An approach to sustainable agriculture by untangling the fate of contrasting nitrogen sources in double-season rice grown with and without biochar

Saif Ullah | Izhar Ali | He Liang | Quan Zhao | Shanqing Wei
Ihsan Muhammad | Min Huang | Amanullah | Nawab Ali | Ligeng Jiang

Abstract
Excessive use of inorganic nitrogen (N) fertilizers is the primary anthropogenic cause of low N use efficiency and environmental damage in wetland rice agriculture. However, little is known about the performance of traditional inorganic N sources used in paddy rice production. Biochar (BC) is considered to be a climate change mitigation tool that can enhance N uptake and utilization in N-fertilized crops. To test this hypothesis, we performed a pot experiment to study the fate of 15N-labeled urea, ammonium nitrate, and ammonium sulfate with and without BC at tillering, heading, and maturity stages of rice in the early and late seasons of 2019. Fertilizer N leaching was significantly reduced by 75.69% and 110.32% in BC vs. non-BC treatments across growth stages in the early and late seasons. The rate of leaching was lower for urea than for ammonium nitrate and ammonium sulfate. Furthermore, the addition of BC resulted in 55.58% and 41.33% higher soil 15N concentrations in the early and late season, respectively, indicating that BC increased N adsorption. 15N uptake by roots, stems, leaves, panicles, and grains averaged 52.39%, 37.14%, 40.86%, 36.37%, and 29.94% higher in BC-amended pots than in BC-free pots in both seasons. There were significant differences (p < 0.05) among N sources in terms of fertilizer N loss, residual N, and N uptake, and performance was ranked in the order urea > ammonium sulfate > ammonium nitrate. Overall, our results indicate that urea with BC is a preferable N source for double rice cropping systems compared with ammonium nitrate and ammonium sulfate.

1 Key Laboratory of Crop Cultivation and Farming System, Guangxi University, Nanning, China
2 College of Urban and Environmental Science, NorthWest University, Xian, China
3 Southern Regional Collaborative Innovation Center for Grain and Oil Crops (CICGO), Hunan Agricultural University, Changsha, China
4 Department of Agronomy, The University of Agriculture Peshawar, Peshawar, Pakistan

Correspondence
Ligeng Jiang, Key Laboratory of Crop Cultivation and Farming System, Guangxi University, Nanning 530004, China.
Email: jiang@gxu.edu.cn

Funding information
This research was financially supported by the National Key Research and Development Project of China (Grant No. 2018YFD20030503).

1 INTRODUCTION

Rice is a fundamental staple food in the diet of more than three billion people worldwide (Van Nguyen & Ferrero, 2006; Xuan et al., 2020). China ranks as the second largest grower of rice and accounts for 19% of the global rice cultivation area; it has the highest rice production in the world, contributing 28% of global rice production (Chauhan et al., 2017).
It is predicted that yield production must increase by 60% relative to current levels by 2025 to satisfy the requirements of a rapidly growing world population. Since the 1980s, synthetic fertilizers have been applied to cereal crops, including rice, to meet increasing global production demands. The over-application of nitrogen (N) to rice fields to drive high yields exceeds the crop's N requirement, causing low N use efficiency (NUE) and high levels of nitrate (NO₃) leaching, ammonia (NH₃) volatilization, and nitrous oxide (N₂O) emission (Dong et al., 2015; Ju et al., 2009). N, a major limiting nutrient for agricultural crops, is needed by rice in amounts greater than those required for any other nutrient (Gamage et al., 2016). The excessive application of N fertilizers during rice production is justified on the basis of maximizing productivity and profit, but high N application rates are not only exorbitantly expensive but they can also harm the environment (Lal, 2015). Therefore, to promote sustainable agriculture, the use of synthetic fertilizer should be evaluated from the standpoint of food production and climate change alleviation.

Biochar (BC) is a natural soil amendment whose use has potential advantages for both climate and agriculture. The addition of BC to croplands has been recommended as a promising approach for reducing N losses to the air and water (Najar et al., 2015). It has the potential in mitigation of greenhouse gases (GHGs) and is useful for carbon sequestration (Vaccari et al., 2011). Multiple studies have demonstrated that the use of BC can be very effective in enhancing soil organic carbon, improving soil water holding capacity (Jaafar, 2014), stimulating soil microorganisms, increasing microbial movement and biomass (Xu et al., 2014), reducing input needs and leaching losses, boosting the availability and adsorption of nutrients (Khan et al., 2020), and improving soil aeration (Laird, 2008). The use of BC together with synthetic fertilizer to enrich soil physical and chemical properties and promote sustainable rice production is therefore a promising avenue for research.

Nitrogen is the key nutrient for rice production; however, excessive application of inorganic fertilizer reduces nitrogen use efficiency and damages the environment. The fate of synthetic N fertilizer initially follows three routes: crop N uptake, retention in the soil, and losses due to leaching and volatilization (Zhu, 2008). All of these routes are interdependent and connected. After the addition of N as fertilizer, to find out the concentration of N that comes from the mineralization and that comes from the fertilizer, isotopically labeled materials are used as tracers (e.g., nitrogen fertilizers artificially enriched in ¹⁵N) (Mondol et al., 2014). Two stable isotopes of N are present naturally in the atmospheric N₂, ¹⁴N is present in an abundance of 99.6337 atom % and ¹⁵N in an abundance of 0.3663 atom %. The advantage of using stable isotopes is that they are almost identical to the more abundant isotopes in chemical and behavioral characteristics. Stable isotopes are consequently a powerful tool in explicating nitrogen cycle in agriculture.

