

Biochar Application Enhanced Post-Heading Radiation Use Efficiency in Field-Grown Rice (*Oryza sativa* L.)

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Abstract: It has been shown that adding biochar to soil can improve nitrogen (N) uptake and utilization in rice (*Oryza sativa* L.). However, there is a lack of research on the physiological alterations of rice as a result of the changes in nitrogen uptake due to the addition of biochar. This study conducted field experiments in 2015 and 2016 with the goal of testing the hypothesis that the application of biochar would enhance radiation use efficiency (RUE) of rice by improving the plant's ability to take in and utilize nitrogen. Our results demonstrated that the application of biochar (20 t ha^{-1}) induced no significant effects on pre-heading specific leaf weight (SLW), nitrogen uptake (NU_{pre}), and leaf area index (LAI) at heading, the ratios of LAI/NU_{pre} and SLW/NU_{pre}, or pre-heading RUE. However, biochar application significantly increased post-heading nitrogen uptake (NU_{post}), ratios of NU_{post}/SLW and NU_{post}/LAI, and post-heading RUE. These results indicate that the application of biochar can improve the plant's nitrogen uptake and RUE in field-grown rice during the post-heading period, which confirms our hypothesis.

Keywords: Biochar; nitrogen uptake; radiation use efficiency; rice

1 Introduction

Biochar is the solid, carbon-rich product resulting from the heating of biomass, while excluding air [1]. The application of biochar can both reduce greenhouse gas emissions [2] as well as induce crop growth by increasing the storage of water, improving nutrient supply, and suppressing disease while increasing beneficial microbial activity. Additionally, it has been hypothesized that biochar could be used as an effective addition to agricultural soil [3–5].

Rice (*Oryza sativa* L.) is a crop essential to the diets of over half the world's population [6]. A number of studies have examined the use of biochar during rice cultivation, demonstrating that it can improve nitrogen (N) uptake and/or utilization in rice [7–9]. However, there is a lack of available research regarding changes in plant N status induced by the application of biochar in rice.

N is not only a critical determinant of the growth and development of leaves and the main plant photosynthetic organs. It is also an essential building block for chlorophyll and photosynthesis-related enzymes. Therefore, a plant's N status is closely related to its photosynthetic capacity [10,11]. The



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Radiation Use Efficiency (RUE) is the volume of biomass that develops for each unit of solar radiation that is absorbed. RUE is an important metric that quantifies the photosynthetic capacity of crops grown under field conditions [12]. We hypothesize the application of biochar could enhance RUE in field-grown rice by improving the plant N status. To test this hypothesis, we conducted two years' worth of field studies in order to determine how the application of biochar affects N uptake, RUE, and leaf characteristics in rice.

2 Materials and Methods

2.1 Site and Soil

We conducted our field experiments in the town of Yongan (28°09'N, 113°37'E, 43 m asl), Hunan Province, China, during the rice-growing seasons of 2015 and 2016. The soil was comprised of clay, and the upper 20 cm possessed the following properties: organic matter = 42.6 g kg⁻¹, pH = 6.30, available N = 207 mg kg⁻¹, available P = 7.11 mg kg⁻¹, and available K = 81.1 mg kg⁻¹. The cumulative incident solar radiation during prior to and following the heading of the rice was 766 and 357 MJ m⁻² in 2015 and 828 and 479 MJ m⁻² in 2016, respectively (Fig. 1). The solar radiation was recorded using an automatic weather station (Vantage Pro2, Davis Instruments Corp., Hayward, CA, USA) positioned at the site of the experiment.

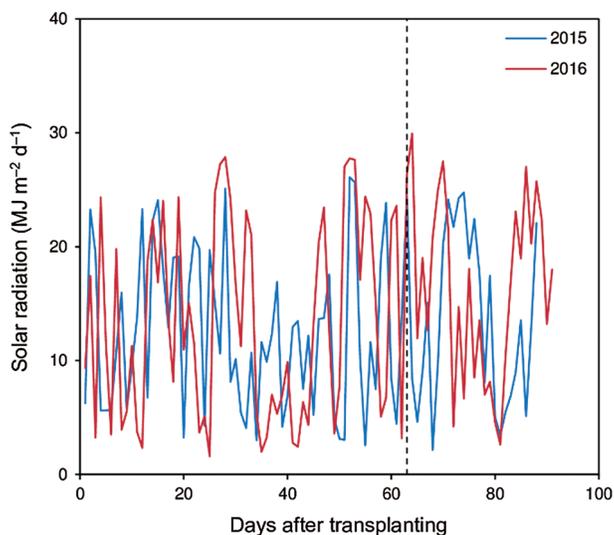


Figure 1: Daily incident solar radiation during the 2015 and 2016 rice-growing seasons. The vertical dashed line represents the heading stage

