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Density-Dependent Fertilization of Nitrogen for Optimal Yield of Perennial Rice

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Abstract: In the absence of tillage, perennial rice is an innovation and supplement to rice production. Proper N fertilizer application connected to planting density has been proposed as an effective way to improve rice yields. The tradeoff between crop N uptake and N supply is essential for optimal N management and soil environment benefit in the perennial rice cropping system. To assess the response of perennial rice to N fertilizer and planting density, field experiments with four consecutive growing seasons within two years, from 2016 to 2017, were conducted in southern China. Four nitrogen rates (N0, N1, N2, and N3 refer to 0, 120, 180, and 240 kg N ha^{-1}, respectively) combined with three planting densities (D1, D2, and D3 refer to 100 × 10^3, 167 × 10^3 and 226 × 10^3 plants ha^{-1}, respectively) were designed. The results showed that both N rate and planting density significantly affected crop production (p < 0.05), N uptake and soil N balance. Specifically, the N2D3 mode could achieve sustainable and higher dry matter accumulation (15.15 t ha^{-1}) and grain yield (7.67 t ha^{-1}) among all the treatments over the four seasons. A positive relationship between N uptake and dry matter/grain yield was observed. The N2D3 mode showed significantly higher N uptake (201 kg ha^{-1} each season) and less soil N loss (27.1%), relative to C.K. Additionally, the N2D3 mode could reach the optimal N balance (−0.2 kg ha^{-1}) with a low N requirement (23.9 kg N Mg^{-1} grain), resulting in higher N use efficiency (NAE: 26.5 kg N kg^{-1}, NRE: 64.9%). In the perennial rice cropping system, therefore, 180 kg N ha^{-1} integrated with 226 × 10^3 plants ha^{-1} could deliver higher grain yields with less N requirement, higher N use efficiency and less soil N loss. This optimal combination between planting density and nitrogen rate can result in soil N balance for sustainable perennial rice production.

Keywords: N balance; N fertilizer; N uptake; perennial rice cropping system; soil N loss

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1. Introduction

Due to the ongoing growth of the world population, the demand for food is under great pressure [1,2]. Rice is the staple food for more than half of the world's population, which faces more pressure than any other grain [3]. In rice production, chemical fertilizer addition, increasing the planting density and improving cultivated area are proposed to increase yields [4–6]. Due to the restricted arable land [7], fertilizer addition and increasing planting density are proposed as the main ways to improve the yield [8]. In particular, fertilizer has been proposed as the primary method for the strong desire of farmers to pursue high grain yields [9]. Overfertilization has been a common phenomenon for farmers. However, excessive or inappropriate fertilization does not always contribute to a high grain yield, which may result in low fertilizer use efficiency and cause a series of environmental problems [10–12]. The overall rice production mainly relies on annual rice with a plowing cropping system, which is an intensive work for farmers that needs seeds, seedling, plowing, transplanting, crop management and harvest etc. annually, especially in terraces and mountains [13]. Annually plowing in the long term also intensifies soil erosion and degradation, which is not conducive to sustainable soil production and the environment [14].

Perennial rice is bred by the clone characteristics of the rhizome of *Oryza longistaminata* and could survive and produce for several successive seasons or years [13,15]. With the release of perennial rice cultivar 23 (PR23) in 2018, the revolution of rice production caused by perennial crops has started. From the second season or year, perennial rice could ratoon from the rhizome of the stubble of last season and produce for successive years [13,15–17]. Without tillage, seeding and transplanting, perennial rice reduced labor and material input, resulting in considerable economic profit for farmers [13,15]. The absence of tillage always reduces soil erosion and enhances soil properties, would achieve sustainable and environmental rice production and balance ecological and food security [15,18].

Nitrogen (N) is the essential element for perennial rice production. Increasing N fertilizer rate and planting density have been regarded as the most effective ways to improve the rice yield significantly [8,19]. However, unreasonable N management would result in low crop yields, along with severe environmental problems [20]. Generally, the averaged N application was 225 kg N ha^{-1} and N fertilizer utilization efficiency was 35% in croplands of China [21], which often caused serious N loss and pollution. The optimal N fertilizer of perennial rice is often highly dependent on the planting density and soil productivity. Due to the short term of perennial rice release, the response of perennial rice to nitrogen and planting density in the perennial cropping system is still unclear. Based on grain yield increase, evaluating the response of perennial rice to N rate and planting density, soil N balance and loss, and N requirement could help us formulate optimal N management and access the soil environment in the perennial rice cropping system. Formulation of optimized N and planting density management would provide scientific guidance for farmers to plant rice environmentally. Thus, a field experiment with four N rates integrated with three planting densities was conducted to assess the dry matter accumulation and grain yield, plant nitrogen uptake and requirement, soil nitrogen balance and loss of perennial rice. The objective of this paper was to explore the response of perennial rice to N fertilizer and planting density, evaluate the productivity and soil nitrogen balance and loss in a perennial rice field, and formulate and provide proper N fertilizer management in a no tillage-perennial rice cropping system.

