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High Sink Capacity Improves Rice Grain Yield by Promoting Nitrogen and Dry Matter Accumulation

Fangwei Cheng [1], Shiyou Bin [2], Anas Iqbal [1], Lijian He [2], Shanqing Wei [1], Hao Zheng [2], Pengli Yuan [1,3], He Liang [1], Izhar Ali [1], Dongjie Xie [1], Xinxin Yang [1], Anjie Xu [1], Saif Ullah [1] and Ligeng Jiang [1,3,*]

1 Key Laboratory of Crop Cultivation and Farming Systems College of Agriculture, Guangxi University, Nanning 530004, China; sddycfw@163.com (F.C.); anasiqbal@gxu.edu.cn (A.I.); wwssqq@163.com (S.W.); pengliyuan@gxu.edu.cn (P.Y.); lianghe@gxu.edu.cn (H.L.); izharali48@gmail.com (I.A.); 13367819702@163.com (D.X.); yangxinxin0707@163.com (X.Y.); xu15778108232@163.com (A.X.); saif2012aup@gmail.com (S.U.)
2 Department of Agriculture and Rural Affairs of Gaungxi Zhuang Autonomous Region Guangxi, Nanning 530004, China; binshiyou@163.com (S.B.); hlj915@126.com (L.H.); haozhengbeyond@163.com (H.Z.)
3 Guangxi Key Laboratory of Agro-Environment and Agro-Products Safety, Guangxi University, Nanning 530004, China
* Correspondence: jiang@gxu.edu.cn; Tel.: +86-137-6831-1375

Abstract: Sink capacity, nitrogen (N), and dry matter accumulation (DMA) all play essential roles in promoting high rice grain yield, but their relationship is unclear. Here, a field experiment was conducted from 2020 to 2021 with Zhuangxiangyou Baijin 5 as the test cultivar. Two rates of N (T1 = 90 kg ha^{-1} N and T2 = 180 kg ha^{-1} N) and three transplanting densities (272,000 hills ha^{-1} (M1), 238,000 hills ha^{-1} (M2), and 206,000 hills ha^{-1} (M3)) were used to investigate rice grain yield and corresponding yield attributes. The results showed significant differences in rice yield, sink capacity, N and DMA, and the leaf area index (LAI) at the heading stage among the different treatments. The results showed that the output of T2M1 was the highest in 2020, increasing by 16.6% compared with the lowest output, while the output of T2M2 was the highest in 2021, increasing by 11.9% compared with the lowest output. During 2020, the highest sink capacity, LAI at the heading stage, and maximum dry matter accumulation at the maturity stage of rice were recorded in T2M1, while the highest N accumulation was recorded in T2M2. Furthermore, the sink capacity, as well as levels of N and DMA, of rice in 2020 was higher in T2M2, and the LAI was higher in T2M1 at the heading stage. Correlation analyses showed that yield was significantly positively correlated with N and DMA. In addition, a significant positive correlation between sink capacity and DMA was observed during both years, while a significant positive correlation between sink capacity and N accumulation was observed in 2021. Thus, we conclude that a high sink capacity can increase rice yield by increasing N and DMA because a high sink capacity is the internal driving force of high rice grain yield. In conclusion, the T2M1 regimen is a promising approach for improving the grain yield of paddy rice.

Keywords: *Oryza sativa* L.; planting density; grain yield; sink capacity; nitrogen; dry matter accumulation

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1. Introduction

Rice is one of the most important staple food crops worldwide, sustaining more than 50% of the world's population and 90% of Asia's population [1]. China is the largest producer and consumer of rice, and its production plays a vital role in the food security of China as well as other countries [2]. Sustainable approaches are needed to meet the increasing demand for food due to the rapid increase in the global population [3,4]. The improvement of rice yield per unit area in China is mainly attributed to two factors: (i) the genetic modification and application of rice varieties, especially the utilization of dwarf genes and heterosis and (ii) the improvement in farming systems, including fertilizer management, cultivation methods, and conditions that help to achieve the yield potential of rice varieties [4–6].

Previous studies reported that the yield potential of rice varieties depends on the balance between source capacity, sink strength, and transport capacity [7]. It was observed that rice varieties with a larger sink capacity had a higher yield potential [8–11]. Therefore, researchers tend to attach importance to improving the sink capacity by using improved varieties or regulated cultivation. For example, the sink capacity per unit area of rice increases with the increase in the panicle number or grains per panicle [12]. Furthermore, there is an important compensatory effect between the panicle number and grains per panicle [13], while grains per panicle often plays an essential role in increasing the sink capacity and yield [14–20].

Planting density and nitrogen fertilizer use are among the factors that can affect rice production in traditional agro-climatic conditions [21]. Likewise, nitrogen is an essential nutrient for rice growth and metabolism, thereby improving plant canopy, photosynthetic capacity, sink capacity, and yield formation [3,22], whereas planting density affects tillering, dry matter accumulation, sink capacity, and yield formation of rice plants [23]. A very high planting density can decrease the grain yield due to large number of non-productive tillers, empty spikelets, and a lower number of grains per spike [24,25]. Despite these factors, an optimal planting density is still critical for increasing rice yield by increasing the sink capacity [26]. A previous study showed that increasing the sink capacity can increase the seed density in panicles, as well as rice yield [27].

The nitrogen application rate and planting density are essential for improving the sink capacity and yield of rice. Both the nitrogen application rate and planting density can affect nitrogen uptake and dry matter accumulation, which are important for yield formation of rice plants [21]. However, the relationship between sink capacity and nitrogen and dry matter accumulation, as well as their influence on the rice yield, is still unclear. Therefore, a field experiment was conducted with two nitrogen fertilizer levels and three planting densities to measure the rice yield, sink capacity, and nitrogen and dry matter accumulation. The objectives of our study were: to determine the differences in rice yield, sink capacity, nitrogen absorption, and dry matter accumulation under different nitrogen application rates and planting densities and to clarify the relationship among them.

2. Material and Methods

2.1. Experimental Location and Materials

The two-season field experiment was carried out in 2020 (late season, July–December) and 2021 (early season, January–June) on the experimental farm (23°06′ N, 108°59′ E) of the Rice Science and Technology Institute in Gula Town, Binyang County, Nanning, Guangxi. The average temperature during the experiment in 2020 was 22.38 °C, and the lowest temperatures from 5 October to 11 October were 19 °C, 17 °C, 16 °C, 17 °C, 18 °C, and 19 °C. The climate is categorized as subtropical with a monsoon zone, with a mean annual precipitation of 1190 mm (Table 1). The soil properties of the experimental site (0–15 cm soil layer) prior to the experiment were as follows: pH 5.4, measured in KCl; organic carbon 18.5 g kg^{-1}; total nitrogen 1.9 g kg^{-1}; available nitrogen 157.4 mg kg^{-1}; total phosphorus 1.5 g kg^{-1}; and total potassium 10.1 g kg^{-1}. The tested rice variety was Zhuangxiangyou Baijin 5, which was provided by Guangxi Baijin Seed Co., Ltd. (Nanning, China). This variety is the main indica hybrid rice variety in Guangxi, which the company bred in 2017, and its parents are Zhuangxiang A and Baijin 5. The growth period of early-season rice was 119 days, and that of late-season rice was 113 days. The effective panicle number was 185,000 ha^{-1}, the plant height was 107.5 cm, and the total number of grains per panicle was 159.0.