Assessment of N fate is essential for the development of optimal N management strategies that support crop production while ensuring that the environment is protected (Zhu & Chen, 2002). To date, there have been few studies on different traditional nitrogen fertilizers such as urea (U), ammonium nitrate (AN), and ammonium sulfate (AS) and their interactive effects with BC on plant N uptake, residual N, and N leaching. To our knowledge, this is the first comprehensive study to investigate these phenomena at different growth stages to fill this gap in our understanding of paddy rice cultivation. Here, we evaluated the effectiveness of U, AN, and AS as N sources in paddy rice culture and compared fertilizer recovery, retention in soil, and leaching losses for each source with and without the addition of BC.

2 | MATERIALS AND METHODS

2.1 | Crop management and experimental design

Greenhouse pot experiments were set up in the early (March–June) and late (August–November) seasons of 2019 at an experimental farm of Guangxi University located at 22°49′20″N 108°17′04″E and an altitude of 75 m. The soil used for pot culture was an ultisol type collected from the paddy field, and its basic physical and chemical properties were as follows: moisture content 11.24%, bulk density 1.32 g cm⁻³, pH 5.92, total N 1.64 g kg⁻¹, total phosphorus (P) 0.62 g kg⁻¹, total potassium (K) 11.24 g kg⁻¹, available N 132.31 mg kg⁻¹, available P 23.15 mg kg⁻¹, available K 124.35 mg kg⁻¹, and carbon to nitrogen ratio (C:N) 6.53. The rice cultivar Zhenguiai was used as the test crop, and three tillers per hill of uniformly sized seedlings were transplanted to each pot on 22 March in early season and 15 August in late season. The experiment included six treatments: T1, ¹⁵N-urea; T2, ¹⁵N-urea +BC; T3, ¹⁵N-ammonium nitrate; T4, ¹⁵N-ammonium nitrate +BC; T5, ammonium sulfate; and T6, ammonium sulfate +BC. All six treatments were replicated nine times in 54 pots in a completely randomized design (CRD).

The experimental pots were made of polyvinyl chloride (PVC) with an inner diameter of 30 cm, a height of 50 cm, and a cumulative surface area of 706 cm². All ¹⁵N-labeled N fertilizers were applied at the rate of 315 kg ha⁻¹ split across three applications (5:3:2) as a basal dose and at the tillering and panicle initiation stages. A detailed description of ¹⁵N enrichment, application rate, and timing for each N source is given in Table 1. Additionally, BC was applied at a rate of 211.8 g per
pot (equivalent to 30 t ha\(^{-1}\)) 10 days before transplant in both the early and late seasons. The BC was derived from cassava straw according to the method described by Ullah et al. (2020), and its nutrient contents were as follows: pH 8.83, carbon 674.00 g kg\(^{-1}\), N 5.43 g kg\(^{-1}\), P 46.33 g kg\(^{-1}\), K 48.33 g kg\(^{-1}\), sulfur 2.39 g kg\(^{-1}\), hydrogen 3.81 g kg\(^{-1}\), and C:N ratio 124.12. The specific surface area was 2.46 m\(^2\) g\(^{-1}\), and the average carbon pore diameter was 3.37 nm. Each pot received 3.17 g P (90 kg ha\(^{-1}\)) as a basal dose and 1.57 g K (134 kg ha\(^{-1}\)) in split applications (6:4) as a basal dose and at the tillering stage.

To examine leaching concentrations, each pot was set up in a pierced box, and a net cloth was placed above the holes in the box to prevent soil loss. The pots were first filled with 12 kg sand to evade net blockage, and the remaining volume was filled with 32 kg soil up to 45 cm. The soil had been air-dried, pulverized, and compacted to ensure that the soil in the pots was consistent with that in the field. The circular gap between the lower box and the PVC pipe was filled with clay to prevent water leakage. Each pot was linked by a plastic pipe with stopcock to the leachate collection bottle (see Appendix S1). All pots were uniformly irrigated twice a day with tap water, once in the morning and once in the evening, to maintain consistent moisture levels. Throughout the experiment, standard agronomic practices were applied similarly to all treatments.

### 2.2 Sampling, analysis, and calculations

Soil and plant materials were collected at tillering, heading, and maturity. At each growth stage, three replicates per treatment were destructively harvested for a total of 18 pots. Whereas, \(^{15}\text{N}\) concentrations in the leachate were measured by collecting 1 L subsamples from the 10 L leachate collection bottles once a week till their representative stages (tillering, heading, and maturity); samples were taken to the laboratory and stored at 4°C. The collected subsamples were mixed together at the end of each growth stage, filtered through a 5 μm filter, and poured into a 500 ml glass beaker to which 5 ml of sulfuric acid (98%, W/W) was added. The beakers were placed in a high temperature blast oven at 85°C until all but 25 ml of the water had evaporated. The 25 ml samples were then transferred to 75 ml glass triangle bottles. The entire process of leachate volume reduction by evaporation took approximately 7 days.

