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^{15}N Tracer-based Analysis on Nitrogen Utilization Laws of Rice Under Different Irrigation Modes

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Abstract In order to promote the efficient and coordinated utilization of water and nitrogen in rice, the ^{15}N missing technology experiment was adopted to study the effects of nitrogen application rate (N_0, N_{160}, N_{200}, N_{240}) and irrigation mode (Wet-dry alternation, conventional irrigation) on nitrogen absorption, transport, residue, loss and nitrogen utilization of super rice. The results showed that with the increase of nitrogen application rate, the accumulation of fertilizer nitrogen and total nitrogen in rice plants at different growth stages, and the accumulation of nitrogen in various organs at mature stage increased significantly, and the accumulation of nitrogen per panicle at mature stage was the highest, reaching 67.20%~69.02%. The transport amount of nitrogen from different sources in different vegetative organs of rice plants increased with the increase of nitrogen application rate, and the comparison of nitrogen transport amount in organs was as follows: leaf > stem > root; After full heading, the accumulation of nitrogen from different sources and its contribution rate to grain nitrogen per spike increased with the increase of nitrogen application rate, but with the increase of nitrogen application rate, the residual amount of soil fertilizer nitrogen increased significantly, the residual rate showed a downward trend, and the nitrogen loss rate increased significantly. Dry-wet alternate irrigation treatment can increase nitrogen accumulation in different organs of rice in different periods, improve nitrogen use efficiency and rice yield to a certain extent. This study summarized the laws of nitrogen absorption, utilization, residue and loss of rice under different irrigation conditions and different nitrogen application conditions, which provided a theoretical basis for efficient cultivation of rice.
Keywords Rice; ^{15}N; Nitrogen application rate; Alternate wetting and drying (AWD); Nitrogen uptake and utilization

Rice is one of the three most important staple foods in the world and plays an important role in global food production and consumption (Nguyen and Ferrero, 2006; Cheng and Hu, 2008). In order to feed the growing population, modern agriculture in East Asia, especially China, is more highly intensive and highly productive, and Dongting Lake area is an important rice-producing area in China, which can be attributed to the use of new technologies and new crop varieties, as well as the high input of nitrogen and pesticides (Xiong et al., 2008; Ji et al., 2011). However, excessive nitrogen input in the intensive agroecosystem leads to the decline of nitrogen utilization efficiency and the increase of nitrogen loss to the environment (Zhu and Chen, 2002). Efficient nitrogen uptake and utilization can effectively improve rice yield and quality and reduce pollution from agricultural sources (Duan et al., 2017; Li and Yang, 2017).

Previous studies have shown that nitrogen fertilizer losses in different ways, such as absorption by crops, soil residue, pollution of air and water systems through ammonia volatilization, surface runoff, leaching and denitrification (Ji et al., 2006; Li, 2020). The absorption, residue and loss of nitrogen fertilizer are closely related. The research of Ke et al. (2017) and Liu et al. (2019) showed that with the increase of fertilization, the absorption rate and residue rate of nitrogen fertilizer will decrease and the loss rate will increase. Different irrigation modes also affect nitrogen utilization efficiency to a certain extent (Pan et al., 2009). Sun et al. (2010) proposed that under the condition of conventional irrigation and flooded irrigation, the optimal fertilization amount is 180 kg/hm^2. Wu et al. (2020) proposed that under the condition of alternate wetting and drying, the application of 180 kg/hm^2 nitrogen fertilizer will achieve a synergistic and efficient utilization of water and fertilizer.

Hunan Province is the main rice-producing area in China, and different nitrogen application rates can significantly affect the soil nitrogen mineralization capacity, nitrogen supply capacity, and nitrogen uptake and utilization capacity. However, there are still few quantitative research reports on the fate of nitrogen fertilizer under the comprehensive utilization of water and fertilizer. ^{15}N tracer-based technology can quantitatively study the fate of nitrogen in the soil-rice system (Wang et al., 2007). In this experiment, ^{15}N tracer-based technology was used to study the effects of different nitrogen application rates on the absorption, transport, residue and loss of potted rice in soil and plants under different irrigation conditions, in order to achieve green, efficient, and high-yield rice production.

