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Localized nitrogen supply facilitates rice yield and nitrogen use efficiency by enabling root-zone nitrogen distribution and root growth

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Introduction: Localized nitrogen (N) supply affects rice N uptake by influencing N release, and few studies have examined the effects of root zone N distribution and root growth on rice yield under localized N supply (LNS).

Methods: A two-year field experiment was conducted with six treatments: no N application, farmers' fertilizer practice (FFP), and four LNS treatments, including two types of N fertilizer with urea (U) and controlled release urea (CRU) were mechanically side deep fertilized (SDF) or root zone fertilized (RZF) at 10 cm soil depth (US, UR, CRUS and CRUR treatments, respectively).

Results: Compared with FFP, the dry matter accumulation, N uptake, and yield of LNS increased by 27%, 21%, and 17%, respectively. For N fertilizer type, compared with U, the NH_4^+-N concentration, total root surface area, volume, average diameter, and root biomass of CRU were significantly increased by 50%, 43%, 53%, and 23%, respectively, which resulted in a significant increase in yield by 12%. Regarding the N application methods, the total surface area, volume, average diameter, and root biomass of SDF were significantly increased by 32%, 24%, 10%, and 25% compared with RZF, respectively. However, the NH_4^+-N under RZF was more stable and lasted longer, with a significant increase in NH_4^+-N concentration of 21% compared to the SDF. Moreover, CRUR increased yield, N agronomic use efficiency, and gross return by 3.15%, 5.62%, and 2.81%, respectively, compared to CRUS.

Conclusion: CRU should be selected as the recommended N fertilizer types, and the combination of CRU and RZF was the most effective choice for rice production.

KEYWORDS

rice, yield, controlled release urea, mechanical side deep fertilization, root zone fertilization

1 Introduction

Rice (*Oryza sativa* L.) is the dominant staple food for more than 50% of the world's population (Yuan et al., 2021). China produces 28% of the global rice supply and still needs to further increase its rice production to close the gap with the target yield (Deng et al., 2019). Chemical fertilizers, especially N fertilizers, have been extensively applied in paddy fields to maintain high rice yields (Zhuang et al., 2022). Excessive N fertilizer inputs with lower N use efficiency (NUE) lead to environmental pollution such as air pollution (Menegat et al., 2022), water eutrophication (Wang et al., 2021), and soil degradation (Jin et al., 2021), thus increasing yields and NUE while reducing environmental pollution has become one of the primary objectives of modern agriculture (Waqas et al., 2023). Extensive studies have been conducted to assess the effects of different fertilization methods on NUE and yield. Localized N supply (LNS) is a method of placing N fertilizer near the root system that allows the localized release of N fertilizer, which increases N uptake by the crop and mainly consists of band or hole applications. Compared to broadcast or mixed, LNS can increase grain yield and NUE (Zhu et al., 2019; Chen et al., 2023).

Mechanical side deep fertilization (SDF) and root zone fertilization (RZF), as two primary fertilization methods for LNS, have received widespread attention in recent decades for their effectiveness in reducing greenhouse gas emissions and achieving increased rice yields (Li et al., 2022). Some studies believe that this is because fertilizer can be delivered precisely to the rice root zone under SDF and RZF, and the concentration of ammonium N (NH_4^+-N) in the root zone increases, while rice is an ammonia-loving crop, so it promotes N uptake by rice root and increases the aboveground biomass of rice, thus obtaining high yields (Zhu et al., 2019; Ding et al., 2022). However, it has also been suggested that the increase in rice yield under SDF and RZF is due to the root system. The root system plays a crucial role in obtaining water and nutrients. Root development and trait distribution strongly influence rice's nutrient uptake and growth (Ma et al., 2021). Moreover, Cheng et al. (2020) reported that higher N supply can improve the spatial structure of the root system and thus promote plant growth (Cheng et al., 2020). Thus, for SDF and RZF, does the concentration of NH_4^+-N affect the growth of rice or roots more? Alternatively, both work together to affect rice growth, which needs further exploration.

Although SDF and RZF are preferable for one-time applications, there is a lack of exploration of corresponding complementary N fertilizer products and mechanisms. Urea (U) and controlled release urea (CRU) are the two main types of N fertilizers being promoted for application in the market today and play a vital role in sustaining rice production (Yang et al., 2021). For U, due to its rapid release of nutrients, a single local application can expose the rice root system to the risk of poisoning and affect rice growth. Hu et al. (2022) showed that applying CRU near the rice root system does not harm the root system but rather is more favorable to root growth (Hu et al., 2022). Moreover, CRU has been reported to promote photosynthesis by increasing the effective N in the soil and seedlings. In addition, a single application of CRU can meet the N requirements of rice throughout the growth stage (Ding et al., 2022). A key question is whether CRU improves N distribution and root growth to promote yield compared with U. It is critical to explore the combination of agricultural machinery and agronomy.

This study examined two types of N fertilizer (U and CRU) and two N application methods (SDF and RZF) with 2-year field experiments to investigate the mechanism of rice yield and NUE. The aims were the following: (1) to compare the effects of different types of N fertilizers on rice N supply and root growth on rice yield and NUE under LNS; (2) to analyze the mechanism of yield increase from N supply and root growth under SDF and RZF; and (3) to provide both theoretical and data support for simplified fertilization and nutrient management by choosing a suitable type of N under LNS.

2 Materials and methods

2.1 Experiment site

A 2-year field experiment was conducted in the Meteorological Bureau of Jingzhou City, Hubei Province, China. The experimental site was located at the Jianghan Plain (N 29° 26′-31° 37′, E 111° 14′-114° 36′). The study site is in a subtropical humid monsoon climate zone. Weather data were obtained from the Jingzhou Weather Bureau. In 2020/2021, the average temperature and total precipitation in the rice season were 23.2/23.8°C and 2,130/1,385 mm, respectively (Figure 1). The region has planted rice for years, and the soil is classified as a silty medium loam, which developed from the deposition of islands and lacustrine. The initial soil test results for the site were pH 7.90, soil bulk density 1.40 g/cm^3 in the 0–20 cm soil layer, organic carbon 26.88 g/kg, total N 1.09 g/kg, Olsen-P 9.4 mg/kg, and available K 56.30 mg/kg. Soil total N, Olsen-P, and available K content were measured using Kjeldahl, Mo-Sb colorimetric, and flame photometer methods, respectively (Qin et al., 2015). Soil organic matter was determined by the wet combustion method.

2.2 Experimental material

YangLiangYou No.6, a widely grown rice cultivar., was transplanted in late May and harvested in September 2020 and 2021. Two types of N fertilizer that were utilized included U (46% N) and CRU (43% N), and CRU had a release longevity of 120 days (d), which was manufactured by the National Engineering Technology Research Center for CRF (Shandong, China) (Supplementary Figure S1).

2.3 Experimental treatments

The field plot experiment was conducted with six treatments: (i) CK: a control test without N fertilizer; (ii) FFP: farmers' fertilizer practice, U was applied three times, 50% as basal fertilizer, 30% as tillering fertilizer, and 20% as booting fertilizer. After the basal fertilizer is applied, the soil is covered and buried, and the tillering fertilizer and booting fertilizer are spread on the surface of the soil; (iii) US: U was applied by SDF; (iv) UR: U was applied by RZF; (v) CRUS: CRU was applied by SDF; and (vi) CRUR: CRU applied by RZF. A map of SDF and RZF is shown in Figure 2. For SDF, a wooden stick was used to cut a 10-cm deep trench, which is 5 cm from the rice root, and then U and CRU were scattered evenly inside the trench, respectively, and finally covered with soil immediately. For RZF, a hollow pipe with an inner diameter of 2 cm was used 5 cm from the root side, and after being pressed vertically into the soil for 10 cm. U and CRU along the inner wall into the bottom of the pipe. The pipe was immediately removed, and the hole was full of mud to cover the applied U and CRU. Fertilizer is applied on the same side of the rice row, and the distance between the two application points is equal to the row spacing (18 cm). The U and CRU were applied deeply as a

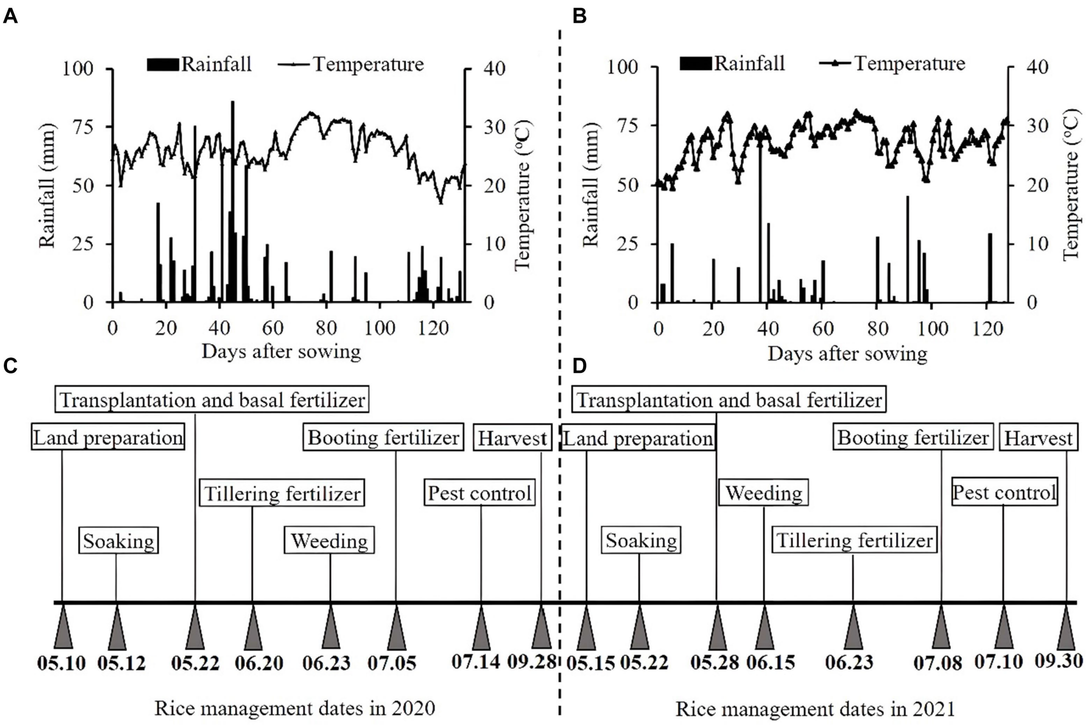

FIGURE 1
The rainfall, average temperatures, and rice management dates. **(A,B)** Represent rainfall and average temperatures in 2020 and 2021, respectively. **(C,D)** Represent rice management dates in 2020 and 2021, respectively.

