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Panicle Nitrogen Strategies for Nitrogen-Efficient Rice Varieties at a Moderate Nitrogen Application Rate in the Lower Reaches of the Yangtze River, China

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Abstract: Nitrogen (N) management is of great importance in rice production, but most previous studies have focused on high N rates and there is a lack of research on management plans under a moderate N rate. This study aimed to explore the agronomic and physiological traits of N-efficient rice varieties (NEVs) and to optimize the management strategy at an N rate below the inflection point of the parabolic curve between N rate and grain yield. Two NEVs and two N-inefficient rice varieties (NIVs) were planted, and three treatments were designed according to the panicle N application method. A larger amount of N applied at panicle initiation (PI) led to higher rice yield and N-use efficiency (NUE). This was mainly due to increases in the total number of spikelets per unit area, root oxidation activity, leaf area duration, and leaf photosynthesis rate as well as to the increased carbon (C) and N utilization rates. Compared with NIVs, NEVs exhibited improved root and shoot functions and higher C and N transport characteristics at the moderate N rate. We suggest that increasing the application of N at PI and that planting of NEVs are important ways to increase rice yield and NUE when adopting moderate N rates.

Citation: Ju, C.; Liu, T.; Sun, C. Panicle Nitrogen Strategies for Nitrogen-Efficient Rice Varieties at a Moderate Nitrogen Application Rate in the Lower Reaches of the Yangtze River, China. *Agronomy* **2021**, *11*, 192. <https://doi.org/10.3390/agronomy11020192>

Received: 25 December 2020

Accepted: 19 January 2021

Published: 20 January 2021

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Keywords: rice (*Oryza sativa* L.); N-efficient varieties; N-use efficiency; panicle fertilizer; N management

1. Introduction

China is the world's largest producer and consumer of N fertilizer [1,2]. For a long time, increasing N application rate has been one of the most effective measures to increase the yield of rice (*Oryza sativa* L.) [3,4]. Since 2000, rice yields have increased slowly, with an average annual growth rate of less than 0.5% (Figure 1a). However, the N rates in China and Jiangsu province are 75% and 200% higher than the world average, respectively [5,6]. According to statistics from the National Bureau of Statistics (NBS) of China, the N inputs in China and Jiangsu province have been gradually reduced since 2014 and 2009, respectively (Figure 1b). Over the past 10 years, the N input in Jiangsu province has decreased by 20%, but the rice planting area has stabilized at 2.21 to 2.26 million hectares [7], meaning that the amount of N applied per unit area has decreased. The relationship between grain yields and N rate can be described by parabolic equations: in general, the grain yields reached maximums when the N rate reached the inflection point [8,9]. Thus the level of N application in many paddy fields has decreased below the optimal N rate. To stabilize and improve rice production at moderate N levels, N-fertilizer strategies must be improved by growing rice varieties with increase grain yields and N-use efficiency (NUE). This will aid in mitigating environmental costs while meeting the food demand of a growing population [10].

N is quantitatively the most important mineral nutrient for plant growth and development [11]. In China, the use of N fertilizer is generally inefficient and causes serious damage to the environment, resulting in significant amounts of greenhouse gas emissions [10]. A

high level of N fertilization can drive soil acidification and degrade soil quality [12]. Overuse of N fertilizers contributes substantially to regional water pollution [10–12]. The selection of N-efficient rice varieties (NEVs) has been known as a promising strategy to maximize grain yield and NUE in crop production and as an essential approach for sustainable agriculture [13,14]. Studies have found that high-yielding rice varieties can achieve a relatively high yield under sufficient N supply [15]. However, some varieties are highly dependent on the input of N fertilizer and their yield shows a large decrease after the N rate is reduced; therefore, these varieties are known as N-inefficient varieties (NIVs). In contrast, some varieties have a relatively high yield under a low N rate and they are known as NEVs [15,16]. The main varieties used in rice production in Jiangsu province are mostly NEVs, indicating that some NIVs relying on high N input have been gradually eliminated under the constantly decreased N application level [17]. Many NEVs possess a high tillering ability and belong to the multi-spike type [17], especially in the case of mechanical transplanting [18]. Peng et al. found that green super rice (GSR) also had the same plant type [19].

