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Responses of Wheat Production, Quality, and Soil Profile Properties to Biochar Applied at Different Seasons in a Rice-Wheat Rotation

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ABSTRACT

In the rice-wheat rotation system, biochar (BC) can be applied at the initiation of the rice or wheat season. Here, we compared the effects of BC that were applied at two different crop seasons on wheat production, quality, and soil profile properties in a rice-wheat rotation system with nitrogen (N) fertilizer applied at 280 kg/ha rate. Results showed that both wheat grain production and N recovery use efficiency were influenced by BC applied at two crop seasons. Biochar application did not affect the total non-essential amino-acid, but when applied during wheat season, BC significantly ($p < 0.05$) increased total essential amino acid in grain by 12.3%, particularly for the valine (+48.2%), methionine (+43.8%), and isoleucine (+10.3%). We found that BC significantly ($p < 0.05$) decreased the pH of soil at 0–6 cm and 20–30 cm by 0.14–0.18 and 0.05–0.08 units, respectively. The NH_4^+ -N content of the whole observed soil profile were reduced by BC application, however, the effect of BC on NO_3^- -N content varied with the application season and profile depth. Interestingly, BC applied at wheat and rice season significantly ($p < 0.05$) improved topsoil N contents by 48.4% and 19.7%, respectively. In addition, data suggested that BC applied during wheat season performed better in enhancing soil available phosphorus, potassium, and organic matter contents. In conclusion, we suggest that the optimum application time of BC for enhancing crop production and quality (take amino-acid content for example) and improving soil fertility is at the initiation of the wheat season.

KEYWORDS

Amino-acid; biochar; paddy soil; rice-wheat rotation; soil fertility; soil organic matter

1 Introduction

The use of chemical fertilizers, in particular nitrogen (N), is a key to food security all over the world [1], where grain production increased by 74% between 1982 and 2020 [2]. However, with the dramatically increasing use of N fertilizer, Chinese policymakers and landowners recognize the interconnections between water and air environmental pollution, low N use efficiency (NUE), and degraded soil fertility [3,4]. It is therefore vital to improve the NUE and soil properties for stable food crops such as maize (*Zea mays*), wheat (*Triticum aestivum* and *Triticum durum*), and rice (*Oryza sativa*) that receive approximately 45% of the applied chemical N fertilizer all over the world [3,5]. Previous works demonstrated that optimizing N fertilizer and irrigation management and amending soil additives could help to address the forgoing issues in stable crop and vegetable productions [6–10].



Biochar (BC) is a typical carbon-rich product that is derived from the thermochemical conversion of agriculture and forest biomass or others under oxygen-limited conditions [11]. Its use in agricultural systems comes with numerous benefits such as improving stable crop yields, ameliorating key soil properties, and combating global climate change [11,12]. Agronomic BC research is a rapidly evolving research field, with >15,000 publications at the end of 2020 but with only <100 publications in 2010 [13,14]. There is no doubt that BC will be broadly used in future agronomic practices. In field management of farmland owners, BC is always incorporated with the topsoil or plow layer while conducting tillage. In China, rice-wheat, maize-wheat, and rice-rape are three typical crop rotations [3,15]. To improve crop production and increase the net benefits of farmland systems, landowners always practice tillage management at one of the crop seasons of rotation. In the rice-wheat rotation system, it means that BC might be incorporated into the paddy soil at the initiation of the rice or wheat growth cycle. Under this context, the alternations of aerobic and anaerobic environments as a result of overlying water flooded and drained change the properties of BC that are applied before either rice transplanting or wheat sowing. These changes in the properties of aged BC affect its functions on crop production and soil characteristics [16,17]. Nevertheless, studies comparing the effects of BC applied at different stages of the crop growth cycle of a rice-wheat rotation system are lacking.