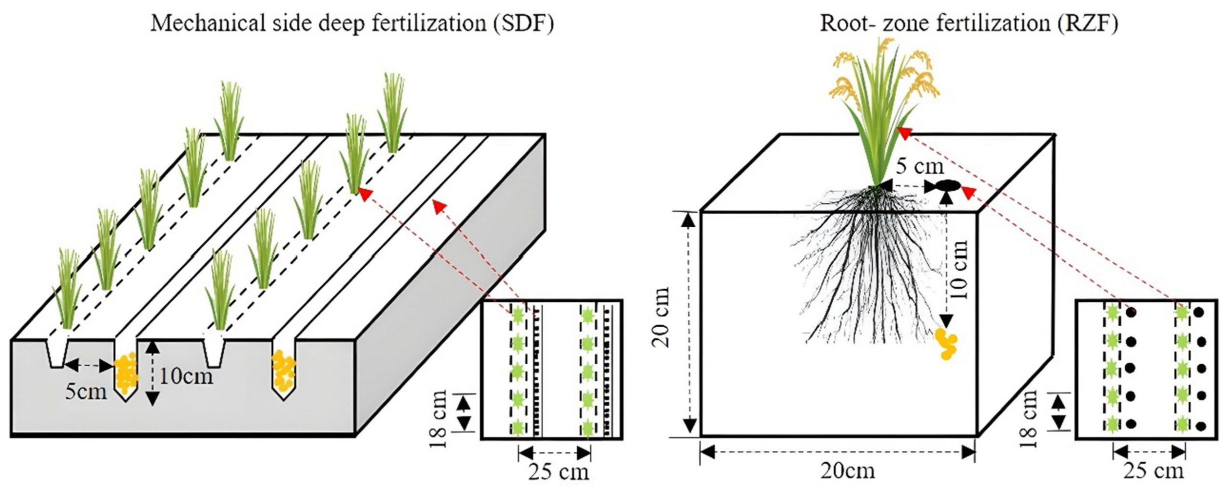

FIGURE 2
The sketch map of SDF and RZF.

basal fertilizer at one time after transplanting (1.76 g/hill for US and UR and 1.88 g/hill for CRUS and CRUR).

The six treatments were randomly arranged, and each was replicated three times. The plot dimensions were 5 m × 5 m, and each plot was separated by a 30 cm-wide earth bank covered with plastic film to prevent the lateral flow of water and nutrients. The LNS treatment plot (US, UR, CRUS, and CRUR) was divided into three plots, one of which was used to calculate N uptake and yield at the harvest stage without any disturbance, and the remaining two were used for destructive sampling to study N diffusion and root growth experiments, respectively. PVC plastic frames were placed in 20 cm deep soil and protruded 10 cm from the soil surface to prevent N fertilizer removal or addition due to runoff or water runoff. Water pipes were placed in the frames to ensure synchronization of irrigation.

The N rate was 180 kg/ha for all the treatments except the CK. The amounts of phosphorus (P_2O_5) and potassium fertilizer (K_2O) were established for each treatment, and the rates were 90 kg/ha and 120 kg/ha, respectively. Superphosphate (5.24% P) was used as phosphorus fertilizer, and potassium chloride (49.59% K) was used as potassium fertilizer. All phosphorus and potassium fertilizers were applied at one time, and the field was then irrigated with a small amount of water to mix the soil and fertilizer before transplanting. The transplanting density was 25 cm × 18 cm for each plot, with 3–5 seedlings in each hole. The field water depth of all plots was maintained between 10 and 80 mm until it was drained approximately 10 d before harvest. All irrigation events were coordinated with precipitation events, and the same irrigation pattern was utilized in 2020 and 2021. Applications of pesticides and herbicides in all the treatments were consistent with the management of local farmers.

2.3.1 Monitoring of ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) diffusion dynamics under LNS

Fresh soil samples were collected from the US, UR, CRUS, and CRUR plots at 30, 60, and 90 d after transplanting in 2020 and 2021. The water was drained before sampling. Four fresh soil samples were then collected in four directions (up, down, left, and right) of the U and CRU placement positions at an interval of 3 cm with a soil sampler that was 3 cm in diameter (Yao et al., 2017). For the CK and FFP, three soil samples were collected from a 0–20 cm soil depth and thoroughly mixed to calculate the NH_4^+-N and NO_3^--N concentrations. The soil samples that had been collected were extracted with two mol/L KCl solution (50 mL) to extract the mineral N using 5 g of field moist soil. The soil mixture was then shaken at 200 rpm for 1 h in an oscillating incubator and filtered with filter paper (Yao et al., 2017). AUV spectrophotometer (UV-5300PC, Shanghai Metash Instruments Co., Ltd., Shanghai, China) was used to measure the concentration of NH_4^+-N and NO_3^--N.

2.3.2 Root sample collection and the measurement of root traits

Bottomless plastic buckets 20 cm long, comprehensive, and 30 cm high were buried in the US, UR, CRUS, and CRUR plots before the rice was transplanted. The roots were collected during different rice growth periods (60 d and 90 d after transplanting in 2020 and 30 d and 60 d after transplanting in 2021) by destructive sampling. The aboveground parts of rice were cut flush by digging the bucket out of the soil and keeping the roots as intact as possible when separating the root system from the soil in the bucket. The roots obtained were immediately rinsed with tap water, and then part of the roots was scanned at 300 dpi (Epson Perfection C700 Photo Scanner, Los Alamitos, CA, United States). After scanning, 0.5 g of root apices were sampled to test the root activity (RA) using triphenyl tetrazolium chloride (TTC). The rest of the unscanned roots continued to be scanned, then analyzed with WinRHIZO PRO 2009 software (Regent Instruments, Inc., Quebec, Canada) to obtain the morphological indices of rice root systems, total surface area (TRS), total root volume (TRV), and root average diameter (RAD). Finally, the roots were oven-dried at 105°C for 30 min to deactivate enzymes, and they were then dried to constant weight at 75°C and weighed.

2.3.3 Plant height and tiller number

A total of 15 representative hills of rice were selected in each plot to measure the plant height and tiller number per hill at the tillering, booting, and heading stages. The plant height was considered the distance between the soil surface and the top of the highest leaf within one hole. In addition, the main stem or mother tiller was counted as a tiller and included in the total tillers.

2.4 Sampling and analyses

2.4.1 Dry matter accumulation

Three hills of plants were sampled from each plot to calculate the dry matter accumulation at the tillering, booting, and maturity stages. First, the stems, leaves, and panicles were separated, and the fresh samples were dried at 105°C for 30 min to deactivate the enzymes and then dried at 75°C to constant weight, considering the dry matter accumulation. The weighed samples were milled and sieved to calculate their total N content using the Kjeldahl method.

The dry matter exportation from vegetative-organs (DME, t/ha) and transportation rate of dry matter from vegetative-organs (TRDV, %) was calculated using the following formulae (Liu et al., 2017):

$$\mathrm{DME} = \mathrm{DMH} - \mathrm{DMM} \tag{1}$$

$$\mathrm{TRDV} = \mathrm{DME} / \mathrm{DMH} \tag{2}$$

Where DMH represents the amount of dry matter for vegetative organs at the heading stage, and DMM represents the amount for vegetative organs at the maturity stage.

2.4.2 N uptake and N use efficiency

The N uptake (kg/ha) was calculated using the following equation (Zhu et al., 2019):

$$\mathrm{N\ uptake} = \mathrm{TDM} \times \mathrm{NC} \tag{3}$$

Where TDM represents the total dry matter accumulation of panicles, leaves, and stems with leaf sheaths. NC represents the content of N in the panicles, leaves, and stems with leaf sheaths.

