Compensation of high nitrogen toxicity and nitrogen deficiency with biochar amendment through enhancement of soil fertility and nitrogen use efficiency promoted rice growth and yield

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Abstract
The quality and nutritional status of agricultural soils are depleting gradually, and biochar is widely used in soil quality improvement. A sustainable approach of biochar application needs a better understanding of its interaction with nitrogen application and the final effect on crop growth. In our study, the effect of different biochar application levels (0, 15, 30, and 60 tons ha⁻¹) in combination with nitrogen fertilizer levels (0, 150, 300, and 450 kg ha⁻¹) on soil properties, rice growth, and nitrogen use efficiency were investigated. The results showed that sole application of biochar (B60) did not promote the rice growth except the soil organic carbon (SOC), total nitrogen, total phosphorous, and available potassium compared with no biochar addition, while a single application of 450 kg N ha⁻¹ (N450) adversely affected the rice physiological and yield traits by destroying rice leaf ultrastructure and reducing the stomatal length by 13%, stomatal width 3% and density 12%, plant height 5%, dry biomass plant⁻¹ 4%, spike weight plant⁻¹ 8%, and grain weight plant⁻¹ 9% compared to N300 unless it was treated in combination with biochar. Biochar can significantly compensate the toxicity of excessive nitrogen and deficiency of low nitrogen fertilizer on rice growth. Compared to a single application of B0+N450 (450 kg N ha⁻¹), combined application of biochar and nitrogen B30+N450 could elevate rice plant nitrogen content by 13%, plant height 30%, aboveground dry biomass plant⁻¹ 136%, spike weight plant⁻¹ 34%, and grain weight plant⁻¹ 36% while increments in total nitrogen accumulation (TNA 79%) and nitrogen use efficiency (NUE 35%) were noted in B30+N300 treatment. These results suggest that biochar amendment combined with a proper amount of nitrogen fertilizer is an effective approach to promote soil conditions, manage nitrogen utilization, improve plant growth and increase crop yield on a sustainable basis.

KEYWORDS
biochar, nitrogen use efficiency, rice, soil quality
Depletion of soil nutrients declines soil fertility, reduces nitrogen use efficiency, contaminates the groundwater, increases fertilizer application cost, and decreases crop yield. Rice is the most important cereal crop in China, and farmers often apply a large amount of nitrogen (N) fertilizer on an average of 300 kg N ha\(^{-1}\) in rice fields in China (Feng et al., 2017), which is surplus than the rice production demand (Lin et al., 2007). More than 50% of the world’s population use rice as a staple food (Muthayya et al., 2014), and the world’s economic growth and growing population need an increase of 1% annually in global rice production to meet the food requirements (Normile, 2008), and sustainable crop production strategies are required for this increase in rice yield.

Nitrogen is an important nutrient for plant growth and development (Xiong et al., 2018), and its efficient use is critical for feeding the world’s human population (Sylvestre-Bradley & Kindred, 2009). However, reduced nitrogen use efficiency and low soil fertility due to soil acidity adversely affect the plant’s physiological, biochemical, and morphological processes and create many hurdles for lower crop production and food security in many developing countries (Agegnehu et al., 2016; Jones et al., 2013). Supplement of nitrogen fertilizer to the field can reduce the risk of crop failure and promote the growth and yield, but lower chemical, physical, and biological properties of soil may limit the fertilizer response to plant. It is quite alarming that half of the applied nitrogen fertilizers did not return during crop harvesting and lost from the agricultural system through surface runoff, ammonia leaching, or nitrous oxide emission (Billen et al., 2013; Bouchet et al., 2016). Recently, many studies have suggested different soil management practices to improve nutrients utilization; however, the application of biochar as an organic amendment seems a good agronomic and sustainable approach (Khan et al., 2020; Ur Rahim et al., 2020) to improve soil fertility status, crop yield, and reduce environmental pollution.

Biochar is a carbon-rich and porous material obtained by thermal decomposition of organic matter under limited or no oxygen at relatively low temperature, and it is highly reluctant to decomposition due to its complex and condensed structure (Lehmann & Joseph, 2012; Spokas et al., 2012). Biochar can be added to soils as organic amendments to improve soil health, nutrients utilization, sustainability of agriculture and environment, and boost up the revenue of bioenergy and economy. It possesses a negative charge, high surface area, and stable carbon (Oladele et al., 2019), which promote its beneficial role in soil properties improvement (Vaccari et al., 2011), retention of soil water and nutrients, reduction in greenhouse gasses emission, and enhancing carbon sequestration and crop yield (Schulz et al., 2013). Previous studies conducted by Gaskin et al. (2010) observed that incorporation of biochar for 2 years elevated soil organic carbon and total nitrogen without promoting soil available phosphorus and Zhang et al. (2012) found that rice yields were increased by 10% and 10%–29%, respectively, in first and second crop cycle under the application of biochar.

The soil nitrogen and phosphorus leaching is reduced with biochar amendment through absorption and retention of soil NO\(_3^-\) - N, NH\(_4^+\) - N, and phosphate (Thangarajan et al., 2018). In addition, biochar acts as a soil reservoir to retain nitrogen and provide carbon to improve soil organic carbon (Prasad et al., 2018) and provide habitat to the beneficial bacteria of nitrogen fixation (Yu et al., 2018). However, negative and adverse effects of biochar amendment are also possible that could adversely affect the nitrogen supply and plant growth, as the decline in soil organic matter priming (SOM; Zimmerman et al., 2011) and lessen enzymatic activities (Song et al., 2020), low mineral nitrogen availability (Gao et al., 2019), and decreased crop growth and development (Van Zwieten et al., 2010). A large surface area and high pH of biochar can reduce the toxicity of highly fertilized soil (Cao et al., 2018), and it can control nutrient stress and enhance plant growth and biomass (Pandit et al., 2018). Biochar can retain nitrogen through increasing N absorption with the help of capillary, complexation, and electrostatic forces present on the surface and associated pores of soil particles (Ding et al., 2010; Yu et al., 2018).

Despite many research studies on the positive role of biochar in improving crop growth and development through promoting soil properties, there is still a lack of information to understand the mutual interactive effect of biochar and nitrogen on rice crop physiological status and growth altered by high nitrogen fertilizer application. The different positive and negative impacts of a single application of biochar and nitrogen encourage new research study to evaluate their mutual and interactive effect for better understanding the synergistic role of biochar in crop growth, soil dynamics, and environment. Therefore, the effects of single and combined application of biochar and nitrogen were investigated on leaf anatomical, morphophysiological, biochemical, and yield traits of rice crop to reveal the significance of biochar. The findings of this research study will emerge new perspectives regarding the excessive application of inorganic fertilizers in combination with biochar.

2 | MATERIALS AND METHODS

2.1 | Plant materials and site conditions

An outdoor experiment was conducted in plastic pots to investigate the integral effect of biochar and nitrogen on soil quality and morphology, physiology, and yield.
of direct-seeded rice. Pored bottom plastic pots of 27 cm height and 25.5 cm and 22.5 cm above and below diameter were filled with sun-dried and sieved (2 mm) mixture of clay and sand (2:1) weighed 14 kg. Initially, five seeds of direct-seeded rice cultivar (Shengtai you 018) were sown and then finally thinned to three plants per pot after seedling establishment. Pots were irrigated with tap water throughout the growing period to maintain the oversaturated condition and fulfill the water requirements. Proper agronomic management techniques were adapted at specific and required times of the trial duration.

