

RESEARCH ARTICLE

Synergistic Effects of Rice Husk Biochar and Urea Fertilizer on Rice Growth Performance, Soil Properties, and Nitrogen Use Efficiency

K.H.C.H. Kumara¹, K.M.C. Fernando², H.K.M.S. Kumarasinghe², D.M. Withanawasam³ and D.L. Wathugala^{2*}

¹Rice Research Station, Department of Agriculture, Ambalanthota, Sri Lanka

²Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

³Regional Rice Research and Development Centre, Department of Agriculture, Bombuwala, Sri Lanka

Abstract: This study evaluated the effect of the combined application of rice husk biochar (RHB) and urea fertilizer on soil properties, plant growth, yield, grain quality, and nitrogen use efficiency metrics in the Low Country Dry Zone of Sri Lanka. A pot experiment, arranged in a completely randomized two-factor factorial design with 4 replicates, was used for the study. The experiment consisted of five biochar carbon rates B0: 0%, B1: 1%, B2: 2%, B3: 3% and two urea levels (U1: 157.5 kg/ha, U2: 225 kg/ha), with treatments repeated across the Yala (from June to September 2023) and Maha (November 2023 to March 2024) seasons. Partially burnt paddy husk (PBPH; 625 kg/ha which corresponds to the 0.01% carbon rate in soil) is a recommended type of biochar-based soil amendment for rice cultivation; therefore, a treatment with the recommended level of PBPH and two urea levels was also included in the study. Results showed that RHB significantly improved soil chemical properties, rice plant growth, yield and nitrogen use efficiency metrics. The B3U2 treatment exhibited an 11% higher grain yield per plant and a 16% increase in nitrogen use efficiency compared to the existing conventional practice (B0U2). Moreover, grain iron content (IC), protein content (PC), and amylose content (AC) in the B3U2 treatment were 7%, 1%, and 15% higher, respectively, compared to the B0U2 treatment. Some growth and yield parameters, as well as grain physical properties, did not significantly differ between the low nitrogen (B3U1) and high nitrogen (B3U2) treatments. Compared to PBPH, RHB demonstrated superior effects on all measured parameters, although its preparation is comparatively more difficult. The findings of the present study revealed that the combined application of RHB and urea fertilizers is beneficial in rice cultivation. However, further research under field conditions is needed to understand the long-term impacts of RHB application.

Keywords: Low Country Dry Zone of Sri Lanka, Nitrogen use efficiency, Rice cultivation, Rice husk biochar, Soil properties, Urea,

Introduction

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population, playing a vital role in global food security and nutrition (Anwari *et al.*, 2019, Asadi *et al.*, 2021). In many Asian countries, rice cultivation is a major component of the country's economy, daily diet, and the livelihoods of rural communities (Ali *et al.*, 2022, Karam *et al.*, 2022). Water limitations, declining soil fertility and acidification of paddy soils are some of the major issues faced in this sector.

Therefore, finding solutions to overcome these challenges is a priority area in rice research.

Achieving higher rice production often necessitates the application of synthetic fertilizers, especially those nitrogen-based, as nitrogen frequently emerges as the most yield-limiting nutrient in many agricultural fields (Lai *et al.*, 2017, Ning *et al.*, 2022, Selvarajh *et al.*, 2024). Therefore, farmers use a considerably high amount of nitrogen-based fertilizers, which create many environmental issues such as water pollution, greenhouse gas emissions and soil degradation due to

*corresponding author: wgdakmini@crop.ruh.ac.lk

 <https://orcid.org/0000-0003-1276-8060>



This article is published under the Creative Commons CC-BY-ND License (<http://creativecommons.org/licenses/by-nd/4.0/>). This license permits commercial and non-commercial reuse, distribution, and reproduction in any medium, provided the original work is not changed in any way and is properly cited.

soil acidification and reducing beneficial soil microbial population (Cassman *et al.*, 2003, Ali *et al.*, 2022, Qin *et al.*, 2023). Due to poor soil conditions, the plant's nitrogen uptake efficiency will also be reduced, and the excess nitrogen will be lost in different ways, leading to economic losses for farmers. Therefore, finding ways to increase rice yield while reducing negative impacts is crucial to feeding a growing population.

Rice husk, a substantial byproduct of rice production, constitutes about 20% of the total rice weight (Asadi *et al.*, 2021, Tan *et al.*, 2024). Because of a high C/N ratio, rice husk can remain soil for a long time without decomposing (Barman and Kandpal, 2019). The production of biochar through the process called pyrolysis is one promising avenue for utilizing this byproduct (Barman and Kandpal, 2019, Asadi *et al.*, 2021, Adebajo *et al.*, 2022). Another cost-effective method of using this resource is converting it to partially burnt paddy husk (PBPH) also known as rice husk charcoal by onsite open burning after harvest (Koyoma *et al.*, 2016). In Sri Lanka, it is recommended to apply PBPH to rice fields at a rate of 625 kg/ha (Yaya programme, 2019) and there is evidence that other Asian countries do the same (Ogawa and Okimori, 2010). Further, the application of PBPH has been reported as a popular practice in wheat cultivation for about 100 years (Ogawa and Okimori, 2010). However, unlike RHB, limited information is available on the effect of PBPH on rice yield and the environment. Further, the application of RHB is not a popular activity in rice cultivation in Sri Lanka. Therefore, conducting research to compare the benefits associated with applying RHB and PBPH to rice is important to provide recommendations.

Numerous studies have reported beneficial effects on rice plant growth, yield, soil properties and nitrogen use efficiency with the application of RHB and nitrogen fertilizer. For instance, a significant increase in rice yield (60%) was observed by Ali *et al.* (2020a) with the application of 60 t/ha RHB with 270 kg N/ha, compared to a control treatment applied only 270 kg N/ha. In 2021, again, Ali *et al.*, (2021) reported a 30% yield increment with the application of 30 t/ha RHB with 135 kg N/ha. The same study also reported a 104% increase in chlorophyll content, a 64% increase in net photosynthetic rate, and a 12% increase in

biomass. According to them, these increases were attributed to enhanced soil properties, improved root characteristics, and increased photosynthetic rates. A 2-year field experiment conducted by Liu *et al.*, (2016) highlighted an 8.5-10.7% increase in grain yield with the application of rice straw biochar. Further, Oladele *et al.*, (2019) also observed an increase in rice yield with the application of different levels of RHB and N fertilizer.

Not only rice yields, but also improvement in various growth parameters of rice were observed in these studies. As an example, a 62% increase in tillers per hill, a 4% increase in plant height, a 19% increase in thousand-grain weight, a 23% increase in panicle length, with a significant 30% increase in grain yield were reported by Ali *et al.*, (2021) all compared to a control group. All previous studies suggest that biochar can increase the efficiency of nitrogen fertilizer and thereby increase the nitrogen uptake, leading to higher yield. However, some studies reported that biochar application alone might negatively impact rice yield in nitrogen-deficient soils due to the nutrient immobilizing effect and high C/N ratio (Asai *et al.*, 2009, Qian *et al.*, 2014, Oladele *et al.*, 2019). These findings suggest that the effectiveness of biochar as a soil amendment depends on initial nitrogen levels of the soil.

Numerous studies focusing on soil properties have reported that the combined application of rice husk biochar and nitrogen fertilizer can enhance various soil health indicators, such as increased soil pH (Ali *et al.*, 2020a, Ali *et al.*, 2020b, Zhao *et al.*, 2023) suggesting its potential to ameliorate soil acidity, a major constraint in rice cultivation regions in tropical countries. Elevated levels of soil organic carbon (Butnan and Vityakon, 2023, Williams *et al.*, 2023) enhanced total nitrogen content by reducing nutrient leaching (Deniel *et al.*, 2018, Ali *et al.*, 2022) and enhancing nutrient release (Ali *et al.*, 2020b, Linh *et al.*, 2023), enhanced water-holding capacity (Lai *et al.*, 2017, Karam *et al.*, 2022), improved cation exchange capacity (Barman and Kandpal, 2019), promotion of aggregate formation (Barman and Kandpal, 2019, Ali *et al.*, 2022), increased microbial activity (Abukari *et al.*, 2019, Williams *et al.*, 2023) are the other soil properties affected by the application of biochar. However, it is important to note that the effect of

biochar on soil properties can vary depending on factors like conditions of biochar production (Karam *et al.*, 2022), soil type (Butnan and Vityakon, 2023), application rate (Ning *et al.*, 2022) and interactions with other soil amendments.

Considering the extensive benefits observed in previous research and the persistent challenges faced in rice cultivation in Low Country Dry Zone in Sri Lanka, this study aims to investigate the effects of different combinations of rice husk biochar and partially burnt paddy husk combined with nitrogen fertilizer urea on rice growth, yield, soil properties, and nutrient use efficiency in a pot experiment.

Materials and Methods

Experimental site and climate details

A two-season outdoor pot experiment was conducted during the Yala season (S1, from June to September 2023) and the Maha season (S2, from November 2023 to March 2024) at the Rice Research Station, Ambalantota, Sri Lanka (6° 13' 18.00'' N, 80° 03' 14.40'' E). Soil was collected from the top horizon (0 - 20 cm) of a rice field. The area experiences a tropical monsoon climate with an average annual rainfall of 1250 mm. The mean maximum and minimum temperatures during S1 and S2 were 31.5 °C and 26.5 °C in 2023 Yala and 2023/24 Maha, respectively. The average relative humidity ranged from 77.37% to 98.52% in S1 and from 81.22% to 100% in S2 (Figure 1).

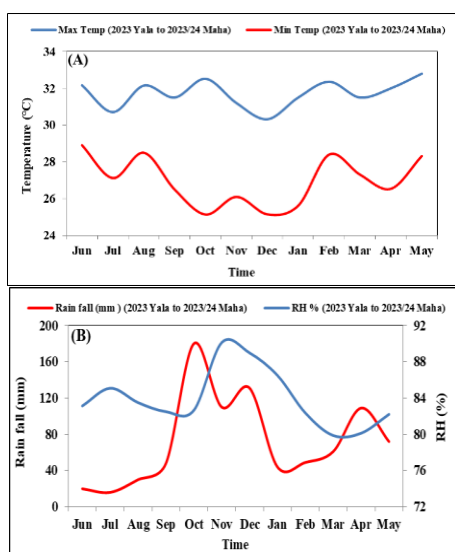


Figure 1: Mean average maximum and minimum temperature (A), relative humidity, and total rainfall (B) during the experimental period.

