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RESEARCH ARTICLE

Single-time fertilization with controlled release blended fertilizer optimizes soil nitrogen distribution and root characteristics to increase conventional *japonica* rice (*Oryza sativa* L.) grain yield and nitrogen use efficiency

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Highlights

- Single-time fertilization with controlled release blended fertilizer (CRBF) optimized soil N distribution and maintained a soil NH_4^+-N concentration that was synchronized with rice N requirements.
- Single-time fertilization with CRBF improved root biomass and shaped root morphology.
- Single-time fertilization with CRBF increased rice grain yield and nitrogen use efficiency (NUE) by increasing plant N uptake, shaping root morphology and increasing soil inorganic N.

Abstract

Single-time fertilization (STF) with controlled release blended fertilizer (CRBF) improves grain yield and nitrogen use efficiency (NUE) in rice production. However, the impact of soil nitrogen (N) distribution and root growth on rice yield and NUE under STF with CRBF remains unclear. Here, a two-year field experiment investigated the effects of two fertilizer types (normal urea (U) and CRBF) and two single-time fertilization methods (broadcast and side-deep fertilization) on the soil N distribution, plant N uptake, root characteristics, grain yield, and NUE. The results showed that CRBF under STF increased the averages of plant dry matter accumulation, N uptake, grain yield, nitrogen recovery efficiency (NRE), and nitrogen agronomic efficiency (NAE) by 8.29, 21.85, 10.57, 79.28, and 74.8% compared to the other treatments, respectively. Side-deep fertilization with CRBF further increased NUE by 12.78% compared to broadcast. Moreover, CRBF under STF increased the leaf SPAD value and glutamine synthetase (GS)/glutamine oxoglutarate aminotransferase (GOGAT) activity by 5.93 and 25.58%, respectively. CRBF under STF increased the soil inorganic N concentration and showed a "rising early and stabilizing later" pattern. In addition, CRBF under STF improved rice root growth and increased the averages of root biomass, total root number, root average diameter, total root length, total root surface area, and total root volume by 28.30, 28.56, 18.64, 13.38, 35.26, and 37.06%, respectively, at the tillering and heading stages. Partial least squares path modeling indicated that CRBF under STF increased the soil inorganic N concentration which improved root morphology, thereby increasing N uptake and improving the rice yield and NUE. Taken together, our findings show that CRBF with single-time fertilization is the preferred N fertilizer strategy for achieving high yield and efficiency in rice, and that side-deep fertilization is the optimal fertilization method.

Keywords: yield, nitrogen use efficiency, controlled release blended fertilizer, soil nitrogen distribution, root

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1. Introduction

Rice is one of the top three staple food crops globally, and it significantly fulfills the increasing food demands. Although China leads global rice production, yield improvements are still needed to support its growing population. In recent decades, substantial investment in N fertilizers has significantly increased China's rice production (Cheng *et al.* 2021). N fertilizer plays a crucial role in enhancing both the yield and quality of rice, but its overuse causes significant environmental damage and economic losses (West *et al.* 2014). In traditional N management to achieve high yields, N application rates in Chinese rice farming can vary widely, commonly ranging from 150 to 250 kg ha^{-1} and occasionally exceeding 300 kg ha^{-1} in intensive farming regions to ensure high yields, which considerably exceeds the global average N application rate (Peng *et al.* 2010; Zhang *et al.* 2015). This excessive application of N fertilizer is not conducive to high nitrogen use efficiency (NUE) (Liu L *et al.* 2023). Thus, a new N fertilization management strategy is clearly needed to improve NUE, ensure the growth of rice yields, and promote the sustainable development of agricultural production.

Controlled release N fertilizer (CRNF), which is a type of fertilizer that prolongs N availability through the controlled release of N, has been shown to improve rice yield and NUE (Wu *et al.* 2021). Some types of CRNFs combined with appropriate fertilization methods, especially mechanical side-deep fertilization, not only significantly improves crop yield and NUE but also can serve as an effective alternative to the split applications of normal urea (Min *et al.* 2021; Zhang *et al.* 2022). However, studies have shown that using certain types of CRNFs, such as sulfur-coated urea and polymer-coated urea (4% coating), cannot increase rice yield and even show reductions to some extent (Li *et al.* 2023). This occurs because the N release characteristic of a single CRNF cannot adequately meet the N requirements of rice, so it has a poor effect on increasing rice yield and NUE (Wu *et al.* 2021). However, different types of CRNFs have been innovatively combined in a certain proportion to obtain controlled release blended fertilizer (CRBF), which can better satisfy the N requirement of rice throughout its entire growth period. The creation of CRBF represents a significant advancement in rice-specific N fertilizer technology as it addresses the limitations of single CRNF and provides a more balanced N supply, which can steadily increase rice yields. Although CRBF can significantly increase rice yield and NUE as a base fertilizer combined with single-time fertilization (STF) (Ke *et al.* 2018), the mechanism by which CRBF combined with single-time fertilization improves yield and NUE is still unclear.

Ammonium (NH_4^+) and nitrate (NO_3^-) are the predominant forms of N absorbed by rice, with NH_4^+ being a critical form of the paddy soil N supply (Miller and Cramer 2005). The uptake of N by rice has obvious temporal differences throughout the growth cycle, and it is mainly concentrated from transplanting to the jointing stage with additional requirements in the heading stage. As the primary N absorbing organs (Wei *et al.* 2018), root development and morphological traits strongly influence the nutrient uptake and growth of rice (Fan *et al.* 2010). The synergistic effect of optimal root growth and a uniform and appropriate soil N supply that aligns with crop N requirements is essential for achieving high yields and high NUE (Peng *et al.* 2015; Yang *et al.* 2023). Proper N fertilizer management enhances the soil NH_4^+ and NO_3^- levels and also improves crop roots, thereby promoting N uptake in rice and improving NUE (Bijay and Singh 2017; Wang *et al.* 2023). CRNF has a controlled rate of N release that better matches crop N needs, which reduces the risk of N loss and increases the soil inorganic N content (Ke *et al.* 2018; He R *et al.* 2023). Consistent with this, the use of CRNF can significantly increase the soil N content of wheat and double-cropping rice compared to normal urea application (Li *et al.* 2021; Su *et al.* 2024), which substantially promotes the root growth of rice seedlings after sowing (He *et al.* 2023) and improves root morphology to promote wheat growth (Li *et al.* 2021). These findings demonstrate that the application of CRNF is an effective method for simultaneously improving crop root development and soil N content.

Numerous studies have demonstrated the effects of CRNF or STF on rice yield and NUE (Yang *et al.* 2021; Li *et al.* 2023). Due to the varying N release rates of different fertilizers (Wu *et al.* 2021), combined with different fertilization methods, soil N distribution in time and space is expected to vary. In this study, CRBF was designed by combining different types of CRNFs in specific proportions to achieve a tailored release pattern. This formulation aims to synchronize N release with the dynamic N requirements of rice during its growth stages. Especially under single-time side-deep fertilization, the slow-release characteristics of CRBF help to minimize N losses through leaching and volatilization, while maintaining higher levels of soil available N (Priya *et al.* 2024). Compared with the individual effects of fertilizer type and STF, less is known about their interactive effects on soil N distribution, root growth, grain yield, and NUE in rice. One key question is whether CRBF with STF improves soil N distribution and root growth to promote rice grain yield and NUE.

