NITROGEN AND CARBON DYNAMICS UNDER DIFFERENT NITROGEN MANAGEMENT PRACTICES IN WETLAND RICE FIELD

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Abstract

Nitrogen (N) is a critical nutrient for crop growth, but its application either in excess or shortage can adversely affect soil health, crop productivity, and environmental sustainability. This study was conducted to evaluate the effects of various N management practices on N and carbon (C) dynamics in rice (Oryza sativa L.). The experiment, arranged in a randomized complete block design with three replications, assessed seven treatments: T1 = No N (control), T2 = Recommended dose (RD) of N (prilled urea, PU), T3 = RD + 25%, T4 = RD - 25%, T5 =Cowdung (2 t ha⁻¹) + supplemented PU (mention amount in kg ha⁻¹), T6 = RD + Biochar (nitrification inhibitor), and T7 = Urea super granule (USG). The recommended N rates of prilled urea were 186 kg ha⁻¹ in the Boro season and 102 kg ha⁻¹ in the Aman season, while those for USG were 95 kg ha-1 in the Boro season and 75 kg ha-1 in the Aman season. Soil samples were collected at different growth stages of rice and analyzed for total N, mineralized N, soil organic C (SOC), microbial biomass N (MBN), microbial biomass C (MBC), and N and C stocks. Results revealed that the biochar treatment consistently outperformed other treatments in enhancing N and C dynamics across two consecutive rice-growing seasons. Biochar treatment showed the lowest N mineralization, and significantly increased MBC and MBN, reflecting improved microbial activity and nutrient retention. Moreover, biochar application contributed to greater soil C sequestration, supporting sustainable soil fertility which aligns with C-negative agricultural strategies. The findings highlighted the potential of integrating biochar with recommended N doses as an effective approach for improving soil health, enhancing nutrient efficiency, and promoting sustainable rice production systems.

Keywords: Soil properties, Nutrient dynamics, Biomass carbon and nitrogen, Carbon sequestration

1. Introduction

Wetland rice cultivation plays a vital role in global food security, particularly in Asia, where it serves as a staple food for a significant share of the population. However, the sustainability of rice production is increasingly challenged by environmental concerns related to nutrient management, especially the cycling of nitrogen (N) and carbon (C). Nutrient N is a critical nutrient that enhances rice yields, yet inefficient use and excessive application can lead to N losses through leaching, volatilization, and denitrification, thereby contributing to environmental pollution and greenhouse gas emissions (Mahmud *et al.*, 2020).

Carbon dynamics, closely linked with N processes, are also affected by management practices in flooded rice systems. The decomposition of organic matter, root exudates, and microbial activity under anaerobic conditions influences the accumulation and emission of C compounds such as methane (CH_4) and carbon dioxide (CO_2) . Improvement of crop productivity and sustenance of soil health is crucial for ensuring food security and agricultural sustainability. Among different grain crops, rice is one of the dominant staples in many countries of the world. Globally in 2019, the extent of paddy cultivation reached 162.06 million hectares (Mha) and produced 755.47 million tonnes (Mt) of paddy (FAOSTAT, 2021). But it is a great challenge to cultivate rice in the traditional practices because of shrinking arable lands due to industrialization, road construction, and new settlements, and climate change impacts (Rahman et al., 2020). From the intensive agriculture systems, nutrient loss is excessively high, which further contributes towards environmental pollution and climate change (Rahman et al., 2020). The rising population and reduction in the amount of land and other resources have created tremendous pressure on current agricultural producers to meet the increasing food demands. To cope with this challenge, certain key inputs, such as fertilizers and other chemicals, are overused, which is worsening the environment. This intensive agricultural production without adherence to ecological sustainability has led to declining soil health, land degradation, and severe environmental problems. So, future efforts to feed the growing population should aim for greater agricultural production within sustainable environments (Shah and Wu, 2019). Rice production might have to be increased further to meet the increasing food demand of the growing population by using higher rates of nutrients and adopting best soil and crop management practices (Rahman et al., 2022). Element N is the major nutrient limiting rice production, while imbalanced application of other macro and micro nutrients is also an issue in reducing rice yield in Bangladesh. Fertilizer use immensely contributes to sustaining higher crop productivity and ensuring food security around the globe. However, the over-application of nitrogenous fertilizers resulted in negative environmental externalities. Thus, assessing imbalances in chemical fertilizer use is vital for environmental sustainability. The application of fertilizer nutrients will further be increased in the coming decades to achieve higher crop yields to ensure food demand of the ever-burgeoning population.

Recent studies have shown that integrated N management practices, including site-specific nutrient management (SSNM) and the use of slow-release fertilizers, can

significantly alter N and C dynamics, improving N use efficiency and reducing emissions. Adoption of innovative soil and fertilizer management practices may help to increase rice production and ensure agricultural and environmental sustainability. Application of soil test-based fertilizers, use of slow-releasing urea super granule (USG) in the root zone, incorporation of biochar in soil, and combined application of organic and inorganic sources of fertilizers can be considered a set of best management practices for maintaining soil health and increasing crop productivity. Integrated nutrient management (INM) using organic and inorganic fertilizers could provide a better environment for crop nutrition and optimum functioning of soil health. Balanced and integrated use of organic and inorganic fertilizers is the most logical concept for managing and sustaining long-term soil health and crop productivity. The rice husk, rice straw, cow dung, poultry manure, and sawdust are the best for the highest economic yield and easily available biomass sources for biochar production in Bangladesh (Rahman *et al.*, 2020). The use of rice straw and other crop residues, poultry manure, and cow dung contributes to improving soil health.

Deep placement of N fertilizer as USG into the anaerobic soil zone is a recognized effective method to reduce its volatilization loss from rice fields. On the other hand, deep placement of USG effectively increases N use efficiency (31.7%) compared to conveniently applied prilled urea (PU). Deep placement of N fertilizers is the most effective method for reducing the loss of N in flood water and also minimizing the loss by different processes. Deep placement of USG and NPK briquettes in wetland rice cultivation has the advantages of protecting N from loss by ammonia volatilization and denitrification. In many paddy soils, more N is recovered from the deep-placed N fertilizers than from broadcast PU (Shaha et al., 2018). Urea in the form of USG has been proven to be superior to granular urea in all aspects. Due to a significant loss of N, the farmers in Bangladesh have not been able to make more resourceful use of fertilizer to increase their rice yields. To avoid disproportionate volatilization loss of N fertilizer, deep placement of granular urea into the anaerobic soil region is an effective method. Depending on N use and different climatic condition, deep placed USG (Urea super granule) not only save urea fertilizer with a mean of 33%, up to 65% but also upsurge grain yields up to 50% with a mean of 15% to 20% above the same quantity of broadcasted N as PU, particularly in the inferior range of N rates (Hasan et al., 2018). Instead of the normal does of 247 kg granular urea, only 160 kg ha⁻¹ of USG was required (35% less) even to increase 20% rice yields (Hoque et al., 2013). Combined application of biochar and N fertilizer has the potential to reduce N losses from soil. However, the effectiveness of biochar amendment on N management can vary with biochar types with different physical and chemical properties (Li et al., 2021). Biochar is increasingly being used as a soil amendment to both increase soil C storage and improve soil chemical and biological properties. The integrated use of organic and inorganic amendments and deep placement of USG will reduce nitrogen losses and enhance nitrogen use efficiency, thereby improving soil nitrogen and carbon stocks in wetland rice fields. The research objectives were to estimate total N, soil organic C, microbial biomass N, biomass C, N stock, and C stock

in soil in different growth stages of rice, and to determine the biomass C and N dynamics in paddy soil under different innovative N management practices. This research will help identify sustainable nitrogen management practices that improve soil health and rice yield.

