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Enhancing Rice Yield and Nitrogen Utilization Efficiency through Optimal Planting Density and Reduced Nitrogen Rates

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Abstract: Rice yields can only be achieved by selecting a high-quality population. Nitrogen rates and transplanting density play a significant role in determining population quality and yield. Field experiments were conducted in Heshan District, Hunan Province, China, to explore suitable nitrogen rates and appropriate transplanting densities for rice production in 2021 and 2022. In this study, three levels of nitrogen, e.g., N1, N2, and N3 (0, 150, and 210) kg ha^{-1}, and three transplanting densities, e.g., B1, B2, and B3 (1, 2, and 3) seedlings per hill were used to study their individual or combined effects on rice (Taiyou 390) population quality, yield, and yield components, nitrogen-related enzyme activities, and nitrogen absorption and utilization efficiency. The results showed that N2B3 had the highest yield, with an average yield of 9.30 t ha^{-1} in two years, which was 3.7~49.6% higher than other treatments. This increase was attributed to higher dry matter accumulation (1538.22 g m^{-2}) and effective panicle number (435.2 × 10^4 ha^{-1}), influenced by increased nitrate reductase activity at the booting stage and glutamine synthase at the heading stage, along with maintenance of higher SPAD value and leaf area index. Nitrogen rates and transplanting densities significantly affected nitrogen use efficiency, with the contribution rate of nitrogen fertilizer to yield decreasing as nitrogen rates increased. However, N2B3 improved nitrogen use efficiency and stabilized rice yield by reducing nitrogen fertilizer application. This study suggested that N2B3 treatment could enhance rice yield by improving plant nitrogen use efficiency under low nitrogen supplementation.

Keywords: rice; planting densities; nitrogen rates; grain yield; nitrogen use efficiency

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1. Introduction

Rice is one of the staple foods worldwide and has been used as a model plant globally in numerous research for many years [1,2]. However, with rapid economic development and continuous population growth, the demand for food constantly increases, and food security is becoming a significant challenge worldwide [3]. Therefore, it is essential to adopt suitable cultivation techniques to improve rice grain yield, as traditional rice cultivation methods do not yield as much as expected.

Nitrogen rate and transplanting density are two important factors affecting rice yield [4]. Appropriate nitrogen rates and seedling numbers per hill can significantly increase rice yield [5]. However, excessive or insufficient nitrogen fertilization can adversely affect rice growth and yield. Excessive nitrogen fertilizer leads to the absorption of excess nitrogen by rice, resulting in nitrogen waste in the soil [6]. On the other hand, insufficient nitrogen fertilizer affects rice photosynthesis, which results in reduced yield [7,8]. While higher nitrogen fertilizer input can help to increase rice yield, it reduces nitrogen use efficiency, adversely affecting biodiversity, human health, and climate and posing a significant challenge to the nitrogen cycle [9,10]. China is the world's largest consumer of nitrogen fertilizer and has a nitrogen use efficiency of only 30–40%, which is 15–20% lower than other major rice-producing countries [11]. In addition to nitrogen rates, transplanting density is a significant factor affecting rice growth and yield. Lower transplanting densities allocate more resources to each plant, while higher transplanting densities reduce resource allocation per plant. Several studies have shown that appropriate transplanting density can improve rice's light energy utilization efficiency and hence increase growth and yield [12,13].

Maintaining a reasonable fertility level is crucial in enhancing rice yield and efficiency. Optimizing transplanting density and nitrogen rate based on soil fertility and organic matter levels is essential to achieve maximum yield and nitrogen use efficiency [12,14]. Appropriate transplanting density can allocate more resources per plant, increasing the adequate panicle number and 1000-grain weight. Similarly, proper nitrogen fertilizer can stimulate plant growth and development, boost grain yield, and improve nutrient utilization efficiency, resulting in higher rice yield and more significant economic benefits [15].

The middle and lower reaches of the Yangtze River have consistently been among China's primary regions for grain production, annually supplying a significant amount of grains to different parts of the country. The cultivation and widespread adoption of high-yield and fertilizer-tolerant rice varieties and reduced rural labor have resulted in fewer seedlings. High nitrogen is becoming a new fertilizer and density management measure in the middle and lower reaches of the Yangtze River [16]. Although this approach promotes the growth advantage of individual plants, it requires a substantial amount of fertilizer, significantly increasing planting costs, decreasing fertilizer utilization efficiency, and exacerbating diseases, insect pests, and soil pollution. Moreover, the yield potential is not realized if the transplanting density is too small or too large [17,18].