2.2 | Treatments combination

The pot experiment was performed in a completely randomized design having four biochar levels (0, 15, 30, and 60 t ha⁻¹) and four nitrogen levels (0, 150, 300, and 450 kg ha⁻¹). There were total 16 different treatments (B0+N0, B0+N150, B0+N300, B0+N450, B15+N0, B15+N150, B15+N300, B15+N450, B30+N0, B30+N150, B30+N300, B30+N450, B60+N0, B60+N150, B60+N300, and B60+N450) and 15 replicates of each treatment. Biochar of different levels was thoroughly mixed in the top 20 cm soil in the pot at the soil filling stage. Rice straw biochar was provided by Hubei Jinzhi Eco-Energy Co., Ltd, prepared by pyrolysis under a high temperature of 600°C (Table 1). Basic essential fertilizers (phosphorus and potassium) were applied as basal fertilizers at the time of sowing. Nitrogen was applied in two doses (50% at the time of sowing and 50% at the tillering stage).

2.3 | Determination of photosynthetic processes

The measurement of photosynthesis (Pn), transpiration (E), stomatal conductance (gs), and intercellular CO₂ (CO₂ ci) was performed at two different intervals (tillering stage and panicle initiation stage). The full expanded fourth flag leaf from bottom to top was selected each time by using a portable photosynthesis system (Li-6400, Li-COR Inc.) during the daytime from 10:00 am to 03:00 pm in full sunshine under the following conditions: CO₂, 400 µmol mol⁻¹; leaf temperature, 23°C; light intensity, 1000 µmol m⁻² s⁻¹; and air humidity, 70%.

2.4 | Soluble sugar, crude protein, chlorophyll contents, and carotenoid determination

Plant leaf samples were taken at tillering and panicle initiation stages to measure the chlorophyll content with 80% acetone. The samples were kept overnight, and absorbance of 663 nm, 645 nm, and 470 nm was read spectrophotometrically for chlorophyll a, b, and carotenoids, respectively. The values of chlorophyll a, b, total chlorophyll, and carotenoids were calculated according to the method of Pei et al. (2010). Plant leaf samples taken at tillering and panicle initiation stages and soluble sugar kit and crude protein kit (Suzhou Grace Biotechnology co., Ltd) were used, and absorbance was noted at 620 nm and 562 nm, respectively, to determine soluble sugar and crude protein contents expressed as mg g⁻¹ FW.

2.5 | Scanning electron microscopy (SEM) analysis and transmission electron microscope (TEM) analysis

A uniform portion of (1 mm²) from the middle parts of leaves was taken in three replicates for each selected treatment. Distilled water was used to wash the samples before the analysis on electron microscopy. Solution of 4% glutaraldehyde and 0.2 M sodium phosphate buffer (pH 6.8) was used to fix the collected samples (6 h, 4°C). Onwards, the samples were washed four times with 0.1 M sodium phosphate buffer having (pH 6.8). After this, samples were washed with diluted ethanol; afterwards, rinsed the samples two times with isomyl acetate and then freeze-dried. The leaf fragments were fixed firmly on stubs with double-sided tape, and samples were sputter-coated using gold (Kong et al., 2013). Finally, JEOLJSM-6390LV Scanning Electron Microscope was run to analyze the samples. For transmission electron microscopy, samples were post-fixed in 1% osmic acid in 0.2 M phosphate buffer (pH 6.8); dehydration was done by graded series of ethanol and dried through critical point drying. Finally, the lead citrate and 2% uranyl acetate were used to stain the thin

| TABLE 1 | Chemical properties of biochar used and soil before the experiment. |
|-----------------|-----------------|-----------------|
| **Properties of** | **Values** | **Properties of** | **Values** |
| **biochar**     |         | **soil**     |         |
| pH              | 9.42    | pH            | 6.04    |
| N               | 0.74%   | EC            | 1.08    |
| C               | 47.14%  | TN            | 0.49 g kg⁻¹ |
| H               | 1.62%   | TP            | 0.34 mg kg⁻¹ |
| O               | 11.85%  | AK            | 76.24 mg kg⁻¹ |
| K               | 18.9%   | SOC           | 4.62 g kg⁻¹ |
| P               | 0.32%   | Ash           | 19.43% |

Abbreviations: AK, available potassium; C, carbon; EC, electric conductivity; H, hydrogen; K, potassium; N, nitrogen; O, oxygen; P, phosphorus; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.
fragments of leaves, and Hitachi 500 electron microscope was used to observe the samples (Kong et al., 2013).

2.6 | Determination of plant nitrogen content, total nitrogen accumulation (TNA) and nitrogen utilization efficiency (NUtE)

Plant nitrogen content was observed using colorimetric analysis with a discrete analyzer (SmartChem 200, Unity Scientific, Brookfield, CT) following Kjeldahl digestion. Total nitrogen accumulation and nitrogen utilization efficiency was determined by the following equations.

\[
\text{TNA} = \text{N concentration} \times \text{total plant dry weight (mg N)} \\
\text{NUtE} = \frac{\text{Plant dry weight}}{\text{Nitrogen concentration (g TDW mg}^{-1}\text{N)}}
\]

(TNA = N concentration × total plant dry weight (mg N) (Abenavoli et al., 2016). 

NUtE = Plant dry weight/Nitrogen concentration(g2 TDW mg−1 N) (Khan et al., 2020).

2.7 | Plant morphological and yield contents

Different morphological parameters (plant height, tillers number, aboveground biomass, and leaf area) were measured at two different intervals of the tillering stage (TS) and panicle initiation stage (PIS) and yield contents (seed weight plant−1, panicle length, dry biomass, and 1000 grain weight) were measured after harvesting the plants of each pot.

2.8 | Soil samples analysis

Three soil samples were taken before the start of the experiment for the measurement of soil basic chemical properties of the trial soil. After the harvesting of rice plants, three replicate soil samples from 20 cm top of the pot were taken and oven-dried for further investigation of the chemical properties of soil. To measure soil pH and EC by digital pH meter (B-212, HORIBA, Ltd.) and EC meter (B-173, HORIBA, Ltd.), 10 g of dry soil samples was mixed at the ratio of 1:2 in distilled water and mixed properly by shaking at 180 rounds/min for 20 min. To measure soil total nitrogen TN and total phosphorus TP content after Kjeldahl digestion, colorimetric analysis was performed using a discrete analyzer (SmartChem 200, Unity Scientific). Soil available potassium AK was measured with a flame photometer. Indophenol methods (Kovar & Pierzynski, 2009) and the Catalado method (Cataldo et al., 1974) were used to measure \( \text{NH}_4^+ - \text{N} \) and \( \text{NO}_3^- - \text{N} \), respectively. Soil of 0.3 g was weighed to measure the soil organic carbon with wet digestion according to the standard protocol (Cataldo et al., 1975).

2.9 | Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine significant differences between different treatments by analyzing four replicates of each treatment with statistix 8.1 (\( p < 0.05 \)). The individual means of different treatments were compared by LSD test. Figures were drawn using GraphPad Prism version 8.0.