Table 1. Maximum and minimum temperature and rainfall at the experimental site.

Month	Max. Temp (°C)	Min. Temp (°C)	Precipitation (mm)
Late season			
August	36	26	270
September	33	24	70
October	29	17	140
November	26	16	55
December	23	15	50
Early season			
March	24	16	180
April	29	21	80
May	31	23	260
June	35	25	125
July	35	26	270

Note: Max—maximum, Min—minimum, Temp—temperature.

2.2. Experimental Design

The experiment investigated two factors: nitrogen fertilizer level and planting density. A split-plot design with three replications was used, with the nitrogen level as the main plot and the planting density as the subplot. Two nitrogen application rates of treatment (T1: 90 kg N ha^{-1} and T2: 180 kg N ha^{-1}) and three planting density patterns (M1, 30 cm × 12 cm; M2, 30 cm × 14 cm; and M3, 30 cm × 16 cm, corresponding to 27.8, 23.8, and 20 hills per square meter, respectively) with three seedlings per hill were employed. The treatment combinations were as follows: T1M1: 90 kg N ha^{-1} + 278,000 hills ha^{-1}; T1M2: 90 kg N ha^{-1} + 238,000 hills ha^{-1}; T1M3: 90 kg N ha^{-1} + 206,000 hills ha^{-1}; T2M1: 180 kg N ha^{-1} + 278,000 hills ha^{-1}; T2M2: 180 kg N ha^{-1} + 238,000 hills ha^{-1}; and T2M3: 180 kg N ha^{-1} + 206,000 hills ha^{-1}. PVC plates were used to separate the main plots to prevent the infiltration of fertilizer and water. The single plot size was 12.6 m^2 (length × width, 4.2 m × 3 m). Uniform seedlings (25 days old, four-leaf stage) were transplanted manually with ten rows in each plot and 35 hills (M1), 30 hills (M2), and 26 hills (M3) in each row. Nitrogen fertilizer (urea) and potassium chloride (191 kg ha^{-1}) were applied in at three stages as follows: 50% basal dose, 30% at the tillering stage (14 days after transplantation), and 20% at the panicle initiation stage (45 days after transplantation), while calcium superphosphate was used at a rate of 102.72 kg ha^{-1} (basal dose). All other agronomic practices, such as irrigation (flooded water condition), as well as the use of pesticides and insecticides (i.e., omethoate and chlorantraniliprole), were uniformly applied among the different treatments.

2.3. Measurements and Analyses

2.3.1. Nitrogen and Dry Matter Accumulation

Five plants from each replication during heading and maturity stages were selected randomly to determine the dry matter accumulation. Plants were collected and separated manually using scissors into three parts: namely, stem, leaf and sheath, and panicle. All samples were oven-dried at 105 °C for 30 min, dried at 75 °C to constant weight, and weighed [3].

To determine the nitrogen accumulation in plants, dry plant samples were crushed using a multifunctional pulverizer (model: 200T; Dongyi (Jinhua, China)), sieved through a 60-mesh sieve, and sterilized by the concentrated H_2SO_4–H_2O_2 method. The nitrogen content of the plant samples was determined using a flow chemistry analyzer (model: BDF1A-9000; Beijing Baode Instrument Co. (Beijing, China)). The nitrogen accumulation, nitrogen harvest index, nitrogen dry matter production efficiency, nitrogen rice production efficiency, and partial productivity of nitrogen fertilizer were determined according to the following calculations:

1. Plant (organ) nitrogen accumulation (kg ha^{-1}) = plant (organ) dry matter accumulation (kg ha^{-1}) × plant (organ) nitrogen content (%) [28];
2. Nitrogen harvest index (kg kg^{-1}) = nitrogen accumulation in mature panicles (kg ha^{-1})/ total nitrogen accumulation in mature plants (kg ha^{-1}) [29];
3. Nitrogen dry matter production efficiency (kg kg^{-1}) = total dry matter accumulation of mature plants (kg ha^{-1})/total nitrogen accumulation of mature plants (kg ha^{-1}) [30];
4. Nitrogen production efficiency (%) = rice yield (kg ha^{-1})/total nitrogen accumulation of mature plants (kg ha^{-1}) [31];
5. Partial productivity of nitrogen fertilizer (kg kg^{-1}) = rice yield (kg ha^{-1})/nitrogen application rate (kg ha^{-1}) [29].

2.3.2. Harvest Index and Leaf Area Index

The harvest index was measured as the ratio of dry matter accumulation in the ear to total dry matter accumulation in the shoot [32]. For the leaf area index, five hills of representative plants were collected at the heading stage. The rice leaf area was measured as length × width × coefficient (0.75) and then converted into the leaf area index [33].

2.3.3. Growth and Grain Yield Attributes

Rice grain yield and corresponding yield attributes were calculated for each treatment. The crop was harvested manually. The clear water screening method was used to distinguish full grains from empty grains, and the effective number of panicles, total grains per panicle, seed setting rate, and 1000-grain weight were determined and then the rice yield was calculated. Sink capacity per [34] unit area (×10^4/ha) was defined as the product of the total grains per panicle and the effective panicle per hectare.

2.3.4. Chemical Properties of Soil

The pHs of soil were determined after shaking the soil and manure with distilled water at a 1:2.5 (*w*/*v*) solid-to-water ratio for 1 h with the help of a digital pH meter (ThunderboltPHS-3C, Shangai, China) [35]. For total organic carbon, sub-samples were ground and again made to pass through a 0.25 mm sieve. Total organic carbon was determined by the method described in Rich et al. [36]. Soil organic matter was measured by multiplying total organic carbon by 1.72. For total N (TN) analysis, 200 mg samples were weighted and digested using the salicylic acid–sulfuric acid–hydrogen peroxide method [37], then TN was analyzed using the micro-Kjeldahl procedure [38], and total phosphorous (TP) was tested using the ascorbic acid method [39]. Standard stock solution was prepared by dissolving KCl in distilled water. Potassium was determined by using an atomic absorption spectrophotometer (Z-5300; Hitachi, Tokyo, Japan) after samples were digested. Available N (AN) was extracted (Z-5300; Hitachi, Tokyo, Japan) after samples were digested. Available N (AN) was extracted from the soil samples using the hot water extraction method [40].