2. Materials and Methods

2.1. Site Description

This study was performed over four successive seasons from 2016 to 2017 at the Perennial Rice Research Station of Yunnan University, located in Gasa town (20°57′22″ N, 100°45′43″ E, altitude 555 m), Jinghong, southwestern China—a typically double rice area, which is characterized by a tropical monsoon climate. The average sum of rainfall recorded in the years 2016 and 2017 was 927.7 mm and 1342.6 mm (Figure 1), respectively, and most rainfall occurred from June to October. The average monthly temperature was 23.8 °C (Figure 1).

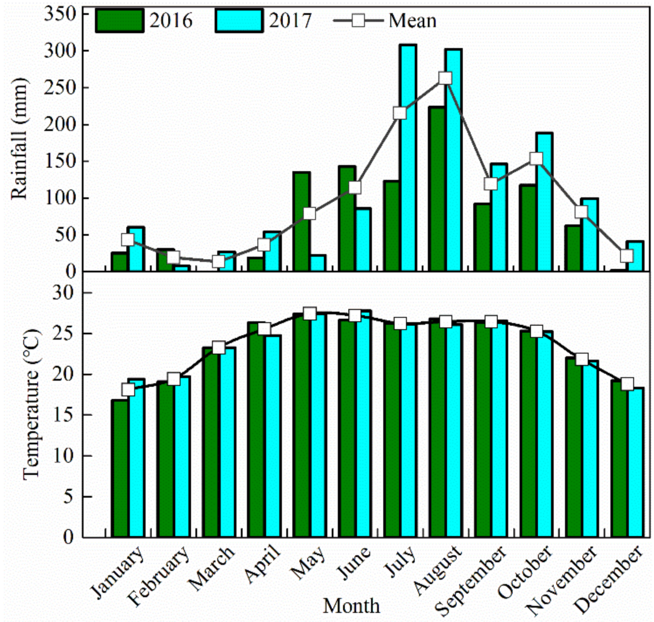

Figure 1. Rainfall and temperature in experimental site Jinghong from 2016 to 2017.

Before 2016, the prevailing rice system-annual rice with plowing annually was conducted in the trail field. The soil was classified as a ferritic soil with 5.05 pH, 34 g kg^{-1} soil organic matter, 2.1 g kg^{-1} soil total nitrogen, 156 mg kg^{-1} available soil nitrogen, 7.6 mg kg^{-1} available soil phosphorus and 139 mg kg^{-1} available soil potassium.

2.2. Experimental Design

A spilt-plot experiment with three replicates was applied over four successive seasons from 2016 to 2017, as 2016F (first season) and regrowth seasons 2016S, 2017F and 2017S. Four N rates, N0, N1, N2 and N3, with 0, 120, 180, and 240 kg N ha^{-1} applied respectively were used as the main plots. Three planting densities included D1, D2 and D3 with 100 $\times$ 10^3, 167 $\times$ 10^3 and 226 $\times$ 10^3 plants ha^{-1}, respectively, were used as subplots (Figure 2a). These four N rates and three planting densities generated the following twelve combinations: N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2 and N3D3 (Figure 2a), each of them was with an area of 20 m^2 size.

The cultivar perennial rice 23 (PR23) was selected as the material that was sowed on 15 Dec 2015 and transplanted in a plowing and level field on 30 January 2016, and harvested in late June and October each year (Figure 2c). After the harvest of each season, the rice stubble was cut back 5–10 cm above the ground to maintain the uniformity of new tillers arising from rhizomes and to depress tillers from the stem. The new tillers that emerged from the rhizome of the rice stubble were only maintained for successive regrowth seasons (2016S, 2017F and 2017S). Meanwhile, no-tilling was conducted across the successive regrowth seasons. During winter, perennial rice lies dormant in the soil and emerges when warmer temperatures return in the next year.

N fertilizer (urea) was manually and evenly spread at four stages 50% at the transplanting time for 2016F or new tillers emerging for regrowth seasons (2016S, 2017F and 2017S), 20% at the tilling stage, 20% at the heading stage and 10% at the filling stage, respectively. For all treatments, the fertilizer P and K were 90 kg ha^{-1} and 180 kg ha^{-1}, respectively. The P fertilizer was once applied as the base fertilizer each season. The K fertilizer was used as a rate of 4:4:2 at the transplanting or cutting stubble, heading stage and 20 days after heading (to keep the root activity and promote the new tillers of perennial rice. For different planting densities, the plant spacing for D1, D2 and D3 were 27, 20 and 17 cm, respectively, and row spacings for these were 37, 30 and 26 cm, respectively.

