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Article

Influence of Biochar on Nitrogen Use Efficiency and Root Morphology of Rice-Seedling in Two Contrasting Paddy Soils

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Abstract: Biochar may affect the root morphology and nitrogen (N) use efficiency (NUE) of rice at seedling stage, which has not been clearly verified until now. To clarify it, we conducted a pot experiment regarding to two soil types (Hydragric Anthrosol and Haplic Acrisol), two biochar application rates (0.5 *wt*% and 1.5 *wt* %) and two rice varieties (common rice var. Xiushui134 and hybrid super rice var. Zhongkejiayou12-6) meanwhile. Seedling NUE of common rice Xiuhui134 was significantly increased ($p < 0.05$) by 78.2% in Hydragric Anthrosol and by 91.4% in Haplic Acrisol following biochar addition with 1.5 *wt*%. However, biochar addition exerted no influence on seedling NUE of super rice Zhongkejiayou12-6 in both soils. Overall, 0.09–0.10 units higher soil pH and 105–116% higher soil NH_4^+-N were observed in Xiushui134 growing two soils with 1.5 *wt*% biochar. In addition, improved root morphology (including longer root length, larger root surface area, bigger root volume, and more root tips) contributed to the higher seedling NUE of Xiushui134 in two soils. The soil pH and NH_4^+-N content, also the root morphology were influenced by biochar, which though could not thoroughly explained the NUE of Zhongkejiayou12-6. In conclusion, biochar application to paddy soil changed soil pH and NH_4^+-N content, root growth, and the consequent seedling NUE of rice, which effects are relative with rice cultivar, biochar addition rate, and soil type.

Keywords: Ammonium; biochar; nitrogen management; rice paddy soil; root morphology; super rice

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops, feeding more than half of the world's population. However, in order to improve yields, rice paddy fields are often over fertilized with nitrogen (N) [1], which is a serious problem in Asia, especially China. Excessive N application not only reduces N use efficiency (NUE), but also causes N leaching and runoff losses, increases ammonia volatilization and nitrous oxide emission [2,3]. Therefore, effective improvement the NUE of rice under optional N input rate is of great significance in ensuring food security and protecting the environment meanwhile [4]. To reach this aim, we should firstly try to enhance the NUE of rice seedling and ideal root morphological traits [5]. As for rice, root morphological characteristics are closely associated with NUE and yield [6].

Biochar is a kind of pyrolysis material with large specific surface area and rich pore structure mainly prepared from plant materials under no or limited oxygen condition [7]. Recently, biochar is widely studied and used to rice paddy soil, attributing to its functions in improving soil properties [8], reducing greenhouse gas emission and ammonia volatilization [9,10], and enhancing NUE and thereby the grain production [11]. Thus we can reduce the application of N and keep grain yields in biochar-added rice paddy soil. What is more, there are reports showing that increases in grain yield and NUE was mainly attributed to root biomass, root length, root tips, and root-oxidizing activity [5,12]. According to previous studies, biochar could improve soil physical structure, which consequently impact the rice root development [13,14]. Field with application of biochar increased root biomass (+32%), root volume (+29%) and root surface area (+39%) of crops [13]. It has been proved that biochar can increase lateral roots and fine roots of crops [14]. For rice crops, ammonium (NH_4^+-N) and nitrate (NO_3^--N) are the main forms of N available to the young rice plants [5,15]. However, root morphology of rice affects NUE and biochar also has an effect on the conversion of N forms [16]. Therefore, it is necessary to explore the response mechanism between rice root morphology and biochar application. However, whether biochar affects NUE by changing rice seedling root morphology has been little documented.

Asia, as the main rice production area all over the world, has many soil types distributed with varied properties, and also many rice varieties, including traditional cultivated and super hybrid rice cultivars, which was also in China [5,17]. Therefore, the hypothesis of current work is biochar can influence the NUE of rice seedling via changing the root morphology, which effect might depended on soil type, rice variety and biochar application rate. Here, we conduct a pot experiment to clarify the responses of rice seedling NUE and root morphology to biochar application with low/higher rates and the potential mechanisms.