2.2 Plant and Treatments

Zhongzao 39, an inbred rice cultivar, were grown under two different levels of biochar application: 0 t ha⁻¹, C0, and 20 t ha⁻¹, C20. This cultivar was selected because it is widely used by farmers cultivating rice in the region of study. We obtained the biochar for the experiment from husks of rice that we placed in a fluidized bed reactor at 600°C for 3 h, inducing pyrolysis. The resulting biochar possessed the following properties: total N = 7.8 g kg⁻¹, total C = 512 g kg⁻¹, and pH = 10.4. The biochar rate of C20 was based on our previous work [13]. The experiment was arranged in a randomized block with three replications each. Each plot size was 20 m².

Pre-germinated seeds were sown in a seedbed to raise seedlings. On April 22 of both years, 25-day-old seedlings were transplanted, with a spacing of 20.0 cm × 16.7 cm between hills. Three seedlings were

transplanted per hill. The biochar was applied to the soil a day before the transplant. N fertilizer (150 kg N ha⁻¹) was applied in three stages: 50% as basal (1 day before transplanting), 30% at early-tillering (7 days after transplanting), and 20% at panicle initiation. P fertilizer (75 kg P₂O₅ ha⁻¹) was applied as basal. K fertilizer (150 kg K₂O ha⁻¹) was applied equally between basal and panicle initiation. The field was flooded with 5-10 cm of water from the day seedlings were transplanted until 7 days prior to maturity. Chemicals were used to control diseases and pests to avoid yield loss.

2.3 Sampling and Measurements

The ratio of solar radiation absorbed by the rice was determined using a system of canopy analysis (SunScan, Delta-T Devices Ltd., Burwell, Cambridge, UK). The radiation intercepted during pre- and post-heading were both calculated according to the method used by Huang et al. [14]. Ten hills of rice plants were tested for each field at both maturity and heading. Leaf area index (LAI = leaf area/land area), specific leaf weight (SLW = leaf dry weight/leaf area), pre-heading N uptake (NU_{pre} = pre-heading biomass production × N content), and pre-heading biomass production, were determined using the plants sampled at their heading. The plants tested at maturity were used to determine total biomass production and total N uptake. A leaf area meter (LI-3000C, LI-COR, Lincoln, NE, USA) was used to determine the leaf area. Biomass was the aboveground dry weight, which was measured after drying it to a constant weight in an oven at 70°C. A segmented flow analyzer (Skalar SAN Plus, Skalar Inc., Breda, The Netherlands) was used to determine the nitrogen content. Pre-heading RUE (RUE_{pre} = pre-heading biomass production/pre-heading intercepted radiation), post-heading RUE [RUE_{post} = (total biomass production–pre-heading biomass production)/post-heading intercepted radiation], post-heading N uptake (NU_{post} = total N uptake–NU_{pre}), and the ratios of LAI/NU_{pre}, SLW/NU_{pre}, NU_{post}/LAI, NU_{post}/SLW, and N use efficiency for biomass production (total biomass/total N uptake) were calculated.

Grain yield was determined from a 5-m² section of each plot, after adjusting to a moisture content of 0.14 g g⁻¹ at fresh weight.

2.4 Data Analysis

We analyzed the data using an analysis of variance (Statistix 8.0, Analytical Software, Tallahassee, FL, USA), and compared the means at the 0.05 probability level the using the least significant difference test (LSD) test.

3 Results

NU_{pre} was not significantly different between C20 and C0 in 2015 or 2016 (Fig. 2A). NU_{post} was 56% higher under C20 than C0 in 2015 and 25% higher under C20 than C0 in 2016 (Fig. 2B).