3 | RESULTS

3.1 | Chemical properties of post-harvest soil

Biochar amendment positively affected the soil status and significantly elevated soil chemical properties while nitrogen fertilizer application was only significant for \( \text{NH}_4^+ \), \( \text{NO}_3^- \), TN%, and TP% while the interaction of biochar and nitrogen for soil chemical properties was not significant (Table 2). The results revealed that biochar application at B15N0 level increased soil pH by 6%, EC by 4%, and SOC by 123% compared to control treatment B0N0, while the effect of nitrogen was not significant for these indices. Similarly, applying biochar at B30N0 level enhanced soil pH, EC, and SOC by 7%, 19%, and 243%, respectively. Likewise, using biochar at B60 induced increments of 9%, 29%, and 417% in soil pH, EC, and SOC, respectively (Table 2). These high and induced values of such quality parameters of soil fertility indicate the importance and significant role of biochar application. Compared to B0+N0 treatment, increasing the level of nitrogen alone also increased the \( \text{NH}_4^+ \) by 29%, 41%, and 57%; \( \text{NO}_3^- \) by 19%, 49%, and 87%; TN by 30%, 66%, and 94%; and TP by 3%, 15%, and 65%; and AK by 23%, 47%, and 64% in B0+N150, B0+N300, and B0+N450 treatments, respectively (Table 2). Moreover, integration of biochar application with nitrogen enhanced soil \( \text{NO}_3^- \) by 115%, 149%, and 172%; \( \text{NH}_4^+ \) by 124%, 139%, and 93%; TN by 388%, 391%, and 398%; TP by 28%, 51%, and 65%; and AK by 23%, 47%, and 64% in B15+N450, B30+N450, and B60+N450 treatments, respectively (Table 2).

3.2 | Nitrogen concentration, accumulation, and use efficiency traits

The positive effects of biochar on soil chemical properties improved the soil condition and thus elevated rice plant nitrogen content (%), total nitrogen accumulation (TNA mg N), nitrogen use efficiency (NUE %), and nitrogen utilization efficiency (NUtE g2 TDW mg−1 N) in combine treatments of biochar and nitrogen than a single application of both (Figure 1). The plant N content increased
**TABLE 2**  Integrated effect of biochar and nitrogen application on chemical properties of soil.

| Biochar | Nitrogen | Soil pH  | EC (dS m\(^{-1}\)) | SOC (g kg\(^{-1}\)) | NH\(^+_4\) (mg kg\(^{-1}\)) | NO\(^-3\) (mg kg\(^{-1}\)) | TN (g kg\(^{-1}\)) | TP (mg kg\(^{-1}\)) | AK (mg kg\(^{-1}\)) |
|---------|----------|---------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| B0      | N0       | 6.72 f  | 1.14 f            | 3.92 e            | 5.62 gh           | 13.13 i           | 0.36 e            | 0.34 j            | 112.83 g         |
|         | N150     | 6.84 e  | 1.15 f            | 4.30 e            | 7.31 defg         | 15.69 h           | 0.47 e            | 0.35 ij           | 117.88 g         |
|         | N300     | 6.75 ef | 1.16 ef           | 4.30 e            | 7.98 def          | 19.58 f           | 0.60 d            | 0.39 ghi          | 113.34 g         |
|         | N450     | 6.83 e  | 1.14 f            | 4.37 e            | 8.84 cd           | 24.61 d           | 0.71 cd           | 0.41 fgh          | 121.38 fg        |
| B15     | N0       | 7.17 cd | 1.19 de           | 8.75 d            | 6.49 fgh          | 16.94 gh          | 1.41 c            | 0.37 hij          | 135.06 e         |
|         | N150     | 7.18 cd | 1.20 de           | 8.42 d            | 7.89 def          | 21.98 e           | 1.68 b            | 0.41 fgh          | 132.96 ef        |
|         | N300     | 7.17 cd | 1.18 def          | 9.09 d            | 10.39             | 24.56 d           | 1.72 ab           | 0.41 fgh          | 141.79 e         |
|         | N450     | 7.19 bcd| 1.20 d            | 9.23 d            | 12.63 a           | 28.32 c           | 1.77 ab           | 0.43 efg          | 139.76 e         |
| B30     | N0       | 7.24 abcd| 1.36 bc         | 13.47 c           | 6.56 fgh          | 18.33 fg          | 1.46 c            | 0.44 def          | 157.40 d         |
|         | N150     | 7.27 abc | 1.39 b         | 12.77 c           | 8.46 de           | 24.65 d           | 1.73 ab           | 0.49 bc           | 169.31 bc        |
|         | N300     | 7.26 abcd| 1.34 c         | 13.27 c           | 11.79 ab          | 28.95 c           | 1.76 ab           | 0.50 bc           | 163.49 cd        |
|         | N450     | 7.24 abcd| 1.35 c         | 13.24 c           | 13.45 a           | 32.73 b           | 1.78 ab           | 0.51 bc           | 166.89 cd        |
| B60     | N0       | 7.33 a  | 1.48 a           | 20.30 a           | 5.57 h            | 19.94 ef          | 1.47 c            | 0.48 cd           | 179.10 ab        |
|         | N150     | 7.28 ab | 1.46 a           | 19.64 ab          | 7.12 efg          | 25.88 d           | 1.76 ab           | 0.48 cd           | 188.23 a         |
|         | N300     | 7.31 a  | 1.45 a           | 18.42 ab          | 8.13 def          | 31.82 b           | 1.77 ab           | 0.54 ab           | 183.17 a         |
|         | N450     | 7.30 a  | 1.46 a           | 19.56 ab          | 10.88 b           | 35.80 a           | 1.81 a            | 0.56 a            | 185.47 a         |

*F* values and significance level

| Factor                | *F* Value | Significance Level |
|-----------------------|-----------|--------------------|
| Biochar               | 165.61*** | *p* < 0.001        |
| Nitrogen              | 240.11*** | *p* < 0.001        |
| Biochar × Nitrogen    | 1717.75***| *p* < 0.001        |
| Nitrogen              | 17.00**   | *p* < 0.01         |
| Biochar × Nitrogen    | 256.74*** | *p* < 0.001        |
| Nitrogen              | 65.91***  | *p* < 0.001        |
| Biochar × Nitrogen    | 55.01***  | *p* < 0.001        |
| Nitrogen              | 184.25*** | *p* < 0.001        |

Different letters within the columns show a significant difference between the treatments at *p* = 0.05 according to LSD test, and asterisks represent a significant difference at *p* < 0.05, **p < 0.01, and ***p < 0.001 level; ns: not-significant.

