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The nitrogen topdressing mode of *indica-japonica* and *indica* hybrid rice are different after side-deep fertilization with machine transplanting

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Determination of the optimal fertilization method is crucial to maximize nitrogen use efficiency and yield of different rice cultivars. Side-deep fertilization with controlled-release nitrogen, in conjunction with machine transplanting and subsequent topdressing, was applied to *Indica-japonica* hybrid rice 'Yongyou1540' (YY1540) and *indica* hybrid rice 'Tianyouhuazhan' (TYHZ). Four nitrogen treatments were applied in 2018 and 2019: traditional nitrogen application with quick-release nitrogen (T_1), single-dose deep fertilization at transplanting with 100% controlled-release nitrogen (T_2), and deep fertilization of 70% controlled-release nitrogen and topdressing of 30% quick nitrogen at tillering (T_3), or at panicle initiation (T_4). Side-deep fertilization reduced the fertilizer application frequency without causing yield loss, T_4 enhanced the yield of YY1540 by increasing the number of productive tillers and number of spikelets per panicle compared with T_1 , T_2 and T_3 . The yield of TYHZ showed no significant difference among treatments. The T_4 treatment decreased the number of tillers at the tillering peak stage and increased the percentage productive tillers and number of differentiated spikelets. Compared with the other treatments, T_4 increased dry matter accumulation and leaf area index during panicle initiation and grain ripening, and contributed to enhanced nitrogen uptake and nitrogen utilization in YY1540. On average, nitrogen uptake and utilization in YY1540 were highest in T_4 , but no significant differences among treatments were observed in TYHZ. Dry matter accumulation and nitrogen uptake from panicle initiation to heading of YY1540 were correlated with number of spikelets per panicle, but no significant correlations were observed for TYHZ. Supplementary topdressing with quick-release nitrogen at the panicle initiation stage was required to increase yield of *indica-japonica* hybrid rice, whereas single-dose deep fertilization with controlled-release nitrogen is satisfactory for the *indica* hybrid cultivar.

Abbreviations

TNT	Nitrogen amount transport from stem, sheaths and leaves to panicles
NTE	Apparent nitrogen translocation efficiency of stem, sheaths, and leaves
NCR	Rate of contribution of transferred nitrogen into grains
NMP	Nitrogen dry matter production efficiency
NU _c E	Nitrogen utilization efficiency
NRF	Apparent nitrogen recovery fraction
NAE	Nitrogen agronomic efficiency

Rice is the dominant food crop in China. Generally, more than 50% of the population consumes rice as a staple food. In traditional rice production in China, the amount of nitrogen fertilizer applied is relatively high and the nitrogen use efficiency is low¹. Traditional fertilization methods typically apply nitrogen fertilizer at three stages: as a basal fertilizer, at the tiller stage, and during panicle initiation. Excessive application of nitrogen fertilizer

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causes ecological problems, such as soil degradation and pollution², which also influence rice quality by increasing the protein content of grains³. In the 1990s, deep placement of nitrogen was reported to enhance nitrogen use efficiency⁴. More recent studies show that deep placement of nitrogen stimulates root growth to increase nutrient uptake, and thereby enhance the early stages of crop growth^{5,6}. Under machine transplanting, deep placement of nitrogen fertilizer enhances rice seedling growth and development of more highly productive tillers⁷. In addition, deep application of nitrogen fertilizer has ecological benefits, by reducing emission of greenhouse gases in the paddy field^{8,9}, improving soil nitrogen function and the community structure of microorganisms¹⁰, and maintaining the ecological sustainability of the rice paddy environment.

With the acceleration of urbanization, the rural population is gradually transitioning to cities, therefore rice machine transplanting technology has developed rapidly to overcome the problem of resulting labor shortage¹¹. Furthermore, side-deep fertilization simultaneous with machine transplanting was developed, which resulted in large-scale alleviation of the labor shortage, improvement in rice production efficiency, and increase in nitrogen use efficiency^{12,13}. With the development of controlled-release fertilizers, side-deep fertilization with machine transplanting has replaced traditional fertilization practices in China in which the quick-release nitrogen fertilizer was used widely.

In China, single season crops accounts for approximately 70% of the rice grown. Hybrid rice comprises 50% of rice production in China, which produces higher yields owing to the strong tillering ability and large spikelets¹⁴. In recent years, *indica/japonica* hybrid rice has been widely grown in the middle and lower reaches of the Yangtze River of China. These cultivars produce significantly improved yields on account of the larger panicles and longer growth season compared with typical *indica* hybrid rice^{15,16}. The nitrogen fertilizer application rate is important for dry matter accumulation, a high application rate is necessary in *indica-japonica* hybrid rice to obtain a higher yield than that attained with inbred hybrid rice^{17,18}. In particular, nitrogen application must be sufficient to meet the demand for spikelet differentiation and the longer grain-ripening period¹⁹.

Controlled-release fertilizer reduces nitrogen loss. Single-dose deep fertilization with controlled-release nitrogen fertilizer and machine transplantation improves rice yield and nitrogen use efficiency in double-season rice production²⁰. Owing to differences in cultivar characteristics, a single application of slow-release fertilizer seems inadequate to fulfill the nitrogen needs of some high-yielding cultivars¹², which is caused by mismatch between the growth period and fertilizer release. However, differences in the response to side-deep fertilization with machine transplanting between *indica-japonica* hybrid and *indica* hybrid rice are unclear.

In 2018, the *indica-japonica* hybrid rice ‘Yongyou1540’ (YY1540) and *indica* hybrid rice ‘Tianyouhuazhan’ (TYHZ) were subjected to ten treatments to study differences in the response of *indica-japonica* hybrid rice and *indica* hybrid rice to controlled-release nitrogen application. Nitrogen topdressing at panicle initiation was beneficial for yield formation in YY1540, whereas a single-dose nitrogen application satisfied the nitrogen needs of TYHZ. The nitrogen application ratio of 70% controlled-release nitrogen applied as side-deep fertilization with machine transplanting to 30% quick-release nitrogen applied as a topdressing at panicle initiation induced the highest yield-increasing effects in YY1540 (Table S1). Therefore, in 2019, we repeated the experiment with four nitrogen application treatments, consisting of traditional nitrogen application (T₁), a single-dose controlled-release nitrogen application (T₂), and 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage (T₃) or the panicle initiation stage (T₄). The aim was to ascertain the difference in response to nitrogen fertilization mode between the large-panicle *indica-japonica* hybrid rice and medium-panicle *indica* hybrid rice, elucidate the factors that contributed to the response, and propose an appropriate controlled-release nitrogen application mode to accompany machine transplanting for *indica-japonica* hybrid rice and *indica* hybrid rice.

Results

Yield and yield components. The change in grain yield of the two the cultivars differed in response to controlled-release nitrogen application (Table 1). In contrast to the traditional fertilization mode (T₁), single-dose fertilization (T₂) decreased the yield of YY1540 by 9.5% ($p > 0.05$) in 2018 and slightly increased the yield in 2019, whereas T₂ slightly increased the yield of TYHZ in 2018 and 2019. The T₄ treatment increased YY1540 yield by 10.5%, 22.1%, and 17.2% compared with the T₁, T₂, and T₃ treatments in 2018, and by 8.0%, 8.0%, and 18.4% in 2019, respectively, which reflected an increase in spikelet number. The T₃ treatment caused a reduction in YY1540 yield in 2019. Regarding TYHZ, a slight decrease ($p > 0.05$) in yield was observed under the T₃ treatment compared with T₁, T₂ and T₄, whereas no significant difference in yield was observed between T₁, T₂, and T₄.

Among the different treatments, yield changes depended on the number of productive tillers and number of spikelets per panicle. The highest number of tillers at the tillering peak stage was observed in the T₃ treatment for YY1540 and T₂ treatment for TYHZ both in 2018 and 2019 (Fig. 1). However, the highest percentage productive tillers was achieved in the T₄ treatment in YY1540 and TYHZ except for the no-nitrogen application control (T₀) in 2019 (Fig. 2). The T₃ treatment resulted in the lowest productive tillers percentage for YY1540 in 2018 and 2019, whereas no significant difference in productive tillers percentage was observed between T₁, T₂, and T₃ for TYHZ.

