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## Effect of biochar on nitrogen use efficiency, grain yield and amino acid content of wheat cultivated on saline soil

HAIJUN SUN<sup>1,2\*</sup>, HUANCHAO ZHANG<sup>1,2</sup>, WEIMING SHI<sup>3\*</sup>, MENGYI ZHOU<sup>4</sup>,  
XIAOFANG MA<sup>4</sup>

<sup>1</sup>College of Forestry, Nanjing Forestry University, Nanjing, P.R. China

<sup>2</sup>Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, P.R. China

<sup>3</sup>School of Food Science and Engineering, Foshan University, Foshan, Guangdong, P.R. China

<sup>4</sup>Advanced Analysis and Testing Center, Nanjing Forestry University, Nanjing, P.R. China

\*Corresponding authors: [hjsun@njfu.edu.cn](mailto:hjsun@njfu.edu.cn); [wmshi@fosu.edu.cn](mailto:wmshi@fosu.edu.cn)

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**Abstract:** Biochar can potentially increase crop production in saline soils. However, the appropriate amount of biochar that should be applied to benefit from resource preservation and increase both grain yield (GY) and quality is not clear. A pot experiment was conducted to evaluate the effects of biochar applied at various rates (i.e., 0, 5, 10, 20, 30, 40 and 50 t/ha) on the nitrogen use efficiency (NUE), GY and amino acid (AA) contents of wheat plants in saline soils. The results showed that the application of 5–20 t/ha biochar increased wheat NUE by 5.2–37.9% and thus increased wheat GY by 2.9–19.4%. However, excessive biochar applications (more than 30 t/ha) had negative effects on both the NUE and GY of wheat. Biochar had little influence on leaf soil and plant analyzer development (SPAD) values, the harvest index or yield components. The AAs were significantly affected by biochar, depending on the application rate. Among the application rates, 5–30 t/ha biochar resulted in relatively higher (by 5.2–19.1%) total AA contents. Similar trends were observed for each of the 17 essential AAs. In conclusion, the positive effects of biochar occurred when it was applied at appropriate rates, but the effects were negative when biochar was overused.

**Keywords:** biochar application rate; biomass resource; food safety; plant nutrition; sustainable agriculture

Salinity can reduce soil and crop productivity, making it one of the major threats to sustainable agricultural development. In recent years, biochar has increasingly become a subject of scientific interest as an amendment for soil improvement (Wiedner et al. 2015, Kraska et al. 2016). Biochar amendment could potentially alleviate the negative effects of salt stress on crops and thus improve crop production (Akhtar et al. 2015, Saifullah et al. 2018). For example, the yield of wheat was shown to be positively affected by biochar amendments (Akhtar et al. 2015). Nevertheless, knowledge of its appropriate application

rates in salt-affected soils is scarce, which limits the sustainable use of biochar and saline soil resources.

Wheat (*Triticum aestivum* L.) is one of the most important crops worldwide. Amino acid (AA) composition and the content are important features for determining the nutritional value of wheat grain for both human and animal diets (Zhang et al. 2016). Studying the quantitative ratio of wheat protein fractions is important for evaluating and using grain and determining its nutritive value. The total protein content in wheat grain varies from 8–25.8% and depends on the genotype and growth conditions

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(Gafurova et al. 2002). Management practices are known to influence protein quantity as well as grain production and, in turn, AA composition (Lošák et al. 2010, Zemanová et al. 2017). Compared with that in plants in control treatments, the total content of free AAs in plants in biochar-amended treatments was shown to be greater (Zemanová et al. 2017). Among the management practices used for wheat production, biochar application was often found to be the most emerging regarding yield and grain quality and seems to be an important strategy for improving both grain yield (GY) and protein quality in winter wheat. As a resource, biochar is not inexhaustible. The high cost associated with the production of biochar and the high application rates are significant challenges to its widespread use in areas affected by salinity (Saifullah et al. 2018). Thus, determining the amount of biochar that should be applied to obtain economically beneficial crop production and quality is highly important. There may be an optimum range of biochar application rates that can both preserve biochar resources and ensure food production, quality and security.