Abbreviations: NH\(^+_4\), ammonium nitrogen; NO\(^-3\), nitrate nitrogen; AK, available potassium; EC, electric conductivity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus.
significantly by 40%, 101%, and 141% in B0+N150, B0+N300, and B0+N450 treatments compared to B0+N0. When biochar and nitrogen were applied in combination compared to control treatment (B0N0), increments of 150%, 173%, and 155% were recorded in B15+N450, B30+N450, and B60+N450 treatments compared to B0+N0 (Figure 1). In comparison with B0+N150, increments in TNA by 359%, 588%, and 403% were recorded in B15, B30, and B60 in combination with N450, NUE by 41%, 59%, and 28% in B15+N300, B30+N300, and B60+N450 treatments, respectively, and NUtE by 77%, 91%, and 52% were noted in B15+N150, B30+N300, and B60+N450 treatments, respectively (Figure 1). Although the amount of nitrogen was high in the soil in the pots treated with N450 treatment, however; due to the negative and toxic effect of high nitrogen on rice growth and physiology, a decline was observed in the nitrogen traits; nonetheless, positive adsorptive effect of biochar increased the values of the mentioned nitrogen traits at N150, N300, and N450 and showed a significant interaction between biochar and nitrogen levels (Figure 1).

3.3 Stomatal and ultrastructure traits of rice leaf

Based on the results obtained for morphological, physiological, biochemical, and yield traits, it was observed that the combination of B30+N450 was more significant and effective than other combinations. Therefore, B0+N0, B0+N450, and B30+N450 treatments were selected for SEM and TEM analysis of rice leaves. These treatments were analyzed to record stomatal number, width and length, and changes in ultrastructure cells. High nitrogen
treatment B0+N450 reduced the stomatal length by 8.17%, stomatal width by 12.05%, and density by 40.02% compared to B30+N0 treatment. However, plants treated with B30+N450 had higher stomatal traits than the control and single high nitrogen treatments. The results showed that treatment B30+N450 increased the stomatal length, width, and density by 8.9%, 13.7%, and 66.74%, respectively, compared to B0+N450 treatment (Table 3; Figure 6).

Cell elongation and cell division in plants play an important role in the growth and development of the plant. The leaf ultrastructure analysis revealed that high nitrogen treatment B0+N450 distorted the shape and size of the cell compared to B0+N0 and B30+N450 treatments (Figure 3b,e). Nevertheless, biochar amendment along with N450 improved the cell structure and shape, and the cells were well defined and established than B0+N450 treatment (Figure 3c,f). Chlorophyll exists inside the chloroplast to run photosynthesis and produce photosynthates for plant growth and development. Therefore, changes in the structure of chloroplast will affect chlorophyll and photosynthesis. In control treatment B0+N0, the chloroplast shape was oval, well established, and adhered to the cell wall (Figure 3a). A single application of high nitrogen treatment B0+N450 destroyed the cell structure, and the shape of the chloroplast became distorted (Figure 3b). In comparison, the integration of biochar and nitrogen B30+N450 compensated the cell injury and enhanced the shape of chloroplast (Figure 3c). Under the single application of high nitrogen treatment B0+N450, reduced starch grains and uncleared and deformed cell wall were observed compared to control treatment B0+N0 (Figure 3a,b). However, the combined application of biochar and nitrogen B30+N450 enhanced the starch grains and improved the structure of the cell wall with fine, clear, and complete edges (Figure 3c).

### 3.4 Response of physiological attributes to biochar application

The physiological status of plants mostly depends on the moisture and nutrients availability of soil. The application of sole nitrogen at B0+N150 increased chlorophyll a content by 5% and 6%; chlorophyll b by 72% and 67%; carotenoids by 5% and 7%; and total chlorophyll by 13% and 16%, respectively, at TS and PIS compared to B0+N0. Similarly, the application of sole nitrogen at B0+N300 increased chlorophyll a content by 16% and 9%; chlorophyll b by 163% and 77%; carotenoids by 82% and 12%; and total chlorophyll by 41% and 20%, respectively, at TS and PIS compared to B0+N0. Similarly, the application of sole nitrogen at B0+N300 increased chlorophyll a content by 11% and 9%; chlorophyll b content by 31% and 10%; carotenoids by 27% and 6%; and total chlorophyll by 18% and 9%, respectively, at TS and PIS compared to N300 (Figures 4 and 5). However, the combination of biochar and nitrogen B30+N450 reduced the adverse effect

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**Table 3** Integrated effect of biochar and nitrogen application on stomatal morphology

| Treatment | Stomatal length (µm) | Stomatal width (µm) | Stomatal number (0.049 mm²) |
|-----------|----------------------|---------------------|-----------------------------|
| B0N0      | 12.37 b              | 1.20 b              | 21.43 b                     |
| B0N450    | 14.37 a              | 1.24 b              | 24.66 b                     |
| B30N450   | 15.65 a              | 1.41 a              | 41.12 a                     |

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**Figure 2** Effect of integrated biochar and nitrogen on rice leaf stomatal length, width, and density compared to control and sole nitrogen treatments. (a–c) in the figure represent the effects of treatments (B0+N0, B0+N450, and B30+N450) on stomatal length and width. (a–c) ×5000 magnification, scale bars = 5 µm. Similarly, in the figure, (d–f) represent the effects of treatments (B0+N0, B0+N450, and B30+N450) on a stomatal number. (d–f) ×500 magnification, scale bars = 50 µm. SG: stomatal guard cell; WP: wart-like protuberance; SW: stomatal width, and SL: stomatal length
FIGURE 3  Effect of integrated biochar and nitrogen on the ultrastructure of rice leaf cells, chloroplast, and cell wall compared to control and sole nitrogen treatments. (a–c) in the figure represent the effects of treatments (B0+N0, B0+N450, and B30+N450) on leaf ultrastructural variations in chloroplast and cell wall. (a–c) ×5000 magnification, scale bars = 5 µm. Similarly, in the figure, (d–f) represent the effects of treatments (B0+N0, B0+N450, and B30+N450) on leaf cells. (d–f) ×500 magnification, scale bars = 50 µm. OP: osmophilic plastoglobuli; CW: cell wall; SG: starch granule; CH: chloroplast; G: grana, and C: cell.

FIGURE 4  Integrated effects of biochar and nitrogen on rice plant chlorophyll a (mg g⁻¹ FW) (a) TS and (b) PIS and chlorophyll b (mg g⁻¹ FW) (c) TS and (d) PIS. TS: tillering stage, and PIS: panicle initiation stage; B0: 0 tons ha⁻¹ biochar; B15: 15 tons ha⁻¹ biochar; B30: 30 tons ha⁻¹ biochar; N0: 0 kg ha⁻¹ nitrogen; N150: 150 kg ha⁻¹ nitrogen; N300: 300 kg ha⁻¹ nitrogen, and N450: 450 kg ha⁻¹ nitrogen, error bars represent standard error for each treatment in three replicates. Different letters on each bar represent a significant difference according to the LSD test (p < 0.05).
of nitrogen and enhanced the chlorophyll \(a\) by 120% and 188%; chlorophyll \(b\) 140% and 117%; carotenoids 69% and 182%; and total chlorophyll 127% and 172%, respectively, at TS and PIS compared to a single application of high nitrogen B0+N450.

The application of single nitrogen at B0+N150 increased soluble sugar by 5% and 64%, and crude protein by 3% and 38%, respectively, at TS and PIS compared to B0+N0. Likewise, sole nitrogen at B0+N300 increased soluble sugar by 59% and 103%, and crude protein by 53% and 67%, respectively, at TS and PIS compared to B0+N0 treatment (Figure 6). On the other hand, the high nitrogen B0+N450 reduced the soluble sugar by 20% and 13% and crude protein by 17% and 11%, respectively, at TS and PIS compared to B0+N300 treatment (Figure 6). Interestingly, compared to control treatment B0+N0, the integral application of biochar with nitrogen compensated the high nitrogen toxicity and low nitrogen deficiency and increased the soluble sugar by 95%, 148%, and 105% and crude protein by 86%, 121%, and 90% at TS and soluble sugar by 134%, 161%, and 154% and crude protein by 79%, 135%, and 133% at B15+N450, B30+N450, and B60+N450 treatments, respectively (Figure 6).

