

Peanut shell biochar increases rice yield in highly saline-alkali paddy fields by regulating of leaf ionic concentration and improving leaf photosynthesis rate

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Research Article

Keywords: Biochar, Saline-alkali stress, Ionic balance, Photosynthesis characteristics, Rice

Posted Date: July 29th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1884188/v1>

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Abstract

Aims

Saline-alkali soil seriously restricts the crop growth and production. Biochar addition could alleviate the adverse impacts of saline-alkali stress in crops. However, little information is available on the ionic accumulation and photosynthesis rate of rice in highly saline-alkali paddy fields. This study aimed to evaluate the influence of the peanut shell biochar on leaf ionic concentration, stress physiology indices, photosynthesis related parameters and rice yield in highly saline-alkali paddy field condition.

Methods

Field experiment was conducted using two nitrogen applied rate treatments (0 and 225 kg N ha⁻¹) and four biochar applied rate treatments (0%, 1.5%, 3.0% and 4.5% w/w). The field experiment was arranged in a complete randomized design with three replications.

Results

The results show that peanut shell biochar significantly reduced the leaf Na⁺ concentration, Na⁺/K⁺ ratio, abscisic acid and malondialdehyde concentration of rice, while enhanced leaf K⁺ content, and improved leaf water status and relative electrical leakage in treatments both with or without N fertilization. Furthermore, peanut shell biochar could provide beneficial effects on chlorophyll content, leaf N content, leaf area index, photosynthetic potential, stomatal conductance, and transpiration rates, which is of great benefit to the enhancement of leaf photosynthesis rate and net assimilation rate of rice population. In addition, the biomass, grain yield and harvested index were remarkably increased.

Conclusions

These results indicated that peanut shell biochar could effectively ameliorate saline-alkali stress and increase rice yield by regulating of leaf ionic concentration and improving leaf photosynthesis rate.

Introduction

Saline-alkali soil is a major ecological threat to crop growth and production. The Songnen Plain, located between 42°30' to 51°20'N and 121°40' to 128°30'E, is the main saline-alkali area in Northeast China, and approximately 3.42 million hectares of soda saline-alkali soil (Gong et al. 2021). The main salt components of this kind of soils are Na₂CO₃ and NaHCO₃. Soda saline-alkali soils are characterized by high level of soluble salt, exchangeable Na⁺, high pH, poor structure and low nutrients level, which severely restricts its agricultural development and utilization. Osmotic stress, ionic toxicity, oxidative

damage and nutritional disorders in soda saline-alkali soil are the main factors that inhibit crop growth (Yan and Guo 2018). Low water potential caused by excessive salt in soda saline-alkali soil limit the uptake of essential plant nutrients and water resulting in water shortage and osmotic stress similar to physiological drought (Ling et al., 2020). Due to the excessive amassing of Na^+ inside and outside in plant cells, the ionic equilibrium of sodium-potassium in the cytoplasm was disrupted, and then causes ionic toxicity effect. (Wong et al. 2010; Santos et al., 2021;). The oxidative stress in crop cells was caused by the accumulation of reactive oxygen species, which was triggered by salt stress (Munns and Tester 2008). These negative effects can cause nutritional disorders, limit the uptake of essential plant nutrients and water, change the structural integrity and function of cell membrane, restrict the leaf photosynthesis, thus reducing the yield (Wong et al. 2010; Chaganti and Crohn 2015; Song et al., 2022). Therefore, how to reduce soda saline-alkali stress and promote the growth and development of crops in saline-alkali land have important meaning to ensure food security. Large number of studies have shown that planting rice is an effective mean to improve soda salinized lands (Gupta et al. 1990; Huang et al. 2022). However, with the global climate change, the ecological system of saline-alkali lands is facing severe problems such as soil barren, increasingly serious salinization degree and water resource scarcity, these serious threats to the development of rice production (Jin et al., 2018).

Photosynthesis is an important physiological and biochemical process in crops and provides energy for crops. Increasing photosynthetic rate can significantly increase crops yield and improve crops quality (Yang et al., 2019; Kamran et al., 2020). Prior studies shows that crop's photosynthesis is very sensitive stress to saline-sodic stress, which can cause photoinhibition and decrease the photosynthetic rate, thus resulting in crops yield loss and even death (Nishiyama and Murata 2014; Abbasi et al., 2015; Ling et al., 2020). Ashraf (2007) found that water loss by transpiration were decreased due to closing stomata of leaves under saline-sodic stress, and the CO_2 concentration of intercellular were also reduced. Salt stress has been shown to decrease the chlorophyll content by destroying the lamellar structure of chloroplast grana (Juan et al. 2005). Many researches have shown that saline-sodic stress remarkably suppressed the activity of Rubisco and photosynthetic electron transport, decreased the consumption of NADPH (Gill and Tuteja, 2010; Abbasi et al. 2016). In addition, salt stress has been shown to remarkably increase the accumulation of ROS, inhibited the transport and utilization of photosynthetic products, and ultimately reduce crops yields (Abbasi et al., 2015). Therefore, how to reduce saline-alkali stress and improve photosynthetic efficiency of crops are very important to promote crops yield.

Many researches showed that biochar application can significantly alleviate saline-alkali stress and promote crops growth (Drake et al., 2016; Jin et al., 2018; Zhu et al., 2022). Our previous study has shown that biochar addition to saline-alkali lands to a large extent increased rice yield by the release of fundamental macro-and micro- nutrients, which can help neutralize the negative effects of salinization (Yao et al., 2021). Biochar can also alleviate ionic toxicity and osmotic stress for crops through reducing leaf Na^+ accumulations, Na^+/K^+ ratio and improving leaf water status due to enhancing K^+ availability (Chakraborty et al., 2016; Ran et al., 2019). In addition, Farhangi-Abriz and Torabian, (2017) found that biochar significantly reduced the oxidation stress through degradation the concentration of superoxide

anion and hydrogen peroxide under salt stress. Biochar posed an effective effect on improving the physicochemical and biological properties of salted soils, ameliorating root environment and enhancing root absorption activity for nutrients and water (Chaganti and Crohn et al., 2015; Agbna et al., 2017; Elshaikh et al., 2018; Li et al., 2022). Moreover, the improvement in stomatal density and conductance were found after biochar application to salt-affected soils due to the decreased production of ABA (Amini et al., 2016; Ali et al., 2017). Our previous research also found that biochar remarkably offset the saline-sodic stress and enhance rice yield by decrease sodium ion concentration in rhizosphere soil and sodium ion accumulation in different rice organs (Jin et al. 2018; Li et al., 2022). For the above reasons, many studies also found biochar application is beneficial to increase the photosynthetic rate of plants under salted stress (Song et al., 2022; Elshaikh et al., 2018; Abbas et al., 2017). However, the effect of biochar on photosynthetic rate were related to experimental conditions, such as soil type, biochar manufacturing process and application rate, thus presenting variable results. Most of the existing studies focused on the dry crops such as cotton, maize, soybean and Pennisetum under laboratory or pot conditions, but how biochar affects rice leaf ionic concentration and leaf photosynthesis rate, and the deep mechanism of regulating photosynthesis rate of rice in highly saline-alkali paddy fields have not yet been investigated. Therefore, field experiments on the impact of biochar addition in highly saline-alkali paddy fields are necessary.

In the present study, we investigated the impacts of peanut shell biochar on leaf ionic accumulation, leaf water status, relative electrical leakage, ABA and MDA content, photosynthetic parameters and photosynthate accumulation in rice under highly saline-alkali stress, the mechanism of peanut shell biochar regulating of leaf ionic concentration and improving leaf photosynthesis of rice in highly saline-alkali paddy field was elucidated by measuring salt-related characteristics, concentrations of Na^+ and K^+ and key photosynthetic parameters of leaves. The results provide a new insight for regulation of rice saline-alkali tolerance by biochar.