The objectives of this study were therefore to evaluate the effects of biochar application management on the nitrogen use efficiency (NUE), GY and AA contents of winter wheat growing in saline soils. It was hypothesized that appropriate rates of biochar added to saline soil would enhance the NUE, GY and AA composition of wheat plants in reclaimed saline soils.

## MATERIAL AND METHODS

### Selected properties of the tested soil and biochar.

Cultivation-layer (i.e., top 0–20 cm) soil samples were collected from a typical coastal saline field in the Sheyang City (33°46'N, 120°15'E), Jiang province, China. The pre-processing of soil samples was the same as that in Sun et al. (2017). The tested soil was classified as a typical saline alluvial soil and its physicochemical properties were as follows: pH (water:soil = 2.5:1) of 8.00, 1.02 g/kg total N, 3.28 g/kg organic carbon, 0.99 dS/m electrical conductivity, 14.4‰ salt content, 20.3% sand, 60.5% silt and 19.2% clay. Selected properties of the applied biochar were as follows: pH (water:biochar = 2.5:1) of 9.58, 16.8 g/kg total N, 675 g/kg total C, 165 mmol<sub>c</sub>/kg cation exchange capacity and 104.5 m<sup>2</sup>/g specific surface area.

**Experiment and management.** A pot experiment involving wheat plants in saline soils was conducted in a greenhouse at Nanjing, China. The soil sam-

ples and different amounts of biochar were mixed thoroughly to prepare 7 biochar application rates (0, 5, 10, 20, 30, 40 and 50 t/ha, which were equal to 0, 15.7, 31.4, 62.8, 94.2, 125.6 and 157.0 g/pot, via pot area, respectively); these prepared samples were subsequently repacked into pots whose depth was 15 cm (20 cm inner diameter and 19 cm deep, each pot contained 6.5 kg of soil). A control treatment (CK) receiving no biochar or N fertilizer was also included. In total, eight treatments (named CK; urea; urea + BC5; urea + BC10; urea + BC20; urea + BC30; urea + BC40, and urea + BC50) with three replicates each were evaluated.

Wheat was planted in the pots at a density of 12 plants/pot. In this study, wheat experiment was conducted from November 10, 2017, to May 20, 2018. Nitrogen was applied at 200 kg N/ha (equal to 0.628 g N/pot) and was split-applied at a ratio of 3:3:4 as a basal fertilizer and two supplementary applications on November 10, 2017, and January 12 and March 15, 2018, respectively. On the basis of soil test recommendations, P and K were applied at 39.6 kg P/ha (equal to 0.126 g P/pot, in the form of calcium superphosphate) and at 99.6 kg K/ha (equal to 0.313 g K/pot, in the form of potassium chloride), respectively, as basal fertilizer for wheat. Other management practices were in accordance with those of local farmers.

**Samplings and measurements.** The leaf soil and plant analyzer development (SPAD) values were determined via a SPAD 502 device (Konica Minolta. Inc., Tokyo, Japan) during the jointing and filling stages of wheat. The plants were manually harvested from each pot at physiological maturity to determine the wheat straw biomass, GY and total N content of the plants. Other yield-related agronomic traits including the effective number of panicles, the number of grains per panicle and thousand-seed weight were determined at the same time. After they were oven dried, the straw and grain samples were finely ground into powder small enough to pass through a 0.25-mm sieve. The plant total N content was determined according to the Kjeldahl method, and the NUE was calculated as the difference between the percentages of applied N fertilizer taken up in the aboveground biomass in each biochar treatment minus that in the CK treatment. The free AA content was determined as described by Kovács et al. (2011). The total amino acid (TAA) content was the sum of the individual free AAs.