2.4. Statistical Analysis

Data were analyzed using analysis of variance (DPS 2020.03.25, Analytical Software), and the means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. Origin software was used to generate the graphs, and Pearson's linear correlation was used to calculate the relationship among the response variables.

3. Results

3.1. Rice Yield and Sink Capacity

We found the lowest grain yield in the T2M3 treatment in both years. The difference between the T2M3 and other treatments was significant; however, the other treatments were observed to correlate non-significantly with each other. In 2020, the yield was significantly affected by planting density ($p < 0.05$), and, in 2021, it was significantly affected by nitrogen application rate and planting density ($p < 0.05$). Compared to T2M2, the grain yields of T1M1, T1M2, T1M3, T2M1, and T2M3 decreased by 11.6%, 6.6%, 11.9%, 7.6%, and 9.5%, respectively, in 2021, while, during the late season of rice in 2020, the grain yield increased in T2M1, and the yields of T1M1, T1M2, T1M3, T2M2, and T2M3 were decreased by 4.6%, 15.9%, 9.7%, 8.0%, and 16.6%, respectively. The yield was higher in 2021 than in 2020 (Figure 1). Between the years, the results showed that there was an increase in grain yield in 2021 compared to 2020, while the treatments behaved the same way during both years.

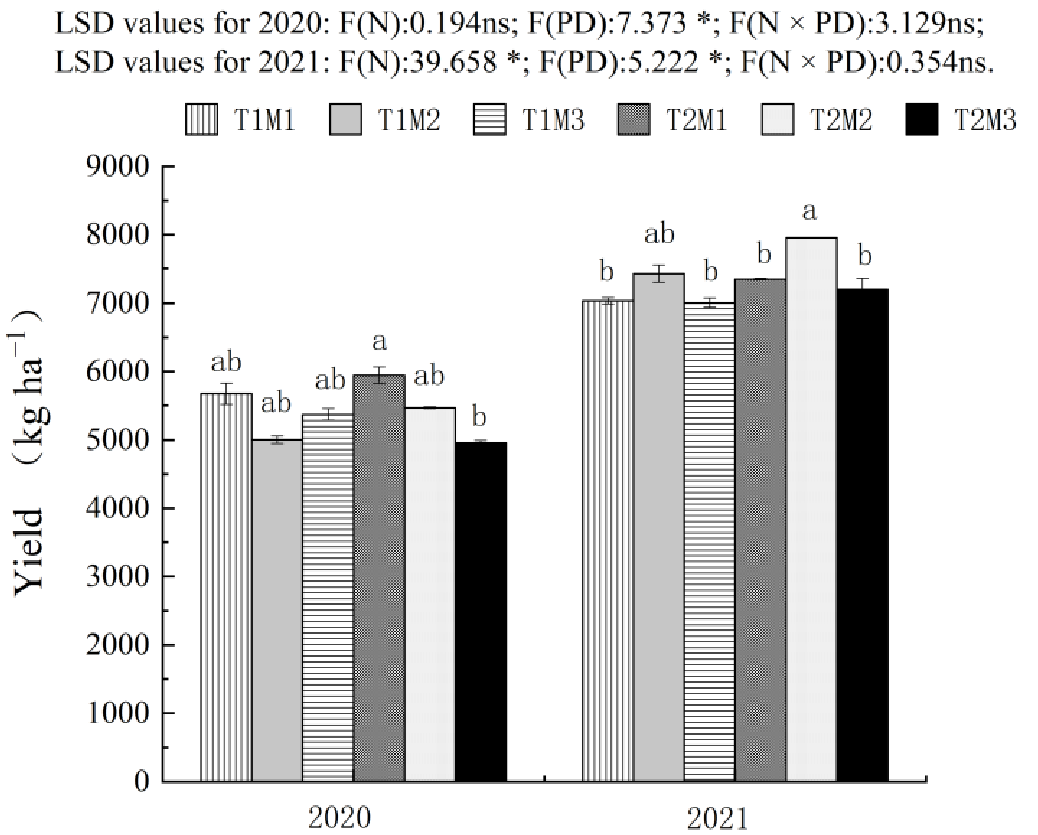

Figure 1. Response of rice grain yield to different nitrogen fertilizer application rates and planting densities. Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. Different lowercase letters indicate a significant difference. * indicates a significant level of 0.05 ($p < 0.05$), ns—non-significant.

Figure 2 shows that, in 2020, the sink capacity was significantly affected by the nitrogen application rate and the interaction between the planting density and nitrogen application rate ($p < 0.05$), and, in 2021, it was significantly affected by nitrogen application rate ($p < 0.05$). Among the treatments, T2M2 resulted in the largest sink capacity in 2021. Compared with T2M2, the sink capacity of rice in T1M1, T1M2, T1M3, T2M1, and T2M3 decreased by 9.4%, 10.8%, 10.9%, 3.2%, and 8.0%, respectively. Similarly, in 2020, the sink capacity in T2M1 was the highest, and the sink capacity in T1M1, T1M2, T1M3, T2M2, and T2M3 decreased by 7.7%, 24.9%, 11.3%, 9.3%, and 27.1%, respectively. Furthermore, sink capacity was found to be higher during 2021 than 2020, and the treatments behaved in the same way during both years.

3.2. Leaf Area Index of Rice

Table 2 shows that the leaf area index (LAI) at the heading stage of the T2M1 treatment was the highest in early rice in 2021 and late rice in 2020. A higher LAI was recorded in T2M1 compared with the other treatments during both years. Compared with T2M1, nitrogen accumulation in T1M1, T1M2, T1M3, T2M2, and T2M3 was decreased by 34.6%, 18.0%, 36.9%, 15.4%, and 12.4%, respectively, in 2021, whereas grain yield was decreased by 25.8%, 23.6%, 30.1%, 12.5%, and 25.5%, respectively, in 2020. Analysis of variance showed that the difference in the LAI between T1 and T2 in 2021 reached a 5% significance level, but there was no significant difference among the other treatments. Among the years, no significant differences were recorded.