Materials And Methods

Study site and experimental soil properties

A field experiment was conducted in Sheli Town, Da'an County, Jilin Province, Northeast China (45°35'N, 123°50'E). This area has a typical dry-cold monsoon climate, with an average annual air temperature of 4.7 °C, average precipitation of approximately 413.7 mm, and average evaporation of approximately 1696.9 mm. The soil texture in this experiment is 23.23% sand, 38.14% silt and 37.60% clay according to the international Society of Soil Science classification. The basic physicochemical characteristics of the soil in this experiment are shown in Table 1. The average value of EC, SAR, pH in saturated paste and ESP is $24.08 \mu\text{s m}^{-1}$, $368.11 (\text{mmolc L}^{-1})^{1/2}$, 10.10, and 71.11%. Based on the texture classification system of USDA (1954), the soil in this experimental area is characterized as saline-alkali and had a three years history of rice planting.

Experimental design

The field experiment was conducted in 2021. The trial was established in a split-plot design with nitrogen as the main plot and biochar as the subplot, with three replicates. There are include a total of 24 plots (each 5 m by 6 m) with separated by buffer rows (60 cm in width). Each plot has an independent irrigation and drainage. There are two nitrogen treatments and four biochar applied rate treatments. The two nitrogen treatments include no nitrogen applied (N0) and conventional nitrogen applied level (N225). Four biochar rates were applied into soil layer (0-20cm), refereed as T0 (zero biochar), T1 (1.5% biochar, w/w), T2 (3.0% biochar, w/w) and T3 (4.5% biochar, w/w), respectively.

The rice variety planted in this field study was japonica rice Changbai 9, one of the elite cultivars used in saline-alkali paddy soil in Northeast China. On 12 April, 2021, rice seeds were sown in a greenhouse. Rice seedlings were transplanted to the field plots on May 21, 2021. The transplanting density (per hill) was 30 cm × 16.5 cm, and each hill contained three seedlings. The rice was harvested on September 30, 2021. In the treatment of N225, the application rates of chemical (NPK) fertilizer were as follows: 225 kg N per hectare, 75 kg P per hectare, and 100 kg K per hectare, respectively. In the treatment of N0, the application rates of chemical (NPK) fertilizer were as follows: 0 kg N per hectare, 75 kg P per hectare, and 100 kg K per hectare, respectively. Field management was the same as that used in local production fields to minimize yield loss.

Preparation of biochar

The biochar was produced from peanut shells using a vertical kiln, manufactured by Jinhefu Agricultural Development Company, Liaoning Province, China, and the pyrolysis temperature was 350 to 550 °C for 4 h. The peanut shells were obtained from Jinhefu Agricultural Development Company, AnShan city, Liaoning Province, China. The physiochemical properties of the biochar and peanut shells presented in Table 2. Biochar was applied in the spring of 2021 and uniformly spread on the surface of saline-alkali paddy soils before rice transplanting, and then thoroughly ploughed into the topsoil (0 to 20 cm) using a wooden rake.

Leaf concentration of Na⁺, K⁺, Na⁺ / K⁺ ratio

All leaves of five rice plants were collected in each treatment at maturity stage. These leaves are dried in an oven to constant weight at 60°C and then ground to fine power. The leaf samples were digested with 1% nitric-perchloric acid (Bastías et al. 2004). The concentration of Na⁺ and K⁺ was measured using the flame meter method (M410, Sherwood Scientific Ltd., Cambridge, England), and then calculated the Na⁺/K⁺ ratio.

Leaf malondialdehyde content (MDA) and abscisic acid (ABA)

For each treatment, flag leaves from 3 rice plants were analyzed at heading stage (August 12) and filling stage (September 1). MDA was quantified using the method of Stewart and Bewley (1980). Fresh leaves (1 g) were homogenized with quartz and 10 ml of 10% trichloroacetic acid, and then centrifuged at 4000 rpm for 10 min. The supernatant of 2 ml was extracted, add 2 ml of 0.6% thiobarbituric acid and shake

well. After react in boiling water for 15 min, and then cooled on ice and centrifuged at 4000 rpm for 10 min. The absorbance of supernatant was read at 532, 600 and 450 nm. MDA content was calculated using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and the content was expressed as $\text{nmol g}^{-1} \text{ FW}$.

Leaf abscisic acid content (ABA) was quantified using the method described by Liquid chromatography-Mass Spectrometry (Liu et al., 2021). Fresh leaves (0.5 g) were ground to a powder with liquid nitrogen. The powder was then pooled (100-200 mg) and placed into a 1.5 mL centrifuge tube, and 750 μL of freeze solution A [methanol/water/acetic acid (89/10/1 v/v/v)] containing 30 ng of 2H-ABA [(-)-5,80,80,80 -d4 ABA] was added. After thorough vortexing, each sample was centrifuged at 13000 rpm for 10 min. The supernatant was placed into a new 1.5 mL centrifuge tube, and 450 μL of solution B [methanol/water/acetic acid (89/10/1 v/v/v)] was added to the precipitate, after which each sample was vortexed thoroughly for 4 h. The samples were then centrifuged at 13000 rpm for 10 min, after which the supernatant was combined with the previous supernatant. The mixed supernatant was used to quantify the ABA contents by an LC-MS system (Ultimate TSQ Quantia, Thermo Fisher Scientific).

Leaf water status, relative electrical leakage

At heading stage (August 12) and filling stage (September 1), based on the method of Dionisio-Sese and Tobita (1998), the relative electrical leakage of leaf was measured. The HR-33T DEW Point Mikrovoltmeter (Wescor Inc., Logan, UT, USA) was used to determine the leaf water potential (Ψ_w) of rice at 9:00 - 11:00 am in both heading and filling stage.

Leaf chlorophyll content and leaf N content

At heading stage (August 12) and filling stage (September 1), the chlorophyll content of upper canopy three fully expanded leaves from main stem of rice was determined using a portable CCM-200 (Opti-Science, Tyngsboro, MA, USA). The content of total N (% DW) was measured from finely check leaf samples using CHNS/O analyzer (Flash 2000, Thermo Fisher Scientific, Cambridge, UK). The operating procedure was according to the dynamic flash combustion method.

Leaf area index (LAI), Photosynthetic potential, Leaf area decreasing rate (LAD), and Net assimilation rate (NAR)

At heading stage (August 12) and filling stage (September 1), five hills were selected from each treatment for measuring dry matter weight. The leaf area was measured by length \times width $\times 0.75$. The leaf area index (LAI) was calculated after the determination of determined.

Leaf area index (LAI) = total leaf area / land area.

Photosynthetic potential = $1/2 (L_1 + L_2) (t_2 - t_1)$, where L_1 and L_2 are the leaf areas (m^2) measured before and after, t_1 and t_2 are the time (d) measured before and after.

Leaf area decreasing rate (LAD) = $(LAI2-LAI1)/(T2-t1)$, where LAI1 and LAI2 are the Leaf area index measured before and after, t1 and t2 are the time (d) measured before and after.

Net assimilation rate (NAR) = $[\ln(LAI2)-\ln(LAI1)]/(LAI2-LAI1) \times (W2-W1)/(t2-t1)$, where LAI1 and LAI2 are the Leaf area index measured before and after, W1 and W2 are the dry matter weight ($g \cdot m^{-2}$) measured before and after, and t1 and t2 are the time (d) measured before and after.

Leaf photosynthesis rates (Pn), stomatal conductance (GS), transpiration rates (Tr) and intercellular CO₂ concentrations (Ci)

Photosynthesis-related parameters were measured at heading stage (August 12) and filling stage (September 1). The net photosynthesis rates, stomatal conductance (GS), transpiration rates (Tr) and intercellular CO₂ concentrations (Ci) of flag leaves from main stem of rice were measured using a portable photosynthesis system Li-6400 (Li-COR 6400, Li-COR Inc., Nebraska, USA) at 9:00-11:00 am. Measurement conditions in leaf chamber were kept consistent: photosynthetically active radiation (PAR) of $1200 \text{ mmol m}^{-2} \text{ s}^{-1}$, CO₂ concentration of 370 ppm and LED light source. Nine leaves were measured for each treatment.