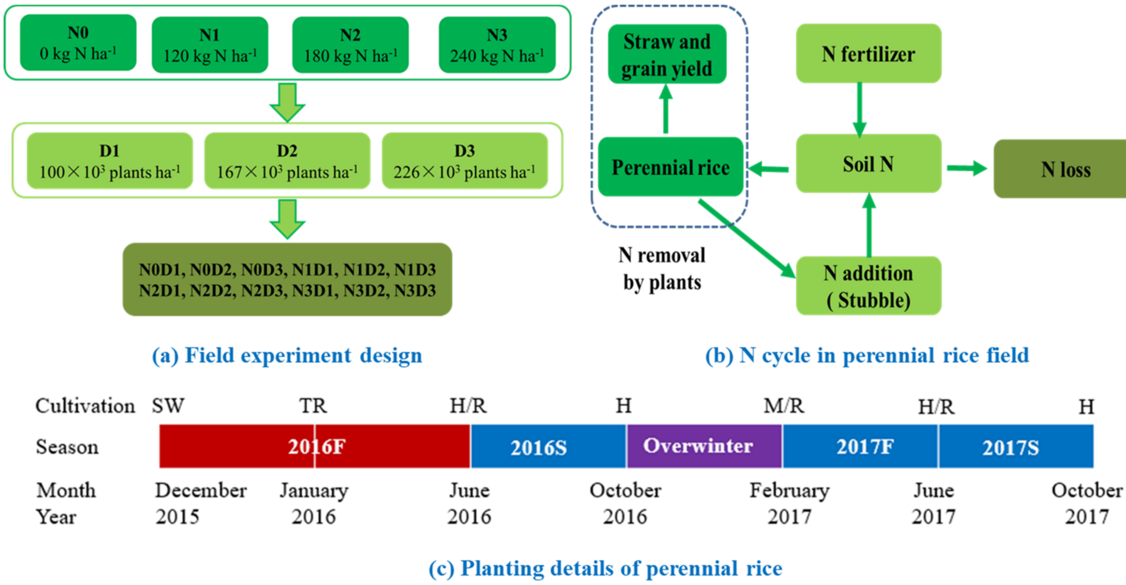

Figure 2. Field experiment design, planting details, and N cycle in the perennial rice field. (**a**) Field experiment design of different N rates and planting density. (**b**) N cycle in the perennial rice cropping system. (**c**) Planting details of perennial rice. SW, sowing. TR, transplanting. H, harvest. R, regrowth. M, stubble management (cutting back). 2016F, first season (red color) from sowing to the first harvest. 2016S, 2017F, and 2017S, three regrowth seasons (blue color) from regrowth to harvest each season. Overwinter, from the last harvest in the first year in winter to the first regrowth in the second year.

2.2.1. Irrigation Regime

The field was irrigated intermittently, and the details are as follows.

In the transplanting season of 2016, 3 cm water above ground was kept for 2 days and then plowed. Perennial rice was transplanted 2 days after plowing, and the field was kept in 3 cm water for 10 days. For the regrowth seasons of 2016 and 2017, the field should be kept 3 cm in water for 10 days after cutting rice stubble. When the rice leaf turns green in the transplanting season or the stubble regrowth is 1–2 tillers, the field should be kept in 1–2 cm water until the tillering stage to promote the tillering of perennial rice. When the tillers reached 75% of the objective total tillers, the field would be naturally dried to control the tillers. When the jointing stage is reached, 2–3 cm water should be kept until the heading stage of perennial rice. Fifteen days after heading, the rainy month arrives and the rice does not need more irrigated water for growth, meaning we naturally dried the field until harvest. In the winter, the field needs certain moisture to keep perennial rice alive and overwinter.

2.2.2. Field Management

Weeds Control

In general, we sprayed herbicide 5–7 days after transplanting in the first season or after tiller emergence in the regrowth seasons. Prometryn was applied to soil to control the gramineous weeds, broadleaf weeds and *Cyperaceae* weeds. Cyhalofop-butyl or fluroxypyr was used to control perennial weeds.

Pest Control

The main pests in this area are rice planthopper and Cnaphalocrocis. Thiamethoxam and pymetrozine were used to control the rice planthopper. Dursban and indoxacarb were used to control Cnaphalocrocis. The usage of pesticides was according to the emergence and condition of pests each year.

Disease Control

Perennial rice has high resistance to rice blast. This is derived from the parent of *Oryza longistaminata,* which has high resistance to rice blast and a strong rhizome. Therefore, the main disease in perennial rice field are *Xanthomonas oryzae*, *Riziocotinia solani* and *Ustilaginoidea virens*. We controlled these three diseases at the tillering stage, metaphase differentiation of the young panicle or the start of heading. Azoxystrobin and tricyclazole were used to control *Xanthomonas oryzae*, chloroisobromine cyanuric acid and thiediazole copper were used to control *Riziocotinia solani*, validamycin and isoprothiolane were used to control *Ustilaginoidea virens*.

2.3. Sampling and Analytical Methods

2.3.1. Grain Yield

At harvest time, grain yield and dry matter were manually harvested at an area greater than 5 m^2, and grain yield was weighted and adjusted to a 14% water content.

2.3.2. Soil and Plant Nitrogen

Soil nitrogen (N) and plant N were determined by using the Kjeldahl method [22]. Soil samples were taken at five points as "S" at 0–20 cm soil and dried naturally without sunshine, then milled by a grinding mill and sieved through a 0.25 mm screen for soil nitrogen analysis. The plant samples were collected and divided into grain, stem, leaf in three sections at harvest time, fixed at 105 °C and dried at 75 °C by using an air dry oven, then milled by a grinding mill and crushed and sieved through a 0.25 mm screen for plant nitrogen analysis. Plant N uptake, soil N loss, N balance, N requirement and N physical effect were calculated by the formulae as follows [23–25]:

$$\text{N uptake (kg ha}^{-1}\text{)} = \text{N\% in grain} \times \text{Yg} + \text{N\% in stem} \times \text{Ys} + \text{N\% in leaf} \times \text{Yl} \tag{1}$$

$$\text{N input (kg ha}^{-1}\text{)} = \text{N application} + \text{N addition by stubble} \tag{2}$$