1 Results and Analysis

1.1 Effects of nitrogen application rate on nitrogen accumulation of rice plants in different growth stages with different irrigation methods

At tillering stage, the fertilizer nitrogen and total nitrogen accumulation in rice plants increased with the increase of nitrogen application rate (Table 1), and the soil nitrogen accumulation decreased first and then increased; Under N_{200} and N_{240} treatments, the fertilizer nitrogen accumulation with G2 treatment was significantly higher than that with G1 treatment. Under different nitrogen application conditions, the soil nitrogen accumulation with G2 treatment was significantly higher than that with G1 treatment. Under N_{160} and N_{200} treatments, the total plant nitrogen with G2 treatment was significantly higher than that with G1 treatment. At booting stage, with the increase of nitrogen application rate, the fertilizer nitrogen and total nitrogen accumulation in rice plants increased significantly. There was no significant difference in soil nitrogen accumulation under each nitrogen application rate treatment. There was no significant difference in the fertilizer nitrogen and soil nitrogen accumulation with G1 and G2 treatment. The total plant nitrogen accumulation with G2 treatment was higher than that with G1 treatment, but the difference was significant only under N_{160} and N_{200} treatment. At full heading stage, the fertilizer nitrogen and total nitrogen accumulation in rice plants increased significantly with the increase of nitrogen application rate, and the soil nitrogen accumulation was $N_{240} > N_{200} > N_0 > N_{160}$; The fertilizer nitrogen accumulation with G1 treatment under N_{240} treatment was significantly higher than that with G2 treatment, and there was no significant difference among other treatments. The soil nitrogen and total plant nitrogen accumulation with G2 treatment were higher than that with G1 treatment, but there was no significant difference in the total plant nitrogen accumulation. At mature period, the fertilizer nitrogen and total nitrogen accumulation in rice plants increased with the increase of nitrogen application rate. The soil nitrogen accumulation under N_{240} treatment was significantly higher than that under other nitrogen application treatments, N_{200} treatment was significantly higher than N_{160} and N_0 treatments, and there was significant difference between N_{160} and N_0 treatments. The fertilizer nitrogen accumulation with G1 treatment was higher than that with G2 treatment under different treatments, which was significant under N_{200} and N_{240} treatments, while the soil nitrogen accumulation with G2 treatment was higher than that with G1 treatment under different treatments, which was significant under N_{160} and N_{200} treatments, and the total plant nitrogen accumulation with G2 treatment was higher than that with G1 treatment under different nitrogen treatments, but there was no significant difference. It can be seen from the analysis of the whole growth period that with the increase of nitrogen application rate, the fertilizer nitrogen and total plant nitrogen accumulation increased significantly, the soil nitrogen accumulation basically showed the trend of $N_{240} > N_{200} > N_0 > N_{160}$, and compared with G1 treatment, G2 treatment can improve plant nitrogen absorption capacity under different nitrogen application treatments.

1.2 Effect of nitrogen application rate on nitrogen distribution in rice plants at maturity stage

With the increase of nitrogen application rate, different nitrogen accumulation in various organs of rice plant increased significantly (Table 2). Under different nitrogen application conditions, the accumulation of different sources of nitrogen is the highest in grain, and the distribution ratio was 67.20% ~ 69.02%. In terms of vegetative organs, the distribution of fertilizer nitrogen accumulation was leaf > stem > root, and the distribution of soil nitrogen accumulation and total plant nitrogen accumulation was stem > leaf > root, indicating that in rice plant nitrogen distribution, there are some differences between fertilizer nitrogen and soil nitrogen. The distribution of fertilizer nitrogen accumulation in rice leaves was higher in G1 than G2 under average nitrogen treatments, The distribution of soil accumulation in rice leaves showed that G2 was significantly higher than G1 under each nitrogen application treatment, and the distribution of total nitrogen accumulation in rice leaves was higher in G2 than G1 under average nitrogen treatments; There was no significant difference in fertilizer nitrogen accumulation between G1 and G2 at different nitrogen application levels, but soil nitrogen showed that G2 was significantly higher than G1 under N_{200} and N_{240} treatment, and the total nitrogen accumulation with G2 treatment was higher than that with G1 treatment under all nitrogen application levels, but the difference was significant only under N_0 and N_{160} treatment; The distribution of fertilizer accumulation in rice stems showed that G1 was significantly higher than G2 only under N_{240} treatment, while under N_{160}, N_{200} and N_{240} treatments, the soil nitrogen and total nitrogen accumulation with G2 treatment was higher than that with G1 treatment. In the nitrogen distribution of rice grain, there was basically no significant difference in fertilizer nitrogen accumulation, soil nitrogen accumulation and total nitrogen accumulation with G1 and G2 treatments under different nitrogen application rates, but the nitrogen accumulation with G2 treatment was higher than that with G1 treatment. Overall, G1 and G2 treatments had gains in nitrogen distribution of fertilizer nitrogen accumulation and soil nitrogen accumulation in rice organs, and G2 treatment was higher than G1 treatment in total nitrogen accumulation.