Although the changes in characteristics of soil from BC-amended soil had been well documented [18], understanding the changes in soil fertility caused by BC application at soil profile is limited. Factors influencing soil fertility including available nutrients, pH, and soil organic matter (SOM) can vary dramatically along with profile depth [19,20]. Liu et al. evaluated the ability of BC that is derived from maize straw to mitigate the N and phosphorus (P) losses and improve the distribution of BC in soil profile [21]. In addition, Ingunn et al. carried out a NIR hyper-spectral reflectance imaging and identified the distribution pattern of BC particles along with the soil profile [22]. The vertical movement of BC particles no doubt changes some soil properties at profile.

In the light of above, we conducted a pot experiment with PVC columns to investigate how crop production, quality, and soil profile properties respond to BC that is applied at the initiations of the rice or wheat growth cycle. We hypothesized that BC applied at different crop seasons would exert varying effects on soil fertility at the profile level. Results obtained from current work would help us to comprehensively clarify how BC management affects crop production and soil fertility in the rice-wheat rotation system.

2 Materials and Methods

2.1 Soil Column Preparation and BC Properties

In a greenhouse with a top-shed at Yixing, China, a soil pot experiment using a PVC column (with 30 cm in inner diameter and 60 cm in height) was conducted. To keep the same conditions with the ambient environment such as the wind, temperature, and moisture, no walls were installed in the greenhouse during crop growing cycle. The study area had an average annual precipitation of 1120 mm and an annual temperature of 16.0°C. Tested soil was classified as a typical Hydragric Anthrosol, sampled from 0–20 cm topsoil and 20–50 cm subsoil in a rice-wheat rotation located at Zhoutie Town (31° 28'N, 119° 59'E), Yixing, Jiangsu Province, China. After air drying and sieved by a 2-mm nylon mesh, the 0–20 and 20–50 cm soil samples were separately repacked into the soil columns. The 0–20 cm topsoil had the following basic properties: pH 7.7 (soil:water = 1:5), bulk density 1.31 g/cm³, available N 96 mg/kg, available P 53 mg/kg, available potassium (K) 188 mg/kg, and organic matter 23.2 g/kg.

The BC used in the current study was provided by Nanjing Forestry University and derived from dried wheat straw, using a biogas-energy pyrolysis system at 500°C continuing 8 h. The physicochemical characteristics of the fresh BC before being incorporated into the soil were pH 9.1 (BC:H₂O = 1:5), CEC (cation exchange capacity) 23 cmol/kg, total carbon 480 g/kg, total N 7.3 g/kg, and BET surface

area 32.0 m²/g. The BC application rate was 35 g/pot, approximately equivalent to 5 t/ha under field condition. We mixed the BC and 0–20 topsoil before rice transplanting and wheat sowing in June and November 2019, respectively. The corresponding experimental treatments were labeled as R and W, respectively. The same management was done consistently during each crop season until the wheat was harvested on May 16, 2021.

2.2 Experiment Design and Management

The current pot experiment included three treatments: (1) chemical N fertilizer (urea) applied at 280 kg/ha rate (abbreviated as N280) for each rice-wheat rotation (180 and 100 kg/ha for rice and wheat, respectively); and (2, 3) urea N plus 5 t/ha BC at initiations of rice [N280+BC(R)] or wheat [N280+BC(W)] growth cycles. For all treatments, besides urea-N, calcium superphosphate, and potassium sulfate were applied into 0–20 cm topsoil to supply P and K nutrients for plant growth at rates of 120 kg/ha P₂O₅ and 100 kg/ha K₂O, respectively. Both P and K fertilizers were applied as basal fertilizers.

2.3 Soil and Plant Sampling and Determinations

For the current study, we used a soil auger (5 cm inner diameter) to collect the 0–6, 6–12, 12–20, 20–30, 30–40, and 40–50 cm depth soil samples from 5 random and different positions in each pot of the control and BC-added treatments) on June 10, 2021, after wheat harvest. The soils from five positions were pooled together to form a composite sample. We first picked out the roots in fresh soils and then sieved them through 2 mm nylon mesh. The soil samples were stored at a 4°C refrigerator before determining their properties.

