INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0478/2018/20–11–2529–2536
DOI: 10.17957/IJAB/15.0797
http://www.fspublishers.org

Full Length Article

Effect of Rice-Straw Biochar Application on Rice (*Oryza sativa*) Root Growth and Nitrogen Utilization in Acidified Paddy Soil

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Abstract

Soil acidification and low nitrogen (N) utilization efficiency are serious problems in rice production. Biochar has the potential to provide a liming effect and strong nutrient adsorption, leading to soil improvement. This study was conducted to investigate specific root traits in rice and to assess the effect of rice-straw biochar amendment on nitrogen efficient utilization in acidified soil. Addition of 20 g kg^{-1} biochar and washed biochar significantly promoted rice growth and the yield increased by over 35% and 24%, respectively, when compared with the control. Application of equivalent lime did not increase the rice yield in either low or high N treatments. Biochar application alleviated soil acidity and improved the available nutrient content. Biochar maintained a high available N during the N depletion period by regulating N adsorption and release in the acidified paddy soil. Biochar or washed biochar amendment was found to significantly improve root growth when compared with the control, particularly root mass and adventitious root length. However, application of equivalent lime only significantly promoted the growth of root system before panicle initiation stage. When compared with the liming effect, the adsorption properties of biochar provided a persistent effect in improving acidified soil. Further studies on long-term effects of biochar addition on crop growth as well as its behavior in soil are required in future. © 2018 Friends Science Publishers

Keywords: Biochar; Liming effect; Adsorption; Root; Nitrogen efficient utilization

Introduction

Soil acidification and low nitrogen utilization efficiency are the serious problems for crop production and seriously restricts sustainable agricultural development (Janssen, 1998). Long-term observations have shown that the average pH value of paddy soil declined by 0.17-0.83 units in the past 30 years in China (Li *et al*., 2014). The excessive use of nitrogen (N) fertilizer could be responsible for regional soil acidification (Guo *et al*., 2010). Nitrogen plays an important role in rice growth and development, and increases in N fertilizer application have been a major management strategy for high-yielding rice cultivation (Cassman *et al*., 2003). Finally, the average amount of N applied in paddy fields was 300 kg N ha^{-1} in some countries (Peng *et al*., 2006). Nevertheless, N utilization efficiency in crop production was relatively lower, and more than 55% of the applied N was not taken up by rice (Raun and Johnson, 1999). The residue of excessive N in the soil accelerated soil acidification of agricultural ecosystems. The main direct effect of soil acidification is to inhibit the growth of root system (Zhang *et al*., 2015), which reduces the N absorption of plants, thereby decreasing the N use efficiency of chemical fertilizers. It is therefore desirable to find efficient and comprehensive strategies that reduce N residue in the soil and improve N use efficiency while mitigating soil acidification.

Biochar produced via the pyrolysis of crop residues is a novel material with potential for soil improvement (Ibrahim *et al*., 2013; Zhang *et al*., 2016). Some reports suggest that adding biochar to soil was an effective approach to gain agronomic benefits including enhancing water holding capacity (Basso *et al*., 2013), increasing the availability of nutrients (Borchard *et al*., 2012) improving the soil physical properties (Major *et al*., 2010), promoting the activities of soil microorganisms (Pereira *et al*., 2015), and controlling contaminant availability (Brennan *et al*., 2014). Differing from most conventional soil organic materials, biochar as a soil amendment material could maintain higher productivity in the long-term (Chan *et al*., 2008). Biochar still contains high quantities of ash, which directly provides some nutrients (Xu *et al*., 2013). Additionally, ash content provides the liming effect of biochar. The direct effect of biochar on the soil is that the pH value of the soil will also increase, which may decrease aluminum (Al) bioavailability and toxicity of acidified soil and increase the nutrients, especially the availability of phosphorus, calcium and potassium (Yuan and Xu, 2011). Moreover, the large surface area of biochar