$$\text{N balance (kg ha}^{-1}\text{)} = \sum \text{N}_{\text{input}} - \text{N}_{\text{uptake}} \tag{3}$$

$$\text{N loss (kg ha}^{-1}\text{)} = \text{soil based N variation (sowing-harvest)} + \text{N input} - \text{N uptake} \tag{4}$$

$$\text{N requirement (kg Mg}^{-1}\text{ grain)} = \text{plant N uptake/Yg} \tag{5}$$

$$\text{N agronomic efficiency (NAE) (kg N kg}^{-1}\text{)} = \text{grain yield (Ni-N0)/N application} \tag{6}$$

$$\text{N recovery efficiency (NRE) (kg N kg}^{-1}\text{)} = \text{pant N uptake (Ni-N0)/N application} \tag{7}$$

where Yg is the grain yield, Ys is the stem yield, Yl is the leaf yield, $i \geq 1$.

2.4. Statistical Analysis

Split-plot analysis with three-way ANOVA (N rate and planting density were set as two fixed factors, and the season was set as a random factor) was used to assess differences of the significance of the main plot and subplot and interactions of the treatments. Before ANOVA, tests on normality (by a Shapiro–Wilks test of the residuals) and homoscedasticity (by a Bartlett test) were conducted. In cases when homogeneity of variances was not given by the original data, we classified the data, recombined the data in SPSSAU, and then the data met the requirements of ANOVA. Three replications were calculated for each measurement, and one-way ANOVA was used to compare the effects of the different treatments on the measured variables [15]. F-tests were conducted, and multiple comparisons were performed using the least significant difference test (L.S.D.) ($p \leq 0.05$). We analyzed the experimental data with the IBM SPSS statistical package v.20.0 (SPSS, Inc., Chicago, IL, USA), and the figures were generated using Origin 2015 (Sys Software, Inc., Northampton, MA, USA, 2015).

3. Results

3.1. Yield

3.1.1. Grain Yield

There was a significant difference in the grain yield of different treatments ($p < 0.05$) (Table 1). Season ($p < 0.001$), nitrogen ($p < 0.001$), density ($p < 0.05$) and the interaction effects of nitrogen with density ($p < 0.05$) and season, nitrogen and density ($p < 0.01$) all decided the grain yield of perennial rice. For the effects of N fertilizer, the N1, N2 and N3 significantly increased the grain yield by 82.2%, 148% and 141% compared with N0 (2.69 t ha^{-1}) ($p < 0.05$). For the planting densities, the D2 (5.34 t ha^{-1}) and D3 (5.64 t ha^{-1}) showed significantly higher grain yields than the D1 (4.59 t ha^{-1}) ($p < 0.05$). In the four seasons, N2D3 resulted in a significantly higher average grain yield, which was 7.67 t ha^{-1}.

Table 1. Dry matter accumulation of perennial rice under different N rates and planting densities over four seasons of 2016–2017.

Treatment	Leaf (t ha^{-1})	Stem (t ha^{-1})	Panicle (t ha^{-1})	Dry Matter (t ha^{-1})	Grain (t ha^{-1})
Season					
2016F	1.08 ± 0.43 b	3.96 ± 1.18 b	7.50 ± 2.39 a	12.54 ± 3.96 a	7.26 ± 2.15 a
2016S	1.13 ± 0.33 b	4.65 ± 1.76 a	4.71 ± 1.84 c	10.49 ± 2.76 b	4.42 ± 1.27 b
2017F	1.50 ± 0.13 a	4.00 ± 1.64 b	5.17 ± 1.43 bc	10.68 ± 2.54 b	4.76 ± 1.74 b
2017S	1.36 ± 0.47 a	3.28 ± 1.04 c	5.79 ± 1.89 b	10.43 ± 2.75 b	4.32 ± 2.05 b
N rates					
N0	0.91 ± 0.23 c	2.55 ± 1.01 d	3.75 ± 1.00 c	7.22 ± 2.45 c	2.69 ± 0.97 d
N1	1.16 ± 0.33 b	3.88 ± 1.56 c	5.56 ± 1.46 b	10.59 ± 2.77 b	4.90 ± 1.66 c
N2	1.47 ± 0.46 a	4.59 ± 2.11 b	7.07 ± 1.92 a	13.14 ± 4.37 a	6.68 ± 1.76 a
N3	1.54 ± 0.47 a	4.87 ± 1.79 a	6.78 ± 2.14 a	13.19 ± 4.09 a	6.48 ± 1.48 b
Planting density					
D1	1.16 ± 0.41 c	3.40 ± 1.69 c	4.94 ± 2.03 c	9.49 ± 4.15 c	4.59 ± 1.82 c
D2	1.26 ± 0.43 b	3.96 ± 1.72 b	5.80 ± 2.01 b	11.02 ± 4.03 b	5.34 ± 2.24 b
D3	1.40 ± 0.45 a	4.56 ± 1.79 a	6.63 ± 2.08 a	12.59 ± 4.15 a	5.64 ± 2.36 a
ANOVA	F-value				
S (df = 3)	7.330 **	9.120 **	5.792 *	2.729 (ns)	15.599 ***
N (df = 3)	17.185 ***	54.708 ***	11.529 **	28.399 ***	36.502 ***
D (df = 2)	12.319 **	22.538 **	10.116 *	25.797 ***	9.332 *
N × S (df = 9)	4.303 **	4.041 **	5.639 ***	9.051 ***	9.047 ***
D × S (df = 6)	1.391 (ns)	4.129 **	2.701 *	4.018 *	4.071 **
N × D (df = 6)	1.524 (ns)	2.979 *	2.554 (ns)	5.088 **	7.399 ***
N × D × S (df = 18)	1.757 *	1.040 (ns)	2.967 ***	1.916 *	2.521 **