With regard to spikelet formation, the main stem was sampled to determine spikelet differentiation and degeneration. The number of spikelets that survived depended primarily on the number of differentiated spikelets. The T₃ treatment resulted in the minimum number of differentiated spikelets for YY1540 both in 2018 and 2019. The number of differentiated spikelets for YY1540 was highest in the T₄ treatment, which was 8.6% and 9.8% higher than those of T₁ in 2018 and 2019, respectively, 18.6% and 20.0% higher than those of T₂, and 24.3% and 18.7% higher than those of T₃ in 2018 and 2019, respectively. The T₄ treatment significantly promoted spikelet degeneration in 2018, but the highest number of spikelets was observed in T₄ for YY1540. The highest number

Year	Cultivar	Treatment	The number of productive tillers (10 ⁵ ha ⁻¹)	The number of spikelet per panicle	Filled grain rate (%)	Grain weight (mg)	Grain yield (t-ha ⁻¹)	
2018	YY1540	T ₁	15.8 ± 0.1 bc	350.7 ± 6.6 ab	80.9 ± 2.3 a	23.3 ± 0.3 a	10.5 ± 0.3 b	
		T ₂	15.3 ± 0.2 c	341.0 ± 32.3 ab	78.3 ± 4.4 a	23.1 ± 0.2 ab	9.5 ± 0.4 c	
		T ₃	16.5 ± 1.0 b	327.7 ± 15.4 b	80.0 ± 1.2 a	22.8 ± 0.2 b	9.9 ± 0.7 c	
		T ₄	17.5 ± 0.2 a	357.5 ± 10.5 a	80.1 ± 0.3 a	23.1 ± 0.2 a	11.6 ± 0.2 a	
	TYHZ	T ₁	19.4 ± 0.3 a	241.0 ± 10.3 a	73.7 ± 1.3 a	22.9 ± 0.4 ab	7.9 ± 0.4 a	
		T ₂	19.9 ± 0.4 a	232.1 ± 4.4 a	75.5 ± 1.6 a	23.4 ± 0.5 a	8.1 ± 0.2 a	
		T ₃	18.8 ± 0.5 a	239.4 ± 11.8 a	74.9 ± 1.4 a	22.1 ± 0.4 b	7.4 ± 0.4 a	
		T ₄	18.6 ± 0.4 a	250.0 ± 10.8 a	76.0 ± 1.4 a	22.4 ± 0.4 b	7.9 ± 0.3 a	
2019	YY1540	T ₀	12.4 ± 0.6 b	227.7 ± 17.6 d	82.0 ± 3.3 a	23.3 ± 0.0 a	5.4 ± 0.8 d	
		T ₁	16.6 ± 0.9 a	360.7 ± 6.6 b	80.9 ± 3.8 a	23.1 ± 0.1 a	11.3 ± 0.9 b	
		T ₂	17.5 ± 0.3 a	352.9 ± 6.4 b	79.0 ± 5.3 a	22.8 ± 0.3 ab	11.3 ± 0.8 b	
		T ₃	16.6 ± 1.0 a	327.0 ± 7.7 c	78.3 ± 2.8 a	22.4 ± 0.4 b	10.3 ± 0.5 c	
		T ₄	16.9 ± 0.7 a	380.7 ± 12.2 a	81.8 ± 5.1 a	23.5 ± 0.3 a	12.2 ± 1.3 a	
	TYHZ	T ₀	16.2 ± 1.0 c	169.1 ± 10.1 b	78.2 ± 3.0 a	23.1 ± 0.3 a	5.0 ± 0.5 c	
		T ₁	19.7 ± 0.6 b	233.8 ± 10.8 a	76.7 ± 1.8 a	22.9 ± 0.4 a	8.1 ± 0.4 ab	
		T ₂	21.1 ± 0.5 a	237.6 ± 17.7 a	74.9 ± 2.5 a	22.2 ± 0.3 ab	8.3 ± 0.7 a	
		T ₃	18.7 ± 0.6 b	234.3 ± 14.8 a	74.9 ± 1.4 a	22.1 ± 0.1 b	7.5 ± 0.3 b	
		T ₄	19.3 ± 0.6 b	243.4 ± 10.9 a	75.0 ± 1.0 a	22.9 ± 0.1 a	8.0 ± 0.5 ab	
		Year		9.96**	1.17	0.09	0.97	9.63**
		Cultivar		299.45**	461.09**	34.24**	20.71**	222.00**
Treatment		51.68**	41.99**	1.35	3.37*	72.32**		
Year × Cultivar		0.01	3.06	0.01	0.8	4.92*		
Year × Treatment		4.91**	0.09	0.35	4.13*	0.9		
Cultivar × Treatment		7.06**	4.64**	0.35	0.65	10.32**		
Year × Cultivar × Treatment		1.84	0.42	0.64	6.05**	0.69		

Table 1. Effect of nitrogen fertilization mode on grain yield and yield components of *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ). Values followed by different lower-case letters within the same column are significantly different among treatments. * $p < 0.05$; ** $p < 0.01$.

of differentiated spikelets for TYHZ was observed in the T₄ treatment, which in 2018 was 1.1%, 4.7%, and 3.0% higher than those in the T₁, T₂, and T₃ treatments, respectively, and in 2019 was 1.3%, 3.0%, and 5.2% higher than those in T₁, T₂ and T₃ treatments, respectively. No significant difference in the proportion of degenerated spikelets was observed among treatments for TYHZ (Table 2).

These results indicated that, after side-deep fertilization with controlled-release nitrogen, topdressing at the panicle initiation stage with quick-release nitrogen was necessary to increase yield of the large-panicle *indica-japonica* hybrid YY1540. Single-dose side-deep fertilization with machine transplanting was effective for the medium-panicle *indica* hybrid cultivar TYHZ.

Dry matter accumulation. The highest amount of accumulated total dry matter was observed in the T₄ treatment for YY1540 in 2018 and 2019, and the lowest total dry matter accumulation was observed in the T₃ treatment for YY1540 (Table 3). No significant differences were observed among the T₁, T₂, T₃, and T₄ treatments, although all treatments differed significantly from the T₀ control for TYHZ in 2019.

Significant differences in dry matter accumulation for YY1540 were observed between the panicle initiation and heading stages and between the heading and maturity stages. Dry matter accumulation from panicle initiation to heading in the T₄ treatment was 2.4%, 6.7%, and 11.5% higher than that observed in the T₁, T₂, and T₃ treatments, respectively, on average in 2018 and 2019. Dry matter accumulation from heading to maturity was 41.9%, 13.5%, and 63.0% higher than that observed in the T₁, T₂, and T₃ treatments, respectively, on average in 2018 and 2019. No difference in dry matter accumulation for TYHZ between the T₁, T₂, T₃, and T₄ treatments in 2018 and 2019 was observed.

Given that the amount of dry matter accumulation depends on leaf photosynthesis activity, we determined that the leaf area index at the heading stage for YY1540 was higher in the T₄ treatment than in the T₁, T₂, and T₃ treatments in 2018 and 2019, and the T₃ treatment resulted in the lowest leaf area index. With regard to TYHZ, except for the leaf area index in T₀, no significant difference in leaf area index was observed between the T₁, T₂, T₃, and T₄ treatments (Fig. 3). To measure the relative changes in leaf chlorophyll content, the SPAD value at the maturity stage was determined. The SPAD value of the T₃ treatment was lower than those of the T₁, T₂, and T₄ treatments for YY1540 in 2018 and 2019 (Fig. 4). No significant difference in SPAD value was observed between the T₁, T₂, T₃, and T₄ treatments, except for the lower SPAD of T₀ treatment in 2019, for TYHZ.

These results suggested that quick-release nitrogen application at the panicle initiation stage in YY1540 was important for dry matter accumulation from panicle initiation to heading and from heading to maturity. In