2 Material and Methods

2.1 Background Information and Soil Pots Installation

Two types of typical paddy soils were collected from Yixing (31°28’ N, 119°59’ E), Jiangsu province, and from Yingtan (28°12’ N, 117°10’ E), Jiangxi province, respectively. The Yixing soil is classified as Hydragic Anthrosol paddy soil and the Yingtan soil is Haplic Acrisol paddy soil according to WRB [18]. These two soils represented two dominant soil types in Chinese rice production systems. After being dried and crushed passing 2-mm sieve, soil samples were correspondingly repacked to transparent pot (inner diameter 12.5 cm, height 20 cm) with 2.4 kg soil each. Biochar was produced by continuous slow pyrolysis of wheat straw without oxygen at 500°C. The biochar has a BET (Brunauer-Emmett-Teller) surface area of 51.5 m^2/g and contains 174 g/kg ash. The quantitative biochar (biochar: soil = 0.5 *wt*% and 1.5 *wt*%) were homogeneously mixed with the soil sample during repacking practice. The selected properties of tested soils and the biochar are shown in Tab. 1.

Table 1: Selected properties of two tested soils and wheat straw biochar

Soils/biochar	pH	Total N	Total P	Total K	SOC	CEC
		g/kg				cmol/kg
Hydragric anthrosol	6.38	1.56	0.96	4.12	22.8	15.0
Haplic acrisol	5.05	1.90	1.29	4.44	18.1	8.89
Wheat straw biochar	9.51	13.3	4.40	20.9	–	27.5

Note: SOC: soil organic carbon; CEC: cation exchange capacity.

A common rice variety Xiushui134 (XS) and a hybrid super rice variety Zhongkejiayou12-6 (ZK), according to previous work [5], were selected for the current experiment. The 28-day rice seedlings were transplanted to the pots (2 seedlings per pot).

2.2 Experimental Design and Management

There were three treatments for each soil type planted with each rice variety: Urea (receiving urea N fertilizer), Urea + 0.5%BC (receiving urea N fertilizer plus 0.5 *wt*% biochar), and Urea + 1.5%BC (receiving urea N fertilizer plus 1.5 *wt*% biochar). Every type of soil planted with each rice variety included one control treatment (no application of N and biochar) to calculate the NUE of rice seedling. Three replicates were maintained for each treatment.

Pre-flooding irrigation for each pot was formed one week prior to rice transplanting. Floodwater was continuously maintained at a depth of 3–5 cm in all soil pots. We homogeneously broadcasted 0.25 g urea to each pot (approximately equal to 240 kg N/ha under field condition).

2.3 Sample and Measurement

Fourteen days after transplanting, all treated rice seeding were harvested for the measurements of shoot biomass, N content. At the same time, the rice seedling roots were washed with deionized water and immediately for observation of root morphology. Soils were sampled for pH (soil: water = 1: 5), NH_4^+-N and NO_3^--N determinations.

The harvested rice seedling samples were placed in a drying oven at 105°C and then were dried at 75°C to a consistent weight, when we recorded the shoot biomass (dry weight). Dry plant samples were ground into powder and digested with H_2SO_4-H_2O_2, then the total N content of rice plant was determined using the Kjeldahl method [5]. The NUE was calculated as the percentage of applied fertilizer N recovered in above-ground biomass minus that of the control treatment. The soil NH_4^+-N and NO_3^--N were extracted by 2.0 mol/L KCl, then the NH_4^+-N and NO_3^--N concentrations of the KCl extracted soil solution were measured by a San^{++} Continuous Flow Analyzer (Skalar, Netherlands).

Rice roots and shoots were separated and washed with deionized water. Root morphology including total root length, root volume, root surface area, average root diameter, and root tip number in the two rice cultivars were measured using a root analysis instrument WinRhizo-LA1600 (Regent Instruments Inc., Quebec, Canada) [5].