Soil Analysis

Soil samples were collected before and after the experiment from each treatment in both seasons to assess physical and chemical properties. Initial soil samples were analyzed for pH (water), soil electrical conductivity (EC), organic carbon (OC), available nitrogen (SN), available phosphorus (SP), and exchangeable potassium (SK) (Table 1). The SN, SP, SK, and OC contents were assessed using the Kjeldahl (Bremner, 1960), Olsen (FAO, 2021), flame emission (Ullah *et al.*, 2022), and Walkley and Black (FAO, 2019) methods, respectively. The Kjeldahl method was employed to determine the soil nitrogen content through sample digestion with sulphuric acid (98%), which converts organic nitrogen into ammonium sulfate. Released ammonia during the distillation was captured and measured by titrating with 4% (w/v) boric acid and determined the nitrogen content. Available phosphorus content was determined by soil samples extracted with 0.05M sodium bicarbonate. Absorbance values of the colorimetric method were used to measure (880 nm wavelength of the spectrophotometer) the Olsen phosphorus content of soil samples. Furthermore, soil available potassium was measured by extracting soil samples with 1M ammonium acetate. Intensity of the potassium flame emission was measured for each extracting solution, which was used to calculate the soil available potassium. Also, soil organic carbon (OC) content was determined using the Walkley and Black method. During the process, soil samples were treated with 1N potassium dichromate and sulfuric acid, which facilitated to oxidize of the organic carbon, and the amount of dichromate ion consumed was titrated with 0.5N ferrous sulfate, and calculations were done by using titration readings.

Biochar and partially burnt paddy husk preparation

Biochar was derived from rice husk (RHB), burnt in a pyrolyzer at approximately 500 °C for 8 hours in the absence of oxygen (Munasinghe *et al.*, 2018). Partially burnt paddy husk (PBPH) was prepared by open burning without temperature control, which obtained more ash and a relatively low amount of biochar carbon compared to RBH. Product outcome of PBPH was obtained relatively low amount of pore spaces and

absorptive surfaces than RHB (Munasinghe *et al.*, 2018, Wickramasinghe and De Silva, 2019)

Experimental design and crop management

The outdoor experiments were conducted in plastic pots (15 cm radius, 32 cm height). Equal weights of biochar and urea were applied based on the treatment combination of the experimental unit of each block. Also, a commonly used research field was selected as the experimental site, and a homogeneity test was conducted during the 2022/23 Maha season, and homogenized blocks were then selected for experimental establishment, which confirmed the homogeneity of the microclimate around the experimental unit. The soil was air-dried, pulverized, and mixed with biochar until homogenized before filling the pots, each containing 6.5 kg of soil. The pots were flooded with water to a depth of 4 cm, and watering was maintained regularly from transplanting until 85% physiological maturity of the rice. The rice variety 'AT 362' was used, with fourteen-day-old seedlings transplanted at four per pot.

The experiment included 10 treatment combinations, replicated 4 times across 40 pots, in a completely randomized two-factor factorial design. Treatments involved two urea fertilizer levels (U1: 70% and U2: 100% of the recommended level by the Department of Agriculture (DOA), Sri Lanka, equivalent to 157.5 and 225 kg/ha, corresponding to 4.45 and 6.35 g/pot) and five biochar carbon levels (B0: 0%, B1: 1%, B2: 2%, and B3: 3%, corresponding to 140, 280, and 420 g/pot), with a DOA recommended level of partially burnt paddy husk (Yaya I programme, 2004) carbon (625 kg/ha which obtained the 0.01% carbon rate in soil) with urea as additional treatments (PU1 and PU2). Urea was applied in four stages: 20% at 2 weeks after planting (WAP), 32% at 4 WAP and 6 WAP, and 16% at 7 WAP. Potassium was applied at 60 kg/ha (0.75 and 1 g/pot at 4 and 6 WAP), and phosphorus was applied at 55 kg/ha (1.7 g/pot) as a basal dose in all treatments. Standard agronomic practices recommended by the Department of Agriculture were followed for all pots during both seasons.

Sampling and measurements

Four weeks after seedling establishment, plant leaves were harvested for pigment analysis. Chlorophyll-a (CLa), chlorophyll-b (CLb), total chlorophyll (TCL),

and carotenoids (CAD) were extracted using 96% methanol as the solvent. The levels of these pigments were determined using a spectrophotometer (Genesis-10S Vis) at specific wavelengths: CLa at 666 nm, CLb at 659 nm, and CAD at 480 nm, following the methods described by (Şükran *et al.*, 1998).

Measurement of plant growth and yield parameters: Growth parameters such as plant height (PH), number of tillers per plant (NT), number of leaves per plant (NL), and internodal length per plant (IL) were measured 10 WAP to get an idea of the biochar effect on early vegetative growth. Grain yield (GY) was measured after harvesting at a 14% moisture level. As other yield parameters, panicle length (PL) (used 5 randomly selected panicles per plant) and the number of panicles per plant (NP) were recorded. After harvesting and drying at 60 °C until a constant weight, shoot dry matter (SDM) and root dry matter (RDM) were determined. Additionally, 4 weeks after harvesting, the contents of shoot nitrogen (StN), shoot phosphorus (StP), and shoot potassium (StK) were assessed using the Kjeldahl, Olsen, and flame emission spectroscopy methods, respectively.

Measurement of grain quality parameters: Physical grain quality parameters, i.e. thousand grain weight (TGW), grain length (L), grain width (W), grain equivalent diameter (De) (equation 1), sphericity (Ø) (equation 2), grain volume (V) (equation 3), and surface area (S) (equation 4 & 5), were calculated using the mathematical models described by Varnmkhasti *et al.* (2008).

$$D_e = [L \frac{(W+T)^2}{4}]^{1/3} \dots\dots\dots (1)$$

$$\emptyset = \frac{(LWT)^{1/3}}{L} \dots\dots\dots (2)$$

$$V = 0.25 \left[\left(\frac{\pi}{6} \right) L(W+T)^2 \right] \dots\dots\dots (3)$$

$$S = \frac{\pi BL}{(2L-B)} \dots\dots\dots (4)$$

$$B = \sqrt{WT} \dots\dots\dots (5)$$

Where: De - equivalent diameter, L-grain length, W-grain width, T-grain thickness, Ø- grain sphericity, V-grain volume, S-grain surface area, π - 22/7

The amylose content (AC) and iron content (IC) of the grains were also assessed. AC was determined by using the spectrophotometric method by measuring

UV absorption at 620 nm and analyzing the color development of the starch solution, following the method described by (Juliano, 1971). IC was also measured using a spectrophotometric method by evaluating the reduction reaction and color development under UV light at 480 nm, as outlined by (Pauel, 1995). Standard curves for iron and amylose were utilized to determine the precise values.

Protein content (PC) was calculated using the Kjeldahl method, with the factor 6.25 applied to the nitrogen content. Ash content (ASC) was measured using the procedure described by (Park and Bell, 2004). Additionally, the contents of nitrogen (GN), the grains were assessed using the Kjeldahl method.

Measurement of nitrogen use efficiency: Nitrogen use efficiency (NUE) was calculated using the method described by Le Gouis *et al.*, (2000) (Equations 6-8). NUE was divided into two components: N uptake efficiency (NUpE; N off per unit availability of N from the soil or fertilizer) and N utilization efficiency (NUtE; grain yield per unit plant N off). N off was calculated using only shoot N.

$$NUpE = \frac{N \text{ off}}{N \text{ available}} \dots\dots\dots (6)$$

$$NUtE = \frac{\text{Grain yield}}{N \text{ off}} \dots\dots\dots (7)$$

$$NUE = NUpE \times NUtE \dots\dots\dots (8)$$

Statistical Analysis: ANOVA was performed using the General Linear Model (GLM) for two factors with Minitab 21 software for Windows 10. The strength of the relationship of each parameter was determined using Pearson's correlation coefficient. The means of the treatments were compared using the least significant difference test at the 0.05 probability level.

Results

Soil chemical properties

The combined application of urea and RHB significantly affected the soil's chemical properties (Table 1). While both biochar and urea slightly reduced soil pH, treatments involving biochar showed the least change compared to those with PBPH and urea. Other soil properties, except SN, improved significantly, especially in the highest biochar and urea treatment (B3U2), where SP, SK, and OC

contents increased substantially (Table 1). The trends were similar for the treatments in both seasons. Significant increases in soil OC (Table 1) and organic matter (OM) (Figure 2) were noted, particularly with the highest RHB application. The B3U2 treatment showed a 79% increase in OC and a 129% increase in OM over the B0U2 treatment. At the end of both seasons, SP and SK levels increased from the initial values (Table 1), with the B3U2 treatment showing average increases of 2% in both SP and SK compared to B0U2.

Table 1: Changes in soil chemical properties as influenced by different levels of rice husk biochar and urea compared to their initial values.

Treatments	pH	EC (dS/m)	OC (%)	SN (ppm)
S1 (Yala - 2023)				
Initial value	6.98	0.34	0.10	2.32
B0U1	- 0.23 ^{ab}	0.05 ^d	0.73 ^f	-1.74 ^c
B0U2	- 0.24 ^{ab}	0.05 ^d	0.75 ^f	-1.73 ^c
B1U1	- 0.26 ^{ab}	0.07 ^c	0.88 ^d	-1.73 ^c
B1U2	- 0.30 ^b	0.06 ^c	0.88 ^d	-1.73 ^c
B2U1	- 0.23 ^{ab}	0.09 ^b	0.94 ^c	-1.68 ^b
B2U2	- 0.23 ^{ab}	0.09 ^b	0.95 ^c	-1.68 ^b
B3U1	- 0.20 ^a	0.12 ^a	1.08 ^b	-1.63 ^{ab}
B3U2	- 0.21 ^a	0.13 ^a	1.08 ^a	-1.60 ^a
PU1	- 1.03 ^c	0.04 ^d	0.84 ^e	-1.76 ^d
PU2	- 1.04 ^c	0.05 ^d	0.84 ^e	-1.75 ^d
S2 (Maha - 2023/24)				
Initial value	7.12	0.42	0.14	1.68
B0U1	- 0.30 ^b	0.03 ^e	0.75 ^e	-1.20 ^d
B0U2	- 0.30 ^b	0.04 ^d	0.75 ^e	-1.17 ^d
B1U1	- 0.24 ^{ab}	0.07 ^c	0.85 ^d	-1.09 ^c
B1U2	- 0.24 ^{ab}	0.07 ^c	0.85 ^d	-1.09 ^c
B2U1	- 0.23 ^b	0.10 ^b	0.96 ^c	-1.05 ^b
B2U2	- 0.21 ^a	0.10 ^b	0.95 ^c	-1.07 ^b
B3U1	- 0.23 ^a	0.12 ^a	1.13 ^b	-0.99 ^a
B3U2	- 0.23 ^a	0.12 ^a	1.14 ^a	-0.99 ^a
PU1	- 0.93 ^c	0.05 ^d	0.86 ^d	-1.11 ^c
PU2	- 0.98 ^c	0.05 ^d	0.86 ^d	-1.11 ^c
B	s	s	s	s
U	ns	ns	s	s
S	s	ns	ns	ns
B×U	s	s	s	s
B×S	ns	ns	ns	ns
U×S	ns	ns	ns	ns
B×U×S	ns	ns	ns	s

N.B: Means of treatments of each season within the same column followed by different letters indicate significant difference between them by LSD test at $p < 0.05$, B: Biochar carbon, P: Partially burnt paddy husk, U: Urea, pH: Acidity, EC: Electrical conductivity (dS/m), OC: Organic carbon (%), SN: Soil nitrogen (ppm), SK: Soil potassium (ppm), SP: Soil phosphorus (ppm).