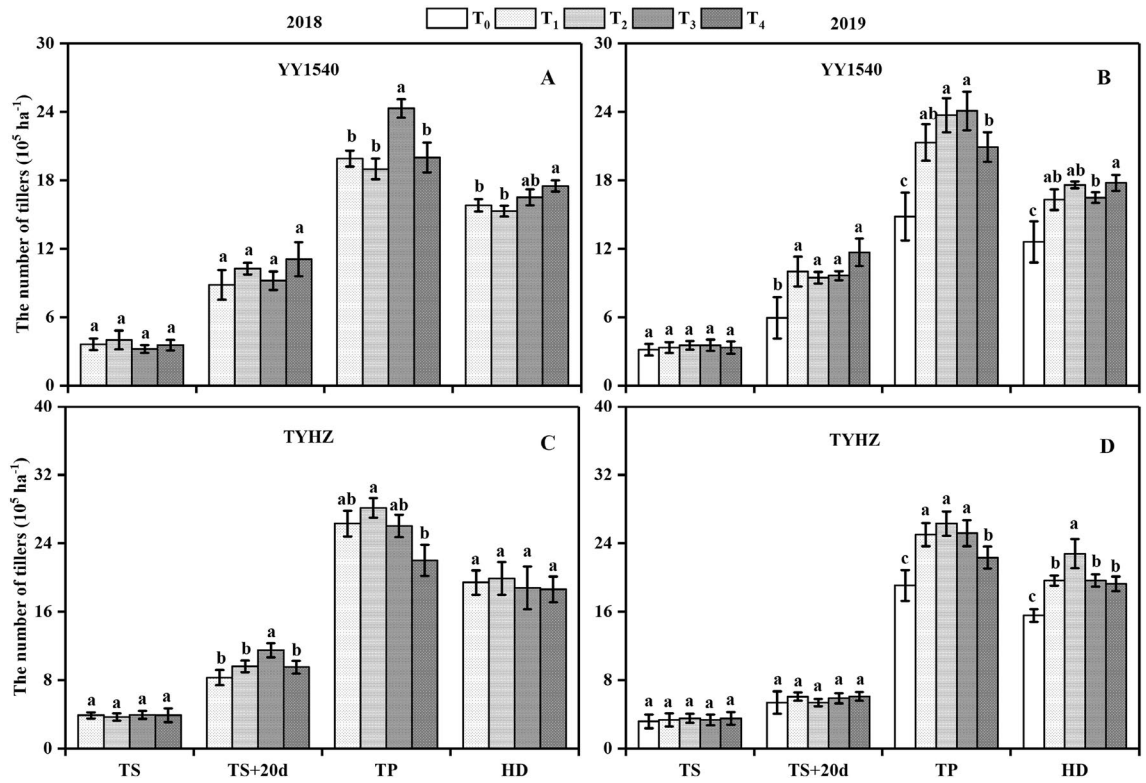


Figure 1. Number of tillers of *indica-japonica* hybrid rice (‘Yongyou1540’, YY1540) and *indica* hybrid rice (‘Tianyouhuazhan’, TYHZ) at four developmental stages under four nitrogen fertilization treatments. (A) YY1540 in 2018; (B) YY1540 in 2019; (C) TYHZ in 2018; (D) TYHZ in 2019. Bars with lower-case letters are significantly different at the 0.05 probability level among treatments. TS transplanting stage, TS + 20d 20 days after transplanting, TP tillering peak stage, HD panicle heading stage.

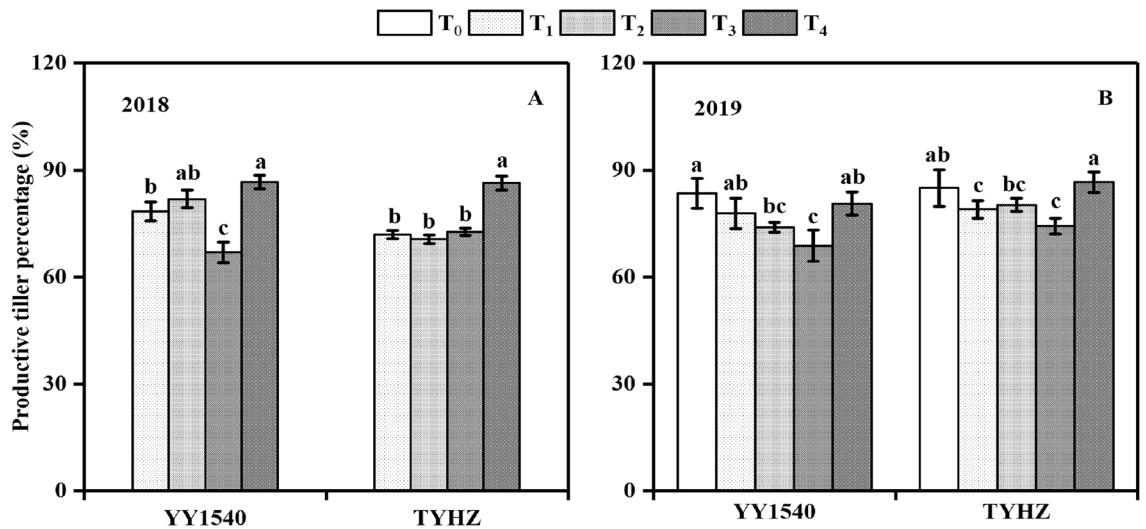


Figure 2. Productive tiller percentage of *indica-japonica* hybrid rice (‘Yongyou1540’, YY1540) and *indica* hybrid rice (‘Tianyouhuazhan’, TYHZ) under four nitrogen fertilization treatments. (A) 2018; (B) 2019. Bars with different lower-case letters are significantly different at the 0.05 probability level among treatments. T₀ no-nitrogen application control, T₁ traditional nitrogen application, T₂ single-dose controlled-release nitrogen application, T₃ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage, T₄ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the panicle initiation stage.

Year	Cultivar	Treatment	The number of spikelets	The number of differentiated spikelets	The proportion of degenerated spikelets (%)	
2018	YY1540	T ₁	396.7 ± 11.5 ab	485.3 ± 9.8 b	18.3 ± 1.8 b	
		T ₂	387.5 ± 20.3 bc	444.2 ± 12.6 c	12.8 ± 3.9 c	
		T ₃	366.2 ± 10.0 c	423.7 ± 20.3 c	13.5 ± 3.1 c	
		T ₄	415.2 ± 9.4 a	526.8 ± 12.0 a	21.1 ± 3.6 a	
	TYHZ	T ₁	294.3 ± 9.5 a	368.5 ± 9.3 a	20.1 ± 0.6 a	
		T ₂	296.7 ± 23.0 a	356.0 ± 13.3 a	16.6 ± 6.3 a	
		T ₃	280.0 ± 6.8 a	361.8 ± 9.2 a	22.6 ± 3.8 a	
		T ₄	312.5 ± 27.9 a	372.7 ± 18.5 a	16.3 ± 3.4 a	
2019	YY1540	T ₀	244.6 ± 5.2 c	285.4 ± 13.3 c	14.2 ± 2.2 c	
		T ₁	396.8 ± 18.5 ab	491.2 ± 32.0 ab	19.2 ± 1.8 b	
		T ₂	373.5 ± 19.6 b	449.2 ± 8.0 b	16.9 ± 3.0 bc	
		T ₃	369.5 ± 4.4 b	454.2 ± 3.4 b	18.8 ± 0.7 b	
		T ₄	406.5 ± 18.3 a	539.2 ± 32.2 a	24.2 ± 1.9 a	
	TYHZ	T ₀	200.3 ± 12.9 b	232.4 ± 15.3 b	13.8 ± 0.9 b	
		T ₁	295.5 ± 14.3 a	375.2 ± 14.9 a	21.2 ± 1.3 a	
		T ₂	298.0 ± 22.4 a	368.8 ± 16.2 a	19.3 ± 2.8 a	
		T ₃	289.3 ± 22.1 a	361.3 ± 8.6 a	20.0 ± 1.5 a	
		T ₄	307.3 ± 7.0 a	380.0 ± 12.1 a	19.1 ± 1.4 a	
		Year		417.29**	587.88**	2.37
		Cultivar		83.11**	182.79**	8.55
Treatment		0.3	5.56*	7.35*		
Year × cultivar		3.72*	18.41**	5.66**		
Year × treatment		0.55	0.63	2.12		
Cultivar × treatment		0.52	0.19	0.54		
Year × cultivar × treatment		0.13	0.97	1.34		

Table 2. Effect of nitrogen fertilization mode on spikelet differentiation and degeneration of primary stems of *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ). Values followed by different lower-case letters within the same column are significantly different among treatments. * $p < 0.05$; ** $p < 0.01$.

contrast, single-dose side-deep fertilization with controlled-release nitrogen satisfied the demand for dry matter accumulation in TYHZ.

Nitrogen accumulation and transport. Nitrogen accumulation showed a significant difference between the T₁, T₂, T₃, and T₄ treatments (Fig. 5). Except in T₀, the nitrogen uptake at the panicle initiation of T₃ was higher than that of the T₁, T₂, and T₄ treatments for YY1540, the maximum nitrogen uptake was observed in T₁ in 2018 and 2019 at the heading stage, and the total nitrogen uptake at maturing stage were observed in T₄ treatment. And in TYHZ, the nitrogen uptake at different developmental stages in 2018 showed no significant differences, whereas significantly lower nitrogen uptake at the maturity stage was observed in T₃ compared with that in the T₁, T₂, and T₄ treatments. Except for the T₀ treatment in 2019, the highest proportion of nitrogen accumulation in panicles at the heading stage for YY1540 was higher in the T₄ treatment in 2018 and 2019 (Fig. 6A,B). An identical trend was observed at the maturity stage. In contrast, the proportion of nitrogen accumulation in panicles for TYHZ not differ significantly among the treatments at the heading stage in 2018 (Fig. 6C), whereas in 2019, the highest proportion was observed in the T₂ treatments at the heading and maturity stages disregarding the T₀ control (Fig. 6D). These results indicated that the T₄ treatment promoted nitrogen accumulation in YY1540 panicles, whereas the T₂ treatment fulfilled the panicle nitrogen need demand of TYHZ.