Biochar integration improved the soil quality (Table 2), nutrients utilization (Figure 1), leaf ultrastructure traits of rice (Table 3; Figures 2 and 3), chlorophyll contents (Figures 4 and 5), and thus synergistically enhanced photosynthesis (A), transpiration (E), stomatal conductance (gs), and carbon dioxide exchange (Ci) rates under combined application of biochar and nitrogen than the sole application of nitrogen levels (Figures 7 and 8). The results showed that sole application of nitrogen B0+N150 increased A by 32% and 17%; E by 42% and 31%; gs by 84% and 22%; and Ci by 15% and 10%, B0+N300 increased A by 49% and 34%;
E by 66% and 56%; gs by 101% and 88%; and Ci by 20% and 18%, respectively, at TS and PIS, while a single application of high nitrogen B0+N450, A, E, gs, and Ci reduced by 4% and 1%, 9% and 14%, 2%, and 0%, respectively, at TS and PIS due to the adverse effect of high nitrogen compared to B0+N300 treatment (Figures 7 and 8). The application of biochar in combination with nitrogen positively affected the photosynthetic attributes by absorbing the excess nitrogen from the soil to reduce nitrogen toxicity and releasing the stored nitrogen to reduce nitrogen deficiency. The highest values of these indices were observed in the combined application of B30+N450 and increased A by 44% and 40%; E by 63% and 53%; gs by 60% and 58%; and Ci by 26% and 27% at TS and PIS compared to control treatment (Figures 7 and 8).

3.5 Plant growth and morphological traits

The morphological growth and development reflect the actual plant physiological status and condition of a growing media of a plant. Biochar and nitrogen both significantly altered the rice growth and morphology (Tables 4 and 5). Rice growth and biomass were enhanced with increments in biochar and nitrogen levels compared to control treatment; however, there was a decline in rice growth at N450 (nitrogen at 450 kg ha⁻¹). As compared to control B0+N0, sole nitrogen N150 increased plant height by 46% and 27%; tiller number by 218% and 230%; leaf area by 594% and 477%; aboveground biomass 356% and 385%; stem biomass by 260% and 353%; and leaf biomass by 542% and 458% at TS and PIS, respectively. Similarly,
the sole application of nitrogen N300 also followed the same trend and enhanced the rice morphological traits; however, a single application of high nitrogen B0+N450 negatively affected plant growth and decreased the plant height by 7% and 7%; tiller number by 8% and 7%; leaf area by 11% and 17%; aboveground biomass by 17% and 17%; stem biomass by 24% and 11%; and leaf biomass by 85 and 26% at TS and PIS, respectively, compared to B0+N300 treatment (Tables 4 and 5). A two-way analysis of variance showed a significant interaction between biochar and nitrogen for rice biomass, which indicates the positive role of biochar on plant growth and nitrogen compensation (Table 5).

### 3.6 Rice yield contents

Plant biomass and yield traits are the end products of a crop which indicates the capability and productivity of soil and plant in a specific season. Biochar and nitrogen application showed a significant effect and interaction on plant height, fresh biomass plant$^{-1}$, dry biomass plant$^{-1}$, spike weight plant$^{-1}$, and grain weight plant$^{-1}$ (Table 6). Nitrogen levels increased the rice growth yield by increasing plant height by 15% and 21%; fresh biomass plant$^{-1}$ by
271% and 594%; dry biomass plant$^{-1}$ by 390% and 564%; spike weight plant$^{-1}$ by 40% and 64%; and grain weight plant$^{-1}$ by 52% and 80%, respectively, at B0+N150 and B0+N300 treatments compared to B0+N0, while B0+N450 reduced plant height by 5%; fresh biomass plant$^{-1}$ by 2%; dry biomass plant$^{-1}$ by 4%; spike weight plant$^{-1}$ by 8%; and grain weight plant$^{-1}$ by 9% compared to B0+N300, unless it was treated in combination with different biochar levels.

Among the different levels of biochar, B30 was more effective and significant to increase the rice yield contents when treated with high nitrogen level N450. Integration of biochar along with nitrogen decreased the nitrogen toxicity and enhanced the nitrogen utilization by absorbing excess nitrogen in excessive nitrogen soil and released stored organic nitrogen in nitrogen-deficient soil and improved plant height by 30%, fresh biomass by 90%, dry biomass by 136%, spike length by 16%, spike weight by 34%, grain weight by 36%, and 100-grain weight by 10% in treatment B30+N450 when compared to a high single application of nitrogen B0+N450 (Table 6). These positive results indicate the prominent role of biochar in the compensation and utilization of nitrogen fertilizer for better plant growth and yield contents. The results revealed that sole application of high nitrogen N450 suppressed the growth of rice and resulted in short stature and yellow colored plants as compared to other treatments; however, integration of biochar along nitrogen was found to be a significant organic amendment to improve nitrogen utilization and rice growth (Figure 9).

4 | DISCUSSION

Recently, various organic amendments have been applied alone or in combination with inorganic fertilizers to reduce
their loss and enhanced their availability and utilization for plant growth. However, biochar is an industrial bio-product, which can be exploited in agriculture, the energy sector and industries for the sustainability of the environment and crop production. In the current study, the residual effect of biochar was evaluated on soil fertility, nitrogen utilization, and rice growth (Table 2; Figures 2 and 10).

The results revealed that biochar treatments significantly increased SOC, TP, and AK compared to treatments without biochar (Table 2). The rice straw biochar contains nitrogen, phosphorus, and large amount of potassium and it can elevate the soil essential nutrients dynamics (Khan et al., 2021). This positive impact of biochar could be attributed to the presence of various organic and inorganic forms of mineral elements; however, concentration of these nutrients varies with the source and production temperature of biochar (Gul & Whalen, 2016; Jin et al., 2016; Khan et al., 2020). The increase in soil TP under the application of biochar could be attributed to the High P holding capacity of biochar that could decrease the surface runoff and increase the soil TP (Blanco-Canqui et al., 2020; Dong et al., 2014).