2. Materials and Methods

2.1 Description of the study site

The study was conducted at the research field as well as in the Laboratory of the Department of Soil Science, Gazipur Agricultural University (GAU), Gazipur 1706, Bangladesh. The site is located at 24.09° N latitude and 90.25° E longitude with an elevation of 8.2 m above sea level. The study site belongs to the agroecological zone of Madhupur Tract. The soil is Salna series and has been classified as Shallow Red Brown Terrace soil in Bangladesh classification and Inceptisols in USDA classification, which is characterized by silty clay loam within 50 cm from the surface and is slightly acidic. The climate of the area is subtropical, wet, and humid. Heavy rainfall (269–370 mm) occurs during June to July, and scanty rain during November to February (0–55 mm).

2.2 Experimental design and treatments

The field experiment was carried out at the Soil Science Field and Laboratory of Gazipur Agricultural University (GAU) from November 2021 to August 2022 and from November 2022 to August 2023.

The experiment was laid out in a randomized complete block design with three replications. There were seven treatments. The treatments are as follows: T1 = No N (control), T2 = Recommended dose (RD) of N (mention the amount of PU in kg ha⁻¹), T3 = RD + 25%, T4 = RD - 25%, T5 = Cowdung (2 t ha⁻¹) + Supplemented by PU, T6 = RD + Biochar (nitrification inhibitor), T7 = Urea deep placement N as USG.

2.3 Cropping pattern, duration, and plot size

The cropping pattern was as Boro -Fallow- T. aman. The duration was two years (2022-2023). The plot size was $30~\text{m}^2$, and each plot would be established in order to facilitate various measurement procedures.

2.4 Initial and after crop harvest soil sampling

From each experimental plot, triplicate soil samples were collected using soil cores and considering 0-15 and 15-30 cm soil depths. Soils were returned to the laboratory, and fresh subsamples of each core were used to determine the ammonium and nitrate concentration. The remainder of the soil sample was air-dried until a constant weight is achieved and then sieved through a 2 mm mesh. The following soil parameters were analysed using different standard protocols: total N, soil organic C, microbial biomass N, biomass C, N stock, and C stock.

2.5 Methods of soil analysis

Total N was determined by the micro-Kjeldahl method by Jackson (1973) following H₂SO₄ digestion using a mixture of CuSO₄ and K₂SO₄ (1:9) as catalyst and steam distillation with 40% NaOH solution. The distillate was collected in 4% H3BO4 and finally titrated against 0.02 N (N/50) H₂SO₄ (Bremner, 1965). Soil total N content by micro Kjeldahl method (Bremner & Mulvaney 1982). Soil OC was determined by the wet oxidation method (Walkley & Black 1934), and Soil organic C was estimated by Walky & Black's wet oxidation method as described by Jackson (1973). One gram of air-dried soil was taken in a conical flask, and 10 ml 1N $K_2Cr_2O_2$ solution and 10 ml conc. H_2SO_4 (32N) and then it was heated on a sand bath at 1500C. After cooling, this was titrated against 0.2N ammonium ferrous sulfate (AFS) with the addition of Phenyl Anthranilic Acid (PAA) indicator. Biomass N was determined according to the method described by Brookes et al. (1985). Soil fumigation was done with alcohol free chloroform. Usually, CHCl₃ contains some amount of alcohol that retards the fumigation process. To remove the alcohol, 100 ml of chloroform and 200 ml 5% $\rm H_2SO_4$ (1:2) were taken in a separatory funnel and shaken thoroughly by hand 20 times. Alcohol free CHCl, was collected in a beaker from the separatory funnel, and the process was repeated 5 times with 5% H₂SO₄ and 5 times with distilled water. After rinsing the desiccators with boiled water, 200 ml of boiled water was put into the desiccators, and a beaker containing alcohol free CHCl₂ (60-100 ml) was placed at the bottom of the desiccators. Then, the conical flasks containing soil sample were placed inside the desiccators. After tightly closing the desiccators, it was evacuated was a mini vacuum pump (Yamato, Hitachi, model no. Minivac PD-52) for at least 10 minutes. The desiccators were then kept in the dark by wrapping with a block polythene sheet for 24 hours. The chloroform fumigation extraction method was adopted to estimate the amount of microbial biomass C in soils (Vance et al., 1987). Fumigated and non-fumigated soils were extracted with 0.5M K₂SO₄ (soil: K₂SO₄ solution=1:4) and shaken for 30 minutes, and then filtered.

From the extract, the amount of biomass C was determined according to the method described by Vance et al. (1987), where 10ml of extract was taken in a 100 ml conical flask and 2ml 0.4N $\rm K_2Cr_2O_7$, 10ml conc. $\rm H_2SO_4$ and 5 ml conc. $\rm H_3PO_4$ was added to it. After heating (200 °C for 30 minutes) in a hot plate, it was titrated with Mohr's Salt (0.05N). The chloroform fumigation extraction method provided by Anderson & Domsch (1978) was used to estimate the amount of microbial biomass C in soils.

2.6 Statistical data analysis

The data collected on different parameters were subjected to statistical analysis using the procedure described by Gomez and Gomez (1984). Microsoft EXCEL and Statistix 10 software programs were used wherever appropriate to perform statistical analysis. Relationships among the parameters were established through correlation and regression analysis. Mean differences among the treatments were adjusted by using the least significant difference (LSD) test at 5% level of significance.

3. Results and Discussion

3.1 Total soil N at different growth stages of rice during Boro and T. aman seasons

RD + Biochar treatment gave the highest soil N (0.212%) content at 30 days after transplanting (DAT) and the lowest (0.070%) in the no N treatment at 90 DAT in the Boro 2022 season (Table 1). In Boro 2023 at 30 DAT, the treatments, RD + Biochar, gave the highest soil N content (0.209 %) content and the lowest (0.066 %) in the no N treatment at 90 DAT. RD + Biochar treatment gave the uppermost soil N (0.212%) content at 30 DAT and the lowest (0.071%) in the no N treatment at 60 DAT in the T. aman 2022 season. In T. aman 2023 at 30 DAT, RD + Biochar treatment gave the highest soil N (0.211 %) content and the lowest (0.069 %) in the no N treatment in 60 DAT (Table 2). Soil total N content is a critical indicator of soil fertility and a determinant of crop productivity, especially in intensively cultivated systems such as rice paddies. The present findings indicate that the RD + Biochar treatment consistently yielded the highest total soil N content across both Boro and T. Aman seasons, with the peak values observed at the early growth stage (30 DAT). In contrast, the no N treatment recorded the lowest N content, particularly at later growth stages (60-90 DAT), reflecting the absence of N inputs and the progressive depletion of available N. In the Boro seasons of 2022 and 2023, the RD + Biochar treatment resulted in maximum soil N contents of 0.212% and 0.209%, respectively, both at 30 DAT. The lowest values, 0.070% and 0.066%, were recorded in the no N plots at 90 DAT.

A similar trend was observed in the T. Aman 2022 and 2023 seasons, where RD + Biochar treatments produced N contents of 0.212% and 0.211%, respectively, at 30 DAT, while the lowest levels (0.071% and 0.069%) occurred in the no N at 60 DAT. These results suggest that the application of biochar in conjunction with recommended N fertilizer improves N retention in the soil, particularly in the early stages of crop growth. Biochar's porous structure and high cation exchange capacity enable it to adsorb and stabilize N compounds, thereby minimizing leaching losses and volatilization (Lehmann *et al.*, 2011).