Different letters within a column represent significant differences at $p < 0.05$ (LSD). S: season. N: nitrogen rate. D: planting density. N × S: interaction effect between nitrogen rate and season. D × S: interaction effect between planting density and season. N × D: interaction effect between nitrogen rate and planting density. N × D × S: interaction effect between nitrogen rate, planting density, and season. * represents significance at $p < 0.05$, ** represents significance at $p < 0.01$, *** represents significance at $p < 0.001$, ns represents no significance.

3.1.2. Dry Matter Accumulation

In 2016–2017, the dry matter accumulation of perennial rice is shown in Figure 3. The dry matter of regrowth seasons (2016S, 2017F and 2017S) remained stable with the transplanting season (2016F), which was significantly affected by season ($p < 0.01$), nitrogen ($p < 0.001$) and density ($p < 0.01$) and interact effect of nitrogen and density ($p < 0.001$) (Table 1). When the N rate and planting density increased, the dry matter of leaf, stem and panicle increased. N2D3 showed the highest aboveground dry matter accumulation (15.46 t ha^{-1}) in four seasons (Table 1); the leaf, stem, and panicle weight were 1.67, 5.34 and 8.45 t ha^{-1}, respectively, followed by N3D3 (15.15 t ha^{-1}). For the effect of N fertilizer, N1, N2 and N3 significantly improved aboveground dry matter accumulation (leaf, stem and grain weight) compared to N0. The increments were by 43.2%, 77.5% and 77.1%, respectively ($p < 0.05$). For the effect of planting density, the D3 and D2 significantly increased the aboveground dry matter accumulation by 38.6% and 19% when compared to D1 ($p < 0.05$).

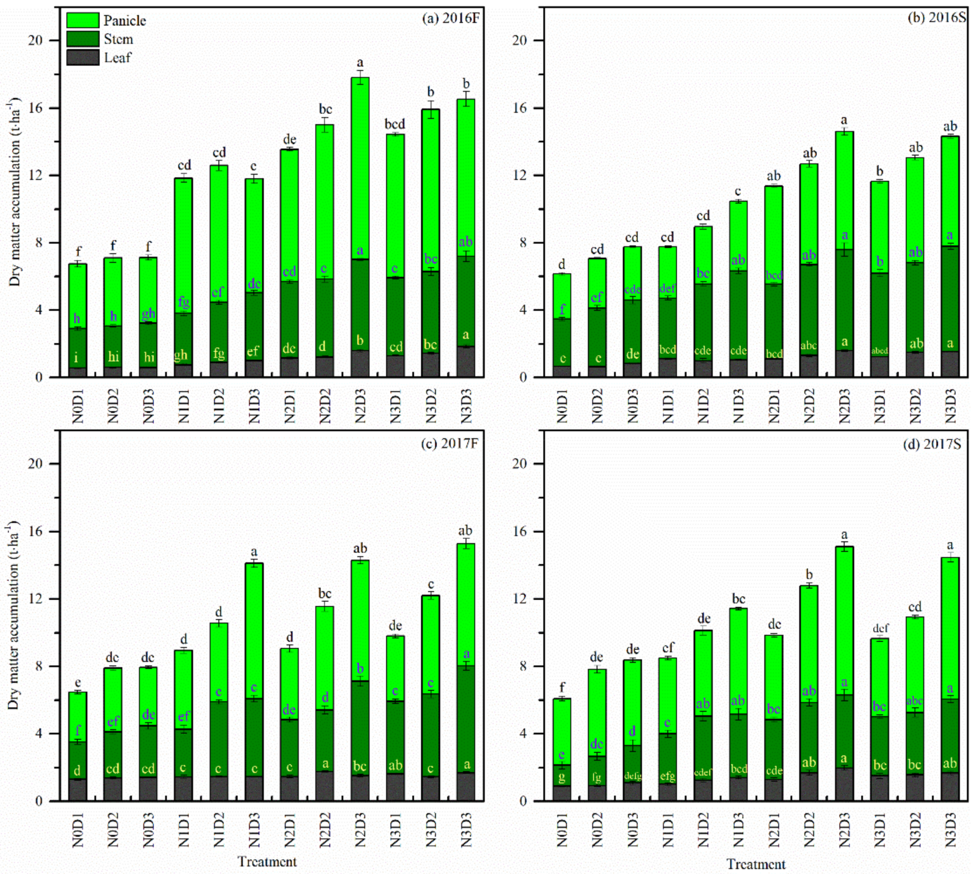

Figure 3. Dry matter accumulation of different treatments. (**a**) Dry matter accumulation in 2016F. (**b**) Dry matter accumulation in 2016S. (**c**) Dry matter accumulation in 2017F. (**d**) Dry matter accumulation in 2017S. Dry matter accumulation, including the dry matter of stem, leaf, and grain. 2016F, the first season of 2016 (transplanting season). 2016S, the second season of 2016 (regrowth season). 2017F, the first season of 2017 (regrowth season). 2017S, the second season of 2017 (regrowth season). Bars with different letters represent a significant difference at $p < 0.05$. The yellow letter represents the difference in the leaf. The purple letter represents the difference in the stem. The dark letter represents the branch and a difference in the panicle.