Table 1 Effects of nitrogen application rate on nitrogen accumulation of rice plants in different growth stages with different irrigation methods

N source	Water treatment	N treatment	Tillering stage	Booting stage	Full heading stage	Mature period
FN	G1	N_0	-	-	-	-
		N_{160}	29.57±0.45^c	44.18±9.51^b	46.63±1.91^c	49.84±3.66^d
		N_{200}	39.00±1.21^b	49.95±6.12^{ab}	53.43±3.42^{bc}	66.17±2.23^{bc}
		N_{240}	44.40±4.18^{ab}	57.29±2.42^a	67.72±10.87^a	81.97±10.25^a
	G2	N_0	-	-	-	-
		N_{160}	29.78±1.79^c	42.20±6.46^b	43.21±4.38^c	48.09±1.38^d
		N_{200}	41.22±3.41^{ab}	52.00±2.00^{ab}	53.43±3.51^{bc}	59.01±12.52^{cd}
		N_{240}	46.00±3.20^a	59.79±0.36^a	64.72±5.78^{ab}	80.27±6.76^{ab}
SN	G1	N_0	31.47±5.90^{bc}	57.54±3.80^a	75.98±1.49^{de}	88.06±3.32^{cd}
		N_{160}	25.63±2.81^c	56.62±1.43^a	58.78±3.57^f	78.02±9.27^d
		N_{200}	30.22±2.86^{bc}	63.55±2.25^a	82.72±2.57^{bcd}	100.35±5.32^{bc}
		N_{240}	38.02±1.4^{ab}	65.03±4.41^a	92.83±7.83^{ab}	121.22±5.99^a
	G2	N_0	37.59±1.89^{ab}	61.77±5.60^a	78.27±4.71^{cd}	91.6±6.56^{cd}
		N_{160}	32.31±0.50^{bc}	61.91±3.05^a	65.62±6.09^{ef}	84.57±11.65^{cd}
		N_{200}	37.91±2.15^{ab}	62.37±3.27^a	88.39±7.58^{bc}	115.88±12.69^{ab}
		N_{240}	41.29±4.31^a	65.66±3.99^a	101.34±3.79^a	131.61±5.74^a
PN	G1	N_0	31.47±5.90^d	57.54±3.80^d	75.98±1.49^d	88.06±3.32^d
		N_{160}	55.20±3.09^c	100.79±9.07^c	105.40±3.66^c	127.85±6.44^c
		N_{200}	69.22±1.70^b	113.49±4.53^{abc}	136.15±5.46^b	166.52±3.23^b
		N_{240}	82.43±2.84^a	122.32±4.09^a	160.55±6.98^a	203.19±4.32^a
	G2	N_0	37.59±1.89^d	61.77±10.60^d	78.27±4.72^d	91.6±6.56^d
		N_{160}	62.09±2.29^{bc}	104.11±4.88^{bc}	108.83±3.55^c	132.66±10.45^c
		N_{200}	79.13±7.83^a	114.37±4.09^{ab}	141.81±8.20^b	174.89±9.1^b
		N_{240}	87.29±2.89^a	125.45±4.34^a	166.06±4.91^a	211.88±10.2^a

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level ($P<0.05$); FN, SN and PN represent fertilizer nitrogen, soil nitrogen and total plant nitrogen, respectively

1.3 Effect of nitrogen application rate on post-anthesis nitrogen transfer from different sources of vegetative organs

The amount of nitrogen transformation from different sources in each vegetative organ of rice plant increased with the increase of nitrogen application rate (Table 3). In the comparison of amount of nitrogen transformation in organs: leaf > stem > root. The amount of nitrogen transformation from different sources in different vegetative organs of rice plants with G1 and G2 treatments did not show great difference under different nitrogen application treatments. In the transfer of total plant nitrogen, G1 treatment was higher than G2 treatment.

Table 2 Effect of nitrogen application rate on nitrogen distribution in rice plants at maturity stage