This study aimed to investigate the mechanism by which CRBF with STF influences rice grain yield and NUE through a two-year experiment, by investigating three complementary aspects: (1) the effects of CRBF with STF on rice grain yield and NUE; (2) the soil N supply characteristics and its regulatory effects on rice root growth and plant N uptake; and (3) the potential mechanism of increasing rice yield and NUE under STF with CRBF.

2. Materials and methods

2.1. Experimental location and weather conditions

The two-year field experiment was conducted from 2022–2023 at Danyang Experimental Base of Nanjing Agricultural University (32°00′N, 119°32′E), China. This site had been using rice–wheat rotation as its cultivation system for a long term. The weather conditions and the properties of the topsoil at the beginning of the experiment are shown in Appendices A and B, respectively.

2.2. Experimental materials and design

The experiment comprised six treatments, i.e., two single-

time fertilization methods and two fertilizer types including a control (CK), and an additional treatment without N (N0) was added to evaluate the NUE. Each treatment was arranged randomly with three replications. Two single-time fertilization methods were used: i) broadcast, with a single-time broadcast on the soil surface while transplanting rice seedlings; and ii) side-deep fertilization, with a 5 cm deep trench created 5 cm adjacent to the rice seedlings and a single uniform application of N fertilizer into the trench. The diagram of the fertilization methods is shown in Appendix C. The two types of fertilizer used were normal urea (U, 46% N) and controlled release blended fertilizer (CRBF, 41.5% N). CK was a four-way split application of normal urea (as basal, tillering, first panicle, and second panicle fertilizer, 3:2:3:2), with a total N amount of 210 kg N ha^{-1}. CRBF consisted of five types of N fertilizer: 2-month polymer-coated urea (43% N), 3-month polymer-coated urea (43% N), 4-month polymer-coated urea (43% N), sulfur-coated urea (37% N), and granular urea (U, 46% N). The N release rates (%) and time intervals of N release rate of CRBF buried in paddy soil after transplanting in 2022 and 2023 are shown in Fig. 1. CRBF and CK had the same N rate of 210 kg N ha^{-1}. All treatments were supplied with 42 kg P_2O_5 ha^{-1} and 84 kg K_2O ha^{-1} at rice planting as the basal fertilizers. The area of each single plot was 72 m^2 (18 m×4 m). Each plot was surrounded by ridges and isolated with plastic film.

The LK-1250 Plant Root Box Imaging System (Eco-mind Technology Co., Ltd., Beijing, China) was used to determine the traits of rice roots (Appendix D). The seedlings were transplanted into root boxes (60 cm in height, 30 cm in length and 10 cm in width) at the same time as the field experiment, and the boxes were incline cultivated (45°) in an artificial climatic chamber. The treatments in the root box experiment were consistent with the field experiment, and the fertilizer amounts, including N, P_2O_5, and K_2O, were converted in proportion to the soil weight. Each treatment had five replicates.

A late *japonica* rice variety, Ningxiangjing 9, was used in this experiment. Ningxiangjing 9 is widely planted locally, and its growth duration is approximately 150 days, with little variation across years. Sowing occurred on 25 May with manual transplantation on 14 and 15 June in 2022 and 2023, respectively. Harvests were conducted on 25 October, 2022 and 30 October, 2023. For field water management, the water level in each plot was maintained at 1–2 cm in the first wk after transplanting to promote seedling and root development. Then, 2–3 cm of flooding was maintained by irrigation during the tillering stage, followed by drainage during the mid-term aeration period. Thereafter, a wetting and drying cycle was performed to achieve the necessary drainage before harvest. The plots were separated to allow for separate water management, and the same water level was maintained in all plots. All additional agronomic practices adhered to the regional standards for high-yield rice cultivation management.

2.3. Sampling and measurements

Sampling and measurement methods Plant dry matter samples were gathered during the four critical growth stages critical growth stages of tillering, panicle initiation, heading, and maturity. At each stage, five plants were selected for sampling according to the average tillering number. At maturity, plants from each whole plot were machine-harvested for grain yield measurement. The grain yield was calibrated to a standard moisture level of 14.5%.

Initially, the samples were subjected to drying at 105°C for 30 min, followed by additional drying at 80°C until they reached a stable weight. Once dried, the samples were weighed, ground, and then sifted through a 2 mm sieve. N uptake (kg ha^{-1}) was quantified by the N content (%) and plant aboveground dry matter weight. The N uptake, nitrogen recovery efficiency (NRE) and nitrogen agronomic efficiency (NAE) were calculated using eqs. (1), (2) and (3), respectively.

$$\text{N uptake}=N_{\%}\times DW\times 10 \quad (1)$$

$$\text{NRE}\ (\%)=(N_t-N_0)/Nr\times 100 \quad (2)$$

$$\text{NAE}\ (\text{kg kg}^{-1})=(GY_t-GY_0)/Nr \quad (3)$$

where $N_{\%}$ is the plant aboveground N content (%) at each stage, assessed *via* the Kjeldahl method established by Bremner (1960). DW is the weight of plant aboveground dry matter (t ha^{-1}) at each stage; N_t is plant aboveground N uptake (kg N ha^{-1}) at the maturity stage of each treatment; N_0 is plant aboveground N uptake (kg N ha^{-1}) at the maturity stage of the N_0 treatment; GY_t is the grain yield (kg ha^{-1})

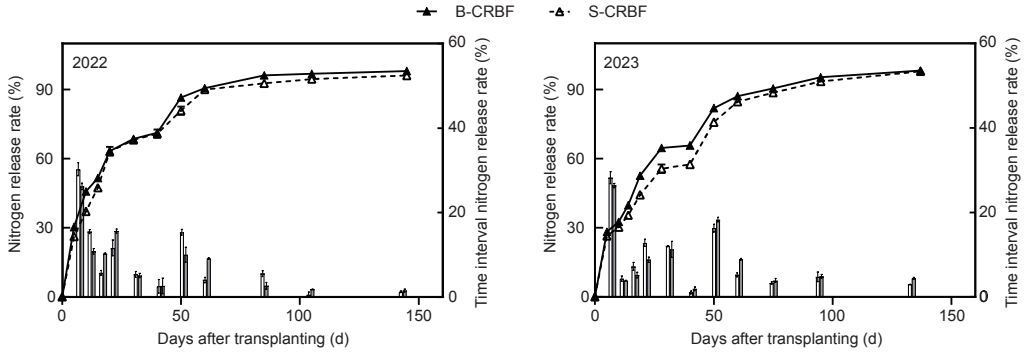


Fig. 1 Nitrogen release rate (curves) and time interval N release rate (bars) of controlled-release blended fertilizer (CRBF) with two single-time fertilization methods in the paddy field during 2022 and 2023. Water management was consistent with the field experiment, and a mid-term aeration was carried out at 35–45 d. B, broadcast; S, side-deep fertilization. Bars mean SE (*n*=3).

of each treatment; GY_0 is the grain yield (kg ha^{-1}) of the N_0 treatment; and Nr is the rate of N fertilizer input (kg N ha^{-1}).