Rice Biomass yield (BY), grain yield (GY) and harvest index (HI)

At the mature stage, 15 rice plants were randomly harvested in each treatment. These plants were oven-dried at 105 °C for 30 min and then at 60 °C to a constant weight. The biomass was recorded. The rice plants were selected from 5 m² for each experimental plot, and then the rice grain yield was calculated. The harvest index (HI) is the ratio of grain yield to biomass.

Statistical analysis

The data were analyzed using SPSS 18.0 software (IBM Corp., Armonk, NY, USA) based on the trial design. One-way ANOVA and Tukey tests were employed to analyze the effect of biochar on the relevant test indicators. The mean value was determined with the least significant difference at the $p < 0.05$ level.

Results

Leaf concentration of Na⁺, K⁺, Na⁺ / K⁺ ratio

Data describing the leaf concentration of Na⁺, K⁺, and Na⁺ / K⁺ ratio as affected by different biochar treatments are presented in Figure 1. Biochar amendments resulted in significantly decreases in leaf Na⁺ concentration (Fig. 1A) and Na⁺/K⁺ ratio (Fig. 1C), but increases in leaf K⁺ concentration (Fig. 1C) in treatments both with or without N fertilization, and with the relative changes varies with the biochar applied rate. Leaf Na⁺ concentration decreased 37.28% in T3N0 treatment as compared to T0N0, and by 22.814% under T3N225 and by 20.57% under T2N225 as compared to T0N225, respectively. Similarly, leaf Na⁺/K⁺ ratio was reduced by 82.16% under T3N0, 68.70% under T2N0 and 46.32% in T1N0 treatment

as compared with T0N0, and by 81.256%, 77.61%, and 64.76% in the T3N225, T2N225 and T1N225 treatments as compared to T0N225. Without N fertilizer condition, enhances in leaf K⁺ concentration following the biochar amendment were 251.10%, 145.01% and 68.29% in the T3N0, T2N0 and T1N0, as compared with T0N0. Biochar amended treatments showed the similar effect under N fertilizer.

Leaf water status and Leaf relative electrical leakage

Biochar amendments resulted in significantly decreases in leaf water status (Fig. 2A) and relative electrical leakage (Fig. 2B) with or without N fertilization ($P < 0.05$). With N fertilizer condition, leaf water status was shown in the order T2>T3>T1>T0 at both growth stage. T2, T3 and T1 were increased by 35.36%, 27.55% and 20.61% as compared to no biochar amendment treatment at heading stage, and by 53.90%, 52.33% and 34.93% at filling stage. Without N fertilizer condition, leaf water status was shown in the order T3>T2>T1>T0 at both growth stage. Leaf relative electrical leakage was reduced by 20.42% under T3N225, 25.03% under T1N225 and 31.42% in T2N225 as compared to T0N225 at heading stage, and by 19.33% under T3N225, 21.36 under T1N225 and 28.03% in T2N225 at filling stage. But no remarkable difference was found among biochar treatments at heading and filling stage. Biochar amendment treatments showed the similar effect under without N fertilizer.

Leaf abscisic acid (ABA) and malondialdehyde content (MDA)

The effect of biochar on leaf proline (ABA) and malondialdehyde content (MDA) are shown in Figure 3. It found that leaf ABA (Fig. 3A) and MDA (Fig. 3B) of biochar amendments was significantly decreased ($p < 0.05$) both with or without N fertilization at both growth stage. With N fertilizer, ABA was decreased by 36.37%, 40.54%, and 19.22% in T3N225, T2N225 and T1N225 as compared to T0N225 at heading stage, all the biochar amendment treatments obvious difference was observed compared to zero-biochar treatment (T0). A similar effect was also achieved in biochar amended soils at filling stage. Without N fertilization, it was shown in the order T3 < T2 < T1 < T0 at both rice growth stage, with the relative changes consistent with the biochar applied rate. Compared with T0N225, MDA was reduced by 54.72% under T3N225, 59.08% under T2N225, and 48.67% under T1N225 at heading stage, and by 57.39%, 66.15% and 56.78% at filling stage, respectively. Without N fertilizer, MDA was shown in the order T3 < T2 < T1 < T0 at both rice growth stage, with the relative changes consistent with the biochar applied rate.

Leaf chlorophyll content and leaf N content

Figure 4. reveals that the leaf chlorophyll content and leaf N content as impacted by different biochar treatments. Biochar amendments resulted in significantly increase in leaf chlorophyll content (Fig. 4A) under both with or without N fertilization. Leaf chlorophyll content increased by 11.87% under T3N225, 10.53% T2N225 and 9.51% under T1N225 as compared to T0N225 at heading stage, and by 6.47%, 13.50% and 5.56% in T3N225, T2N225 and T1N225 as compared to T0N225 at filling stage, while there was no significant difference was found among biochar amendment treatments at both growth stage. Without N fertilizer, leaf chlorophyll content was shown in the order T3 < T2 < T1 < T0 at both rice growth stage, and with the relative changes consistent with the biochar applied rate. Leaf N content was

increased by 5.34% under T3N225, 5.50% under T2N225 and by 4.85% under T1N225 at heading stage, while decreased by 6.77% under T3N225, 6.38% under T2N225 and by 2.50% under T1N225 at filling stage, respectively.

Leaf area index (LAI), Photosynthetic potential (LAD), Leaf area decreasing rate, and Net assimilation rate (NAR)

Data describing the production of rice dry matter production related parameters as affected by biochar treatments are showed in Table 3. With or without N fertilization, biochar amendments resulted in significantly increases in leaf area index (LAI), photosynthetic potential (LAD) and net assimilation rate (NAR), but decreases in leaf area decreasing rate. Without N fertilizer condition, LAI, LAD and NAR were shown as $T3 > T2 > T1 > T0$ at heading stage to filling stage, and the remarkably difference was found among all experimental treatments. In contrast, the leaf area decreasing rate was shown in the order $T0 > T1 > T3 > T2$, and the significant difference ($P < 0.05$) was found between all biochar treatments and B0 (zero-biochar), but no remarkably difference among biochar amendment treatments. Under N fertilizer condition, biochar amendments resulted in 91.26%, 77.05% and 75.96% increase in LAI in the T2N225, T3N225 and T1N225 as compared with T0N225 at heading stage, and 146.09%, 121.86% and 121.09% as compared with T0N225 at filling stage. Photosynthetic potential increased 95.21% in T2N225 treatment as compared to T0N225, and by 77.13% under T3N225 and by 64.36% under T1N225 at heading stage to filling stage, respectively. Similarly, NAR was increased by 32.43% under T2N225, 29.73% under T3N225 and 10.81% under T1N225 as compared to T0N225 at heading stage to filling stage. However, LAD was reduced by 39.29% under T2N225, 32.14% under T3N225 and 28.57% in the T1N225 as compared to T0N225 at heading stage to filling stage, respectively.