Constructing a viable rice population requires an appropriate transplanting density to control population size, stabilize adequate panicle number, increase grains per panicle, and ultimately lead to high yield [19]. In this study, we aim to investigate the effect of the fertilizer–density interaction on rice yield and nitrogen recovery efficiency in the middle and lower Yangtze River basin. We established three nitrogen fertilizer application rates and three transplanting densities to evaluate yield and yield components, nitrogen-related enzyme activities, and nitrogen use efficiency of hybrid rice in the main rice-producing areas in northern Hunan.

2. Materials and Methods

2.1. Field Experiments

The experiment was conducted in Oujiangcha village (28°29′ N 112°35′ E, 12 m asl), Oujiangcha town, Yiyang city, in 2021 and 2022, using farmers' rice fields. Before transplanting in 2021, the chemical properties of the 0~20 cm soil layer in the rice field were measured and are presented in Table 1. Throughout the rice growing season in 2021 and 2022, the daily average temperature was 27.35 °C and 29.81 °C, respectively. In 2021, the daily average rainfall was 3.45 mm, while in 2022, it was 0.89 mm 2022. Additionally, the daily average solar radiation was 14.50 MJ m^{-2} in 2021 and 17.17 MJ m^{-2} in 2022, as shown in Figure 1. Growth duration from SS to MS was 1 d shorter in 2022 than in 2021, respectively (Table 2).

Table 1. Soil pH, organic matter, total nitrogen, and available nutrients (N, P, and K) of the experimental field.

pH	Organic Matter (gkg^{-1})	Total Nitrogen (gkg^{-1})	Olsen Phosphorus ($mgkg^{-1}$)	Alkali-Hydrolysis Nitrogen ($mgkg^{-1}$)	Exchangeable Potassium ($mgkg^{-1}$)
5.2	36.6	1.1	132.5	186.6	136.5

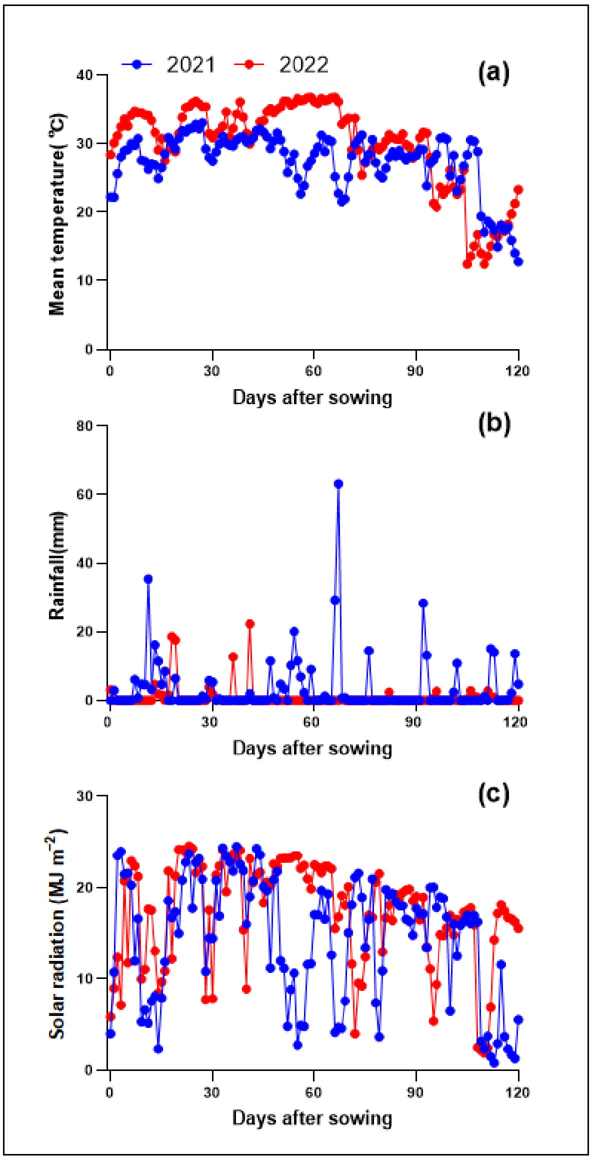

Figure 1. Daily mean temperature (**a**), daily mean rainfall (**b**), and solar radiation (**c**) during the rice-growing season in 2021 and 2022.

Table 2. Growth duration of rice cultivars grown in 2021 and 2022.

Cultivar	Growth Duration (d)		
	SS-HS	HS-MS	SS-MS
2021	88	32	120
2022	84	35	119

SS, HS, and MS represent sowing, heading, and maturity stages, respectively.