In the first season of 2016 and 2017, the panicle accounted for a large proportion of dry matter, which was 50.46–56.03%, and the straw (leaf and stem) accounted for 43.97–49.54% (Figure 3). In the second season (2016S, 2017S), the straw accounted for a large proportion of dry matter, which was 54.39–62.67%, and the panicle accounted for 37.33–45.61%.

3.2. Plant N Uptake

N rate and planting density significantly affected the N uptake of perennial rice ($p < 0.05$) (Figure 4); N uptake of perennial rice was stable in the first and second season, respectively. Compared to the second season (28.7–59.9%), perennial rice uptake and transfer of N in grain was higher in the first season (49.5–78.3%). The N uptake of grain accounted for 54.5–59.7% of aboveground plant N content and in 2016F, 2016S, 2017F and 2017S, these values were 73.5%, 50.6%, 65.1% and 37.9%, respectively. When the N rate and planting density increased, N uptake by stem, leaf and grain increased (Figure 4). For the N uptake by grain, N0, N1, N2 and N3 were 43, 73, 95 and 95 kg ha^{-1}, respectively and D1, D2 and D3 were 66, 74 and 89 kg ha^{-1}, respectively. For the N uptake by stem, N3 and D3 showed the highest value, 57 and 55 kg ha^{-1}, respectively. N uptake by leaf, N3, and D3 showed the highest value, 18 and 16 kg ha^{-1}. N3D3 and N2D3 showed the highest averaged N uptake values across the four seasons, which were 204 and 201 kg ha^{-1}, respectively.

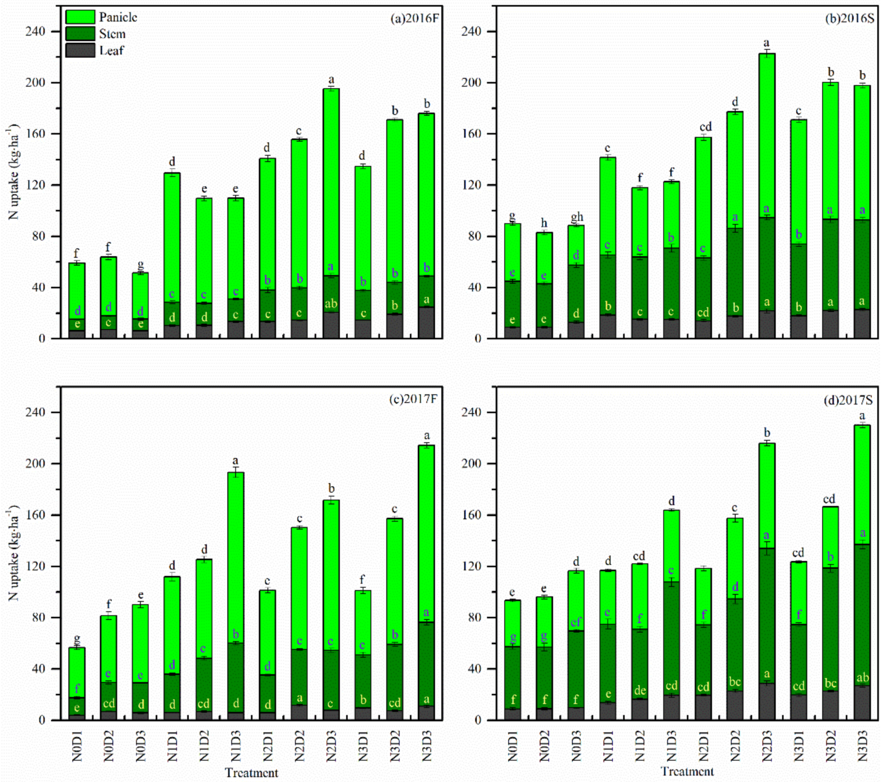

Figure 4. N uptake of aboveground dry matter. (**a**) N uptake in 2016F. (**b**) N uptake in 2016S. (**c**) N uptake in 2017F. (**d**) N uptake in 2017S. Plant N uptake, including the N uptake of stem, leaf, and grain. 2016F, the first season of 2016 (transplanting season). 2016S, the second season of 2016 (regrowth season). 2017F, the first season of 2017 (regrowth season). 2017S, the second season of 2017 (regrowth season). Bars with different letters represent a significant difference at $p < 0.05$. The yellow letter represents a difference in the leaf. The purple letter represents the difference in the stem. The dark letter represents the difference in the panicle.

After accounting for all treatments, the dry mater, straw (stem and leaf) and grain yield were significantly and positively related to the N uptake ($p < 0.01$) (Figure 5). The high N uptake of grain, straw yield and dry matter in N2D3 and N3D3 resulted in high dry matter and grain yield.