**Statistical analysis.** The results were expressed as the means ( $n = 3$ ) and standard deviations (SDs).

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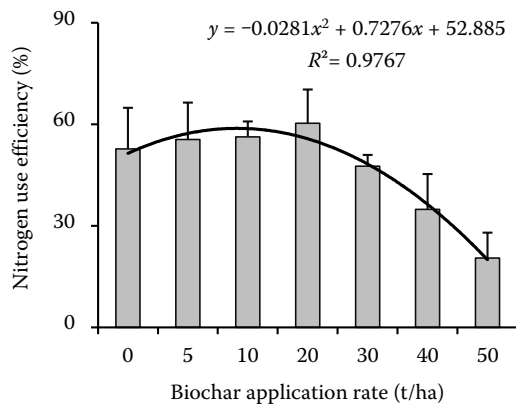


Figure 1. Nitrogen use efficiency of wheat plants in saline soil amended with biochar applied at various rates

Single-factor ANOVA was used to determine differences between the treatments. The least significant difference (*LSD*) multiple comparison methods was applied at a 0.05 probability level. The data were analysed via the SPSS 16.0 software (SPSS Inc., Chicago, USA) for Windows.

## RESULTS AND DISCUSSION

**NUE of wheat.** The data from our pot experiment suggested that biochar addition at a rate up to 20 t/ha increased the NUE of wheat by 5.2–38%, although the differences were not statistically significant. Nevertheless, the addition of 30–50 t/ha biochar to saline soils reduced the NUE (Figure 1). The relationship between wheat NUE and biochar application rate was analysed, and a significant ( $R^2 = 0.9767$ ) correlation was found between them. Interestingly, a positive effect of biochar application on NUE was observed when biochar was applied at a rate of less than 20 t/ha, but the effect was negative when the application rate was more than 20 t/ha. According to the fitted equation, the optimal biochar application rate for the highest NUE is 13.0 t/ha.

Chan et al. (2007) conducted a pot trial and highlighted the role of biochar (applied at 10, 50, and 100 t/ha) in improving the NUE of radish, which was confirmed by our data in the treatments that received 5–20 t/ha biochar (Figure 1). The increased NUE of wheat in response to biochar addition could be attributed to the improved soil physiochemical properties, water-holding capacity and available nutrients within the biochar (Chan et al. 2007, Bu et al. 2017). Notably, excessive (more than 20 t/ha) biochar application was not necessary and even reduced the N uptake capacity of wheat plants in saline soils. In

general, the addition of straw biochar (regardless of pyrolysis temperature and residence time) increases soil pH because of its liming effect (Feng et al. 2017, Si et al. 2018). Consequently, Sun et al. (2017) suggested that biochar must be used at an appropriate rate; otherwise, it would increase N losses via  $\text{NH}_3$  volatilization, which consequently lowered the NUE of plants. Also, heavy metals within biochar hindered wheat plants when biochar was excessively applied (> 30 t/ha in the present work) (Kraska et al. 2016), the effect of which was less significant when lower rates of biochar were applied.

SPAD values can reflect leaf chlorophyll concentrations. The SPAD values of flag leaves measured in the present experiment are shown in Figure 2. Nitrogen fertilizer significantly ( $P < 0.05$ ) increased the SPAD values measured during the jointing stage of wheat. In contrast to the positive effects of N fertilizer on SPAD values, biochar did not strongly influence the SPAD values (Figure 2). Similar trends in flag leaf SPAD values were observed during the filling stage of wheat. These results indicated that the leaf SPAD values were not influenced by biochar addition, which confirms the results of Asai et al. (2009).