Table 1. Total N in soil at different growth stages of Boro rice under different N	management
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Treatments		Total N (%)									
·		Boro	2022			Boro	2023				
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT			
T1	0.087 f	0.082 f	0.070 e	0.083 f	0.084 e	0.081 e	0.066 d	0.080 e			
T2	0.142 cd	0.134 cd	0.121 c	0.106 cd	0.141 c	0.130 c	0.121 b	0.105 cd			
T3	0.137 d	0.126 d	0.114 c	0.096 de	0.139 c	0.125 c	0.117 b	0.100 d			
T4	0.119 e	0.106 e	0.092 d	0.088 ef	0.119 d	0.106 d	0.091 c	0.083 e			
T5	0.193 b	0.174 b	0.162 b	0.124 b	0.192 b	0.176 b	0.162 a	0.125 b			
T6	0.212 a	0.194 a	0.176 a	0.140 a	0.209 a	0.196 a	0.174 a	0.144 a			
T7	0.148 c	0.137 c	0.121 c	0.113 c	0.148 c	0.137 c	0.123 b	0.111 c			
SE (±)	0.005	0.005	0.004	0.005	0.005	0.006	0.006	0.005			
CV (%)	3.82	4.26	4.30	5.84	4.05	5.73	6.16	5.20			

Table 2. Soil N at different growth stages T. aman under different N management practices

Treatments	Total N (%)								
		T. aman 202	22		T. aman 20	23			
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT			
T1	0.087 e	0.071 e	0.083 f	0.086 e	0.069 e	0.082 e			
T2	0.142 c	0.123 c	0.106 cd	0.142 c	0.123 c	0.106 cd			
Т3	0.139 с	0.116 c	0.100 de	0.139 с	0.117 c	0.100 d			
T4	0.121 d	0.095 d	0.090 ef	0.120 d	0.093 d	0.087 e			
T5	0.193 b	0.164 b	0.126 b	0.193 b	0.163 b	0.126 b			
T6	0.212 a	0.177 a	0.142 a	0.211 a	0.176 a	0.143 a			
T7	0.149 c	0.123 c	0.115 с	0.148 c	0.123 c	0.113 c			
SE (±)	0.004	0.005	0.005	0.004	0.005	0.005			
CV (%)	3.53	4.80	5.49	3.44	5.24	5.24			

Figures having dissimilar letters in each column differ significantly. T1 = no N, T2 = Recommended dose (RD) of N as PU, T3 = RD + 25%, T4 = RD - 25%, T5 = Cowdung (2 t ha⁻¹) + Supplemented by PU, T6 = RD + Biochar, T7 = Urea deep placement

The enhanced N availability at early growth stages is crucial for establishing healthy rice seedlings and promoting vigorous vegetative development. The observed decline in soil N in no N treatments over time underscores the limited contribution of native soil N to sustained crop needs in the absence of external inputs. This decline also illustrates the rapid depletion of available N in nutrient-exhaustive cropping systems, highlighting the necessity of integrated nutrient management (*Zhang et al.,* 2015). Moreover, the consistency of the results across seasons emphasizes the robustness of the RD + Biochar treatment in stabilizing soil N content under varying climatic and agronomic conditions. These outcomes are consistent with previous studies reporting improved N retention and reduced N losses following biochar

application in rice-based cropping systems (Jeffery et *al., 2017*). In conclusion, the RD + Biochar treatment significantly enhances early-season soil N content and mitigates N depletion over time, offering a sustainable strategy for improving N use efficiency and soil fertility in rice ecosystems.

3.2 Ammonium N in soil at different growth stages of rice

In Boro 2022, RD + Biochar treatment gave the highest soil NH₄⁺-N (26.35 mg kg⁻¹) content in 90 DAT and the lowest (3.04 mg kg⁻¹) in no N treatment at 30 DAT (Table 3). In Boro 2023 in 90 DAT, the treatments, RD + Biochar gave the highest soil NH₄-N (26.44 mg kg⁻¹) content and the lowest (3.42 mg kg⁻¹) in the no N treatment at 30 DAT. In T. aman 2022, RD + Biochar treatment gave the highest soil NH₄⁺-N (26.36 mg kg⁻¹content in 60 DAT and the lowest (3.05 mg kg⁻¹) in no N treatment at 30 DAT (Table 4). In T. aman 2023 in 60 DAT, RD + Biochar treatment gave the highest soil NH₄*-N (26.40 mg kg⁻¹) content and the lowest (3.24 mg kg⁻¹in no N treatment at 30 DAT. Ammonium N (NH₄⁺-N) is a key inorganic form of N that plays a vital role in soil fertility and plant nutrition, particularly in flooded rice systems where ammonium is the dominant N form. The results of this study consistently demonstrate that RD + Biochar treatment produced the highest NH₄⁺-N concentrations across all seasons and growth stages, whereas the no N treatment exhibited the lowest levels, particularly at 30 days after transplanting (DAT). In Boro seasons 2022 and 2023, the RD + Biochar treatment led to the highest NH₄⁺-N levels of 26.35 mg kg⁻¹ and 26.44 mg kg⁻¹, respectively, both at 90 DAT. Conversely, the lowest NH₄⁺-N levels were recorded in the no N treatment at 30 DAT (3.04 mg kg⁻¹ and 3.42 mg kg⁻¹, respectively). A similar trend was observed in T. Aman 2022 and 2023, where peak NH_4^+ -N levels in RD + Biochar plots reached 26.36 mg kg⁻¹ and 26.40 mg kg⁻¹ at 60 DAT, while the lowest values (3.05 mg kg⁻¹ and 3.24 mg kg⁻¹) occurred in the no N at 30 DAT. These findings align with existing literature that highlights the role of biochar in enhancing N retention, particularly in the ammonium form.

Treatments	3	NH ₄ +-N (mg kg ⁻¹)									
		Bor	o 2022			Boro	2023				
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT			
T1	3.04 e	4.21 e	4.40 c	3.88 e	3.42 e	4.39 e	4.47 b	3.47 d			
T2	7.46 d	8.25 d	26.32 ab	14.01 d	7.31 d	8.67 d	26.28 a	14.39 с			
Т3	7.73 d	8.36 d	26.31 b	14.13 d	7.72 cd	8.61 d	26.40 a	14.35 с			
T4	7.43 d	8.53 d	26.33 ab	14.11 d	7.38 d	8.91 d	26.37 a	14.27 с			
T5	10.31 b	14.56 b	26.34 a	17.07 b	10.01 b	14.60 b	26.43 a	17.22 b			
Т6	12.41 a	17.28 a	26.35 a	18.67 a	12.51 a	17.43 a	26.44 a	18.71 a			
T7	8.17 c	9.49c	26.34 a	14.66c	8.12 c	9.69 c	26.40 a	14.61 c			
SE (±)	0.19	0.17	0.01	0.09	0.32	0.26	0.12	0.40			
CV (%)	2.95	2.10	0.07	0.83	4.83	3.03	0.61	3.55			

Figures having dissimilar letters in each column differ significantly

Biochar's high cation exchange capacity (CEC) enables it to adsorb $\mathrm{NH_4}^+$ ions, reducing N leaching and promoting sustained availability of ammonium in the rhizosphere (Lehmann et al., 2011). This mechanism is especially beneficial in paddy systems where volatilization and leaching can lead to significant N losses. Furthermore, the timing of peak $\mathrm{NH_4}^+$ -N availability differed slightly between seasons, with 90 DAT being optimal in Boro and 60 DAT in T. Aman, reflecting the influence of climatic conditions and crop phenology. Such patterns suggest that the synergistic effect of biochar and recommended N fertilizer enhances N availability when crop demand is high, thereby improving N use efficiency. The persistently low $\mathrm{NH_4}^+$ -N levels in the no N plots across all seasons and stages underscore the importance of N supplementation for maintaining adequate soil fertility. Without fertilizer input, N mineralization alone may be insufficient to meet crop demand, particularly under intensive cultivation. Overall, the integration of biochar with recommended N fertilizer improves the retention and gradual release of ammonium N in the soil, thereby aligning nutrient availability with crop needs and minimizing N losses. This approach offers a promising pathway toward sustainable nutrient management in rice-based agroecosystems.