The B3U2 treatment had the highest reduction in SN levels and the highest increase in SK levels, but there was no significant difference compared to B3U1 in both seasons. Higher SP was noted in B3U2 in both seasons, but no significant difference was observed

compared to B3U1, B2U2, and B2U1 treatments in the Yala season.

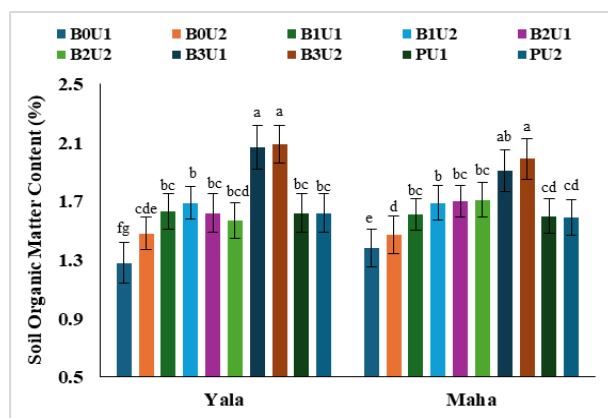


Figure 2: Effect of rice husk biochar and urea levels on soil organic matter content.

Leaf pigment concentration

As shown in Table 2, the combined application of RHB and urea significantly impacted leaf total chlorophyll (TCL) and carotenoid (CAD) contents. In this study, TCL and CAD were measured at 14, 28, 42, and 56 days after transplanting. The addition of RHB combined with urea significantly increased TCL and CAD levels, suggesting that RHB can positively affect leaf pigment concentration. The behavior of pigment concentration was similar in both seasons. On average across the season, B3U2 treatment increased TCL and CAD contents by 12% and 5%, respectively, compared to conventional practice. The data indicates that the effect on TCL is greater than the effect on CAD. However, to solidify this claim, further experiments are needed.

Table 2: Total Chlorophyll (mg/g) and Carotenoids (mg/g) contents as influenced by rice husk biochar and urea levels during four growth stages and two seasons.

Treatments	14 DAT		28 DAT		42 DAT		56 DAT	
	TCL	CAD	TCL	CAD	TCL	CAD	TCL	CAD
S1 (Yala - 2023)								
B0U1	3.32 ^g	0.73 ^{bc}	3.44 ^g	0.74 ^c	3.94 ^f	0.75 ^b	4.36 ^f	0.75 ^b
B0U2	3.39 ^c	0.73 ^{bc}	3.66 ^f	0.74 ^c	4.05 ^f	0.75 ^b	4.36 ^f	0.75 ^b
B1U1	3.55 ^{bc}	0.70 ^{cd}	3.79 ^c	0.75 ^b	4.36 ^c	0.75 ^b	4.46 ^d	0.75 ^b
B1U2	3.44 ^d	0.72 ^c	3.88 ^d	0.76 ^b	4.62 ^b	0.75 ^b	4.46 ^d	0.75 ^b
B2U1	3.57 ^b	0.72 ^c	4.25 ^{bc}	0.76 ^b	4.56 ^{bc}	0.75 ^b	4.43 ^c	0.75 ^b
B2U2	3.58 ^b	0.75 ^b	4.25 ^{bc}	0.76 ^b	4.43 ^d	0.75 ^b	4.28 ^g	0.76 ^a
B3U1	3.58 ^b	0.79 ^a	4.29 ^b	0.80 ^a	4.56 ^c	0.76 ^a	4.51 ^b	0.76 ^a
B3U2	3.62 ^a	0.79 ^a	4.35 ^a	0.80 ^a	4.78 ^a	0.76 ^a	4.59 ^a	0.76 ^a
PU1	3.43 ^d	0.75 ^b	3.90 ^d	0.77 ^b	4.44 ^d	0.75 ^b	4.48 ^c	0.75 ^b
PU2	3.39 ^f	0.74 ^b	3.90 ^d	0.75 ^b	4.44 ^d	0.74 ^c	4.48 ^c	0.75 ^b
S2 (Maha - 2023/24)								
B0U1	3.38 ^c	0.73 ^c	3.44 ^g	0.74 ^c	3.94 ^c	0.75 ^b	4.18 ^g	0.75 ^b
B0U2	3.39 ^c	0.73 ^c	3.58 ^f	0.74 ^c	4.15 ^d	0.75 ^b	4.20 ^f	0.75 ^b
B1U1	3.53 ^c	0.73 ^c	3.72 ^c	0.75 ^b	4.36 ^c	0.75 ^b	4.46 ^c	0.75 ^b
B1U2	3.54 ^c	0.73 ^c	3.78 ^c	0.76 ^b	4.41 ^b	0.79 ^a	4.45 ^c	0.75 ^b
B2U1	3.57 ^b	0.75 ^b	4.23 ^{bc}	0.76 ^b	4.41 ^b	0.79 ^a	4.24 ^c	0.75 ^b
B2U2	3.58 ^b	0.75 ^b	4.25 ^{bc}	0.76 ^b	4.43 ^{ab}	0.79 ^a	4.34 ^d	0.76 ^b
B3U1	3.58 ^b	0.78 ^a	4.37 ^b	0.80 ^a	4.40 ^b	0.79 ^a	4.51 ^b	0.78 ^a
B3U2	3.62 ^a	0.79 ^a	4.55 ^a	0.80 ^a	4.45 ^a	0.74 ^c	4.59 ^a	0.78 ^a
PU1	3.43 ^d	0.74 ^b	4.07 ^d	0.77 ^b	4.39 ^{bc}	0.75 ^b	4.45 ^c	0.76 ^b
PU2	3.43 ^d	0.75 ^b	3.60 ^f	0.77 ^b	4.36 ^{bc}	0.75 ^b	4.35 ^d	0.76 ^b
B	s	s	s	s	s	ns	ns	ns
U	s	ns	s	ns	s	ns	s	ns
S	ns	ns	ns	ns	ns	ns	ns	ns
B×U	s	s	s	s	s	s	s	s
B×S	ns	ns	s	ns	s	ns	ns	ns
U×S	ns	ns	ns	ns	ns	ns	ns	ns
B×U×S	ns	ns	ns	ns	ns	ns	ns	ns

N.B: Means of treatments in different seasons within the same column followed by different letters indicate significant difference between them by LSD test at $p < 0.05$. B: Biochar carbon, U: Urea, P: Partially burnt paddy husk, S: Season, TCL: Total chlorophyll (mg/g), CAD: Carotenoids (mg/g), DAT: Days after transplanting.

Rice plant growth and yield parameters

RHB application combined with urea significantly ($p < 0.05$) increased rice plant growth and yield parameters in both seasons (Table 3). The B3U2 treatment, with the highest levels of biochar and urea, showed the most substantial improvements, compared to the treatment applying urea only (B0U2). The increments observed in B3U2 treatment compared to the B0U2 were plant height (PH) (8%), tiller number (TN) per plant (24%), number of leaves (NL) per plant (17%), first internode length (IL) (20%), root dry weight (RDW) (87%), shoot dry weight (SDW) (64%), panicle number (PN) per plant (21%), panicle length (PL) (17%), and grain yield (GY) per plant (11%). The treatment with a high amount of urea and PBPH (PN2) did not show promising results like the treatments with RHB.

Grain quality

In this study, grain chemical and nutritional properties were also influenced by the combined application of RHB and urea (Table 4). The B3U2 treatment resulted in significantly higher values across all measured grain chemical and physical parameters compared to the control treatment. Average percentage increases in grain length (GL), grain width (GW), grain volume (GV), grain nitrogen (GN), grain ash content (ASC), iron content (IC), amylose content (AC), and protein content (PC) were 1.6%, 2.4%, 5%, 6.5%, 1%, and 15% respectively, with grain protein content showing the greatest increase. All other parameters showed a significant but less than 1% increment with the application of 3% RHB and the recommended level of urea (B3U2). Whereas PU2 treatment showed slight increments of GL, SP and ASC compared to the control treatment, indicating again that the application of RHB is more beneficial than the application of PBPH.

Table 3: Effect on rice plant growth and yield parameters as influences by different levels of rice husk biochar and urea.