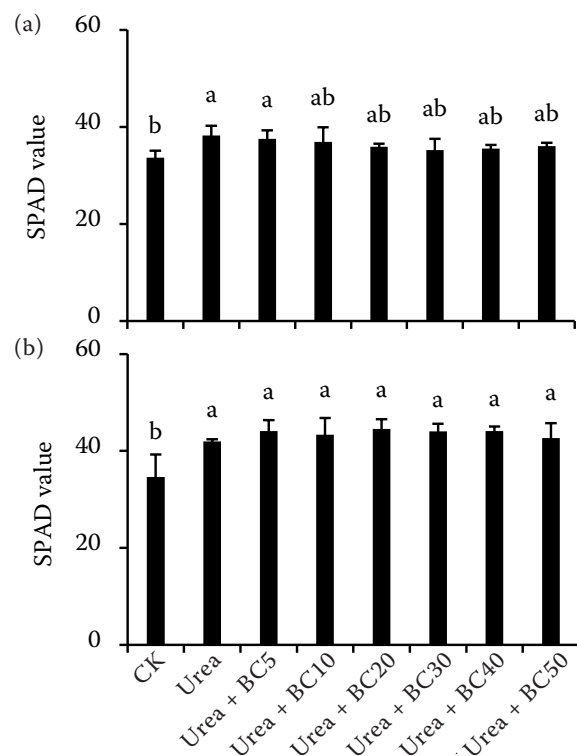


Figure 2. Soil and plant analyzer development (SPAD) values of flag leaf determined during the (a) jointing stage and (b) filling stage of wheat plants in saline soil amended with biochar applied at various rates

Table 1. Wheat straw/grain yield, harvest index and yield components under different treatments

| Treatment   | Straw yield               | Grain yield               | Harvest index (%)        | Yield component           |                           |                           |
|-------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
|             | (g/pot)                   |                           |                          | effective panicles (/pot) | grains per panicle        | thousand-grain weight (g) |
| CK          | 6.53 ± 0.17 <sup>d</sup>  | 3.57 ± 0.68 <sup>c</sup>  | 35.2 ± 4.5 <sup>b</sup>  | 7.67 ± 0.58 <sup>a</sup>  | 16.3 ± 3.6 <sup>b</sup>   | 28.8 ± 3.1 <sup>bc</sup>  |
| Urea        | 13.6 ± 1.32 <sup>a</sup>  | 10.3 ± 1.17 <sup>a</sup>  | 39.5 ± 7.8 <sup>ab</sup> | 10.0 ± 1.00 <sup>a</sup>  | 26.0 ± 5.7 <sup>ab</sup>  | 34.0 ± 4.4 <sup>ab</sup>  |
| Urea + BC5  | 13.3 ± 0.86 <sup>a</sup>  | 10.6 ± 1.82 <sup>a</sup>  | 43.1 ± 4.5 <sup>ab</sup> | 10.7 ± 2.08 <sup>a</sup>  | 30.0 ± 8.8 <sup>a</sup>   | 33.2 ± 1.1 <sup>abc</sup> |
| Urea + BC10 | 12.0 ± 1.26 <sup>ab</sup> | 11.4 ± 1.92 <sup>a</sup>  | 48.5 ± 5.0 <sup>a</sup>  | 11.3 ± 1.15 <sup>a</sup>  | 30.9 ± 3.7 <sup>a</sup>   | 32.7 ± 4.6 <sup>abc</sup> |
| Urea + BC20 | 13.4 ± 1.32 <sup>a</sup>  | 12.3 ± 0.92 <sup>a</sup>  | 48.1 ± 1.1 <sup>a</sup>  | 10.7 ± 2.08 <sup>a</sup>  | 33.1 ± 6.4 <sup>a</sup>   | 35.8 ± 1.9 <sup>a</sup>   |
| Urea + BC30 | 13.1 ± 0.16 <sup>a</sup>  | 10.2 ± 0.95 <sup>a</sup>  | 41.5 ± 4.4 <sup>ab</sup> | 10.0 ± 1.73 <sup>a</sup>  | 26.1 ± 12.3 <sup>ab</sup> | 31.8 ± 1.7 <sup>abc</sup> |
| Urea + BC40 | 10.6 ± 0.74 <sup>b</sup>  | 6.90 ± 2.00 <sup>b</sup>  | 38.7 ± 5.4 <sup>b</sup>  | 8.67 ± 4.16 <sup>a</sup>  | 22.4 ± 5.16 <sup>ab</sup> | 27.6 ± 3.4 <sup>c</sup>   |
| Urea + BC50 | 8.51 ± 0.44 <sup>c</sup>  | 5.20 ± 0.67 <sup>bc</sup> | 37.9 ± 3.7 <sup>b</sup>  | 8.00 ± 1.00 <sup>a</sup>  | 22.2 ± 3.21 <sup>ab</sup> | 29.6 ± 2.3 <sup>bc</sup>  |