We did not observe a significant difference in LAI between C0 and C20 and in 2015. In 2016, C20 displayed 18% lower LAI than C0 (Fig. 2C). We observed no significant difference in SLW between C0 and C20 in 2015 or 2016 (Fig. 2D).

There was no significant difference in LAI/NU_{pre} between C20 and C0 in 2015 or 2016 (Fig. 2E). SLW/NU_{pre} was not significantly different between C20 and C0 in 2015, while under C20 it was 22% higher than under C0 in 2016 (Fig. 2F). C20 had 64% and 52% higher NU_{post}/LAI than C0 in 2015 and 2016, respectively (Fig. 2G). Under C20, NU_{post}/SLW was higher than C0 by 57% in 2015 and by 24% in 2016 (Fig. 2H).

We did not observe a significant difference in RUE_{pre} between C0 and C20 in 2015 or 2016 (Fig. 3A). C20 had 50% and 20% higher RUE_{post} compared to C0 in 2015 and 2016, respectively (Fig. 3B).

The total production of biomass under C20 was 18% higher than under C0 in 2015, while it was not significantly different between C20 and C0 in 2016 (Tab. 1). There was no significant difference in the

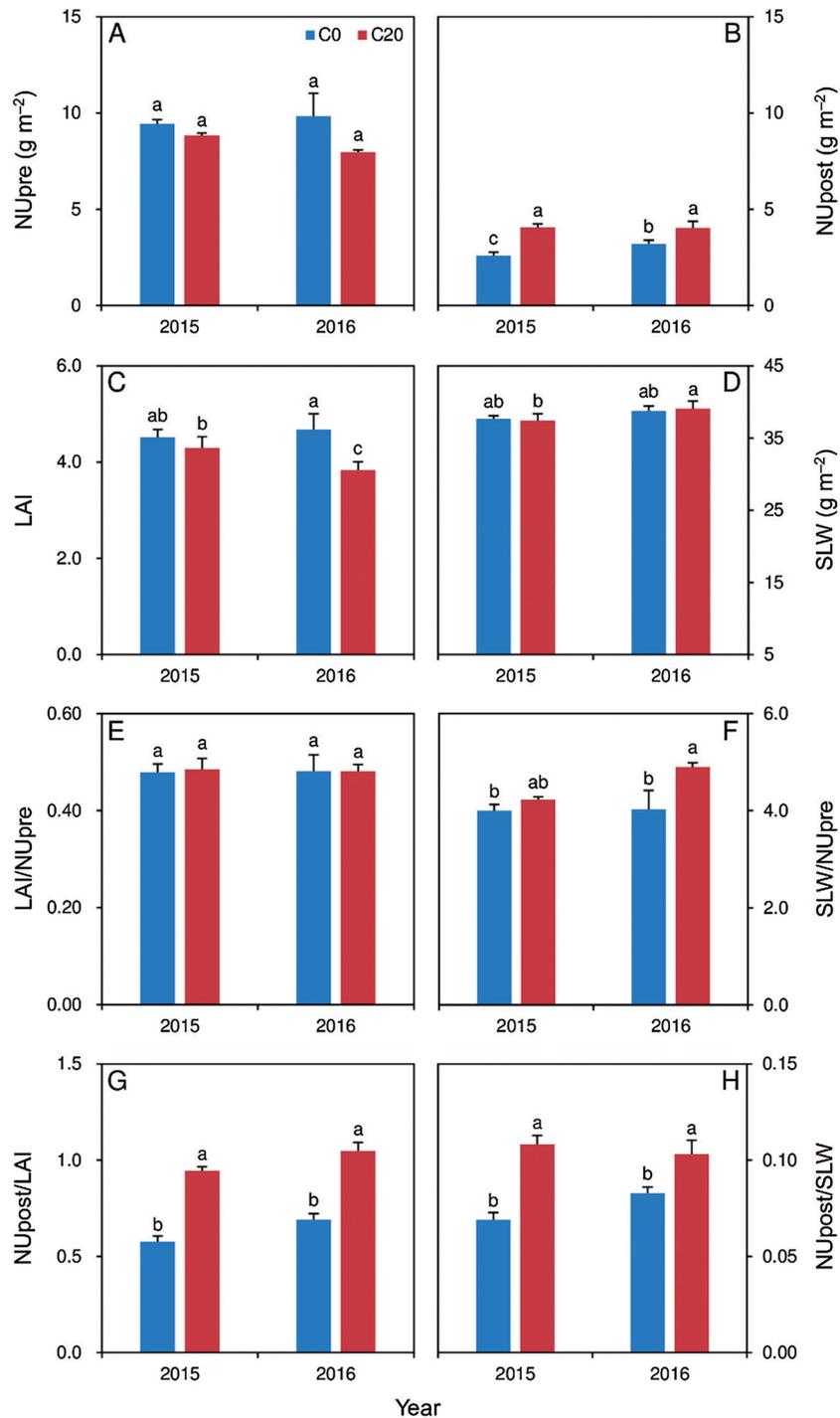