Organ	Water treatment	N treatment	FN	SN	PN	
					Accumulation (kg·hm^{-2})	Distribution ratio (%)
Leaf	G1	N_0	-	-	10.50±0.69^{c}	11.92
		N_{160}	6.84±0.58^{c}	7.88±0.93^{f}	13.49±1.12^{c}	12.14
		N_{200}	8.94±0.92^{bc}	10.13±1.49^{de}	19.54±1.97^{b}	10.55
		N_{240}	12.09±1.08^{a}	12.67±0.46^{bc}	24.25±1.71^{a}	10.59
	G2	N_0	-	-	11.12±0.96^{c}	11.73
		N_{160}	6.91±1.81^{c}	8.76±1.87^{ef}	14.05±1.69^{c}	13.67
		N_{200}	8.51±1.73^{bc}	14.95±0.36^{a}	23.91±2.22^{a}	11.94
		N_{240}	11.00±1.35^{ab}	13.58±1.24^{ab}	24.31±2.83^{a}	11.47
Root	G1	N_0	-	-	3.23±0.64^{e}	3.66
		N_{160}	1.77±0.55^{b}	3.58±0.48^{d}	5.14±0.60^{cd}	3.87
		N_{200}	2.32±0.20^{b}	4.36±0.55^{cd}	7.13±0.66^{b}	4.02
		N_{240}	3.44±0.05^{a}	5.59±1.15^{bc}	10.06±0.26^{a}	4.73
	G2	N_0	-		3.55±0.32^{de}	4.28
		N_{160}	1.65±0.24^{b}	3.55±0.32^{d}	6.28±0.37^{bc}	4.31
		N_{200}	2.12±0.87^{b}	5.05±1.12^{c}	7.53±2.06^{b}	4.95
		N_{240}	3.52±0.20^{a}	7.2±0.55^{a}	10.31±0.83^{a}	4.87
Steam	G1	N_0	-	-	15.16±1.38^{d}	17.22
		N_{160}	7.03±0.83^{b}	14.41±0.84^{bc}	20.98±1.01^{c}	15.00
		N_{200}	7.13±1.35^{b}	15.12±2.28^{bc}	23.51±4.09^{c}	16.41
		N_{240}	10.93±0.60^{a}	18.38±3.09^{ab}	28.9±3.29^{ab}	17.48
	G2	N_0	-	-	13.74±2.04^{d}	14.12
		N_{160}	6.68±1.01^{b}	16.59±2.42^{bc}	23.19±2.09c	14.52
		N_{200}	7.45±2.30^{b}	17.71±2.3^{abc}	25.39±2.71^{bc}	14.24
		N_{240}	10.15±0.76^{b}	21.02±1.43^{a}	31.55±2.13^{a}	14.89
Grain	G1	N_0	-	-	59.17±1.89^{d}	67.19
		N_{160}	19.27±4.26^{b}	58.81±3.04^{c}	88.25±5.60^{c}	68.98
		N_{200}	24.17±3.83^{b}	70.32±5.77^{bc}	116.35±5.52^{b}	69.02
		N_{240}	30.25±0.91^{a}	83.38±3.05^{a}	139.95±6.39^{a}	67.20
	G2	N_0	-		63.19±5.07^{d}	69.87
		N_{160}	20.78±2.25^{b}	61.26±9.17^{c}	89.14±8.10^{c}	67.50
		N_{200}	23.55±2.48^{b}	77.63±11.32^{b}	118.05±6.31^{b}	68.88
		N_{240}	30.75±2.34^{a}	89.97±4.07^{a}	145.71±8.14^{a}	68.77
Total	G1	N_0	-	-	88.06±3.32^{d}	-
		N_{160}	34.91±3.52^{c}	84.68±4.31^{c}	127.85±6.44^{c}	-
		N_{200}	42.56±4.20^{b}	100.35±5.32^{bc}	166.52±3.23^{b}	-
		N_{240}	56.70±0.79^{a}	121.22±5.99^{a}	203.19±4.32^{a}	-
	G2	N_0	-	--	91.6±6.56^{d}	-
		N_{160}	36.02±2.70^{bc}	91.24±13.44^{c}	132.66±10.45^{c}	-
		N_{200}	41.63±5.90^{bc}	115.88±12.69^{ab}	174.89±9.1^{b}	-
		N_{240}	55.42±1.37^{a}	131.61±5.74^{a}	211.88±10.2^{a}	-

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level (P<0.05); FN, SN and PN represent fertilizer nitrogen, soil nitrogen and total plant nitrogen, respectively

Table 3 Effect of nitrogen application rate on post-anthesis nitrogen transfer from different sources of vegetative organs