Soil pH (1:5 soil-water ratio) was determined using a pH probe (Quark Ltd., Nanjing, China). Soil total N and SOM were measured by a CN analyzer (Perkin Elmer, 2400 II, Waltham, USA) and wet digestion method with H₂SO₄-K₂Cr₂O₇, respectively. We used 2 M KCl (1:5 soil-solution ratio) to extract the exchangeable NH₄⁺-N and NO₃⁻-N and then quantified them by a continuous-flow San++ System analyzer (Skalar Analytical BV, Breda, Netherlands).

Wheat shoots were manually harvested after plants reached their maturity (end of May 2021) and divided into grain and straw, respectively. After being oven-dried, their dry weights were recorded. Meanwhile, separately grain and straw (approx. 300 g for each) were oven dried at 105°C for 0.5 h firstly and then at 75°C to a constant weight. The total N contents of the dry plant sample were determined by the Kjeldahl method, after being digested with H₂O₂ and H₂SO₄ solutions. Wheat N uptake capacity was calculated as the multiplied product of the dry biomass weight and total N contents. The percentage of wheat biomass (straw plus grain) N accumulation increases because urea application to the N rate was used as wheat NUE [23]. The free amino-acid content determination and the total amino-acid content calculation of wheat grain were conducted as described elsewhere [23].

2.4 Statistical Analysis

Data in tables and figures were presented as the means ($n = 3$) ± standard deviations (SDs). We determined the statistically significant differences between the treatments using the single-factor ANOVA method. The least significant difference (LSD) multiple comparison method in SPSS 16.0 software was applied at $p < 0.05$ level.

3 Results and Discussion

3.1 Wheat Production and NUE

Wheat plant height, straw dry biomass, and grain production under three treatments were 68.7–69.3 cm, 45.0–47.9 g/pot, and 29.7–31.9 g/pot, respectively, which were not influenced by BC, regardless of the crop seasons in a rice-wheat rotation (Table 1). Increasing crop production is always the first commonly reported

positive effect of amending BC into farmland soils [13]. Improvements in the physical properties of soil such as bulk density and water holding capacity might decide the effects of BC on grain yield. However, some contrasting experimental results have been published, which are dependent on their experimental scale, soil characteristics, and BC type [24]. Unlike our result, Hu et al. reported a remarkable after-effect of straw-derived BC in improving crop plant growth and final production [15]. No positive effect in wheat grain yield improvement following BC amendments may be explained by the sufficient N nutrient in tested soil [5,25]. What was more, our data showed that applying BC during the wheat season, i.e., the N280+BC(W) treatment, significantly ($p < 0.05$) decreased the thousand-seed weight of wheat grain by 11.6%, compared with N280. However, three yield-related agronomic traits of wheat grain were not changed by BC and were amended before rice seedlings were transplanted.

Table 1: Impacts of biochar (BC) that being applied at rice (R) or wheat (W) season on the grain production and the agronomic traits of wheat and the N use efficiency (NUE)

Treatment	Plant height (cm)	Straw (g/pot)	Grain (g/pot)	Yield-related agronomic traits			Nitrogen use efficiency (NUE)
				Panicles	Grains per panicle	Thousand-seed weight (g)	
N280	68.7 ± 0.8 a	45.0 ± 6.2 a	31.9 ± 0.5 a	33 ± 3 a	30 ± 1 a	31.1 ± 1.5 a	57.5 ± 9.4 a
N280 +BC(R)	69.3 ± 0.9 a	45.8 ± 2.1 a	32.4 ± 1.4 a	34 ± 1 a	33 ± 2 a	29.8 ± 0.7 a	59.7 ± 1.6 a
N280 +BC(W)	69.3 ± 1.9 a	47.9 ± 1.1 a	29.7 ± 1.3 a	35 ± 1 a	34 ± 5 a	27.5 ± 0.5 b	52.2 ± 5.2 a

Note: Data was mean ± SD ($n = 3$); the different small letters in the same column refer to the significant difference between the experimental treatments ($p < 0.05$).