To cite this paper: Zhang, Y., H. Chen, G. Ji, Y. Zhang, J. Xiang, S. Anwar and D. Zhu, 2018. Effect of rice-straw biochar application on rice (*Oryza sativa*) root growth and nitrogen utilization in acidified paddy soil. *Int. J. Agric. Biol*., 20: 2529–2536

results in increased adsorption capacity as well as cation exchange capacity (CEC) of soils. This might contribute to prevention of nutrient leaching, particularly with regard to N mobilization. Lehmann *et al.* (2003) reported that ammonium-N (NH_4^+-N) leaching was significantly decreased after biochar amendment, while nitrate-N leaching in biochar modified soil was higher than that without biochar soil. Similarly, Yoo *et al.* (2014) observed a significant reduction in NH_4^+-N content in rice paddy soil treated with biochar. By increasing CEC, fertilizers could be sorbed to the surface of the biochar and thereby be used more efficiently by plants. However, few studies have been carried out to investigate the effect of the liming and sorption characteristics of biochar in improvement of acidified soil.

Rice is one of the main crops grown under acidic soil conditions in China. When the pH of paddy soil is below 5.0, rice yields decreased dramatically (Zeng *et al*., 2014). It has been showed that biochar had great potential for acidic soil improvement (Marris, 2006). In this paper, the effects of biochar in acidified soil on root morphology and nitrogen utilization from two aspects of the liming and adsorption properties of biochar are studied, respectively, which provide a theoretical basis for the application of biochar in acidified soil.

Materials and Methods

Plant Cultivation and Experimental Design

The soil sample was taken from Langya town of Jinhua, located in Eastern China (N 29°00'17.37", E 119°29'54.84"). The acidified paddy soil basic chemical characteristics are showed in Table 1. Prior to use, the soils were screened by a 2-mm sieve. The biochar (pH 10.13, total N 11.39 g kg^{-1} and total C 602 g kg^{-1}) was produced from rice straw at 500°C in a muffle furnace under oxygen-limited conditions for 2 h. The washed biochar was obtained by rinsing with 0.1 mol L^{-1} H_2SO_4 until the aqueous phase reached the same pH value as the acidic soil to remove of lime characteristics of biochar, and then it was rinsed thoroughly with distilled-deionized water. The treated biochar was dried at 80°C.

A pot experiment was conducted using the acidified paddy soils in a greenhouse at the fuyang base of China National Rice Research Institute. Plants were grown in plastic columns (25 × 35 cm, diameter × height) filled with 15 kg soil. The amount of nitrogen applied as $(NH_4)_2SO_4$ was 20 mg kg^{-1} (low N) or 300 mg kg^{-1} (high N), and each fertilizer trial consist of four treatments: the soil without biochar (CK), the soil ameliorated with 20 g kg^{-1} biochar (B), the soil ameliorated with 20 g kg^{-1} washed biochar (WB, alkaline removal and showing the adsorption characteristics of biochar) and the soil ameliorated with lime equivalent to 20 g kg^{-1} biochar (LM). 50% of N fertilizer was applied before transplanting, 30% at the tillering stage, and the remaining 20% at the panicle stage. All the other fertilizers were applied just before transplanting. The biochar, washed biochar and lime were thoroughly incorporated into the soil. Other nutrients were provided as follows (mg kg^{-1} soil): 200 P (as $Ca(H_2PO_4)_2$), 250 K (as KCl), 50 Mg (as $MgSO_4$), 100 Ca (as $CaCl_2$).

Rice seeds (*Oryza sativa* L., cv. Zhongzheyou 1) disinfected with 30% hydrogen peroxide for 20 min and soaked in deionized water for 24 h. Seeds sprouted at 32°C in the dark on wet filter paper for 12 h and were cultivated on the seedling bed on May 15, 2015. The seedling was transplanted on May 30, 2015 with two seedlings per pot and harvested on 28 September, 2015. The crop management was performed according to following the routine rice field production. Every treatment was repeated by 6 times. All the pots were placed in a glass greenhouse and were re-positioned randomly every week. The test was set up at 25-32°C (daytime) and temperature 18-22°C (night) under natural light.

Plant Sampling and Analysis

Plant sampling was carried out at the key growth stages of tillering, panicle initiation (PI), heading and maturity. The shoots were collected and separated into stems, leaves and spikes (from heading) before root sampling. The leaf area was determined by a leaf area analyzer (Li-Cor 3100, USA). Plant samples dried at 70°C for 4 days. N contents of shoot were analyzed by micro Kjeldahl to calculate N uptake in the aboveground.