N source	Water treatment	N treatment	Amount of nitrogen transformation		
			FN	SN	PN
Root	G1	N_0	-	-	1.31±0.49[a]
		N_{160}	0.47±0.26[a]	1.35±0.21[a]	1.82±0.46[a]
		N_{200}	0.5±0.83[a]	2.12±1.04[a]	2.2±1.43[a]
		N_{240}	1.52±0.58[a]	2.93±2.53[a]	4.29±2.41[a]
	G2	N_0	-	-	1.4±1.74[a]
		N_{160}	0.83±0.39[a]	0.48±0.52[a]	1.73±0.61[a]
		N_{200}	1.04±0.37[a]	1.41±1.38[a]	2.45±1.61[a]
		N_{240}	1.35±0.69[a]	2.32±1.53[a]	3.83±2.46[a]
Steam	G1	N_0	-	-	5.61±2.72[bc]
		N_{160}	1.68±0.99[a]	4.23±1.85[bc]	5.91±1.87[bc]
		N_{200}	1.59±2[a]	11.45±3.01[a]	13.04±5.01[ab]
		N_{240}	7.29±4.06[a]	10.91±3.01[ab]	18.2±1.4[a]
	G2	N_0	-	-	6.89±0.91[bc]
		N_{160}	2.36±1.44a	1.11±5.16[c]	3.47±3.99[c]
		N_{200}	5.75±2[a]	7.44±3.41[abc]	13.19±2.4[ab]
		N_{240}	5.82±4.59[a]	12.18±3.19[a]	18±7.78[a]
Leaf	G1	N_0	-	-	21.36±4.35[c]
		N_{160}	12.75±0.53[b]	17.94±2.45[b]	30.69±2.08[bc]
		N_{200}	13.92±1.24[b]	28.61±1.82[a]	42.53±1.99[a]
		N_{240}	23.79±4.32[a]	24.2±4.91[ab]	47.99±3.61[a]
	G2	N_0	-	-	21.73±0.16[c]
		N_{160}	12.93±2.14[b]	18.6±6.25[ab]	31.53±4.18[bc]
		N_{200}	17.2±6.55[ab]	20.5±5.95[ab]	37.7±10.9[ab]
		N_{240}	22.43±4.38[a]	24.95±5.29[a]	47.38±7.93[a]
Total	G1	N_0	-	-	28.27±3.78[d]
		N_{160}	14.9±1.72[b]	23.52±3.45[bc]	38.41±2.76[cd]
		N_{200}	16.01±2.13[b]	42.18±0.31[a]	57.77±2.96[ab]
		N_{240}	32.6±8.93[a]	38.05±9.68[ab]	70.49±7.06[a]
	G2	N_0	-	-	30.03±2.46[d]
		N_{160}	16.12±3.74[b]	20.19±11.65[c]	36.73±7.43[d]
		N_{200}	23.99±8.48[ab]	29.36±10.05[abc]	53.34±13.88[bc]
		N_{240}	29.6±8.69[a]	39.45±7.68[ab]	69.2±14.57[ab]

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level. FN, SN and PN represent fertilizer nitrogen, soil nitrogen and total plant nitrogen, respectively

1.4 Nitrogen accumulation and its contribution to grain nitrogen after full heading

Increasing nitrogen application rate can significantly increase the nitrogen accumulation from different sources and improve its contribution to grain nitrogen after full heading (Table 4), indicating that increasing nitrogen application rate can promote the nitrogen accumulation after full heading. The contribution rate of nitrogen accumulation to grain nitrogen after full heading was 20.42% ~ 31.45%. The contribution rate of fertilizer nitrogen accumulation to grain fertilizer nitrogen after full heading was 16.66% ~ 50.57%, and the contribution rate of soil nitrogen accumulation to grain soil nitrogen after full heading was 32.72% ~ 37.38%, indicating that nitrogen transfer in vegetative organs was one of the important sources of fertilizer nitrogen, soil nitrogen and plant nitrogen in grains. G1 and G2 treatments had no significant difference in fertilizer nitrogen accumulation, but G2 treatment increased the soil nitrogen and plant nitrogen accumulation of rice plants after full heading.

Table 4 Nitrogen accumulation and its contribution to grain nitrogen after full heading

Water treatment	N treatment	FN		SN		TN	
		Accumulation	Contribution rate (%)	Accumulation	Contribution rate (%)	Accumulation	Contribution rate (%)
G1	N0	-	-	-	-	12.08±4.81[c]	20.42
	N160	3.21±3.66[a]	16.66%	19.24±11.5[a]	32.72	22.45±9.35[bc]	25.44
	N200	12.74±4.16[a]	52.71%	17.63±4.34[a]	25.07	30.37±6.48[abc]	26.10
	N240	14.25±7.55[a]	47.11%	28.39±7.84[a]	34.05	42.64±11.21[ab]	30.47
G2	N0	-	-	-	-	13.33±2.44[c]	21.10
	N160	4.87±5.4[a]	23.44%	18.95±7.6[a]	30.93	23.83±12.42[bc]	26.73
	N200	5.59±5.81[a]	23.74%	27.49±7.05[a]	35.41	33.08±16.88[abc]	28.02
	N240	15.55±12.31[a]	50.57%	30.27±7.69[a]	37.38	45.82±14.64[a]	31.45

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level (P<0.05); FN, SN and PN represent fertilizer nitrogen, soil nitrogen and total plant nitrogen, respectively

1.5 Effects of nitrogen application rates on 15N in soil residual and loss at mature period of rice.

Under different irrigation modes, the trend of soil fertilizer nitrogen residue and nitrogen loss was consistent (Table 5). Average two irrigation treatments, the soil fertilizer residue rates of rice with N_{160}, N_{200} and N_{240} treatments were 39.85%, 35.36% and 31.99% respectively, and the nitrogen loss rates were 29.56%, 33.35% and 34.22% respectively. With the increase of nitrogen application rate, the soil fertilizer nitrogen residue increased significantly, but the residue rate showed a downward trend, and the nitrogen loss increased significantly.