With regard to nitrogen transport TNT, NTE and NCR were significantly higher in TYHZ than in YY1540. The different treatments more strongly influenced TNT, NTE, and NCR in YY1540 than those of TYHZ. The highest TNT was observed in the T₁ treatment in 2018 and 2019 for YY1540 and in comparison, TNT in the T₂, T₃ and T₄ treatments was 29.3%, 29.7% and 34.7% lower respectively, than that of the T₁ treatment on average in 2018 and 2019. The highest TNT for TYHZ was observed in the T₄ treatment, but there were no significant difference observed in among the T₁, T₂, T₃ and T₄ treatments. The NTE was significantly higher in the T₃ treatment than that in T₁, T₂, and T₄ in 2018, whereas no significant differences were observed in 2019 for YY1540, and for TYHZ in 2018 and 2019. The T₁ treatment showed the highest NCR for YY1540 in 2018 and 2019, which was higher than that of T₂, T₃, and T₄ by 22.3%, 14.9%, by 73.0% in 2018, by 42.5%, 10.1%, and 62.0% in 2019, respectively (Table 4). No significant difference was observed among the T₁, T₂, T₃, and T₄ treatments for TYHZ.

Year	Cultivar	Treatment	Total dry matter accumulation (t·ha ⁻¹)	Dry matter accumulation from Sowing to panicle initiation (t·ha ⁻¹)	Dry matter accumulation from panicle initiation to heading (t·ha ⁻¹)	Dry matter accumulation from heading to maturing (t·ha ⁻¹)	
2018	YY1540	T ₁	15.7±0.8 a	1.2±0.1 a	7.9±0.8 b	6.7±1.7 a	
		T ₂	16.7±0.6 a	1.2±0.1 a	7.4±0.5 b	8.0±0.8 a	
		T ₃	13.5±0.3 b	1.1±0.1 a	7.5±1.0 b	4.9±1.0 b	
		T ₄	17.8±1.4 a	1.1±0.1 a	8.5±0.3 a	8.2±1.3 a	
	TYHZ	T ₁	13.1±0.7 a	1.5±0.2 a	8.0±0.6 a	3.5±0.5 a	
		T ₂	12.9±0.3 a	1.5±0.1 a	8.0±0.7 a	3.4±0.6 a	
		T ₃	13.2±0.6 a	1.5±0.1 a	8.0±0.6 a	3.7±0.3 a	
		T ₄	13.4±0.8 a	1.5±0.2 a	7.8±0.3 a	4.1±0.6 a	
2019	YY1540	T ₀	13.0±0.4 c	0.6±0.1 b	6.9±1.1 c	5.5±0.7 c	
		T ₁	15.8±0.1 b	1.1±0.0 a	9.1±0.6 a	5.7±0.6 bc	
		T ₂	17.4±0.5 b	1.1±0.0 a	8.9±2.0 bc	7.5±1.8 b	
		T ₃	15.2±0.5 b	1.1±0.0 a	8.1±0.2 ab	5.9±0.6 c	
	TYHZ	T ₀	9.4±0.9 b	0.9±0.1 b	8.0±0.6 ab	0.5±0.6 b	
		T ₁	12.0±0.8 a	1.2±0.1 b	8.2±0.5 ab	2.6±1.1 a	
		T ₂	12.5±1.0 a	2.0±0.2 a	7.9±0.2 b	2.6±1.1 a	
		T ₃	13.1±0.2 a	1.3±0.2 b	9.3±0.8 a	2.5±0.8 a	
	TYHZ	T ₄	13.7±1.1 a	1.5±0.1 b	8.5±1.1 ab	3.7±1.2 a	
		Year		205.70**	143.36**	0.31	208.73**
		Cultivar		35.58**	33.23**	2.38	13.09**
		Treatment		2.08	2.08	8.01**	2.42
Year × cultivar		9.14**	5.36**	1.85	2.87		
Year × treatment		7.25*	0.8	0.50	4.88*		
Cultivar × treatment		1.63	5.88**	0.09	1.1		
Year × cultivar × treatment		0.07	7.76**	1.10	1.73		

Table 3. Effect of nitrogen fertilization mode on dry matter accumulation by *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ). Values followed by different lower-case letters within the same column are significantly different among treatments. **p* < 0.05; ***p* < 0.01.

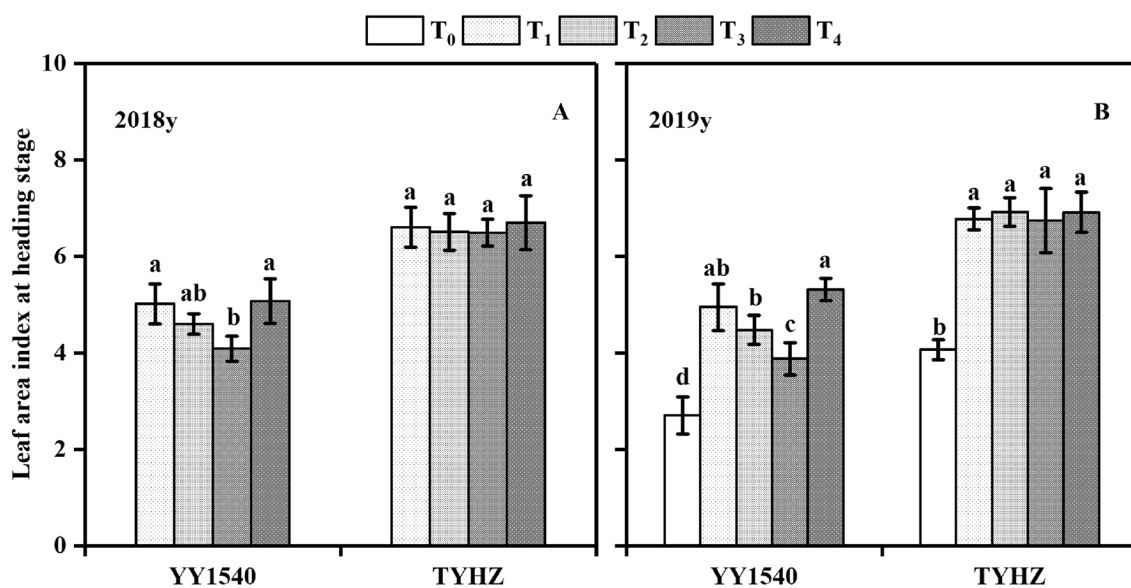


Figure 3. Leaf area index at the heading stage under different treatments. (A) 2018; (B) 2019. Bars superscripted by different lower-case are significantly different among treatments. T₀ no-nitrogen application control, T₁ traditional nitrogen application, T₂ single-dose controlled-release nitrogen application, T₃ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage, T₄ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the panicle initiation stage.

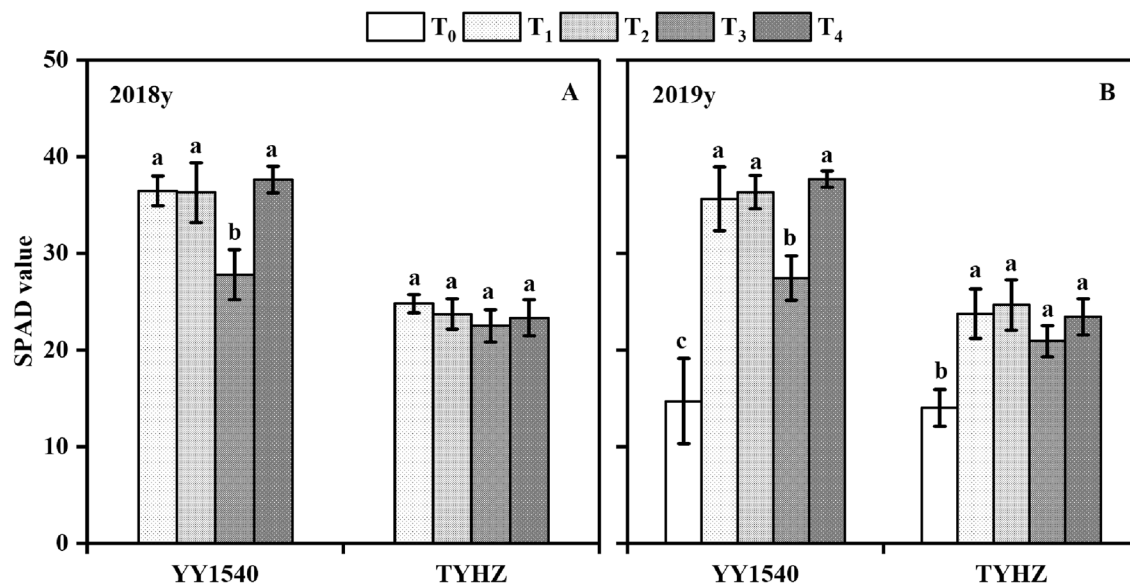


Figure 4. SPAD value at maturing stage of *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ) under four nitrogen fertilization treatments. (A) 2018; (B) 2019. Bars with different lower-case are significantly different at the 0.05 probability level among treatments. T₀ no-nitrogen application control, T₁ traditional nitrogen application, T₂ single-dose controlled-release nitrogen application, T₃ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage, T₄ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the panicle initiation stage.