Leaf photosynthesis rates (Pn), transpiration rates (Tr), stomatal conductance (Gs) and intercellular CO₂ concentrations (Ci)

With or without N fertilizer condition, biochar amendment exerted a remarkable effect on the leaf photosynthesis related parameters, such as Pn (Fig. 5A), Tr (Fig. 5C), Gs (Fig. 5B) and Ci (Fig. 5D). With N fertilizer, Pn was shown in the order $T2 > T3 > T1 > T0$ at both growth stage, T2, T3 and T1 were increased by 16.81%, 15.44% and 5.98% as compared to no biochar amendment treatment at heading stage, and by 26.23%, 23.77% and 13.65% at filling stage. There was no significant difference between T2N222 and T3N225. Without N fertilizer condition, leaf water status was shown in the order $T3 > T2 > T1 > T0$ at both growth stage, and the differences among all treatments reached significant level. Compared with T0N225, Tr was increased by 71.64%, 63.67%, and 57.70% in T2N225, T3N225 and T1N225 at heading stage. A similar effect was also achieved in biochar amended soils at filling stage. Without N fertilization condition, Tr was shown in the order $T2 < T3 < T1 < T0$ at heading stage, while $T3 < T2 < T1 < T0$ at filling stage. With N fertilizer, Gs was shown in the order $T2 > T3 > T1 > T0$ at both growth stage, T2, T3 and T1 were increased by 82.94%, 77.35% and 13.43% as compared to no biochar amendment treatment at heading stage, and by 34.06%, 31.88% and 10.09% at filling stage. Without N fertilization condition, Gs was shown in the order $T2 < T3 < T1 < T0$ at heading stage,

while $T3 < T2 < T1 < T0$ at filling stage. In contrast, biochar amendments significantly decreased the leaf C_i with or without N fertilizer conditions ($P < 0.05$) at both growth stage (Fig. 5D). At heading stage, C_i showed trend as $T3 < T2 < T1 < T0$ with or without N fertilizer conditions, but no significant difference was found among biochar amendment treatments. At filling stage, C_i shown trend as $T2 < T3 < T1 < T0$ with N fertilizer condition, while $T3 < T1 < T2 < T0$ without N fertilizer condition. There was no significant difference was found among biochar amendment treatments at without N fertilizer condition.

Rice biomass yield (BY), grain yield (GY) and harvest index (HI)

Data describing the rice biomass yield (BY), grain yield (GY) and harvest index (HI) as affected by different biochar treatments are presented in Table 4. Biochar amendments resulted in significantly increases in BY, HY and HI in treatments both with or without N fertilization. Without N fertilizer condition, the BY, HY and HI were shown in the order $T3 > T2 > T1 > T0$ at both growth stage, with the relative changes varies with the biochar applied rate, but no significant difference was observed among biochar amendment treatments. With N fertilizer condition, increases in rice biomass yield following the biochar amendment were 83.38% under T3N225, 75.81% under T2N225 and 76.49% under T1N225 as compared to no biochar amendment treatment, respectively. A similar effect was also achieved in biochar amended soils with without N fertilizer. Grain yield was increased by 153.21% under T2N225, 145.66% under T3N225 and 144.15% under T1N225 as compared to T0N225, respectively. HI showed trend as $T2 > T3 > T1 > T0$ with N fertilizer, and all the biochar amendment treatments significantly differences ($p < 0.05$) were observed as compared to T0N225, but no significant difference was found among biochar amendment treatments.

Discussion

Effect of biochar amendment on leaf ionic concentration and physiological properties

Sodium ion toxicity, osmotic stress, oxidative damage and nutritional disorders in soda saline-alkali soil are the main factors that inhibit crops growth and productivity (Chi et al., 2012; Yan and Guo 2018). High Na^+ concentration not only interferes with the absorption of K^+ via roots, but also disturbed the physio-biochemical process of crop cells, limiting the uptake of basic nutrients in crops, and ultimately leading to crop yield loss (Oster et al., 1999; Ghafoor et al., 2001; Munns 2002). Reduce Na^+ content and increase K^+ content in plant tissues is an important way to ameliorate saline-alkali stress and reuse saline-alkali lands (Chakraborty et al., 2106). Many priors had reported biochar amendment can significantly decrease the entry of Na^+ in to crop cells and alleviate salinity stress caused damage to crops due to the porous structure and high specific surface area of biochar (Torabian et al. 2018; Ran et al., 2019; Li et al., 2022). In this study, peanut shell biochar amendment obviously decreased the leaf Na^+ concentration (Fig. 1A) and Na^+/K^+ ratio (Fig. 1C) in treatment both with or without N fertilization. At the meanwhile, the relative changes varied with the biochar applied rates. It well known that K is a kind of inorganic osmotic

regulating substance, and increase K^+ concentration of plant tissues was considered a critical factor for counteracting salted stress and enhancing plant growth (Chakraborty et al., 2016; Drake et al., 2016; Saifullah et al., 2018). Song et al. (2022) indicated that increased K^+/Na^+ ratio by improving K availability is an effective approach to enhance growth and yield of peanut in saline-sodic soil. Consistent with this, the leaf K^+ concentration (Fig. 1B) was remarkably increased following peanut shell biochar amendment in saline-alkali paddy soil. In addition, we found a higher leaf K^+ content, lower leaf Na^+ content and Na^+/K^+ ratio in rice leaf with peanut shell biochar amendment under both with or without N fertilization compared with no biochar treatment, such conditions can improve leaf water status (Fig. 2A) and relative electrical leakage (Fig. 2B). The results of this study show peanut shell biochar's great potential for protect cell integrity of rice tissues and alleviate Na^+ toxicity in highly saline-alkali paddy field. This may be due to (i) the high sodium ion adsorption potential of biochar can fasten Na^+ from the soil solution (Table 2; Akhtar et al., 2015; Huang et al., 2019; Yao et al., 2021); (ii) biochar is considered direct sources of mineral nutrients of Ca^{2+} and Mg^{2+} (Table 2), that replace Na^+ at the exchange site (Amini et al., 2016; Ali et al., 2017; Zheng et al., 2018). The immobilization of Na^+ and increase in K^+ uptake caused an ionic balance in cell tissues (Fig. 1) and alleviated oxidative stress (Akhtar et al. 2015; and Fig. 3). Similar findings were shown by Song et al., (2022) on Hybrid Pennisetum. In addition, we also found that from this study, the beneficial effect of peanut shell biochar (extent of the increase in K^+ accumulation and decline of Na^+/K^+ ratio) under N fertilizer treatment was significantly greater than without N fertilizer (Fig. 1). The reasons can be contributed to the saline-alkali soils are less fertility and poor structure and that means under no N fertilizer, rice in saline-alkali soils growth worse than that with N fertilizer, thus bring about lower increase in the uptake of K^+ by roots following biochar addition (Li et al., 2022).

Malondialdehyde is one of the most important products of membrane lipid peroxidation when plants suffer from stress injury, which can denature proteins and nucleic acids, resulting in reduced membrane fluidity, enhanced membrane permeability, decreased cell function, and even cell death in severe cases. Therefore, MDA content is widely used to express the extent of damage in response to abiotic stress (Feng et al., 2021). Previous studies shown that ABA is a good indicator of the osmotic stress and acts as a long-distance signal molecule to close stomata under abiotic stress, for example, drought stress, salted-stress and high temperature stress (Liu et al., 2005; Saifullah et al., 2018; Lu et al., 2019). Zelm et al. (2020) study shown that plants under saline-alkali stress can promote ABA and MDA production. In the present study, we found that the ABA (Fig. 3A) and MDA (Fig. 3B) concentration of rice at heading and filling stage were significantly reduced after biochar amended in treatment both with or without N fertilization in saline-alkali paddy fields. The decreased production of ABA and MDA concentration in rice could be ascribed to a biochar-induced (i) reduction in leaf Na^+ concentration (Fig. 1A) and increase in leaf K^+ concentration (Fig. 1B), (ii) improvement in leaf water status (Fig. 2A) and leaf relative electrical leakage (Fig. 2B), (iii) amelioration in soil physicochemical properties, which would result ultimately in promoted the uptake of water and nutrients by roots (Huang et al., 2019; Yao et al., 2021; Li et al., 2022). Thus, it is clear from the present study that peanut shell biochar can effectively ameliorate effect of

osmotic stress on rice by reducing leaf ABA and MDA concentration under saline-alkali paddy field. Similar to our results, existing studies have expressed that biochar amendment decreases the ABA and MDA of maize (Feng et al., 2021), soybean (Mehmood et al., 2020; Liu et al., 2021), hybrid Pennisetum (Song et al., (2022), and tomato (Juan et al., 2005) under salted-stress conditions. Consequently, the current findings will be useful in future for the improvement of rice in saline-alkali paddy fields.