SPAD and leaf enzyme activities The SPAD values of the top 3rd leaf on the main stem (from five representative plants) were measured during the tillering, panicle initiation, heading, and grain-filling stages (20 days post-heading) using a SPAD-502 chlorophyll meter (Minolta, Japan).

To measure the activities of glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT), leaf samples were collected from the top 2nd leaf (fully expanded) at the tillering stage and the top 1st leaf at the heading stage. For each treatment, 10 leaves from the main stem were selected. Enzyme activities were measured using the GS Assay Kit (BC0915) and the GOGAT Assay Kit (BC0075) from Solarbio (Beijing, China), following the manufacturer's protocols.

Root characteristics In this field study, soil blocks measuring 60 cm long, 30 cm wide, and 20 cm deep, which included the roots of three selected plants, were extracted from each plot during the tillering and heading stages for assessing root biomass. These blocks were placed within 40-mesh nylon bags and thoroughly rinsed with running water to separate the roots. Each collected root sample was first dried at 105°C for 30 min, further dried at 80°C until a constant weight was obtained and then the dried root samples were weighed.

Five root boxes (LK-1250 Plant Root Box Imaging System) were selected from each treatment at the tillering and heading stages to photograph the rice roots, and rhizoTrak was used to analyze the root morphology (total root number, total root length, total root surface area, and total root volume) according to the method of Möller *et al.* (2019).

Soil inorganic N During the growing season, soil samples from a depth of 0–60 cm were collected at 5-d intervals for the first 3 wk after rice transplantation, and subsequently every 10 days until harvest. Additional soil samples were collected from the 0–20 cm depth with two zones (root-zone soil and bulk soil) at the tillering and heading stages. The root-zone soil was sampled at about 1 cm from both sides of the plant, and the samples from both sides were mixed evenly. The bulk soil was sampled at about 15 cm from the plant. Five replicates were taken for each treatment. The soil sampling method is shown in Appendix E. These fresh soil samples were promptly extracted using a 2 mol L^{-1} potassium chloride (KCl) solution. The concentrations of NH_4^+ and NO_3^- in the extracts were quantified using an auto-analyzer (Model AA3, Bran-Luebbe, Hamburg, Germany).

2.4. Data statistical analysis

To evaluate the statistical significance of the mean differences, the least significant difference (LSD) test was applied at $P<0.05$ or $P<0.01$, using SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA). All graphs were generated using GraphPad Prism 10.1.2 (GraphPad Prism, San Diego, CA, USA). Correlation analysis was performed using the Apps Correlation Plot in OriginPro 2021 (OriginLab, Northampton, MA, USA). Partial least squares path modeling (PLS-PM) was conducted with SmartPLS 3.0 (SmartPLS GmbH, Pinneberg, Germany).

3. Results

3.1. Grain yield and NUE

Both B-CRBF and S-CRBF increased grain yields by 1.45 and 2.70% in 2022, and significantly increased yields by 4.90 and 6.17% in 2023 compared to CK, respectively (Fig. 2). Compared to U under STF, B-CRBF and S-CRBF significantly increased grain yields by 11.72 and 13.09% in 2022, and by 21.49 and 22.95% in 2023, respectively. There were no significant differences between broadcast and side-deep fertilization methods.

Regarding NRE and NAE, the effects of different treatments in 2022 and 2023 are shown in Fig. 3. Compared to CK, B-CRBF and S-CRBF increased the NRE by 5.65 and 17.81% in 2022, and by 18.40 and 33.70% in 2023, respectively, and they increased the NAE by 7.71 and 14.36% in 2022, and by 18.11 and 22.79% in 2023, respectively. Compared to U under STF, B-CRBF and S-CRBF significantly increased the NRE by 138.11 and 166.84% in 2022, and

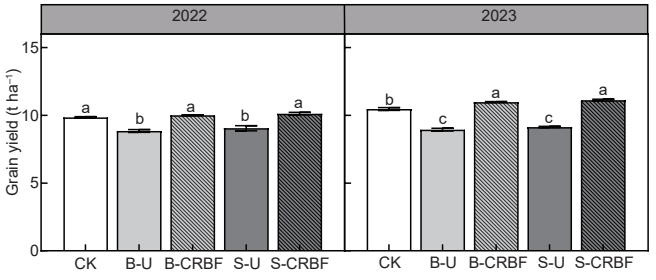


Fig. 2 Effects of different fertilizer types with different single-time fertilization methods on yield in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. Bars mean SE (n=3). Different letters indicate significant differences at the 0.05 level.

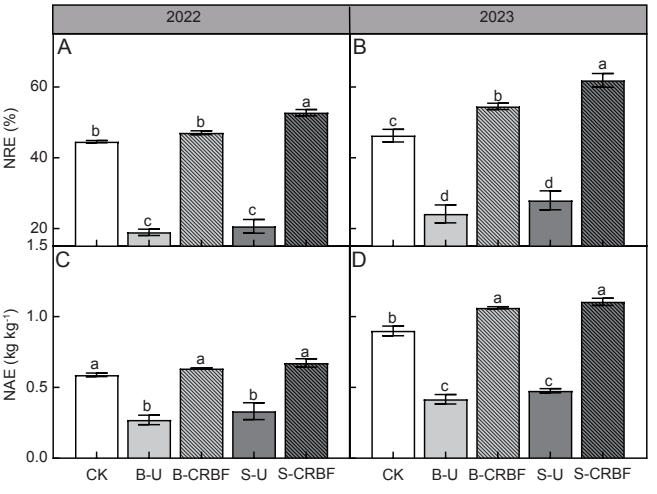


Fig. 3 Effects of different fertilizer types with different single-time fertilization methods on nitrogen recovery efficiency (NRE) (A and B) and nitrogen agronomic efficiency (NAE) (C and D) in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. Bars mean SE (n=3). Different letters indicate significant differences at the 0.05 level.

by 109.40 and 137.64% in 2023, respectively, and they significantly increased the NAE by 110.73 and 123.74% in 2022, and by 138.28 and 147.72% in 2023, respectively. Finally, S-CRBF significantly increased the NRE by 12.06% in 2022 and 13.49% in 2023 compared to B-CRBF, but there was no significant difference in NAE.

3.2. Plant dry matter accumulation and N uptake

For CRBF under STF versus CK, no significant improvement was observed in total plant aboveground dry matter accumulation at the maturity stage during the two years (Fig. 4-A and B). For CRBF under STF compared to U under STF, the total plant aboveground dry matter accumulation significantly increased by 11.21–17.62% in 2022 and 11.92–18.57% in 2023, and these differences were mainly concentrated in the SS-TS and TS-PI stages. During the SS-TS stage and compared to CK, S-CRBF enhanced dry matter accumulation by 30.51% in 2022 and 15.91% in 2023, while B-CRBF did not show any significant enhancement. For CRBF under STF, compared to S-CRBF, B-U and S-U significantly reduced the dry matter accumulation by 14.78–24.14% in 2022, and by 12.58–19.41% in 2023, but there were no significant differences compared to B-CRBF. During the TS-PI stage, CRBF under STF did not differ significantly from CK in dry matter accumulation.