Trets	PH (cm)	TN plant ⁻¹	NL plant ⁻¹	INL (cm)	RDW (g/plant)	SDW (g/plant)	PN plant ⁻¹	PL (cm)	GY (g/plant)	TGW (g)
S1 (Yala--2023)										
B0U1	89.13 ^h	9.23 ^g	29.56 ^f	4.37 ^e	6.29 ^h	11.59 ^e	8.25 ^e	19.30 ⁱ	51.57 ^{ef}	25.09 ^e
B0U2	90.79 ^{ef}	10.25 ^f	39.22 ^{cd}	4.49 ^{de}	6.37 ^g	11.63 ^e	9.00 ^{cd}	19.48 ^{hi}	51.63 ^{ef}	25.29 ^{ab}
B1U1	92.08 ^{de}	11.00 ^c	39.15 ^{cd}	4.45 ^{de}	8.65 ^e	17.31 ^{cd}	8.91 ^{cd}	21.10 ^{efg}	54.70 ^d	25.16 ^{bc}
B1U2	93.57 ^{cd}	10.39 ^{de}	43.03 ^{bc}	4.87 ^{cd}	9.76 ^{cd}	15.29 ^{de}	9.83 ^{bc}	21.67 ^{cde}	58.83 ^{bc}	25.20 ^b
B2U1	95.60 ^{cd}	11.02 ^c	32.22 ^e	4.91 ^{bc}	8.23 ^f	18.04 ^{bc}	8.82 ^{cd}	23.03 ^{bc}	57.66 ^c	25.37 ^{ab}
B2U2	96.02 ^{bc}	11.00 ^c	43.00 ^{bc}	4.28 ^{def}	9.91 ^c	17.96 ^{bc}	9.02 ^{bc}	22.24 ^{bcd}	58.80 ^{bc}	25.35 ^{ab}
B3U1	97.63 ^{ab}	12.97 ^{ab}	44.05 ^{ab}	5.28 ^{ab}	13.05 ^b	21.04 ^a	10.33 ^{ab}	23.50 ^b	58.83 ^b	25.51 ^a
B3U2	98.28 ^a	13.33 ^a	45.15 ^a	5.47 ^a	13.22 ^a	19.98 ^{ab}	11.66 ^a	24.14 ^a	60.11 ^a	25.54 ^a
PU1	89.51 ^g	10.30 ^f	37.89 ^e	4.44 ^{de}	9.94 ^c	15.22 ^{de}	8.71 ^d	19.41 ^{hi}	51.52 ^c	25.28 ^{bc}
PU2	89.63 ^{ef}	10.40 ^{de}	39.27 ^{cd}	4.47 ^{de}	9.72 ^{cd}	15.46 ^{de}	8.83 ^{cd}	21.34 ^{def}	52.50 ^c	25.31 ^{cd}
S2 (Maha- 2023/2024)										
B0U1	86.23 ^h	10.58 ^f	33.45 ^f	4.31 ^c	7.30 ^c	10.63 ^h	8.42 ^f	20.12 ^c	45.78 ^g	25.12 ^c
B0U2	90.59 ^f	11.00 ^d	39.22 ^{cd}	4.48 ^d	7.35 ^c	12.63 ^g	9.60 ^c	21.28 ^{de}	54.23 ^f	25.31 ^{bc}
B1U1	94.28 ^{cde}	11.00 ^d	39.15 ^{cd}	4.49 ^d	9.65 ^{de}	17.71 ^{cd}	8.72 ^d	21.60 ^{cd}	54.20 ^f	25.46 ^{abc}
B1U2	94.51 ^{cd}	10.99 ^d	43.03 ^{bc}	4.80 ^{cd}	9.96 ^{cde}	17.89 ^{cd}	9.65 ^c	21.66 ^{cd}	55.03 ^e	25.50 ^{ab}
B2U1	96.20 ^{bc}	12.72 ^{bc}	39.21 ^{cd}	4.96 ^b	10.13 ^{cd}	18.54 ^{bc}	8.99 ^d	22.99 ^b	55.36 ^d	25.47 ^{ab}
B2U2	96.13 ^{bc}	12.80 ^b	44.01 ^{bc}	4.28 ^{def}	11.21 ^c	18.66 ^{bc}	9.62 ^c	22.94 ^b	55.51 ^c	25.55 ^{ab}
B3U1	97.13 ^{ab}	12.95 ^a	45.15 ^b	5.28 ^a	12.31 ^b	19.89 ^a	10.23 ^b	22.90 ^b	56.65 ^b	25.76 ^a
B3U2	97.38 ^a	12.97 ^a	46.25 ^a	5.29 ^a	12.42 ^a	19.88 ^a	10.86 ^a	23.54 ^a	56.99 ^a	25.79 ^a
PU1	90.46 ^f	10.78 ^c	37.59 ^e	4.45 ^d	9.55 ^{de}	15.42 ^{ef}	8.54 ^c	21.76 ^{cd}	54.19 ^f	25.31 ^{bc}
PU2	91.23 ^g	10.25 ^g	37.67 ^{cde}	4.51 ^{cde}	7.22 ^c	15.47 ^{ef}	8.63 ^c	21.81 ^{cd}	51.31 ^c	25.23 ^{bcd}
B	s	s	s	s	s	s	s	s	s	ns
U	s	s	s	s	s	s	s	s	s	s
S	s	s	ns	ns	ns	ns	s	ns	ns	ns
B×U	s	s	s	s	s	s	s	s	s	s
B×S	ns	ns	s	ns	ns	s	ns	s	s	ns
U×S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B×U×S	s	s	ns	s	s	s	s	s	s	ns

N.B: Means of treatments in different seasons within the same column followed by different letters indicate significant difference between them by LSD test at $p < 0.05$. B: Biochar carbon, U: Urea, P: Partially burnt paddy husk, S: Season, PH: Plant height (cm), TN: Number of tillers/plant, NL: Number of leaves/plant, INL: 1st internodal length/plant, RDW: Root dry weight (g/plant), SDW: Shoot dry weight (g/plant), PN: Number of panicles/plant, PL: Panicle length (cm), GY: Grain yield (g/plant), and TGW: 1000 grain weight (g)

Table 4: Effect on grain physical and chemical properties as influenced by different levels of rice husk biochar and urea.

Tret.	GL (mm)	GW (mm)	L/W Ratio	ED (mm)	GSP (%)	GV (mm ³)	GSA (mm ²)	GN (ppm)	ASC (%)	IC (µg/g)	AC (%)	PC (%)
S1 (Yala - 2023)												
B0U1	6.64 ^{bc}	1.85 ^{bc}	3.73 ^c	2.75 ^{bc}	42.37 ^{ab}	11.33 ^{bc}	3.27 ^{cd}	1.24 ^{bc}	11.49 ^e	20.66 ^{cde}	29.69 ^d	0.25 ^c
B0U2	6.64 ^{bc}	1.84 ^{bc}	3.80 ^b	2.75 ^{bc}	42.45 ^a	11.33 ^{bc}	3.29 ^{ab}	1.24 ^{bc}	12.49 ^{bc}	20.08 ^c	31.08 ^b	0.26 ^b
B1U1	6.64 ^{bc}	1.84 ^{bc}	3.77 ^b	2.75 ^{bc}	42.45 ^a	11.33 ^{bc}	3.29 ^{ab}	1.24 ^{bc}	11.73 ^d	20.08 ^c	29.72 ^d	0.26 ^b
B1U2	6.66 ^{ab}	1.85 ^{bc}	3.80 ^b	2.76 ^{ab}	42.45 ^a	11.39 ^{ab}	3.31 ^{ab}	1.25 ^b	12.40 ^{bc}	21.09 ^{bc}	31.12 ^b	0.26 ^b
B2U1	6.64 ^{bc}	1.85 ^{bc}	3.80 ^b	2.76 ^{ab}	42.37 ^{ab}	11.39 ^{ab}	3.29 ^{ab}	1.23 ^d	12.40 ^{bc}	21.05 ^{bc}	29.88 ^d	0.28 ^a
B2U2	6.67 ^{ab}	1.85 ^{bc}	3.80 ^b	2.76 ^{ab}	42.45 ^a	11.41 ^a	3.31 ^{ab}	1.25 ^b	12.50 ^b	21.47 ^b	30.54 ^c	0.28 ^a
B3U1	6.67 ^{ab}	1.87 ^a	3.80 ^b	2.76 ^{ab}	42.45 ^a	11.42 ^a	3.29 ^{ab}	1.28 ^a	12.40 ^{bc}	21.57 ^a	31.45 ^a	0.28 ^a
B3U2	6.67 ^a	1.87 ^a	3.81 ^a	2.77 ^a	42.45 ^a	11.42 ^a	3.33 ^a	1.28 ^a	12.60 ^a	21.58 ^a	31.58 ^a	0.28 ^a
PU1	6.64 ^{bc}	1.85 ^{bc}	3.77 ^b	2.75 ^{bc}	42.37 ^{ab}	11.39 ^{ab}	3.27 ^{cd}	1.24 ^{bc}	11.70 ^d	20.60 ^c	30.12 ^c	0.24 ^c
PU2	6.64 ^{bc}	1.85 ^{bc}	3.77 ^b	2.75 ^{bc}	42.45 ^a	11.39 ^{ab}	3.29 ^{ab}	1.24 ^{bc}	12.43 ^{bc}	20.10 ^c	30.20 ^c	0.24 ^{ac}
S2 (Maha - 2023/24)												
B0U1	6.66 ^c	1.82 ^{bcd}	3.75 ^{ab}	2.71 ^{cd}	42.30 ^b	10.64 ^{cde}	3.27 ^{bc}	1.21 ^d	11.30 ^e	20.10 ^e	29.61 ^d	0.26 ^c
B0U2	6.67 ^b	1.86 ^{ab}	3.77 ^{ab}	2.72 ^{cd}	42.30 ^b	10.72 ^{cd}	3.27 ^{bc}	1.24 ^b	11.30 ^e	20.22 ^d	30.05 ^c	0.26 ^c
B1U1	6.66 ^c	1.84 ^{bc}	3.76 ^{ab}	2.72 ^{cd}	42.30 ^b	10.76 ^{cd}	3.27 ^{bc}	1.24 ^b	11.75 ^d	20.93 ^c	30.10 ^b	0.28 ^b
B1U2	6.67 ^b	1.86 ^{ab}	3.76 ^{ab}	2.73 ^{cd}	42.30 ^b	11.29 ^a	3.27 ^{bc}	1.25 ^{ab}	12.25 ^c	20.72 ^c	31.13 ^b	0.31 ^{ab}
B2U1	6.67 ^b	1.84 ^{bc}	3.76 ^{ab}	2.74 ^{abc}	42.33 ^{ab}	11.12 ^{ef}	3.27 ^{bc}	1.25 ^{ab}	12.25 ^c	20.93 ^c	30.15 ^b	0.3 ^{ab}
B2U2	6.67 ^b	1.89 ^a	3.76 ^{ab}	2.74 ^{abc}	42.33 ^{ab}	11.10 ^{ab}	3.29 ^{ab}	1.26 ^a	12.44 ^b	21.24 ^b	30.12 ^b	0.30 ^{ab}
B3U1	6.67 ^b	1.85 ^{abc}	3.77 ^{ab}	2.75 ^{ab}	42.35 ^a	11.03 ^b	3.29 ^{ab}	1.26 ^a	12.47 ^b	21.29 ^{ab}	30.15 ^b	0.32 ^a
B3U2	6.69 ^a	1.89 ^a	3.79 ^a	2.76 ^a	42.35 ^a	11.31 ^a	3.30 ^a	1.26 ^a	12.54 ^a	21.34 ^a	30.30 ^a	0.32 ^a
PU1	6.67 ^b	1.84 ^{bc}	3.75 ^{ab}	2.70 ^{cd}	42.31 ^b	10.63 ^{cde}	3.26 ^{bc}	1.23 ^c	11.72 ^d	20.13 ^e	29.78 ^d	0.28 ^b
PU2	6.69 ^a	1.85 ^{abc}	3.76 ^{ab}	2.71 ^{cd}	42.31 ^b	10.63 ^{cde}	3.25 ^c	1.23 ^c	11.73 ^d	20.12 ^e	30.09 ^c	0.28 ^b
B	s	s	s	s	s	s	s	s	s	s	s	s
U	s	ns	s	ns	s	s	ns	s	s	s	s	s
S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B×U	s	s	s	s	s	s	s	ns	s	s	s	s
B×S	ns	ns	ns	ns	ns	s	ns	ns	ns	ns	ns	ns
U×S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B×U×S	ns	ns	ns	ns	ns	s	ns	ns	ns	ns	ns	ns

N.B: Means of treatments in different seasons within the same column followed by different letters indicate significant difference between them by LSD test at p< 0.05, B: Biochar carbon, U: Urea, P: Partially burnt paddy husk, S: Season, GL: Grain length (mm), GW: Grain width (cm), L/W: Grain length and width ratio, E: Grain equivalent diameter (mm), GSP: Grain sparcity (%), GV: Grain volume (mm³), GSA: Grain surface area (mm²), GN: Grain nitrogen content (ppm), ASC: Grain ash content (%), IC: Grain iron content (µg/g), AC: Grain amylose content (%) and GP: Grain protein content (%).