Figure 1. The average grain yield of rice (a) and total agricultural N application (b) of China and Jiangsu province in the past 30 years (data from the National Bureau of Statistics of China).

Studies have shown that it is most effective to apply N fertilizer at panicle initiation (PI) for multi-spike varieties [18]. However, it remains unknown whether increasing the proportion of N fertilizer applied at PI can alleviate the impact of reduced N application on rice yield after reducing the amount of applied N fertilizer. Therefore, we designed three N treatments based on the amount of N fertilizer applied at PI to study the effects of different amounts of N fertilizer applied at PI on NEV yield and NUE with reduced N application. It would be very meaningful to understand the agronomic and physiological characteristics of NEVs under these different N managements. The purpose was to investigate rice yield and NUE of NEVs under different treatments and to understand their agronomic and physiological bases by determining root biomass and length, root oxidation activity (ROA), leaf area index (LAI), leaf area duration (LAD), photosynthetic rate of leaves, nonstructural carbohydrate (NSC) accumulation in the stems and N accumulation in the stems and leaves, and its remobilization during grain filling. The results of this study should reveal the high-yield and high-efficiency source and sink mechanism of rice and to provide a reference for NEV breeding.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Field experiments were conducted during the rice growing seasons of 2017 and 2018 at a research farm located in Yizheng Country, Jiangsu Province (32°50' N, 119°23' E), which was an alluvial plain that is one of the major rice production regions in China. The soil properties of the field were as follows: organic matter of 23.5 g kg⁻¹, total N of 1.08 g kg⁻¹, Olsen P of 31.7 mg kg⁻¹, exchangeable K of 74.4 mg kg⁻¹ at 0 to 20 cm soil

depth, 0.188 g g^{-1} of soil moisture content, and 1.32 g cm^{-3} of soil bulk density. The meteorological data during the rice-growing season across the study years were collected at a weather station close to the field site, shown in Figure 2.

Figure 2. Precipitation (a), sunshine hours (b), and temperature (c) during the growing season of rice in 2017 and 2018 at the field site of Yangzhou, China.

Two N-efficient varieties, Huaidao5 (HD-5) and Lianjing7 (LJ-7), and two N-inefficient varieties, Zhongdao1 (ZD-1) and Yangjing4038 (YJ-4), were grown in the paddy field. The equations between the N rate and yield, inflection point, and max yield of tested varieties under a N split proportion of 4:2:2 are shown in Table 1. The tested varieties have a similar growth period, ranging from 152 to 155 days from sowing to maturity. The seeds of ZD-1 and LJ-7 were provided by Lianyungang Agricultural Research Institute (Lianyungang, China); the other two rice varieties were obtained from Lixiahe Agricultural Research Institute (Yangzhou, China). The classification of spike type was generally according to the number of spikelets: small-spike type or multi-spike type with less than 140, large-spike type with more than 200, and medium-spike type with 140–200 spikelets per panicle. With the increasing N rate, the spikelets per panicle improved. The spikelet number of the tested varieties in this study were mostly less than 140, so they could be considered multi-spike types. The seedlings were raised on 22 May of 2017 and 20 May of 2018 and transplanted on 12 June of 2017 and 10 June of 2018 at a transplanting spacing of $0.33 \text{ m} \times 0.12 \text{ m}$, with three seedlings per hill, by using a rice transplanter. Phosphorus was applied as a single superphosphate at a rate of 40 kg ha^{-1} , and potassium was applied as KCl at a rate of 50 kg ha^{-1} before transplanting. To avoid yield loss, water, weeds, diseases, and insects were intensively controlled. The heading date and harvest date of the tested varieties were 23–25 August and 16–18 October, respectively.

Table 1. Equations between N rate and yield, inflection point, and max yield of the tested varieties.

Variety ¹	Equations between N Rate and Yield	R ²	Inflection Point (kg hm ⁻²)	Max Yield (kg hm ⁻²)
HD-5	$y = -0.0550x^2 + 28.69x + 6356$	0.934	260.8	10,097
LJ-7	$y = -0.0535x^2 + 28.58x + 6519$	0.927	267.1	10,336
ZD-1	$y = -0.0418x^2 + 27.33x + 5859$	0.939	327.3	10,326
YJ-4	$y = -0.0443x^2 + 28.11x + 5729$	0.955	308.5	10,185

¹ HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038.