During the key growing periods, the rhizosphere soil was collected. Soil mineral nitrogen including ammonium nitrogen and nitrate nitrogen in the soil was extracted from soil with 2 *M* potassium chloride (soil/water=1:10) and shaken for 1 h at 25°C. Then, the suspensions were filtered through filter paper, and the concentrations of ammonium nitrogen and nitrate nitrogen in the filtrate were measured by a Continuous Flow Analyzer (FOSS FIAstar 5000, Denmark). Roots were carefully washed and detached from the nodal bases. To measure root length and surface area, the root was placed in the glass tray (30×30 cm) with shallow water and scanned with a scanner (Epson V700, Japan), and then use the WinRHIZO root system to analyze data from digital images (Regent Instruments Inc., Quebec, Canada). Measurement of grain production and yield components followed Yoshida's procedures.

Statistics

All the data were average values and standard errors of average values. Using variance analysis and least significant difference (LSD) test, the effect of biochar, washed biochar, and lime treatment on root growth, N uptake, soil mineral N, and rice yield were determined the statistical significance using SAS ver. 9.1 (SAS Institute, Cary, NC, USA). Difference between the values at $P < 0.05$ was considered statistically significant.

Results

Soil Properties

The application of biochar significantly improved the soil pH value by 0.77 units, while washed biochar and lime amendment increased pH values by 0.33 and 0.28 units, respectively, when compared with the control. The content of total N, available P, exchangeable K, and organic matter increased under biochar amendment when compared with the control. Biochar significantly increased the content of total N in soil by 51%, while soil organic matter also increased by 86%. There were no significant differences between washed versus unwashed biochars except for the available P and exchangeable K. In particular, biochar amendment significantly increased soil available P and exchangeable K when compared with washed biochar. Lime amendment significantly increased available P by four times when compared with the control (Table 1).

Rice Root Growth

Biochar and washed biochar amendment significantly affected root growth (Fig. 1 and Table 2); root biomass, total root length, lateral root length, adventitious root length and root surface area per plant were significantly higher in the biochar amended soil compared to control with N addition except at the tillering stage. However, total root length, lateral root length, adventitious root length and root surface area per plant were significantly greater in the lime treatment when compared with the control before the PI stage. Root average diameter per plant was thicker in both biochar and washed biochar amended soils when compared with the control. The difference in root diameter was mainly attributed to adventitious roots (Fig. 1).

Mineral N in Rhizosphere Soil and its Uptake by Rice

Biochar amendment affected the soil N availability (Tables 2 and 3). No significant differences in NO_3^--N content in the biochar treatment were observed when compared with the control under low N supply. The contents of NO_3^--N in soil significantly increased throughout the growth season with the application of biochar or washed biochar compared to the control under sufficient N supply, especially in the PI period. Meanwhile, lime application also significantly increased the NO_3^--N content of soil. No significant differences in NH_4^+-N content in biochar or washed biochar amended rhizosphere soils were observed when compared with the control under low N. NH_4^+-N content of soil decreased at the tillering and PI stages in biochar or washed biochar treatments when compared with the control with N addition ($P < 0.05$). However, NH_4^+-N content of soil significantly increased at the heading stage in biochar or washed biochar treatments when compared with the control ($P < 0.05$). There were no significant differences in NH_4^+-N content of soil between the lime and control treatments under high N.

Nitrogen contents of rice were significantly affected by either biochar or washed biochar amendment with N addition (Fig. 2), whereas there were no significant differences in N uptake of rice between equivalent lime and control treatments under high N. No influence of the amendments on N uptake was observed when compared with the control under low N supply except at maturity.

Rice Growth and Yield

The application of biochar on the growth of rice had a positive effect and significantly increased shoot biomass with high N supply (Fig. 3). Similarly, rice plants under rice straw biochar addition exhibited significantly higher leaf area than in the control with or without N application ($P < 0.05$). The application of lime had significant impacts on rice growth at the tillering and PI stages. Rice grain yield with biochar and washed biochar treatments with high N supply increased by 35% and 24%, respectively, when compared with the control (Table 2 and 4). Similar to the high N treatments, rice grain yields in biochar and washed biochar treatments were significantly higher at low N by 35% and 28%, respectively, when compared with the control. Lime amendment did not increase the rice yields under either low or high N supply. The panicle number per plant was higher in both biochar and washed biochar amended soil when compared with the control. The percentage of filled grains increased by 12% and 11%, and grain number per panicle increased by 17% and 10% for biochar and washed biochar treatments than the control, respectively. The high grain yields in biochar and washed biochar treatments were mainly due to a high percentage of filled grains, panicle number per plant and grain number per panicle. No significant differences in the panicle number, percentage of filled grains and grain number per panicle between equivalent lime and control treatments were observed.