Nitrogen use efficiency metrics and nutrient contents of rice straw

As indicated in figures 3A, B, and C, the application of biochar combined with urea significantly influenced nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUE), and nitrogen uptake efficiency (NUE) in rice. The highest values for these parameters were observed in treatments with the highest amount of RHB and urea fertilizer. Notably, NUpE during the Yala season and NUtE during both seasons showed no significant differences between the B3U1 and B3U2 treatments, despite the higher urea application in B3U2. On average, B3U2 treatment showed increases of 9% in NUpE, 9% in NUtE, and

16% in NUE compared to the urea-only treatment. In PU2 treatment, there were no changes in the NUpE, but there were reductions in NUtE and NUE of 3% and 2%, respectively.

Interestingly, in this experiment, higher amounts of NPK levels were observed in the straw, 4 weeks after harvesting (Table 5). The combined application of RHB and urea led to significant increments in straw nitrogen (StN), potassium (StK), and phosphorus (StP) contents in the B3U2 treatment than in all other treatments, with increments of 13%, 6% and 5% respectively. In the PU2 treatment, a 5% increment in StN, 4% reduction of StP, and no change in StK were observed compared to the urea-only treatment (B0U2)

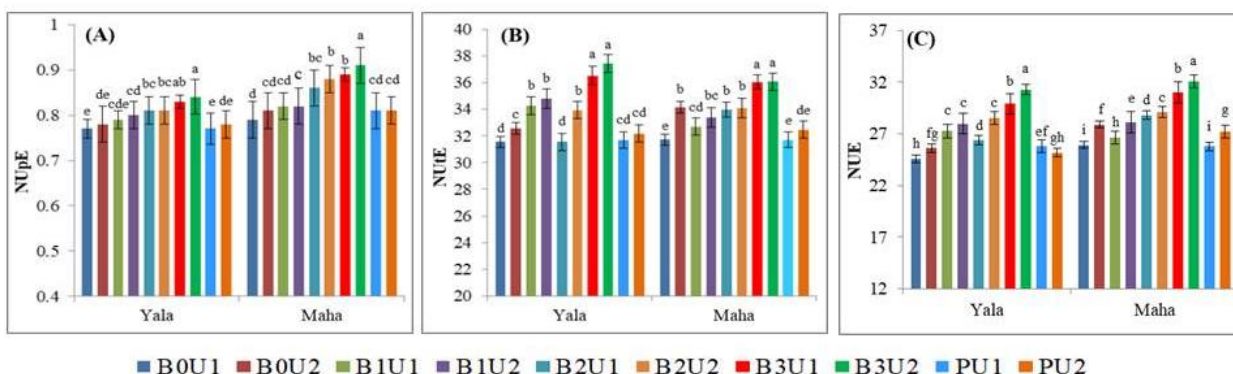


Figure 3: Effect of rice husk biochar and urea levels on nitrogen uptake efficiency (NUpE) (A), nitrogen utilization efficiency (NUE) (B) and nitrogen use efficiency (NUE) (C) for Yala 2023 and Maha 2023/24.

Table 5: Changes in shoot phosphorus (StP), potassium (StK), and nitrogen (StN) levels after harvesting as influenced by different levels of rice husk biochar and urea.

Treatments	S1 (Yala - 2023)			S2 (Maha - 2023/24)		
	StP (ppm)	StK (ppm)	StN (ppm)	StP (ppm)	StK (ppm)	StN (ppm)
B0U1	1.55 ^d	0.73 ^{cde}	26.01 ^f	1.56 ^f	0.74 ^{bcd}	24.11 ^g
B0U2	1.56 ^{cd}	0.73 ^{cde}	26.57 ^e	1.58 ^{cd}	0.78 ^b	25.22 ^f
B1U1	1.56 ^{cd}	0.73 ^{cde}	26.43 ^e	1.57 ^{de}	0.76 ^{bc}	26.43 ^e
B1U2	1.58 ^{bc}	0.76 ^{bc}	27.27 ^d	1.59 ^{bc}	0.78 ^b	26.43 ^e
B2U1	1.54 ^{de}	0.78 ^b	28.60 ^c	1.57 ^{de}	0.76 ^{bc}	28.67 ^c
B2U2	1.59 ^b	0.78 ^b	28.70 ^c	1.59 ^{bc}	0.78 ^b	28.70 ^c
B3U1	1.56 ^{cd}	0.78 ^b	29.09 ^b	1.61 ^b	0.78 ^b	29.09 ^b
B3U2	1.65 ^a	0.79 ^a	29.37 ^a	1.65 ^a	0.81 ^a	29.37 ^a
PU1	1.42 ^f	0.72 ^{de}	26.43 ^e	1.55 ^{ef}	0.76 ^{bc}	27.27 ^d
PU2	1.46 ^g	0.73 ^{cde}	27.27 ^d	1.56 ^{ef}	0.78 ^b	27.27 ^d

NB: Means of treatments within the same column followed by different letters indicate significant difference between them by LSD test at $p < 0.05$, B: Biochar carbon, U: Urea, P: Partially burnt paddy husk, StP: Shoot phosphorous content (ppm), StK: Shoot potassium content (ppm), StN: Shoot nitrogen content (ppm).

Discussion

Soil quality: The soil properties mentioned in Table 1 and Figure 1 were significantly improved by the combined application of urea and RHB, particularly in the highest application of urea and RHB (B3U2), while slight reduction in soil pH and SN was observed compared to the initial values. Previous studies have documented biochar's benefits extensively. Deniel *et al.*, (2018) found that RHB increased soil pH due to its alkaline nature. However, Abrishamkesh *et al.*, (2015) and Asadi *et al.*, (2021) noted that in alkaline soils, RHB did not affect on soil pH, but in acidic soils, RHB raised pH by 1 to 2 units at rates of 3 to 12 t/ha of biochar (Asadi *et al.*, 2021). Ding *et al.*, (2022)

reported a 0.34 increase in pH and significant boosts in total organic carbon, total nitrogen, and available phosphorus by 114.22%, 17.21%, and 45.51%, respectively. Conversely, nitrogen alone decreased pH by 0.32 (Linh *et al.*, 2023). Research by Oladele *et al.*, (2019) and Ding *et al.*, (2022) supports that biochar can increase soil pH in acidic soils due to its high buffering capacity and nutrient-rich composition. However, while RHB generally raises pH, nitrogen fertilizers like urea tend to lower it (Singh *et al.*, 2018). In this study, urea's significant pH reduction may outweigh RHB's pH increase. The quality of urea fertilizers also significantly affects the soil pH (Fageria *et al.*, 2010).

Nawaz *et al.*, (2018) found biochar could reduce soil electrical conductivity (EC), but this study observed a slight increase. Urea increases soil EC through hydrolysis, converting to ammonium and carbonate ions, while biochar might reduce EC by immobilizing sodium ions (Hammer *et al.*, 2015). Fahad *et al.*, (2016) suggested biochar might mitigate salinity effects, but combined biochar and nitrogen applications may create complex nutrient interactions, warranting further study (Asai *et al.*, 2009, Albuquerque *et al.*, 2013). Similar to the results of this study, several other studies indicate biochar enhances OC and OM due to its stable carbon form, which improves soil structure and microbial activity (Asai *et al.*, 2009, Yuxue *et al.*, 2016, Ali *et al.*, 2020b).

Oladele *et al.*, (2019) reported that biochar and nitrogen fertilizer together enhance soil total nitrogen, available phosphorus, and potassium although a reduction in SN was reported in this study. Biochar's porous structure and surface area retain and slowly release nutrients, reducing leaching (Oladele *et al.*, 2019, Qin *et al.*, 2023). However, biochar can decrease nitrogen availability due to nitrate adsorption and immobilization (Clough *et al.*, 2013, Zheng *et al.*, 2013). Apart from nitrogen adsorption by biochar, nitrogen assimilation by plants, enhanced leaching, volatilization, and denitrification due to various conditions and high initial nitrogen levels can also contribute to SN reduction in a pot experiment.

Biochar usually increases available phosphorus by preventing its fixation onto soil colloids, benefiting crop production (Hammer *et al.*, 2015, Ali *et al.*, 2020b). Its impact on potassium and other nutrient dynamics varies with biochar type and application rates. Some studies suggest that biochar combined with nitrogen may not significantly improve soil cation exchange capacity (CEC) or nutrient levels (Ning *et al.*, 2022), indicating a need for long-term monitoring.

Analysis was revealed that biochar and urea have significantly impacted on soil pH, EC, OC, SN, SK and SP. However, there were not significant interactions between biochar, urea and season (B:U:S) for most of soil parameters except the soil nitrogen as well as pH was significantly differed according to the season.

Results revealed that, combining RHB and urea fertilizer appears to enhance soil chemical properties. However, further research is needed to understand long-term impacts and optimize application methods across different agricultural contexts. Biochar can improve plant growth by enhancing soil nutrient retention, but effects vary based on feedstock, pyrolysis conditions, soil type, and fertilizer management (Asai *et al.*, 2009, Clough *et al.*, 2013, Hammer *et al.*, 2015, Oo *et al.*, 2018)

Leaf pigment traits: The combined application of RHB and urea in this study significantly impacted leaf total chlorophyll (TCL) and carotenoid (CAD) contents. Previous studies provided limited information on direct measurements between the application of RHB and N fertilizer on TCL and CAD concentration. However, several studies (Ali *et al.*, 2020b, Ali *et al.*, 2021) have reported increased concentrations of CLa and CLb with the application of RHB and N fertilizer. In some studies (Haque *et al.*, 2022) SPAD meter reading were measured instead of chlorophyll, and increased values were observed with the application of biochar. These increments might be the indirect effect of increased physicochemical properties, nutrient availability (Ali *et al.*, 2020a, Ali *et al.*, 2020b) and enhanced activity of nitrogen metabolism enzymes in rice plants, such as nitrate reductase, glutamine synthetase, and glutamine oxoglutarate aminotransferase (Ali *et al.*, 2021) due to the application of biochar. Additionally, the positive effects of biochar and nitrogen fertilizer on leaf pigment concentrations are not limited to rice. For example, increased chlorophyll content in the leaves was observed by Wang *et al.*, (2014) in *Malus hupehensis* Rehd. seedlings, similarly, Ogawa and Okimori, (2010) in soybean plants and (Gao *et al.*, 2017) in peanut plants. Moreover, seasonal effects were not impacted on leaf pigment synthesis as well as three factors of biochar, urea and season also was not significant on chlorophyll and carotenoid production of rice leaves.