Different lowercase letters within a column indicate a significant difference ( $P < 0.05$ )

**Wheat GY and yield components.** The mass of the harvested wheat grain ranged from  $5.20 \pm 0.67$  g/pot to  $12.3 \pm 0.92$  g/pot (Table 1). When biochar was applied at a rate of no more than 20 t/ha, the wheat GY was 2.91–19.4% higher than that in the urea treatment. Also, wheat production significantly increased ( $P < 0.05$ ) with an increasing biochar application rate (lower than 20 t/ha) (Figure 3). However, the urea + BC30 treatment produced the same wheat GY as did the urea treatment. Unfortunately, the wheat production in response to the biochar application rate of 40–50 t/ha was markedly (33.0–49.5%) lower ( $P < 0.05$ ) than that in response to no biochar (Table 1). When biochar was applied at rates lower than 30 t/ha, increased NUE was highlighted as the explanation for the maintenance of wheat GY (Table 1, Figure 1).

Plant growth in salt-affected soils is challenging in many direct and indirect ways. Biochar applications

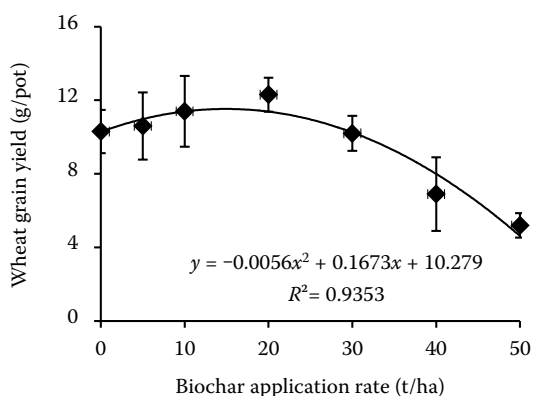


Figure 3. Wheat grain yield as a function of biochar addition rate. The wheat grain yield in the control treatment was far lower than that in the presented treatments and was only  $3.57 \pm 0.68$  g/pot

to soils are known to increase agricultural productivity (Akhtar et al. 2015, Sun et al. 2016). There are several reasons why biochar might be expected to increase the GY of food crops. Laboratory and field studies have shown that biochar additions to salt-affected soils largely alleviated salt stress and improved plant growth directly via the release of essential macro- and micronutrients such as Ca, K, N, P and Zn in the soil, which helped offset the adverse effects of salts (Saifullah et al. 2018). Increased NUE was frequently cited as an explanation for this result (Sohi et al. 2010). Huang et al. (2014) showed that rice GY increased by 8–10% in response to biochar applications, but there was no significant difference in GY between the biochar application rates of 150 and 300 g/pot.

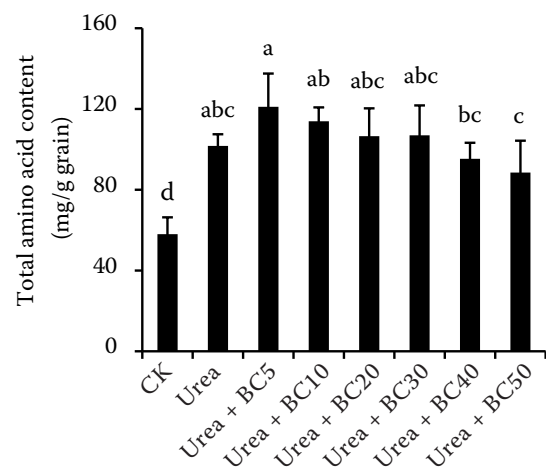


Figure 4. The total amino acid content in wheat grain under treatments receiving biochar applied at various rates