Soil samples were collected from the 30 cm layer in each pot at five randomly selected points to measure \(^{15}\text{N}\) retention. Visible root fragments were hand-picked from each soil sample, and the samples were air-dried and sieved (<1 mm). All plants from each pot were separated into roots, stems, leaves, and panicles at each growth stage. At the maturity stage, panicles were hand threshed and the grains were collected. The plant samples were dried to constant weight in a 70°C oven for approximately 48 h. The oven-dried plant samples and air-dried soil samples were ground to a fine powder. A mass spectrometer (Delta V Advantage IRMS, Thermo-Fisher) was used to measure \(^{15}\text{N}\) in soil and plant samples, and a stable isotope ratio mass spectrometer (Isoprime100, Elementar Analysensysteme GmbH) was used to measure \(^{15}\text{N}\) in the water samples. The amount of \(^{15}\text{N}\) was calculated according to the method of Huang et al. (2014) and Junk and Svec (1958): \(\text{total N uptake} \times \frac{\text{total } {^{15}\text{N}} \text{ abundance in samples}}{\text{total } {^{15}\text{N}} \text{ abundance in fertilizer}}\).

All data were analyzed by analysis of variance (Statistix 8.0, Analytical Software), and significant differences among means were assessed by the least significant difference (LSD) test at the \((p < 0.05)\) probability level. SigmaPlot 12.0 was used to create figures, and MS Excel was used for tables.

### 3 RESULTS

#### 3.1 \(^{15}\text{N}\) leaching

Fertilizer \(^{15}\text{N}\) leaching was significantly reduced \((p < 0.05)\) in BC-applied treatments at each growth stage in both seasons, with the exception of the heading stage in the early season (Table 2).
BC application improved soil $^{15}$N retention similarly in both seasons. Averaging both seasons across the growth stages, $^{15}$N leaching was 27.14 mg per pot for urea fertilizer with BC (T2) compared with 53.51 mg per pot for urea alone (T1). In the early season, the addition of BC (T2, T4, and T6) was associated with leaching reductions of 93.09%, 59.56%, and 73.41% compared with the corresponding BC-free fertilizer treatments (T1, T3, and T5). Likewise, in the late season, treatments with fertilizer alone exhibited greater $^{15}$N leaching at all growth stages than treatments with added BC. The highest $^{15}$N leaching was observed at the heading and maturity stages. Among the BC treatments, the lowest $^{15}$N leaching, an average of 22.34 mg per pot, was recorded in T6 (BC + AS). Similarly, among BC-free treatments, the lowest $^{15}$N leaching (45.87 mg per pot) was recorded in T5 (AS alone) across growth stages. The relative amounts of leaching among the BC treatments (T2, T4, and T6) were similar at all growth stages, and there were no significant differences among BC treatments with the exception of T2 and T4 at the late season heading stage. Treatments T2, T4, and T6 reduced $^{15}$N leaching by an average of 106.37%, 117.65%, and 106.95% compared with T1, T3, and T5, respectively.

3.2 | $^{15}$N retention in soil

Residual fertilizer nitrogen in the soil decreased progressively with growth stage in both the early and late seasons (Table 3). Soil nitrogen retention was consistently higher in BC treatments than in fertilizer-only treatments, and this difference was significant for a number of treatments and growth stages in each season. The order of $^{15}$N retention was T2 > T6 > T4 > T1 > T5 > T3 in the early season and T2 > T6 > T4 > T5 > T1 > T3 in the late season. In the early season, the effect of BC on $^{15}$N retention was not significant at the maturity stage, but at the tillering and heading stages, $^{15}$N retention was significantly higher in response to BC addition. The BC + U treatment (T2) exhibited the highest $^{15}$N retention (0.65 g per pot), which was 68.26% higher than that observed for U alone (T1). There were no significant differences between T2 and T4 or among T1, T3, and T5 ($p < 0.05$). In the late season, soil $^{15}$N retention was again highest in the BC treatments. Across the seasons and growth stages, $^{15}$N retention averaged 0.50 g per pot for T2 (BC + U), 0.48 g per pot for T6 (BC + AS), and 0.43 g per pot for T4 (BC + AN). Compared with the fertilizer-only treatments (T1, T3, and T5), the corresponding BC treatments (T2, T4, and T6) exhibited 55.81%, 37.49%, and 30.44% greater N retention across all growth stages, respectively. Moreover, $^{15}$N concentration did not differ significantly among the BC or BC-free treatments.