Table 5 Effects of nitrogen application rates on 15N in soil residual and loss at mature period of rice

Water treatment	N treatment	Soil fertilizer nitrogen residue	Residual rate (%)	Nitrogen loss	Loss rate (%)
G1	N_{160}	63.43±0.98[c]	39.64	46.74±2.91[c]	29.21
	N_{200}	70.05±1.07[b]	35.02	63.78±2.27[b]	31.89
	N_{240}	75.69±1.44[a]	31.54	82.34±11.18[a]	34.31
G2	N_{160}	64.08±0.08[c]	40.05	47.84±1.46[c]	29.90
	N_{200}	71.4±2.22[b]	35.70	69.59±14.5[ab]	34.80
	N_{240}	77.84±1.51[a]	32.43	81.89±5.26[a]	34.12

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level

1.6 Effects of nitrogen application rate on the yield and composition of early rice under different irrigation patterns

The increase of nitrogen fertilizer can significantly increase the panicle number and grains per spike and reduce the seed setting rate. The 1000-grain weight increased first and then decreased with the increase of nitrogen application rate (Table 6). N_{200} treatment was the highest, and with an average of two irrigation treatments, the theoretical grain yield with N_{160}, N_{200} and N_{240} treatment was 47.86%, 61.69% and 51.87% higher than that with N_0 treatment respectively. The actual yield was 56.40%, 68.18% and 62.20% respectively, and N_{200} treatment was also the highest. It shows that when a certain amount of nitrogen application was reached, increasing fertilization will not only not increase yield, but even affect the seed setting rate and 1000-grain weight, resulting in yield reduction. The theoretical yield and actual yield of G1 treatment were higher than those of G2 treatment under N_0, N_{160}, N_{200} and N_{240} treatment. Although it was not significant, it showed that G1 treatment could increase production.

Table 6 Effects of nitrogen application rate on the yield and composition of early rice under different irrigation patterns

Water treatment	N treatment	Paniclenumber ($\times 10^4 \cdot hm^{-2}$)	Grains per spike	Seed setting rate (%)	1000-grain weight (g)	Theoretical yield (kg/hm^2)	actual yield (kg/hm^2)
G1	N0	185.83±3.04^{d}	112.04±3.17^{f}	88.68±0.62^{a}	25.57±0.02^{c}	4720.06±64.54^{d}	4112.25±151.08^{d}
	N160	266.11±2.87^{c}	119.65±2.41^{e}	85.71±0.65^{b}	25.58±0.01^{c}	6981.31±122.11^{c}	6556.18±55.68^{c}
	N200	281.72±1.36^{b}	123.57±0.87^{cd}	84.91±0.66^{bc}	25.82±0.02^{a}	7631.7±55.29^{a}	7038.16±60.76^{a}
	N240	296.3±3.1^{a}	128.6±1.82^{ab}	74.28±0.95^{d}	25.3±0.01^{d}	7160.02±10.33^{b}	6786.76±91.47^{b}
G2	N0	183.32±1.9^{d}	113.05±1.84^{f}	89.3±0.47^{a}	25.6±0.02^{bc}	4737.06±50.42^{d}	4271.67±182.95^{d}
	N160	266.13±2.74^{c}	119.29±1.57^{de}	86.08±1.16^{b}	25.63±0.01^{b}	7002.16±59.84^{c}	6555.96±124.22^{c}
	N200	283.06±1.76^{b}	125.28±0.62^{bc}	83.62±1.08^{c}	25.83±0.02^{a}	7659.33±73.48^{a}	7061.75±79.75^{a}
	N240	293.94±4.33^{a}	131.08±2.94^{a}	74.04±0.59^{d}	25.25±0.01^{e}	7202.16±63.27^{b}	6811.76±38.57^{b}

Note: Different small letters in the same column meant significant difference among treatments for the same N source at 0.05 level ($P<0.05$)