The experiment was conducted in a split-plot design with three replications, using a plot area of 60 m^2 and a split-zone area of 20 m^2. The main plots were divided into three different N application treatments: N1 (0 kg ha^{-1}), N2 (150 kg ha^{-1}), and N3 (210 kg N ha^{-1}). Additionally, the secondary plot was planted at three different densities, a hill spacing of 20 cm × 20 cm with 1, 2, and 3 seedlings per hill. The N1 treatment served as the low N treatment. The rice variety "Taiyu 390," provided by the Heshan District Agricultural and Rural Bureau, was used as the test crop. The pre-germinated seeds were sown in seedbeds on June 19, and the seedlings were raised according to local practices.

The seedlings were manually uprooted and transplanted at 16.5 cm × 26.5 cm on July 19. Urea(46%) as the N fertilizer was applied at three stages, e.g., before transplanting (base fertilizer), 7 days after transplanting (tiller fertilizer), and at the beginning of young spike differentiation (spike fertilizer) at a ratio of 5:3:2, respectively. All treatments received 750 kg ha^{-1} of calcium superphosphate (12% P_2O_5) and 150 kg ha^{-1} of potassium chloride (60% K_2O) before transplanting and at the tillering stage. All plots were flooded with a water depth of 5 cm from transplanting to 7 days before maturity, after which they were drained in preparation for harvest. Locally recommended plant protection measures and chemicals were used to strictly control pathogens, insects, and weeds according to the local farmers' practices for the rice cultivation system in this region. Rice sheath blight and false smut were controlled by validamycin. Rice borer, planthopper, and leaf roller were controlled by triazophos, buprofezin, and phoxim, respectively. Weeds were controlled by herbicide and hand-pulling.

2.2. Sampling and Measurement

The field sampling was conducted at five distinct stages of rice growth: tillering stage (TS), booting stage (BS), heading stage (HS), grain filling stage (FS), and mature stage (MS). Ten rice plants were selected from each plot based on the average number of stems and tillers in a single hill. The stems, leaves, and spikes were put in paper bags separately, dried in an oven at 105 °C for 30 min, and dried at 80 °C until they reached a constant weight. The total aboveground dry weight (TDW) was then measured using a balance. After drying, the samples with dry matter weight were crushed and sieved through a 100-mesh sieve. The sample of 0.5000 g powder was weighed, and the total nitrogen was extracted from the plants using the H_2SO_4-H_2O_2 method [20].

To measure the leaf area index (LAI), a Plant Canopy Analyzer (LAI-2200, LI-COR, Lincoln, NE, USA) was used at the TS, BS, HS, and FS. The LAI was estimated at three different heights (0, 0.5, and 1 m) in each plot, and the average of the three observations was considered as the LAI of the plot [21].

The SPAD value was measured using a portable chlorophyll meter (SPAD-502 PLUS, Minola Osaka Company, Japan) at four stages: TS, BS, HS, and FS. Five plants with consistent growth were randomly selected from each plot, and the SPAD value of the sword leaves was measured. The average value was used to determine the SPAD value for the respective treatment [22].

At the maturity stage (MS), one hundred effective rice spikes were examined in each plot. Then, 12 hills were randomly selected from each plot based on the average value of effective spikes. These samples were transported to the laboratory to investigate the following parameters: panicle number × 104 ha^{-1}, spikelets per panicle, spikelet filling, and 1000-grain weight. The harvested rice was sun-dried, threshed, and cleared of straw and empty grains. The grain weight was measured, and the moisture content was determined using the drying method to convert the actual yield to a moisture content of 14% [20]. Nitrogen grain production efficiency (NGE), nitrogen harvest index (NHI), agronomic nitrogen use efficiency (AE), nitrogen recovery efficiency (NRE), partial factor productivity of applied fertilizer nitrogen (PFP), and physiological nitrogen use efficiency (PE) was calculated according to the method published by Meng et al. [23].

A total of 12 flag leaves were randomly selected from each plot to determine N-metabolism enzyme activities. These leaves were immediately placed in liquid nitrogen and stored at ultra-low temperature (−80 °C) for subsequent measurements [24]. Nitrate reductase (NR) and glutamine synthetase (GS) were extracted from the samples. Enzyme activities and complementary products related to nitrogen metabolism were determined using assay kits provided by Beijing Solabao Technology Co., Ltd, Beijing, China.

2.3. Data Analysis

The data were analyzed using analysis of variance (ANOVA, SAS Version 9.1.2), the means of the nitrogen rate and transplanting density treatments were compared using the least significant difference test (LSD) with a 0.05 probability level, and graphs were constructed using Microsoft Excel 2017 (Microsoft Corp., Redmond, WA, USA).