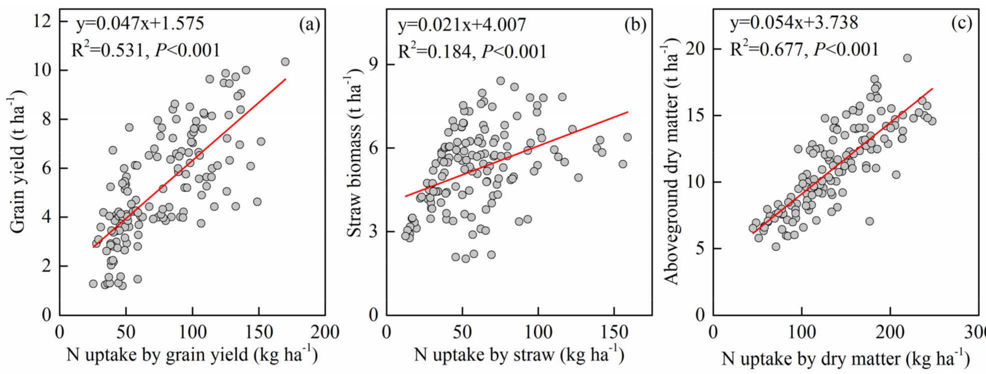

Figure 5. The relationship of N uptake with grain yield, straw biomass, and aboveground dry matter. (**a**) Relationship of N uptake by grain and grain yield. (**b**) N uptake by straw (stem and leaf) and straw biomass. (**c**) Relationship of N uptake by dry matter and aboveground dry matter.

3.3. Soil N Cycle

3.3.1. Soil N

In the four seasons, the soil N was significantly affected by season ($p < 0.001$) and the interactional effects of season. The interactional effect of season, N rate with planting density ($p < 0.001$) and N rate with planting density ($p < 0.05$) significantly affected the soil N (Table 2), neither N rate nor planting density had a significant effect on soil N (Figure 6). As the growth season continued, the soil N declined significantly ($p < 0.05$).

Table 2. N uptake and loss of perennial rice under different N rates and planting densities over four successive seasons of 2016–2017.

Treatment	N Uptake (kg ha^{-1})	N Loss (kg ha^{-1})	Soil N (g kg^{-1})
Season			
2016F	124.67 ± 47.33 a	98.42 ± 54.93 a	2.20 ± 0.05 a
2016S	147.33 ± 47.57 a	96.46 ± 56.88 a	2.14 ± 0.10 b
2017F	128.50 ± 48.85 a	104.81 ± 65.72 a	1.92 ± 0.07 c
2017S	143.50 ± 44.28 a	90.40 ± 68.74 a	1.83 ± 0.05 d
N rate			
N0	79.92 ± 19.10 d	12.29 ± 4.83 d	2.00 ± 0.17 a
N1	130.25 ± 24.93 c	93.52 ± 21.18 c	2.03 ± 0.18 a
N2	163.67 ± 35.65 b	109.67 ± 30.90 b	2.03 ± 0.17 a
N3	170.17 ± 37.68 a	171.60 ± 21.05 a	2.02 ± 0.17 a
Planting density			
D1	115.44 ± 42.45 c	109.31 ± 57.50 a	2.01 ± 0.17 a
D2	133.31 ± 40.93 b	100.67 ± 56.05 b	2.01 ± 0.16 a
D3	159.25 ± 41.59 a	82.58 ± 51.20 c	2.04 ± 0.17 a
ANOVA	F-value		
S (df = 3)	1.096 (ns)	0.987 (ns)	58.506 ***
N (df = 3)	38.063 ***	129.932 ***	0.612 (ns)
D (df = 2)	8.054 *	24.154 **	0.759 (ns)
N × S (df = 9)	3.514 *	5.008 **	0.876 (ns)
D × S (df = 6)	6.307 ***	1.616 (ns)	1.783 (ns)
N × D (df = 6)	7.758 ***	17.010 ***	2.673 *
N × D × S (df = 18)	2.585 **	2.289 **	4.560 ***

Different letters within a column represent significant differences at $p < 0.05$ (LSD). S: season. N: nitrogen rate. D: planting density. N × S: interaction effect between nitrogen rate and season. D × S: interaction effect between planting density and season. N × D: interaction effect between nitrogen rate and planting density. N × D × S: interaction effect between nitrogen rate, planting density, and season. * represents significance at $p < 0.05$, ** represents significance at $p < 0.01$, *** represents significance at $p < 0.001$, ns represents no significance.

3.3.2. Soil N Removal and Loss

In the perennial rice cropping system, soil N is mainly taken by the plant removal (N uptake by plants) (Figure 2b), and there were significant differences among the different treatments (Figure 7 and Table 2). With the increment in N rate, the N removal by perennial rice significantly increased ($p < 0.05$) and N0, N1, N2 and N3 were 80, 130, 164 and 170 kg ha^{-1}, respectively, but there was no significance between N2 and N3 ($p < 0.05$). For different planting density, when the density increased, N removal by plants increased significantly ($p < 0.05$); D1, D2 and D3 were 154, 178 and 212 kg ha^{-1}, respectively. In the four seasons, N3D3 and N2D3 resulted in the highest N removal values, which were 204 and 201 kg ha^{-1}, respectively.