The findings of the current study indicate that increments in soil pH, EC, and nitrogen dynamics, such as TN, \(\text{NH}_4^+\), \(\text{NO}_3^-\), were directly proportional to an increase in biochar levels (Table 2). The application of biochar can regulate soil temperature, aeration, and moisture content which helps to absorb inhibitors (phenolics) of nitrifiers to facilitate the activities of nitrifiers and thus enhance nitrification (Mukherjee & Lal, 2014; Nguyen et al., 2017). Nitrifiers are very vulnerable to change in soil pH, and nitrification terminates when pH is less than 5.0 and increase if pH is above 6.0 (Sahrawat, 2008). Interestingly, biochar induced liming effect in soil and changed the nature of soil from acidic to alkaline by increasing soil pH and EC (Table 2), which could be correlated with the existence of organic carbon, oxygen-containing organic functional groups, CaCO\(_3\), and base soluble cations in biochar, which are major forms of alkalinity (Liu et al., 2019; Singh et al., 2010). The high contents of soil nitrogen indices could be related to that biochar application can reduce the \(\text{NH}_3\) loss from soil by direct adsorption of \(\text{NH}_3\) and prevents the conversion of \(\text{NH}_4^+\) to \(\text{NH}_3\) due to the high surface area and increased cation exchange capacity (CEC) of biochar (Clough & Condron, 2010; Mandal et al., 2016). Previously, Khan et al. (2020) reported that biochar played a dual role to provide nutrients and retained the nutrients

### Table 4 Integrated effect of biochar and nitrogen application on rice morphology

| Biochar | Nitrogen | Plant height (cm) | Tiller number | Leaf area (cm\(^2\)) |
|---------|----------|-------------------|---------------|----------------------|
|         |          | TS | PIS | TS | PIS | TS | PIS |
| B0      | N0       | 44.00 d | 61.00 f | 3.67 f | 4.33 h | 103.54 g | 257.78 g |
|         | N150     | 64.33 c | 78.00 de | 11.67 e | 14.33 fg | 719.14 def | 1489.15 ef |
|         | N300     | 72.67 b | 85.33 cd | 16.00 cd | 19.00 bcd | 904.00 bcde | 2418.87 bc |
|         | N450     | 67.33 c | 79.33 de | 14.67 d | 17.67 de | 803.94 cdef | 2003.82 cd |
| B15     | N0       | 46.33 d | 62.33 f | 4.00 f | 5.67 h | 105.74 g | 459.44 g |
|         | N150     | 65.33 c | 80.33 de | 15.33 d | 17.00 def | 695.31 def | 1713.93 de |
|         | N300     | 75.00 ab | 89.33 bc | 18.33 bc | 20.00 bcd | 875.21 cdef | 2132.31 cd |
|         | N450     | 74.00 b | 91.67 abc | 20.00 ab | 22.33 ab | 860.67 cdef | 2466.18 bc |
| B30     | N0       | 45.00 d | 63.33 f | 4.67 f | 5.00 h | 108.71 g | 415.95 g |
|         | N150     | 64.00 c | 78.00 de | 14.67 d | 15.00 efg | 609.33 ef | 1666.95 def |
|         | N300     | 76.67 ab | 91.33 abc | 19.67 ab | 21.00 bc | 1037.27 bc | 2636.71 b |
|         | N450     | 79.00 a | 98.33 a | 22.33 a | 24.33 a | 1421.05 a | 3189.76 a |
| B60     | N0       | 43.33 d | 60.33 f | 5.00 f | 5.33 h | 98.50 g | 329.74 g |
|         | N150     | 63.00 c | 76.67 e | 10.67 e | 12.67 g | 585.80 f | 1183.96 f |
|         | N300     | 74.00 b | 85.33 cd | 16.67 cd | 18.33 cd | 971.51 bcd | 2324.00 bc |
|         | N450     | 76.33 ab | 94.33 ab | 18.33 bc | 21.33 abc | 1193.60 ab | 2674.32 b |

F values and significance level

| Biochar  | Nitrogen | Plant height (cm) | Tiller number | Leaf area (cm\(^2\)) |
|----------|----------|-------------------|---------------|----------------------|
|          |          | TS | PIS | TS | PIS | TS | PIS |
| B0       | N0       | 5.14* | 14.54*** | 5.12* | 10.55*** | 3.58 ns | 11.28** |
|          | N150     | 297.11*** | 87.15*** | 161.45*** | 153.34*** | 64.83*** | 235.13*** |
|          | N300     | 2.30 ns | 1.95 ns | 1.96 ns | 1.63 ns | 2.05 ns | 2.82*** |
|          | N450     | 1.95 ns | 1.96 ns | 1.63 ns | 2.05 ns | 2.82*** | 2.82*** |

Different letters within the columns show a significant difference between the treatments at \(p = 0.05\) according to LSD test, and asterisks represent a significant difference at \(*p < 0.05, **p < 0.01, and ***p < 0.001\) level; ns: not-significant.

Abbreviations: PIS, panicle initiation stage; TS, tillering stage.
that increased the contents of nitrogen, phosphorus, and potassium in soil for later uptake and utilization by the plants. Increments in these soil nutrients due to biochar addition will probably increase the nutrients uptake by plants and decrease their leaching and improve the nutrients use efficiency in crop production.

The enhancement of soil fertility with biochar addition in our study compensated the nitrogen supply and ensured proper plant growth. The plant N accumulation and utilization dynamics responded positively to the integrated application of biochar and nitrogen compared to their single application (Figure 1). In our findings, the nitrogen level N300 showed high NUE and NUtE than N450 in contrast to the high N content in N450 level than the N300 level (Figure 1). These low N indices in our study without the presence of biochar could be related to soil N losses through leaching of NO$_3^-$, ammonia volatilization, and denitrification. Biochar is known to decrease denitrification and prevent the leaching of NO$_3^-$ by converting it to molecular nitrogen (N$_2$). This mechanism is attributed to the production of redox potential in the biochar pores and the neutralization capacity of biochar particles to help and promote the shift of nitrogen from N$_2$O to N$_2$ (Dörsch et al., 2012; Oh et al., 2013). Our results are in line with previous findings of high N uptake and lower nitrogen use efficiencies in higher nitrogen applied fertilizer (Agegnehu et al., 2016) and the addition of biochar to soil either alone or in combination with nitrogen fertilizer enhanced N uptake in plants, proving that absorbed nitrogen in biochar is plant available (Taghizadeh-Toosi et al., 2012).

The variations in leaf gas exchange are related to changes in epidermal traits and stomata that regulates the transport of water and CO$_2$ which can be interfered by different environmental components (Xu & Zhou, 2008), which can affect the plant physiology and growth in different ways. Our results revealed that the B0+N0 and B0+N450 treatments exhibited the deficit and surplus N, respectively, that adversely affected rice leaf stomatal traits and ultrastructure (Figures 2 and 3). The supply of soil N in B0+N0 treatment was not sufficient for plant demand, while in B0+N450, the supply of soil N was excessive than plant N requirement. Interestingly, the combined application of different levels of biochar with N450 compensated and absorbed the excess nitrogen, which improved the rice leaf ultrastructure, nitrogen utilization efficiency, and rice