Effect Of Biochar Amendment On Leaf Photosynthetic Related Parameters

Chlorophyll content has been proved to be an important reference index for plant response to environmental stress, nutrient status and photosynthetic efficiency (Zou et al., 2018). Previous studies have shown that the salinization of soil could adversely affect chlorophyll synthesis by accelerating the degradation of chlorophyll and decreasing the uptake of N (Van Hoorn et al. 2001; Liu et al., 2018; Song et al., 2022). Many studies have confirmed that biochar addition increases the chlorophyll through decreased the manufacture of reactive oxygen species and DPPH activity of leaf cells in saline soil (Torabian et al., 2018; Bashir et al., 2020). Similarly, here peanut shell biochar addition remarkably enhanced the chlorophyll content at heading stage in treatment both with or without N fertilization (Fig. 4A). In addition, the relative changes varied with the biochar applied rates. This may be due to the increase of rice leaf N content (Fig. 4B) and the decrease the ABA and MDA (Fig. 3). Consistent with our result, the authors (Song et al., 2022) concluded that biochar addition increased chlorophyll a and chlorophyll b content of hybrid pennisetum through reducing the impacts oxidative damage caused by salination stress on leaf pigments. In contrast to the chlorophyll content, biochar amendment tended to decrease N content in leaves at the filling stage (Fig. 4B). Similar results were shown by Akhtar et al. (2014, 2015) on tomatoes and potatoes under salt stress. During grain filling stage when the formation of storage proteins is most rapid, N is needed in considerable amounts. Large amounts of N stored in leaves before anthesis were then transferred to grains (Papakosta and Gagianas 1991; Ladha et al. 1998; Rasse et al., 2022). This may be the reason for the decrease of N content in leaves at filling stage (Fig. 4B) compared with that under no biochar treatment in treatment both with or without N fertilization. On another hand, the chlorophyll and nitrogen content at the filling stage was lower than that at heading stage (Fig. 4). This may be due to the transfer of nutrients, especially nitrogen, to the powerful and new sink like grains (the related results have not yet been published). Furthermore, the gain yield was obviously enhanced after biochar application, and resulted in a significant increase in nitrogen requirement (Table 4).

Excessive amounts of salt enter the shoot and eventually rise to toxic levels in the transpiring leaves; this toxicity causes leaf premature senescence, decreasing photosynthetic leaf area, reducing net assimilation rate, and ultimately resulting in biomass losses of plant to a level that cannot sustain growth (Brugnoli and Lauteri, 1991; Rahnema et al., 2010). Biochar application to salt-stressed soil can effectively increase photosynthetic leaf area, improve leaf photosynthetic capacity, and increase crop biomass as evident in soybean (Song et al., 2022), wheat (Akhtar et al., 2015), maize (Kamran et al., 2020) and halophyte plants

(Zheng et al., 2018). Here, biochar amendment significantly increased the LAI of rice population (heading and filling stage), the photosynthetic potential (heading to filling stage) and NAR (heading to filling stage) in treatment both with or without N fertilization (Table 3). In contrast, the leaf area decreasing was remarkably reduced compared to no-biochar treatment (Table 3). Our results indicate that peanut shell biochar significantly enhanced the net assimilation rate from heading to filling stage in highly saline-alkali paddy field, mainly through (i) ensuring the stable degradation of photosynthetic system of rice population by alleviating leaf area decreasing after heading stage, which benefit to optimized rice canopy structure; (ii) maintaining a thicker photosynthetic layer, higher photosynthetic potential and net assimilation rate. The following reasons could be used for explanation of this result. First, peanut shell biochar could effectively ameliorate effect of ionic toxicity and osmotic stress on rice by reducing leaf Na^+ accumulations (Fig. 1), Na^+/K^+ ratio (Fig. 1) and improving leaf water status (Fig. 2) due to enhancing K^+ availability (Fig. 1). Secondly, peanut shell biochar could improve leaf net photosynthetic capacity (Fig. 5A) by increasing chlorophyll content, nitrogen status (Fig. 4) and alleviating osmotic stress (Fig. 3). Moreover, our previous pot experiment showed that biochar application obviously optimized the root structure, increased the root volume, and improved the root activity and absorption capacity, which indicated that biochar could benefit to uptake of water and nutrients in saline-alkali paddy soil (Li et al., 2022).

Saline-alkali stress induced damage in photosynthetic apparatus in plants due to stomatal closure (Abbasi et al., 2015; Ling et al., 2020), destruction of chlorophyll pigment system (Juan et al. 2005), damage to the reaction centre of photosystem (Gill and Tuteja, 2010; Abbasi et al. 2016), either or all, which results in a significant decrease in photosynthetic capacity, peroxidation damage and suppresses plant growth and development. In this present study, saline-alkali stress significantly inhibited the Pn (Fig. 5A), Tr (Fig. 5B) and Gs (Fig. 5C), while increasing the Ci (Fig. 5D) at heading and filling stage in treatment both with or without N fertilization. However, the addition of peanut shell biochar obviously ameliorated these effects in both growth stage (Fig. 5). Our results indicated that peanut shell biochar increased photosynthesis in rice by alleviating the stomatal limitation (Fig. 5B), membrane permeability of rice leaves (Fig. 2B), enhancing chlorophyll synthesis and leaf N content (Fig. 4), hence promoting net assimilation rate (Table 3). Similar findings have been observed in Hybrid Pennisetum amended with biochar (Song et al., 2022). From this study, we also found that among biochar treatments, T2 was the most effective. Several previous studies reported the same results and suggested appropriate addition rate of biochar (Jin et al., 2018; Saifullah et al., 2018; Huang et al., 2019; Zhao et al., 2020). The increased competition of high C input in soil caused by excess biochar and crop for nutrients elements may be the potential reason for lower increase in Pn (Elshaikh et al., 2018; Ran et al., 2019; Yao et al., 2021).

Effect Of Biochar Amendment On Rice Yield

Crop growth and yield in saline-sodic soils are restricted due to (i) reduced leaf photosynthetic rate caused by high accumulation of ROS (Abbasi et al., 2015); (ii) resulted in metabolic disorder caused by a high concentration of Na^+ (Al-Karaki 1997; Santos et al., 2021), (iii) reduced availability of water to crops

caused by high osmotic pressure of the soil solution (Naidu and Rengasamy 1993; Palansooriya et al., 2019), (iv) restrained absorption of indispensable nutrients (K, Ca, P, etc.) caused by a high concentration of Na^+ (Chaganti and Crohn 2015), and (v) limited root growth caused by poor physical characteristics (Sumner 1993). Many studies have shown that biochar applications can alleviate saline-alkali stress and promote crops growth (Drake et al., 2016; Jin et al., 2018; Zhu et al., 2022). In this field experiment, the biomass yield and grain yield of rice were significantly promoted with biochar application under highly saline-alkali paddy field in treatments both with or without N fertilization (Table 4). The treatment with biochar applied at T2 (3.0% biochar, w/w) had the most positive effect on yield performance in the authors' field experiment under normal N fertilization application (N225). However, there were no significant difference among T1(1.5% biochar, w/w), T2 (3.0% biochar, w/w) and T3 (4.5% biochar, w/w) in terms of grain yield. The mechanisms for biochar to increase rice yield can be attributed to the following four aspects. First, peanut shell biochar remarkably alleviated leaf Na^+ concentration (Fig. 1A), Na^+/K^+ ratio (Fig. 1C), and elevated leaf K^+ concentration (Fig. 1B) of rice by transient Na^+ binding due to its high adsorption capacity and by releasing mineral nutrients into the soil solution (Huang et al. 2019; Mehdizadeh et al. 2020), which can alleviate oxidative stress and osmosis stress (Fig. 3; Zhao et al. 2020; Song et al., 2022) and protect cell integrity of rice tissues (Fig. 2). In addition, the peanut shell biochar applied to the highly saline-alkali paddy field can improve the chloroplast activities (Feng et al., 2021) and net assimilation rate (NAR) through increases in chlorophyll content (Fig. 4A) and leaf N content (Fig. 4B). Furthermore, peanut shell biochar increased the LAI, photosynthetic potential, NAR, while decreases leaf area decreasing rate (Table 3), thus increasing leaf photosynthesis rates (Fig. 5A). A similar result was also found by Zhao et al. (2020), who added corn straw biochar to saline-sodic upland soils, which significantly increased corn yield 50% under the 20 t ha^{-1} biochar application rate. Peanut shell biochar greatly increased the harvest index (HI) in the current study (Table 4), which led to an increase in the conversion rate of photosynthetic C source to rice grain. This may be another reason for the increase in grain yield after peanut shell biochar was added to the saline-alkali paddy soils. This clearly showed the potential of peanut shell biochar to promote rice productivity in highly saline-sodic paddy soils, and the most economical application rate of biochar was 3.0% biochar (w/w).