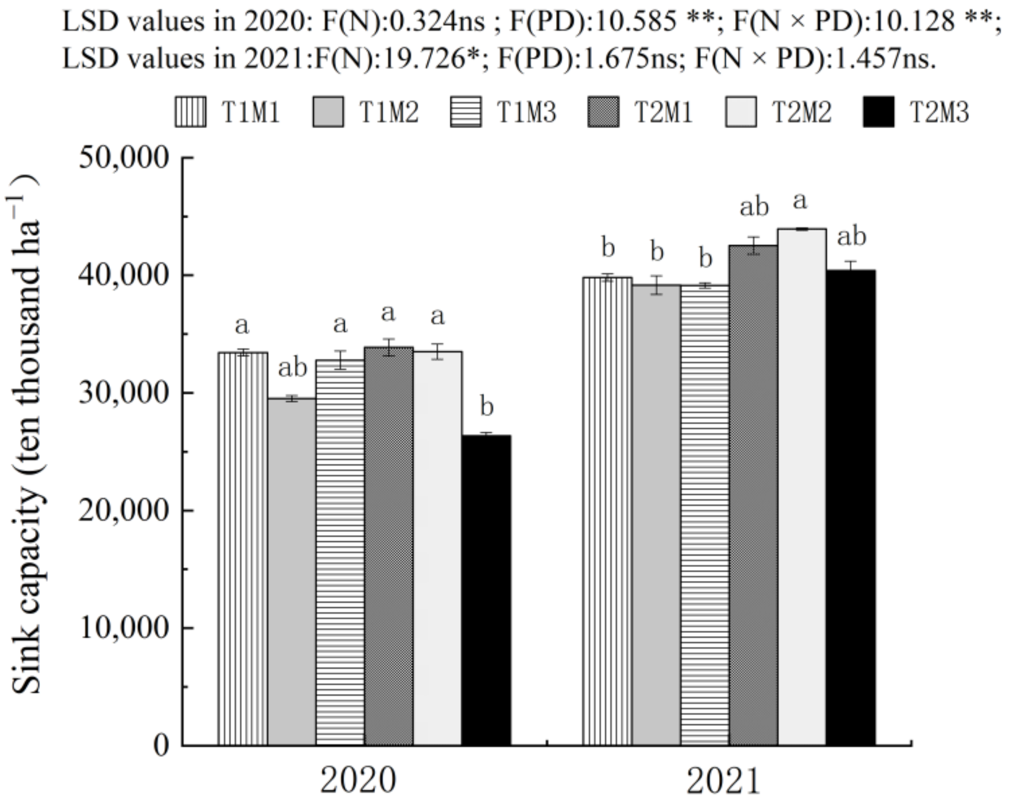

Figure 2. Sink capacity of rice under different nitrogen application rates and planting densities. Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. In the same year, different lowercase letters indicate a significant difference. ns—non-significant. ** indicates a significant level of 0.01 ($p < 0.01$), and * indicates a significant level of 0.05 ($p < 0.05$).

Table 2. Effects of the nitrogen application rate and planting density on the LAI of rice at the heading stage.

Nitrogen Application Rate	Planting Density	2020	2021
T1	M1	5.88 ± 0.14^{ab}	4.83 ± 0.24^{b}
	M2	6.05 ± 0.30^{ab}	6.06 ± 0.28^{b}
	M3	5.54 ± 0.22^{b}	4.66 ± 0.28^{b}
T2	M1	7.92 ± 0.61^{a}	7.39 ± 0.34^{a}
	M2	6.93 ± 0.20^{ab}	6.47 ± 0.23^{ab}
	M3	5.90 ± 0.07^{ab}	6.25 ± 0.23^{ab}
F (N)		28.246 *	25.77 *
F (PD)		2.120^{ns}	0.66^{ns}
F (N × PD)		0.621^{ns}	1.63^{ns}

Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. * and different lowercase letters after the same column of data represent significant differences at a probability level of 5% ($p < 0.05$), ^{ns}—non-significant.

3.3. Dry Matter Accumulation and Harvest Index

Dry matter accumulation at the heading and maturity stages during both seasons are presented in Table 3. The results show that dry matter accumulation at the heading stage was higher in T2M1 and lower in T1M2, and no significant differences were observed among the nitrogen rates in 2021, while, at the maturity stage, dry matter accumulation in T2M2 was the highest, and total dry matter accumulation in T1M1, T1M2, T1M3, T2M1, and T2M3 was decreased by 13.7%, 15.5%, 11.8%, 5.6%, and 13.4%, respectively, compared with that in T2M2. Analysis of variance showed no significant differences in dry matter accumulation among the planting densities and significant differences among the nitrogen treatments ($p < 0.05$). In 2020, dry matter accumulation in T2M1 was the highest at the heading and maturity stages, but no significant differences were observed among the nitrogen treatments. From the heading to maturity stages, dry matter accumulation was the highest in T2M2 and the lowest in T1M2, and there were no significant differences among the nitrogen rates.

Table 3 also shows that the differences in the harvest index among the nitrogen treatments reached significant levels in 2021 and 2020. In 2021, T1M2 had the largest harvest index and T2M1 had the smallest, and, in 2020, T1M1 had the largest harvest index and T2M2 had the smallest. The difference among the years showed that, compared to 2020, DMA was higher in 2021 during both the heading and maturity stages.

3.4. Nitrogen Uptake and Utilization Efficiency

Table 4 shows that T2M2 had the highest nitrogen accumulation during both years. Total N accumulation was at its maximum in 2021 as compared to 2020. Among the treatments compared with T2M2, nitrogen accumulation in T1M1, T1M2, T1M3, T2M1, and T2M3 decreased by 19.2%, 19.1%, 19.2%, 6.5%, and 9.1%, respectively, in 2021, while, in 2020, it decreased by 36.3%, 39.7%, 31.5%, 5.8%, and 24.8%, respectively. The difference and interaction in nitrogen accumulation between nitrogen application rates and planting densities were significant or highly significant. In 2021, the nitrogen harvest index of T2M3 was the largest, and the nitrogen harvest indexes of T1M1, T1M2, T1M3, T2M1, and T2M2 were 4.4%, 1.5%, 3.4%, 10.0%, and 9.0% lower than that of T2M3, respectively. There were no significant differences among the nitrogen treatments, although the differences among the planting densities were highly significant ($p < 0.01$), and the interactions between nitrogen application rates and planting densities were significant ($p < 0.05$). In 2020, the nitrogen harvest index of T1M2 was the largest, and the nitrogen harvest indexes of T1M1, T1M3, T2M1, T2M2, and T2M3 were 3.5%, 0.1%, 11.8%, 11.2%, and 6.8% lower than that of T1M2, respectively. There were no significant differences among the planting densities, and the differences among the nitrogen application rates were highly significant ($p < 0.01$).

Table 4 also shows that the leaf nitrogen content of T2M2 was the highest during both years. Compared with T2M2, the leaf nitrogen content in T1M1, T1M2, T1M3, T2M1, and T2M3 decreased by 28.6%, 27.8%, 39.6%, 18.0%, and 12.7%, respectively, in 2021, while, in 2020, it decreased by 27.9%, 26.8%, 30.9%, 11.1%, and 11.1%, respectively. The difference and interaction between nitrogen application rates and planting densities were significant or highly significant. The leaf nitrogen content in T2M2 was the highest in both years, and the difference was significant in 2020, while there was no significant difference in 2021.

Table 5 shows that the nitrogen application rate had a significant or highly significant effect on the partial productivity of nitrogen fertilizer, nitrogen rice production efficiency, and nitrogen dry matter production efficiency, but the effect of planting density and the interaction between nitrogen application rate and planting density were not significant. Compared with the nitrogen application rates, the partial productivity of nitrogen fertilizer, nitrogen rice production efficiency, and nitrogen dry matter production efficiency of T1 were significantly higher than those of T2.