Discussion

Acidification, artificial processes and farming methods cause soil degradation and reduce the fertility rate of agro-ecosystems. Biochar application to soil improved crop growth in acidic or acidified soil (Zhu *et al*., 2014; Bakar *et al*., 2015; Yu *et al*., 2015). Results from the current study indicate that biochar application significantly increased rice yields in acidified paddy soil (Table 4). The contribution to rice yield was closely related to the improvement in soil properties. Biochar application resulted in a higher K content in the soil solution (Olmo *et al*., 2015). Also, P availability could be enhanced with the increase in pH with biochar application (pH increased from 4.6 to 5.2). Al is very toxic to plant roots, and an increase in pH alleviates

Table 1: Effect of biochar application on acidified paddy soil chemical properties*

	pH	Total N (g kg^{-1})	Available P (mg kg^{-1})	Exchangeable K (mg kg^{-1})	Organic matter (g kg^{-1})
Acidified soil	4.59±0.01c	1.03±0.04b	3.95±0.54c	54.95±2.78c	21.05±1.09b
Lime + soil	4.87±0.03b	1.02±0.07b	16.52±1.45a	56.13±1.68c	21.59±1.88b
B + soil	5.36±0.08a	1.56±0.27a	8.26±0.54b	728.12±10.49a	39.24±3.06a
WB + soil	4.92±0.02b	1.34±0.24a	5.28±0.70c	120.36±2.30b	35.94±3.38a

* pH value: 2.5: 1 (water/soil); total N content: Kjeldahl method; available P content: Bray II method; Exchangeable K: NH_4OAC extraction; organic matter: $K_2Cr_2O_7$-H_2SO_4 digestion

Table 2: Variance analysis of effects of N, biochar treatment, and N×biochar treatment interaction on root growth, dry matter accumulation, N uptake, grain yield and yield components parameters of rice at maturity

Source of variation	N level (N)	Biochar treatment (B)	N×B
Total root length	8.15^{*}	5.29^{*}	2.52^{NS}
Lateral root length	0^{NS}	3.96^{*}	2.58^{NS}
Adventitious root length	282.53^{***}	5.54^{*}	0.17^{NS}
Root surface area	184.93^{***}	4.35^{*}	1.42^{NS}
Root diameter	92.28^{***}	11.60^{***}	1.14^{NS}
Root dry weight	354.52^{***}	9.33^{**}	0.75^{NS}
Shoot dry weight	803.38^{***}	19.43^{***}	5.88^{*}
N uptake	2009.27^{***}	21.45^{***}	9.41^{**}
Content of NH_4^+-N	0.85^{NS}	1.87^{NS}	0.10^{NS}
Content of NO_3^--N	23.22^{***}	6.35^{**}	2.77^{NS}
1000 grain weight	0.10^{NS}	3.08^{NS}	2.10^{NS}
Percentage of filled grains	3.34^{NS}	3.22^{NS}	4.31^{*}
Panicle number	551.12^{***}	3.73^{*}	2.24^{NS}
Grain number/panicle	78.90^{***}	13.35^{***}	1.06^{NS}
Grain yield	665.12^{***}	19.32^{***}	5.76^{**}

Note: *denotes significance at the $P \leq 0.05$ level, **denotes significance at the $P \leq 0.01$ level, ***denotes significance at the $P \leq 0.001$ level. NS: not significant

Table 3: Effects of biochar and N level on the content of NH_4^+-N (mg kg^{-1}) and NO_3^--N (mg kg^{-1}) in the rhizosphere soil