2.2. Treatments

The experiments were laid out in a split-plot design with N management as the main plots and varieties as the subplots with three replicates. The plot size was 72 m^2 ($12 \text{ m} \times 6 \text{ m}$), and plots were separated by 1-m-wide ridges. In the high-yield area of lower reaches of the Yangtze River, the average N input was 300 kg per hectare, which is 67% higher than N application in the single rice cropping system in China. There was no standard criteria to classify high, moderate, and low N rates. An application rate of

200 kg hm⁻² was considered a relatively moderate level in the lower reaches of the Yangtze River, China. Treatments consisted of four N managements; for each treatment, N as urea was split into four applications at four growth stages (Table 2).

Table 2. The amount of N applied to four treatments at different growth stages in the experiment.

N Split	Growth Stage ¹	N Rate (kg hm ⁻²)			
		N0 ²	N1	N2	N3
1st application	Pre-transplanting	-	80	80	80
2nd application	Mid tillering (V9)	-	40	40	40
3rd application	Panicle initiation (R0)	-	0	40	80
4th application	Spikelet differentiation (R2)	-	80	40	0
Total nitrogen		0	200	200	200

¹ The classification of growth stages was according to the methods described by Counce et al. [20]. ² N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI).

2.3. Sampling and Measurements

Root biomass and root length were determined at about 40, 75, and 125 days after transplanting of growth, shown as the stages of panicle initiation (PI), heading time (HT), and maturity (MA), respectively. Plant samples were separated into four parts: roots, leaves, stems, and panicles (at HT and MA). The dry matter of each part was recorded after drying at 75 °C to constant weight and weighed. The root length was measured with a scanner (Epson Expression 1680, SeikoEpson, Suwa City, Japan) and analyzed using a root analyzer system developed by regent instruments incorporation, Canada.

The root oxidation activity (ROA) and leaf photosynthetic rate were measured at the same time. The ROA was determined according to the method of Chu et al. [21]. The photosynthetic rate of fully expanded leaves was measured by a portable photosynthetic instrument (Li-Cor 6400, LI-COR, Lincoln, NE, USA). The leaves were measured during 09:00–11:00 h when photosynthetic active radiation above the canopy was 1300–1500 µmol m⁻² s⁻¹ and the parameter of CO₂ level of leaf chamber and temperature were 380 µmol mol⁻¹ and 28–30 °C, respectively. Each gas exchange analyzer was used in one treatment replicate, and eight leaves were measured for each treatment.

The leaf area index (LAI) was measured at PI, HT, and MA. Six plants (per hill was considered as per plant) were sampled from each treatment for each measurement. Leaf area was determined by scanning the projected area and measured with an area meter (LI-3000 C, LI-COR, Lincoln, NE, USA). The leaf area duration (LAD) was calculated using the following formula:

$$LAD = 1/2(L1 + L2) \times (t2 - t1)$$

where L1 and L2 are the previous and next measurements of the leaf area index (m² m⁻²), respectively, and t1 and t2 present the previous and next times (d), respectively, of the measurement.

N concentration was determined by microwave disintegration, distillation, and titration to calculate aboveground N uptake [21]. The methods for calculating NUE indexes were according to Ju et al. [15], including the internal N-use efficiency (IE, the ratio of grain yield to the total N uptake in plants at maturity), agronomic N-use efficiency (AE, the ratio of yield difference between N application and no N application treatment to the amount of N applied), nitrogen partial factor productivity (PFP, the ratio of grain yield to the amount of N applied), apparent recovery efficiency (RE, the percentage of fertilizer N recovered in aboveground plant biomass at the end of the cropping season), and nitrogen physiological efficiency (PE, the ratio of yield difference between N application and no N application treatment to the N uptake difference between N application and no N application treatment). The

amount of nonstructural carbohydrate in the stem was determined at the stages of heading and maturity according to the method described by Yoshida et al. [22].

Plants were harvested manually on 18 October in 2017 and 16 October in 2018. Yield was determined from a harvest area of 6.0 m² in each plot and adjusted to the standard moisture content of 0.14 g H₂O g⁻¹. The aboveground biomass and yield components were determined from plants sampled randomly from each plot. Filled grains were separated by submerging them in salt water (specific gravity ≥ 1.06 g cm⁻³).