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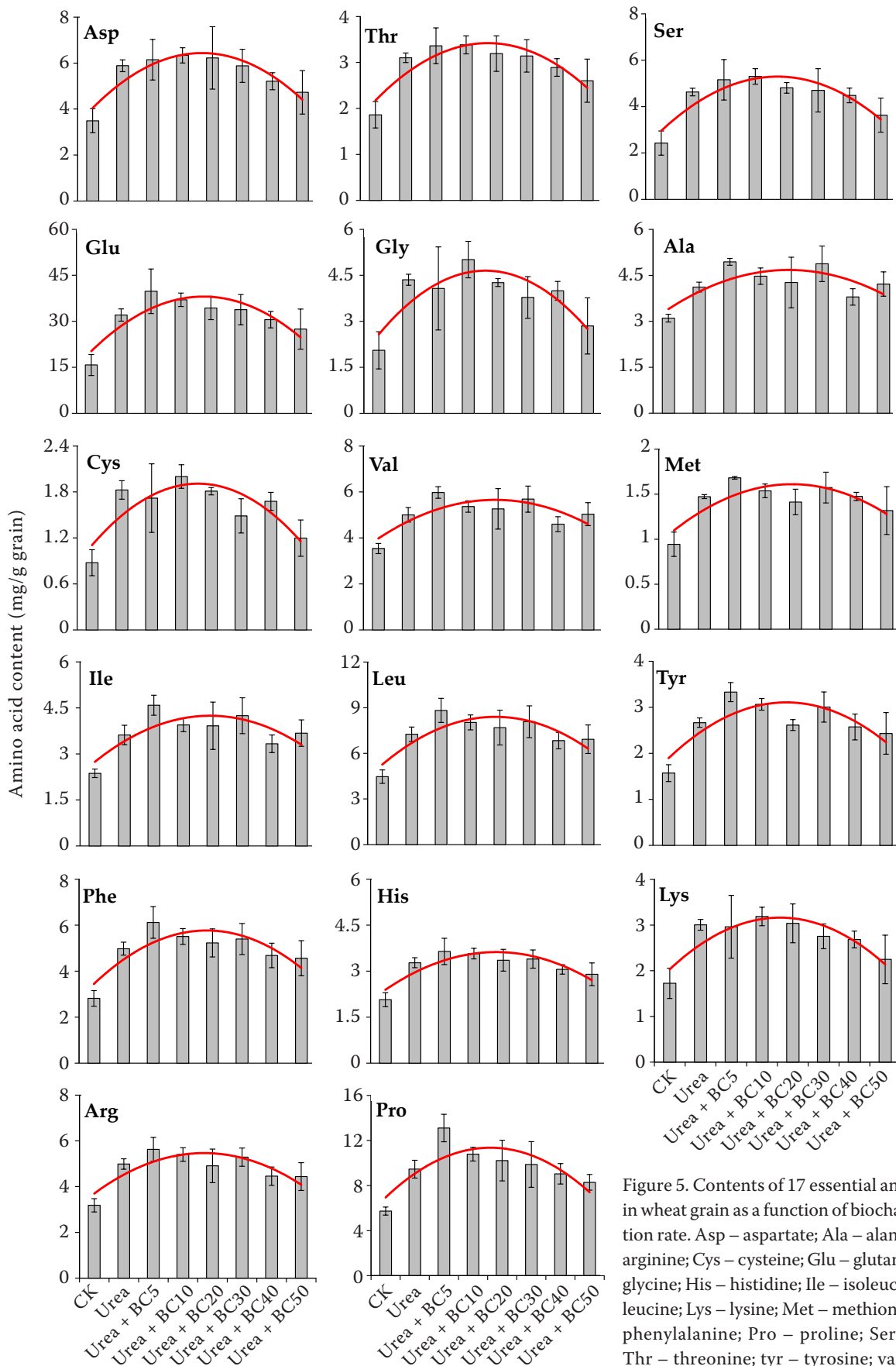


Figure 5. Contents of 17 essential amino acids in wheat grain as a function of biochar application rate. Asp – aspartate; Ala – alanine; Arg – arginine; Cys – cysteine; Glu – glutamate; gly – glycine; His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Met – methionine; Phe – phenylalanine; Pro – proline; Ser – serine; Thr – threonine; tyr – tyrosine; val – valine