The plant N uptake levels at various growth stages for different treatments in 2022 and 2023 are shown in Fig. 4-C and D. Compared to CK, B-CRBF and S-CRBF increased N uptake by 13.62 and 26.16% at SS-TS, 8.13 and 14.05% at TS-PI, and 24.63 and 37.37% at HS-MS in the two years, respectively. Compared to CK, S-CRBF significantly increased total plant N uptake by 7.79% in 2022 and 13.66% in 2023; while B-CRBF significantly increased it by 13.66% in 2023 with no significant difference in 2022. Both S-CRBF and B-CRBF significantly increased total N uptake compared with U under STF. Comparing the differences between the different fertilization methods, S-CRBF significantly increased the total plant N uptake by 5.27% in 2022 and 6.01% in 2023 compared to B-CRBF.

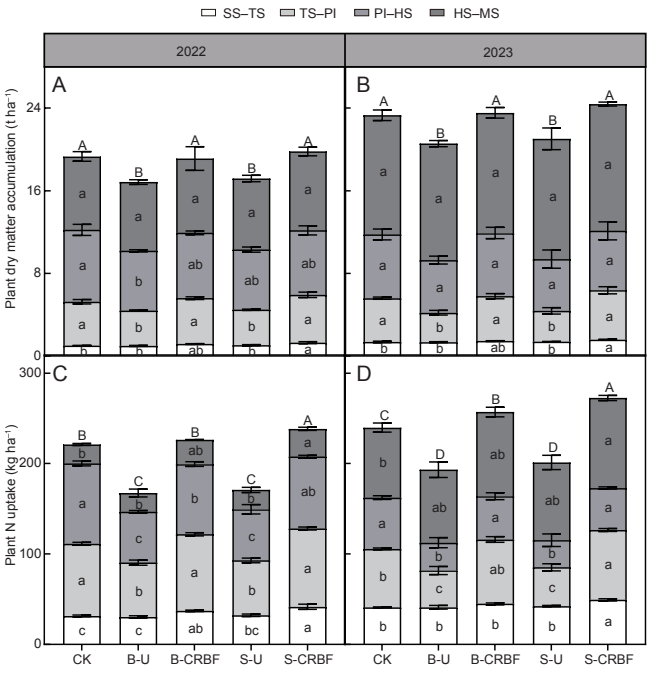


Fig. 4 Effects of different fertilizer types with different single-time fertilization methods on plant dry matter accumulation (A and B) and N uptake (C and D) in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. SS, seedling stage; TS, tillering stage; PI, panicle initiation; HS, heading stage; MS, maturity. Bars mean SE (n=3). Different lowercase and uppercase letters indicate significant differences (P<0.05) at different stages and in the total dry matter accumulation, respectively.

3.3. Leaf SPAD value and N assimilation enzyme activities

The top 3rd leaf SPAD value declined from the tillering stage to the panicle initiation stage, then increased from the panicle initiation to heading stages and finally declined to the grain-filling stage for CK and CRBF under STF in 2022 and 2023 (Fig. 5). Compared to CK, CRBF under STF significantly increased the SPAD values by 4.92–6.52% and 5.36–6.22%

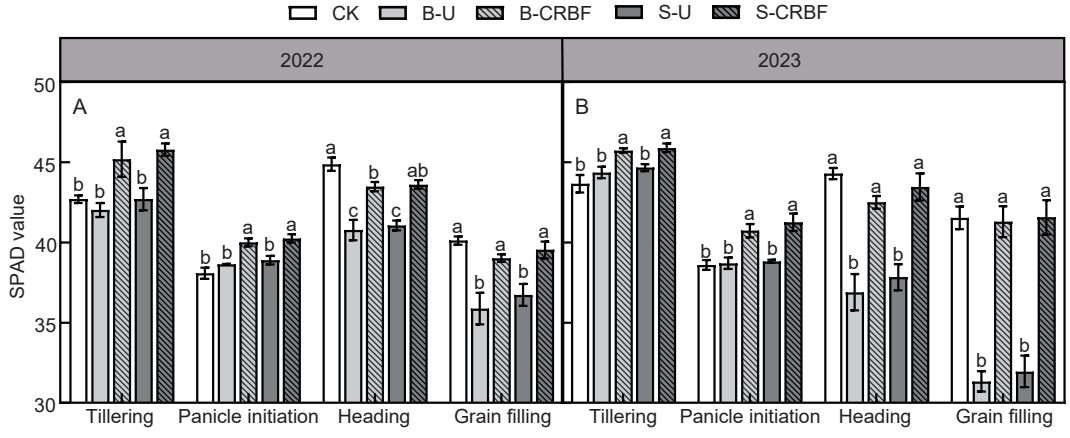


Fig. 5 Effects of different fertilizer types with different single-time fertilization methods on the SPAD values at different stages in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. Bars mean SE (n=5). Different letters indicate significant differences at the 0.05 level.

at the tillering and panicle initiation stages, respectively, while there were no significant differences in U under STF. S-CRBF did not differ significantly from CK at the grain filling stage and there was no significant difference between S-CRBF and CK, but B-CRBF significantly reduced the SPAD values at the heading stage by 3.14% in 2022 and 4.06% in 2023.

Fertilizer type and fertilization method affected the GS and GOGAT activities at the tillering and heading stages (Fig. 6). For the same fertilizer type, there were no significant differences in the GS and GOGAT enzyme activities between broadcast and side-deep fertilization at the tillering and heading stages. For GS activity, there were no significant improvements in CRBF under STF compared to CK at the tillering and heading stages, while U under STF significantly reduced the GS activity at heading stage by 22.51% in 2022 and by 20.31% in 2023. For GOGAT activity, there was no significant improvement in CRBF under STF compared to CK at the tillering stage, but it increased by 40.17% in 2022 and 8.34% in 2023 at the heading stage.

3.4. Root characteristics

The root characteristics under different fertilizer types and fertilization methods at the tillering and heading stages are shown in Fig. 7. Significant differences were observed for root biomass, TRN, RAD, TRL, TRS, and TRV at the tillering and heading stages, and compared to U under STF, with CRBF under STF they significantly increased by 19.13–32.75%, 28.33–46.54%, 36.22–40.34%, 6.43–8.91%, 45.10–52.50%, and 60.56–81.43% at the tillering stage, respectively, and by 34.45–58.59%, 36.21–57.53%, 17.79–

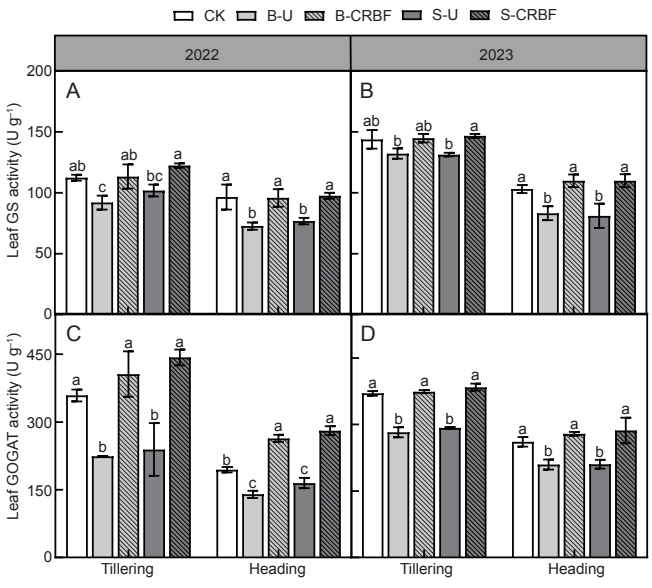


Fig. 6 Effects of different fertilizer types with different single-time fertilization methods on leaf glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) activities at different stages in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. Bars mean SE (n=3). Different letters indicate significant differences at the 0.05 level.