2.4 Statistical Analysis

Analysis of variance (one-way ANOVA) was used to determine the significance of the difference between treatments. Ducan multiple-comparison test was conducted to determine the differences among treatments ($p < 0.05$) (SPSS Ver. 16.0 for Windows, SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Shoot Biomass and NUE of Rice at Seedling Stage

Compared to the control, application of N fertilizer significantly increased ($p < 0.05$) shoot biomass of two rice seedlings planted in two soils (Tab. 2). Generally, biochar amendment exerted no remarkable effect on rice shoot biomass. Exceptionally, averaged 19.0% higher shoot biomass was observed under Urea + 1.5%BC than that under Urea only treatment for Xiushui134 planted in Haplic Acrisol. The NUEs of Xiushui134 seedlings were only 6.62–11.8% and 4.89–9.36%, when grown in Hydragric Anthrosol and Haplic Acrisol, respectively. Zhongkejiayou12-6 had relatively higher NUEs, reached 10.7–12.1% in Hydragric Anthrosol and 16.2–18.2% in Haplic Acrisol. Data in Tab. 1 suggested that biochar amendment did not improve the NUE of Zhongkejiayou tested under both soils. However, NUEs of Xiushui134 under biochar-added treatments were higher than that under Urea treatment. Particularly, Urea

+ 1.5%BC had 78.2% and 91.4% significantly higher ($p < 0.05$) NUEs than that of Urea, in Hydragric Anthrosol and Haplic Acrisol, respectively.

Table 2: Impacts of biochar addition on shoot biomass and nitrogen use efficiency (NUE)

Soil type	Rice variety	Treatment	Shoot biomass (g)	NUE (%)
Hydragric Anthrosol	Xiushui134	Control	0.22 ± 0.02 b	–
		Urea	0.27 ± 0.03 a	6.62 ± 3.21 b
		Urea + 0.5%BC	0.28 ± 0.02 a	9.09 ± 2.72 ab
		Urea + 1.5%BC	0.29 ± 0.03 a	11.8 ± 1.21 a
	Zhongkejiayou12-6	Control	0.21 ± 0.03 b	–
		Urea	0.29 ± 0.04 a	12.1 ± 7.10 a
		Urea + 0.5%BC	0.27 ± 0.02 a	10.7 ± 6.18 a
		Urea + 1.5%BC	0.29 ± 0.02 a	11.7 ± 1.99 a
Haplic Acrisol	Xiushui134	Control	0.15 ± 0.01 c	–
		Urea	0.21 ± 0.01 b	4.89 ± 0.84 b
		Urea + 0.5%BC	0.23 ± 0.02 ab	5.39 ± 2.25 ab
		Urea + 1.5%BC	0.25 ± 0.02 a	9.36 ± 2.43 a
	Zhongkejiayou12-6	Control	0.19 ± 0.03 b	–
		Urea	0.34 ± 0.04 a	18.2 ± 5.34 a
		Urea + 0.5%BC	0.32 ± 0.04 a	16.5 ± 3.37 a
		Urea + 1.5%BC	0.29 ± 0.02 a	16.2 ± 3.30 a

Note: Values shown are the means ± SD of three replicates. Different letters in same column for single rice variety indicate significant differences between each treatment within same soil type and rice variety according to Duncan's multiple range test at $p < 0.05$.

3.2 Soil pH and NH_4^+ -N Content

The soil pH and NH_4^+-N content after two-week seedlings harvested were presented in Figs. 1 and 2, respectively. Urea fertilizer input slightly increased soil pH, excepted for Haplic Acrisol planted with Xiushui134. Overall, soil pH increased following the application of biochar, particularly with higher rate (1.5 *wt*%). When applied with 1.5%, biochar increased soil pH by 0.07–0.18 units, compared to the urea only treatments.