Rice plant growth and yield traits: The B3U2 treatment, with the highest levels of biochar and urea, showed the most substantial significant improvements in rice plant growth and yield parameters in both seasons (Tables 3), compared to the treatment applying urea only (B0U2). In previous studies, Ali *et*

al., (2021) found an increase in plant height, number of tillers, panicle length, and grain weight in 4%, 62%, 23%, and 30% respectively, with the application of 30t/ha RHB and 180 kg/ha N fertilizer. However, the amount of RHB and N fertilizer used in this experiment was greater than in the present study. Further, Daniel *et al.* (2018) reported 4% and 35% increments of TN and RDW, respectively with the application of RHB. Lai *et al.*, (2024) have also noted that a significant increment of rice yield, plant biomass, tiller number, panicle length, grains per panicle, and grain weight per panicle with combined application of chemical fertilizers and biochar. Oladele *et al.*, (2019) and (Zhao *et al.*, 2023) also reported an increase in rice yield, plant height, productive tiller numbers, and leaf area index with the application of biochar and nitrogen fertilizer. According to these researchers these increments are attributed to enhanced soil properties, including increased pH and nutrient availability. The significant increase in RDW compared to SDW in the present study supports observations by Abrishamkesh *et al.*, (2015), who reported increased root biomass in lentils with RHB application. Although in the present study, soil physiological properties like bulk density and porosity have not been measured, biochar's ability to improve these properties is well documented (Lehmann *et al.*, 2015). Decreased bulk density and improved porosity can create favourable environment for root growth. In contrast, the combined application of PBPH and high amount of urea (PU2) improved RDW, SDW, and PL but resulted in a 2% GY reduction compared to the control (B0U2), across two seasons suggesting that RHB is more beneficial than PBPH. In the process of preparation of PBPH, incomplete combustion takes place. Therefore, the properties of PBPH and RHB are different, which may be the reason why PBPH does not yield similar benefits as RHB. Barman and Kandpal, (2019) have documented that the effectiveness of biochar differs with the production process and the type of biomass used. Interestingly, PH, TN, IL, SDW, and TGW values in the B3U2 treatment did not significantly differ from those in the lower nitrogen treatment (B3U1) in both seasons. However, NL and PN in the B3U2 treatment did not significantly differ from the B3U1 treatment only in the Yala season. RDW, PL, and GY of B3U2 were significantly different from B3U1 in both seasons. Furthermore, seasonal effects

were significantly impacted on plant height and number of tillers per plant of the rice crop, but were not significant on other growth and yield parameters. Three factors as biochar, urea, and season were significantly impacted INL, RDW, SDW, PN, PL, and GY, although TGW and NL were not significantly different according to three-factor variation. Therefore, further experiments are needed to explore the potential for reducing the recommended amount of urea fertilizer for rice by applying RHB.

Changes in grain physical and nutritional properties:

In this study, grain chemical and nutritional properties were also influenced by the combined application of RHB and urea (Table 4). Consistent with the results of the present study, previous studies also reported increments of grain protein, nitrogen, and amylose contents with the application of RHB and urea (Ali *et al.*, 2020b, Haque *et al.*, 2022). The ash portion of the biochar consists of oxides, hydroxides, and carbonates of various cations like potassium, sodium, calcium, and silicon (Butnan and Vityakon, 2023). These components become available for plant uptake when biochar is applied to the soil, and these components can influence the final composition of rice grains. While previous studies do not directly address the impact of biochar on iron content in rice grains, they do indicate that biochar can influence iron availability in the soil. As an example, biochar can influence soil pH, which in turn affects iron solubility and plant uptake. Contradictory findings were reported Butnan and Vityakon, (2023) in relation to the application of RHB and iron uptake by rice. They found that prolonged RHB incorporation periods led to iron deficiency in rice plants, while Anwari *et al.*, (2019) suggested that biochar itself could be a source of iron for plants.

Few studies have examined biochar's impact on the amylose content of rice grains. Ali *et al.*, (2020b) found that biochar application can increase amylose content of noodle rice. However, Gong *et al.*, (2020) reported a decrease in AC in two japonica rice varieties with the application of biochar. The influence on start-related enzymes and gene expression was attributed to this decrease.

The percentage of changes in grain physical parameters due to different levels of RHB and urea are considerably lower than the chemical properties

(Table 4). However, biochar, alone or combined with urea, significantly impacted grain physical parameters across seasons. In previous studies, Ali *et al.*, (2022) found a 23% increase in GL with biochar and nitrogen fertilizer than treatment without biochar, but no significant differences in GW. However, Fahad *et al.*, (2016) reported increased GW and grain size in the rice cultivar “Huanghuazhan”, but not in the cultivar “IR-64”.

Previous studies did not directly address the effect of biochar and nitrogen fertilizer on grain ED, Ø, GV, and GSA, however they do suggest that the combined application of RHB and urea can affect on grain size and weight. Biochar with N fertilizer has been found to increase TGW of rice (Lai *et al.*, 2017, Gowthami *et al.*, 2022). However, Zhao *et al.*, (2023) found that biochar application did not significantly affect TGW in the study conducted on maize straw and RHB with nitrogen fertilizer. As discussed earlier multiple studies showed the increase in grain yield with the application of RHB and N fertilizer than the treatments alone nitrogen fertilizer. Except for grain volume, all other grain quality attributes were not significant according to the season or three factors as biochar, urea and season. Further experiments are needed to fill the research gap on effect of biochar and N fertilizer on grain physical parameters.

Nitrogen use efficiency metrics and nutrient contents of rice straw

There are several studies to prove that the RHB combined with nitrogen fertilizer can improve NUE of rice with enhanced nitrogen uptake and reduced nitrogen losses by adsorbing nitrogen ions (Deniel *et al.*, 2018, Haque *et al.*, 2022, Lai *et al.*, 2024) and improved soil properties like soil pH, organic matter content, cation exchange capacity (Ali *et al.*, 2021, Haque *et al.*, 2022, Selvarajh *et al.*, 2024). This NUpE is more prominent in nitrogen-poor soils (Fahad *et al.*, 2016, Ali *et al.*, 2020b). Further biochar can increase NUpE by influencing soil microbial activities, affecting nitrogen mineralization and immobilization (Feng *et al.*, 2014, Huang *et al.*, 2018). However, the rate of RHB and urea fertilizer for maximizing NUE

and rice yield is highly dependent on soil type, environmental conditions, rice cultivar, and the method of pyrolyzing (Lai *et al.*, 2017, Qin *et al.*, 2023, Lai *et al.*, 2024). The results of this experiment, along with previous studies, emphasize that biochar can enhance the effectiveness of N fertilizer, but not reduce the amount required. Therefore, rather than reducing the application of N fertilizer, increasing its effectiveness by adding biochar as a sustainable soil management strategy will reduce the environmental impact of N fertilizer while increasing crop productivity.

None of the studies focused on the nutrient content of rice straw with the effect of application of RHB and urea fertilizer. In this experiment, higher amounts of NPK levels were observed in the straw, 4 weeks after harvesting with the application of RHB. Rice straw is a common agricultural residue used for many purposes in agriculture, such as mulching and organic manure, both in its raw form and after composting. Therefore, the increase of nutrient content finally affects the quality of the organic manure.

Correlations analysis

The relationship between rice grain yield, soil chemical properties, nitrogen use efficiency, rice growth and yield parameters, and grain chemical and physical properties is depicted in Figure 4. The correlation analysis of the heat map reveals that variations in rice grain yield are influenced by a multitude of parameters measured in the study. Specifically, rice grain yield exhibited positive correlations with several factors: soil properties (i.e. EC, SK, OC, and OM), plant growth and yield parameters: (i.e. StP, StK, GN, TGW, RDM, SDM, and TCL), grain chemical and physical properties (i.e. AMC, ASC, IC, GV, and SP) and nitrogen use efficiency metrics (i.e. NutE and NUE). Conversely, grain yield showed a negative correlation with soil pH, indicating that higher pH levels were associated with lower yields. Notably, no significant correlation was observed between grain yield and grain ED. All other measured properties demonstrated varying degrees of positive correlation with rice grain yield.