2.4. Statistical Analysis

Statistical data analysis was performed using analysis of variance (SAS Institute, Cary, NC, USA). The statistical model used included sources of variation caused by variety (V), N treatment, and the interaction between V and N treatment. Data from each sampling date were analyzed separately. Means were compared by the multiple comparison tests method at the 0.05 probability level. Since root biomass, root length, ROA, and leaf photosynthetic rate exhibited similar patterns at the stages of PI, HT, and MA, data at HT were only presented for conciseness.

3. Results

3.1. Differences in Experimental Factors

Table 3 shows the computed F values for the differences in grain yield, yield components, total N uptake, and agronomic NUE between two study years, the varieties, and the N treatments. The results showed a significant difference ($p < 0.05$) in plant traits among the varieties, N treatments, and the interaction between variety and N treatment (Table 3) but no significant difference between years or the interactions year $\times$ variety and year $\times$ N treatment. Similar results were obtained for other measurements, such as root biomass, LAI, LAD, and remobilized NSC reserve. Since the year was not a significant factor in this study, means of both years were presented.

Table 3. Analysis of variance for grain yield, yield components, total N uptake, and agronomic N-use efficiency of rice among varieties for N treatments.

Source of Variation	df	Grain Yield	Spikelets per Panicle	Filled Grains Rate	Grain Weight	Total N Uptake	Agronomic NUE
Year(Y)	1	NS	NS	NS	NS	NS	NS
Variety(V)	3	**	**	**	**	**	**
N treatments (N)	3	**	**	**	**	**	**
Y $\times$ V	3	NS	NS	NS	NS	NS	NS
Y $\times$ N	3	NS	NS	NS	NS	NS	NS
V $\times$ N	9	**	**	**	*	**	**

NS, not significant at the $p = 0.05$ level. * Significant at the $p = 0.05$ level. ** Significant at the $p = 0.01$ level.

3.2. Grain Yield and NUE

Under the same N treatment, the yield difference between the two NEVs or the two NIVs was not significant and the yield of NEVs was significantly higher than that of NIVs. The yield of N3 was significantly higher than those of N1 and N2, mainly because, under N3, the number of spikelets per panicle increased significantly, although the filled grain percentage and grain weight decreased slightly (Table 4).

Similar to yield (Table 5), NUE indexes of NEVs were significantly higher than those of NIVs under the same N treatment. With the increasing amount of N applied at PI from N1 to N3, the amount of N uptake by plants gradually increased. Compared with N1 and N2, the N3 treatment increased the AE, PFP, and RE of rice. NIVs had relatively high IE and PE under the N1 treatment but showed low AE, PFP, and RE, indicating that N absorbed by plants under the N1 treatment could not be efficiently transported to the grain.

Table 4. Grain yield and yield components of rice under various N managements.

Variety ¹	Treatment ²	Grain Yield (t ha ⁻¹)	Panicles per m ²	Spikelets per Panicle	Total Spikelets (×10 ³ m ⁻²)	Filled Grain (%)	Grain Weight (mg)
HD-5	N0	5.7d ³	203 b	105 ef	21.3 g	93.1 a	28.6 a
	N1	9.2 abc	286 a	122 cd	34.9 de	92.1 ab	28.6 a
	N2	9.4 ab	288 a	125 c	36.0 cd	91.7 ab	28.5 a
	N3	9.9 a	287 a	138 b	39.6 a	89.5 de	27.9 ab
LJ-7	N0	5.6 d	198 b	112 de	22.2 g	92.8 a	27.3 b
	N1	9.2 abc	275 a	133 bc	36.6 bcd	92.0 ab	27.2 b
	N2	9.3 ab	276 a	136 b	37.5 bc	91.8 ab	26.9 bc
	N3	9.8 a	279 a	147 a	41.0 a	89.4 de	26.6 c
ZD-1	N0	5.2 e	201 b	103 f	20.7 g	91.3 abc	27.4 b
	N1	8.4 c	289 a	113 de	32.7 f	91.4 abc	28.1 a
	N2	8.5 bc	288 a	117 d	33.7 ef	90.3 bcd	27.8 ab
	N3	8.6 bc	284 a	127 c	36.1 cd	86.8 f	27.5 b
YJ-4	N0	5.1 e	195 b	109 e	21.3 g	89.7 cde	26.5 c
	N1	8.3 c	284 a	124 c	35.2 de	88.9 de	26.4 c
	N2	8.4 c	282 a	128 c	36.1 cd	88.4 ef	26.2 c
	N3	8.6 bc	281 a	135 b	37.9 b	87.1 f	26.0 c

¹ HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038. ² N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI). ³ No shared letter indicates statistical significance at the $p = 0.05$ level within the same column.