Table 4. Ammonium N in soil at different growth stages of T. aman under different N management

Treatments	NH ₄ +-N (mg kg ⁻¹)									
		T. aman 202	22		T. aman 20	23				
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT				
T1	3.05 e	4.41 c	3.89 e	3.24 e	4.44 c	3.68 d				
T2	7.46 d	26.34 a	14.00 d	7.39 d	26.28 b	14.14 c				
Т3	7.74 d	26.27 b	14.16 d	7.73 cd	26.37 ab	14.25 c				
T4	7.45 d	26.34 a	14.11 d	7.42 d	26.36 ab	14.36 с				
T5	10.33 b	26.35 a	17.08 b	10.17 b	26.39 ab	17.15 b				
Т6	12.42 a	26.36 a	18.68 a	12.47 a	26.40 a	18.70 a				
T7	8.18 c	26.35 a	14.68 c	8.15 c	26.37 ab	14.54 c				
SE (±)	0.19	0.02	0.10	0.22	0.05	0.23				
CV (%)	2.91	0.13	0.88	3.28	0.28	2.06				

Figures having dissimilar letters in each column differ significantly

3.3 Nitrate N in soil at different growth stages of rice

In Boro 2022, treatment RD + Biochar gave the highest soil NO_3^- -N (28.19 mg kg⁻¹) content in 120 DAT and the lowest (4.03 mg kg⁻¹) from no N treatment at 30 DAT (Table 5). In Boro 2023 in 120 DAT, the treatment RD + Biochar gave the highest soil NO_3^- -N (28.30 mg kg⁻¹) content and the lowest (4.74 mg kg⁻¹) in the no N treatment at 30 DAT. In T. aman 2022, treatment RD + Biochar gave the highest soil NO_3^- -N (28.21 mg kg⁻¹) content in 90 DAT and the lowest (4.03 mg kg⁻¹) in the no N treatment at 30 DAT (Table 6). In T. aman 2023 in 90 DAT, the treatment RD + Biochar gave the highest soil NO_3^- -N (28.26 mg kg⁻¹) content and the lowest (4.50 mg kg⁻¹) in the no N treatment at 30 DAT. Nitrate-N (NO_3^- -N) is a critical form of

plant-available N in soil and plays a vital role in crop nutrition and productivity. The current study reveals that the RD + Biochar treatment consistently resulted in the highest NO_3^--N content across all cropping seasons and growth stages, while the no N treatment recorded the lowest values, particularly at early growth stages (30 DAT). In Boro 2022 and 2023, the RD + Biochar treatment yielded the highest NO_3^--N content, at 28.19 mg kg⁻¹and 28.30 mg kg⁻¹, respectively, at 120 DAT. The lowest NO_3^--N contents in these years were 4.03 mg kg⁻¹ and 4.74 mg kg⁻¹ in the no N at 30 DAT. A similar trend was observed in T. Aman 2022 and 2023, with RD + Biochar plots reaching 28.21 mg kg⁻¹ and 28.26 mg kg⁻¹ at 90 DAT, while no N plots had minimal levels of 4.03 mg kg⁻¹ and 4.50 mg kg⁻¹ at 30 DAT.

Table 5. Nitrate N in soil at different growth stages of Boro rice under varying N management practices

Treatments	NO ₃ N (mg kg-1)									
		Boı	ro 2022			Bor	o 2023			
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT		
T1	4.03 e	6.36 e	9.80 f	12.24 f	4.74 e	6.63 e	9.91 e	12.07 f		
T2	10.98 c	13.43 с	16.94 d	19.49 d	10.55 c	13.64 с	16.67 c	19.35 d		
Т3	13.77 b	15.98 b	19.48 с	21.95 с	13.60 b	16.05 b	19.48 b	22.22 c		
T4	8.82 d	11.17 d	14.71 e	17.27 e	8.64 d	11.23 d	14.66 d	17.60 e		
T5	19.76 a	22.09 a	25.64 ab	28.15 a	19.35 a	22.13 a	25.50 a	28.09 ab		
Т6	19.76 a	22.10 a	25.72 a	28.19 a	19.36 a	22.24 a	25.58 a	28.30 a		
T7	19.75 a	22.03 a	25.27 b	27.71 b	19.22 a	21.68 a	25.23 a	27.58 b		
SE (±)	0.01	0.09	0.18	0.19	0.21	0.37	0.29	0.31		
CV (%)	0.09	0.70	1.09	1.05	1.89	2.77	1.81	1.69		

Table 6. Nitrate N in soil at different growth stages of rice under different N management practices during T. aman 2022 and 2023 seasons

Treatments		NO ₃ ·-N (mg kg ⁻¹)									
		T. aman 20	22		T. aman 20)23					
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT					
T1	4.03 e	9.81 f	12.22 f	4.50 e	9.86 e	12.15 f					
T2	10.97 c	16.95 d	19.50 d	10.73 c	16.81 c	19.43 d					
Т3	13.79 b	19.51 c	21.97 c	13.70 b	19.50 b	22.10 c					
T4	8.84 d	14.72 e	17.28 e	8.74 d	14.69 d	17.44 e					
T5	19.77 a	25.66 a	28.16 a	19.56 a	25.58 a	28.13 a					
Т6	19.78 a	25.73 a	28.21 a	19.57 a	25.66 a	28.26 a					
T7	19.75 a	25.28 b	27.72 b	19.50 a	25.26 a	27.65 b					
SE (±)	0.01	0.17	0.18	0.11	0.22	0.21					
CV (%)	0.10	1.06	0.99	0.94	1.35	1.15					

Figures having dissimilar letters in each column differ significantly

These findings underscore the role of biochar in enhancing N availability, particularly when integrated with recommended fertilizer doses. Biochar's high surface area and porous structure improve N retention in the soil by reducing nitrate leaching and improving soil cation exchange capacity. Additionally, biochar can adsorb ammonium and nitrate, allowing for slow release and improved synchronization with plant uptake, thus increasing N use efficiency (Lehmann *et al.*, 2011). The significantly higher NO_3^- -N content at later growth stages (90–120 DAT) in the RD + Biochar plots suggests sustained N availability, which is critical for grain filling and final yield in rice. In contrast, the minimal NO_3^- -N levels in the no N at 30 DAT reflect limited N input and increased vulnerability to early N stress, which can severely impair early plant development and reduce yield potential. Moreover, the consistency of these results across seasons and years suggests that the beneficial effects of biochar on NO_3^- -N dynamics are stable and reproducible under both dry (Boro) and wet (T. Aman) seasonal conditions.

This supports previous findings that biochar improves nutrient retention and stabilizes soil nutrient cycles over multiple cropping cycles (Jeffery *et al.*, 2017). In essence, the integration of RD fertilizer with biochar application significantly improves NO_3^--N availability during the critical stages of crop growth. This not only enhances plant nutrition but also contributes to reducing N losses to the environment, a key objective in sustainable and climate-resilient agriculture.