	Treatment	Tillering	PI	Heading	Mature
NH_4^+-N	CK	3.94±0.63b	3.44±0.29a	2.19±0.17a	1.90±0.88a
	LM	3.56±0.18b	3.25±0.33a	2.85±0.77a	1.79±0.53a
	B	5.30±0.29a	3.92±0.23a	2.37±0.51a	2.68±0.19a
	WB	4.26±0.36b	4.34±0.46a	2.22±0.10a	2.09±0.29a
	CKF	24.50±1.92a	85.87±1.77ab	3.01±0.08b	2.07±0.17ab
	LMF	20.57±1.08ab	96.48±2.54a	2.83±0.30b	1.96±0.09b
	BF	17.03±1.18c	78.15±4.26bc	4.15±0.37a	2.89±0.26a
	WBF	18.92±2.12bc	69.86±2.67c	4.56±0.18a	2.65±0.27ab
NO_3^--N	CK	0.29±0.07a	0.23±0.02b	0.23±0.04b	0.26±0.05b
	LM	0.31±0.09a	0.30±0.10ab	0.59±0.18a	0.32±0.05ab
	B	0.34±0.04a	0.24±0.02b	0.31±0.06ab	0.53±0.10a
	WB	0.34±0.05a	0.46±0.08a	0.28±0.08ab	0.47±0.02ab
	CKF	0.33±0.02a	0.23±0.02b	0.21±0.03c	0.43±0.04c
	LMF	0.61±0.10a	0.30±0.02b	0.60±0.08a	0.74±0.06bc
	BF	0.53±0.10a	0.87±0.07a	0.48±0.05ab	1.42±0.17ab
	WBF	0.92±0.29a	0.63±0.14a	0.33±0.06bc	1.68±0.51a

Note: Tillering (the active tillering stage), PI (the panicle initiation stage), Heading (80% of the first spike stage), Mature (the filling ripening stage). CK without biochar, lime addition (LM), biochar (B), washed biochar (WB) with low N fertilization and CKF, LMF, BF, WBF refer to high N fertilization application. Each value is the average ± standard error of four replicates. Different letters indicate a significant different meaning ($P < 0.05$)

this toxic effect in acidic soil after biochar application because of the liming effect as well as by the adsorption of Al^{3+} on the biochar (Qian *et al*., 2013; Zhu *et al*., 2014). Biochar improves the physical properties of soil, which is beneficial to the growth of root system and the crops (Olmo *et al*., 2015).

In the present study, biochar amendment improved rice root growth in acidified paddy soil, and the total root length, lateral root length and adventitious root length significantly increased (Fig. 1). The lateral roots account for the vast majority of the total root length, and their spatial distribution and density play an important role in the acquisition of nutrients (Li *et al*., 2006). Olmo *et al*. (2015) reported that biochar addition promoted the increase of the specific root length and reduction of root diameter, indicating fine root proliferation. Nevertheless, average root diameter was thicker in biochar amended soils, and the difference in root diameter was mainly attributed to adventitious roots (Fig. 1). The distribution of adventitious roots determines the architecture of the root system. Long adventitious roots can increase the distribution of

Table 4: Effects of biochar application and N level on rice yield and its components

	1000 grain weight (g)	Percentage of filled grains (%)	Panicle number/plant	Grain number/panicle	Grain yield (g/plant)
CK	25.3±0.31a	89.9±1.43a	16.6±0.33a	79.5±0.84b	33.3±1.13b
LM	25.8±0.21a	90.8±1.27a	16.9±0.67a	85.5±3.38b	36.6±1.46b
B	25.2±0.17a	88.7±2.19a	18.3±1.20a	97.4±3.24a	44.9±1.52a
WB	25.3±0.14a	88.5±2.18a	16.6±0.67a	100.9±2.65a	42.6±2.00a
CKF	24.1±0.53b	80.5±2.32b	34.0±1.15b	101.9±4.63b	93.1±7.09b
LMF	26.0±0.27ab	89.1±1.28a	38.0±1.00ab	110.8±1.97ab	106.2±3.13ab
BF	26.1±0.46a	90.2±0.50a	40.1±1.76a	118.8±2.67a	125.7±6.02a
WBF	25.7±0.74ab	89.3±1.00a	40.3±1.86a	112.2±3.32ab	115.5±3.64a

Note: CK without biochar, lime addition (LM), biochar (B), washed biochar (WB) with low N fertilization and CKF, LMF, BF, WBF refer to high N fertilization application. Each value is the average ± standard error of four replicates. Different letters indicate a significant different meaning ($P < 0.05$)