3. Results

3.1. Effect of Nitrogen Rate and Transplanting Density on Grain Yield

Both in 2021 and 2022, the grain yield of rice was significantly affected by different transplanting densities and nitrogen application rates (Figure 2). The N2B3 treatment resulted in the highest grain yield of 9.11 t ha^{-1} and 9.49 t ha^{-1} in 2021 and 2022, respectively. In contrast, the N1B1 treatment showed the lowest rice yield, producing only 6.16 t ha^{-1} and 6.28 t ha^{-1} in 2021 and 2022, respectively. The highest and lowest yields in the two years were 2.96 t ha^{-1} and 3.21 t ha^{-1}, respectively.

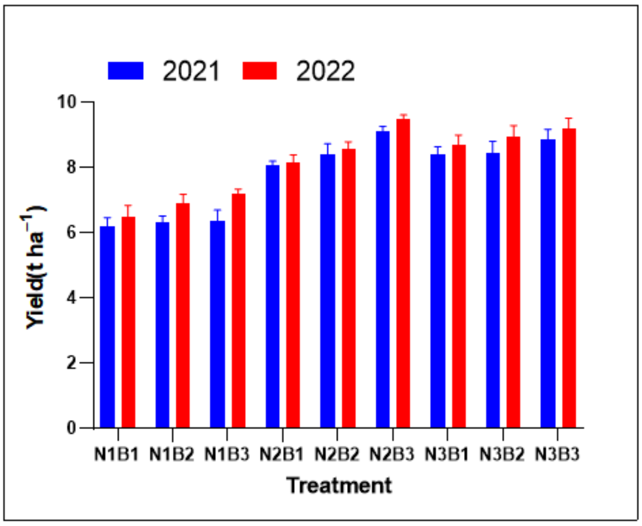

Figure 2. Effect of nitrogen rate and transplanting density on grain yield in 2021 and 2022. N1, N2, and N3 (0, 150, and 210) kg ha^{-1}, and three transplanting densities, e.g., B1, B2, and B3 (1, 2, and 3) seedlings per hill.

3.2. Effect of Nitrogen Rate and Transplanting Density on Yield Components

Table 3 demonstrates the significant effects of transplanting density and nitrogen rate on grain yield components of rice in 2021 and 2022. Notably, there was a consistent and significant interaction between transplanting density and nitrogen rate across both crop years (i.e., no significant interactions between transplanting density, nitrogen rate, and year), indicating that yield was increased more strongly when an appropriate nitrogen rate was combined with an increase in transplanting density. The interaction between transplanting density and nitrogen rate had a very significant effect on effective panicle number and a significant effect on spikelet filling and 1000-grain weight, but no effect on spikelets per panicle. However, the interaction between transplanting density, nitrogen rate, and year had no significant effect on effective panicles, spikelets per panicle, spikelet filling, and 1000-grain weight.

Table 3. Effect of nitrogen rate and transplanting density on yield components.

Year	Treatment	Panicles (×104 ha−1)	Spikelet Filling (%)	Grain Weight (mg)	Spikelets Panicle^{-1}
2021	N1B1	204.7 e	78.8 a	25.9 a	157.5 b
	N1B2	247.6 d	75 b	25.5 b	141.9 cd
	N1B3	268.5 d	73.5 bc	25.3 c	133.9 d
	N2B1	252.6 b	76.7 b	25.9 a	174.3 a
	N2B2	335.5 d	71.8 c	25.4 c	145.9 c
	N2B3	426.2 a	70.5 d	25.4 c	125.4 d
	N3B1	296.6 c	76.6 b	25.4 c	150.7 c
	N3B2	365.5 b	70.9 cd	25.3 c	131 d
	N3B3	423.4 a	69.4 e	25.1 c	122 d
2022	N1B1	216.6 e	84.7 a	25.4 a	147.9 b
	N1B2	258.8 d	81.1 bc	25.2 b	140.2 c
	N1B3	284.5 d	80.3 c	24.9 c	133.7 c
	N2B1	269.3 d	80.1 c	25.2 b	160.4 a
	N2B2	336.8 c	80.8 c	24.9 c	132.6 c
	N2B3	444.2 a	80.1 c	24.7 cd	113.2 e
	N3B1	324.5 c	82.2 b	24.7 cd	135.3 c
	N3B2	399.4 b	75.5 d	24.5 d	121.6 d
	N3B3	426 ab	76.3 d	24.8 cd	116.9 de
SOV	N	**	ns	ns	**
	B	**	**	**	**
	N × B	**	*	*	ns
	N × B × Y	ns	ns	ns	ns

Within a column for each year, means followed by the same letters are not significantly different according to LSD (0.05). SOV, source of variation. Significant treatment effects are indicated by * ($0.01 < p \leq 0.05$), or ** ($0.001 < p \leq 0.01$), the non-significant is indicated by ns ($p > 0.05$).