3.3 | $^{15}$N concentration in roots

The effects of fertilizer source and BC addition on $^{15}$N concentrations in roots are shown in Figure 1. The addition of BC significantly increased root $^{15}$N at most growth stages in both seasons. The effect of BC addition varied among fertilizer sources. Averaged across both seasons, the highest root $^{15}$N concentration (0.10 g per pot) was observed in T6 (BC + AS), and this value was not significantly different from that of T2 (BC + U). The lowest $^{15}$N concentrations were observed in the fertilizer-only treatments. Compared with the fertilizer-only treatments (T1, T3, and T5), the corresponding BC treatments (T2, T4, and T6) showed moderate increases in root $^{15}$N concentration of 57.43%, 36.01%, and 60.84% in the early season and 58.78%, 45.26%, and 56.04% in the late season. The variation among treatments was similar in both seasons. Nitrogen fertilizer source had no significant ($p < 0.05$) effect on $^{15}$N concentrations with or without BC application.

| SOS | Early season | Late season |
|-----|--------------|-------------|
| GS  | Tillering | Heading | Maturity | Tillering | Heading | Maturity |
| N leached (mg per pot) | N leached (mg per pot) | N leached (mg per pot) |
| T1  | 39.96 ab | 85.83 a | 55.69 abc | 35.01 a | 50.55 a | 54.05 ab |
| T2  | 23.21 c  | 44.77 a | 25.85 c  | 17.21 b | 21.09 c | 30.69 bc |
| T3  | 47.47 a  | 67.48 a | 65.56 ab | 38.51 a | 51.79 a | 72.62 a |
| T4  | 26.98 bc | 51.15 a  | 38.37 bc | 18.29 b | 29.57 b | 27.16 c |
| T5  | 50.60 a  | 62.95 a  | 71.39 a  | 37.75 a | 45.75 a | 54.11 ab |
| T6  | 29.69 bc | 42.61 a  | 34.81 bc | 17.68 b | 25.85 bc | 23.48 c |

Note: T1: $^{15}$N-urea 315 kg ha$^{-1}$, T2: $^{15}$N-urea 315 kg ha$^{-1}$ + BC 30 t ha$^{-1}$, T3: $^{15}$N-ammonium nitrate 315 kg ha$^{-1}$, T4: $^{15}$N-ammonium nitrate 315 kg ha$^{-1}$ + BC 30 t ha$^{-1}$, T5: $^{15}$N-ammonium sulfate 315 kg ha$^{-1}$, T6: $^{15}$N-ammonium sulfate 315 kg ha$^{-1}$ + BC 30 t ha$^{-1}$. Within a column, values followed by different letters are significantly different at $p < 0.05$.

Abbreviations: GS, growth stage; SOS, sowing season; Treat, treatments.
Differences in stem $^{15}$N concentrations at different growth stages and seasons were similar to those observed in roots (Figure 2). Regardless of N source, BC amendment significantly enhanced stem $^{15}$N concentrations at maturity in the early season. By contrast, BC amendment significantly increased ($p < 0.05$) stem $^{15}$N concentrations at all

### 3.4 $^{15}$N concentrations in stems

Differences in stem $^{15}$N concentrations at different growth stages and seasons were similar to those observed in roots.
growth stages in the late season. Compared with the una-
mended treatments (T1, T3, and T5), stem $^{15}$N concentra-
tions averaged 30.59%, 42.93%, and 39.45% higher in the
BC-amended treatments in the early season. Likewise, late
season application of N fertilizers with BC enhanced stem
$^{15}$N concentrations by an average of 34.31%, 27.18%, and
48.41% in T2, T4, and T6, respectively. Averaged across
both seasons, the greatest stem $^{15}$N was observed when BC
was applied with AS (0.37 g per pot), and the lowest was
observed for AN alone (0.22 g per pot). There were signifi-
cant differences ($p < 0.05$) between the BC treatments (T2
and T4) at maturity stage or between the BC-free treatments
(T1 and T3) at heading stage. The total stem $^{15}$N concentra-
tions in fertilizer-only treatments for all growth stages in
both seasons were lower than those in BC treatments. The
average $^{15}$N concentration (g per pot) in rice stems followed
the order T6 (0.37 g per pot) > T2 (0.34 g per pot) > T4
(0.29 g per pot) > T1 (0.26 g per pot) > T5 (0.26 g per
pot) > T3 (0.22 g per pot).

3.5 $^{15}$N concentration in leaves

$^{15}$N concentrations in rice leaves of the BC-amended ferti-
lizer treatments were clearly higher than those of the ferti-
lizer-only treatments (Figure 3). No significant differences
were observed among the fertilizer sources applied without
biochar during the entire growth period in both early and
late seasons ($p < 0.05$). However, there was a significant
difference between N sources applied with BC. In both sea-
sons, similar trend in leaf $^{15}$N concentrations was observed
with all N sources, and substantial decreases were recorded
from the tillering to the maturity stage. In the early season,
the averaged $^{15}$N concentration in the BC treatments at till-
ering and maturity was 0.66 and 0.23 g per pot, compared
with 0.38 and 0.15 g per pot in the BC-free treatments. The
averaged maximum $^{15}$N concentration was recorded
in urea applied with BC (0.63 g per pot). Among the ferti-
lizer-only treatments, AS (T5) showed the highest leaf $^{15}$N
concentration (0.36 g per pot). The BC treatments T2, T4,
and T6 had greater leaf $^{15}$N concentrations by an average
of 75.02%, 62.71%, and 68.41% compared with their cor-
responding BC-free treatments. Moreover, in the late sea-
son, a similar trend was observed for $^{15}$N concentrations.
AS + BC (T6), exhibited the highest average concentration
of 0.63 g per pot, and AS alone (T5) exhibited 0.37 g per
pot. However, T6 (BC + AS) did not differ significantly
from T2 (BC + U). The lowest leaf $^{15}$N concentrations were
observed for ammonium nitrate application with or without
BC. BC-amended treatments (T2, T4, and T6) demonstrated
markedly higher $^{15}$N concentrations by 56.23%, 42.41%, and
62.97% compared with their corresponding BC-free treat-
ments across the growth stages.