NUE: N recovery efficiency (NRE, %), N agronomic use efficiency (NAE, kg/kg), the N partial factor productivity (PFPN, kg/kg), N dry matter production efficiency (NDMPE, kg/kg), and N grain production efficiency (NGPE, kg/kg) were calculated using the following formulae (Zhu et al., 2019):

$$\mathrm{NRE} = \left(\mathrm{Nup} - \mathrm{N0up}\right) / \mathrm{FN} \tag{4}$$

$$\mathrm{NAE} = \left(\mathrm{GY} - \mathrm{GY0}\right) / \mathrm{FN} \tag{5}$$

$$\mathrm{PFPN} = \mathrm{GY} / \mathrm{FN} \tag{6}$$

$$\mathrm{NDMPE} = \mathrm{TBup} / \mathrm{TNup} \tag{7}$$

$$\mathrm{NGPE} = \mathrm{GY} / \mathrm{NUP} \tag{8}$$

Where GY and GY_0 represent the grain yield in the N application and CK plots, respectively, Nup and N_0up represent the total N uptake of aboveground in the N application and CK plots, respectively. FN represents the total N application rate, and TBup and TNup represent the total dry matter accumulation of the aboveground and the uptake of N in the aboveground, respectively.

2.4.3 Yield and its components

Six hills of rice from each plot were collected to investigate the yield components at the maturity stage. Manual threshing separated each grain from the rachis and immersed in tap water to separate the filled grains. Floating grains were determined to be unfilled grains. All the spikelets of the panicle were taken off to weigh the total weight. Then 30 g of them were manually separated the filled and unfilled spikelets, and the filled and unfilled spikelets was to measured. Finally, the number of filled and unfilled spikelets from total weight wan calculated by the weight conversion, and the grain-filling percentage (100 × filled spikelets number/total spikelets number) can be calculated. The rice plants were harvested from each plot, and the moisture content of grain yield was adjusted to 14%.

2.4.4 Economic benefits

The gross return (GR, \$/ha), gross margin (GM, \$/ha), and benefit-to-cost ratio (BCR, %) were calculated as follows (Yang et al., 2020):

$$GR = GY \times \text{Rice price} \tag{9}$$

$$GM = GR - \text{Cost} \tag{10}$$

$$BCR = GM / \text{Cost} \tag{11}$$

Where the average rice price was 0.42 \$/kg in 2020 and 2021. The cost of cultivation was added by all the inputs, including urea (374.51, \$/t), controlled release urea (494.35, \$/t), superphosphate (179.76, \$/t), potassium chloride (479.37, \$/t), labor fertilization for FFP and LNS (17.98, 29.96, \$/ha, respectively), and seedling (202.24, \$/ha), all price was provided by local market.

2.5 Data analysis

Microsoft Excel 2010 (Microsoft, Redmond, WA, United States) was used for data processing.

The pairwise means among treatments were compared using the Tukey's test at the 0.05 level of probability (SAS 9.3, SAS Institute Inc., Cary, NC, United States), and SPSS 26.0 (IBM, Inc., Armonk, NY, USA) was used for path coefficient of the indicators affecting yield. The NH_4^+-N and NO_3^--N diffusion dynamics were described by Surfer 8.0 (Golden Software, Inc., Golden, CO, USA), and the graphs were drawn with Origin 8.5 (Origin Lab, Northampton, MA, USA).

3 Results

3.1 Analysis of variance of the concentration of NH_4^+-N, root traits, and yield

There were significant differences between different types of N fertilizer and N supply methods on the concentration of NH_4^+-N, root traits, yield, NRE, and GM in 2020 and 2021. The interaction of different types of N fertilizer and N supply methods significantly affects the concentration of NH_4^+-N ($p < 0.01$) (Table 1).

3.2 NH_4^+-N diffusion dynamics under LNS treatments

The movement of NH_4^+-N was slow under LNS, and it primarily occurred 4–13 cm below the soil surface. The N primarily moved downward from the placement site (Figures 3A,B). The distribution of NH_4^+-N diffusion was strip for US and CRUS and punctiform for UR and CRUR. CRUS and CRUR provided higher NH_4^+-N concentrations at different stages compared with US and UR, and the release of NH_4^+-N under CRUS and CRUR was relatively stable and lasted longer. The highest concentration of NH_4^+-N occurred at 9–12 cm around the placement site of CRUR, with 229.46 mg/kg for 30 d, 165.83 mg/kg for 60 d, 127.83 mg/kg for 90 d in 2020, and 258.41 mg/kg for 30 d, 217.89 mg/kg for 60 d, 145.31 mg/kg for 90 d in 2021. There were no significant differences in NO_3^--N diffusion dynamics under LNS on different days in 2020 and 2021 (Figure S3).

3.3 NH_4^+-N and NO_3^--N concentrations in soil

As shown in Figure 4A, the N application treatment significantly increased the concentration of NH_4^+-N by 33.01% ~ 203.86% compared with the CK ($p < 0.05$). Compared with FFP, the concentrations of NH_4^+-N under the LNS treatment at 30 and 60 d significantly increased by 59.60% ~ 148.27 and 26.11% ~ 140.48% on average of 2 years ($p < 0.05$), respectively, indicating that LNS was beneficial to increasing the concentration of NH_4^+-N in the soil at different periods. Under the LNS, the concentration of NH_4^+-N significantly increased by 48.14% (2020) and 47.65% (2021) under the CRU ($p < 0.05$), respectively, compared with the U. This indicated that the CRU is more favorable to promote the concentration of soil NH_4^+-N. When the same type of N fertilizer was applied, the concentration of NH_4^+-N significantly increased by 13.09% (2020) and 13.19% (2021) under RZF ($p < 0.05$), respectively, compared with SDF.

The NO_3^--N concentration was also significantly affected by the N application treatment at 30 d and 60 d (Figure 4B). The NO_3^--N concentration of the FFP, US, UR, CRUS, and CRUR at 60 d significantly increased by 49.01% ~ 56.95% in 2020 and 43.15% ~ 57.43% in 2021 compared with that in the CK ($p < 0.05$). There were no significant differences in the concentration of NO_3^--N between the N application treatments at 90 d in either year.

3.4 Root traits indices, biomass, root activity, and root/shoot ratio under LNS

The root trait indices under different N application treatments at LNS are shown in Figure 5. Significant differences were observed for TRS, TRV RAD, and root biomass, and compared with U, the TRS, TRV RAD, and root biomass of CRU significantly increased by 42.59, 52.68, 22.63, and 47.08% ($p < 0.05$), respectively. This indicated that CRU was beneficial to promote the growth of rice roots. In addition, when the same N fertilizer was applied, compared with RZF, the TRS,

TABLE 1 Analysis of variance of NH_4^+-N concentration, root characteristic, and yield under different N fertilization treatments.

Source of variation	Degree of freedom	NH_4^+-N concentration (mg/kg)	TRS (cm^2)	TRV (cm^3)	RAD (mm)	Root biomass (g)	Yield (t/ha)	NRE (%)	GM ($/ha)
T	1	1660.9**	540.4**	358.8**	317.8**	122.8**	43.7**	105.3**	8.4**
S	2	323.7**	123.8**	95.1**	64.7**	39.5**	8.9**	22.6**	33.1**
Y	1	ns	93.4**	429.6**	20.6**	100.9**	25.2**	ns	25.2**
T×S	1	18.0**	ns	ns	ns	ns	ns	ns	ns
T×Y	1	ns	26.4**	ns	6.7*	6.0**	ns	13.4**	ns
S×Y	2	ns	ns	ns	ns	ns	ns	ns	ns
T×S×Y	1	ns	ns	ns	ns	ns	ns	ns	ns

The "TRS, TRV, RAD, NRE, GM" indicated total surface area, total root volume and root average diameter, N recovery efficiency, and gross margin, respectively. S, T, and Y indicated the N supply methods, N-type, and year, respectively. "*" and "**" represented statistical significance at $p < 0.05$ and $p < 0.01$, respectively. "ns" indicated statistical significance at $p > 0.05$ within a column.

TRV RAD, and root biomass of SDF significantly increased by 22.08, 23.33, 22.63, and 24.45% ($p < 0.05$), respectively, which indicated that SDF was more conducive to construct good root morphology.

The RA and RSR are shown in Table 2. Compared with U at 30 d and 60 d, CRU increased RA by 35.87 and 34.61%, respectively, which indicated that CRU facilitates RA. For RSR, CRU was significantly higher than that of U. Moreover, the RSR under CRUS significantly increased by 28.06 and 22.63% at 60 d and 90 d in 2020, and by 33.33 and 20.00% at 30 d and 60 d in 2021, compared with CRUR, respectively ($p < 0.05$).

3.5 Plant height and tiller number

As shown in Figure 6A, the plant height of rice increases with time. Compared with the CK, the N application treatments (FFP, US, UR, CRUS, and CRUR) increased the plant height by 9.57% ~ 15.17% (2020) and 8.74% ~ 15.41% (2021). The highest plant height was observed of FFP at the tillering stage in 2020 and 2021, but the plant height of CRUS and CRUR exceeded that of FFP, US, and UR at the heading stage. This indicated that CRU is the most efficient fertilizer in the rice's later stages.

The application of N significantly increased the number of tillers, as shown in Figure 6B, and different N application methods and types resulted in significant differences at different stages. In 2020 and 2021, the number of tillers of U was significantly higher than that of CRU at the tillering stage but was lower than that of CRU at the booting and heading stages. Compared with the US at the booting and heading stages in 2021, CRUS caused a significant increase in the number of tillers by 26.51 and 20.0% ($p < 0.05$). Compared with UR, CRUR significantly increased the number of tillers by 32.74% ($p < 0.05$).