Conclusion

Peanut shell biochar significantly alleviated the effect of ion toxicity, osmotic stress and oxidative damage on rice in the highly saline-alkali paddy field, which resulted from the decreased of leaf Na^+ concentration, Na^+/K^+ ratio, enhanced leaf K^+ concentration, and improve leaf water status and relative electrical leakage. In addition, peanut shell biochar could provide beneficial effects on chlorophyll content, leaf N content, LAI, photosynthetic potential, Gs, and Tr, which is of great benefit to the enhancement of leaf photosynthesis rate, ultimately led to the increasement of net assimilation rate of rice population. Furthermore, the greatest positive effect on rice yield performance was observed in the treatment with a biochar application rate of 3.0% biochar (w/w). To our knowledge, this is the first report on examining the biochar-induced leaf photosynthesis rate response to the biochar-enhanced yield for rice growth in the highly saline-alkali paddy fields. These findings provide a new insight for regulation of

rice saline-alkali tolerance by peanut shell biochar. However, further investigation is needed to identify the effects of biochar on rice physiological and biochemical changes and environmental benefits in the saline-alkali ecosystem under the changing climate.

Declarations

Funding: This study was funded by the National Natural Science Foundation of China (No. 32071951) and Jilin Province Education Department Planning Project (No. JJKH20200340KJ).

Conflict of Interest: The authors declare that they have no conflict of interest.

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Tables

Table 1
Physicochemical Properties of the Soil

Soil Properties (0 to 25 cm Soil Layers)	Value
Sand content (%)	23.13 ± 1.11
Silt content (%)	38.14 ± 1.31
Clay content (%)	37.60 ± 2.09
Bulk density (g cm ⁻³)	1.61 ± 0.13
ECe (µs m ⁻¹)	24.08 ± 0.71
pH	10.10 ± 0.24
SARe (mmolc L ⁻¹) ^{1/2}	368.11 ± 4.03
ESP (%)	71.11 ± 2.17
Organic matter (%)	0.64 ± 0.04
Total N (g kg ⁻¹)	0.27 ± 1.11
Alkali-hydrolysable N (mg kg ⁻¹)	16.30 ± 1.11
Available P (mg kg ⁻¹)	9.13 ± 0.68
Available K (mg kg ⁻¹)	107.25 ± 5.68
Notes: ECe: electrical conductivity of soil saturation extract, SARe: sodium adsorption ratio of soil saturation extract, ESP: exchangeable sodium percentage, N: nitrogen, P: phosphorus, K: potassium	

Table 2
Basic properties of raw peanut shell and biochar

pH and Elemental Component	Peanut Shell	
	Raw Material	Biochar
pH	5.56 ± 0.11	7.94 ± 0.32
CEC (cmol·kg ⁻¹)	—	78.69 ± 11.32
EC (dS·m ⁻¹)	—	7.88 ± 0.59
C (mg·g ⁻¹)	429.19 ± 13.05	540.64 ± 26.58
N (mg·g ⁻¹)	10.85 ± 0.61	15.93 ± 1.01
S (mg·g ⁻¹)	2.58 ± 0.05	6.85 ± 0.34
P (mg·g ⁻¹)	0.29 ± 0.00	0.74 ± 0.03
Mg (mg·g ⁻¹)	1.46 ± 0.01	0.25 ± 0.00
K (mg·g ⁻¹)	5.51 ± 0.21	12.53 ± 0.51
Ca (mg·g ⁻¹)	6.32 ± 0.43	2.01 ± 0.02
Na (mg·g ⁻¹)	1.79 ± 0.39	1.17 ± 0.21
Notes: CEC: cation exchange capacity, EC: electrical conductivity, C: carbon, N: nitrogen, S: Sulfur, Mg: magnesium, P: phosphorus K: potassium, Ca: calcium, Na: sodium		

Table 3

Effects of different biochar treatments on leaf area index (LAI), photosynthetic potential, leaf area decreasing rate (LAD), and net assimilation rate (NAR)

N applied level	Biochar applied rates	LAI		Heading stage to Filling stage		
		Heading stage	Filling stage	Photosynthetic potential ($\text{m}^2 \cdot \text{d}$)	Leaf area decreasing ($\text{LAI} \cdot \text{d}^{-1}$)	NAR ($\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$)
N0	T0	0.38 ± 0.01 d	0.15 ± 0.00 d	0.28 ± 0.01 d	0.013 ± 0.00 a	0.12 ± 0.00 d
	T1	0.47 ± 0.02 c	0.28 ± 0.01 c	0.35 ± 0.01 c	0.011 ± 0.00 b	0.13 ± 0.00 c
	T2	0.63 ± 0.02 b	0.41 ± 0.01 b	0.47 ± 0.02 b	0.010 ± 0.00 b	0.25 ± 0.00 b
	T3	0.72 ± 0.03 a	0.50 ± 0.02 a	0.59 ± 0.02 a	0.011 ± 0.00 b	0.28 ± 0.00 a
N225	T0	1.83 ± 0.11 c	1.28 ± 0.14 c	1.88 ± 0.18 c	0.028 ± 0.00 a	0.37 ± 0.03 c
	T1	3.22 ± 0.13 b	2.83 ± 0.12 b	3.09 ± 0.13 b	0.020 ± 0.00 b	0.41 ± 0.02 b
	T2	3.50 ± 0.18 a	3.15 ± 0.26 a	3.67 ± 0.17 a	0.017 ± 0.00 b	0.49 ± 0.03 a
	T3	3.24 ± 0.14 b	2.84 ± 0.17 b	3.33 ± 0.31 ab	0.019 ± 0.00 b	0.48 ± 0.06 a

Different letters indicate significantly different values ($P < 0.05$).

Table 4

Effects of different biochar treatments on rice biomass yield (BY), grain yield (GY) and harvest index (HI)

N level	Parameters	T0	T1	T2	T3
N0	BY (t/hm^2)	2.94 ± 0.36 c	5.73 ± 0.43 b	7.06 ± 0.89 a	7.83 ± 0.51 a
	GY (t/hm^2)	0.30 ± 0.01 d	0.91 ± 0.02 c	1.52 ± 0.02 b	1.83 ± 0.09 a
	HI	0.10 ± 0.00 c	0.16 ± 0.00 b	0.22 ± 0.01 a	0.23 ± 0.01 a
N225	BY (t/hm^2)	7.40 ± 1.11 b	13.06 ± 1.26 a	13.01 ± 1.03 a	13.57 ± 0.94 a
	GY (t/hm^2)	2.65 ± 0.16 b	6.47 ± 0.25 a	6.71 ± 0.68 a	6.51 ± 0.28 a
	HI	0.36 ± 0.03 b	0.50 ± 0.04 a	0.52 ± 0.06 a	0.48 ± 0.06 a

Different letters indicate significantly different values ($P < 0.05$).