Nitrogen utilization. Based on the nitrogen accumulation analysis in 2018, we added the no-nitrogen treatment (T₀) in 2019 to calculate nitrogen utilization (Table 5). In YY1540, NMP was highest in T₀ and in comparison, the NMP was decreased by 31.4%, 25.4%, 26.0%, and 24.4.0% in T₁, T₂, T₃, and T₄ respectively. The NU_E of YY1540 was highest in the T₂ treatment, but did not differ significantly from T₀, and that of the T₁, T₃ and T₄ treatments decreased by 9.7%, 4.2%, and 9.5% compared with T₀. The highest NRF of YY1540 was observed in the T₄ treatment, which was 11.6%, 30.3% and 40.2% higher than those of the T₁, T₂ and T₃ treatments, respectively. The NAE of the T₄ treatment was 12.7%, 4.5%, and 32.2% higher than those of T₁, T₂, and T₃ treatments, respectively, for YY1540. In contrast, no significant difference among the T₁, T₂, T₃, and T₄ treatments were observed for NMP, NU_E, NRF, and NAE of TYHZ.

Correlation analysis. To assess the relationship between dry matter accumulation and nitrogen uptake, correlation analysis of the relevant variables at different developmental stages and number of productive tillers, number of spikelets per panicle, or yield was performed on data from 2018 and 2019 combined (Table 6). Under the different nitrogen application treatments, dry matter accumulation from panicle initiation to heading was significantly correlated to the number of spikelets per panicle and yield in YY1540, whereas nitrogen uptake from panicle initiation to heading was significantly correlated to number of spikelets per panicle. However, for TYHZ, no significant correlations were observed between dry matter accumulation and number of productive tillers and number of spikelets per panicle, in the period from sowing to panicle initiation and from panicle initiation to heading, however a significant correlation was observed between the nitrogen uptake from panicle initiation to heading and number of productive tillers and yield. These results suggested the dry matter accumulation and nitrogen uptake from panicle initiation to heading were more important for panicle formation to increase yield in YY1540 than in TYHZ, and that the different fertilization treatments had little effect on TYHZ.

Discussion

Effect of nitrogen application treatments on yield formation. Compared with the traditional fertilization treatment (T₀), nitrogen topdressing at the panicle initiation stage (T₄) resulted in increases in yield and nitrogen utilization efficiency over those attained with controlled-release nitrogen application. The present results verified that application of controlled-release nitrogen can improve the nitrogen utilization efficiency of rice, and that side deep fertilization with machine transplanting significantly improves fertilization efficiency^{21,22}. However, the yield under single-dose controlled-release nitrogen application (T₂) and quick-release nitrogen top dressing at the tillering stage (T₃) showed a tendency to reduce yield in YY1540 compared with the T₁ treatment, whereas no significant difference in yield of TYHZ was observed between the T₁, T₂, T₃, and T₄ treatments. These results suggested that controlled-release nitrogen application did not necessarily promote an increase in yield, and that the advantage of controlled-release nitrogen fertilizer application depended on cultivar characteristics and fertilization methods.

The yield of *indica-japonica* hybrid rice is considerably higher than that of *indica* hybrid rice (Table 1). The principal advantage of the former is the production large panicles under high biomass²³, which was correlated

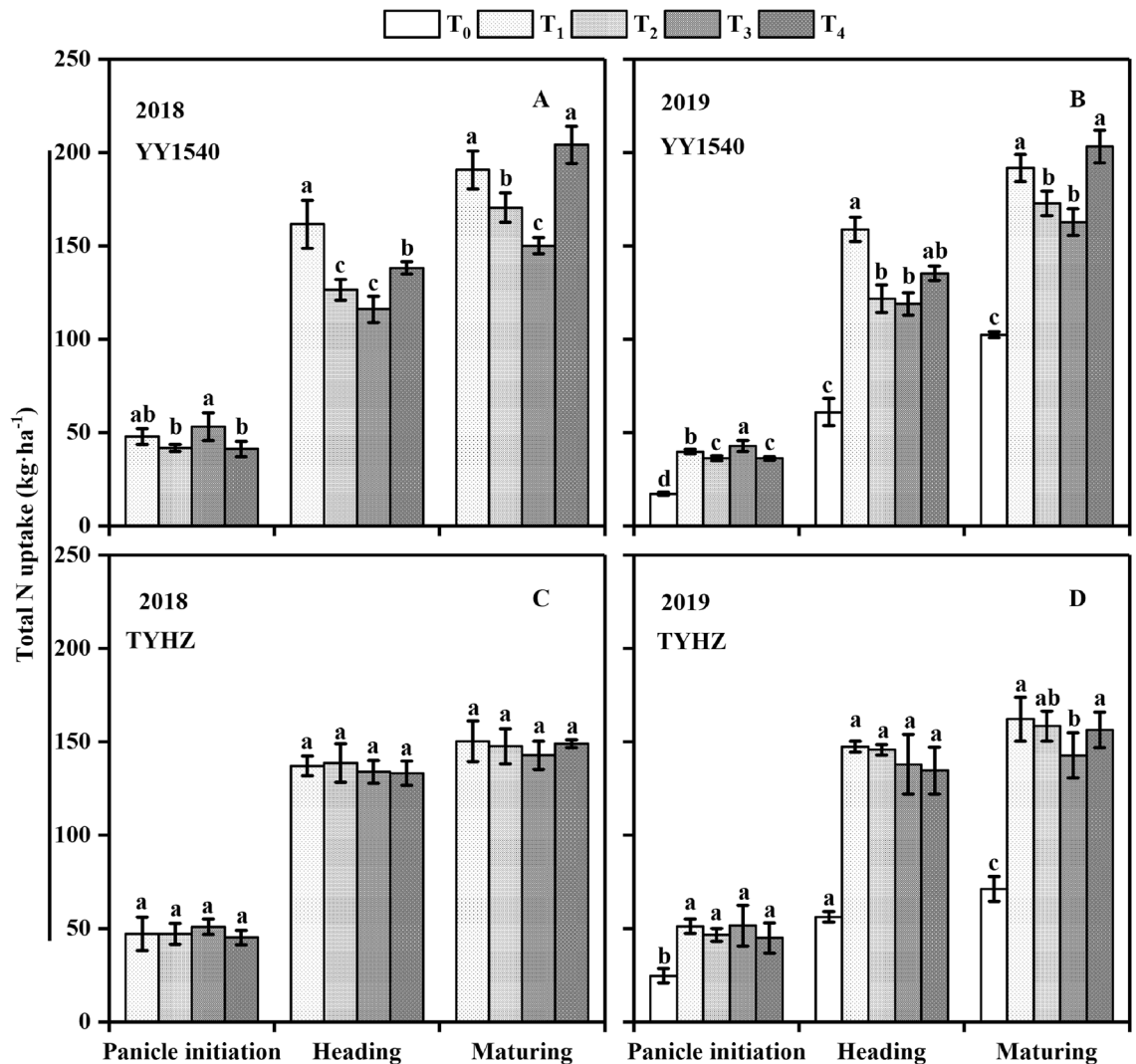


Figure 5. Nitrogen uptake of *indica-japonica* hybrid rice ('Yongyou1540; YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ) plants under four nitrogen fertilization treatments. (A) YY1540 in 2018; (B) YY1540 in 2019; (C) TYHZ in 2018; (D) TYHZ in 2019. Bars with different lower-case letters are significantly different at the 0.05 probability level among treatments. T₀ no-nitrogen application control, T₁ traditional nitrogen application, T₂ single-dose controlled-release nitrogen application, T₃ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage, T₄ 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the panicle initiation stage.

with the grain yield among the four treatments applied to YY1540²⁴. In the present study, the release cycle of the controlled-release fertilizer was about 120 days, Correlation analysis showed that dry matter accumulation and nitrogen uptake from the panicle initiation stage to the heading stage was correlated with spikelet number per panicle in YY1540 (Table 6), which suggested that the slow release of nitrogen by the controlled-release fertilizer did not meet the nitrogen demand of *Indica-japonica* hybrid rice. The productivity advantage of *indica-japonica* hybrid rice is dependent on the development of large panicles through enhanced by the spikelet differentiation, which may be associated with the cytokine in synthesis^{25,26}. However, in the present study, the nitrogen application rate was 195 kg·ha⁻¹, which was slightly lower than the traditional nitrogen application rate used for *indica-japonica* hybrid rice²⁷. Whether an increased rate of nitrogen application without topdressing can meet the nitrogen needs for panicle development requires further study, However increased nitrogen application at the tillering stage, as applied in the T₃ treatment, resulted in a significant reduction in productive tiller percentage and reduced the number of spikelets per panicle, whereas the productive tiller percentage in the T₄ treatment was significantly increased. Therefore, substantially increased nitrogen application at the tillering stage was not suitable to enhance yield of YY1540. For TYHZ, a slight reduction in yield was also observed in the T₃ treatment, which was consistent with previous results that postponement of nitrogen topdressing may increase rice yield²⁸. In addition, the release of controlled-release nitrogen at two stages is required to meet the nitrogen requirements at different growth stages of *indica-japonica* hybrid rice. For TYHZ, the T₄ treatment slightly increased ($P > 0.05$)