Table 5. Nitrogen (N) uptake and N-use efficiency (NUE) of rice under various N managements.

Variety ¹	Treatment ²	N Uptake (kg ha ⁻¹)	IE (kg kg ⁻¹)	AE (kg kg ⁻¹)	PFP (kg kg ⁻¹)	RE (%)	PE (kg kg ⁻¹)
HD-5	N0	70.2 d ³	80.9 a				
	N1	139.8 bc	65.7 c	17.6 bc	46.0 c	34.8 ef	50.4 ab
	N2	147.1 ab	64.0 cd	18.7 b	47.1 bc	38.5 b	48.5 bcd
	N3	151.3 a	65.4 c	21.1 a	49.5 a	40.6 a	51.9 a
LJ-7	N0	70.3 d	79.9 a				
	N1	142.2 bc	64.3 cd	17.7 bcd	45.8 c	36.0 de	49.1 bc
	N2	148.4 ab	62.5 d	18.3 bc	46.4 c	39.1 b	46.7 de
	N3	153.0 a	63.7 cd	20.7 a	48.8 ab	41.4 a	49.9 abc
ZD-1	N0	67.7 d	76.5 b				
	N1	134.8 c	62.2 d	16.1 d	42.0 d	33.6 fg	47.8 cd
	N2	140.8 bc	60.1 de	16.4 d	42.3 d	36.6 d	44.9 e
	N3	144.3 ab	59.7 e	17.2 cd	43.1 d	38.3 bc	44.8 e
YJ-4	N0	67.5 d	74.8 b				
	N1	133.9 c	61.8 d	16.1 d	41.4 d	33.2 g	48.5 bcd
	N2	140.5 bc	59.5 e	16.6 cd	41.8 d	36.5 e	45.3 e
	N3	141.6 bc	60.7 de	17.7 bc	43.0 d	37.1 cd	47.8 cd

¹ HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038. ² N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI). ³ No shared letter indicates statistical significance at the $p = 0.05$ level within the same column.

3.3. Root Biomass, Root Length, Root Oxidation Activity, and Leaf Photosynthetic Rate

The root biomass and ROA of each variety at HT were the highest under the N3 treatment, followed by the N2 and N1 treatments. Under the same N treatment, the root biomass, root length, and ROA of NEVs were significantly higher than those of NIVs (Figure 3A–C). The change of photosynthetic rate was consistent with ROA ($R^2 = 0.93$, $n = 16$), indicating an interaction between roots and shoots.

Figure 3. Root biomass (a), root length (b), root oxidation activity (c), and leaf photosynthetic rate (d) of rice at heading under different N treatment: the vertical bars represent the $\pm$ standard error of the mean ($n = 5$), where these exceed the size of the symbol. No shared letter above the column indicates statistical significance at the $p = 0.05$ level within the same variety. N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI). HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038.

3.4. LAI and LAD

From N1 to N3, the LAI gradually increased at HT, and the LAI of N3 was significantly higher than that of N1. At the maturity stage, the difference in LAI for N1, N2, and N3 was not significant (Figure 4). The LADs from PI to HT and from HT to MA under the N3 treatment were significantly higher than those under the N1 and N2 treatments, and the LAI and LAD of NEVs were higher than those of NIVs.

3.5. Carbon and Nitrogen Remobilization

At heading, the NSC accumulated by NEVs was significantly higher than that of NIVs under the N0, N2, and N3 treatments, and the difference was not significant under the N1 treatment (Table 6). Between different N treatments, the NSC at MA was not significantly different, but the NSC levels at HT decreased in the order $N3 > N2 > N1$. Therefore, the NSC transport rate under N3 was higher than those under N2 and N1. Compared with N1 and N2, the amount of N accumulated in the stem and leaf at HT was highest under N3, and the amount of residual N in MA was lowest in this treatment. Increased fertilizer amount applied at the stage of PI increased the N transport rate from stems and leaves to grain during the grain filling stage.