Figure 2: The effects of the application of biochar on nitrogen uptake and leaf characteristics in rice grown in the field in 2015 and 2016. (A) pre-heading nitrogen uptake (NU_{pre}); (B) post-heading nitrogen uptake (NU_{post}); (C) leaf area index (LAI) at heading; (D) specific leaf weight (SLW) at heading; (E-H) ratios of LAI/NU_{pre}, SLW/NU_{pre}, NU_{post}/LAI, and NU_{post}/SLW. C0 represents 0 t biochar ha⁻¹ and C20 represents 20 t biochar ha⁻¹. Error bars represent standard errors. In the sub-tables, the levels that don't share a letter are significantly different at the 0.05 level, per the LSD test

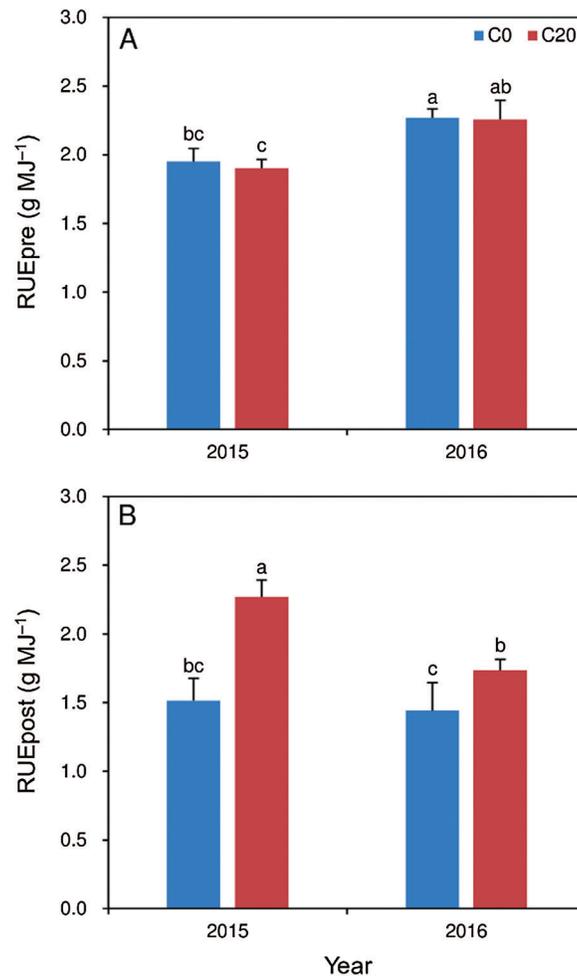


Figure 3: The effects of the application of biochar on radiation use efficiency on field-grown rice in 2015 and 2016. (A) pre-heading radiation use efficiency (RUE_{pre}); (B) post-heading radiation use efficiency (RUE_{post}). C0 represents 0 t biochar ha⁻¹ and C20 represents 20 t biochar ha⁻¹. Error bars represent standard errors. In the sub-tables, the levels that don't share a letter are significantly different at the 0.05 level, per the LSD test

Table 1: The effects of the application of biochar on total biomass production, total nitrogen uptake, the efficiency of nitrogen usage in biomass production, and yield of grain in field-grown rice in 2015 and 2016