The results showed that the N2B3 treatment produced higher grain yield in the 2021 and 2022 growing seasons than other treatments due to its larger storage capacity. The average effective panicle number of N2B3 was 435.2 × 104 ha^{-1} over two years, which was 2.5~106.6% higher than other treatments. The super-high rice yield was achieved by greatly expanding the yield sink capacity (i.e., increasing the total spikelet number).

3.3. Effect of Nitrogen Rate and Transplanting Density on SPAD

The SPAD value of rice flag leaves was significantly influenced by transplanting density and nitrogen fertilization, as shown in Figure 3. The SPAD value increased from the TS, peaked at the HS, and gradually declined during the later growth stages. At the HS, the N2B3 and N3B2 treatments showed significantly higher SPAD values than the other treatments. Furthermore, the SPAD values of the N2B3 and N3B2 treatments remained consistently higher during the later growth stages.

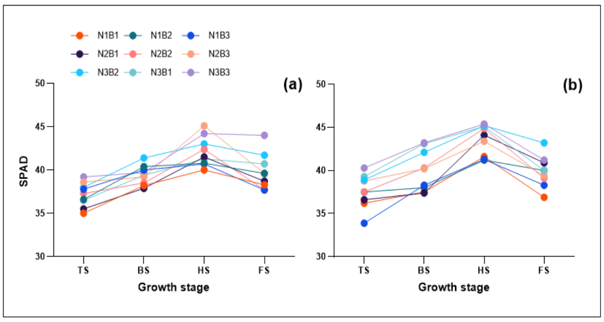

Figure 3. Effect of different planting densities and nitrogen levels on SPAD values under different growth stages in 2021 (**a**) and 2022 (**b**). Note: TS—tillering stage, BS—booting stage, HS—heading stage, and FS—grain filling stage.

3.4. Effect of Nitrogen Rate and Transplanting Density on Leaf Area Index

Transplanting density and nitrogen rates significantly affected the LAI of rice (Figure 4). The LAI of all treatments increased from the TS, peaked at the HS, and then declined gradually during later growth stages. At effective tillering, the LAI was approximately 3.5, while at BS, HD, and FS, it ranged from 6.0 to 8.0. The LAI of the N2B3 treatment was significantly higher than that of the other treatments at all stages, indicating a larger photosynthetic leaf area. Notably, N2B3 had the highest green leaf area at HS, suggesting a higher leaf quality under this cultivation mode.

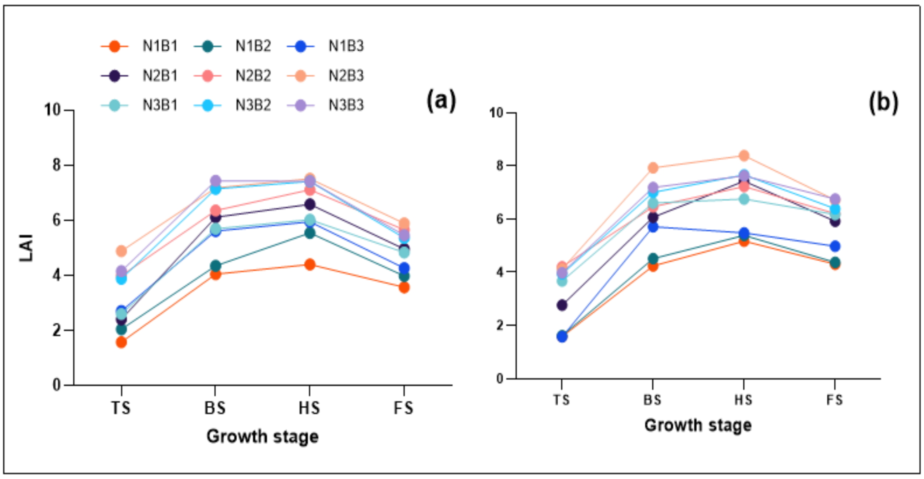

Figure 4. LAI in different growth stages in 2021 (**a**) and 2022 (**b**). TS represents the tillering stage, BS represents the booting stage, HS represents the heading stage, and FS represents the grain-filling stage.