Figure 4. Leaf area index (LAI) of rice at heading time (a) and maturity (c), and Leaf area duration (LAD) from panicle initiation to heading (b) and from heading to maturity (d) under various N management strategies: the vertical bars represent the $\pm$ standard error of the mean, where these exceed the size of the symbol. No shared letter above the column indicates statistical significance at the $p = 0.05$ level within the same variety. N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI). HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038.

Table 6. Nitrogen (N) uptake and NUE of rice under various N managements.

Variety ¹	Treatment ²	NSC at Heading (g m^{-2})	NSC at Maturity (g m^{-2})	Remobilized NSC Reserve (%) ³	N Accumulation at Heading (kg ha^{-1})	N Accumulation at Maturity (kg ha^{-1})	N Translocation Rate (%) ⁴
HD-5	N0	159 d ⁵	58 b	63.3 a	63 d	22 d	65.1 a
	N1	267 c	148 a	44.6 g	137 bc	65 a	52.4 de
	N2	298 ab	144 a	51.7 d	142 ab	62 ab	56.3 c
	N3	303 ab	144 a	52.5 d	150 a	55 c	63.3 ab
LJ-7	N0	162 d	59 b	63.8 a	59 d	21 d	64.1 ab
	N1	265 c	148 a	44.2 g	136 bc	64 ab	53.5 cde
	N2	302 ab	147 a	51.3 d	140 ab	63 ab	55.2 cd
	N3	311 a	143 a	54.0 c	150 a	56 c	63.0 ab
ZD-1	N0	143 d	61 b	57.5 b	56 d	22 d	61.6 b
	N1	265 c	149 a	43.8 g	123 c	63 ab	48.9 f
	N2	268 c	141 a	47.4 f	126 c	62 ab	50.8 ef
	N3	283 bc	145 a	48.8 ef	130 bc	57 c	56.4 c
YJ-4	N0	146 d	62 b	57.8 b	55 d	22 d	61.9 b
	N1	264 c	146 a	44.7 g	124 c	64 ab	48.5 f
	N2	272 c	142 a	47.8 ef	129 bc	62 b	52.5 de
	N3	287 abc	144 a	49.8 e	130 bc	58 c	56.0 c

¹ HD-5, Huaidao 5; LJ-7, Lianjing 7; ZD-1, Zhongdao 1; and YJ-4, Yangjing 4038. ² N0, zero-N control; N1, panicle N applied at spikelet differentiation (SD); N2, panicle N split at PI and SD; and N3, panicle N applied at panicle initiation (PI). ³ (NSC accumulation in stems at heading – NSC accumulation in stems at maturity)/NSC accumulation in stems at heading $\times 100$. ⁴ (N accumulation in stems and leaves at heading – N accumulation in stems and leaves at maturity)/N accumulation in stems and leaves at heading $\times 100$. ⁵ No shared letter indicates statistical significance at the $p = 0.05$ level within the same column.

4. Discussion

Nitrogen management is a key step in improving the NUE of rice fields, and it is of great importance in rice production and environmental protection [23,24]. In this study, we observed that, at a moderate N rate, a higher amount of N applied at PI led to higher yield and NUE of NEVs, and it was not necessary to apply further N fertilizer at the spikelet differentiation stage. The results showed that improving N management at a moderate N rate could achieve both high yield and high efficiency. After the N applied at PI was increased, the yield and NUE increases were more significant for NEVs (Tables 4 and 5). We observed that NEV had better root and shoot traits than NIV at the N rates in this study (Figures 3 and 4). However, we also found that there was no significant difference between NEV and NIV at the N rate of 300 kg ha⁻¹ [15]. This suggests that NEVs could play a better role under moderate or low N rates. The response of NEVs was asymptotic, with a maximum value already reached at about 260 kg ha⁻¹, whereas the NIVs still showed a strong response to N above 300 kg ha⁻¹.