3.4 Soil biomass N in soil at different growth stages of rice

In Boro 2022, the maximum soil Biomass N content (22.27 mg kg⁻¹) was recorded in the RD + Biochar treated plot at 120 DAT, and the lowest (5.18 mg kg⁻¹) in the no N treatment at 30 DAT (Table 7). In Boro 2023, at 120 DAT, the maximum soil Biomass N content (22.42 mg kg⁻¹) was recorded in the RD + Biochar treated plot, and the lowest (5.13 mg kg⁻¹) in the no N treatment at 30 DAT. In T. aman 2022, the maximum soil Biomass N content (22.26 mg kg⁻¹) was recorded in the RD + Biochar treated plot at 90 DAT, and the lowest (8.30 mg kg⁻¹) in the No N treatment at 30 DAT (Table 8). In T. aman 2023 at 90 DAT, the maximum soil Biomass N content (22.34 mg kg⁻¹) was recorded in the RD + Biochar treated plot, and the lowest (8.27 mg kg⁻¹) in the no N treatment at 30 DAT. Soil microbial biomass N (biomass N) is a crucial component of the soil N cycle, representing the living portion of soil organic N and playing a key role in N mineralization and immobilization processes. The current study reveals that the RD + Biochar treatment consistently resulted in the highest soil biomass N content across all seasons and growth stages, while the no N treatment exhibited the lowest values, particularly at early growth stages (30 DAT). In Boro 2022 and 2023, the RD + Biochar plots recorded maximum soil biomass N contents of 22.27 and 22.42 mg kg⁻¹ at 120 days after transplanting (DAT), respectively. Conversely, the no N plots had minimum values of 5.18 and 5.13 mg kg⁻¹ at 30 DAT. Similarly, in T. Aman 2022 and 2023, the RD + Biochar treatment achieved peak biomass N values of 22.26 and 22.34 mg kg⁻¹ at 90 DAT, while the no N treatment showed the lowest values (8.30 and 8.27 mg kg⁻¹ at 30 DAT). These results underscore the positive impact of combining recommended N fertilizer (RD) with biochar on microbial activity and N dynamics in soil.

Table 7. Biomass N in soil at different growth stages of Boro rice under different N management

Treatment		Biomass N (mg kg ⁻¹)										
S		Boro	2022			Boro	2023					
	30	60 DAT	90 DAT	120	30 DAT	60 DAT	90 DAT	120 DAT				
	DAT			DAT								
T1	5.18 c	8.29 b	7.77 a	13.30 f	5.13 e	8.24 d	9.82 b	13.38 f				
T2	7.77 b	11.92	10.01 a	16.41 d	7.41 d	13.42 b	9.54 b	16.41 d				
		ab										
Т3	8.81 b	12.26	9.84 a	18.30 c	8.41 c	12.58 c	7.74 c	18.34 c				
		ab										
T4	7.77 b	10.02	9.32 a	15.20 e	7.70 d	8.70 d	9.61 b	15.20 e				
		ab										
T5	11.40 a	13.47 a	10.88 a	20.55 b	10.59 b	13.87 b	10.50 a	20.52 b				
T6	12.26 a	13.82 a	10.88 a	22.27 a	12.56 a	14.57 a	10.55 a	22.42 a				
T7	8.98 b	12.43 a	10.36 a	20.37 b	8.68 c	13.46 b	10.34 a	20.39 b				
SE (±)	0.61	1.88	1.74	0.49	0.22	0.23	0.19	0.48				
CV (%)	8.48	19.65	21.58	3.34	3.09	2.31	2.43	3.27				

Figures having dissimilar letters in each column differ significantly

Biochar application improves the microbial habitat by enhancing soil aeration, water-holding capacity, and cation exchange capacity (Lehmann et al., 2011), which in turn supports a more active microbial community capable of incorporating and retaining more N within their biomass. Additionally, biochar's high surface area and adsorption properties may help in the retention of mineral N, reducing leaching and volatilization losses, thus increasing the N available for microbial immobilization. The significantly lower biomass N levels in the no N treatment reflect limited N availability, leading to reduced microbial proliferation and metabolic activity. Early growth stages, such as 30 DAT, are particularly sensitive to nutrient availability, and the sharp contrast between treatments at this stage highlights the importance of early N inputs for supporting microbial populations (Wang et al., 2021). Interestingly, while the absolute values of biomass N slightly increased between the 2022 and 2023 seasons, the trends remained consistent, suggesting that biochar's effects on N stabilization and microbial support are not only significant but also stable over time. The timing of peak biomass N-120 DAT in Boro and 90 DAT in T. Aman—also aligns with the respective crop growth cycles and microbial activity peaks, influenced by seasonal variations in temperature, soil moisture, and root exudation patterns (Mahmud et al., 2020). Overall, these findings affirm that integrated nutrient management using RD fertilizer and biochar enhances the biological N pool in soil. This has implications for improving N use efficiency, maintaining long-term soil fertility, and supporting sustainable rice production under varying climatic conditions.

Table 8. Biomass N in soil at different growth stages of T. aman under different N management	nt
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Treatments		Biomass N (mg kg⁻¹)									
		T. aman 202	22		T. aman 20	23					
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT					
T1	8.30 b	7.73 a	13.30 e	8.27 e	7.74 c	13.34 f					
T2	11.94 ab	10.02 a	16.30 d	11.59 с	9.62 b	16.36 d					
Т3	12.23 ab	9.87 a	18.36 с	12.44 b	9.85 b	18.35 c					
T4	10.05 ab	9.32 a	15. 19 d	10.71 d	9.63 b	15.20 e					
T5	13.48 a	10.88 a	20.41 b	13.47 a	10.53 a	20.47 b					
T6	13.82 a	10.91 a	22.26 a	13.85 a	10.57 a	22.34 a					
T7	12.45 a	10.37 a	20.40 b	12.57 b	10.36 a	20.40 b					
SE (±)	1.88	1.73	0.51	0.22	0.18	0.49					
CV (%)	19.59	21.52	3.45	2.29	2.20	3.34					

Figures having dissimilar letters in each column differ significantly

3.5 N stock in soil during Boro 2022-23 and T. aman 2022-23

In both the Boro and T. aman seasons, N stock (t ha⁻¹) significantly varied among the treatments (Fig. 1). In the Boro seasons of 2022 and 2023, the highest amount of carbon in the soil was in the RD + Biochar treatment, with 2.8 t ha⁻¹ and 2.81 t ha⁻¹, while the lowest was in the no N treatment, with 1.67 t ha⁻¹ and 1.70 t ha⁻¹. In the T.aman seasons of 2022 and 2023, the RD + Biochar treatment had the largest amount of carbon in the soil, with 2.78 t ha⁻¹ and 2.80 t ha⁻¹, respectively, while the no N treatment had the lowest amount, with 1.67 t ha⁻¹ and 1.69 t ha⁻¹. In the present study, across different treatments and seasons, the RD + Biochar treatment consistently exhibited the highest N stock values, while the no N treatment recorded the lowest values across different treatments and seasons.