2 Discussion

^{15}N tracer-based technology can effectively track the fate of nitrogen in soil-crop system (Bronson et al., 2000). In this study, ^{15}N tracer-based technology was used to study the laws of nitrogen absorption, utilization, residue and loss of rice under different irrigation conditions and different nitrogen application conditions. The nitrogen fertilizer absorbed by rice mainly comes from fertilizer nitrogen and soil nitrogen (Yan et al., 2009). Pan et al. (2012) proposed that 32.58% ~ 38.50% of nitrogen accumulation of rice plants came from fertilizer nitrogen and 61.50% ~ 67.42% from soil nitrogen. Chen et al. (2016) proposed that 34.0% ~ 41.7% of nitrogen accumulation of rice plants came from fertilizer nitrogen and 58.3% ~ 66.0% from soil nitrogen. Under the experimental conditions, 33.74% ~ 40.34% of the nitrogen accumulation of rice plants came from fertilizer nitrogen and 59.66% ~ 66.26% from soil nitrogen, which was consistent with the previous results. The period from full heading to maturity is the key period of nitrogen absorption and distribution in rice. Bai et al. (2019) showed that the transfer of nitrogen stored in vegetative organs before full heading accounts for more than 60% of grain nitrogen. It was found that 2/3 of grain nitrogen accumulation came from nitrogen transport in vegetative organs of rice before full heading, which was consistent with the results of previous studies. Therefore, nitrogen management is one of the effective means to regulate the accumulation, transport and distribution of nitrogen in rice plants. Pan et al. (2012) proposed that about 35.54% of the chemical fertilizer applied to the rice field in that season was absorbed by the rice plant, and about 12.40% ~ 16.61% of the nitrogen remained in the soil, while 48.5% ~ 62.2% was lost to the environment through various ways. In this study, 32.43% ~ 40.05% of nitrogen remained in the soil and 29.21% ~ 34.31% of nitrogen lost. The reason may be that there was no field surface runoff and leaching in the pot experiment, which needs to be verified by field research in the future. At the same time, this study found that with the increase of nitrogen application level, the nitrogen accumulation in rice plants increased, but the nitrogen use efficiency decreased, which was consistent with previous studies (Lin et al., 2012). In conclusion, reasonable nitrogen management can meet the total fertilizer demand of rice during the growth period, balance the demand for nitrogen at different growth stages of rice and the relationship between soil nitrogen supply, reduce the loss rate of nitrogen, and fully ensure the effective utilization of fertilizer nutrients, so as to improve the nitrogen utilization rate and grain yield of rice.

Reasonable water-saving irrigation can improve the nitrogen utilization efficiency of rice (Zhang et al., 2018). The research on the effect of alternate wetting and drying on the nitrogen utilization efficiency of rice has different results. Some researchers believe that soil nitrification and denitrification will be strengthened and N_2O emissions will increase under alternate wetting and drying conditions, resulting in decreased nitrogen accumulation in rice plants and reduced nitrogen utilization efficiency (Sah et al., 1983; Eriksen et al., 1985; Dong et al., 2018). However, Wang (2016) showed that the alternate wetting and drying model can improve the nitrogen absorption, the productivity per unit nitrogen absorption and the partial productivity of nitrogen fertilizer in rice plants. The results showed that alternate wetting and drying could significantly improve the nitrogen accumulation in different organs of rice at different growth stages, the storage nitrogen transport capacity, transport efficiency and the contribution of transported nitrogen to grain nitrogen. This may be because the alternate wetting and drying model can give full play to the coupling effect of water and fertilizer, promote the activity of nitrogen metabolism enzymes in roots, improve the photosynthetic rate and maximum photochemical efficiency of leaves, so as to improve the efficient utilization of nitrogen (Xu et al., 2020). The alternate wetting and drying technology can improve water use efficiency and achieve the purpose of water saving, but the results of its impact on rice yield are different, which may be caused by different degrees of soil drying, different soil texture and different ambient temperature for rice growth (Bouman et al., 2001; Belder et al., 2005; Wang et al., 2011). This study found that the alternate wetting and drying technology can increase production to a certain extent, however, it is not significantly improved, which can be caused by enhancement of root activity, leaf cytokinin content and photosynthetic rate under alternate wetting and drying (Bian et al., 2017). Reasonable water management can save water resources, improve nitrogen uptake and utilization of rice, and meet the requirements of high yield and high efficiency cultivation of rice.

This study summarized the laws of nitrogen absorption, utilization, residue and loss of rice under different irrigation conditions and different nitrogen application conditions, and verified that alternate wetting and drying and nitrogen application of 200 kg/hm were more conducive to improve nitrogen utilization and yield of rice, but there were some limitations due to the similar soil texture and environmental temperature in pot experiment. Therefore, for more reasonable nitrogen application and water management of rice, it is also necessary to increase different nitrogen application gradient treatment in combination with field experiment, and carry out further research by using ^{15}N tracer-based analysis technology.

3 Materials and Methods

3.1 Test materials

The pot experiment soil was selected from the Garden Base of Hunan Agricultural University. The pH value was 5.31, alkali-hydrolyzable nitrogen was 193.4 mg/kg, available phosphorus was 88.6 mg/kg, available potassium 110.8 was mg/kg, total nitrogen was 1.68 g/kg, total phosphorus was 1.82 g/kg, total potassium was 7.15 g/kg and organic matter was 36.7 g/kg. The super rice variety was Zhongjiazao 17 provided by the Agriculture and Rural Bureau of Heshan District of Yiyang City. The nitrogen fertilizer was ^{15}N labeled urea (abundance 10.24%) provided by Shanghai Research Institute of Chemical Industry. The experiment was carried out in the Rice Institute of Hunan Agricultural University from April to August in 2019.