Increasing the number of spikelets per unit area is the key to improving yield and NUE [25]. Therefore, the increased application of N fertilizer at PI is recommended to increase the total number of spikelets in rice production [26,27]. This may be more effective at moderate and low N rates. In this study, the total number of spikelets under the N3 treatment was significantly higher than that under N2 (Table 4). However, when the N rate was further increased, increasing the proportion of N applied at PI could not considerably increase the number of spikelets [28]. This may be because of the source and sink relationship of rice changes at the high N rate. At high N rates, the delay of N-fertilizer application could improve the grain filling efficiency, and this effect was greater on large-spike varieties than on small-spike varieties [18,23]. At high N rates, although the proportion of N fertilizer in the early stage was reduced, the amount of N fertilizer applied after reduction remained higher than the amount of N fertilizer applied in the early stage of the moderate N treatment.

NEVs are mostly multi-spike varieties, and green super rice is also mostly multi-spike types [19]. This may be because multi-spike varieties are more suitable for mechanical operations and have a relatively low requirement for N input [29]. Under the conditions of mechanized and simplified cultivation, the growth period of rice has shortened and the number of spikelets per panicle has been reduced; thus, the advantages of large-spike varieties have weakened [29]. Therefore, multi-spike varieties are more suitable for light and simplified cultivation. Although large-spike cultivars have relatively high biomass and photosynthetic characteristics, the number of spikelets per panicle is large and the quantity of assimilates distributed to each grain is lower than for multi-spike varieties [30]. As a result, higher N-fertilizer input is required, and the late stage with unstable grain filling percentage requires more labor input.

Root morphology and physiology play very important roles in nutrient absorption and growth of the aboveground parts in plants [31]. The earlier the N application period, the greater the promotion effect on the root system [32]. In this study, with an increasing amount of applied N fertilizer from N1 to N3, root biomass, root length, and ROA gradually increased, which is consistent with previous studies (Figure 3). Moreover, Dong et al. proposed that an increase in the number of spikelets will help improve the root characteristics of the plant and the ability to absorb N [31]. The improvement of root characteristics is also conducive to the photosynthetic characteristics of the aboveground parts [20]. In the current study, under the N3 treatment, the LAI and photosynthesis at the heading stage of the aboveground part were significantly improved (Figure 4). LAI is a basic indicator for evaluating the coordination between sources and sinks and for balancing the development of the aboveground and underground parts [33]. The LAI results showed that an improvement in root and canopy functions after increasing the amount of N applied at PI supported increases in yield and NUE. This is likely to be related to increases in source quantity (LAI and LAD) and source quality (ROA and photosynthesis) and to an increase in the sink capacity (total spikelets).

Some researchers have proposed that increasing the accumulation of NSC in the stem before the heading stage can increase the grain filling efficiency [34]. Fu et al. showed that NSC is significantly positively correlated with sink strength [35]. At the heading stage, the NSC content in stems under the N3 treatment was significantly higher than those under the N1 and N2 treatments and that the NSC transport efficiency was also improved (Table 6). C and N metabolism during grain filling is a coupled process [36]. After the N fertilizer is applied at PI, the N transport efficiency is also improved. The increase in the C and N transport rates is an important physiological reason for the increases in grain filling efficiency and rice yield. In summary, increasing the proportion of N fertilizer applied at PI can increase the source, expand the sink, and strengthen the flow at a moderate N rate.

5. Conclusions

Increasing the proportion of N applied at PI under a moderate N rate could not only increase the NEV yield but also increase NUE. Under the N3 treatment, there were increases in root biomass and root length, LAI and LAD, photosynthesis and ROA, and C and N transport, which may help achieve the dual goal of increasing yield and efficiency. Improved root and canopy functions and a more coordinated source and sink relationship will help increase yield and NUE. We suggest that increasing the amount of N fertilizer applied at PI is an effective method to reduce NEV yield loss and to increase NUE when the amount of applied N is reduced below the optimal N rate.

Author Contributions: Methodology, T.L.; validation, T.L. and C.S.; formal analysis, C.J.; investigation, C.J.; resources, C.S.; data curation, C.J.; writing—original draft preparation, C.J.; writing—review and editing, C.J.; visualization, T.L.; supervision, T.L.; project administration, C.S.; funding acquisition, C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (32001465, 31872852, and 31701355), by the National Key Research and Development Program of China (2018YFD0300805), and by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Acknowledgments: We thank the editors and three anonymous reviewers for their constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

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