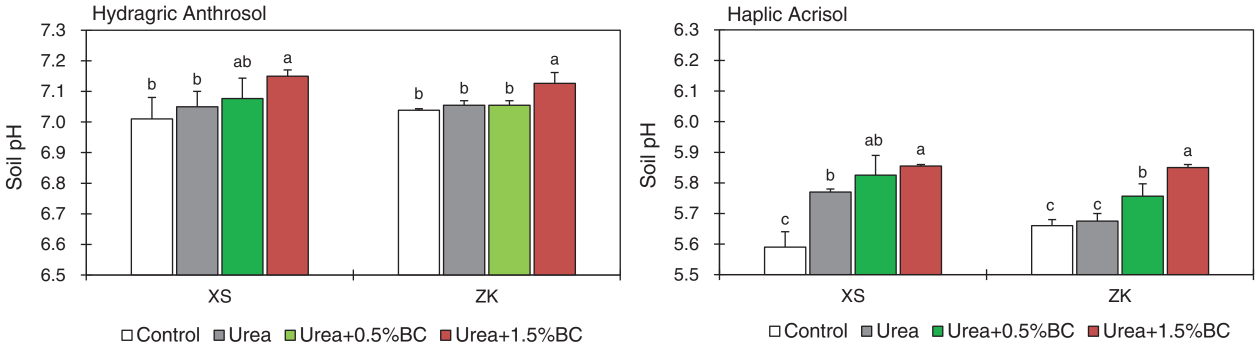

Figure 1: Impacts of biochar addition on soil pH at rice seedling stage. XS and ZK referred to Xiushui134 and Zhongkejiayou12-6, respectively. Error bars represent the standard deviation (SD) for three replicates. Different letters indicate significant differences between each treatment within same rice variety according to Duncan's multiple range test at $p < 0.05$

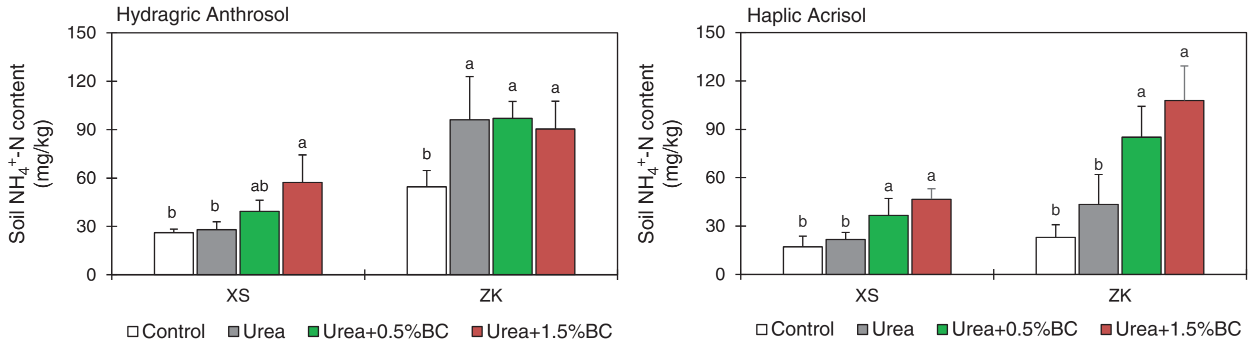

Figure 2: Impacts of biochar addition on soil NH_4^+-N content at rice seedling stage. XS and ZK referred to Xiushui134 and Zhongkejiayou12-6, respectively. Error bars represent the standard deviation (SD) for three replicates. Different letters indicate significant differences between each treatment within same rice variety according to Duncan's multiple range test at $p < 0.05$

Fig. 2 showed that NH_4^+-N contents of two contrasting soil cultivated with Zhongkejiayou12-6 were relatively higher than that cultivated with Xiushui134. For Xiushui134 planted soils, urea N addition did not influence soil NH_4^+-N content. However, soil NH_4^+-N content increased significantly ($p < 0.05$) by 76.3–88.7% following the N fertilization (Fig. 2). Except for Zhongkejiayou12-6 cultivated Hydragric Anthrosol, biochar application raised soil NH_4^+-N contents by 40.9–148.6%, compared with Urea treatment. What is more, this improve effect was stronger when biochar was applied at higher rate (1.5 *wt*%).

3.3 Root Morphology Indexes

Impacts of biochar addition on rice seedling planted in two contrasting soils were presented in Tab. 3. For Xiushui134 in Hydragric Anthrosol, we found that biochar addition promoted the seedling root morphology, with longer root length (+87.6–88.2%), larger surface area (+58.9–64.3%), higher root volume (+38.5–46.2%), and more root tips (+67.9–69.4%), compared with the Urea treatment. This effect was also confirmed by Zhongkejiayou12-6 in Hydragric Anthrosol and Xiushui134 in Haplic Acrisol (Tab. 3). The improvement effect of biochar on seedling root was independent with biochar applied rate for two rice varieties in Hydragric Anthrosol, but not for Xiushui134 in Haplic Acrisol, which had best root morphology when biochar applied with a relative higher rate (1.5 *wt*%).