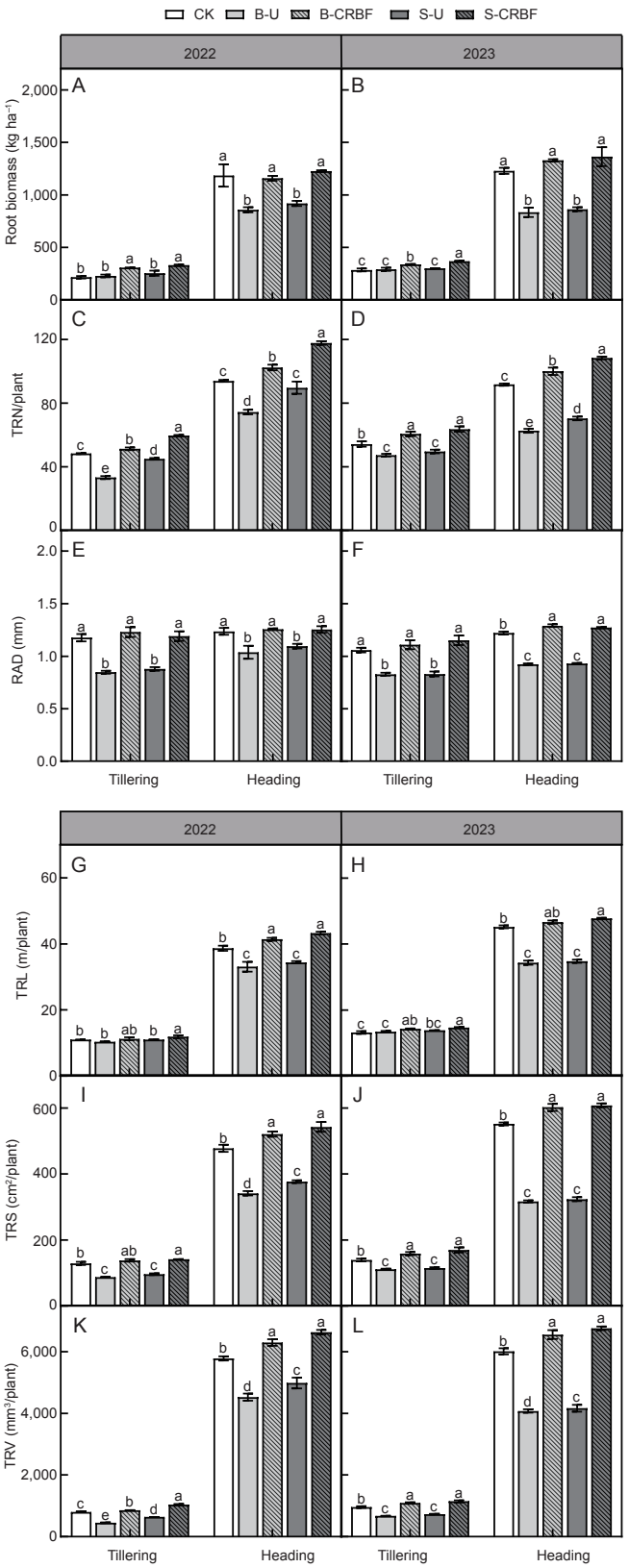


Fig. 7 Effects of different fertilizer types with different single-time fertilization methods on root characteristics at different stages in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. TRN, total root number; RAD, root average diameter; TRL, total root length; TRS, total root surface area; TRV, total root volume. Bars mean SE (n=5). Different letters indicate significant differences at the 0.05 level.

38.40%, 25.54–36.57%, 48.48–89.04%, and 36.4–61.60% at the heading stage, respectively.

3.5. Soil inorganic N concentration

Fertilizer types and fertilization methods had significant effects on the spatial and temporal distribution of soil NH_4^+-N concentration (Fig. 8). In the 0–60 cm soil layer, the soil NH_4^+-N concentration in CRBF under STF showed dynamic changes consistent with the N release characteristics of CRBF under STF (Fig. 1). Throughout the rice growth period, CRBF under STF experienced two soil NH_4^+-N concentration peaks during the early tillering stage (20–30 d) and the panicle initiation stage (50–60 d). This aligns with the changes in the soil NH_4^+-N concentration in CK, which were due to the split application of urea in the tillering fertilizer and panicle fertilizer. For U under STF, where all N fertilizer was supplied at once as a basal fertilizer, the soil NH_4^+-N concentration showed one peak in the early tillering stage (0–10 d) and gradually declined over time. A comparison of the soil NH_4^+-N concentrations in different soil layers during the two critical stages revealed that CRBF under STF significantly increased the soil NH_4^+-N concentrations by 33.28–91.77% and 40.20–111.87% at the tillering stage, and by 144.61–158.69% and 58.55–99.31% at the heading stage in the 0–10 cm soil layer, respectively. Moreover, the concentration of soil NH_4^+-N in the 10–20 cm soil layer under CRBF under STF increased by 113.16–132.77% and 159.34–289.08% at the tillering stage, and by 227.52–231.06% and 95.38–155.01% at the heading stage, respectively.

In the 20–40 and 40–60 cm soil layers, there were no significant differences in the NH_4^+-N concentration between the different treatments at either the tillering or heading stages in either year. As shown in Fig. 9, compared to U under STF, the soil NH_4^+-N and NO_3^--N concentrations in the root-zone soil for CRBF under STF were significantly increased by 56.66–90.78% and 24.18–93.19% at the tillering stage, and by 91.08–296.37% and 15.74–99.27% at the heading stage. The soil NH_4^+-N and NO_3^--N concentrations were also increased in the bulk soil by 39.53–99.32% and 22.58–94.97% at the tillering stage, and by 46.73–215.46%