Figures

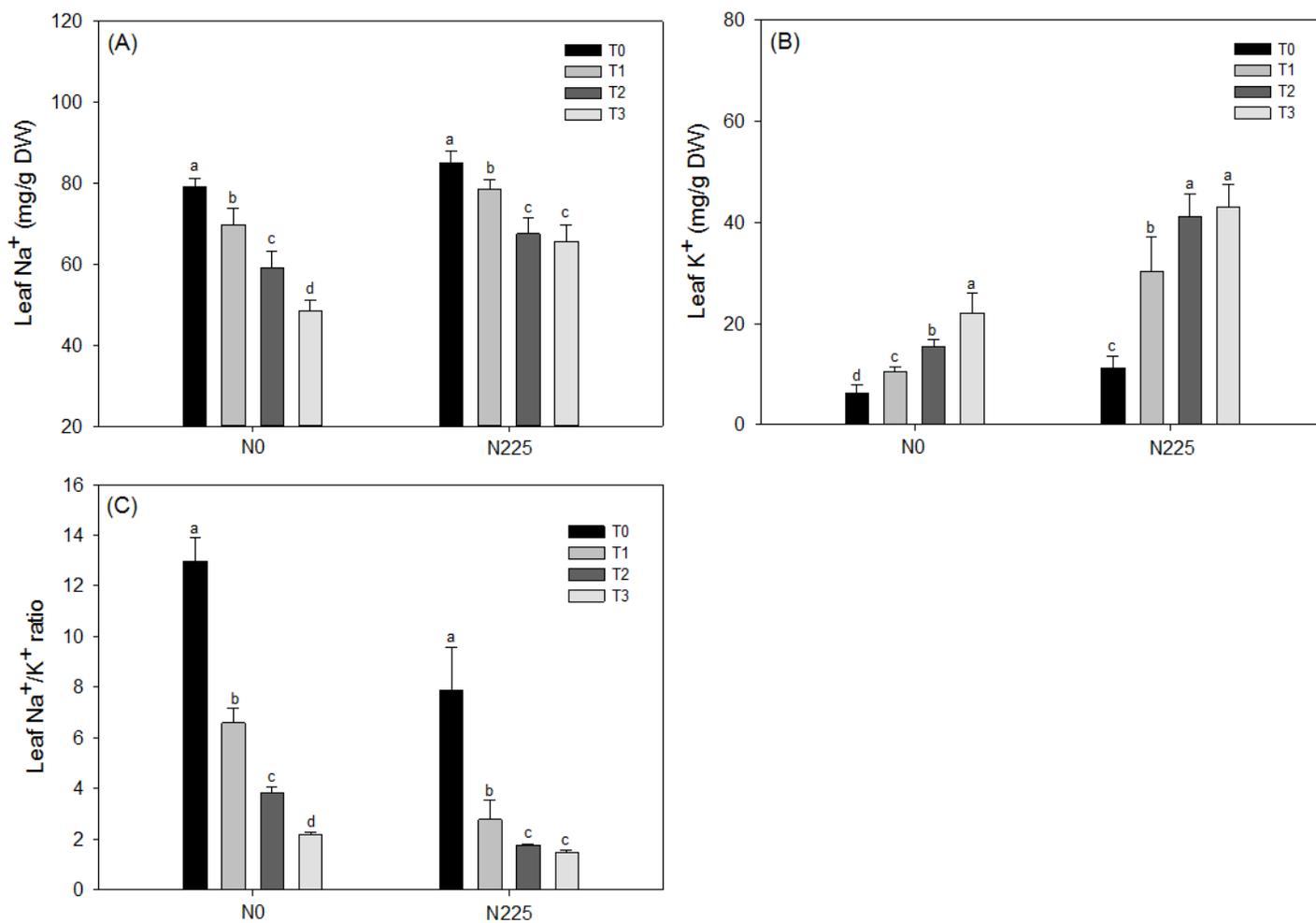


Figure 1

Leaf concentration of Na⁺, K⁺, and Na⁺ / K⁺ ratio as affected by different biochar treatments. Different letters indicate significantly different values (P<0.05).

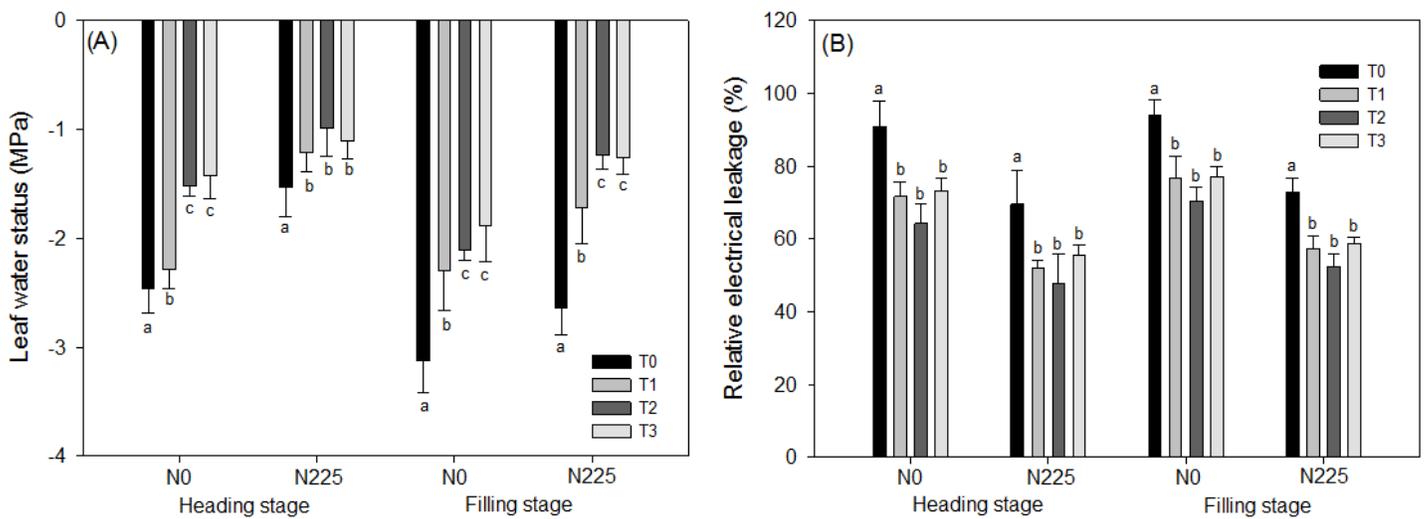


Figure 2

Effects of different biochar treatments on leaf water status and relative electrical leakage at rice different growth stage. Different letters indicate significantly different values (P<0.05).