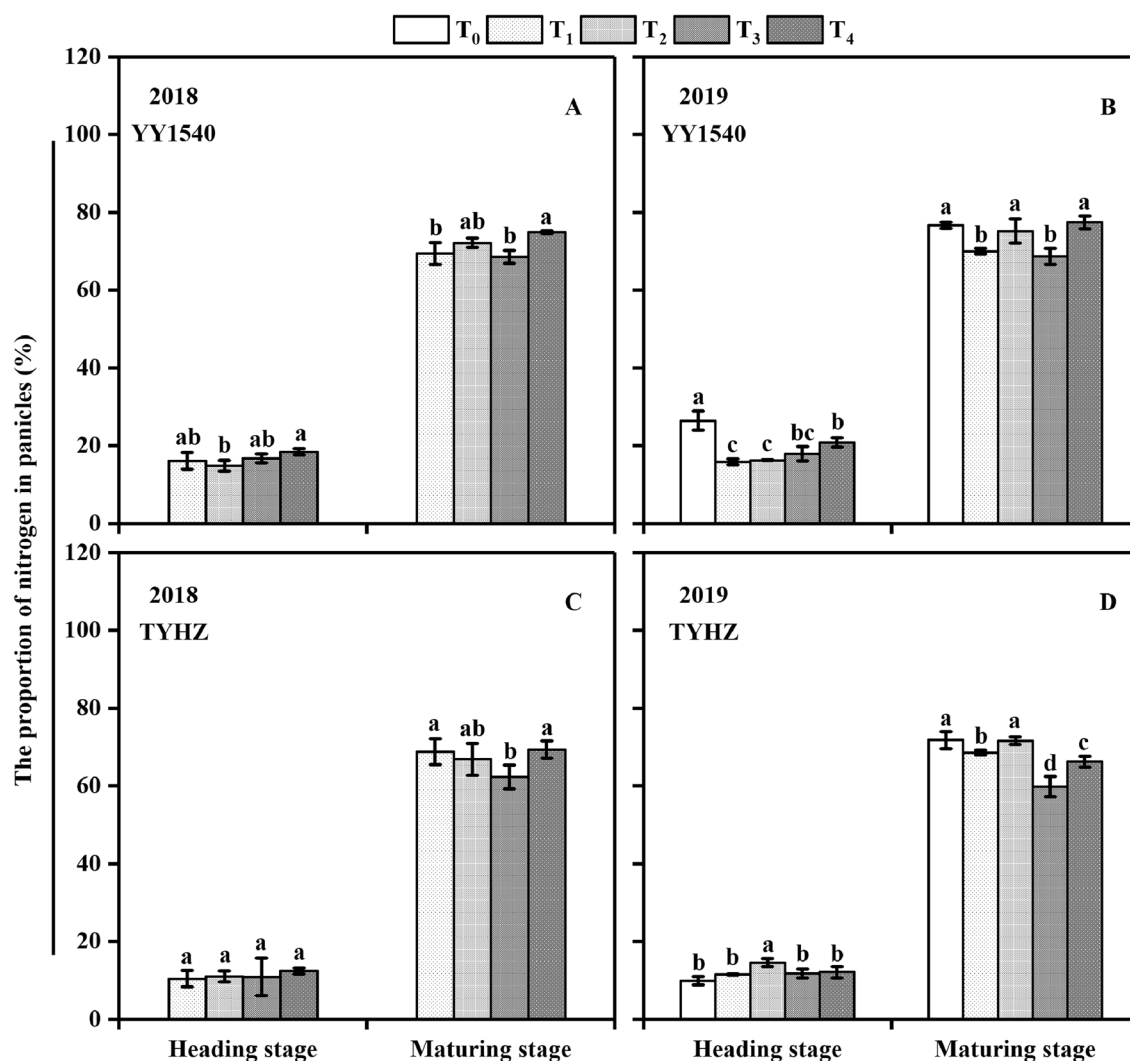


Figure 6. Proportion of nitrogen accumulation in panicles of *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ) under four nitrogen fertilization treatments. (A) YY1540 in 2018; (B) YY1540 in 2019; (C) TYHZ in 2018; (D) TYHZ in 2019. Bars with different lower-case letters are significantly different at the 0.05 probability level among treatments. T_0 no-nitrogen application control, T_1 traditional nitrogen application, T_2 single-dose controlled-release nitrogen application, T_3 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the tillering stage, T_4 70% controlled-release nitrogen as side-deep fertilization with machine transplanting and 30% as topdressing applied at the panicle initiation stage.

the number of spikelets per panicle, but yield showed no significant difference between the T_1 and T_4 treatments owing to the higher tiller number ($P > 0.05$). Thus, a single-dose controlled-release nitrogen application could be adopted for *indica* hybrid rice cultivars such as TYHZ, which are more dependent on the number of productive tillers to attain a high yield than the number of spikelets per panicle²⁶.

Effect of nitrogen application treatments on dry matter accumulation. Dry matter accumulation is significantly influenced by nitrogen application, which plays an important role in maintaining photosynthesis²⁹. In the T_3 treatment, a higher number of tillers were observed at the tillering peak stage compared with that observed in the T_1 , T_2 , and T_4 treatments in YY1540, which was induced by the excessive nitrogen. Dry matter accumulation from sowing to panicle initiation was not significantly influenced by the nitrogen treatment, but the dry matter accumulation of a single tiller was decreased, which led to the low percentage productive tillers (Fig. 2), lower leaf area index (Fig. 3), and lower number of spikelets per panicle (Table 1) in YY1540. These responses may have been induced by carbohydrate competition between tillers^{26,30}. Dry matter accumulation from sowing to panicle initiation was higher in TYHZ than that in YY1540 (Table 3), which may explain why the tiller number at the tillering peak stage was higher in TYHZ. This finding may also account for the stronger tillering ability of TYHZ, whereas *indica-japonica* hybrid rice exhibits intersubspecific heterosis for panicle initiation under similar dry matter accumulation from panicle initiation to heading^{31,32}.

Year	Cultivar	Treatment	TNT (kg-ha ⁻¹)	NTE (%)	NCR (%)		
2018	YY1540	T ₁	77.3 ± 10.6 a	57.0 ± 5.1 b	60.2 ± 3.5 a		
		T ₂	60.1 ± 6.3 ab	55.8 ± 4.8 b	49.2 ± 8.0 bc		
		T ₃	58.9 ± 4.0 b	61.0 ± 1.0 a	52.4 ± 2.1 b		
		T ₄	48.4 ± 6.9 b	42.9 ± 5.8 c	34.8 ± 6.0 c		
	TYHZ	T ₁	69.2 ± 7.9 a	55.8 ± 8.5 a	73.7 ± 8.6 a		
		T ₂	78.8 ± 9.6 a	59.3 ± 8.9 a	75.7 ± 7.5 a		
		T ₃	68.7 ± 7.9 a	61.5 ± 7.3 a	70.7 ± 8.8 a		
		T ₄	74.6 ± 6.6 a	62.3 ± 3.4 a	73.2 ± 4.3 a		
2019	YY1540	T ₀	32.4 ± 5.1 c	57.1 ± 4.3 a	41.9 ± 7.1 b		
		T ₁	76.1 ± 6.9 a	56.9 ± 2.6 a	56.7 ± 4.2 a		
		T ₂	48.3 ± 8.4 b	54.3 ± 7.2 a	39.8 ± 8.3 b		
		T ₃	49.0 ± 2.3 b	52.1 ± 0.3 a	51.5 ± 2.8 a		
		T ₄	51.7 ± 9.3 b	48.2 ± 7.3 a	35.0 ± 6.8 b		
	TYHZ	T ₀	30.5 ± 3.9 c	60.3 ± 5.6 a	60.1 ± 9.1 b		
		T ₁	79.4 ± 2.9 ab	60.9 ± 1.9 a	71.8 ± 7.5 ab		
		T ₂	79.5 ± 3.4 ab	63.9 ± 1.9 a	70.3 ± 6.8 ab		
		T ₃	71.2 ± 5.1 b	59.5 ± 12.0 a	83.7 ± 5.6 a		
		T ₄	86.7 ± 12.6 a	60.8 ± 4.4 a	85.9 ± 10.4 a		
		Year			18.28**	8.26**	64.57**
		Cultivar			20.42**	1.92	2.59
Treatment			1.27	0.42	1.13		
Year × cultivar			6.62**	2.96*	4.06**		
Year × treatment			1.4	0.05	0.19		
Cultivar × treatment			0.51	1.06	0.8		
Year × cultivar × treatment			0.28	0.57	0.72		

Table 4. Nitrogen transport under four nitrogen fertilization treatments in *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ). Values followed by different lower-case letters within the same column are significantly different among treatments. * $p < 0.05$; ** $p < 0.01$. TNT nitrogen amount transport from stem, sheaths and leaves to panicles, NTE nitrogen apparent translocation efficiency of the stem, sheaths and leaves, NCR rate of contribution of transferred nitrogen into grains.