3.6 The dry matter accumulation, N uptake, grain yield, NUE, and economic benefits

The dry matter accumulation of the aboveground was affected by N application methods and types of N fertilizer in 2020 and 2021 (Table 3). The amount of dry matter increased with time. The dry matter accumulation increased by 24.88% ~ 75.70% (2020) and 49.16% ~ 95.66% (2021) under N application treatments in 2020 and 2021 compared with the CK, and dry matter accumulation increased by 6.12% ~ 38.49% of LNS in 2020 compared with the FFP. For LNS, compared with U, the dry matter accumulation of HTM, MS, DME, and TRDV under the CRU treatment increased by 25.08, 17.90, 55.89, and 30.30%, respectively. These results indicate that CRU effectively promotes dry matter accumulation and the translocation of dry matter nutrients in organs compared with U.

The effects of N application methods and N fertilizer types on the total N uptake at different stages are shown in Figure 7. Compared with CK, the total N uptake under FFP significantly increased by 23.26% (2020) and 24.48% (2021). Compared with FFP, the total N uptake of LNS at the maturity stage in 2020 and 2021 increased by 14.06% ~ 29.93 and 7.99% ~ 29.51%, respectively. Compared with the US, the total N uptake of CRUS at different stages significantly increased by 12.66% ~ 28.09% (2020) and 16.98% ~ 38.45% (2021), respectively ($p < 0.05$). Compared with UR, the total N uptake of CRUR at different stages significantly increased by 13.91% ~ 18.99% (2020) and 12.74% ~ 31.84% (2021), respectively ($p < 0.05$). Compared with CRUS, the total uptake in CRUR at maturity stages increased by 1.42% (2020) and 3.36% (2021), respectively.

As shown in Table 4, the N application significantly increased rice yield in 2020 and 2021. Compared with CK, yield under FFP significantly increased by 21.43% (2020) and 24.19% (2021). Compared with FFP, the yield of LNS increased by 7.03% ~ 23.09% and 8.56 ~ 26.72% in 2020 and 2021 ($p < 0.05$), respectively. For CRU under LNS in 2020 and 2021, compared with the US, CRUS significantly increased yield by 12.81 and 13.62%, respectively ($p < 0.05$). Compared with UR, CRUR significantly increased yield by 10.88 and 11.91%, respectively ($p < 0.05$). The effective number of spikes and the total number of spikelets of CRU significantly increased by 13.25 and 18.41%, respectively, compared with those of U. There were no significant differences in the number of filled grains per panicle, seed-setting rate, and 1,000-grain weight between U and CRU. This indicated that the effective number of spikes and total number of spikelets caused the increase in yield. For SDF and RDF under LNS in 2020 and 2021, compared with the US, UR increased yield by 3.72 and 4.30%, respectively, and compared with CRUS, CRUR increased yield by 1.35 and 2.73%, respectively.

NRE, NAE, and PFPN were affected by the methods and type of N fertilizer (Table 5). Compared with FFP in 2020 and 2021, LNS

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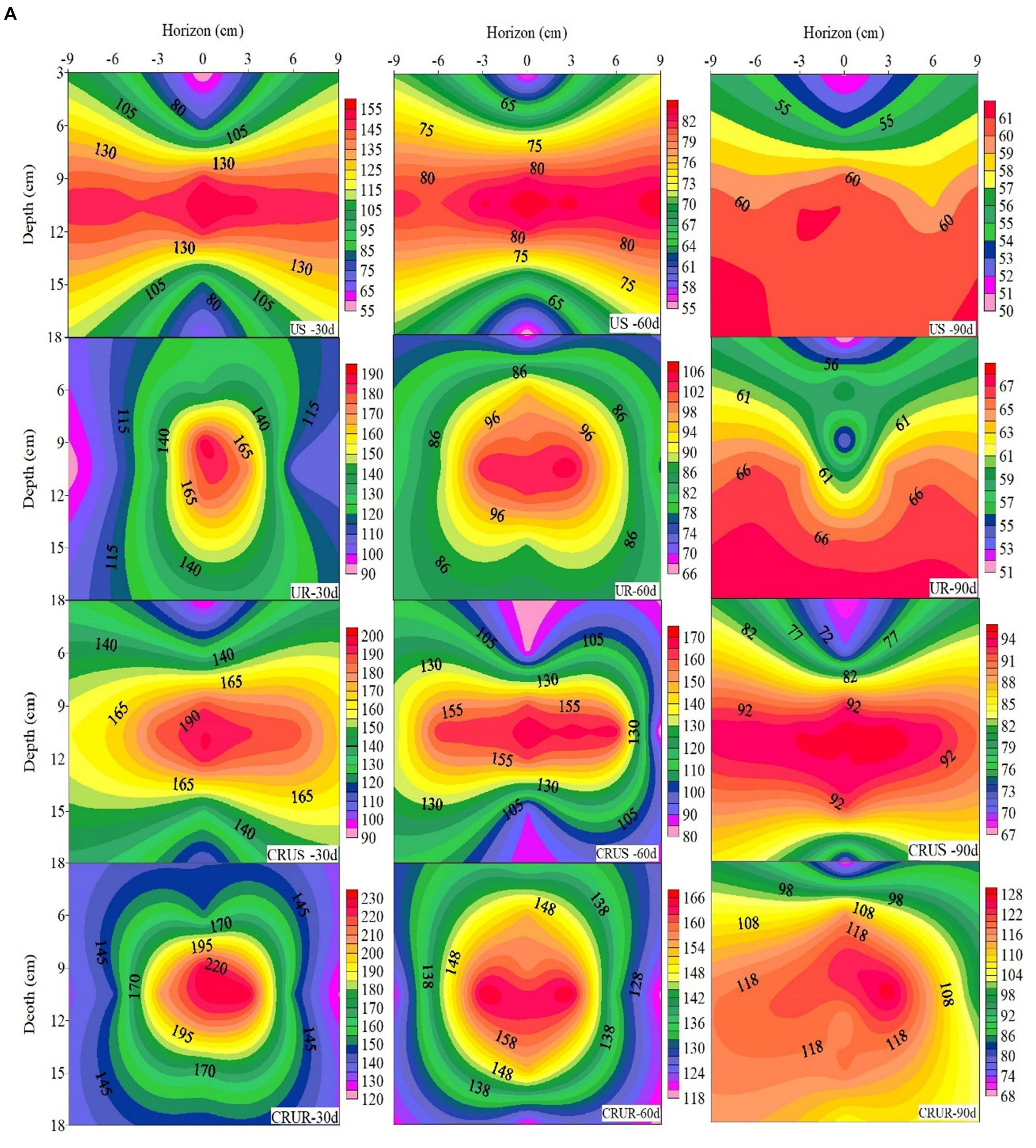

FIGURE 3 (Continued)

increased NRE by 44.43% ~ 96.60 and 26.80% ~ 96.01%, NAE by 40.31 ~ 131.84 and 41.53% ~ 131.00%, PFPN by 7.06% ~ 23.02 and 8.47% ~ 26.72%, respectively. For CRU under LNS in 2020 and 2021, compared with U, CRU significantly increased NRE, NAE, and PFPN by 34.02% ~ 46.54, 40.50% ~ 60.14, and 10.67% ~ 13.74%, respectively ($p < 0.05$). For SDF and RZF under LNS in 2020 and 2021, compared with SDF, RZF increased NRE, NAE, and PFPN by 0.59% ~ 12.51, 3.18% ~ 16.82, and 1.02% ~ 4.30%, respectively.

As shown in Table 6, the N application treatments increased significantly by 23.26% ~ 53.94 and 20.21% ~ 53.01% ($p < 0.05$), respectively, compared to CK. Compared with FFP in 2020 and 2021, LNS increased GR by 7.05% ~ 23.01 and 8.47% ~ 26.71% and GM by 7.96 ~ 24.89 and 9.79% ~ 29.59%. For CRU under LNS in 2020 and 2021, compared with the US, CRUS significantly increased GR by 12.61 and 13.63% and GM by 14.31 and 14.22%, respectively ($p < 0.05$). Compared with UR, CRUR significantly increased GR by 10.67 and 11.58%, respectively ($p < 0.05$). For SDF and RZF under LNS in 2020 and 2021, compared with SDF, RZF increased GR by 10.67% ~ 13.75 and 11.99% ~ 13.63% and GM by 10.69% ~ 14.31 and 12.26% ~ 14.22%, respectively.