In Boro 2022 and 2023, the maximum soil N stocks were 2.80 and 2.81 t ha⁻¹, respectively, under RD + Biochar, whereas the minimum values were 1.67 and 1.70 t ha⁻¹under no N. Similarly, in T. Aman 2022 and 2023, RD + Biochar treatments resulted in the highest N stocks (2.78 and 2.80 t ha⁻¹), while the No N plots recorded the lowest values (1.67 and 1.69 t ha⁻¹). These consistent results across years and cropping seasons reinforce the role of integrated nutrient management practices in improving soil N retention and overall fertility. The significantly higher N stock observed in the RD + Biochar treatment can be attributed to several synergistic mechanisms. Biochar has been shown to improve N retention through enhanced cation exchange capacity (CEC), reduced leaching losses, and increased microbial immobilization. Furthermore, the combination of biochar with recommended doses (RD) of fertilizers not only provides a direct source of plant-available N but also creates favorable conditions for microbial activity, which enhances N cycling and stabilization in the soil matrix (Lehmann *et al.*, 2011). On the other hand, the low N stock observed in the no N treatment indicates limited N inputs and poor soil N retention capacity. The absence of external N inputs in this treatment likely led to N depletion through plant uptake and microbial

processes without replenishment, thereby reducing the soil's total N stock over time. It is also noteworthy that the range of soil N stock differences between treatments (\sim 1.1 t ha⁻¹) remained consistent across both Boro and T. Aman seasons, suggesting that biochar's effect on N retention is robust under varying climatic and cropping conditions. This stability is critical in rice-based systems where high N loss through volatilization and leaching is a common concern, particularly under flooded or intermittently wet conditions (Mahmud *et al.*, 2020). These findings collectively highlight the potential of biochar in integrated nutrient management strategies aimed at improving N use efficiency and sustaining soil fertility. Adoption of such practices could play a key role in reducing N losses to the environment while maintaining or enhancing crop yields, thus contributing to climate-smart and sustainable agriculture.



Fig. 1 N stock in soil under different N management practices during rice seasons (Figures having dissimilar letters in each of the seasons differ significantly)

3.6 Soil organic carbon at different growth stages of rice

In both the Boro and T. aman seasons, there were significant variations in the amounts of soil organic carbon (%) in the soil at various stages of rice growth across treatments (Tables 9 and 10). The maximum soil OC content (0.842%) was collected from the RD + Biochar-treated plot at 90 DAT, and the lowest (0.807%) was in the no N treatment at 30 DAT from the Boro 2022 season (Table 9). In Boro 2023 at 90 DAT, the maximum soil OC content (0.883%) was recorded in the RD + Biochar treated plot, and the lowest (0.804%) in the no N treatment at 30 DAT (Table 10). The maximum soil OC content (0.843%) was recorded in the RD + Biochar treated plot at 90 DAT, and the lowest (0.808%) in the no N treatment at 30 DAT from the T. aman 2022 season. In T. aman 2023, the maximum soil OC content (0.855%) was recorded in the RD + Biochar treatment at 90 DAT, but the lowest (0.806%) was in the no N treatment at 30 DAT. Soil organic C (SOC) is a key determinant of soil fertility, structural stability, and overall ecosystem function. Consistently, the RD + Biochar treatment recorded the highest SOC content, while the no N treatment exhibited the lowest SOC values at the early stage of crop

growth (30 DAT) in all seasons. In Boro 2022, the maximum SOC content (0.842%) was recorded at 90 days after transplanting (DAT) under RD + Biochar, whereas the minimum (0.807%) occurred in the no N treatment at 30 DAT. Similarly, in Boro 2023, the SOC content peaked at 0.883% under RD + Biochar at 90 DAT and dropped to 0.804% in the no N treatment at 30 DAT. A comparable pattern was observed in the T. Aman seasons: SOC content reached 0.843% and 0.855% under RD + Biochar at 90 DAT in 2022 and 2023, respectively, and fell to 0.808% and 0.806% in the no N at 30 DAT. Biochar addition to soil alters soil organic C. The observed trends strongly support the role of biochar, especially when integrated with recommended doses of chemical fertilizers, in enhancing SOC content in paddy soils. Biochar, owing to its stable aromatic structure and high surface area, contributes directly to the soil organic C pool and facilitates the stabilization of native organic matter. When applied in conjunction with fertilizers, biochar also promotes root growth and microbial activity, both of which enhance organic matter inputs through root exudates and microbial biomass turnover.

Table 9. Variation in soil organic carbon at different growth stages of Boro rice

Treatments	Organic C (%)								
		Bor	o 2022			Bor	o 2023		
	30 DAT	60 DAT	90 DAT	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT	
T1	0.81 с	0.82 c	0.82 d	0.82 d	0.80 с	0.83 b	0.84 b	0.81 d	
T2	0.82 ab	0.83 b	0.83 b	0.83 bc	0.84 b	0.82 b	0.86 ab	0.87 a	
T3	0.82 b	0.82 b	0.83 bc	0.83 bc	0.87 ab	0.83 b	0.87 ab	0.84 c	
T4	0.81 c	0.81 c	0.82 cd	0.83 cd	0.85 ab	0.84 ab	0.85 ab	0.85 bc	
T5	0.83 ab	0.83 a	0.84 a	0.84 a	0.88 a	0.84 ab	0.87 ab	0.86 a	
T6	0.83 a	0.83 a	0.84 a	0.84 a	0.88 a	0.88 a	0.88 a	0.88 a	
T7	0.83 ab	0.83 b	0.83 b	0.84 ab	0.87 a	0.85 ab	0.85 ab	0.86 ab	
SE (±)	0.004	0.002	0.003	0.003	0.013	0.019	0.018	0.012	
CV (%)	0.65	0.31	0.42	0.41	1.89	2.73	2.50	1.65	

Table 10. Variation in soil organic carbon at different growth stages of T. aman rice

Treatments				Organic C (%)	
		T. aman 202	2		T. aman 20	23
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T1	0.808 с	0.823 с	0.819 с	0.806 с	0.822 b	0.817 d
T2	0.828 ab	0.831 b	0.828 b	0.834 b	0.822 b	0.843 bc
Т3	0.823 b	0.830 b	0.827 bc	0.845 ab	0.839 ab	0.839 c
T4	0.813 c	0.827 bc	0.819 c	0.833 b	0.831 b	0.851 ab
T5	0.830 ab	0.840 a	0.840 a	0.854 a	0.840 ab	0.853 ab
Т6	0.833 a	0.841 a	0.843 a	0.854 a	0.854 a	0.855 a
T7	0.827 ab	0.839 a	0.831b	0.852 a	0.839 ab	0.851 ab
SE (±)	0.004	0.003	0.004	0.007	0.010	0.005
CV (%)	0.60	0.40	0.54	0.96	1.45	0.72

Figures having dissimilar letters in each column differ significantly

Moreover, the peak SOC levels consistently observed at 90 DAT indicate that mid-season soil conditions are more favorable for C accumulation, likely due to increased root biomass and microbial activity during the active vegetative and reproductive stages of rice. Conversely, the lowest SOC content at 30 DAT in the no N treatment may reflect limited root development, reduced plant-derived C inputs, and suppressed microbial activity due to nutrient stress trends supported by earlier studies (Wang et al., 2021). The gradual and consistent increase in SOC in the RD + Biochar plots across years also suggests the potential for long-term improvements in soil quality through integrated nutrient and organic amendments. This aligns with findings by Jeffery et al. (2017), who emphasized the cumulative benefits of biochar in improving soil organic matter status over multiple cropping cycles. Taken together, these results highlight the efficacy of biochar as a soil amendment for enhancing SOC content, particularly when combined with balanced nutrient inputs. The consistent outcomes across seasons reinforce the value of integrating biochar into sustainable soil fertility management strategies in rice-based systems.