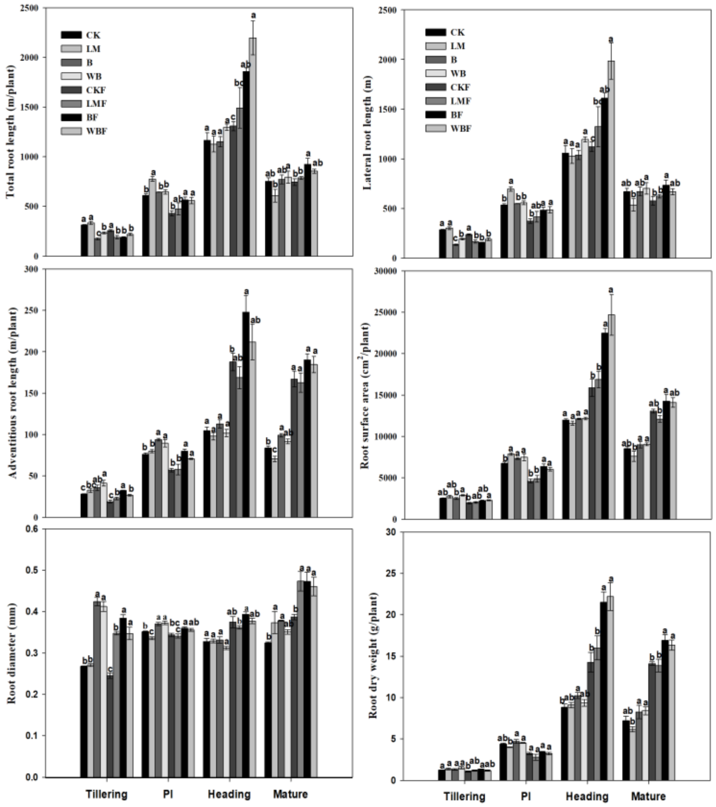

Fig. 1: Effects of biochar application and N level on the root morphology variables during different growth periods. Tillering (the active tillering stage), PI (the panicle initiation stage), Heading (80% of the first spike stage), Mature (the filling ripening stage). CK without biochar, lime addition (LM), biochar (B), washed biochar (WB) with low N fertilization and CKF, LMF, BF, WBF refer to high N fertilization application. Each value is the average ± standard error of four replicates. Different letters indicate a significant different meaning ($P < 0.05$)

vertical or horizontal roots. Abiven *et al*. (2015) showed that biochar amendment resulted in more extensive root systems and improved N uptake in deep soil.

Biochar is relatively inert and decomposes slowly and might not supply N to plants directly (Xu *et al*., 2013); however, it can change the soil mineral N availability because of its porous nature. In this study, biochar amendment increased NO_3^--N content of the rhizosphere in the acidified soil. The level of NO_3^--N was directly influenced by the air-filled pore space as well as neutral pH values of soil (about pH 6.7) (Olness *et al*., 2001). The effect of large macropore area and high pH on biochar may have a role effect on N dynamics. The addition of lime could also promote an increase in the NO_3^--N content (Table 3). However, biochar amendment decreased the content of NH_4^+-N in the acidified soil at the tillering and PI stages,

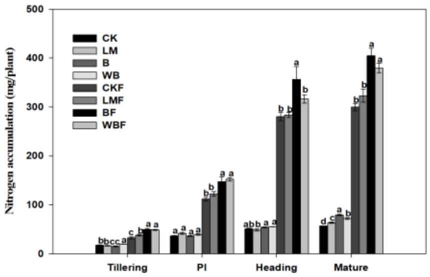

Fig. 2: Effects of biochar application and N level on N uptake by rice during different growth periods. Tillering (the active tillering stage), PI (the panicle initiation stage), Heading (80% of the first spike stage), Mature (the filling ripening stage). CK without biochar, lime addition (LM), biochar (B), washed biochar (WB) with low N fertilization and CKF, LMF, BF, WBF refer to high N fertilization application. Each value is the average ± standard error of four replicates. Different letters indicate a significant different meaning ($P < 0.05$)