Table 3. Dry matter accumulation of rice under different nitrogen applications and planting densities.

Nitrogen Application Rate	Planting Density	2020				2021			
		Heading (kg ha^{-1})	Maturity (kg ha^{-1})	Heading–Maturity (kg ha^{-1})	Harvest Index (%)	Heading (kg ha^{-1})	Maturity (kg ha^{-1})	Heading–Maturity (kg ha^{-1})	Harvest Index (%)
T1	M1	7290 ± 235.4 ^{ab}	12,065 ± 291.2 ^{ab}	4165 ± 299.2 ^{a}	47.2 ± 0.4 ^{a}	9671 ± 71.6 ^{a}	13,842 ± 182.9 ^{b}	4170 ± 253.32 ^{a}	54.3 ± 0.4 ^{ab}
	M2	7052 ± 319.2 ^{ab}	10,748 ± 127.6 ^{b}	3696 ± 446.3 ^{a}	46.5 ± 0.3 ^{a}	9570 ± 322.7 ^{a}	13,550 ± 206.3 ^{b}	3979 ± 459.3 ^{a}	57.6 ± 0.3 ^{a}
	M3	6739 ± 29.8 ^{b}	12,079 ± 270.4 ^{ab}	5340 ± 254.1 ^{a}	44.5 ± 0.8 ^{ab}	9661 ± 150.8 ^{a}	14,145 ± 176.7 ^{b}	4483 ± 318.7 ^{a}	52.6 ± 0.8 ^{b}
T2	M1	8231 ± 291.7 ^{a}	13,332 ± 149.55 ^{a}	5100 ± 188.5 ^{a}	44.7 ± 0.8 ^{ab}	10,555 ± 284.6 ^{a}	15,132 ± 236.0 ^{ab}	4577 ± 88.64 ^{a}	51.3 ± 0.8 ^{b}
	M2	7586 ± 166.5 ^{ab}	13,246 ± 133.3 ^{a}	5660 ± 292.1 ^{a}	41.3 ± 0.4 ^{b}	9760 ± 162.2 ^{a}	16,032 ± 138.3 ^{a}	6271 ± 283.9 ^{a}	51.5 ± 0.4 ^{b}
	M3	7007 ± 104.2 ^{b}	11,113 ± 264.8 ^{b}	4106 ± 358.3 ^{a}	44.8 ± 1.0 ^{ab}	9953 ± 205.3 ^{a}	13,881 ± 350.4 ^{b}	3927 ± 555.7 ^{a}	55.7 ± 1.0 ^{ab}
F (N)		10.346 ^{ns}	4.446 ^{ns}	1.581 ^{ns}	26.483 *	3.885 ^{ns}	19.09 *	2.052 ^{ns}	9.107 ^{ns}
F (PD)		4.197 ^{ns}	2.651 ^{ns}	0.007 ^{ns}	0.805 ^{ns}	0.416 ^{ns}	1.032 ^{ns}	0.668 ^{ns}	0.779 ^{ns}
F (N × PD)		0.056 ^{ns}	6.565 *	2.401 ^{ns}	1.434 ^{ns}	0.278 ^{ns}	3.187 ^{ns}	1.46 ^{ns}	4.993 *

Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. * and different lowercase letters after the same column of data represent significant differences at a probability level of 5% ($p < 0.05$), ^{ns}—non-significant.

Table 4. Effects of different nitrogen application rates and planting densities on nitrogen accumulation, nitrogen harvest index, and leaf nitrogen content of rice.

Nitrogen Application Rate	Planting Density	2020			2021		
		Nitrogen Accumulation at Maturity Stage (kg ha^{-1})	Nitrogen Harvest Index (%)	Nitrogen Content in Leaves at Heading Stage (kg ha^{-1})	Nitrogen Accumulation at Maturity Stage (kg ha^{-1})	Nitrogen Harvest Index (%)	Nitrogen Content in Leaves at Heading Stage (kg ha^{-1})
T1	M1	113.16 ± 1.72 ^{cd}	60.08 ± 0.44 ^{ab}	34.91 ± 0.43 ^{c}	109.86 ± 0.11 ^{c}	61.90 ± 0.11 ^{ab}	43.17 ± 0.01 ^{c}
	M2	106.73 ± 0.86 ^{d}	62.26 ± 0.45 ^{a}	35.45 ± 0.12 ^{c}	110.06 ± 0.61 ^{c}	63.78 ± 0.27 ^{a}	36.48 ± 0.01 ^{c}
	M3	121.22 ± 1.20 ^{c}	62.20 ± 0.22 ^{a}	33.47 ± 0.02 ^{c}	109.95 ± 0.98 ^{c}	62.57 ± 0.67 ^{a}	36.48 ± 0.01 ^{c}
T2	M1	166.61 ± 2.14 ^{a}	54.93 ± 0.64 ^{d}	43.08 ± 0.39 ^{b}	127.18 ± 0.39 ^{b}	58.31 ± 0.36 ^{c}	49.56 ± 1.56 ^{b}
	M2	176.89 ± 1.03 ^{a}	55.30 ± 0.26 ^{cd}	48.45 ± 0.39 ^{a}	136.01 ± 1.30 ^{a}	58.91 ± 0.09 ^{bc}	60.42 ± 0.34 ^{a}
	M3	133.05 ± 0.73 ^{b}	58.05 ± 0.23 ^{bc}	43.06 ± 0.39 ^{b}	123.59 ± 0.52 ^{b}	64.75 ± 0.19 ^{a}	52.77 ± 0.23 ^{b}
F (N)		428.5 **	126.12 **	484.74 **	1041.26 **	5.47 ^{ns}	152.2 **
F (PD)		12.27 **	3.88 ^{ns}	13.19 **	8.45 *	16.39 **	1.26 ^{ns}
F (N × PD)		43.50 **	1.14 ^{ns}	5.34 *	8.06 *	17.73 **	21.65 **

Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. Different lowercase letters after the same column of data represent significant differences at a level of 5% ($p < 0.05$); ** indicates a significant level of 0.01 ($p < 0.01$); and * indicates a significant level of 0.05 ($p < 0.05$), ^{ns}—non-significant.

Table 5. Nitrogen grain production efficiency, nitrogen dry matter production efficiency, and nitrogen partial productivity rice under different nitrogen application rates and planting densities.