3.6 $^{15}$N concentrations in panicles

Panicle $^{15}$N concentration at the heading stage was signifi-
cantly ($p < 0.05$) affected by the interaction of BC with N
sources (Figure 4a). Consistent with the higher $^{15}$N uptake
by stems and leaves in the BC-amended treatments, the
$^{15}$N uptake of rice panicles in the early season was 44.95%,
24.03%, and 22.83% higher in T2, T4, and T6 than in their
corresponding BC-free treatments. The highest $^{15}$N concen-
tration was observed in BC + U (T2, 0.22 g per pot) and did
not differ significantly from that observed in T6. Among the
BC-amended treatments, BC + AN (T4) showed the lowest
panicle $^{15}$N concentration, and AN alone (T3) also produced
the lowest $^{15}$N concentration among the BC-free treatments
(0.12 g per pot). Results from the late season were similar,
and there was a clear difference in panicle $^{15}$N concentra-
tion between BC-amended and BC-free treatments. $^{15}$N
concentrations were 32.64%, 40.21%, and 53.58% higher in treatments T2, T4, and T6 than in treatments T1, T3, and T5, respectively. U alone (T1) produced the highest 15N panicle concentrations (0.17 g per pot) among the BC-free treatments, and BC + U (T2) produced the highest concentrations among the BC-amended treatments (0.22 g per pot). There were no significant differences among T2, T4, and T6. 15N accumulation in rice panicles was ranked in the order T2 > T6 > T4 > T1 > T5 > T3.

3.7 15N concentration in grains

BC amendment of N fertilizers significantly increased (p < 0.05) grain 15N concentrations in the early and late seasons (Figure 4b). The addition of BC to N sources uniformly increased 15N concentrations, although significant differences were recorded in T2 and T4 in early season. In both the early and late seasons, 15N concentrations were highest (0.59 and 0.61 g per pot) in BC + U (T2) and lowest (0.38 and 0.40 g per pot) in AN (T3). The BC treatments (T2, T4, and T6) resulted in 24.84%, 14.28%, and 30.28% higher 15N concentrations in the early season and 38.42%, 34.61%, and 37.27% higher 15N concentrations in the late season compared with their corresponding BC-free treatments. On the basis of these results, the treatments were rated in the order T2 > T6 > T4 > T1 > T5 > T3.

4 DISCUSSION

4.1 N sources, BC, and 15N leaching

N loss from leaching is a major concern for maintaining good water quality. In organic and conventional paddy rice cultivation, N leaching losses constitute a tradeoff with rice yield. To achieve high yields, a relatively large amount of inorganic N must be provided, and some may be lost. In the current investigation, we measured the impacts of different N sources (15N-urea, 15N-ammonium nitrate, and 15N-ammonium sulfate) with or without BC on plant nitrogen fertilizer uptake and loss. The highest level of 15N leaching was observed when fertilizers were applied without BC amendment (Table 2). Treatments with BC markedly decreased 15N leaching by 93.01% across the seasons; urea with BC showed an average reduction of 99.03% in both seasons, the greatest reduction of any BC treatment. As reported by Widowati et al. (2014), BC can influence the leaching of N from litter by affecting water percolation, which is reduced as BC levels increase. BC application to soil can increase soil porosity (Ali et al., 2020). Leached nitrate joins the groundwater as water percolates through the soil column under gravity. The extent of N leaching from fertilizers differs depending on the soil media and the fertilizer application methods (Xing & Zhu, 2000). Therefore, the incorporation of organic amendments with N fertilizers is a sustainable strategy for improving nitrogen use efficiency in a double cropping rice system (Iqbal et al., 2019). In the current study, we observed the greatest amount of 15N leaching with ammonium nitrate fertilizer applied with or without BC. For example, Ullah et al. (2020) found that plant available forms of N (ammonium and nitrate) move through leaching. However, nitrate moves more readily due to its negative charge, whereas ammonium’s strong positive charge allows it to become adsorbed to negatively charged soil particles.

Ullah et al. (2020) reported that field incorporation of crop residues as an organic amendment after harvest can add a substantial amount of SOC to the soil, helping to retain NO₃⁻–N from fertilizer addition and thereby diminishing N leaching. Here, BC addition reduced N loss from all N
sources (urea, ammonium nitrate, and ammonium sulfate), and a significant decrease ($p < 0.05$) in $^{15}$N leaching from the pot soil was observed across the seasons. Nitrogen is the primary limiting nutrient for normal plant growth and development (Zebatn et al., 2009). BC soil amendments have been reported to adsorb, release, and/or stimulate N mineralization (Clough et al., 2013). The present study focused on soil–plant nitrogen dynamics with different N sources with or without BC addition and demonstrated that BC amendment significantly diminished nitrogen leaching across the seasons. These results are consistent with Zheng et al. (2013), who reported that BC addition enhanced soil $N_2$ fixation by 63%, decreased leaching by 26%, and relieved the risk of plant nitrogen deficiency. In the current study, the application of N fertilizers and BC at the basal and tillering stages met the requirements of young plants under leaching conditions and significantly retained fertilizer N in soil. Novak et al. (2009) reported that BC produced from pecan shells was able to reduce soil $NO_3^-$–$N$ leaching at 25 and 67 days. The findings of the present experiment are also consistent with those of Lehmann et al. (2003) and Steiner et al. (2007), who showed that BC promoted nutrient retention, particularly that of N in tropical soils that experience vigorous rainfall. The fertilizer N leaching results in Table 2 for each growth stage and treatment represent possible N pollution. The maximum values were observed for AN alone in both seasons, and the lowest values were observed for the BC-amended fertilizer treatments. The reduction in fertilizer N leaching, accomplished with the BC-amended fertilizers was 75.65% and 110.32% (vs. BC-free treatments) in the early and late seasons, respectively.