Wheat NUE ranged from 52.2% to 59.7% (Table 1). Wheat planted under N280+BC(R) had the highest NUE, which was 12.6% higher than that under N280+BC(W). However, no difference in wheat NUE among the three treatments was statistically significant ($p < 0.05$). According to a previous meta-analysis, BC leads to an average increase in plant N uptake by 11%, which is attributed to a remarkable 12% increase in plant biomass but a minor 2% decrease in plant tissue N content [26].

3.2 Free Amino Acid Content in Wheat Grain

Biochar addition increased the total free amino acid contents in wheat grain (Fig. 1). Compared with N280, N280+BC(W) significantly ($p < 0.05$) improved (+4.0%) the content of free total amino acid in wheat grain. The balance of free amino acid compositions contained in the edible wheat grains determines their nutritional quality and market price [27]. Similar to a study conducted in saline soil [23], the main components of the total amino acids were influenced by BC amendment. Easily understood, the ambient environmental conditions influence crop production, protein quantity, and amino acid compositions [28]. Meanwhile, improvements in soil physical properties such as the bulk density and water holding capacity following the BC amendment might promote the amino acid content of wheat grain. Therefore, BC applied at an optimum time can improve crop grain quality. Additional studies are required to assess the long-term effects of BC application on the amino acid content of wheat and other edible quality, particularly under field conditions.

Crop grain amino acids can be classified into two major kinds: essential amino acids and non-essential amino acids, which vary from 8%–26% and depend on the crop genotype and the field growing conditions [29]. Data from Table 2 showed that the BC being applied before wheat seed sowing significantly ($p < 0.05$)

increased the contents of valine (+48.2%), methionine (+43.8%), and isoleucine (+10.3%), compared with N280. In addition, 26.1% more lysine was recorded from N280+BC(R) than from N280 (significant at $p < 0.05$). Other essential amino acids in wheat grain, i.e., threonine, leucine, and phenylalanine were not impacted by BC applied at either crop season. Overall, BC amendment improved the essential amino-acid content in wheat grain by 0.11–0.26 mg/kg. Particularly, wheat grain harvested from N280+BC(W) had 11.5% more essential amino acid than that from N280 (significant at $p < 0.05$; Table 2). Therefore, from the perspective of improving grain quality, the BC applied during the wheat growth cycle exerted a greater positive effect.

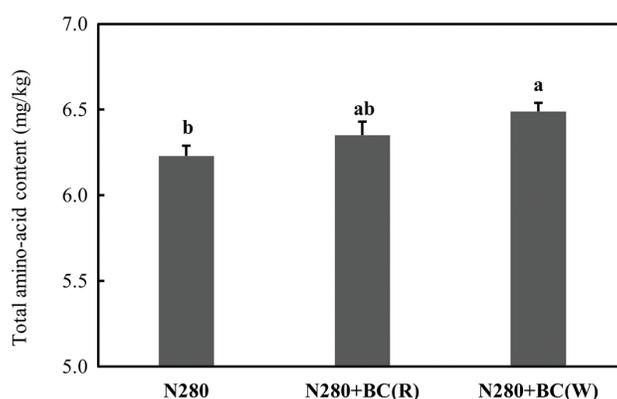


Figure 1: Response of total amino-acid content of wheat to biochar (BC) applied at rice (R) or wheat (W) season. Data were presented as means \pm SD ($n = 3$); data labeled with different small letters is significantly different at $p < 0.05$ level

Table 2: Effects of biochar (BC) applied at rice (R) or wheat (W) season on the contents of essential amino-acid in wheat grain

Treatment	Content of the essential amino acids (mg/kg)							
	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Lysine	Total
N280	0.28 \pm 0.03	0.27 \pm 0.05 b	0.16 \pm 0.01 b	0.29 \pm 0.01 b	0.56 \pm 0.01	0.32 \pm 0.02	0.23 \pm 0.01 b	2.11 \pm 0.09 b
N280+BC(R)	0.26 \pm 0.03	0.32 \pm 0.05 ab	0.16 \pm 0.04 b	0.28 \pm 0.02 b	0.57 \pm 0.04	0.34 \pm 0.02	0.29 \pm 0.03 a	2.22 \pm 0.14 ab
N280+BC(W)	0.27 \pm 0.02	0.40 \pm 0.07 a	0.23 \pm 0.02 a	0.32 \pm 0.02 a	0.55 \pm 0.08	0.32 \pm 0.01	0.27 \pm 0.01 ab	2.37 \pm 0.11 a

Note: Data were presented as means \pm SDs ($n = 3$); data labeled with different small letters is significantly different at $p < 0.05$ level.