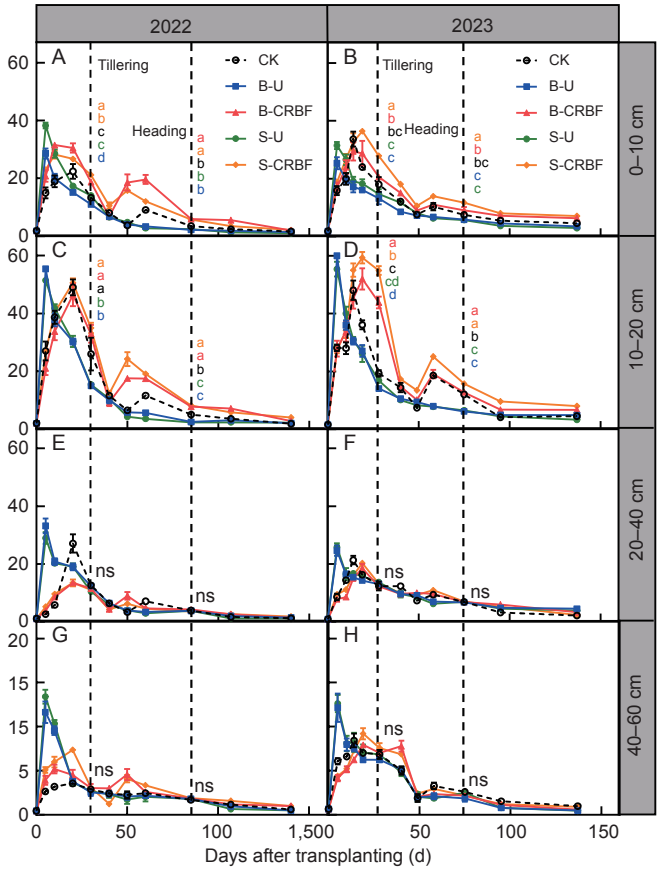


Fig. 8 Soil NH_4^+-N concentrations in the 0–60 cm soil layers under different fertilizer types with different single-time fertilization methods in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization; ns, no significant difference. Bars mean SE (n=3). Different letters indicate significant differences at the 0.05 level.

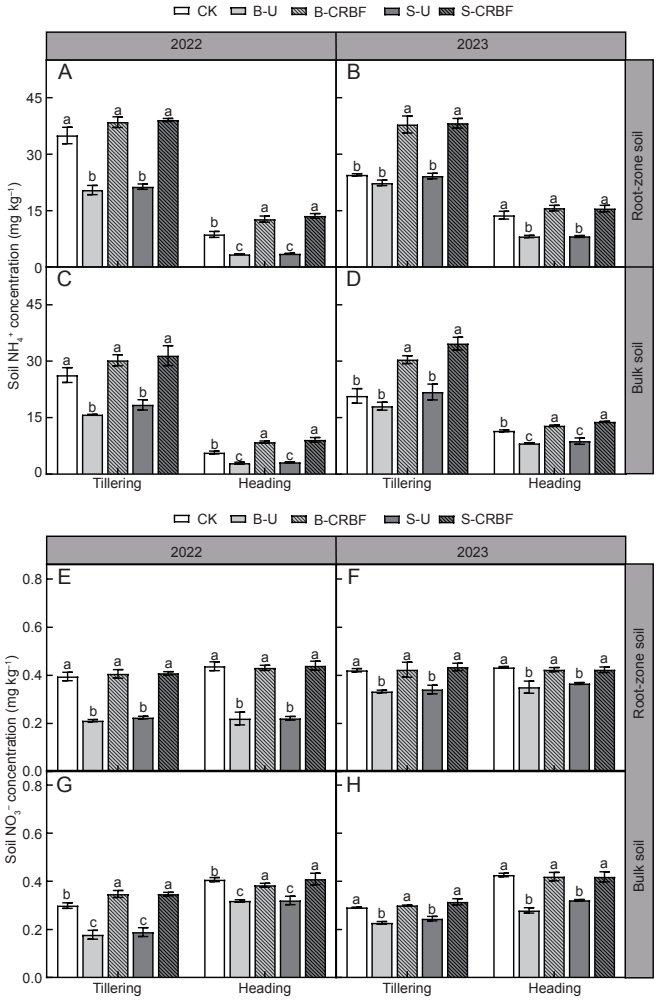


Fig. 9 Effects of different fertilizer types with different single-time fertilization methods on soil NH_4^+-N (A–D) and NO_3^--N (E–H) in the root-zone and bulk soils at different stages in 2022 and 2023. CK, four split applications of normal urea; U, normal urea; CRBF, controlled release blended fertilizer; B, broadcast; S, side-deep fertilization. Bars mean SE (n=3). Different letters indicate significant differences at the 0.05 level.

and 19.66–50.28% at the heading stage, respectively, but there were no significant differences between the different fertilization methods under the same fertilizer type.

3.6. Correlation analysis and PLS-PM

The correlation analysis (Fig. 10) indicated that grain yield and NUE had strong positive correlations with several factors, including plant total dry matter accumulation, plant total N uptake, plant N status (including SPAD value and leaf GS/GOGAT activities), root biomass, root morphology (including TRN, RAD, TRL, TRS, and TRV), and soil inorganic N content (including NH_4^+ and NO_3^- concentrations in root-zone soil and bulk soil). Furthermore, the results of the PLS-PM analysis revealed that soil inorganic N influenced the rice root morphology which consequently affected plant N status (Fig. 11). This, in turn, enhanced plant N uptake, leading to increased dry matter accumulation and, ultimately, improved grain yield and NUE.

4. Discussion

4.1. Single-time fertilization with CRBF improves the spatial and temporal dynamics of soil N

In this study, CRBF under STF showed stable and controllable N release characteristics, and the soil NH_4^+-N concentration was consistent (Figs. 1, 8, and 9). The soil NH_4^+-N concentrations under B-CRBF and S-CRBF showed two obvious peaks at 10–30 and 50–70 d after transplanting (Fig. 8), which match the N demand peaks of rice to ensure a sufficient N supply during the critical stages (Ling 2010). Furthermore, the soil N supply was significantly reduced at about 40 d after transplanting, which can precisely reduce the N uptake of rice in the ineffective tillering stage to meet the requirements of high-yield rice cultivation. Combined with agronomic practices such as mid-season aeration, this can reduce ineffective tillers and increase the productive tiller percentage (Carrijo *et al.* 2017). These controlled N release and soil N supply characteristics contrasted significantly with the N dynamics observed with U under STF, where a rapid rise in the NH_4^+-N concentration occurred shortly after transplanting, followed by a sharp decline. The rapid release of N from U leads to periods of excess N, increasing the risk of N losses, particularly in the early stages after rice seedling transplantation when the seedling N demand is lower (Shi *et al.* 2010). This is because the soil has a limited ability to fix NH_4^+-N, and maximum NH_4^+-N fixation in paddy soils is reached 1–3 d after fertilization. In addition to the portion that is absorbed by the plants and retained in the soil, the remainder is lost through leaching, runoff, and volatilization (Nieder *et al.* 2011). This may explain why the NH_4^+-N concentrations under B-CRBF and S-CRBF were higher than those under B-U and S-U. The continuous release of N during the whole plant growth cycle under STF with CRBF plays a crucial role in minimizing N losses in the soil and mitigating the risks associated with leaching and ammonia volatilization, thereby increasing plant N uptake and reducing N losses. During the later stages of growth, particularly after the heading stage — which is an essential stage for rice yield development — CRBF under STF maintained levels of soil NH_4^+/NO_3^- comparable to CK following the application of panicle fertilizer in both the root-zone and bulk soil and was significantly higher than that of U under STF. This appropriate and sufficient N supply can ensure the continuing growth of rice in the later stages.