4.2 N sources, BC, and soil $^{15}$N retention

Conventional applied sources of N are readily lost from the root zone and percolate into the groundwater (Huang et al., 2015). Because organic amendments (e.g., biochar) increase N retention, they can diminish N losses and enhance NUE (Huang et al., 2014). In the current study, the amendment of N fertilizers with BC appeared to promote nutrient retention, and $^{15}$N concentrations were generally high in root zone soil during all growth periods in both seasons (Table 2). Specifically, soil $^{15}$N concentrations were 48.41% lower in BC-free treatments compared with BC-amended treatments (Table 3). This implies that N fertilizers and BC together can increase inorganic N in the rhizosphere to promote the growth of rice. Consistent with our findings, Nelissen et al. (2015) reported that BC application increased $NH_4^+$–$N$ substrate availability for autotrophic nitrifiers because of quicker mineralization rates, notwithstanding the stimulation of autotrophic nitrification in high pH microsites because of the high alkalinity of the BC. Overall, BC application to soil slowed the N cycle, implying that a large part of mineralized, and therefore accessible, N was consequently immobilized again, either as $NH_4^+$–$N$ or $NO_3^–$–$N$ (Nelissen et al., 2015). In this study, we observed the minimum $^{15}$N concentration in BC-free soil samples treated with ammonium nitrate in both seasons (Table 3). The probable reason was that much of the $^{15}$N from AN was leached into the water. Sole AN application therefore amplified the possibility of belowground water pollution. The majority of inorganic nitrogen in the soil is organically bound. Soil fertility is usually estimated by nitrogen concentration, and crop growth rate closely tracks the extent of available N (Spokas et al., 2012).

In the comprehensive analysis of all growth stages, N status was improved by BC addition to nitrogen fertilizers across both seasons, and soil $^{15}$N concentrations were higher in BC treatments than in BC-free treatments. Similar augmentation of soil nitrogen concentration due to BC addition was noted by Jing et al. (2011). Likewise, Laird et al. (2010) reported that BC amendment was one of the most significant soil fertility management techniques by increasing specific surface areas (up to 18%), higher cation exchange capacities (up to 20%), increasing pH values and significantly improved total N (up to 7%) relative to the un-amended controls. Here, we observed that soil $^{15}$N status changed consistently, decreasing from tillering to maturity in BC-free treatments and from heading to maturity in BC treatments. These outcomes are consistent with earlier studies in which inorganic N retention in the soil was improved by BC addition through its direct adsorption ability and or because of an indirect microbial immobilization effect (Cayuela et al., 2014; Ippolito et al., 2012). By contrast, treatments without BC experienced greater $^{15}$N leaching and volatilization, and their N concentrations differed substantially among fertilizer sources. The soil $^{15}$N concentration for the AN treatment was always lower in both seasons (Table 2). Some laboratory sorption studies have reported that BC has a weak potential to adsorb $NO_3^–$–$N$ but can mitigate $NO_3^–$–$N$ leaching when integrated as an amendment into the soil (Jin et al., 2016). For example, Yao et al. (2012) stated that only 4 out of 13 BCs could adsorb $NO_3^–$–$N$ and that two BCs applied to sandy soil decreased $NO_3^–$–$N$ leaching by 34–34.3%. The addition of BC to soils increases cation exchange and adsorption capacities (Hale et al., 2013), which would be expected to bind $NH_4^+$–$N$ directly. Lehmann et al. (2003) reported a significant reduction in $NH_4^+$–$N$ leaching in fertilized and unfertilized soil after BC addition. The capability of BC to engage in $NH_4^+$–$N$ and $NO_3^–$–$N$ adsorption or desorption, that is, its mineralization and immobilization influence on soil N, are two major processes by which it reduces N leaching. These processes may be affected by abiotic and microbial processes (Bai et al., 2015). In summary, BC
helped to alleviate inorganic N losses of urea, ammonium nitrate, and ammonium sulfate by 52.88%, 46.45%, and 45.91% compared with the application of the same fertilizers alone.