3.5. Effect of Nitrogen Rate and Transplanting Density on the Aboveground Total Dry Weight

Results showed that transplanting density and nitrogen rate significantly affected the TDW of rice (Figure 5). Specifically, the TDW of the rice population at different growth stages varied significantly among the different transplanting densities and nitrogen rates. In both years, the TDW was significantly higher in the nitrogen rate treatments than the no nitrogen treatments at the TS, BS, HS, FS, and MS stages. Moreover, the difference between the treatments became more pronounced with the advancement of the growth process, ultimately leading to higher TDW of the rice population at the MS stage. At the late growth stages, i.e., from flowering to maturity, the N2B3 treatment had significantly higher TDW than other treatments. The average TDW over two years at maturity was 1538.22 g m^{-2} for the N2B3 treatment, 4.3% to 39.1% higher than the other treatments.

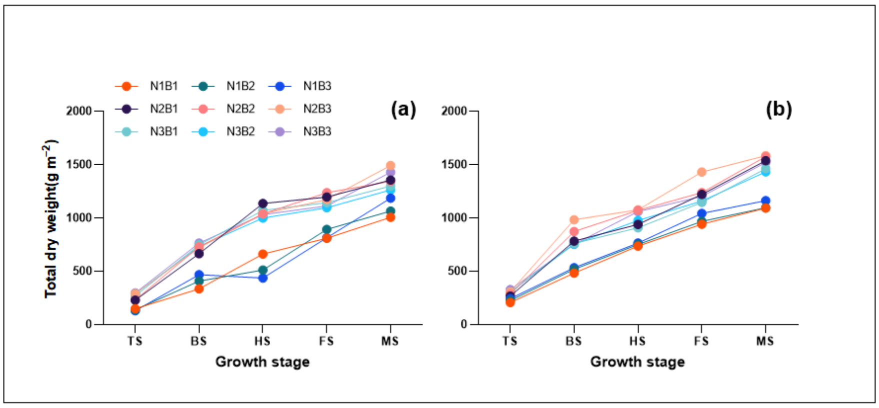

Figure 5. TDW in different growth stages in 2021 (**a**) and 2022 (**b**). Note: TS—tillering stage, BS—booting stage, HS—heading stage, FS—grain filling stage, and MS—mature stage.

3.6. Effect of Nitrogen Rate and Transplanting Density on Nitrate Reductase and Glutamine Synthetase

During the growth process of rice, the activities of NR and GS showed a low–high–low trend (Figure 6). However, the activities of these enzymes varied at different growth stages. The activity of NR was observed higher at the BS stage, while the activity of GS was highest at the HS stage. Notably, the activities of NR and GS in functional leaves increased with nitrogen rates. However, the rate of increase in NR and GS activities slowed significantly when the nitrogen rate reached 240 kg ha^{-1} under three transplanting densities.

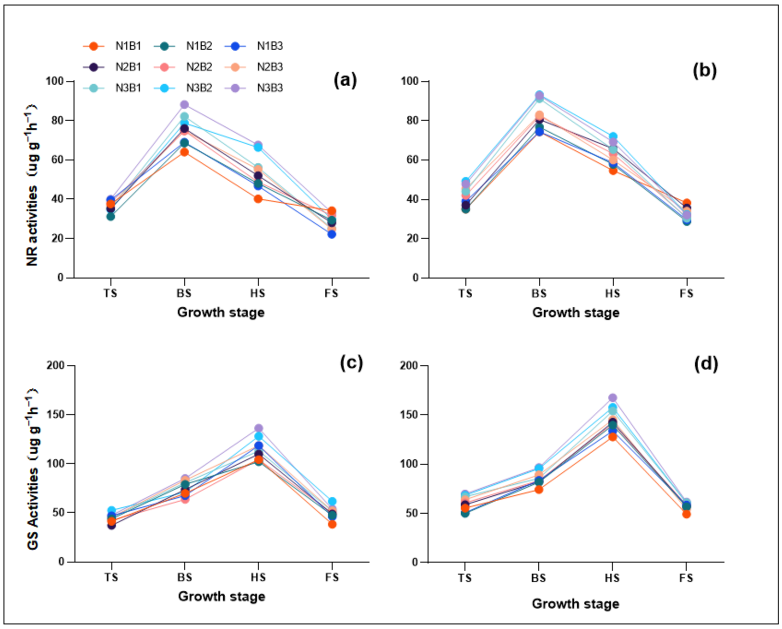

Figure 6. NR (**a**,**b**) and GS (**c**,**d**) in different growth stages in 2021 (**a**,**c**) and 2022 (**b**,**d**). TS—tillering stage, BS—booting stage, HS—heading stage, and FS—grain filling stage.