### TABLE 5  Integrated effect of biochar and nitrogen application on rice morphology

| Biochar | Nitrogen | Aboveground biomass (g) | Leaf biomass (g) | Stem biomass (g) |
|---------|----------|-------------------------|-----------------|-----------------|
|         |          | TS | PIS | TS | PIS | TS | PIS | TS | PIS |
| B0      | N0       | 1.47 h | 4.16 i | 0.50 f | 1.27 h | 0.97 h | 2.89 h |
|         | N150     | 6.72 fg | 20.19 gh | 3.22 e | 7.07 fg | 3.49 g | 13.12 fg |
|         | N300     | 9.36 cdef | 33.01 cd | 4.01 cde | 12.34 bc | 5.34 cdef | 20.68 cd |
|         | N450     | 7.69 efg | 27.35 ef | 3.68 cde | 9.13 ef | 4.01 fg | 18.22 de |
| B15     | N0       | 1.56 h | 6.00 i | 0.52 f | 2.25 h | 1.04 h | 3.75 h |
|         | N150     | 8.64 defg | 25.92 ef | 3.75 cde | 9.22 ef | 4.89 defg | 16.70 e |
|         | N300     | 10.11 cde | 29.40 def | 4.29 cde | 10.47 cde | 5.82 bcd | 18.94 cde |
|         | N450     | 11.03 bcd | 34.21 bcd | 4.54 bcd | 12.38 bc | 6.49 bcd | 21.83 bcd |
| B30     | N0       | 1.64 h | 5.81 i | 0.54 f | 2.06 h | 1.09 h | 3.76 h |
|         | N150     | 8.11 efg | 25.26 fg | 3.41 de | 8.97 ef | 4.70 efg | 16.29 ef |
|         | N300     | 11.44 bc | 38.66 b | 4.77 bc | 14.00 b | 6.67 bc | 24.66 b |
|         | N450     | 15.43 a | 46.78 a | 6.72 a | 17.04 a | 8.71 a | 29.74 a |
| B60     | N0       | 1.50 h | 5.44 i | 0.53 f | 1.79 h | 0.98 h | 3.65 h |
|         | N150     | 7.10 fg | 18.66 h | 3.21 e | 6.50 g | 3.89 fg | 12.16 g |
|         | N300     | 9.60 cde | 32.14 cd | 4.46 bcd | 11.55 cd | 5.14 cdef | 20.59 cd |
|         | N450     | 12.65 b | 35.10 bc | 5.66 ab | 12.66 bc | 6.99 b | 22.44 bc |

**$F$ values and significance level**

| Biochar | Nitrogen | Aboveground biomass (g) | Leaf biomass (g) | Stem biomass (g) |
|---------|----------|-------------------------|-----------------|-----------------|
|         |          | TS | PIS | TS | PIS | TS | PIS | TS | PIS |
| B0      | N0       | 1.47 h | 4.16 i | 0.50 f | 1.27 h | 0.97 h | 2.89 h |
|         | N150     | 6.72 fg | 20.19 gh | 3.22 e | 7.07 fg | 3.49 g | 13.12 fg |
|         | N300     | 9.36 cdef | 33.01 cd | 4.01 cde | 12.34 bc | 5.34 cdef | 20.68 cd |
|         | N450     | 7.69 efg | 27.35 ef | 3.68 cde | 9.13 ef | 4.01 fg | 18.22 de |
| B15     | N0       | 1.56 h | 6.00 i | 0.52 f | 2.25 h | 1.04 h | 3.75 h |
|         | N150     | 8.64 defg | 25.92 ef | 3.75 cde | 9.22 ef | 4.89 defg | 16.70 e |
|         | N300     | 10.11 cde | 29.40 def | 4.29 cde | 10.47 cde | 5.82 bcd | 18.94 cde |
|         | N450     | 11.03 bcd | 34.21 bcd | 4.54 bcd | 12.38 bc | 6.49 bcd | 21.83 bcd |
| B30     | N0       | 1.64 h | 5.81 i | 0.54 f | 2.06 h | 1.09 h | 3.76 h |
|         | N150     | 8.11 efg | 25.26 fg | 3.41 de | 8.97 ef | 4.70 efg | 16.29 ef |
|         | N300     | 11.44 bc | 38.66 b | 4.77 bc | 14.00 b | 6.67 bc | 24.66 b |
|         | N450     | 15.43 a | 46.78 a | 6.72 a | 17.04 a | 8.71 a | 29.74 a |
| B60     | N0       | 1.50 h | 5.44 i | 0.53 f | 1.79 h | 0.98 h | 3.65 h |
|         | N150     | 7.10 fg | 18.66 h | 3.21 e | 6.50 g | 3.89 fg | 12.16 g |
|         | N300     | 9.60 cde | 32.14 cd | 4.46 bcd | 11.55 cd | 5.14 cdef | 20.59 cd |
|         | N450     | 12.65 b | 35.10 bc | 5.66 ab | 12.66 bc | 6.99 b | 22.44 bc |

Different letters within the columns show a significant difference between the treatments at $p = 0.05$ according to LSD test, and asterisks represent a significant difference at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ level; ns: not-significant.

Abbreviations: PIS, panicle initiation stage; TS, tillering stage.
| Biochar | Nitrogen | PH (cm) | FBP (g) | DBP (g) | SL (cm) | SWP (g) | GWP (g) | 100 GW |
|---------|----------|---------|---------|---------|---------|---------|---------|--------|
| B0      | N0       | 71.00 f | 18.76 g | 5.10 g  | 20.33 de| 21.84 k | 18.85 k | 2.19 efg|
| N150    | 81.67 e  | 69.63 f | 24.99 f | 20.00 e | 30.71 gh| 28.82 gh| 2.15 g  |
| N300    | 86.00 d  | 130.22 e| 35.48 de| 20.00 e | 35.86 def| 34.05 def| 2.23 defg|
| N450    | 81.00 e  | 126.92 e| 33.89 def| 20.33 de| 32.85 fg | 30.97 fg | 2.25 cdefg|
| N150    | 82.67 de | 154.47 cd| 40.19 cde| 22.00 abcd| 36.71 de | 34.76 de | 2.40 abc|
| N300    | 94.00 c  | 159.03 cd| 43.92 cd | 22.67 ab | 37.97 cde | 36.07 cde| 2.43 ab  |
| N450    | 95.00 c  | 167.29 c| 49.62 c  | 22.67 ab | 39.02 bcd| 37.18 bcd| 2.47 a  |
| B30     | N0       | 70.00 f | 28.67 g | 9.68 g  | 21.67 bcde| 26.81 ij | 23.48 ij | 2.23 defg|
| N150    | 82.33 e  | 143.01 de| 39.34 de | 22.00 abcd| 34.68 ef | 32.73 ef | 2.34 abcd|
| N300    | 98.67 b  | 222.53 a| 72.34 b  | 22.33 abc | 40.98 abc| 39.15 abc| 2.46 ab  |
| N450    | 105.67 a | 241.84 a| 83.98 a  | 23.67 a  | 44.23 a  | 42.35 a  | 2.47 a  |
| B60     | N0       | 68.67 f | 26.67 g | 8.13 g  | 20.33 de | 23.27 jk | 20.76 jk | 2.18 fg |
| N150    | 84.00 de | 76.38 f | 31.79 ef | 20.33 de | 27.98 hi | 26.27 hi | 2.20 defg|
| N300    | 94.67 c  | 156.61 cd| 39.22 de | 21.00 bcde| 38.27 bcde| 36.43 cd | 2.32 bcdef|
| N450    | 97.33 bc | 189.70 b| 69.14 b  | 20.67 cde| 41.78 ab | 39.87 ab | 2.35 abcd|

F values and significance level

|                      | Biochar | Nitrogen | Biochar × Nitrogen |
|----------------------|---------|----------|--------------------|
|                      | 35.60***| 38.22*** | 156.81***          |
|                      | 379.64***| 402.67***| 133.29***          |
|                      | 18.88***| 14.58*** | 7.81***            |
|                      | 10.10** | 28.76*** | 32.71***           |
|                      | 2.53 ns  | 112.92***| 149.40***          |
|                      | 0.90 ns  | 3.68**   | 4.14**             |
|                      | 9.60*    | 10.02*** | 0.85 ns            |

Different letters within the columns show a significant difference between the treatments at p = 0.05 according to LSD test, and asterisks represent a significant difference at *p < 0.05, **p < 0.01, and ***p < 0.001 level; ns: not-significant.