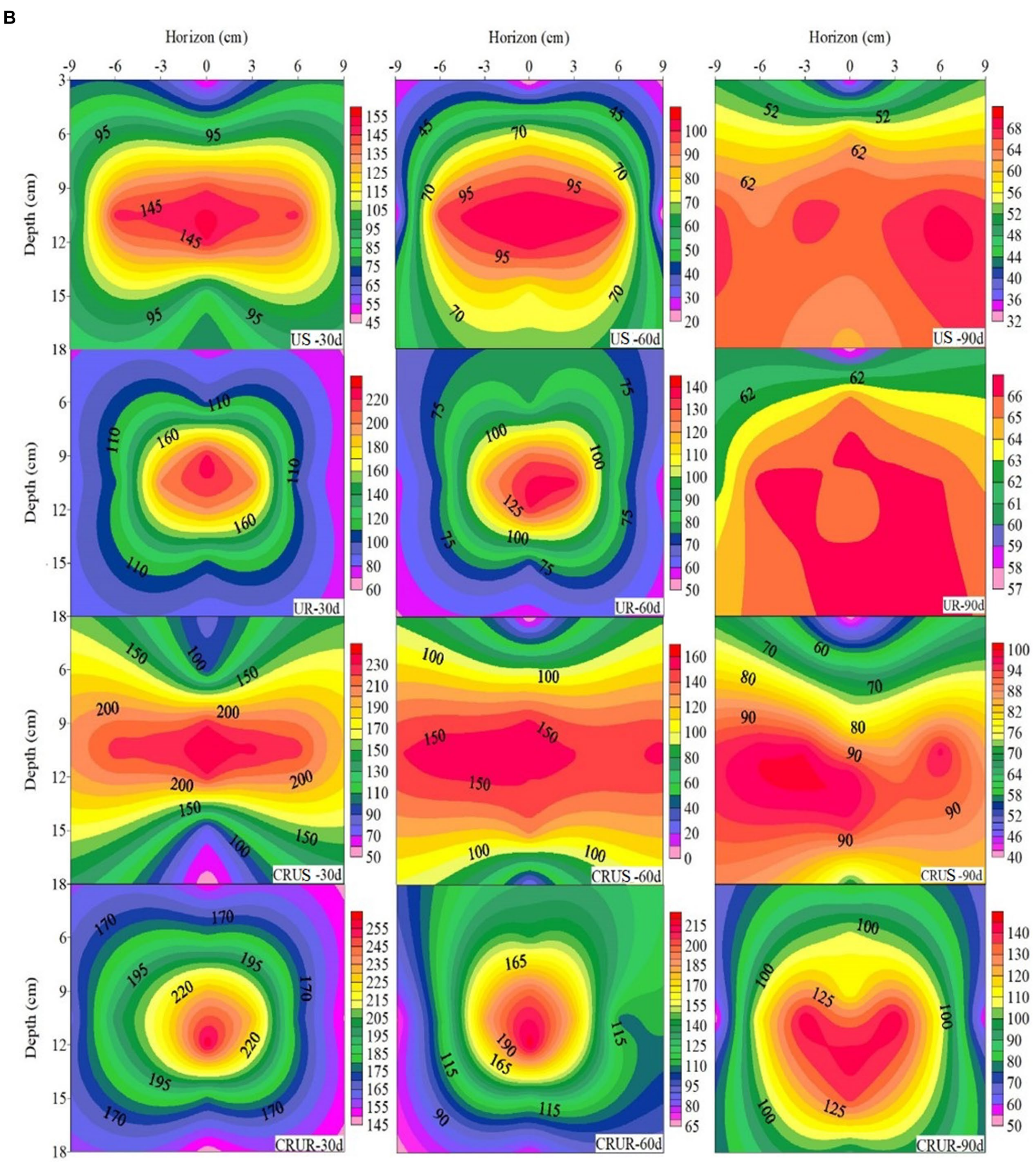

FIGURE 3
The NH_4^+-N diffusion dynamics of LNS in 2020 **(A)** and 2021 **(B)** rice season. NH_4^+-N concentration of the different soil layers at 30 d, 60 d, and 90 d after transplanting, respectively. For US and CRUS treatment, the U and CRU were applied once into a 10-cm deep ditch that were positioned 5 cm from the rice roots as basal fertilizer. For UR and CRUR treatment, the U and CRU were applied once into 10 cm deep holes positioned 5 cm from the rice roots as basal fertilizer.

3.7 Relationship between NH_4^+-N concentration, root traits, and yield

The path coefficient structural model describes the essential relationships among selected traits (Figure 8). The N uptake in rice shoots directly and positively affected NUE and yield. Moreover, NH_4^+-N concentration directly and positively affects N uptake and acts indirectly by promoting root growth, and root traits positively affect N uptake.

4 Discussion

4.1 Yield and NUE under LNS treatments

LNS was reported to increase rice yield by a significant 44.0% compared to conventional broadcasting (Wu et al., 2017); similar results were acquired in this study, especially for UR, CRUS, and CRUR. On the one hand, this is related to the N application methods. The N application of tillering and booting fertilizer under

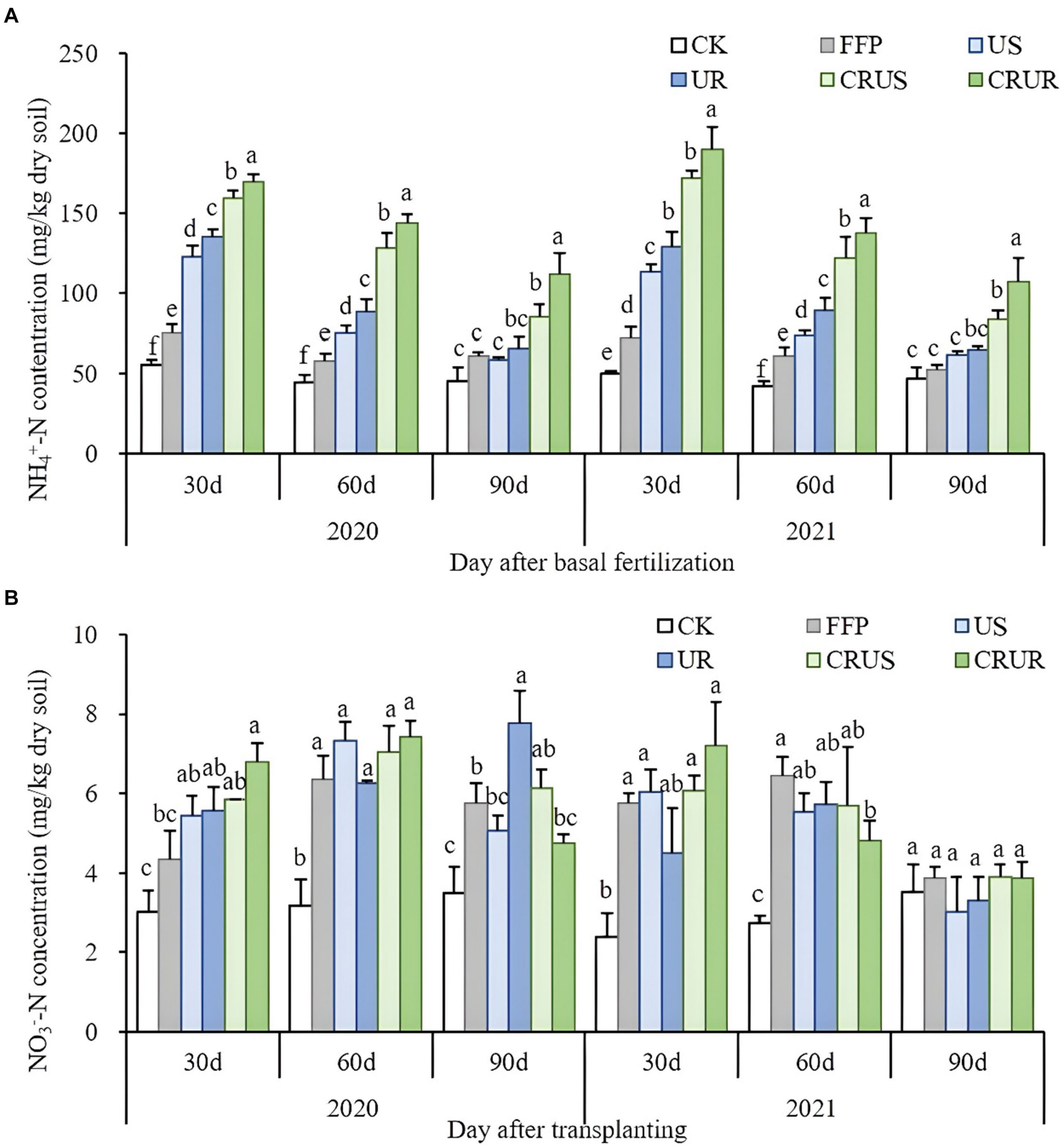

FIGURE 4
Different N fertilization treatments affect NH_4^+-N **(A)** and NO_3^--N **(B)** concentration in soil. Values are means ± SD of three replicates. Different lowercase letters on bars indicate differences by the Tukey test at the 5% significance level; 30 d, 60 d, and 90 d indicate the days after transplanting.

FFP is spread on the soil's surface, which results in being too far away from the rice root. Most fertilizers were not absorbed by the rice root, which undergoes denitrification to produce greenhouse gases that diffuse into the air and cause environmental pollution (Jain, 2023). On the other hand, it was also related to the N fertilizer types. The application of CRU significantly increased the soil NH_4^+-N concentration in the root zone with a duration of 120 d (Figure S1). Moreover, the LNS resulted in a more reasonable distribution of NH_4^+-N in the soil, which satisfied the nutrient needs of rice during the entire growth period (Figure 4) and thus significantly increased rice yield. Ding et al. (2022) also found that a one-time application of CRU under LNS increased the ratoon rice yield (Ding et al., 2022).