Compared to other treatments, the NR and GS activities of N3B2 and N3B3 functional leaves were significantly higher in 2021 and 2022. On average, N3B2 and N3B3 improved NR activity by 4.5–24.6%, 5.2–23.5%, 1.2–31.7%, and 14.5–26.4% at the TS, BS, HS, and FS stages, respectively, compared to other treatments. Similarly, compared to other treatments, the GS activity of the N3B3 treatment was higher by 2.9–22.3%, 8.9–20.6%, 6.3–23.6%, and 5.6–28.3% at the TS, BS, HS, and FS stages, respectively.

3.7. Effect of Nitrogen Rate and Transplanting Density on Nitrogen Uptake and Use Efficiency

Nitrogen rates had a significant effect on nitrogen grain efficiency (NGE), nitrogen harvest index (NHI), nitrogen recovery efficiency (NRE), agronomic efficiency (AE), physiological efficiency (PE), and partial factor productivity (PFP) of rice in 2021 and 2022 growing seasons. Transplanting density significantly affected the NHI of rice in the 2021 and 2022 growing seasons, but it did not significantly affect NGE, NRE, AE, PE, and PFP. The interaction between transplanting density and nitrogen rate significantly affected the NHI of rice in the 2021 and 2022 growing seasons and also significantly affected NGE, NRE, AE, PE, and PFP. The interaction of transplanting densities, nitrogen rate, and the growing seasons had significant effects on the NHI of rice in 2021 and 2022, but it had no significant effects on NGE, NRE, AE, PE, and PFP (Table 4).

Table 4. Effect of nitrogen rate and transplanting density on nitrogen uptake and use efficiency.

Year	Treatment	NGE (kg kg^{-1})	NHI (%)	NRE %	AE (kg kg^{-1})	PE (kg kg^{-1})	PFP (kg kg^{-1})
2021	N1B1	63.7 e	77.5 a				
	N1B2	66.8 e	80.1 a				
	N1B3	66.9 e	80.7 a				
	N2B1	99.2 cd	63.0 c	39.9 b	10.4 b	39.4 b	53.8 a
	N2B2	111.4 c	61.5 c	39.8 b	10.4 b	40.4 a	54 a
	N2B3	114.9 c	80.4 a	41.3 a	11.3a	41.1 a	52.2 b
	N3B1	123.2 c	55.6 d	33.9 c	7.4 d	26.7 d	41.8 d
	N3B2	139.2 b	57.8 cd	34.4 c	7.2 d	28.4 c	44.4 c
	N3B3	168.6 a	68.3 b	34.6 c	8.5 c	28.4 c	44.0 c
2022	N1B1	68.0 e	83.4 a				
	N1B2	61.2 e	82.5 a				
	N1B3	68.2 e	84.4 a				
	N2B1	106.5 d	72.1 b	40.9 b	10.1 a	38.9 b	59.4 a
	N2B2	114.3 d	76.8 b	41.6 ab	10.2 a	41.1 a	60.6 a
	N2B3	127 c	82.6 a	42.5 a	10.6 a	41.4 a	60.6 a
	N3B1	135.6 bc	59.4 d	36.1 c	7.9 b	30.8 c	47.5 c
	N3B2	148.4 b	59.4 d	36.3 c	8.0 b	31.0 c	47.2 c
	N3B3	179.7 a	66.2 c	37.5 c	8.4 b	30.8 c	49.3 b
SOV	N	**	**	**	**	**	**
	B	*	**	ns	ns	ns	ns
	N × B	*	**	*	*	*	*
	N × B × Y	*	ns	ns	ns	ns	ns

Within a column for each year, means followed by the same letters are not significantly different according to LSD (0.05). SOV, source of variation, nitrogen grain production efficiency (NGE), nitrogen harvest index (NHI), agronomic nitrogen use efficiency (AE), nitrogen recovery efficiency (NRE), partial factor productivity of applied fertilizer nitrogen (PFP), and physiological nitrogen use efficiency (PE). Significant treatment effects are indicated by * ($0.01 < p \leq 0.05$), or ** ($0.001 < p \leq 0.01$), the non-significant is indicated by ns ($p > 0.05$).

The PE of rice decreased as the nitrogen rates increased under various transplanting densities, whereas NRE and AE initially increased, reached their maximum at a nitrogen rate of 180 kg ha^{-1}, and then decreased as nitrogen rates continued to increase. The results indicated that increasing nitrogen rates were beneficial for increasing NGE, but it decreased both the NGE and PE. Notably, increasing the nitrogen rate did not always lead to a decline in NRE and AE. However, when increasing the nitrogen rate failed to increase nitrogen accumulation significantly, it would significantly decline uptake and use efficiency.