Furthermore, biochar can increase plant-available water and soluble soil nutrient contents (Novak et al. 2009, Sun et al. 2017). These findings could explain the higher GY in response to the biochar treatments applied at 5–20 t/ha compared with the treatments applied at the other rates. However, too high biochar application rates potentially increased N loss via  $\text{NH}_3$  volatilization and increased the C/N ratio (Feng et al. 2017, Sun et al. 2017), which consequently reduced the NUE of wheat plants and thereby resulted in the lowest GY observed in the current study. Similarly, Si et al. (2018) suggested that positive effects of biochar (derived from apple branches) on the wheat yield occurred only at relatively low application rates.

The straw biomass and harvest index were significantly affected by biochar amendments. Similar trends of the effects of biochar addition on wheat straw biomass and the harvest index were observed in the present study (Table 1). The data in Table 1 also show that the three yield components (effective number of panicles, number of grains per panicle and thousand-grain weight) were not sensitive to biochar amendments.

These results emphasized that the response of wheat production strongly depends on the rate of biochar application before wheat sowing. Also, 15.0 t/ha was the optimum biochar application rate for obtaining the highest wheat GY, and this rate was similar to the application rate that resulted in the highest NUE.

**AA contents in wheat grain.** In general, fertilization can significantly influence the AA content in plant issues (Ježek et al. 2011). Not surprisingly, N fertilizer significantly increased the TAA content in the wheat grain (Figure 4). Of the N addition treatments, the 5 t/ha BC treatment resulted in the highest TAA content with 121.1 mg/g grain. Similar TAA contents were recorded for the 10–30 t/ha BC treatments. In contrast, the TAA content in the 40 and 50 t/ha BC treatments was significantly lower (by 21.5–27.3%) than that in the 5 t/ha BC treatment. In other words, the effects of biochar on the TAA content depended on its application rate.

The balance of AA composition in wheat grain largely determines wheat nutritional quality (Zhang et al. 2016). Figure 5 summarizes the contents of 17 essential amino acids (EAAs) in the wheat grains obtained from the treatments amended with different amounts of biochar. The seven main EAAs were glutamate, proline, leucine, serine, valine, phenylalanine and arginine (Figure 5), which is consistent with previous works (Del Moral et al. 2007, Jiang et

al. 2014). That is to say; the biochar amendments did not alter the main components of TAAs. For these 17 EAAs, biochar addition only slightly changed the percent composition. However, the content of each AA was affected by the biochar application rate. In addition, this effect was similar to that observed for the TAA content in the wheat grain.

Environmental conditions influence grain production as well as protein quantity and, in turn, AA composition (Del Moral et al. 2007, Kovács et al. 2011). In a study, the AA composition was significantly influenced by the N rate, where the greatest yield, EAA, and TAA contents occurred under N application rates of 225 kg/ha at Nanyang and 120–225 kg/ha at Xinyang (Zhang et al. 2016). However, knowledge of the effects of biochar applications on the EAAs in wheat plants under salt-affected conditions is poor. Another study showed that the total content of free AAs was higher in spring spinach harvested from biochar-amended (5% per mass of soil) soils than from control soils (Zemanová et al. 2017).

In the present study, the data suggested that the optimum rate of biochar addition increases the TAA content, whereas an excessive rate of biochar reduces the TAA content. Considering the results presented in Figures 1 and 4, it was found that the TAA content was positively related to the NUE of wheat and, therefore, the N content in the grain. Thus, the mechanism of the influence of biochar on wheat TAA contents was similar to that on wheat NUE.

The data revealed that when the addition rates were within a reasonable range, biochar was beneficial for the improvement of wheat growth and, consequently, the GY, NUE and AA content of the grain. However, these effects were negative when biochar was applied excessively. Therefore, biochar should be applied at appropriate rates to ensure the sustainable reclamation and usage of coastal saline soil resources. Additional studies are needed to assess the long-term effects of biochar applied at various rates on the NUE, GY and AA content of wheat under field conditions.

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