### 4.3 N sources, BC, and crop $^{15}$N uptake

In the present study, BC addition had a substantial effect on the fate of N sources across all growth stages in both seasons; however, no significant differences were found among N sources. The higher tissue $^{15}$N concentrations in BC treatments may be attributed to a promotion of dry matter production. These results are in line with the findings of Huang et al. (2014), who reported that BC amendment resulted in a 23%–27% increase in N derived from fertilizer in rice. Similarly, we found that joint application of BC and nitrogen fertilizers increased fertilizer N utilization by 52.39%, 37.14%, 40.86%, 36.37%, and 29.94% in roots, stems, leaves, panicles, and grains across both seasons compared with the corresponding BC-free controls (Figures 1–4). Based on our data, BC amendment increased total plant $^{15}$N uptake by 0.33% in the first season and 0.37% in the late season compared with BC-free controls. BC not only stimulated N utilization from fertilizer but also improved plant growth. This probably reflects the ability of BC to act as a soil improver, changing soil chemical and microbial properties and subsequently increasing uptake of soil nutrients that are unavailable to plants in low fertility soil (Shen et al., 2016; Van Zwieten et al., 2010). For specific nutrients, soil N may be mineralized into inorganic N when N supply is insufficient for plant needs or when the demand for BC induces microbial activity (Muhammad et al., 2016; Nelissen et al., 2012). Overall, BC amendment combined with different N sources clearly influenced plant N dynamics, as shown by marked improvements in plant $^{15}$N uptake and soil nitrogen status.

BC functions as a soil amendment when combined with inorganic N fertilizers, and several BCs have been reported to increase nutrient uptake, crop yield, and crop productivity (Yao et al., 2017). Furthermore, BC directly influences soil nutrient concentration. Although the pots used in this study were designed to undergo N leaching, plants nonetheless took up $^{15}$N in large amounts because of the ability of BC to retain nutrients. Results of the current investigation are consistent with those of Amin and Eissa (2017), who reported that BC may have a direct influence on nutrient supply, as well as several indirect effects, such as nutrient retention. Interestingly, plant N uptake in the current study was markedly enhanced by joint application of BC and N sources compared with N sources alone. However, each N source acted differently with or without BC, probably because of their different chemical characteristics. Moreover, a significant differences were observed among the N sources applied with or without BC ($p < 0.05$). For example, (i) the addition of BC to AS caused greater $^{15}$N uptake in roots, stems, and leaves, (ii) the addition of BC to U increased $^{15}$N uptake in panicles at the heading stage and in grains at maturity compared with AN and AS, and (iii) similar trend of $^{15}$N uptake was noted among BC-free N fertilizer applications. According to Jones et al. (2011), most N and P utilized for seed filling and grain establishment are derived from the stem, leaves, and panicles rather than from direct soil acquisition. In this study, nitrogen derived from fertilizers was greater in rice plants with BC amendment than those with only fertilizer application, showing that the plant obtains maximum inorganic N from soil treated with BC. Only one-third of nitrogen was derived from fertilizer sources; the rest was derived from the soil. Low acquisition of N from fertilizer and high dependence on soil nitrogen results in quick depletion of N, especially from applied fertilizer (Reddy & Patrick, 1980). This low N uptake efficiency from fertilizer can result in various environmental issues and economic losses. From an economic perspective, Raun and Johnson (1999) estimated that worldwide N loss from fertilizer sources in cereal production costs approximately $15.9 billion annually. Therefore, the addition of BC to N sources may increase nitrogen acquisition from fertilizer in plants as a result of its ability to retain N and reduce leaching losses, thereby potentially enriching the growth and yield of paddy rice.

### 5 CONCLUSION

The combined application of BC and N fertilizers, regardless of N source, markedly decreased fertilizer N leaching, increased soil $^{15}$N retention, and enhanced plant $^{15}$N uptake in rice across three growth stages in the early and late seasons. A significant improvement was observed in grain $^{15}$N content in response to BC amendment. Average fertilizer N concentration in pots that included BC as an organic amendment was higher than that in pots with only N fertilizers. Moreover, there were clear differences among N sources with and without BC. In terms of fertilizer N leaching, soil $^{15}$N retention, and final grain $^{15}$N concentration, urea was more efficient than the other two N sources. The efficacy of N sources was ranked as urea > ammonium sulfate > ammonium nitrate in both seasons. In summary, the application of BC with urea will be an effective strategy for enhancing fertilizer N uptake, reducing leaching, thereby minimizing environmental issues related to chemical N fertilizers in paddy rice.

### CONFLICT OF INTEREST

The authors declare that they have no known competing interests related to this work.
Ihsan Muhammad
Izhar Ali
Chauhan, B. S., Jabran, K., & Mahajan, G. (Eds.). (2017). Rice production worldwide (Vol. 191), 5–16. Springer International Publishing.

Cayuela, M. L., Van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., & Bai, S. H., Reverchon, F., Xu, C.-Y., Xu, Z., Blumfield, T. J., Zhao, Amin, A. E. E. E. A. Z., & Eissa, M. A. (2017). Biochar effects on nitrous oxide emissions: A review and meta-analysis. Agriculture, Ecosystems & Environment, 191, 5–16.

Chauhan, B. S., Jabran, K., & Mahajan, G. (Eds.). (2017). Rice production worldwide (Vol. 247). Springer International Publishing. https://doi.org/10.1007/978-3-319-47516-5

Clough, T. J., Condron, L. M., Kammann, C., & Müller, C. (2013). A review of biochar and soil nitrogen dynamics. Agronomy, 3(2), 275–293.

Dong, D., Feng, Q., Mcgrouther, K., Yang, M., Wang, H., & Wu, W. (2015). Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. Journal of Soils and Sediments, 15(1), 153–162.