By contrast, BC applied at both crop seasons exerted a weak effect on the contents of non-essential amino acids in wheat grain (Table 3). Only glycine was decreased significantly ($p < 0.05$) by 17.2% following BC that was added during the wheat season.

3.3 Soil Properties

3.3.1 Soil pH

For N280, the 0–6 cm topsoil layer had a pH of 7.26, then it decreased to 7.08–7.12 in the subsoil profile. Data from Fig. 2 demonstrated that the BC applied at both crop seasons significantly ($p < 0.05$) decreased the

0–6 and 20–30 cm soil pH by 0.14–0.18 and 0.05–0.08 units, respectively. At 6–12 cm, the BC applied at two varied crop seasons exerted a contrasting influence on soil pH. Only being applied at the rice season, BC reduced the 30–40 cm soil pH by 0.07 units. Furthermore, the pH of soil collected from the 12–20 and 40–50 cm was not influenced by BC, regardless it was added during rice or wheat season. Generally, previous studies have confirmed that BC amending treatments showed remarkable increases in soil pH as compared with the control [30,31]. Reduced pH soil that mixed with BC is the result of its basic characteristic. However, along with the aging process in soil, the pH of BC decreased by 1.6–3.8 units, according to Bakshi et al. [32], which helps to explain the relative lower pH in BC-amended soils in the current work.

Table 3: Effects of biochar (BC) applied at rice (R) or wheat (W) season on the non-essential amino-acid contents of wheat grain

Treatment	Content of the non-essential amino acid (mg/kg)										Total
	Aspartate	Serine	Glutamate	Proline	Glycine	Alanine	Cysteine	Tyrosine	Histidine	Arginine	
N280	0.59 ± 0.02	0.33 ± 0.02	1.34 ± 0.02	0.17 ± 0.01	0.29 ± 0.02 a	0.41 ± 0.00	0.13 ± 0.01	0.19 ± 0.02	0.15 ± 0.02	0.53 ± 0.04	4.12 ± 0.03
N280+BC(R)	0.60 ± 0.05	0.39 ± 0.01	1.32 ± 0.01	0.18 ± 0.03	0.25 ± 0.02 ab	0.40 ± 0.01	0.15 ± 0.02	0.16 ± 0.04	0.12 ± 0.03	0.56 ± 0.04	4.13 ± 0.04
N280+BC(W)	0.60 ± 0.02	0.33 ± 0.03	1.37 ± 0.08	0.20 ± 0.02	0.24 ± 0.02 b	0.40 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.56 ± 0.03	4.12 ± 0.12

Note: Data were presented as means ± SDs ($n = 3$); data labeled with different small letters is significantly different at $p < 0.05$ level.

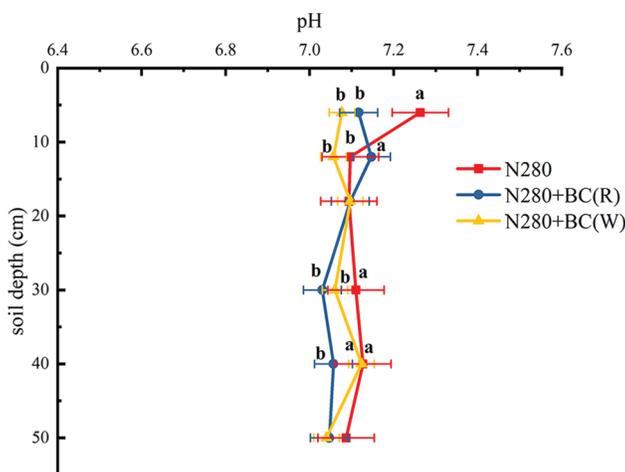


Figure 2: Changes in pH of soil profile caused by biochar (BC) applied at different crop seasons. Data were presented as means ± SDs ($n = 3$); data labeled with different small letters for each depth is significantly different at $p < 0.05$ level