4.2 NH_4^+-N concentration and root traits under U and CRU

In this study, U and CRU applications under LNS increased the concentration of NH_4^+-N in the soil. For US and UR under LNS treatment, this was owing to the rapid conversion of U to NH_4^+-N by microbial urease. Rice roots took up part of it, and the rest was fixed by soil adsorption. NH_4^+-N is not easily leached with water, which results in higher concentrations of NH_4^+-N in the soil under deep urea application (Shi et al., 2022). For CRUS and CRUR, the slow and continuous release of N from CRU, compared with U, helps to reduce the concentration of substrate and soil urease activity. This delays nitrification and reduces the losses of N from the soil (Ma et al.,

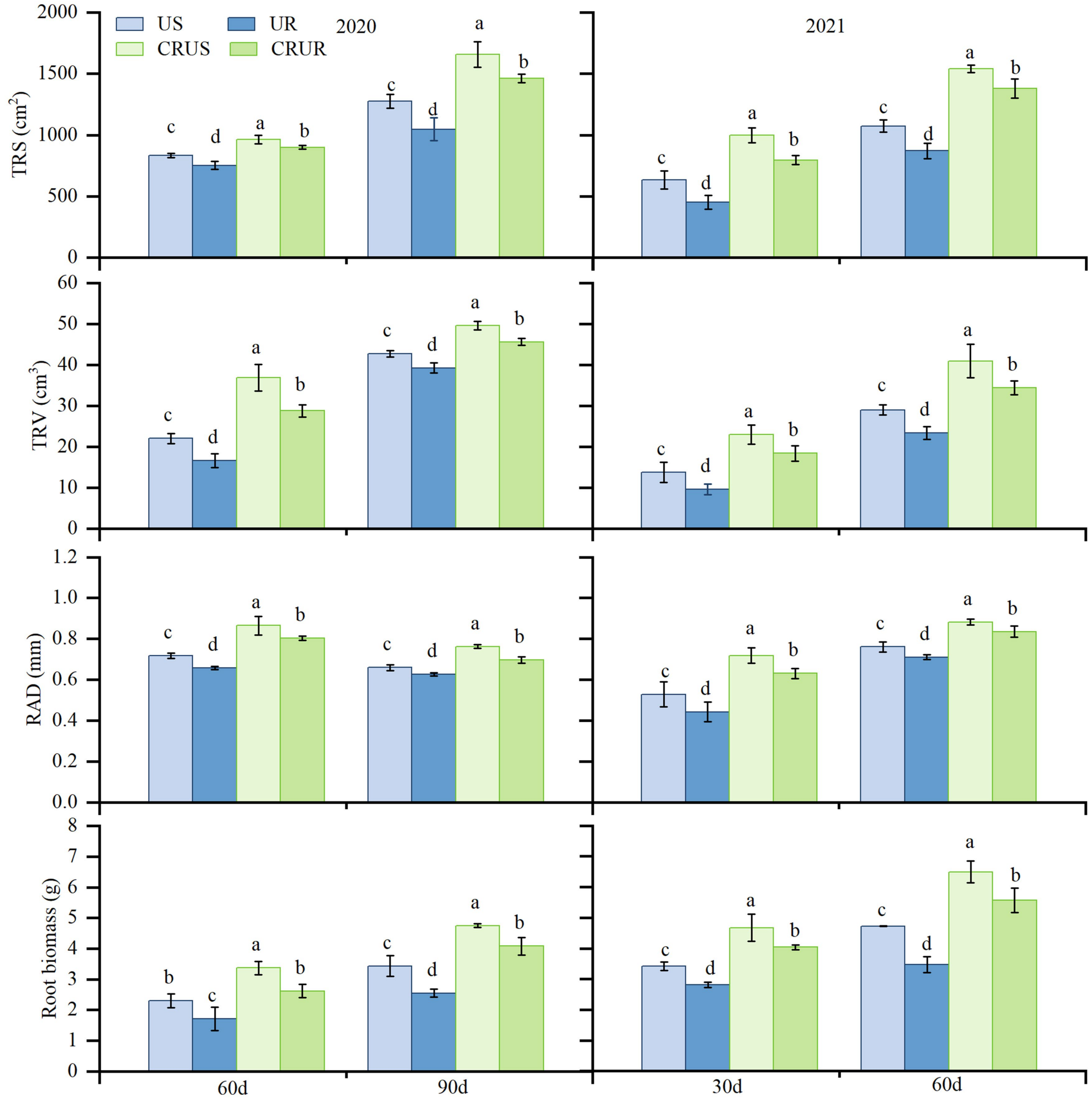

FIGURE 5
Different N fertilization treatments affect root trait indices and biomass at different stages in 2020 and 2021. TRS, TRV, and RAD indicated total surface area, total root volume, and root average diameter, respectively. Values are means ± SD of three replicates. Different lowercase letters on bars indicate differences by the Tukey test at the 5% significance level.

TABLE 2 Effects of different N fertilization treatments on RA and RSR of rice in 2021.

Year	2020				2021			
	60 d		90 d		30 d		60 d	
Treatment	RA (μg/g·h)	RSR	RA (μg/g·h)	RSR	RA (μg/g·h)	RSR	RA (μg/g·h)	RSR
US	-	0.05 ± 0.01b	-	0.04 ± 0.01c	36.50 ± 1.25b	0.11 ± 0.01b	63.62 ± 3.17bc	0.03 ± 0.00c
UR	-	0.04 ± 0.01c	-	0.03 ± 0.01c	35.42 ± 0.77b	0.07 ± 0.01c	57.79 ± 3.12b	0.03 ± 0.00c
CRUS	-	0.08 ± 0.01a	-	0.06 ± 0.01a	50.24 ± 4.81a	0.16 ± 0.01a	82.12 ± 3.12a	0.06 ± 0.00a
CRUR	-	0.06 ± 0.00b	-	0.05 ± 0.00b	47.50 ± 3.11a	0.12 ± 0.00b	80.70 ± 1.86ab	0.05 ± 0.00b

"RA and RSR" indicated root activity and root/shoot ratio, respectively. Values are means of three replicates. Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level.

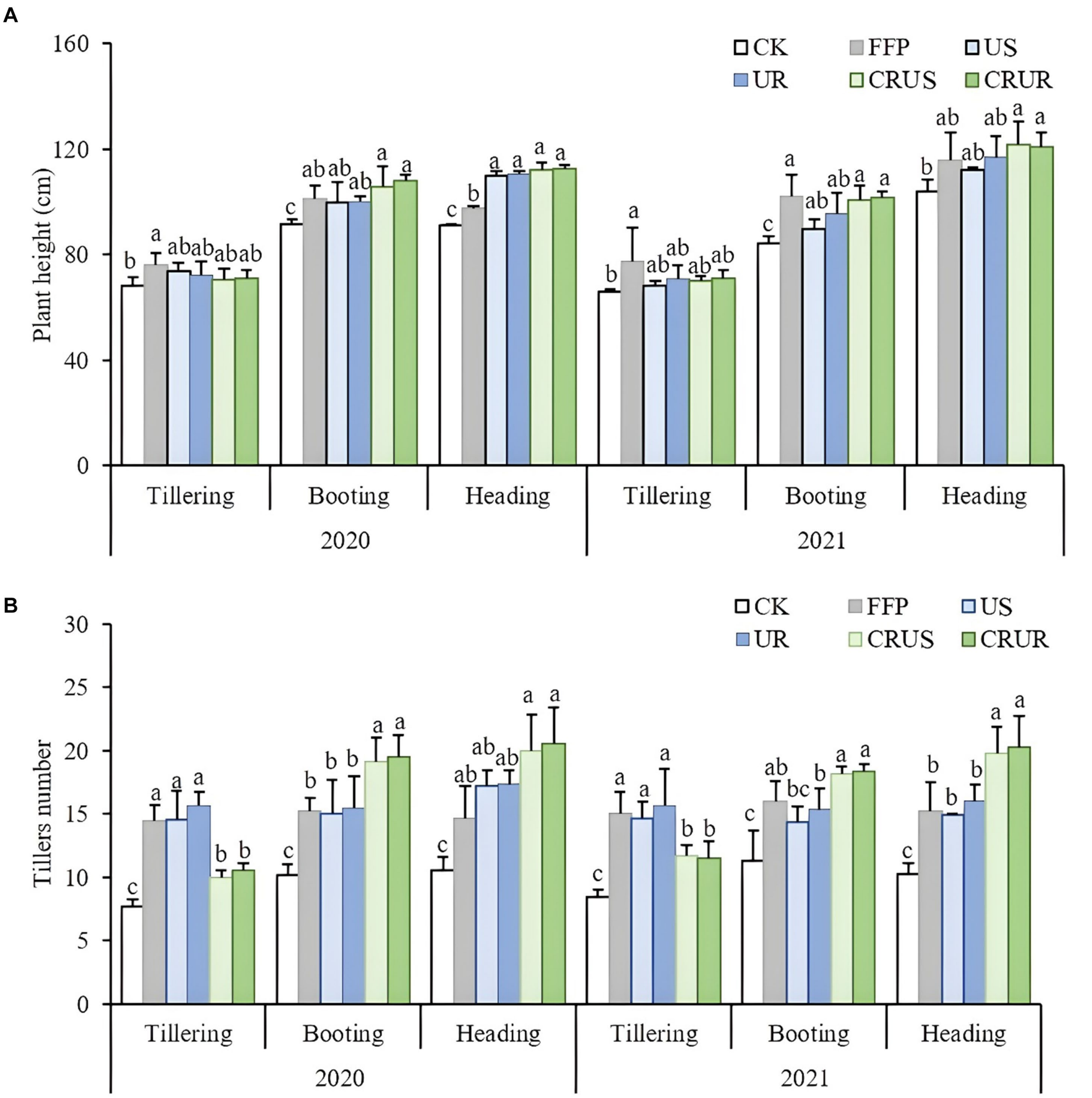

FIGURE 6
Different N fertilization treatments affect plant height **(A)** and tiller number **(B)** at different stages in 2020 and 2021. Values are means ± SD of three replicates. Different lowercase letters on bars indicate differences by the Tukey test at the 5% significance level.

2023). In contrast, because the ability of soil to sorb and fix NH_4^+-N is limited, studies have shown that the highest soil sorption of NH_4^+-N occurs on the first day after fertilizer application, only 34% of the amount of NH_4^+-N applied is taken up, and the rest that is not taken up by plants and fixed by the soil being lost as gas, runoff, or leaching pathways (Sun et al., 2020). This may explain why the concentrations of NH_4^+-N under CRU were consistently higher than those under the U treatment.