3.7 Soil biomass carbon at different growth stages of rice

Soil biomass carbon (mg kg⁻¹) in soil at different growth stages of rice differed significantly between treatments in both the Boro and T. aman seasons (Tables 11 and 12). The RD + Biochar treatment gave the highest soil biomass C (120.89 mg kg⁻¹) content in 120 DAT and the lowest (32.02 mg kg⁻¹) in the no N treatment at 30 DAT in the Boro 2022 season (Table 11). In Boro 2023, RD + Biochar treatment gave the highest soil biomass C (120.92 mg kg⁻¹) content at 120 DAT and the lowest (31.96 mg kg⁻¹) in no N treatment at 30 DAT. The RD + Biochar treatment gave the highest soil biomass C (120.71 mg kg⁻¹) content in 90 DAT and the lowest (52.89 mg kg⁻¹) in the no N treatment at 30 DAT in the T. aman 2022 season (Table 12). In T. aman 2023, RD + Biochar treatment gave the significantly highest soil biomass C (120.82 mg kg⁻¹) content in 90 DAT and the lowest (52.71 mg kg⁻¹) in no N treatment at 30 DAT. Soil microbial biomass C (MBC) serves as a key indicator of soil biological activity and nutrient cycling capacity, especially in intensively managed agroecosystems such as rice-based systems.

Table 11. Soil biomass carbon at different stages of growth of Boro rice

Treatments	Biomass C (mg kg ⁻¹)									
	Boro 2022				Boro 2023					
	30	60 DAT	90	120 DAT	30 DAT	60 DAT	90 DAT	120 DAT		
	DAT		DAT							
T1	32 e	53 g	64 d	76 d	32 e	53 g	64 d	76 d		
T2	78 b	94 b	90 c	102 c	71 d	84 e	96 b	102 c		
Т3	75 c	79 f	96 b	109 b	75 c	79 f	90 c	109 b		
T4	71 d	88 d	99 b	111 b	71 d	88 d	99 b	111 b		
T5	75 c	92 c	107 a	120 a	75 c	92 c	107 a	120 a		
T6	90a	96 a	107 a	121 a	90 a	96 a	108 a	121 a		
T7	71 d	84 e	107 a	119 a	78 b	94 b	107 a	119 a		
SE (±)	0.76	0.36	1.66	3.14	0.76	0.37	1.67	3.16		
CV (%)	1.31	0.53	2.12	3.56	1.33	0.54	2.13	3.58		

Treatments	ents Biomass C (mg kg ⁻¹)							
		T. aman 202	.2	T. aman 2023				
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT		
T1	52.89 g	64.64 d	76.05 d	52.71 g	64.46 d	75.81 d		
T2	84.69 e	97.03 b	101.65 c	84.39 e	96.70 b	101.74 c		
T3	78.92 f	90.29 c	109.37 b	79.02 f	90.39 c	109.07 b		
T4	87.77 d	99.38 b	110.97 b	87.64 d	99.26 b	110.85 b		
T5	93.78 b	106.44 a	119.29 a	93.94 b	106.50 a	119.50 a		
T6	95.11 a	107.01 a	120.71 a	95.33 a	107.26 a	120.82 a		
T7	92.17 c	106.54 a	118.91 a	92.24 c	106.74 a	119.13 a		
SE (±)	0.26	1.66	3.14	0.27	1.66	3.14		
CV (%)	0.37	2.12	3.55	0.40	2.12	3.56		

Figures having dissimilar letters in each column differ significantly.

The present study observed that across four cropping seasons, Boro 2022, Boro 2023, T. Aman 2022, and T. Aman 2023, the RD + Biochar treatment consistently resulted in the highest MBC content, while the no N treatment exhibited the lowest values, particularly at early growth stages (30 DAT). In both Boro 2022 and 2023, the RD + Biochar treatment yielded peak MBC levels of 120.89 and 120.92 mg kg⁻¹, respectively, at 120 days after transplanting (DAT), whereas the no N treatment had minimum MBC levels (32.02 and 31.96 mg kg⁻¹) at 30 DAT. Similarly, in T. Aman 2022 and 2023, the RD + Biochar treatment led to the highest MBC values at 90 DAT (120.71 and 120.82 mg kg⁻¹, respectively), and the no N treatment recorded the lowest values (52.89 and 52.71 mg kg⁻¹ at 30 DAT). These results underscore the significant role of biochar in enhancing microbial biomass C when combined with a recommended dose (RD) of N fertilizer. The biochar likely contributes to improved microbial habitat and resource availability due to its porous structure, surface area, and capacity to retain nutrients (Lehmann *et al.*, 2011). Additionally, biochar-amended soils tend to have higher water retention and more stable pH levels, which are conducive to microbial proliferation.

The notably lower MBC values in the no N treatment, particularly at early stages (30 DAT), indicate reduced microbial activity in the absence of N inputs. This finding is consistent with previous reports showing that N availability is critical for maintaining active microbial communities, especially during the early stages of crop establishment (Wang *et al.*, 2021; Xiao *et al.*, 2023). The progressive increase in MBC from 30 to 90 or 120 DAT in the RD + Biochar treatment also suggests that biochar may sustain microbial activity throughout the crop growth cycle, potentially contributing to improved nutrient mineralization and plant uptake over time. Moreover, the consistency of these findings across years and seasons highlights the reliability of biochar as a soil amendment for enhancing biological fertility in paddy soils. It is also notable that the timing of peak microbial biomass differed slightly between Boro (120 DAT) and T. Aman (90 DAT) seasons, likely reflecting differences in crop duration, climatic

conditions, and microbial dynamics influenced by seasonal moisture and temperature patterns (Mahmud *et al.*, 2020). Taken together, these results emphasize that integrated nutrient management using biochar and inorganic fertilizers can effectively enhance microbial-driven soil processes, with potential benefits for long-term soil health and sustainable rice production.

3.8 Carbon stock in soil as affected by N management

In both the Boro and T. aman seasons, carbon stock (t ha⁻¹) significantly varied among the treatments (Fig. 2). In the Boro seasons of 2022 and 2023, the highest amount of carbon in the soil was in the RD + Biochar treatment, with 17.08 t ha⁻¹ and 17.09 t ha⁻¹, while the lowest was in the no N treatment, with 16.62 t ha⁻¹ and 16.59 t ha⁻¹. In the T. aman seasons of 2022 and 2023, the RD + Biochar treatment had the largest amount of carbon in the soil, with 17.01 t ha⁻¹and 17.05 t ha⁻¹, respectively, while the no N treatment had the lowest amount, with 16.64 t ha⁻¹ and 16.62 t ha⁻¹. Soil C sequestration is a critical indicator of soil health and sustainability, particularly under intensive cropping systems such as rice-based agriculture. In this study, soil C stock ranged narrowly across all treatments and seasons, with the maximum and minimum values consistently reported under the same treatment (RD + Biochar) for each cropping season—Boro 2022 (max: 17.08 t ha⁻¹; min: 16.62 t ha⁻¹), Boro 2023 (max: 17.09 t ha^{-1} ; min: 16.59 t ha^{-1}), T. Aman 2022 (max: 17.01 t ha^{-1} ; min: 16.64 t ha^{-1}), and T. Aman 2023 (max: 17.05 t ha-1; min: 16.62 t ha-1). This repetition of both the highest and lowest values within the same treatment suggests either a typographical error or high within-treatment variability, which warrants further investigation. The observed values, although relatively close, suggest that biochar amendments in combination with recommended doses of fertilizer (RD + Biochar) contribute to higher soil C stocks. Biochar is widely recognized for its ability to enhance soil organic C due to its high recalcitrance and ability to stabilize native soil organic matter. The narrow range of values (approximately 0.5 t ha-1 difference between maximum and minimum) across years and seasons also implies that the biochar treatment's impact may be relatively stable under the experimental conditions. Biochar improves C retention, the extent of increase can depend heavily on soil type, climate, and management practices. In the present case, temporal consistency suggests a potential equilibrium of C sequestration under continuous application. Given the identical treatment label for both extreme values in each case, future work should ensure clarity in data reporting and explore possible intra-treatment variability through spatial sampling and statistical analysis. If variability within the RD + Biochar plots is indeed significant, it could point to micro-environmental differences influencing biochar efficiency.