Parameters	2015		2016	
	C0	C20	C0	C20
Total biomass production (g m ⁻²)	1100 ± 67b	1294 ± 56a	1299 ± 90a	1360 ± 42a
Total nitrogen uptake (g m ⁻²)	12.0 ± 0.2a	12.0 ± 0.2a	13.1 ± 1.4a	12.9 ± 0.5a
N use efficiency for biomass production (g g ⁻¹)	91 ± 5b	100 ± 3b	100 ± 3b	113 ± 2a
Yield of grain (t ha ⁻¹)	5.94 ± 0.15b	5.57 ± 0.08b	8.45 ± 0.09a	8.30 ± 0.55a

Note: C0 represents 0 t biochar ha⁻¹ and C20 represents 20 t biochar ha⁻¹. Data are means ± standard errors. In the rows, the data that don't share a letter are significantly different at the 0.05 level, per the LSD test.

total uptake of nitrogen between C0 and C20 in 2015 or 2016. N use efficiency for biomass production was not significantly different between C20 and C0 in 2015, while it was 13% higher under C20 than C0 in 2016. We did not observe significant differences in the yield of grain between C0 and C20 in either 2015 or 2016.

4 Discussion

Prior studies have determined that N uptake by rice plants can be altered by the application of biochar [8, 9]. However, the previous studies usually focused on the total N uptake (i.e., N uptake at maturity). Our study investigated what effects the application of biochar would have on nitrogen uptake by field-grown rice plants during two growth periods (i.e., pre- and post-heading). We found that applying biochar made no significant difference in NU_{pre} but significantly increased NU_{post} , a novel finding concerning N uptake. Interestingly, in parallel with the increase in NU_{post} due to biochar application, a significant increase in RUE_{post} induced by biochar application was observed in this study. This finding supports our hypothesis that biochar application enhances RUE in field-grown rice by improving the plant N status.

Several studies have investigated what effects the application of biochar would have on plant and soil characteristics related to N uptake by rice crops [7–9]. There are three instances where the application of biochar would have a positive effect on nitrogen uptake in rice: (1) increasing soil N content; (2) reducing fertilizer N loss; and (3) improving root growth and activity. Some or all of these pathways might be responsible for the increase in NU_{post} by biochar application in the present study. In addition, we observed an interesting phenomenon wherein a large amount of *Azolla* occurred in the plot with biochar application during the early growth period (Fig. 4). It is well-known that *Azolla* forms symbiotic relationships with blue-green algae, which fixes nitrogen and can thus fix nitrogen at a higher rate [15]. Applying *Azolla* at the base ($10\text{--}12\text{ t ha}^{-1}$) can increase $50\text{--}60\text{ kg ha}^{-1}$ of soil N [16]. Additionally, *Azolla* reduces the volatilization of ammonia from paddy soils [17]. Thus, in this study, the occurrence of *Azolla* is likely a reason for the increased NU_{post} following biochar application. However, to date, limited information is available regarding the effects of biochar application on the occurrence and growth of *Azolla*, highlighting that further investigations are required on this topic.



Figure 4: The effects of the application of biochar on *Azolla* occurrence in 2016. C0 represents $0\text{ t biochar ha}^{-1}$ and C20 represents $20\text{ t biochar ha}^{-1}$

Post-heading is the period when leaf senescence occurs. Therefore, delaying leaf senescence is important for increasing post-heading photosynthetic capacity. An increase in NU_{post} has been shown to stunt leaf senescence while increasing the level of chlorophyll, the levels of soluble protein, and the net rate of

photosynthesis during the post-heading period in rice crops [18,19]. This might also be why RUE_{post} was enhanced by biochar application in this study, where NU_{post} and ratios of NU_{post}/LAI and NU_{post}/SLW increased alongside biochar application.

This study found that an increase in RUE_{post} following the application of biochar increased post-heading biomass production and overall biomass production. Grain yield did not increase, but a reduction in the harvest index made up for this (data not shown). We determined the harvest index by assessing the use of stored reserves in grain that was growing, as well as photosynthesis while the grain was forming. The decreased harvest index we observed in rice with applied biochar during this study could be related to a lower release of reserves in the growing grain, since RUE during the grain-filling stage is increased by the application of biochar. While an oft-cited benefit of biochar application in soils is an increase in crop yield, these results vary and depend on a number of different factors, including biochar source, the type of soil, fertilization status, as well as climate [7,20,21]. As such, further study is needed to systematically evaluate how to best implement the application of biochar in rice production.

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

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