There are two factors: irrigation mode and nitrogen application rate. The irrigation modes were 2 types, namely conventional irrigation (G1, turning green in deep water, tillering in shallow water, dry the field when enough seedlings, heading with water, dry and wet grouting) and alternate wetting and drying (G2, in the process of rice growth, keep the water layer for a period of time, naturally dry until the soil is not seriously dry and cracked, then irrigate, then dry, and then irrigate, so on); Nitrogen application rates were 4 levels, N_0, N_{160}, N_{200} and N_{240}, respectively 0 kg/hm^2, 160 kg/hm ^{2} (2.4574 g urea per basin), 200 kg/hm^2 (3.071 7 g urea per basin) and 240 kg/hm^2 (3.686 1 g urea per basin).

The pot experiment used a plastic bucket with a diameter of 30 cm and a height of 25 cm. Before potting soil, mix the soil evenly, measure the soil moisture, and then load the basin. Take part of the soil to measure the basic soil nutrients, and load 7 kg of soil into each basin. Isotope nitrogen fertilizer was applied at one time. Other nutrient inputs were as follows: 75 kg/hm^2 potassium fertilizer, 75 kg/hm^2 phosphorus fertilizer. Potassium fertilizer was applied according to the ratio of base fertilizer to panicle fertilizer of 1:1. All phosphorus fertilizer was used as base fertilizer. The urea, calcium superphosphate and potassium chloride were used as nitrogen fertilizer, phosphorus fertilizer and potassium fertilizer respectively. In the pot experiment, rainproof treatment was conducted during the whole growth period.

3.2 Measurement index and method

Changes of the content of soil nitrogen.

Dry matter accumulation: select 3 points at full tillering stage, booting stage, full heading stage, milk mature stage and mature stage to measure the amount of dry matter of the plant. Dig out the whole plant from the field and separate the aboveground part from the underground part. The aboveground part shall be separated according to the stem, leaf and spike, de-enzyme in an oven at 105℃ for 30 minutes, dried to constant weight at 80℃, cooled to room temperature, and then weighed with an electronic balance. Put the underground part into a nylon net bag, wash the roots with running water, gently absorb the adsorbed water with filter paper, and weigh after drying.

Nitrogen utilization: in the Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences, plant total nitrogen was measured by K-05 automatic nitrogen determinator (Shanghai Sonnen Automated Analysis Instrument Co., Ltd.) and ^{15}N abundance was analyzed by Thermo-fisher delta V advantage IRMS.

Yield and yield composition: before maturity, the remaining plants sampled in the early stage were counted the effective panicle number, and the plant height, panicle length, total grains per spike, seed setting rate and 1000-grain weight were measured. Calculate the theoretical output and actual output.

3.3 Calculation method

Nitrogen utilization efficiency = fertilizer nitrogen accumulation in plants / nitrogen application rate × 100%

Plant nitrogen accumulation = plant dry matter weight × plant nitrogen content

Proportion of plant nitrogen from fertilization = ^{15}N atom percentage excess % of plant with fertilizer treatment / ^{15}N atom percentage excess % of fertilizer × 100%

Plant fertilizer nitrogen accumulation = plant nitrogen accumulation × Proportion of plant nitrogen from fertilization

Plant soil nitrogen accumulation = plant nitrogen accumulation - plant fertilizer nitrogen accumulation

Plant nitrogen transport = nitrogen accumulation in vegetative organs at full heading stage - nitrogen accumulation in vegetative organs at mature period

Contribution rate of nitrogen accumulation to grain nitrogen after full heading stage = (plant nitrogen accumulation at mature period - plant nitrogen accumulation at full heading stage) / grain nitrogen accumulation at mature period × 100%

Fertilizer nitrogen residue (kg/hm^2) = unit land area (m^2) × soil layer thickness (cm) × unit weight (g/cm^3) × soil nitrogen content (%) × ten × proportion of soil residual nitrogen from fertilizer

Fertilizer nitrogen loss (kg/hm^2) = nitrogen application rate - plant fertilizer nitrogen accumulation - soil fertilizer nitrogen residue

Nitrogen recovery rate = fertilizer nitrogen accumulation of rice plants at mature period / nitrogen application rate × 100%

Nitrogen residue rate = soil fertilizer nitrogen residue of rice at mature period / nitrogen application rate × 100%

Nitrogen loss rate = fertilizer nitrogen loss of rice at mature period / nitrogen application rate × 100% (Li et al., 2020).

3.4 Data analysis

Microsoft Excel 2010 and SPSS.20 software were used for statistical analysis of data.

Authors' contributions

ZHJ, ZT and WXH were the designers of the experiment and the executors of this study; ZHJ and SAL completed the data analysis and wrote of the first draft of the manuscript; ZHJ and WY participated in the experimental design and analyzed the experimental results; WXH was the conceiver and person in charge of the project, guiding experimental design, data analysis, thesis manuscript and revision. All authors read and approved the final manuscript.

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