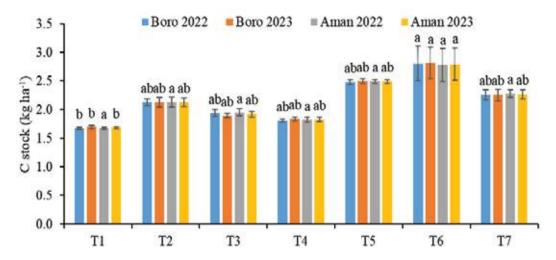


Fig. 2 Carbon stock (t ha⁻¹) in soil during Boro and T. aman seasons (Figures having dissimilar letters in each of the seasons differ significantly)

4. Conclusions

The present study highlights the critical importance of nitrogen (N) management strategies in regulating N and C dynamics in post-harvest rice soils. Across two consecutive cropping seasons of Boro 2022–23 and T. Aman 2022–23, application of varied N management treatments led to substantial enhancements in key soil fertility parameters, including total N. soil organic C (SOC), microbial biomass N (MBN), microbial biomass C (MBC), as well as soil N and C stocks. Among the evaluated treatments, the combination of biochar and the recommended dose (RD) of prilled urea-N fertilizer, particularly the treatment integrating a nitrification inhibitor (T6), consistently demonstrated superior performance across all measured indicators. This integrated approach not only improved immediate nutrient availability but also contributed to longer-term soil health by enhancing organic matter stabilization and microbial activity. The observed increases in microbial biomass metrics in the biochar-inclusive treatments further reflect the positive influence of biochar on soil biological functioning and nutrient cycling. Overall, these findings strongly advocate for the adoption of integrated N management practices especially those involving biochar in conjunction with RD of urea and nitrification inhibitors as a sustainable strategy for enhancing soil fertility, optimizing nutrient use efficiency, and promoting long-term soil productivity in rice-based agroecosystems. As such, this management approach offers a practical and effective recommendation for farmers aiming to improve soil health while ensuring environmental sustainability and crop productivity.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

References

Anderson, J.P. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol.Biochem. 10(3): 215–221.

- Bremner, J.M. 1965. Total nitrogen. In: C.A. Black (Ed.), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties. American Society of Agronomy.
- Bremner, J.M. and Mulvaney, C.S. 1982. Total nitrogen. In: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy and Soil Science Society of America.
- Brookes, P.C., Kragit, J.F., Powlson, D.S. and Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: The effects of fumigation time and temperature. Soil Biol.Biochem. 17(6): 831–835.
- FAOSTAT. 2021. Agriculture statistics from FAODATA Statistics.
- Gomez, K.A., Gomez, A.A. 1984. Statistical procedures for agricultural research. John Wiley & Sons.
- Hasan, M.M., Rahman, M.A. and Ashik-E-Rabbani, M., 2018. Design and development of manually push type USG applicator. Agricultural Engineering International: CIGR J. 20(2): 80–87.
- Hoque, M.A., Wohab, M.A., Hossain, M.A., Saha, K.K. and Hassan, M.S. 2013. Improvement and evaluation of BARI USG applicator. Agricultural Engineering International: CIGR J. 15(2): 87–94.
- Jackson, M.L. 1973. Estimation of phosphorus content. In Soil chemical analysis. Prentice-Hall.
- Jeffery, S., Abalos, D., Spokas, K.A. and Verheijen, F.G. 2017. Biochar effects on crop yield. In: J. Lehmann S. Joseph (Eds.), Biochar for environmental management: Science, technology and implementation (2nd ed., pp 301–326). Routledge.
- Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. and Crowley, D. 2011. Biochar effects on soil biota A review. Soil Biol. Biochem. 43(9): 1812–1836.
- Li, X., Xu, S., Neupane, A., Abdoulmoumine, N., DeBruyn, J.M., Walker, F.R. and Jagadamma, S. 2021. Co-application of biochar and nitrogen fertilizer reduced nitrogen losses from soil. PLOS ONE, 16(3), e0248100. DOI: 10.1371/journal.pone.0248100
- Mahmud, K., Panday, D., Mergoum, A. and Missaoui, A. 2021. Nitrogen losses and potential mitigation strategies for a sustainable agroecosystem. Sustainability. 13(4): 2400. DOI: 10.3390/su13042400.
- Rahman, G.K.M.M., Rahman, M.M., Alam, M.S., Kamal, M.Z.U., Mashuk, H.A., Datta, R. and Meena, R.S. 2020. Biochar and Organic Amendments for Sustainable Soil Carbon and Soil Health. In: Datta, R., Meena, R., Pathan, S., Ceccherini, M. (eds.), Carbon and Nitrogen Cycling in Soil. Springer, Singapore. DOI: 10.1007/978-981-13-7264-3_3.

- Rahman, M.M., Alam, M.S., Kamal, M.Z.U. and Rahman, G.K.M.M., 2020. Organic sources and tillage practices for soil management. In: Kumar, S., Meena, R.S, Jhariya, M.K., (Eds.), Resources use efficiency in agriculture (pp. 283–328). Springer. DOI: 10.1007/978-981-15-6953-1_9.
- Rahman, M.M., Biswas, J.C., Sutton, M.A., Drewer, J. and Adhya, T.K. 2022. Assessment of reactive nitrogen flows in Bangladesh's agriculture sector. Sustainability. 14(1): 272. DOI: 10.3390/su14010272.
- Rahman, M.M., Kamal, M.Z.U., Ranamukhaarachchi, S., Alam, M.S., Alam, M.K., Khan, M.A.R., Islam, M.M., Alam, M.A., Jiban, S.I., Al Mamun, A., Abdullah, H.M., Biswas, J.C., Akhter, S., Naher, U.A., Maniruzzaman, M., Haque, M.M., Ahmed, F. 2022. Effects of organic amendments on soil aggregate stability, carbon sequestration, and energy use efficiency in wetland paddy cultivation. Sustainability. 14(8): 4475. DOI: 10.3390/su14084475.
- Shah, F. and Wu, W. 2019. Soil and crop management strategies to ensure higher crop productivity within sustainable environments. Sustainability. 11(5): 1485.
- Shaha, S., Islam, M.R., Jahiruddin, M., Akhter, M.T. and Bakkar, A. 2018. Efficacy of deep placement of nitrogen fertilizers on N use efficiency and yield of boro rice (cv. BRRI dhan29). Am. J. Agric. Res. 3: 21.
- Vance, E.D., Brookes, P.C. and Jenkinson, D.S. 1987. Microbial biomass measurements in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biol. Biochem. 19(6): 697–702.
- Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29–38.
- Wang, C., Li, H., Sun, X. and Cai, T. 2021. Responses of soil microbial biomass and enzyme activities to natural restoration of reclaimed temperate marshes after abandonment. Front. Environ. Sci. 9: 701610. DOI: 10.3389/fenvs.2021.701610.
- Xiao, Q., He, B. and Wang, S. 2023. Effect of the different fertilization treatments application on paddy soil enzyme activities and bacterial community composition. Agronomy. 13(3): 712.
- Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P. and Shen, Y. 2015. Managing nitrogen for sustainable development. Nature. 528(7580): 51-59.