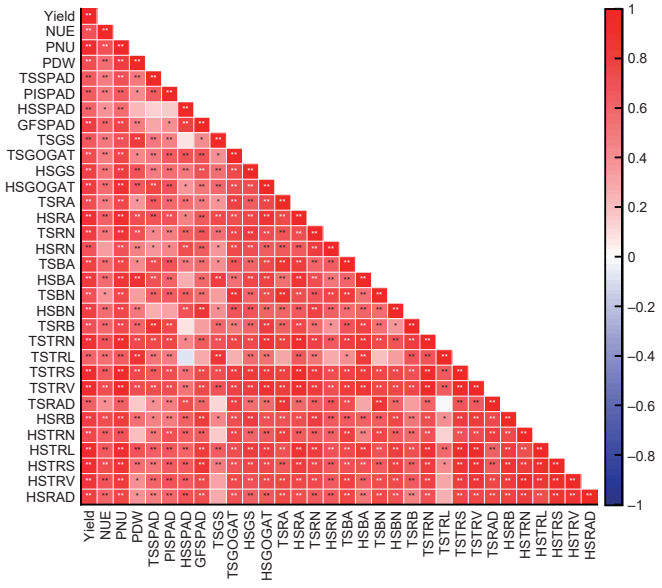


Fig. 10 Correlation analysis illustrating the relationships between grain yield, N use efficiency (NUE), dry matter weight, plant N status, soil inorganic N, and root traits of different fertilizer types with different single-time fertilization methods in 2022 and 2023. Correlation coefficients are shown by scale bars, and the negative correlations are blue while the positive correlations are red. TS, tillering stage; HS, heading stage; PI, panicle initiation stage; GF, grain-filling stage; PNU, plant total N uptake; PDW, plant total dry matter accumulation; SPAD, plant top 3rd leaf SPAD value; GS, glutamine synthetase activity; GOGAT, glutamine oxoglutarate aminotransferase activity; RA and RN, NH_4^+ and NO_3^- concentrations in root-zone soil; BA and BN, NH_4^+ and NO_3^- concentrations in bulk soil; RB, root biomass; TRN, total root number; RAD, root average diameter; TRL, total root length; TRS, total root surface area; TRV, total root volume. *, P<0.05; **, P<0.01.

Our findings also revealed that compared to U under STF, CRBF under STF could increase the NH_4^+-N concentration in the topsoil (0–20 cm) and maintain a level that was not significantly different in the 20–60 cm soil layer, which may be related to the slow release of N from CRBF resulting in reduced downward migration through the soil column (Zhang *et al.* 2018). This aligns with the findings of Li *et al.* (2021) in wheat, who reported that CRNF enhanced the NH_4^+-N levels in the topsoil by 9–11% compared to normal urea, with no significant difference in the 20–40 cm soil layer. Compared to the study by Ke *et al.* (2018), our findings further clarify the spatial and temporal dynamics of the soil NH_4^+-N concentration under STF of CRBF, particularly in terms of spatial distribution. Specifically, our results demonstrated that CRBF significantly increased the NH_4^+-N concentration in the 0–20 cm soil layer. The results also showed that under STF with U NH_4^+-N tended to migrate to the deep soil layer in the early growth stage, which may be related to the excessively high soil NH_4^+-N concentration after the single-time fertilization with U, which made it more likely to cause NH_4^+-N leaching.

Overall, by using STF with CRBF, the paddy soil can always maintain sufficient inorganic N for rice uptake, and its special N supply dynamics of "rising early and stabilizing later" can be synchronized with the N requirements of rice.

4.2. Root growth and N uptake are improved by single-time fertilization with CRBF

The application of CRBF promotes plant growth and enhances N uptake (Liu Y *et al.* 2023). Our results showed that CRBF under STF increased plant dry matter accumulation and N uptake by 8.29 and 21.85% compared to CK and U under STF (Fig. 4), and the PLS-PM analysis demonstrated that CRBF promoted rice root growth to enhance N uptake and promote rice growth (Fig. 11). The root is the crucial nutrient uptake organ of plants (Kiba and Krapp 2016). CRBF combined with STF created a stable soil N supply environment due to the continuous release of N, especially under side-deep fertilization which increased the local N concentration in the soil, while the root responded through morphological plasticity to adapt to the changing soil environment (Robinson 1994; López-Bucio *et al.* 2003). Our findings showed that single-time fertilization with CRBF mainly improved root morphology to promote N uptake and thus increased dry matter accumulation (Fig. 11). We believe this is because STF with CRBF effectively increased the soil NH_4^+-N concentration. An appropriate and optimal soil N level can stimulate the growth and development of rice roots, and rice roots can use morphological plasticity to adapt to the distribution characteristics of NH_4^+-N under the STF with CRBF application (Mou *et al.* 2013). This morphological adaptation results in greater TRN, TRL, TRS, and TRV in soil regions with higher soil NH_4^+-N concentrations. Notably, with side-deep fertilization, N was more concentrated on the fertilization side due to this method, creating N heterogeneity that further promoted root proliferation and optimized root morphology. Similarly, Hu *et al.* (2024) demonstrated through local fertilization experiments that CRNF can increase the TRS, TRV, and RAD of rice and enhance nutrient uptake. Jia *et al.* (2022) and Nacry *et al.* (2013) found that soil inorganic N levels are related to the hormone contents in roots, such as auxin, cytokinin, and strigolactone. For example, NH_4^+ can affect auxin diffusion and accumulation through the ammonium transporter, thereby affecting root system architecture such as root tip number and root length to improve N acquisition (Luo *et al.* 2022). In this study, CRBF under STF increased the TRN, TRL, TRS, TRV, and RAD compared to U under STF (Fig. 7), which may be related to the fact that in order to adapt to different soil N concentrations and distributions, the rice roots used morphological plasticity to change and optimize the root morphology.