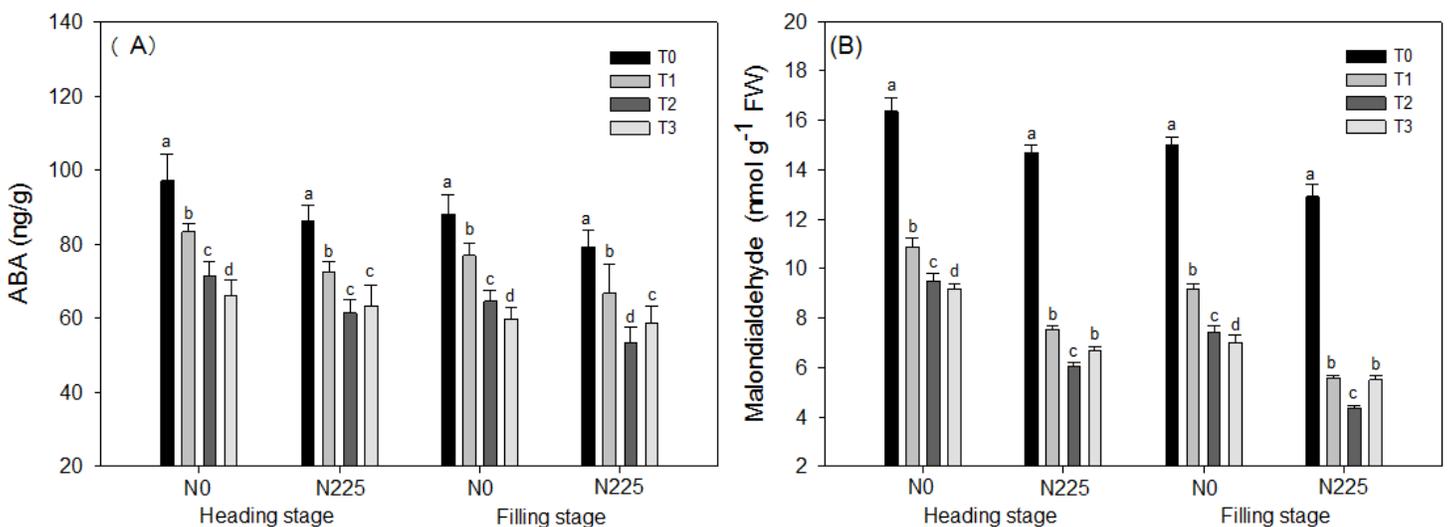


Figure 3

Effects of different biochar treatments on leaf proline content and malondialdehyde content at rice different growth stages. Different letters indicate significantly different values (P<0.05).

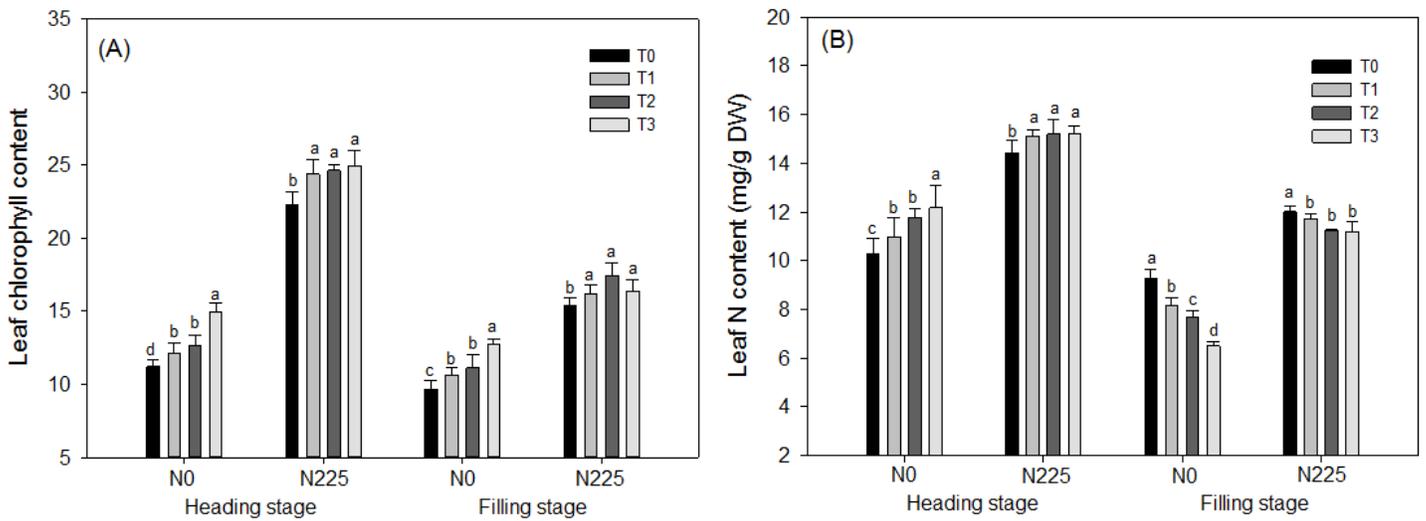


Figure 4

Effects of different biochar treatments on chlorophyll content index and leaf N at rice different growth stages. Different letters indicate significantly different values ($P < 0.05$).

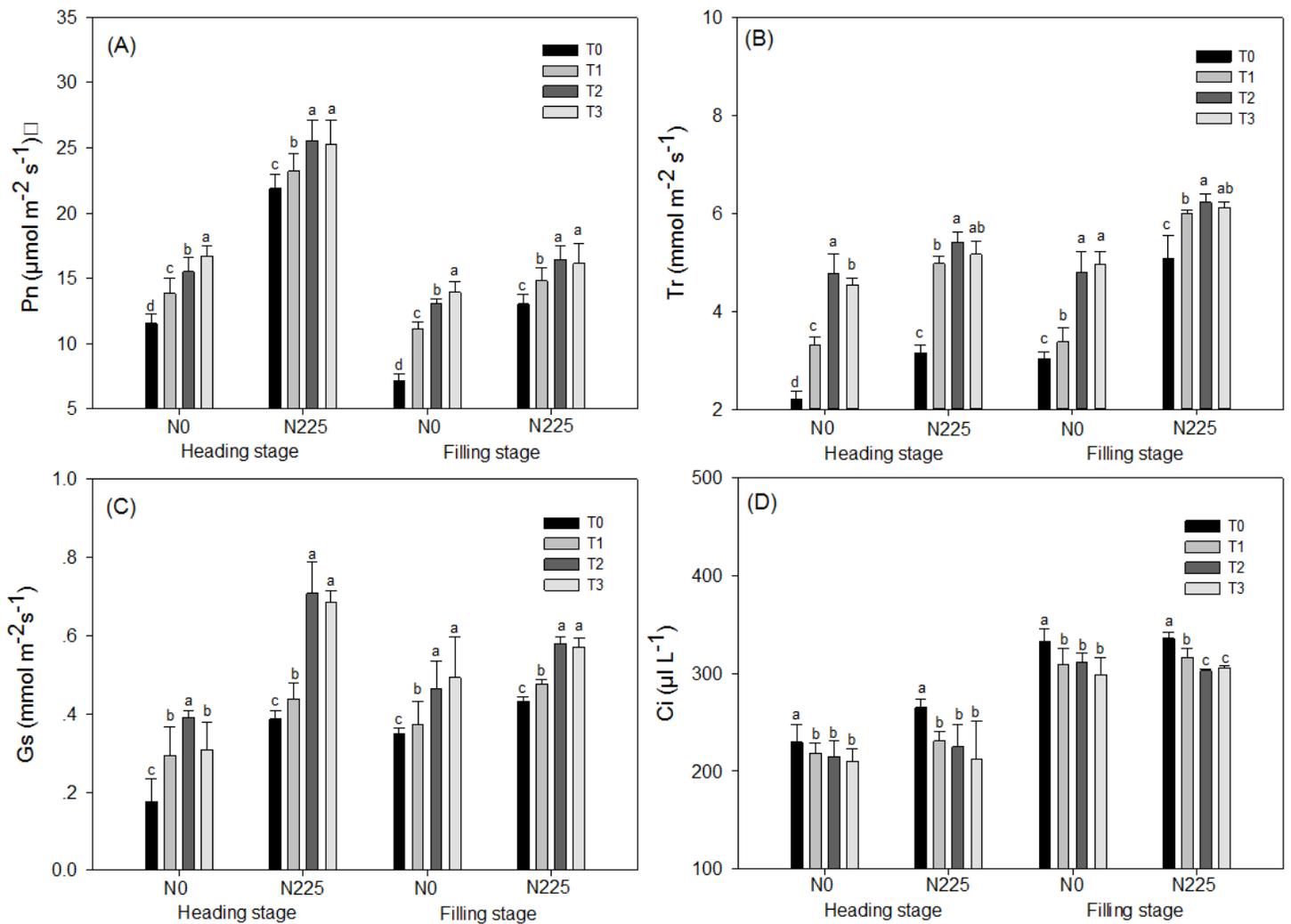


Figure 5

Effects of different biochar treatments on leaf net photosynthesis rates (Pn), transpiration rates (Tr), stomatal conductance (GS) and intercellular CO₂ concentrations (Ci) at rice different growth stages. Different letters indicate significantly different values (P<0.05).