Cultivar	Treatment	NMP (kg·kg ⁻¹)	NU _t E (kg·kg ⁻¹)	NRF (%)	NAE (kg·kg ⁻¹)
YY1540	T ₀	127.2 ± 2.0 a	54.9 ± 2.8 ab		
	T ₁	87.3 ± 1.3 c	49.6 ± 3.3 b	45.8 ± 3.8 a	21.4 ± 2.0 b
	T ₂	94.9 ± 5.5 bc	57.2 ± 4.0 a	36.1 ± 3.4 b	23.4 ± 1.9 ab
	T ₃	94.1 ± 6.9 bc	52.6 ± 3.0 ab	31.0 ± 3.6 b	16.6 ± 0.7 c
	T ₄	96.2 ± 3.7 b	49.7 ± 2.0 b	51.8 ± 4.5 a	24.5 ± 0.4 a
TYHZ	T ₀	132.4 ± 3.6 a	74.1 ± 6.1 a		
	T ₁	78.1 ± 3.1 b	48.9 ± 4.4 b	46.7 ± 6.0 a	13.6 ± 0.8 a
	T ₂	78.6 ± 3.2 b	51.4 ± 2.7 b	44.8 ± 4.1 a	14.8 ± 0.7 a
	T ₃	90.3 ± 15.1 b	50.9 ± 4.8 b	36.7 ± 6.2 a	10.2 ± 2.4 a
	T ₄	87.9 ± 87.9 b	51.0 ± 1.6 b	43.7 ± 4.8 a	14.0 ± 1.3 a
Cultivar		8.58**	3.33	0.88	200.68**
Treatment		59.29**	16.56**	11.04**	21.40**
Cultivar × Treatment		2.6	10.35**	3.70*	2.13

Table 5. Nitrogen utilization of in *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ) under four nitrogen fertilization treatments in 2019. Values followed by different lower-case within the same column are significantly different among treatments. * $p < 0.05$; ** $p < 0.01$. NMP nitrogen dry matter production efficiency, NU_tE nitrogen utilization efficiency, NRF nitrogen apparent recovery fraction, NAE nitrogen agronomic efficiency.

Item	YY1540			TYHZ		
	The number of productive tillers	The number of spikelets per panicle	Theoretical yield	The number of productive tillers	The number of spikelets per panicle	Theoretical yield
Dry matter accumulation from sowing to panicle initiation	-0.699	-0.474	-0.732	0.672	0.187	0.439
Dry matter accumulation from panicle initiation to heading	0.703	0.733*	0.866**	-0.293	-0.356	-0.417
Dry matter accumulation heading to maturing	0.175	0.709*	0.53	-0.483	0.745*	-0.183
N uptake from sowing to panicle initiation	-0.432	-0.68	-0.671	-0.227	-0.629	-0.562
N uptake from panicle initiation to heading	-0.041	0.712*	0.507	0.882*	-0.301	0.904**
N uptake from heading to maturing	0.592	0.558	0.657	0.109	0.588	0.505

Table 6. Correlation analysis between dry matter accumulation and nitrogen uptake in *indica-japonica* hybrid rice ('Yongyou1540', YY1540) and *indica* hybrid rice ('Tianyouhuazhan', TYHZ). * $p < 0.05$; ** $p < 0.01$.

Nitrogen supplementation is important to maintain carbohydrate supply for grain ripening of *indica-japonica* hybrid rice¹⁹. In the present study, a larger reduction in grain weight was observed in YY1540 compared with that of TYHZ under the T₃ treatment. The *indica-japonica* hybrid rice YY1540 has a longer grain-filling stage than that of TYHZ. The significant decrease in dry matter accumulation from heading to maturity may be caused by leaf senescence⁴. The lower SPAD value is consistent with these results (Fig. 4). The SPAD value and dry matter accumulation in the heading-maturity stage were higher in YY1540 than in TYHZ, which indicated that the leaf photosynthetic capacity during the grain-filling stage was higher in YY1540 than in TYHZ. Thus, a large amount of supplementary nitrogen is needed to maintain the chlorophyll content and photosynthesis capacity of YY1540³³, which was the reason that nitrogen topdressing at panicle initiation stage was needed in YY1540.

Effect of nitrogen application treatments on nitrogen utilization. Nitrogen accumulation in YY1540 was higher than that in TYHZ (Fig. 5), which is consistent with the stronger nitrogen absorption capacity in *indica-japonica* hybrid rice than that of *indica* hybrid rice³⁴. Although the total nitrogen uptake at the heading stage was slightly lower in YY1540 than that in TYHZ, the nitrogen accumulation in panicles was higher in YY1540 than in panicles of TYHZ (Fig. 4), which showed that the contribution of nitrogen to panicle development in YY1540 is greater than that in TYHZ. In TYHZ nitrogen was used mainly for leaf growth, which is consistent with the higher NMP of YY1540 compared with that of TYHZ (Table 5).

The NCR was lower in YY1540 compared with that of TYHZ, and the TNT of YY1540 was highest in the T₁ treatment (Table 4). These findings are consistent with a previous study that showed nitrogen accumulation in *indica-japonica* hybrid rice primarily depends on absorption rather than transport³⁵. In addition, an adequate late growth nitrogen supply is extremely important for *indica-japonica* hybrid rice²⁷. The lower NRF on average of YY1540 compared with that of TYHZ also supported this interpretation. However, NRF a lower in the T₂ and T₃ treatments than that in the T₁ and T₄ treatments (Table 5). Furthermore the NRF of YY1540 in the T₂ and T₃ treatments was lower than that in TYHZ, which may be associated with the ability of *indica-japonica* hybrid rice to contribute to nitrogen priming in soil with high microbial activity. The T₂ treatment resulted in the highest N_{UE} in YY1540 and TYHZ (Table 5). In addition, a reduction in N_{UE} was observed at the quick-release nitrogen application treatments, which showed that controlled-release nitrogen is beneficial for improvement of nitrogen utilization efficiency and reduction of nitrogen losses^{36,37}.

Conclusions

Controlled-release nitrogen side-deep application with machine transplanting can reduce the required fertilization frequency. Nitrogen topdressing at the panicle initiation stage is necessary for high yield formation in *indica-japonica* hybrid rice with large panicles. This treatment leads to increase in the number of spikelets per panicle by ensuring sufficient nitrogen fertilizer supply and increase in dry matter accumulation, and results in maximum nitrogen use efficiency in *indica-japonica* hybrid rice. Single-dose controlled-release side-deep fertilization can satisfy the nitrogen absorption demand for high-yield formation in *indica* hybrid rice, which produce medium-type panicles and show enhanced tillering capacity.

Materials and methods

Experimental site and meteorological conditions. The field experiments were conducted from May to October in 2018 and 2019 at the China National Rice Research Institute, Hangzhou, Zhejiang Province (119° 55' 48" E, 30° 2' 24" N), China. The experimental field contained common paddy soil with pH 5.45, organic matter 32.13 g·kg⁻¹, total nitrogen 1.69 g·kg⁻¹, available phosphorus 85.8 mg·kg⁻¹ and available potassium 95 mg·kg⁻¹. Soil analyses were performed on samples collected from the uppermost 20 cm following the methodology of Ke et al.³⁸.

Year	Month	Rainfall (mm)	Average Temperature (°C)	Average relative humidity (%)	Cumulative solar radiation (MJ/m ²)
2018	May	129.6	23.5	83.3	202.9
	June	204.0	26.1	84.5	246.7
	July	113.4	29.4	83.7	332.3
	August	201.2	28.3	85.7	268.0
	September	275.6	24.4	86.7	436.5
	October	17.6	17.1	82.1	150.4
2019	May	109.6	21.5	87.3	195.1
	June	185.0	25.8	86.3	235.5
	July	172.9	28.5	82.6	357.0
	August	180.8	29.5	77.3	409.7
	September	36.0	24.7	78.2	325.3
	October	19.7	19.8	80.1	227.6

Table 7. Meteorological conditions during the rice growth seasons at the study site.