3.3.2 NH_4^+ -N, NO_3^- -N and Total N

Generally, the soil NH_4^+ -N contents were 0.33–0.88 mg/kg at the 0–6 cm topsoil, which increased to 1.70–2.20 mg/kg at the 6–12 cm profile. Data in Fig. 3 suggested that the BC application overall lowered the soil NH_4^+ -N contents. In particular, the NH_4^+ -N contained in the 0–6, 30–40, and 40–50 cm soil from N280+BC(R) and N280+BC(W) were 47.7%–62.5%, 61.1%–77.8%, and 27.8%–40.7% lower than that from N280 (significant at $p < 0.05$). Furthermore, BC applied during rice season reduced the NH_4^+ -N content (–22.7%) of the 6–10 cm soil profile. It is a critical property of BC's sorption on nutrients such as NH_4^+ -N, which helps to explain its positive value as a soil additive [17]. According to Liao et al. [33],

aged BC had a significantly higher NH_4^+ immobilization rate than fresh BC (over 4-folds), which process was primarily dominated by immobilizing NH_4^+ into the recalcitrant organic N pool. This phenomenon helps us to understand the relative lower NH_4^+ content in topsoil as a result of BC amending.

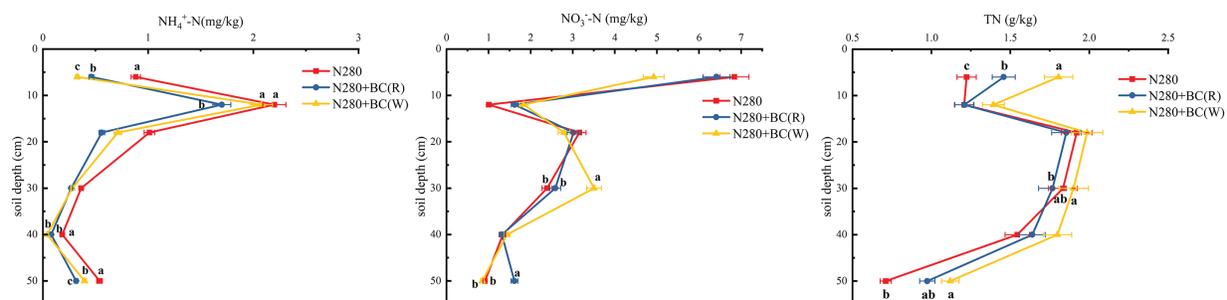


Figure 3: Changes in ammonium (NH_4^+ -N), nitrate (NO_3^- -N), and total N (TN) contents of soil profile caused by biochar (BC) applied at rice (R) or wheat (W) crop season. Data were presented as means \pm SD ($n = 3$); different small letters in the same profile refer to significant differences between treatments at $p < 0.05$ level

The influence of BC application on soil NO_3^- -N content was relatively weak (Fig. 3). Only at 20–30 and 40–50 cm soil profiles, N280+BC(W) and N280+BC(R) had 46.9% and 76.9% more NO_3^- -N content than N280, respectively (both significant at $p < 0.05$). Biochar has shown the potential to reduce N-leaching losses from fertilizer sources in farmland soil [34]. Interestingly, our data suggested that BC amendment generally increased soil total N content in the profile. Particularly, BC amendments enhanced total N content from the 0–6 and 40–50 cm soils by 19.7%–48.4% and 36.6%–57.7%, respectively (Fig. 3). Plants in N280+BC(W) treatment also had 24.0% and 7.3% higher total N than N280+BC(R) at the 0–6 and 20–30 cm soil depth, respectively (significant at $p < 0.05$). This result indicated that wheat season may be the better time to apply BC into paddy soil for N retention. Similarly, Han et al. clarified the optimum BC application schemes in a maize-wheat rotation considering crop production efficiency and soil fertility [35].