Abbreviations: 100 GW, hundred-grain weight; DBP, dry biomass plant\(^{-1}\); ET, effective tillers; FBP, fresh biomass plant\(^{-1}\); GWP, grain weight plant\(^{-1}\); PH, plant height; SL, spike length; SWP, spike weight plant\(^{-1}\); TT, total tellers.

**FIGURE 9** Rice representative phenotypes of each treatment
growth. Biochar porous structure and single condensed aromatic carbon rings can adsorb the surplus nitrogen in the soil (Baronti et al., 2014) to help in alleviating the adverse effect of high nitrogen concentration in the soil and improve the physiological status and growth of rice plants by slowly releasing the absorbed nitrogen to continue the nitrogen supply in the later growth stage of rice.

Regarding the alleviation of nitrogen adversity, the effect of biochar at 30 t ha⁻¹ was much stronger and synergistic to restore and improve the leaf ultrastructure than other biochar levels (Figures 2 and 3). High application of biochar can also affect plants growth negatively due to the toxic effects of a huge amount of salts and metals present in biochar (Mukherjee & Lal, 2014). Moreover, the enhancement in rice physiological traits and leaf ultrastructure due to biochar addition could be accredited to the presence of various nutrients such as N, P, K, Ca, and Mg in biochar, which participates in various growth and developmental processes and especially K regulates the osmotic potential in plants (Laird et al., 2010; Walter & Rao, 2015). The possible mechanism for this improved rice growth could be the liming effect and high pH of biochar which improved the availability and uptake of P and N and reduced the concentration of elements such as Al³⁺ and Mn²⁺ which are harmful to plant growth (Jeffery et al., 2011).

Plant physiological status depends on better nutrients and water uptake from growing media, and improved nutrients utilization efficiency could enhance plant growth and yield. A single application of high nitrogen and high biochar negatively affected the rice plant physiological status; however, the application of biochar in combination with nitrogen significantly elevated the contents of chlorophyll (Figures 4 and 5), soluble sugar and crude protein contents (Figure 6), and rates of photosynthetic processes (Figures 7 and 8). Chlorophyll exists inside the chloroplast, which is the machinery of photosynthesis; however, the high nitrogen treatment negatively affected the chloroplast and cell structure (Figure 2), which might have decreased the chlorophyll content. Soluble sugars are the primary products of photosynthesis and building blocks of macromolecules that control the growth and development of higher plants (Gibson, 2000). Sucrose is the main product of photosynthesis (Zhu et al., 2019); we assumed that biochar, being a rich source of carbon, could increase carbon assimilation during photosynthesis to enhance the production of sugar and provide sufficient carbohydrates to regulates plant metabolism, which can enhance plant growth and yield. The porous structure and high surface area of biochar particles (Sial et al., 2019) adhered to more moisture, causing an increase in water-holding capacity of soil and availability of water to roots for plant uptake that resulted in high stomatal conductance and photosynthesis to produce more hydrated plants as previously reported. The possible mechanism could be that biochar application enhanced plant growth by promoting soil water and nutrients availability and electron transport rate in photosystem II to decrease stomatal injury and increase photosynthesis rate (Haider et al., 2015).

The enhanced or reduced growth and development of a plant reflected the physiological status of a plant treated with applied biochar and nitrogen fertilizer either alone or in combination. Biochar integration can also increase the soil pH, EC, and SOC, enhancing the soil quality and physical structure, and availability of nutrients from coupled inorganic applied fertilizer, thereby improving crop growth and yield (Khan et al., 2020; Liu et al., 2021; Van Zwieten et al., 2010). However, the rice growth and yield were not enhanced and were adversely affected when the biochar level was increased from B30 to B60 (Tables 4, 5 and 6). Furthermore, compared to control, plant height, tillers number, and leaf area were significantly enhanced under the combined application of biochar and nitrogen fertilizer than the single application of either biochar or nitrogen fertilizer, which might be due to high and increased nutrients availability (Table 4). Generally, the availability and uptake of nitrogen greatly influence the aboveground biomass of plants by promoting tillering before stem extension, and the stored nitrogen in plant biomass redistribute from leaves and stem for grain protein formation during the grain filling stage (Abbruzzini et al., 2019; Hgca, 2008; Ullah et al., 2021). The possible mechanism for this might be due to the high binding forces in soil with high biochar concentration that decreased the

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**Figure 10** Daily average temperature (°C) and rainfall (mm) during rice growth (2020)
availability of the nutrients and increased the salt concentration due to its liming effect. Excessive application of biochar can also increase the high volatile matter in the soil, which can decrease the availability and uptake of nitrogen and thereby adversely affecting plant development and yield (Deenik et al., 2010). Similar results of reduced plant growth in high biochar treatment in our study are in line with the previous study (Abideen et al., 2020).

Rice plants treated with biochar in our trial were less affected by high nitrogen, that is, green, denser, and tall compared to sole application of high nitrogen N450 (Figure 9). Previous studies have observed that combined application of biochar and inorganic fertilizer has high nutrients supply than a single application of biochar (Fischer & Glaser, 2012). The mutual stimulating effect of biochar on rice growth, likely due to the addition of free basses such as K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) to the soil by the ash content of biochar that provides nutrients to plant in readily available form (Lehmann et al., 2003). Shi et al. (2012) observed that nitrogen utilization efficiency and grain yield were decreased with increasing the nitrogen application rate. Under this condition, it is possible that surplus nitrogen available from applied inorganic fertilizer increased the nitrogen uptake without increasing the rice biomass and yield and consequently decreased the NUE. Considering the previous studies and our findings of high N content and NUE in plants treated with biochar in combination with nitrogen emphasizes the synergistic and important role of biochar that enhanced the rice physiology, growth, and yield contents.

5 | CONCLUSIONS

In the current study, we demonstrated the synergetic effect of biochar on nutrients availability, nitrogen use efficiency, morphophysiological, and yield traits of rice. The findings of our research revealed new information regarding the ameliorative effect of biochar that decreased the high nitrogen toxicity by direct absorption and improved the nitrogen deficiency by a gradual release of extra nitrogen, and improved the cell injury of rice leaf caused by excessive nitrogen fertilizer application. The biochar application resulted in increments in soil chemical properties, reflecting the positive role of biochar due to its high surface area, high pH, and liming effect. The morphophysiological rice traits were improved, and plant nitrogen content was increased in the combined application of B30+N450. At the same time, TNA and NUE dynamics were higher in the B30+N300 treatment, which shows that improved nitrogen utilization can be achieved with the combined application of less nitrogen and biochar to attain better growth and yield. Thus, combining biochar and nitrogen fertilizer can decrease nitrogen toxicity, deficiency, and fertilizer input through increasing NUE, improving the soil properties and simultaneously improving crop productivity on a long-term sustainable basis.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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