Cultivar	Year	Sowing date	Transplanting date	Date of panicle initiation beginning	Date of panicle heading beginning	Date of harvest
YY1540	2018	5/17	6/12	7/16	8/22	10/21
	2019	5/17	6/12	7/15	8/22	10/25
TYHZ	2018	5/17	6/12	7/12	8/13	9/25
	2019	5/17	6/12	7/9	8/12	9/23

Table 8. Details of the rice growth period in 2018 and 2019.

Treatment	Nitrogen application mode	Conduct year
T ₀	No nitrogen application	2019
T ₁	Traditional nitrogen fertilization with quick-release nitrogen at the 1 days before transplanting (base fertilizer, 40%N), 7 days after transplanting (tillering fertilizer, 30% N) and panicle initiation stage (panicle fertilizer, 30% N)	2018 and 2019
T ₂	Control-release nitrogen by single-dose side deep fertilization with machine transplanting (100% N)	2018 and 2019
T ₃	Controlled-release nitrogen by side deep fertilization machine transplanting (70% N) + Quick-release nitrogen top dressing at 7 days after transplanting (30% N)	2018 and 2019
T ₄	Controlled-release nitrogen by side-deep fertilization machine transplanting (70% N) + Quick-release nitrogen top dressing at panicle initiation stage (30% N)	2018 and 2019

Table 9. Experimental nitrogen treatments applied in the study.

During the 2-year experiment, rainfall, relative humidity, temperature, and solar radiation were measured in the field using a HOBO weather station (MAH-H21, Onset Computer Corporation, Bourne, MA, USA). The average rainfall of 2018 was higher than that of 2019 and the humidity showed a similar trend (Table 7). The monthly average temperature in 2018 ranged from 17.1 °C to 29.4 °C, which was slightly lower than that in 2019 (19.8–32.2 °C). The cumulative solar radiation was 391.7 MJ·m⁻² in 2019, which was higher than that in 2018 (272.8 MJ·m⁻²).

Plant material. We selected two super-rice cultivars grown commercially in China. The *indica-japonica* hybrid rice ‘Yongyou1540’ (YY1540) and *indica* hybrid rice ‘Tianyouhuazhan’ (TYHZ), have been widely cultivated in the middle and lower regions of the Yangtze River for single-season rice production for more than 10 years, and are representative of the *indica-japonica* hybrid rice and *indica* rice cultivars in cultivation^{39,40}.

The growth period of YY1540 from sowing to maturity in 2018 and 2019 was 161 days and 157 days, respectively. The average spikelet number of YY1540 was 338 per panicle. The growth period of TYHZ from sowing to maturity in 2018 and 2019 was 129 days and 131 days, respectively. The average spikelet number of TYHZ was 217 per panicle. Details of the rice growth period in both years of the study are shown in Table 8.

Experimental design. The experiment was conducted using a split-plot design with cultivars as primary plots and nitrogen treatments as subplots. A total of 195 kg N ha⁻¹ was applied during the rice growth period. The treatments are summarized in Table 9.

For all treatments, quick-release nitrogen was applied as common urea (nitrogen content: 46%) and controlled-release nitrogen was applied as a slow release fertilizer (N content: 41.6%, Kingenta International Co., Ltd., Shandong, China). Phosphorus fertilizer was applied as calcium superphosphate at the rate of 510 kg·ha⁻¹ as a basal dressing. Potassium fertilizer was split-applied with 50% as a basal level and 50% at the panicle initiation stage at a rate of 280 kg·ha⁻¹ potassium. The deep fertilization machine used was developed by the Jinhe Agricultural Science and Technology Co, Ltd. (Zhejiang, China) following the methodology of Zhu²⁰. The depth of fertilization was 5 cm. The irrigation method followed local practices established to achieve high-yield crops. The treatments were repeated three times. The subplot size was 216 m².

Determination methods. *Yield and yield components.* Yield and yield components were determined as described by Yoshida⁴¹. The grain yield was determined from a harvest area of 6 m² in each subplot at the rice brown stage and adjusted to 13.5% grain moisture. The yield components (panicle number, spikelets number per panicle, percentage of filled grains and grain weight) were determined from the plants within a 1 m² area randomly chosen in each subplot (excluding border plants) in accordance with the method of Kamiji⁴². The filled-grain percentage was calculated as follows: number of filled grains per panicle/(number of filled grains per panicle + number of unfilled grains per panicle) × 100.

Tiller number dynamics. After transplantation, the number of tillers was determined at 7-days intervals. Thirty plants per plot were selected for investigation. The criterion for tiller recognition was presence of three leaves on each tiller. The proportion of effective panicles was calculated as the number of effective panicles/tiller number at the peak stage.

Spikelet differentiation and degeneration. To maintain a consistent panicle development period for each treatment, ten primary tillers (tagged during rice growth) from each subplot were collected when the panicle was 50% elongated and used to quantify spikelet differentiation and degeneration. The number of degenerated spikelets was calculated by counting the vestiges present on the panicles in accordance with the method of Yao⁴³. The number of differentiated spikelets per panicle is the sum of surviving and degenerated spikelets per panicle. The proportion of degenerated spikelets was calculated as follows: number of degenerated spikelets per panicle/number of total differentiated spikelets per panicle × 100.

Dry matter accumulation, leaf area index and SPAD value. Ten plant hills were sampled from each plot based on the average tiller number at the panicle initiation, heading and maturity stages. To determine level of dry matter accumulation, the sampled plants were dried at 105 °C for 30 min and then dried to a constant weight at 80 °C following the method of Zhu²⁰. Panicles, leaves, and stems with leaf sheaths were separated from the plants after the heading stage. An LI-3100C Area Meter (LI-COR, Inc., Lincoln, NE, USA) was used to measure the leaf area of each green leaf. The leaf area per square meter was calculated as the leaf area index following the method of Wu¹⁹. The SPAD value was determined at the maturity stage using a SPAD chlorophyllII meter (SPAD-502PLUS, Spectrum Technologies, Aurora, IL, USA).

Plant nitrogen content, nitrogen uptake and nitrogen use efficiency. After measurement of the sample dry matter at the panicle initiation, heading and maturity stages, the total nitrogen content of the samples was determined using the micro-Kjeldahl digestion method in accordance to the procedure described by Bremner⁴⁴. To determine the nitrogen content, ground samples (0.20 g) of the panicle, leaf, stem, and sheath were digested in H₂SO₄-H₂O₂ solution at 420 °C for 2 h and distilled with 10 mol·L⁻¹ sodium hydroxide solution. The evaporated NH₃ was absorbed with Na₂B₄O₇ and titrated with 0.01 mol·L⁻¹ sulfuric acid. According to the consumption of the titrant, the percentage of nitrogen was calculated, which was analyzed by the micro-Kjeldahl method (Kjeltec TM 8400, FOSS, Helleröd, Denmark). Nitrogen uptake was calculated using the following formula: dry matter accumulation × nitrogen concentration.

Measures of nitrogen transport and nitrogen use efficiency, namely the amount of nitrogen amount transport from stem, sheaths and leaves to panicles (TNT), apparent nitrogen translocation efficiency of stem, sheaths, and leaves (NTE), rate of contribution of transferred nitrogen into grains (NCR), nitrogen dry matter production efficiency (NMP), nitrogen utilization efficiency (NU_E), apparent nitrogen recovery fraction (NRF), and nitrogen agronomic efficiency (NAE) were calculated using the formulas described by López-Bellido et al.

TNT = Nitrogen amount of leaf and stem sheath at the heading stage – Nitrogen amount of leaf and stem sheath at the maturity stage;

NTE = TNT/Nitrogen amount of leaf and stem sheath at the heading stage × 100;

NCR = TNT/Nitrogen amount of grains at the maturity stage × 100;

NMP = Total dry matter accumulation uptake at the maturity stage/Total nitrogen accumulation uptake at the maturity stage;

NU_E = Grain yield/Total nitrogen accumulation uptake at the maturity stage;

NRF (%) = Nitrogen accumulation above ground with nitrogen treatment – Nitrogen accumulation above ground with no-nitrogen/Total nitrogen application rate × 100;

NAE = Grain yield with nitrogen treatment – Grain yield with no nitrogen treatment/Total nitrogen application rate.

Statistical analyses. The experimental data for YY1540 and TYHZ were analyzed statistically using one-way analysis of variance as implemented in SAS 9.1 software (SAS Corp, Cary, NC, USA). Multiple comparisons

of the different treatments (three replicates) were analyzed with Duncan's multiple range test ($p < 0.05$) using SAS 9.1 (SAS Corp.). Graphs were generated using Origin 9.1 (Origin Lab, Northampton, MA, USA).

Data availability

The data used or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Y.W. and H.C. conceived the project, and designed and coordinated the experiments; X.W., Y.W., H.C., J.X., Y.Z., Y.Z. and D.Z. performed the experiments and analysed the data. X.W. and Y.W. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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