Nitrogen Application Rate	Planting Density	Nitrogen Grain Production Efficiency (kg kg^{-1})		Partial Productivity of Nitrogen Fertilizer (kg kg^{-1})		Nitrogen Dry Matter Production Efficiency (kg kg^{-1})	
		2020	2021	2020	2021	2020	2021
T1	M1	50.42 ± 2.15 ^{a}	64.03 ± 0.36 ^{a}	63.04 ± 1.7 ^{a}	78.16 ± 0.50 ^{a}	107.15 ± 4.15 ^{a}	125.99 ± 1.56 ^{a}
	M2	46.87 ± 0.41 ^{a}	67.47 ± 0.85 ^{a}	55.59 ± 0.6 ^{a}	82.55 ± 1.41 ^{a}	100.71 ± 0.91 ^{ab}	123.08 ± 1.47 ^{ab}
	M3	44.29 ± 0.38 ^{ab}	63.73 ± 0.64 ^{a}	59.68 ± 0.9 ^{a}	77.82 ± 0.76 ^{a}	99.58 ± 1.53 ^{abc}	128.62 ± 0.47 ^{a}
T2	M1	35.74 ± 0.87 ^{bc}	57.80 ± 0.28 ^{b}	33.03 ± 0.6 ^{b}	40.83 ± 0.07 ^{b}	80.23 ± 1.86 ^{cd}	119.04 ± 2.19 ^{ab}
	M2	30.92 ± 0.25 ^{c}	58.53 ± 0.53 ^{b}	30.37 ± 0.0 ^{b}	44.19 ± 0.03 ^{b}	74.90 ± 0.80 ^{d}	117.92 ± 0.90 ^{ab}
	M3	37.27 ± 0.18 ^{bc}	58.24 ± 1.11 ^{b}	27.55 ± 0.1 ^{b}	40.01 ± 0.90 ^{b}	83.45 ± 1.52 ^{bcd}	112.32 ± 2.82 ^{b}
F (N)		29.59 *	345.98 **	197.16 **	3150.36 **	23.29 *	34.56 *
F (PD)		2.04 ^{ns}	1.20 ^{ns}	4.35 ^{ns}	4.13 ^{ns}	0.91 ^{ns}	0.14 ^{ns}
F (N × PD)		2.71 ^{ns}	0.71 ^{ns}	1.80 ^{ns}	0.04 ^{ns}	0.90 ^{ns}	0.92 ^{ns}

Note: F, variance statistic; N, nitrogen; PD, plant density. The means of treatments were compared based on the least significant difference (LSD) test at a probability level of 0.05. Different lowercase letters after the same column of data represent significant differences at a level of 5% ($p < 0.05$); ** indicates a significant level of 0.01 ($p < 0.01$); and * indicates a significant level of 0.05 ($p < 0.05$), ^{ns}—non-significant.

3.5. Correlations among Indicators

Figure 3 shows that there was a very significant positive correlation between grain yield and sink capacity, and its coefficients of determination (R^2) were 0.46 (2020) and 0.58 (2021), respectively.

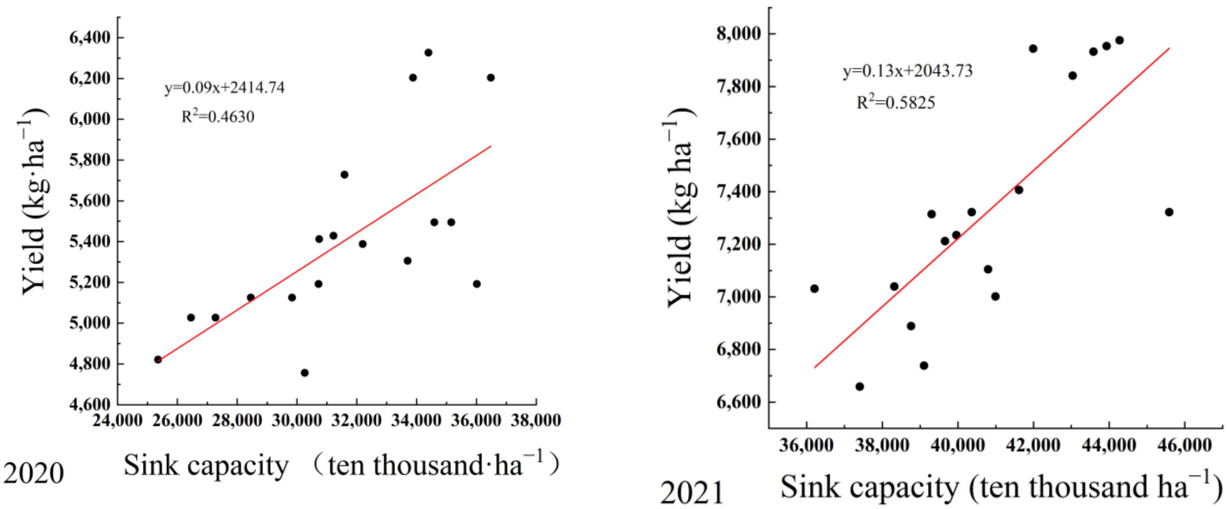

Figure 3. Relationship between sink capacity and grain yield of rice in 2020 and 2021.

Table 6 indicates that the yield was positively correlated with total dry matter accumulation (r = 0.63 **) and nitrogen accumulation (r = 0.71 **) in 2021. In 2020, the yield was positively correlated with total dry matter accumulation (r = 0.76 **) but not with nitrogen accumulation.

Table 6. Correlation analysis between grain yield and nitrogen and dry matter accumulation.

Index	2021	2020
Nitrogen accumulation	0.63 **	0.36 ^{ns}
Total dry matter accumulation	0.71 **	0.76 **

Note: ** indicates a significant level of 0.01, ^{ns}—non-significant.

Figure 4 shows that the dry matter accumulation amount and total dry matter accumulation amount from the heading to maturity stage increased significantly with the increase in the sink capacity, and the coefficients of determination (R^2) were 0.23 and 0.25 (2021) and 0.45 and 0.62 (2020), respectively.