3.3.3 Available P and K Contents in Soil

At the 0–6 cm topsoil, BC amendment lowered soil available P content, particularly under N280+BC(R) treatment, which with 16.8% lower available P content than N280 (Fig. 4). Nevertheless, BC applied at rice season increased soil available P content at the 6–12 cm horizon. At other soil profiles, BC additives overall increased the available P contents. The BC applied before wheat seed sowing increased the available P contents in the 12–20, 20–30, and 40–50 cm soils by 30.4%, 41.5%, and 30.1%, respectively (all significant at $p < 0.05$). Similarly, soil available K contents were enhanced by the BC, particularly that being applied during the wheat season. For instance, the available K contents in N280+BC(W) soils from 0–6, 6–12, and 30–40 cm were 44.2%, 46.3%, and 22.0% higher than that in N280 (Fig. 4). However, two BC amending treatments also significantly ($p < 0.05$) decreased the available K contents of 40–50 cm (on average by 26.4%).

Similarly, the BC additive remarkably raised the available P content in the soil, in comparison with the control condition from a previous indoor incubation experiment [36]. However, the soil available P concentration varied little in BC-amended treatment but decreased in straw-amended treatment according to a 7-year long-term investigation [37]. These results demonstrated that BC amendment is favorable for available P nutrients in the soil, at least compared with straw turnover. Biochar also has been approved to substitute partially P and K fertilizers with appropriate rates in the farming systems [38], which also indicated that BC amendments could enhance soil fertility. Moreover, our results suggested that the positive benefit in improving soil available P and K nutrients of BC applied during the wheat season is better than that applied during the rice season (Fig. 4). Of course, more long-term field experiments should be conducted in the future.

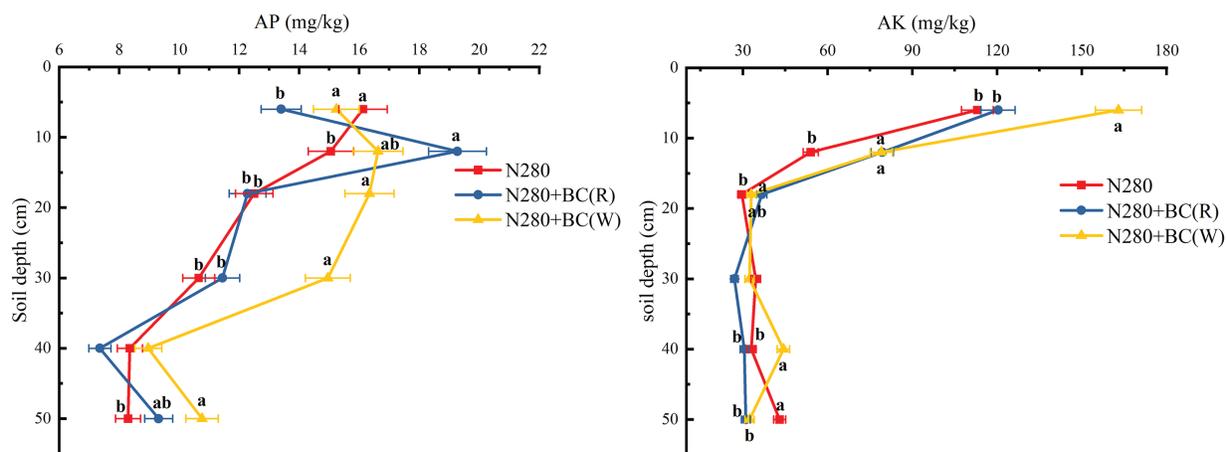


Figure 4: Changes in available P (AP) and K (AK) contents of soil profile caused by biochar (BC) applied at rice (R) or wheat (W) season. Data were presented as means \pm SDs ($n = 3$); different small letters in the same profile refer to significant differences between treatments at $p < 0.05$ level