Table 3: Root total length, surface area, root volume, average diameter and root tips of Xiushui134 and Zhongkejiayou12-6 seedlings planted in Hydragric Anthrosol and Haplic Acrisol with biochar-added

Soil type	Rice variety	Treatment	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Average diameter (cm)	Root tips
Hydragric Anthrosol	Xiushui134	Control	176.3 ± 33.3 b	16.7 ± 2.2 b	0.13 ± 0.01 b	0.30 ± 0.02 a	2068 ± 379 b
		Urea	168.8 ± 56.5 b	16.8 ± 3.7 b	0.13 ± 0.01 b	0.33 ± 0.05 a	1940 ± 726 b
		Urea + 0.5%BC	317.7 ± 44.7 a	27.6 ± 3.2 a	0.19 ± 0.02 a	0.28 ± 0.01 a	3258 ± 185 a
		Urea + 1.5%BC	316.6 ± 74.4 a	26.7 ± 4.2 a	0.18 ± 0.02 a	0.27 ± 0.02 a	3287 ± 842 a
	Zhongkejiayou12-6	Control	252.6 ± 35.0 c	24.4 ± 2.3 b	0.19 ± 0.01 c	0.30 ± 0.02 a	2554 ± 581 b
		Urea	346.7 ± 25.4 b	31.9 ± 9.2 b	0.23 ± 0.06 bc	0.30 ± 0.04 a	3054 ± 509 b
		Urea + 0.5%BC	672.3 ± 53.7 a	51.3 ± 8.1 a	0.31 ± 0.07 ab	0.24 ± 0.02 b	6161 ± 401 a
		Urea + 1.5%BC	739.9 ± 46.7 a	57.4 ± 8.3 a	0.35 ± 0.04 a	0.25 ± 0.02 b	6772 ± 727 a
Haplic Acrisol	Xiushui134	Control	194.1 ± 56.4 b	15.8 ± 3.6 b	0.10 ± 0.02 b	0.26 ± 0.02 ab	1820 ± 867 b
		Urea	219.0 ± 52.9 b	19.0 ± 3.5 b	0.13 ± 0.02 b	0.28 ± 0.02 a	2011 ± 274 b
		Urea + 0.5%BC	293.5 ± 84.9 b	21.6 ± 4.9 b	0.13 ± 0.03 b	0.24 ± 0.02 b	2491 ± 846 b
		Urea + 1.5%BC	422.0 ± 17.9 a	31.0 ± 0.6 a	0.18 ± 0.02 a	0.23 ± 0.01 b	4020 ± 732 a

(Continued)

Table 3 (continued).

Soil type	Rice variety	Treatment	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Average diameter (cm)	Root tips
	Zhongkejiayou12-6	Control	261.6 ± 50.4 c	23.3 ± 4.6 c	0.16 ± 0.04 c	0.28 ± 0.02 a	2500 ± 736 c
		Urea	876.9 ± 61.7 a	68.7 ± 4.4 a	0.43 ± 0.03 a	0.25 ± 0.00 b	6837 ± 678 a
		Urea + 0.5%BC	484.8 ± 72.9 b	38.0 ± 6.8 b	0.24 ± 0.05 b	0.25 ± 0.01 b	4758 ± 206 b
		Urea + 1.5%BC	430.1 ± 81.2 b	33.6 ± 2.6 b	0.21 ± 0.03 bc	0.25 ± 0.01 b	4414 ± 257 b

Note: Values shown are the means ± SD of three replicates. Different letters in same column for single rice variety indicate significant differences between each treatment under same soil type and rice variety according to Duncan's multiple range test at $p < 0.05$.