Gamage, D. V., Mapa, R. B., Dharmakeerthi, R. S., & Biswas, A. (2016). Effect of rice-husk biochar on selected soil properties in tropical Alfisols. Soil Research, 54(3), 302–310.

Hale, S. E., Alling, V., Martinsen, V., Mulder, J., Breedveld, G. D., & Cornelissen, G. (2013). The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. Chemosphere, 91(11), 1612–1619.

Ju, X.-T., Xing, G.-X., Chen, X.-P., Zhang, S.-L., Zhang, L.-J., Liu, X.-J., Cui, Z.-L., Yin, B., Christie, P., Zhu, Z.-L., & Zhang, F.-S. (2009). Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proceedings of the National Academy of Sciences of the United States of America, 106(9), 3041–3046.

Junk, G., & Svec, H. J. (1958). The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. Geochimica et Cosmochimica Acta, 14(3), 234–243.

Khan, Z., Zhang, K., Khan, M. N., Fahad, S., Xu, Z., & Hu, L. (2020). Coupling of biochar with nitrogen supplements improve soil fertility, nitrogen utilization efficiency and rapeseed growth. Agronomy, 10(11), 1661.
fertility of a southeastern coastal plain soil. *Soil Science, 174*(2), 105–112.

Raun, W. R., & Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal, 91*(3), 357–363.

Reddy, K. R., & Patrick, W. H. (1980). Uptake of fertilizer nitrogen and soil nitrogen by rice using 15N-labelled nitrogen fertilizer. *Plant and Soil, 57*(2–3), 375–381.

Shen, Q., Hedley, M., Camps Arbestain, M., & Kirschbaum, M. U. F. (2016). Can biochar increase the bioavailability of phosphorus? *Journal of Soil Science and Plant Nutrition, 16*(2), 268–286.

Spokas, K. A., Novak, J. M., & Venterea, R. T. (2012). Biochar’s role as an alternative N-fertilizer: Ammonia capture. *Plant and Soil, 350*(1–2), 35–42.

Steiner, C., Teixeira, W. G., Lehmann, J., Nehls, T., de Macêdo, J. L., V., Blum, W. E., & Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil, 291*(1–2), 275–290.

Ullah, S., Liang, H., Ali, I., Zhao, Q., Iqbal, A., Wei, S., & Jiang, L. (2020). Biochar coupled with contrasting nitrogen sources mediated changes in carbon and nitrogen pools, microbial and enzymatic activity in paddy soil. *Journal of Saudi Chemical Society, 24*(11), 835–849.

Vaccari, F. P., Baronti, S., Lugato, E., Genesio, L., Castaldi, S., Fornasier, F., & Miglietta, F. (2011). Biochar as a strategy to sequester carbon and increase yield in durum wheat. *European Journal of Agronomy, 34*(4), 231–238.

Van Nguyen, N., & Ferrero, A. (2006). Meeting the challenges of global rice production. *Paddy and Water Environment, 4*, 1–9.

Van Zwieten, L., Kimber, S., Downie, A., Morris, S., Petty, S., Rust, J., & Chan, K. Y. (2010). A glasshouse study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Soil Research, 48*(7), 569–576.

Widowati, W., Asnah, A., & Utomo, W. H. (2014). The use of biochar to reduce nitrogen and potassium leaching from soil cultivated with maize. *Journal of Degraded and Mining Lands Management, 2*(1), 211.

Xing, G. X., & Zhu, Z. L. (2000). An assessment of N loss from agricultural fields to the environment in China. *Nutrient Cycling in Agroecosystems, 57*(1), 67–73.

Xu, H. J., Wang, X. H., Li, H., Yao, H. Y., Su, J. Q., & Zhu, Y. G. (2014). Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. *Environmental Science & Technology, 48*(16), 9391–9399.

Xuan, Y., Yi, Y., Liang, H. E., Wei, S., Chen, N., Jiang, L., Ali, I., Ullah, S., Wu, X., Cao, T., Zhao, Q., & Li, T. (2020). Amylose content and RVA profile characteristics of noodle rice under different conditions. *Agronomy Journal, 112*(1), 117–129.

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., & Wang, G. (2017). Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. *Soil Biology and Biochemistry, 110*, 56–67.

Yao, Y., Gao, B., Zhang, M., Inyang, M., & Zimmermann, A. R. (2012). Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere, 89*(11), 1467–1471.

Zebarth, B. J., Drury, C. F., Tremblay, N., & Cambouris, A. N. (2009). Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: A review. *Canadian Journal of Soil Science, 89*(2), 113–132.

Zheng, H., Wang, Z., Deng, X., Herbert, S., & Xing, B. (2013). Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma, 206*, 32–39.

Zhu, Z. L. (2008). Research on soil nitrogen in China. *Acta Pedologica Sinica, 45*(5), 778–783.

Zhu, Z. L., & Chen, D. L. (2002). Nitrogen fertilizer use in China—Contributions to food production, impacts on the environment and best management strategies. *Nutrient Cycling in Agroecosystems, 63*(2–3), 117–127.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Ullah S, Ali I, Liang H, et al. An approach to sustainable agriculture by untangling the fate of contrasting nitrogen sources in double-season rice grown with and without biochar. *GCB Bioenergy, 2021;13:382–392. https://doi.org/10.1111/gcbb.12789*