3.3.4 SOM Content

There was an average of 108.7% and 46.1% more SOM contained in the 0–6 and 6–12 cm layers under BC-added treatments, respectively, compared with that under N280 (Fig. 5). Overall, N280+BC(W) had more SOM than N280+BC(R), in particular, that was recorded at the 0–6 cm topsoil and 20–30 cm subsoil. Biochar addition increases soil organic carbon content [38]. In addition, a seven-year continuous BC amendment could favor the soil organic carbon by increasing the aromatic [37]. We demonstrated the SOM-increasing effects following the BC amendment could be observed to cover a 0–50 cm soil profile. Similarly, previous work confirmed the potential of BC to improve SOM along with the whole soil profile [39]. Biochar has been widely produced and applied to sequester carbon and remediate degraded soil as described in the introduction section. The increase in SOM because of BC can be explained by the BC-contained carbon itself and it could inhibit the SOM mineralization while promoting the root volume [40,41].

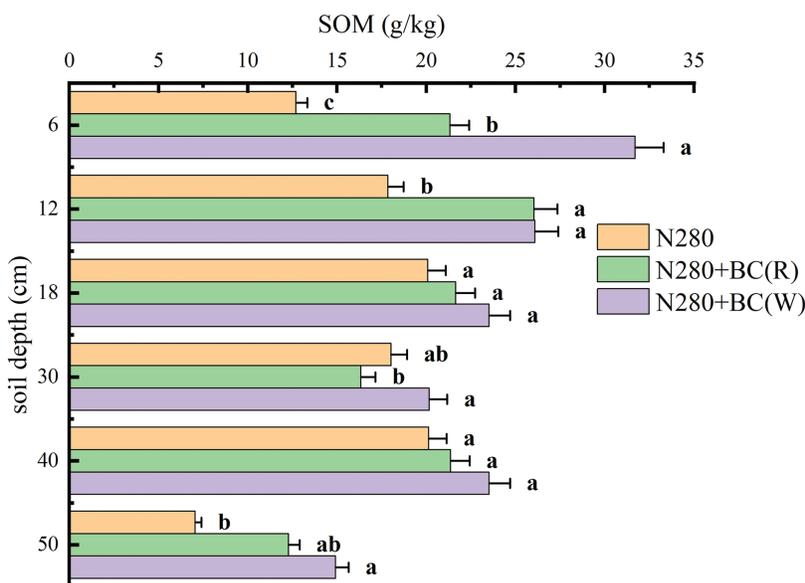


Figure 5: Changes in soil organic matter (SOM) content at different depths caused by biochar (BC) applied at rice (R) or wheat (W) crop season. Data were presented as means \pm SDs ($n = 3$); different small letters in the same profile refer to significant differences between treatments at $p < 0.05$ level

4 Conclusion

We conducted a simulated soil pot experiment using PVC columns to clarify how BC that applied at the initiation of the rice or wheat growth cycle on wheat production, quality (amino acid content), and soil properties at 0–50 cm depth in a rice-wheat rotation. Biochar did not influence the wheat grain yield and recovery NUE, but when applied during the wheat season, BC enhanced some essential amino acids in wheat grain, which helped to improve the grain quality. The changes in the soil characteristics caused by BC were mainly recorded at the 0–12 cm topsoil, and the effects were dependent on the applying time of BC. In particular, the total N and SOM contained in the 0–6 cm topsoil were enhanced by BC application at both crop seasons, which effect was greater in treatment with BC that is applied during wheat season. Biochar amendments still changed the characteristics of the 12–50 cm subsoil, but the effects were weaker than that on the topsoil. Overall, we conclude that it is better to apply BC during the wheat season to improve crop quality and optimize soil fertility along the soil profile. Meanwhile, long-term and field condition studies should be conducted.

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Availability of Data and Materials: All data included in this work are available upon request by contact with the corresponding author.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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