen absorption and utilization of sugarcane. Finally, this study provides a theoretical basis for biochar application in sugarcane cultivation and production.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

GC: Analyzed and interpreted the data, Wrote the original article. JG: Performed the experiments, Contributed reagents, materials, analysis tools or data. C-XQ: Contributed reagents, materials, analysis tools or data. D-LH: revised the article. FL: Conceived and designed the experiments, Performed the experiments, Wrote the original article. LY: conceived and design the experiments. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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