The mechanism of nutrient release by CRU indicates that the nutrients in its envelope are continuously released during crop growth (Liu et al., 2019), which leads to an increase in the local nutrient concentration in the soil, while the rice root system adapts to the change in soil environment through various plastic responses, including root morphological plasticity (Verma et al., 2022). In this study, the TRS, TRV, and RAD were significantly higher under the CRU treatment than the U treatment (Figure 5), which was related to root morphological plasticity. Elevated inter-root NH_4^+-N concentrations under CRU treatment led to changes in the root apoplastic pH and auxin levels, which induced lateral root emergence (Pélissier et al., 2021).

4.3 The NH_4^+-N concentration and root traits under SDF and RZF

There were significant differences in the concentration of NH_4^+-N and root traits under different N application methods when the same types of N fertilizer were applied. We found that the concentration of NH_4^+-N was significantly higher in RZF than in SDF (Figure 4). This was because the contact area between N fertilizer and soil determined the dilution and rate of release of N fertilizer; the more extensive contact area results in higher dilution and rates of release of N

TABLE 3 Effects of different N fertilization treatments on dry matter accumulation and transportation.

Year	Treatment	Dry matter accumulation (t/ha)				DME (t/ha)	TRDV (%)
		TI	HS	HTM	MS		
2020	CK	2.47 ± 0.16d	9.91 ± 0.10c	4.34 ± 0.12c	14.25 ± 0.08d	1.12 ± 0.17c	15.11 ± 1.86b
	FFP	3.65 ± 0.02c	11.25 ± 0.53b	4.83 ± 0.34bc	16.09 ± 0.53c	1.51 ± 0.11bc	18.98 ± 0.51b
	US	3.72 ± 0.12c	12.09 ± 0.50b	5.44 ± 0.50b	17.53 ± 0.46b	1.42 ± 0.20bc	15.66 ± 2.41b
	UR	4.67 ± 0.15b	12.43 ± 0.79b	5.37 ± 0.34b	17.79 ± 0.46b	1.83 ± 0.37b	20.19 ± 3.84ab
	CRUS	5.95 ± 0.38a	14.08 ± 0.07a	6.40 ± 0.57a	20.47 ± 0.50a	2.81 ± 0.41a	25.45 ± 2.33a
	CRUR	5.72 ± 0.68a	14.45 ± 0.82a	6.53 ± 0.93a	20.98 ± 0.12a	2.90 ± 0.18a	24.93 ± 1.77a
2021	CK	1.55 ± 0.09c	8.49 ± 0.24d	4.22 ± 0.24c	12.71 ± 0.43c	1.76 ± 0.23b	25.67 ± 3.07b
	FFP	3.18 ± 0.28b	10.16 ± 0.14c	5.48 ± 0.31bc	15.63 ± 0.21b	2.27 ± 0.37b	28.71 ± 2.76ab
	US	2.76 ± 0.28b	10.55 ± 0.07bc	4.76 ± 0.49b	15.31 ± 0.44b	2.32 ± 0.29b	26.96 ± 2.96ab
	UR	3.36 ± 0.26b	10.83 ± 0.65b	5.07 ± 0.80b	15.90 ± 0.68b	2.36 ± 0.50b	27.34 ± 6.43ab
	CRUS	4.68 ± 0.88a	11.97 ± 0.01a	6.31 ± 0.12a	18.28 ± 1.30a	3.18 ± 0.24a	32.95 ± 0.61a
	CRUR	4.68 ± 0.48a	12.17 ± 0.48a	6.50 ± 0.18a	18.68 ± 1.59a	3.01 ± 0.26a	30.88 ± 0.53ab

TI, HS, HTM, MS, DME, and TRDV indicated tillering, heading, full heading to maturity, maturity stage, dry-matter exportation from vegetative-organs, transportation rate of dry-matter from vegetative-organs, respectively. Values are means of three replicates. Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level.

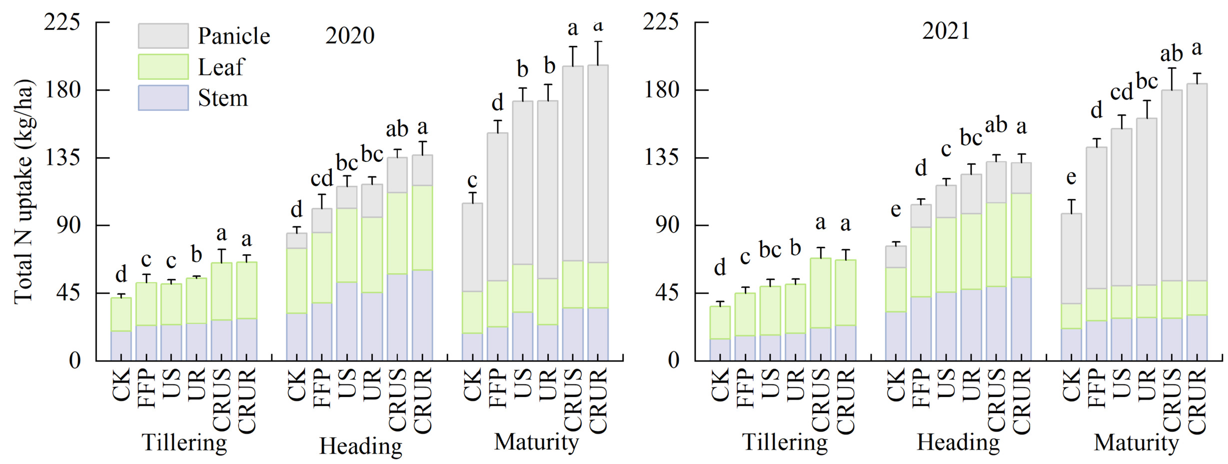

FIGURE 7
Effects of different N fertilization treatments on total N uptake of rice at tillering, heading, and maturity stages in 2020 and 2021. Values are the means ± SD of three replicates. Different lowercase letters on bars indicate differences by the Tukey test at the 5% significance level.

fertilizer. The dilution and rate of release of N fertilizer were the smallest under RZF conditions, and less inorganic N was lost (Jiang et al., 2017).

In contrast, the root traits (TRS, TRV, and RAD) were significantly higher in SDF than in RZF (Figure 5). This is related to the contact area of the fertilizer with the root. By comparing Arabidopsis at 16 different N levels, Jia et al. (2022) showed that root growth is regulated by NH_4^+-N and NO_3^--N, with NH_4^+-N promoting the expression of gene *AMTs* in root epidermal cells and NO_3^--N promoting the expression of gene *AFB3* (Vidal et al., 2010), both of which induce lateral root formation through the modulation of aboveground synthesis of growth hormones in rice (Jia et al., 2022). A fertilizer strip is formed near the rice root system under SDF, whereas RZF has a spot fertilizer near the rice under RZF. For rice roots, the fertilizer strip formed under SDF has a larger contact area with the root and produces more lateral roots.

4.4 The relationship between NH_4^+-N concentration, root traits, and yield

The path coefficient structural model was used to explore further the causal relationship between NH_4^+-N concentration, root traits, N uptake, NUE, and yield (Hu et al., 2023). The results show that increased NH_4^+-N concentration and root traits directly stimulated N uptake, which resulted in an increased rice yield by regulating NUE (Figure 8), supported by the fact that CRU promotes root growth by increasing the soil NH_4^+-N concentration, which further promotes N uptake by rice root. Interestingly, the yield and NUE under CRUR were slightly higher than those under CRUR (Table 3). Slightly higher may be related to the root/shoot ratio (RSR). The RSR is the root biomass ratio to the aboveground biomass, which is the parameter that directly reflects plant biomass allocation (Qi et al., 2019). The RSR under CRUS was significantly higher than that of CRUR (Table 2). Xu et al. (2018) reported that the RSR of rice was highly significant and

TABLE 4 Effects of different N fertilization treatments on yield and yield components in 2020 and 2021.

Year	Treatment	Yield (t/ha)	Effective panicle number (10^4/ha)	Total number of spikelets (10^6/ha)	Number of filled grains per panicle	Seed-setting rate (%)	1,000-grain weight (g)
2020	CK	7.03 ± 0.58d	274.05 ± 12.83d	395.29 ± 10.70c	101.67 ± 6.24ab	61.53 ± 2.24bc	25.73 ± 0.10a
	FFP	8.53 ± 038c	362.93 ± 12.83c	474.43 ± 38.02b	109.33 ± 17.00ab	71.77 ± 5.87ab	25.47 ± 0.71a
	US	9.13 ± 0.12c	385.15 ± 25.66c	524.54 ± 24.040b	83.33 ± 6.02b	53.73 ± 6.35c	26.70 ± 1.57a
	UR	9.47 ± 0.12bc	392.55 ± 28.67bc	534.17 ± 25.63b	115.33 ± 6.94a	77.37 ± 5.34a	26.43 ± 0.62a
	CRUS	10.36 ± 0.52ab	429.59 ± 27.54ab	615.49 ± 17.87a	111.00 ± 3.27ab	70.70 ± 4.06ab	24.93 ± 1.05a
	CRUR	10.50 ± 0.42a	436.99 ± 30.53a	616.09 ± 14.83a	108.00 ± 2.16a	76.53 ± 1.70a	25.40 ± 0.33a
2021	CK	6.15 ± 0.21e	262.32 ± 14.31d	387.00 ± 10.39d	111.54 ± 3.98b	70.37 ± 6.12b	25.74 ± 1.22a
	FFP	7.71 ± 0.35d	311.08 ± 14.70c	444.00 ± 13.08 cd	134.12 ± 4.75a	89.92 ± 7.14a	26.91 ± 0.39a
	US	8.37 ± 0.36 cd	338.86 ± 9.62bc	458.33 ± 23.86 cd	117.04 ± 8.02ab	86.49 ± 4.21a	26.20 ± 0.77a
	UR	8.73 ± 0.19bc	351.82 ± 2.56b	500.67 ± 23.71bc	118.26 ± 5.55ab	82.92 ± 8.03a	25.30 ± 1.73a
	CRUS	9.51 ± 0.42ab	399.96 ± 14.70a	572.33 ± 52.88ab	115.88 ± 7.22ab	81.38 ± 9.75ab	25.91 ± 0.40a
	CRUR	9.77 ± 0.14a	394.41 ± 19.24a	581.33 ± 18.34a	125.86 ± 8.33ab	85.24 ± 6.23a	25.85 ± 1.04a

Values are means of three replicates. Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level.