Our research revealed that CRBF under STF effectively

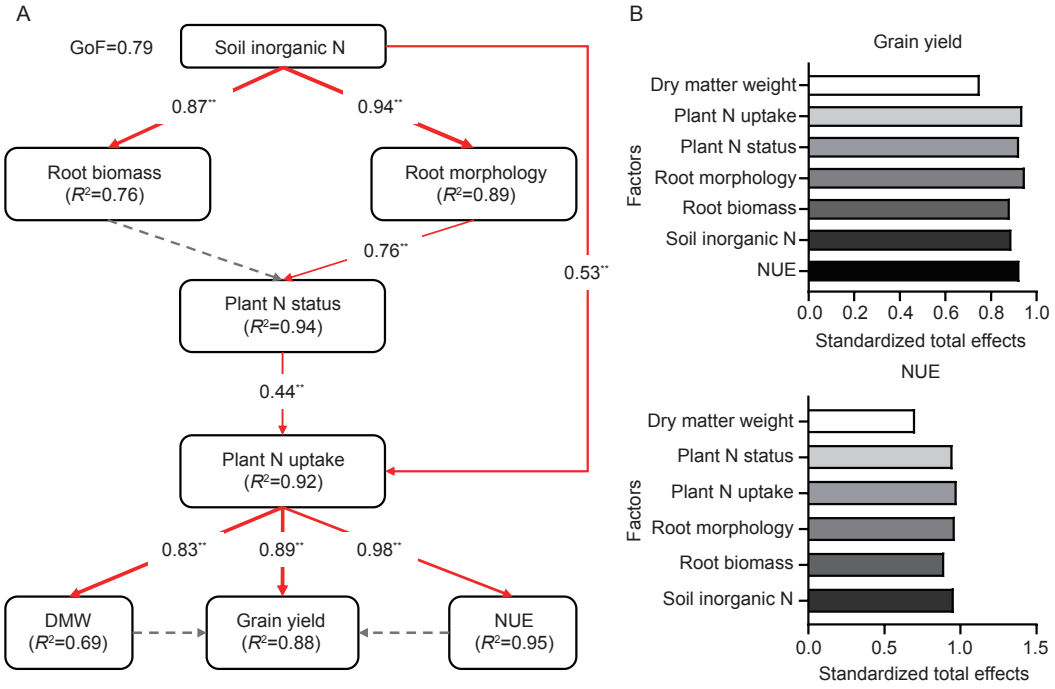


Fig. 11 Partial least squares path models (PLS-PM) illustrating the direct and indirect effects of soil inorganic N, root biomass, root morphology, plant N status, plant N uptake, and plant total dry matter weight (DMW) on the contributions of grain yield and N use efficiency (NUE) (A) and the standardized total effect on grain yield and NUE from PLS-PM (B). The GoF index served as a metric for assessing model quality, with a score exceeding 0.7 signifying outstanding predictive capability. The R^2 values of the latent variables were analyzed to determine the extent of variance in the dependent variables that could be accounted for by the independent latent variables. Positive causal relationships are denoted by red arrows, whereas black arrows illustrate negative causal influences. The thickness and numerical values of the arrows signify normalized path coefficients, while dotted arrows represent paths that are not statistically significant. Root morphology was considered a latent variable and indicated as rice total root number (TRN), root average diameter (RAD), total root length (TRL), total root surface area (TRS), and total root volume (TRV) at the tillering and heading stages. Plant N status was indicated as the plant top 3rd leaf SPAD value, and plant leaf GS/GOGAT activities at the tillering and heading stages. **, P<0.01.

enhanced the dry matter accumulation and N uptake in rice during the early growth stage, and there was no significant difference in the later stage compared to CK (Fig. 4). This improvement could be attributed to the higher soil inorganic N concentration in the tillering stage, which induced an appropriate root morphology in the early stage, thereby creating a good foundation for N uptake capacity. As the rice grew, CRBF continuously released N and maintained an appropriate inorganic N concentration after heading, which delayed root senescence and maintained the root N uptake capacity. The N uptake was significantly greater under side-deep fertilization with CRBF compared to broadcast. The reason for this is that side-deep fertilization expands the interaction zone between the root and the fertilizer, subsequently enhancing the plant N uptake capability. Similar results were reported by Zhang *et al.* (2024) and Zhe *et al.* (2023) in potato and maize, respectively. Meanwhile, in this study, CRBF under STF increased SPAD values at the tillering and heading stages (Fig. 5), and the plant N content also supported this result (Appendix F). This indicates that CRBF under STF can increase the plant N content, probably due to the combination of a stable soil N supply level and appropriate root architecture, which promotes more efficient N utilization. In addition, CRBF under STF increased the leaf GS and GOGAT activities (Fig. 6). GS and GOGAT are key enzymes for plant N assimilation (Kojima *et al.* 2021), and their greater activities indicate that CRBF under STF promoted the intensity of plant N assimilation and increased the N utilization capacity, thus promoting plant N accumulation.

4.3. Single-time fertilization with CRBF increases rice grain yield and NUE

N fertilizer plays a crucial role in enhancing the rice yield and NUE (Cai *et al.* 2023). Our findings revealed that CRBF under STF significantly enhanced the rice grain yield, NRE, and NAE compared to CK and U under STF, with average increases of 10.57, 79.28, and 74.78% over the two years, respectively (Figs. 2 and 3). Some studies have reported that the tillering and heading stages are the peaks of rice N requirement, and can determine the yield and NUE (Ata-Ul-Karim *et al.* 2017; Yi *et al.* 2019). Therefore, ensuring an adequate soil N supply during these two stages is crucial for improving the rice grain yield and NUE. Through PLS-PM analysis, our results showed that CRBF under STF can promote plant N uptake by increasing the soil inorganic N supply, thereby increasing the rice grain yield and NUE (Fig. 11).

The stable increase in the soil N supply, with a pattern of "rising early and stabilizing later" from the CRBF under STF during the tillering and heading stages, aligns closely with the N requirement of rice. An analysis of yield components showed that CRBF under STF increased the rice yield by increasing the panicle number and total spikelets (Appendix G). This was because the greater soil inorganic N during the tillering stage promoted the rice tiller number, ultimately increasing the panicle number and productive tiller percentage at the maturity stage (Appendix H) and significantly increasing the plant N uptake in the early stage through better root architecture. The consistent availability of soil inorganic N throughout the panicle initiation and grain-filling stages contributed to dry matter accumulation at maturity, while the well-developed root system established earlier on delayed senescence in the later stages and maintained continuous N uptake. Therefore, there was no significant difference in N uptake compared to CK after heading (Fig. 4). This N uptake pattern with the characteristics of "promotion early and stability later" ultimately increased the rice grain yield and NUE. These results are consistent with studies indicating that a single application of CRBF increases rice grain yield and NUE (Ke *et al.* 2017; He R *et al.* 2023). Moreover, CRBF concentrated the soil N supply in the root zone under side-deep fertilization, reduced N loss and promoted more efficient N uptake to improve NUE. Zhang *et al.* (2023) and Zhong *et al.* (2024) reported the same conclusions in maize and double-season rice, respectively.

In contrast, the rapid N release of U under STF may lead to an N surplus in the early stage, followed by potential N deficiency after the crop N requirement reaches its peak. This situation is not synchronized with the rice N requirement characteristics, resulting in lower yield and NUE. Therefore, CRBF under STF, especially with side-deep fertilization, provides a sufficient soil N supply in the paddy field with appropriate root growth to improve the rice grain yield and NUE, so it can be recommended as a high-yield and high-efficiency fertilization strategy in rice production.

5. Conclusion

CRBF under STF optimized the soil N distribution and maintained the soil NH_4^+-N concentration throughout all the growth stages, which was manifested as "rising early and stabilizing later" to synchronize with the rice N requirement. CRBF under STF enhanced the soil inorganic N concentration and optimized root morphology to improve plant N uptake, thus increasing rice grain yield and NUE compared to U under STF. Compared to broadcast, side-deep fertilization of CRBF had a slightly higher yield and significantly improved NUE. These findings indicate that CRBF under STF, especially with side-deep fertilization, is an efficient N fertilization strategy for achieving high yield and high efficiency in rice production. Further in-depth studies at the physiological and molecular levels are essential to reveal the mechanistic details of how single-time fertilization with CRBF optimizes rice yield and NUE.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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