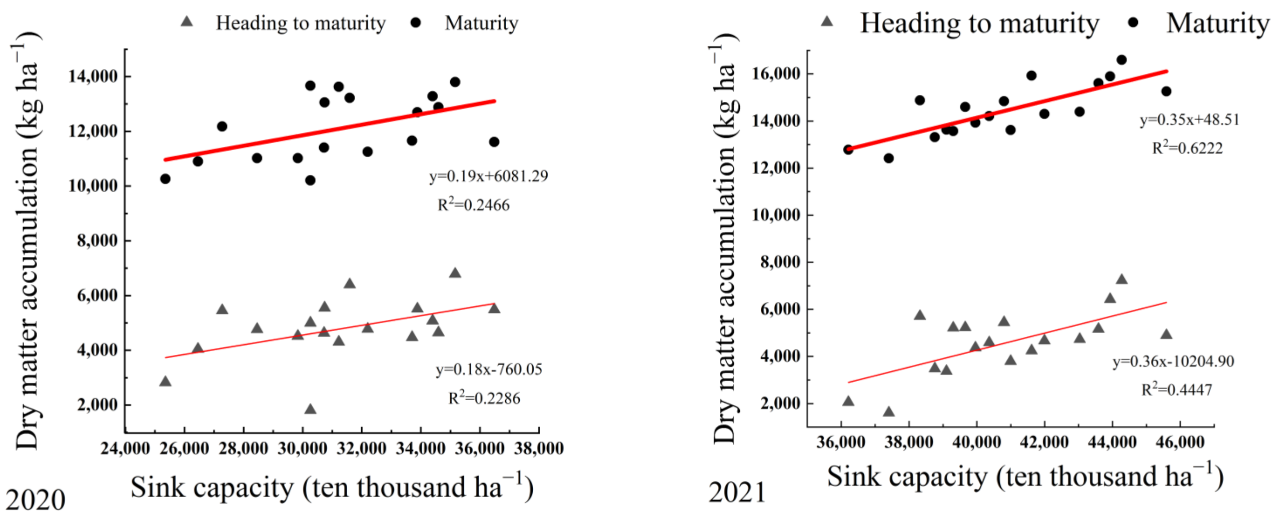

Figure 4. Relationship between sink capacity and dry matter accumulation in rice.

Correlation analysis showed that the sink capacity was significantly positively correlated with the leaf nitrogen content (r = 0.71 **) and nitrogen accumulation (r = 0.71 **) and significantly negatively correlated with the partial productivity of nitrogen fertilizer (r = −0.50 *) and nitrogen harvest index (r = −0.54 *) in 2021. However, no significant correlations were found in 2020 (Table 7).

Table 7. Correlation analysis between rice sink capacity and nitrogen accumulation and utilization.

Index	2020	2021
Nitrogen content in leaves at the heading stage	0.197 ^{ns}	0.707 **
Nitrogen accumulation	0.28 ^{ns}	0.708 **
Nitrogen harvest index	−0.242 ^{ns}	−0.543 *
Partial productivity of nitrogen fertilizer	0.241 ^{ns}	−0.503 *
Nitrogen rice production efficiency	0.061 ^{ns}	−0.255 ^{ns}
Nitrogen dry matter production efficiency	0.095 ^{ns}	−0.023 ^{ns}

Note: ** indicates a significant level of 0.01, and * indicates a significant level of 0.05, ^{ns}—non-significant.

4. Discussion

4.1. Effects of Nitrogen Application Rate and Planting Density on Nutrient Accumulation and Yield

Nitrogen is an essential component required for chlorophyll production, enzyme activity, and growth hormone synthesis in rice [41]. Nitrogen participates in many important metabolic processes in plants and plays important roles in plant growth and yield formation [6,41]. Rational nitrogen application is an important factor for the high yield of rice [42]. Furthermore, planting density plays a crucial role in regulating population structure and plant growth. The application of nitrogen fertilizer in conjunction with an optimal planting density is an effective way to increase grain yield and reduce the amount of nitrogen fertilizer required [43]. Therefore, rational nitrogen application and planting density are critical measures for a high yield of rice [44].

The leaf area index plays an important role in radiation interception and carbon assimilation, significantly affecting yield formation of rice plants. In the present study, increasing nitrogen fertilizer use increased the leaf area index, but there was no significant association with the planting density (Table 2). A possible explanation for this difference is the higher nitrogen application rate, which can improve the utilization rate of carbohydrates in photosynthetic products [45,46]. LAI is regulated by the nitrogen level, and, thus, the interaction between the leaf nitrogen concentration and the number of tillers could be such that, when the plant population becomes too high, tiller death cannot be halted by a very high concentration of leaf nitrogen [47,48]. The yield formation of rice is closely related to the accumulation of nutrients. Optimal planting density and rational nitrogen application are key to establishing a high yield of crops. A high nitrogen application rate (180 kg ha^{-1}) can significantly increase nitrogen accumulation in rice, laying a foundation for a high yield of rice. However, when the nitrogen application rate is low, dense planting can reduce the yield of rice and improve the production efficiency of nitrogen [27,49]. In this study, compared with T2, the reduction in the nitrogen application rate significantly improved the production efficiency of rice, dry matter production efficiency, and partial productivity of nitrogen fertilizer (Table 5). Although the increase in the nitrogen application rate increased nitrogen accumulation, it decreased the nitrogen utilization rate and increased with the decrease of in nitrogen application rate (Tables 4 and 5). These findings indicate that increasing the nitrogen application rate can reduce the proportion of nitrogen transfer to the ear, thereby reducing the nitrogen utilization efficiency. Yuan et al. [50] also reported that the nitrogen uptake of rice plants increased with the increase in the nitrogen application rate, but the proportion of nitrogen uptake in panicles decreased.

Dry matter accumulation is closely related to the yield formation of rice plants (Table 6). In this study, the accumulation of dry matter in rice increased with plant growth, reaching a maximum at the maturity stage. Nitrogen fertilizer application significantly increased total dry matter accumulation from the heading to maturity stages. Dry matter accumulation increased considerably with an increase in planting density (Table 3). The possible explanation for this increment is the increase in nitrogen application rate and planting density, which, consequently, promoted the growth of rice leaves, improved the leaf area index (Table 2), and provided more photosynthetic compounds for rice growth [45,51]. Cheng et al. [52] reported that improved vegetative growth was reflected in a significant increase in dry matter accumulation. Sihag et al. [53] showed that a higher plant density often produced higher accumulation compared to a lower plant density, resulting in higher dry matter yield. Optimal nitrogen application promotes photosynthesis in leaves and the transport of photosynthetic compounds to grains [54]. A low nitrogen concentration in leaves (Table 2) reduces radiation utilization efficiency and biomass productivity, leading to the reduced dry matter yield of rice [43].

The effect of planting density on rice yield showed a consistent trend in both years, while the effect of the nitrogen application rate on rice yield was different. In 2021, the sink capacity and yield of T2M2 were the highest, while, in 2020, there was no significant association between the sink capacity and yield and the nitrogen application rate (Figure 1). This may be due to the fact that Zhuangxiangyou Baijin 5 has strong tillering ability. When the planting density is too high, the population growth is too large, and mutual shading is heavy. When the density is too low, the population is too small, and this is not conducive to the accumulation of nutrients and dry matter, thereby reducing the yield. Therefore, Zhuangxiangyou Baijin 5 best exerts its yield potential at a medium planting density. In the early season of rice in 2021, T1 nitrogen application significantly increased the yield by increasing the harvest index. Therefore, increasing the harvest index or aboveground biomass or both can increase the rice yield [55,56]. From a source-sink perspective, sink and source intensities play important roles in regulating growth and yield formation of rice plants [51]. Rice yield is composed of grains per panicle, effective panicles, seed setting rate, and grain weight, and number of spikelets per unit area is considered as the main determinant [57]. The results showed that the yield and sink capacity of rice changed significantly with the nitrogen application rate and planting density [57–59]. Furthermore, the trends of the two parameters were consistent, so there was a significant positive correlation between the output and the storage capacity (Figure 3).