4. Discussion

Transplanting density and nitrogen application rate are critical factors affecting rice yield, quality, and nitrogen utilization. By optimizing the combination of transplanting density and nitrogen rate, farmers can improve the utilization efficiency of nutrients by crops, increase rice yield, and enhance economic benefits. This study investigated the effects of various transplanting densities and different nitrogen fertilizer rates on yield and its components, including TDW, SPAD, LAI, NR activity, GS activity, and nitrogen recovery efficiency in the middle and lower reaches of the Yangtze River.

A more significant accumulation of photosynthetic matter (dry matter mass) can be achieved using higher planting densities at different stages. Similar results were reported by Liu et al. [25], who reported a more substantial photosynthetic capacity of rice individuals. In the present study, the dry matter at the MS stage of N2B3 treatment was higher. The possible reason for these increments might be due to N2B3 maintaining a higher LAI than other treatments at the BS booting and HS, which helps avoid poor field transmittance caused by excessive LAI of N3B3, thus preventing any negative impact on the photosynthesis of lower leaves. Furthermore, after full heading, the photosynthetic capacity of individual plants under nitrogen rate treatment was significantly higher than those under no nitrogen rate treatment, indicating that individual senescence was relatively fast in the later stages of no nitrogen rate treatment.

Increasing the yield storage capacity (total spikelet amount) is crucial to achieving super-high yields in rice cultivation [26]. This can be accomplished by increasing the number of panicles and spikelets per panicle per unit of land area. The reduction in N application with increasing planting density to increase panicle number could improve the grain yield of inbred rice, which has been reported in machine-transplanted rice by Huang et al. [27]. Although the number of spikelets per panicle increased compared to N2B3, the increase in total spikelets was primarily due to the increase in panicle number. Therefore, expanding the sink capacity in rice yield mainly depends on increasing the panicle number.

Rice yield is determined by the product of TDW and harvest index [28]. The study results indicate that, with suitable early material production, promoting material production capacity in the middle and late stages, and increasing material production from the BS to the HS and from the HS to the MS, exceptionally high-efficiency material production after HS is essential for achieving higher rice yield, which was similar to the results reported by Choi et al. [29]. Therefore, in order to increase rice yields, we can implement measures such as reducing nitrogen fertilizer usage and increasing planting density. It can be achieved by regulating the flow and distribution of dry matter across various organs of the rice plant at different growth stages, establishing a balanced source–sink system, and improving the efficiency of dry matter conversion.

Various plants' nitrogen absorption and utilization must be accomplished through reactions and transformations involving nitrogen-metabolizing enzymes, such as NR and GS [30]. Our results showed that, compared with B1 and B2 treatments, treatment B3 significantly increased the activities of NR and GS throughout the entire growth period. Similar results were reported by Mahmood et al. [31], who reported that the N supply and seed rate increased, and the activities of metabolic enzymes (NR and GS) significantly increased. This may be attributed to the more developed root system and improved soil aeration in treatment B3. Notably, treatment N3B3 exhibited the highest activity of GS at the HS, which could promptly assimilate NH4+ produced by NR catalysis and transfer it into protein. This promoted treatment N3B3 to absorb more nitrogen before and after the HS [32]. With increasing nitrogen rates, the activities of NR and GS in rice leaves at different growth stages tended to increase. However, excessively high nitrogen rates would increase NR and GS activities, which would increase NR and GS activities, leading to protein degradation and transportation [33].

Combining appropriate nitrogen rates and transplanting densities can result in superior agronomic traits in rice, as indicated by measures such as SPAD, LAI, and dry matter accumulation. These indices are closely related to nitrogen rates, transplanting densities, and their interaction. Further research on rice yield has shown that nitrogen rates, transplanting densities, and their synergistic effect significantly impact it. Therefore, using clear and reasonable fertilization and seedling control tillage methods can help to adjust the relationship between land and fertilizer, promote efficient energy use and have practical significance for high-yield cultivation and energy utilization of rice.

5. Conclusions

Appropriate planting density and nitrogen rates can improve rice yield. N2B3, which combines a specific planting density and nitrogen rate, has been found to increase nitrate reductase and glutamine synthetase activities, leading to improved nitrogen fertilizer utilization. As a result, SPAD and leaf area index levels also increased, increasing photosynthesis and dry matter accumulation in leaves. These findings demonstrate that N2B3 can maintain stable rice yield and improve plant nitrogen use efficiency while reducing nitrogen fertilizer application. Thus, optimal planting density and reduced nitrogen rates are promising methods for achieving high rice yield with reduced nitrogen fertilizer input.

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