TABLE 5 Effects of different N fertilization treatments on NUE in 2020 and 2021.

	Treatment	NRE (%)	NAE (kg/kg)	PFPN (kg/kg)	NDMPE (kg/kg)	NGPE (kg/kg)
2020	CK	-	-	-	136.88 ± 9.67a	67.4 ± 5.9a
	FFP	25.93 ± 4.77c	8.26 ± 2.69c	47.31 ± 2.69c	106.16 ± 9.78b	56.0 ± 4.9b
	US	37.45 ± 4.76bc	11.59 ± 0.86bc	50.65 ± 2.53bc	102.10 ± 2.50b	53.2 ± 4.6b
	UR	37.67 ± 6.01b	13.54 ± 1.81b	52.59 ± 3.43b	103.60 ± 7.39b	55.1 ± 2.6b
	CRUS	50.19 ± 0.95a	18.56 ± 1.33a	57.61 ± 3.34a	105.27 ± 3.55b	53.4 ± 4.4b
	CRUR	50.98 ± 3.40a	19.15 ± 1.49a	58.20 ± 1.49a	107.14 ± 4.49b	53.6 ± 3.8b
2021	CK	-	-	-	131.26 ± 17.14a	63.5 ± 1.86a
	FFP	24.59 ± 2.59c	8.74 ± 2.39c	42.85 ± 2.39c	109.88 ± 14.00b	54.2 ± 4.06ab
	US	31.18 ± 5.10bc	12.37 ± 1.15bc	46.48 ± 4.42bc	99.66 ± 8.31b	54.6 ± 5.10ab
	UR	35.08 ± 2.22b	14.37 ± 0.85b	48.48 ± 2.28b	99.00 ± 2.94b	54.5 ± 5.68ab
	CRUS	45.69 ± 3.15a	18.70 ± 1.56a	52.81 ± 3.21a	102.05 ± 8.10b	53.1 ± 2.05b
	CRUR	48.20 ± 3.87a	20.19 ± 1.78a	54.30 ± 4.83a	101.33 ± 4.09b	53.03 ± 4.11b

The NRE, NAE, PFPN, NDMPE, and NGPE indicated N recovery efficiency, N agronomic use efficiency, N partial factor productivity, N dry matter production efficiency, and N grain production efficiency, respectively. Values are means of three replicates. Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level.

negatively correlated with a yield at the heading and maturity stage. Moreover, Passioura (1983) showed that root growth consumes twice as much unit mass of material as aboveground (Passioura, 1983); higher root biomass in the later stages of rice fertility can excessively consume photosynthetically synthesized products above ground, thus adversely affecting grain filling and yield (Zhu et al., 2020). Overall, there was adequate N supply and better root growth under CRUR, which can be used for recommended fertilization management in rice production practice.

5 Conclusion

These results showed that localized N supply significantly increased rice yield and controlled release urea significantly improved NH_4^+-N concentration and roots growth compared with urea, which contributed to nutrient acquisition and improved rice yield. Compared with mechanical side deep fertilization, root zone fertilization had a slightly higher yield. Thus, localized nitrogen supply contributes to higher rice yields, controlled release urea should be selected as the recommended N fertilizer types, and the combination of controlled release urea and root zone fertilization was the most effective choice for rice production.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

TABLE 6 Effects of different N fertilization treatments on economic benefits in 2020 and 2021.

Year	Treatment	Fertilizer cost ($/ha)	Labor input ($/ha)	Seedling cost ($/ha)	GR ($/ha)	GM ($/ha)	BCR (%)
2020	CK	175.8	18.1	203.1	2955.43 ± 245.15d	2558.29 ± 245.15c	6.47 ± 0.23a
	FFP	332.0	36.1	203.1	3577.32 ± 165.76c	3014.82 ± 165.76b	5.38 ± 0.29c
	US	332.0	48.2	203.1	3829.04 ± 205.45b	3254.24 ± 205.45bc	5.69 ± 0.36bc
	UR	332.0	48.2	203.1	3976.00 ± 111.98b	3401.74 ± 111.98b	5.94 ± 0.19ab
	CRUS	383.7	48.2	203.1	4355.40 ± 341.06a	3720.43 ± 341.04a	5.88 ± 0.18bc
	CRUR	383.7	48.2	203.1	4400.20 ± 192.10a	3765.19 ± 192.10a	5.95 ± 0.15ab
2021	CK	175.8	18.1	203.1	2581.22 ± 87.47d	2184.68 ± 87.47d	5.52 ± 0.22a
	FFP	332.0	36.1	203.1	3239.36 ± 147.28c	2677.39 ± 147.53c	4.78 ± 0.30b
	US	332.0	48.2	203.1	3514.40 ± 149.38b	2939.65 ± 149.38b	5.14 ± 0.26ab
	UR	332.0	48.2	203.1	3665.40 ± 138.71b	3090.91 ± 138.71b	5.40 ± 0.24a
	CRUS	383.7	48.2	203.1	3992.84 ± 174.42a	3357.81 ± 174.41a	5.31 ± 0.28a
	CRUR	383.7	48.2	203.1	4104.83 ± 248.13a	3469.79 ± 248.14a	5.48 ± 0.24a

"GR, GM, BCR" indicated gross return, gross margin, and benefit-to-cost ratio, respectively. Values are means of three replicates. Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level.

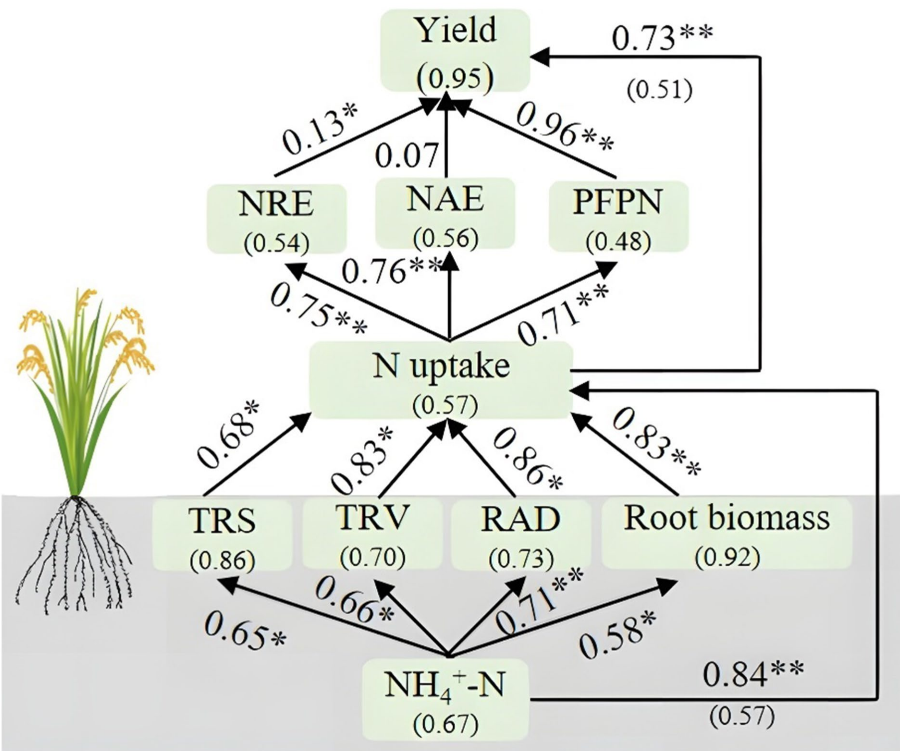

FIGURE 8
Path coefficient structural model of rice yield and the variables. Boxes indicate the variable name, and the numbers in parentheses indicate the variance explained by the model (R^2). The numbers adjacent to the arrows are standardized path coefficients and indicative of the effect size of the relationship. A line with an arrowhead indicates a causal link between the cause (base of the arrow) and effect (end of error). "* and **" represented the significance level of $p < 0.05$ and $p < 0.01$, respectively. "NRE, NAE, and PFPN" indicated N recovery efficiency, N agronomic use efficiency, and N partial factor productivity, respectively. "TRS, TRV, and RAD" indicated total surface area, total root volume, and root average diameter, respectively.

Author contributions

RH: Writing – original draft. ZD: Methodology, Writing – review & editing. YT: Supervision, Writing – review & editing. YC: Formal analysis, Writing – original draft. JH: Project administration, Writing – review & editing. XW: Data curation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2024.1326311/full#supplementary-material

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