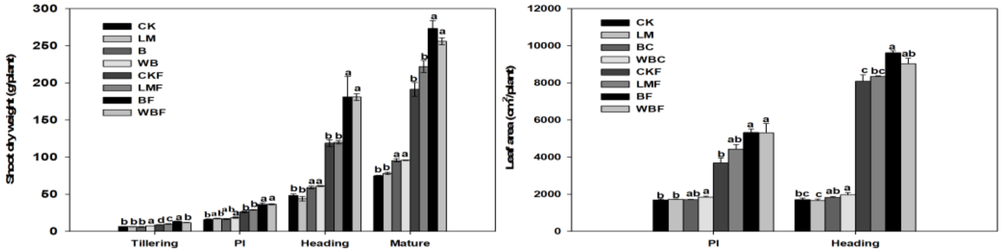

Fig. 3: Effects of biochar application and N level on shoot dry weight and leaf area during different growth periods. Tillering (the active tillering stage), PI (the panicle initiation stage), Heading (80% of the first spike stage), Mature (the filling ripening stage). CK without biochar, lime addition (LM), biochar (B), washed biochar (WB) with low N fertilization and CKF, LMF, BF, WBF refer to high N fertilization application. Each value is the average ± standard error of four replicates. Different letters indicate a significant different meaning ($P < 0.05$)

and maintained higher NH_4^+-N content at the later growth stages. The decrease in NH_4^+-N content might be caused by the increased pH, leading to volatile ammonia formation (Xu *et al*., 2013). Biochar could also absorb NH_4^+-N directly and decrease its solubility by surface binding of ions when the soil NH_4^+-N concentration was high (Chen *et al*., 2012). When N was depleted during the later growth stages of rice, the biochar treatment still maintained a relatively high content of available N in the soil. Taghizadehtoosi *et al*. (2012) showed that NH_4^+-N adsorbed by biochar was readily bioavailable and could be captured by crops.

The addition of biochar could optimize NH_4^+-N and NO_3^--N supply in the soil by regulating N adsorption and release, and improve the nutrient use efficiency of nutrients from soil and fertilization. Biochar application significantly increased N uptake in rice, as noted in earlier findings of Huang *et al*. (2014). Biochar application induced the production of a large root system and higher root activity of rice in late stage, and then p romoted grain filling (Pratiwi and Shinogi, 2016). In our study, higher percentage of filled grains was observed, besides, the increase in panicle number and grain number per panicle may explain the reason of the high grain yields in biochar treatments.

Rice straw biochar provided liming and nutrient adsorption effects, and shows great potential for improving acidified paddy soil. The addition of biochar improved rice yield by 35%, whereas the equivalent lime amendment showed no significant yield increase when compared with the control. The liming effect of biochar contributed to the reduction of acidity and improved the available nutrient content, however, the effects of those processes was short lived when compared with the adsorption. There were no significant differences in the grain yield between biochar and washed biochar amendments. The washed biochar had low mineral nutrient content and lost liming effects, however, the adsorption capacity of biochar would be enhanced by acid treatment because of the decrease in inorganic fractions and dissolved organic C (Peng *et al*., 2016). Qian *et al*. (2013) reported the adsorption effect of biochar showed a more sustainable effect on the mitigation of aluminum toxicity than the lime effect. When compared with the liming effect, the adsorption of biochar markedly improved the growth of rice over the whole growth period in the acidified soil.

Conclusion

Biochar providing both sorption and liming effects alleviated soil acidity and improved the available nutrient content in acidified paddy soil. Addition of biochar or washed biochar maintained high available N in the soil by regulating N adsorption and release during the later rice growth stages. Biochar and washed biochar stimulated N uptake and increased grain yield. However, application of equivalent lime did not increase the rice yield. Root growth in acidified paddy soils was enhanced by biochar amendment, whereas the application of equivalent lime significantly increased the length and surface area of root only before the PI stage. The adsorption of biochar exhibited a persistent effect in improving acidified soil when compared with the liming effect.

Acknowledgments

This research was supported by the National Natural Science Foundation (31701374), Zhejiang Provincial Natural Science Foundation (LY16C130006), National Key Research and Development Plan of China (2016YFD0200801, 2016YFD0300502), Special Funds for the Construction of Modern Agricultural Technology System (CARS-01-26) and Basic Research Foundation of National Commonweal Research Institute (2017RG004-4).

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(Received 28 March 2018; Accepted 11 June 2018)