Among the years, the results showed that LAI, sink capacity, dry matter accumulation, N accumulation, and grain yield were higher in the early season of 2021 compared to the late season of 2020. These seasonal changes might have been due to temperature and other seasonal environmental differences (Table 1). Similar seasonal changes were reported by Iqbal et al. [60]; the LAI was higher in early-season rice compared to late-season rice. Furthermore, Ali et al. [3] also documented that grain yield and DM accumulation was found to be higher in early-season rice compared to late-season rice. Likewise, Zhao et al. [51] reported that panicle top, primary branches, grain filling, and grain yield were improved in the early season of 2015 compared to the late season of 2014. Overall results among the years showed that early rice cropping resulted in higher growth and yield of rice compared to late-season rice.

4.2. Physiological Effect of High Sink Capacity

The sink refers to the plant organs or tissues that use or store assimilates [61]. The rice plant sink system mainly consists of reproductive organs and new tissues, and panicle grain is the main sink [61,62]. Sink capacity is the main indicator used to measure the ability of a plant to store photosynthetic products [63], and a large storage capacity is the basis of a high yield of rice. Therefore, rice varieties with large storage capacity often show high yield potential [10]. As such, increasing the maximum sink capacity of rice by regulating cultivation practices can significantly increase the rice yield [64], but this largely depends on different varieties, or the same varieties cultured under different conditions. Thus, sink capacity plays an important role in the yield formation of rice plants.

The significance of sink capacity for yield is related to the fact that "the sink" itself is a yield organ, and it is closely related to its regulation of plant growth [51]. Furthermore, sink capacity has a significant effect on metabolic processes in rice plants. It was reported that spraying rice plants with spermidine at the heading stage increased the spermine content, grain filling rate, and grain weight [65]. It also increased the photosynthetic rate per unit area of flag leaf and the distribution of photosynthetic products to grains [66]. Another study reported that spraying a low concentration of abscisic acid (ABA) at the filling stage caused abscisic acid to regulate the re-running of assimilates, thereby increasing starch-metabolism-related enzyme activity or fructan degradation and sucrose synthesis in vegetative tissues. This enhanced the loading capacity of the assimilates and increased the activity of key enzymes involved in the conversion of sucrose to starch in sink organs, thereby increasing the unloading capacity of the assimilates and starch synthesis in grains [65,67,68]. Abscisic acid is mainly distributed in root caps and wilting leaves, and a large sink capacity can promote the senescence of leaves and roots [69] and promote the production of abscisic acid, which also shows that a large sink capacity can promote carbon and nitrogen metabolism. As such, the activities of enzymes and hormones in grains at the filling stage are important physiological factors that promote photosynthesis in leaves.

Secondly, the sink plays an important regulatory role in leaf photosynthetic capacity. There is an obvious interaction between rice source and sink, especially during the filling stage in grains [56,70,71]. Makino et al. [72] reported that the photosynthetic capacity of leaves increased with the increase in the leaf nitrogen content and significantly increased the sink capacity. It was reported that increasing the ratio of sink to leaf and expanding the population storage capacity of suitable LAIs at the same time helps to lay the internal physiological foundation of photosynthetic matter productivity after anthesis [73]. If half of the ears are removed at the heading stage and the leaves remain unchanged, the photosynthetic rate of the leaves decreases significantly, indicating that the decrease in the sink inhibits the photosynthetic capacity of the leaves [74]. In addition, under conditions of increasing CO_2 concentrations, an insufficient pool capable of absorbing extra carbon may also lead to the excessive accumulation of photosynthetic products in leaves, eventually decreasing photosynthesis, indicating that a small pool can inhibit the photosynthetic capacity of leaves [75,76]. The photosynthetic rate of flag leaves increased after spermine was applied to ears at the heading stage [66]. This analysis shows that the photosynthetic capacity of leaves is not only closely related to their nitrogen content but is also regulated by the sink–source ratio. A large sink capacity with high activity can improve the photosynthetic capacity of leaves. In this study, the accumulation of dry matter from the heading to maturity stages increased with the increase in the sink capacity (Table 2), indicating that a high sink capacity can improve the photosynthetic capacity of rice plants at the grain filling stage.

The sink capacity also plays an important role in regulating the movement and distribution of photosynthates. It is generally believed that some non-structural carbohydrates (NSCs) stored in the stem sheath of rice plants before the heading stage are transported to the ear after the heading stage, which plays important roles in stabilizing rice seed setting and increasing rice yield. The results showed that the number and ratio of NSC remobilization were jointly adjusted by the size of the sink capacity and sink–source ratio. In other words, the rice varieties with a large sink capacity had stronger vascular bundle systems, and the accumulation of NSCs in the stem and sheath before the heading stage was high, and the apparent transport of NSCs at the filling stage was large, while the apparent contribution of NSCs to yield was higher than that of the rice varieties with a small sink capacity [7,77]; meanwhile, the sink capacity of rice variety NSC with a high sink–source ratio decreased [78]. A ^{14}C isotope tracing study showed that the larger the sink capacity, the greater the number and the larger the ratio of photosynthates transported from leaves to ears [61]. Therefore, rice varieties with a large storage capacity have a larger proportion of dry matter distributed to the ears at the maturity stage and a higher economic coefficient [9].

With the aging of roots and leaves at the late filling stage of rice, the contradiction between sink and source increases. A large sink capacity can accelerate the senescence of roots and leaves, leading to a rapid decrease in the cytokinin content in roots, as well as protein and chlorophyll content in leaves, a rapid increase in the malondialdehyde content, and a rapid decrease in superoxide dismutase activity [69]. These findings show that, while expanding storage capacity during production, attention must also be paid to preventing the occurrence of premature aging in the later stage so as to realize the potential of increased production with a high storage capacity.

5. Conclusions

This study shows that the nitrogen application rate and planting density are two important cultivation factors that affect rice growth and yield. A higher nitrogen application rate combined with a medium or high planting density can significantly increase the leaf area index of rice, as well as nitrogen and dry matter accumulation, and, consequently, increase rice yield. Among the seasons, the early season of 2021 resulted in a higher yield and yield attributes due to environmental factors. Under the conditions of rational nitrogen application and planting density, rice can achieve a maximum sink capacity, thereby significantly promoting nitrogen and dry matter accumulation, improving metabolic processes in rice, and achieving high yield. Therefore, a high sink capacity is the intrinsic driving force for a high rice yield.

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Abbreviations

ABA—bscisic acid; NSCs—non-structural carbohydrates; LAI—leaf area index F—variance statistic; N—nitrogen; PD—plant density; DMA—dry matter accumulation; TN—total nitrogen; TP—total phosphorous; AN—available nitrogen.

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