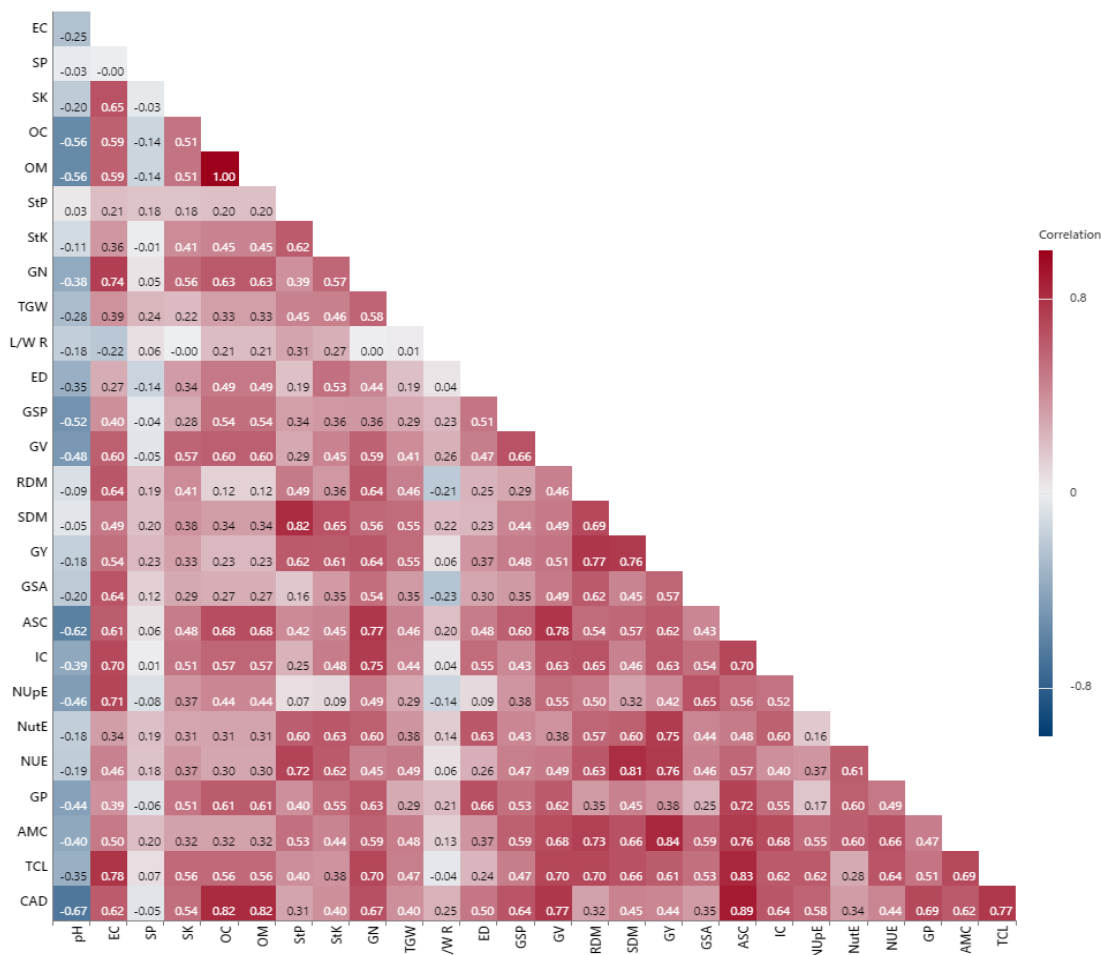


Figure 4: Heatmap correlation matrix representing Pearson correlation coefficients of soil properties, growth, yield and some quality attributes of rice (pH - Acidity, EC - Electrical conductivity, SP - Soil phosphorus, SK - Soil potassium, OC - Organic carbon, OM - Organic matter, StP - Shoot phosphorous, StP - Shoot potassium, GN - Grain nitrogen, TGW - Thousand grain weight, L/W R - Grain length and width ratio, ED - Equivalent diameter, GSP - Grain sphericity, GV - Grain volume, RDM - Root dry matter, SDM - Shoot dry matter, GY - Grain yield, GSA - Grain surface area, ASC – Grain ash content, IC – Grain iron content, NUpE - Nitrogen uptake efficiency, NutE - Nitrogen utilization efficiency, NUE - Nitrogen use efficiency, GP - Grain protein, AMC – Grain amylose content, and TCL - Total chlorophyll level, CAD - Carotenoids content).

Conclusion

The findings of this study clearly demonstrate that the application of RHB carbon at 3%, combined with urea fertilizer at 225 kg/ha (103.5 kg N/ha), leads to significant improvements in soil physicochemical properties, nitrogen uptake, and overall rice grain quality and yield of the AT362 rice variety under low country dry zone conditions in Sri Lanka. Notably, the RHB treatment resulted in enhanced concentrations of total chlorophyll and carotenoid pigments, contributing to better plant health and productivity.

Compared to the PBPH treatment, RHB consistently delivered superior results across all measured parameters. Therefore, the application of 3% RHB carbon in combination with 225 kg/ha urea fertilizer is recommended as an effective strategy for optimizing the yield of the AT 362 rice variety, improving soil health, and enhancing nitrogen use efficiency under low country dry zone conditions in Sri Lanka. Overall, the results of the present study again claim that RHB, particularly combined with urea fertilizer, can positively affect soil properties and rice production. However, it is important to note that these results were

obtained from a pot experiment. The effects of biochar on nitrogen fertilizers might differ under field conditions. Thus, long-term field experiments are necessary to validate these findings and determine the broader applicability of this approach in various agricultural settings. Similarly, further experiments are needed to see the possibility of reducing N fertilizer requirement with the addition of RHB.

Acknowledgement

The authors would like to acknowledge the Department of Agriculture, Sri Lanka for providing the necessary facilities to conduct this research study.

References

- Abrishamkesh, S., M. Gorji, H. Asadi, G. H. Bagheri-Marandi, and A. A. Pourbabae. (2015). Effects of rice husk biochar application on the properties of alkaline soil and lentil growth. *Plant, Soil and Environment* 61:475-482. <https://doi.org/10.17221/117/2015-PSE>.
- Abukari, A., A. A. Abunyewa, and H. Issifu. (2019). Effect of rice husk biochar on nitrogen uptake and grain yield of maize in the Guinea savanna zone of Ghana. *UDS International Journal of Development* 5:1-6. <https://doi.org/10.47740/290.UDSIJD6i>.
- Adebajo, S. O., F. Oluwatobi, P. O. Akintokun, A. E. Ojo, A. K. Akintokun, and I. S. Gbodepe. (2022). Impacts of rice-husk biochar on soil microbial biomass and agronomic performances of tomato (*Solanum lycopersicum* L.). *Scientific Reports* 12:1787. <https://doi.org/10.1038/s41598-022-05757-z>.
- Albuquerque, J. A., P. Salazar, V. Barrón, J. Torrent, M. d. C. del Campillo, A. Gallardo, and R. Villar. (2013). Enhanced wheat yield by biochar addition under different mineral fertilization levels. *Agronomy for Sustainable Development* 33:475-484. <https://doi.org/10.1007/s13593-012-0128-3>.
- Ali, I., L. He, S. Ullah, Z. Quan, S. Wei, A. Iqbal, F. Munsif, T. Shah, Y. Xuan, Y. Luo, L. Tianyuan, and J. Ligeng. (2020a). Biochar addition coupled with nitrogen fertilization impacts on soil quality, crop productivity, and nitrogen uptake under double-cropping system. *Food and Energy Security* 9:e208. <https://doi.org/10.1002/fes3.208>.
- Ali, I., S. Ullah, L. He, Q. Zhao, A. Iqbal, S. Wei, T. Shah, N. Ali, Y. Bo, and M. Adnan. (2020b). Combined application of biochar and nitrogen fertilizer improves rice yield, microbial activity and N-metabolism in a pot experiment. *PeerJ* 8:e10311. <http://dx.doi.org/10.7717/peerj.10311>.
- Ali, I., P. Yuan, S. Ullah, A. Iqbal, Q. Zhao, H. Liang, A. Khan, Imran, H. Zhang, X. Wu, S. Wei, M. Gu, and L. Jiang. (2022). Biochar Amendment and Nitrogen Fertilizer Contribute to the Changes in Soil Properties and Microbial Communities in a Paddy Field. *Frontiers in Microbiology* Volume 13 - 2022. <https://doi.org/10.3389/fmicb.2022.834751>.
- Ali, I., Q. Zhao, K. Wu, S. Ullah, A. Iqbal, H. Liang, J. Zhang, I. Muhammad, Amanullah, A. Khan, A. A. Khan, and L. Jiang. (2021). Biochar in Combination with Nitrogen Fertilizer is a Technique: To Enhance Physiological and Morphological Traits of Rice (*Oryza sativa* L.) by Improving Soil Physio-biochemical Properties. *Journal of Plant Growth Regulation* 41:2406-2420. 10.1007/s00344-021-10454-8.
- Anwari, G., H. Xiaoxuan, Z. Yiming, Y. Tianxu, W. Wenan, Z. Bowen, W. Xin, L. Qingtian, S. Xiwen, and A. A. Moussa. (2019). Effects of rice-husk biochar and aluminum sulfate application on rice grain quality in saline-sodic soil of paddy-field. *International Journal of Biosciences* 15:325-333.
- Asadi, H., M. Ghorbani, M. Rezaei-Rashti, S. Abrishamkesh, E. Amirahmadi, C. Chengrong, and M. Gorji. (2021). Application of Rice Husk Biochar for Achieving Sustainable Agriculture and Environment. *Rice Science* 28:325-343. <https://doi.org/10.1016/j.rsci.2021.05.004>.
- Asai, H., B. K. Samson, H. M. Stephan, K. Songyikhangsuthor, K. Homma, Y. Kiyono, Y. Inoue, T. Shiraiwa, and T. Horie. (2009). Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Research* 111:81-84. <https://doi.org/10.1016/j.fcr.2008.10.008>.

- Barman, R., and G. Kandpal. (2019). Impact of Rice Husk Biochar on Agriculture. *Bulletin of Environment, Pharmacology and Life Sciences* 8:25-27.
- Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science* 55:11-33. <https://doi.org/10.1017/S0021859600021572>.
- Butnan, S., and P. Vityakon. (2023). Lengths of Time of Rice Husk Biochar Incorporation before Planting Affect Soil Properties and Rice Yield. *Agronomy* 13:1445. <https://doi.org/10.3390/agronomy13061445>.
- Cassman, K. G., A. Dobermann, D. T. Walters, and H. Yang. (2003). Meeting Cereal Demand While Protecting Natural Resources and Improving Environmental Quality. *Annual Review of Environment and Resources* 28:315-358. <https://doi.org/10.1146/annurev.energy.28.040202.122858>.
- Clough, T. J., L. M. Condron, C. Kammann, and C. Müller. (2013). A Review of Biochar and Soil Nitrogen Dynamics. *Agronomy* 3:275-293.
- Deniel, S., A. Rosenani, S. Ahmad, and K. Abdul Rahim. (2018). Influences of rice husk biochar (rhb) on rice growth performance and fertilizer nitrogen recovery up to maximum tillering stage. *Journal of Wetlands Environmental Management* 6:32-44.
- Ding, Y., S. Zhu, R. Pan, J. Bu, Y. Liu, and A. Ding. (2022). Effects of Rice Husk Biochar on Nitrogen Leaching from Vegetable Soils by ¹⁵N Tracing Approach. *Water* 14:3563.
- Fageria, N. K., O. P. de Moraes, and A. B. dos Santos. (2010). Nitrogen use efficiency in upland rice genotypes. *Journal of Plant Nutrition* 33:1696-1711. <https://doi.org/10.1080/01904167.2010.496892>.
- Fahad, S., S. Hussain, S. Saud, S. Hassan, M. Tanveer, M. Z. Ihsan, A. N. Shah, A. Ullah, Nasrullah, F. Khan, S. Ullah, H. Alharby, W. Nasim, C. Wu, and J. Huang. (2016). A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. *Plant Physiology and Biochemistry* 103:191-198. <https://doi.org/10.1016/j.plaphy.2016.03.001>.
- FAO. (2019). Soil organic carbon Walkley-black method: Titration and Colorimetric Method. Food and Agriculture Organization of the United Nations. <https://openknowledge.fao.org/server/api/core/bitstreams/e498d73e-1711-4d18-9183>
- FAO. (2021). Standard operating procedure for soil available phosphorus - Olsen method. Food and Agriculture Organization of the United Nations, Rome.
- Feng, L., G.-t. Li, Q.-m. Lin, and X.-r. Zhao. (2014). Crop Yield and Soil Properties in the First 3 Years After Biochar Application to a Calcareous Soil. *Journal of Integrative Agriculture* 13:525-532. [https://doi.org/10.1016/S2095-3119\(13\)60708-X](https://doi.org/10.1016/S2095-3119(13)60708-X).
- Gao, M., X. Liu, N. Li, P. Luo, X. Han, and J. Yang. (2017). The impact of application of biocar on peanuts growing. *IOP Conference Series: Materials Science and Engineering* 274:012156. <https://doi.org/doi:10.1088/1757-899X/274/1/012156>.
- Gong, D., X. Xu, L. a. Wu, G. Dai, W. Zheng, and Z. Xu. (2020). Effect of biochar on rice starch properties and starch-related gene expression and enzyme activities. *Scientific Reports* 10:16917. <https://doi.org/10.1038/s41598-020-73888-2>.
- Gowthami, V., B. Venkateswarlu, P. Prasad, C. S. Rao, S. R. Kumari, and C. N. Jyothi. (2022). Effect of Rice Husk Biochar and Inorganic Nitrogen on Growth and Yield Parameters of Direct Seeded Rice. *The Andhra Agricultural Journal* 69:179-187.
- Hammer, E. C., M. Forstreuter, M. C. Rillig, and J. Kohler. (2015). Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. *Applied Soil Ecology* 96:114-121. <https://doi.org/10.1016/j.apsoil.2015.07.014>.