Nevertheless, the development of seedling root was likely retarded following the biochar amendment. Data in Tab. 3 showed that 44.7–51.0% shorter root length, 44.7–51.1% smaller surface area, 44.2–51.2% lower root volume, and 30.4–35.4% less root tips were recorded under Zhoukejiayou12-6 in Haplic Acrisol added with biochar (both at 0.5 and 1.5 *wt*%) than that under their counterpart Urea treatment. Results in Tab. 3 also suggested that the influences of biochar on average root diameter varied with rice variety and soil type.

4 Discussion

China initiated a "super rice" breeding program in 1996, focusing on hybrid rice lines, with the goals of producing high-yield rice cultivars through morphological improvement and through the utilization of N fertilizer [5]. In the present study, as can be seen from Tab. 2, biochar has different effects on NUE of the two rice varieties, hybrid super rice variety Zhongkejiayou12-6 indeed showed higher NUE than common rice variety Xiushui134. What is more, Zhongkejiayou12-6 developed better root traits than Xiushui134, regardless of soil type and biochar addition rate (Tab. 3).

Nitrogen is one of the key nutrient for rice, and its availability in soil profile or root rhizosphere can influence the plant root morphology, total N uptake, and the NUE [12]. It had been demonstrated that NH_4^+-N a preferred N source, and is the main form of N available to rice seedling [15]. Data in Tab. 2 suggested that biochar addition, particularly when applied at higher rate (1.5 *wt*%), indeed enhanced rice seedling NUE. Improvement on soil physical properties likely explained the higher NUE of Xiushui134 in biochar-added soils [7,13]. Well-know, biochar always has a basic features (biochar tested in current work was with a pH of 9.51), which could increase soil pH, particularly under acidic Haplic Acrisol (Fig. 1). After been transplanted, seedlings are more sensitive to pH changes, so changes in soil pH may be benefit to seedling growth. What is more, biochar can inhibit the soil N nitrification process that more NH_4^+-N could be retained in soil [19,20]. In the present study, except for Zhongkejiayou12-6 in Hydragric Anthrosol, biochar-added soils recorded higher NH_4^+-N contents than that of only urea added soils (Fig. 2). Higher soil NH_4^+-N content provided more N available to youth rice seedling that exerted higher NUE. Moreover, the rich functional groups and large specific surface area of biochar can improve soil CEC [21], absorb more nutrient ions besides N [22]. However, this could not explain why biochar addition had no significant influence on NUE of Zhongkejiayou12-6, a hybrid super rice cultivar, which should be explored in future study.

The change of soil pH has a direct influence on the growth and development of crops, especially the root system [23,24]. That is to say, biochar significantly promoted the growth and development of rice root system during Xiushui134 seedling stage (Tab. 3), which is consistent with a previous report [16]. Similarly, studies have shown that biochar has a catalytic effect on other plants. For example, biochar increased the root growth of citrus rootstock by 50% and the roots prefer to grow around biochar particles [23]. Miller et al. [24] also reported that roots preferentially grow around biochar to extend the rhizosphere. Therefore, the root system of the crop can extend to capture more N in the biochar-added soil. Nevertheless, biochar retarded the

Zhongkejiayou12-6 root growth in acidic Haplic Acrisol, though had not exert negative effect on NUE of seedling (Tabs. 2 and 3). Thus, the long-term effect and the underlying mechanisms of biochar on root morphology of varied rice cultivars in contrasting soils should be studied in future.

5 Conclusion

A soil pot experiment was conducted to observe the effects of biochar application with different rates on NUE, root morphology of rice seedling and the underlying relationship considering varied soil type and rice cultivars. Common rice Xiuhui134's NUE was increased after biochar addition, which effect was significant in higher rate of biochar-added two soils. The enhanced NUE of Xiushui134 was likely attributing to the improved soil pH, increased soil NH_4^+-N content, and developed seedling root morphology. However, biochar addition had no impact on seedling NUE of super rice Zhongkejiayou12-6, which could not be thoroughly explained by the presently observed index. This phenomenon should be further explored in future study. In conclusion, biochar amendment indeed influence the soil pH and NH_4^+-N content, and the seedling root morphology and possibly impact the seedling NUE, which effects are dependent on rice cultivar, soil type and biochar application rate.

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