- Haque, A. N. A., M. K. Uddin, M. F. Sulaiman, A. M. Amin, M. Hossain, Z. M. Solaiman, A. A. Aziz, and M. Mosharrof. (2022). Combined Use of Biochar with 15Nitrogen Labelled Urea Increases Rice Yield, N Use Efficiency and Fertilizer N Recovery under Water-Saving Irrigation. *Sustainability* 14:7622.
- Huang, M., L. Fan, J. Chen, L. Jiang, and Y. Zou. (2018). Continuous applications of biochar to rice: Effects on nitrogen uptake and utilization. *Scientific Reports* 8:11461. <http://doi.org/10.1038/s41598-018-29877-7>.
- Juliano, B. O. (1971). A simplified assay for milled-rice amylose. *Cereal Science Today* 16:334-340.
- Karam, S., Daljit, P. Nagabovanalli, K. Sundara Rajoo, C. Fauziah Ishak, A. Abdu, Z. Rosli, F. Melissa Muharam, and D. Zulperi. (2022). An overview on the preparation of rice husk biochar, factors affecting its properties, and its agriculture application. *Journal of the Saudi Society of Agricultural Sciences* 21:149-159. <https://doi.org/10.1016/j.jssas.2021.07.005>.
- Lai, C., R. Muon, V. Touch, S. Hin, P. Podwojewski, P. Ket, P. Jouquet, A. Degré, and V. Ann. (2024). Impact of Biochar from Rice Husk on Nutrient Distribution and Rice Growth and Yield: A Soil Column Experiment. *Journal of Soil Science and Plant Nutrition* 24:159-171. 10.1007/s42729-023-01539-2.
- Lai, L., M. R. Ismail, F. M. Muharam, M. M. Yusof, R. Ismail, and N. M. Jaafar. (2017). Effects of rice straw biochar and nitrogen fertilizer on rice growth and yield. *Asian Journal of Crop Science* 9:159-166. <https://doi.org/10.3923/ajcs.2017.159.166>.
- Le Gouis, J., D. Béghin, E. Heumez, and P. Pluchard. (2000). Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. *European Journal of Agronomy* 12:163-173. [https://doi.org/10.1016/S1161-0301\(00\)00045-9](https://doi.org/10.1016/S1161-0301(00)00045-9).
- Lehmann, J., Y. Kuzyakov, G. Pan, and Y. S. Ok. (2015). Biochars and the plant-soil interface. *Plant and Soil* 395:1-5. 10.1007/s11104-015-2658-3.
- Linh, D. T. T., C. M. Khoi, K. Ritz, N. V. Sinh, N. T. K. Phuong, H. M. T. My, T. B. Linh, D. D. Minh, T. T. Linh, and K. Toyota. (2023). Effects of Rice Husk Biochar and Compost Amendments on Soil Phosphorus Fractions, Enzyme Activities and Rice Yields in Salt-Affected Acid Soils in the Mekong Delta, Viet Nam. *Agronomy* 13:1593.
- Liu, S., Y. Zhang, Y. Zong, Z. Hu, S. Wu, J. Zhou, Y. Jin, and J. Zou. (2016). Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. *GCB Bioenergy* 8:392-406. <https://doi.org/10.1111/gcbb.12265>.
- Munasinghe, S., R. Dharmakeerthi, P. Weerasinghe, and L. Madusanka. (2018). Preparation of biochar as a soil amendment from rice husk and corn cob by slow pyrolysis process. *Tropical Agriculturist* 166:137-151.
- Nawaz, H., F. Ahmed, W. Abbas, J. Ahmed, Z. Mehmood, H. Ullah, and N. U. Basar. (2018). Soil electrical conductivity as affected by biochar under summer crops. *International Journal of Environmental Sciences & Natural Resources* 14:54-57. <https://doi.org/10.19080/IJESNR.2018.14.555887>.
- Ning, C., R. Liu, X. Kuang, H. Chen, J. Tian, and K. Cai. (2022). Nitrogen Fertilizer Reduction Combined with Biochar Application Maintain the Yield and Nitrogen Supply of Rice but Improve the Nitrogen Use Efficiency. *Agronomy* 12:3039. <https://doi.org/10.3390/agronomy12123039>.
- Ogawa, M., and Y. Okimori. (2010). Pioneering works in biochar research, Japan. *Australian Journal of Soil Research* 48:489-500. <https://doi.org/10.1071/sr10006>.
- Oladele, S. O., A. J. Adeyemo, and M. A. Awodun. (2019). Influence of rice husk biochar and inorganic fertilizer on soil nutrients availability and rain-fed rice yield in two contrasting soils. *Geoderma* 336:1-11. <https://doi.org/10.1016/j.geoderma.2018.08.025>.
- Oo, A. Z., S. Sudo, H. Akiyama, K. T. Win, A. Shibata, A. Yamamoto, T. Sano, and Y. Hirono. (2018). Effect of dolomite and

- biochar addition on N₂O and CO₂ emissions from acidic tea field soil. *PloS one* 13:e0192235.
<https://doi.org/10.1371/journal.pone.0192235>.
- Park, Y. W., and L. N. Bell. (2004). Determination of moisture and ash contents of foods. Page 28 in L. M. L. Nollet, editor. *Food Science and Technology*-New York-Marcel Dekker-. CRC Press.
- Pauel, E., Adams. (1995). Determining Iron Content in Foods by Spectrophotometry. *Journal of Chemical Education* 72:649.
<https://doi.org/10.1021/ed072p649>.
- Qian, L., L. Chen, S. Joseph, G. Pan, L. Li, J. Zheng, X. Zhang, J. Zheng, X. Yu, and J. Wang. (2014). Biochar compound fertilizer as an option to reach high productivity but low carbon intensity in rice agriculture of China. *Carbon Management* 5:145-154.
<https://doi.org/10.1080/17583004.2014.912866>.
- Qin, S., F. Rong, M. Zhang, G. Su, W. Wang, L. Wu, A. Wu, and F. Chen. (2023). Biochar Can Partially Substitute Fertilizer for Rice Production in Acid Paddy Field in Southern China. *Agronomy* 13:1304.
<https://doi.org/10.3390/agronomy13051304>.
- Selvarajh, G., H. Y. Ch'ng, N. Md Zain, L. Seong Wei, J. Y. Liew, S. N. H. Mohammad Azmin, L. Naher, P. S. Abdullah, O. H. Ahmed, M. B. Jalloh, and I. Damrongrak. (2024). Enriched rice husk biochar superior to commercial biochar in ameliorating ammonia loss from urea fertilizer and improving plant uptake. *Heliyon* 10:e32080.
<https://doi.org/10.1016/j.heliyon.2024.e32080>.
- Singh, C., S. Tiwari, V. K. Gupta, and J. S. Singh. (2018). The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils. *CATENA* 171:485-493.
<https://doi.org/10.1016/j.catena.2018.07.042>.
- Şükran, D., G. Tohit, and S. Rıdvan. (1998). Spectrophotometric Determination of Chlorophyll - A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents. *Turkish Journal of Botany* 22:13-18.
- Tan, T., Y. Xu, X. Liao, Z. Yi, and S. Xue. (2024). Formulating new types of rice husk biochar-based fertilizers for the simultaneous slow-release of nutrients and immobilization of cadmium. *GCB Bioenergy* 16:e13142.
<https://doi.org/10.1111/gcbb.13142>.
- Ullah, R., Z. Abbas, M. Bilal, F. Habib, J. Iqbal, F. Bashir, S. Noor, M. A. Qazi, A. Niaz, K. S. Baig, A. Rauf, L. Fatima, I. Akhtar, B. Ali, M. I. Ullah, A. Al-Hashimi, M. S. Elshikh, S. Ali, and H. Saeed-ur-Rehman. (2022). Method development and validation for the determination of potassium (K₂O) in fertilizer samples by flame photometry technique. *Journal of King Saud University - Science* 34:102070.
<https://doi.org/10.1016/j.jksus.2022.102070>.
- Wang, Y., F. Pan, G. Wang, G. Zhang, Y. Wang, X. Chen, and Z. Mao. (2014). Effects of biochar on photosynthesis and antioxidative system of *Malus hupehensis* Rehd. seedlings under replant conditions. *Scientia Horticulturae* 175:9-15.
<https://doi.org/10.1016/j.scienta.2014.05.029>.
- Wickramasinghe, J. I., and C. S. De Silva. (2019). Effect of Partially-burnt Paddy Husk as a Supplementary Source of Potassium on Growth and Yield of Turmeric (*Curcuma longa* L.) and Soil Properties. *OUSL Journal* 13.
<https://doi.org/10.4038/ouslj.v13i2.7412>.
- Williams, R., J. B. Belo, J. Lidia, S. Soares, D. Ribeiro, C. L. Moreira, L. Almeida, L. Barton, and W. Erskine. (2023). Productivity gains in vegetables from rice husk biochar application in nutrient-poor soils in Timor-Leste. *Scientific Reports* 13:10858.
<https://doi.org/10.1038/s41598-023-38072-2>.
- Yaya programme. (2019). Ministry of Agriculture and Department of Agriculture. Sri Lanka.
- Yuxue, L., H. Lu, S. Yang, and Y. Wang. (2016). Impacts of biochar addition on rice yield and soil properties in a cold waterlogged paddy for two crop seasons. *Field Crops Research* 191:161-167.
<https://doi.org/10.1016/j.fcr.2016.03.003>.

- Zhao, Y., H. Jiang, J. Gao, Y. Feng, B. Yan, K. Li, Y. Lan, and W. Zhang. (2023). Effects of nitrogen co-application by different biochar materials on rice production potential and greenhouse gas emissions in paddy fields in northern China. *Environmental Technology & Innovation* 32:103242. <https://doi.org/10.1016/j.eti.2023.103242>.
- Zheng, H., Z. Wang, X. Deng, S. Herbert, and B. Xing. (2013). Impacts of adding biochar on nitrogen retention and bioavailability in agricultural soil. *Geoderma* 206:32-39. <https://doi.org/10.1016/j.geoderma.2013.04.018>.