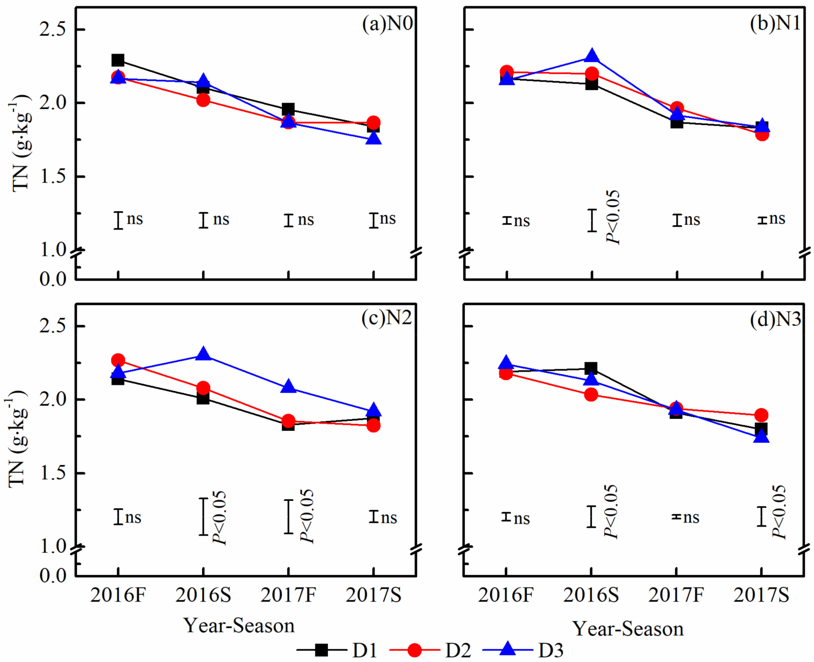

Figure 6. Soil total nitrogen (TN) of different treatments. (**a**) soil total nitrogen in 2016F. (**b**) soil total nitrogen in 2016S. (**c**) soil total nitrogen in 2017F. (**d**) soil total nitrogen in 2017S. 2016F, the first season of 2016 (transplanting season). 2016S, the second season of 2016 (regrowth season). 2017F, the first season of 2017 (regrowth season). 2017S, the second season of 2017 (regrowth season). Vertical bars represent the standard error for different treatments. $p < 0.05$ represent a significant difference among other therapies, and ns mean no difference among treatments.

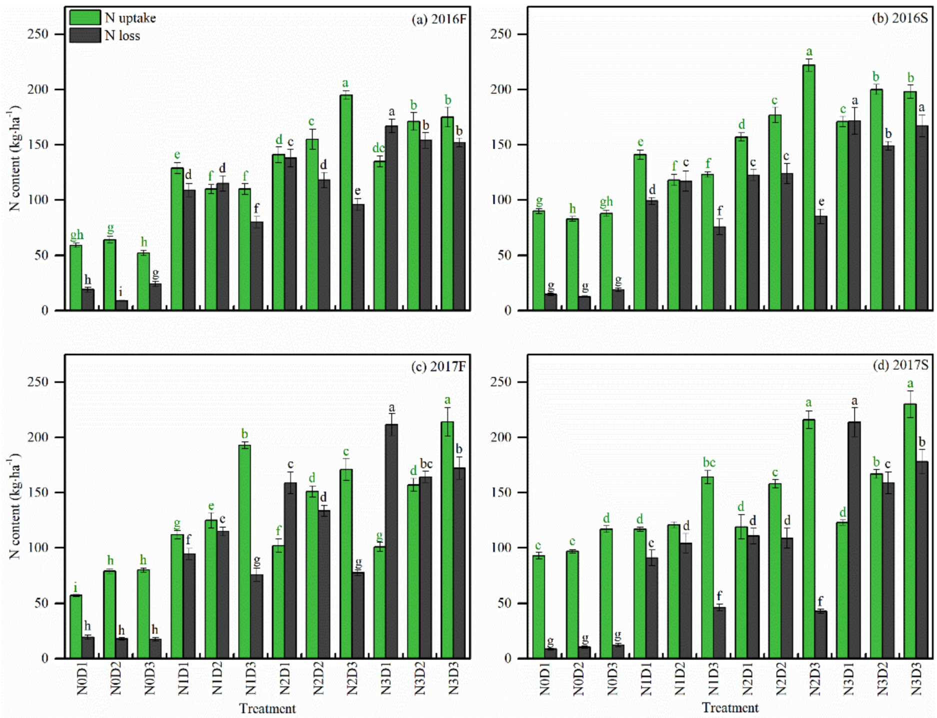

Figure 7. N removal by plants and soil N loss in the perennial rice cropping system. (**a**) N removal and soil N loss in 2016F. (**b**) N removal and soil N loss in 2016S. (**c**) N removal and soil N loss in 2017F. (**d**) N removal and soil N loss in 2017S. Bars with different letters represent significant differences at $p < 0.05$. The green letter represents the difference in N uptake. The dark letter represents a difference in N loss.

Different from the N removal by plants, soil N loss and loss rate increased with the increase in N rate and decrease in planting density (Figure 7 and Table 2). The soil N losses in N0, N1, N2 and N3 were 15, 94, 110 and 172 kg ha^{-1}, respectively, but there was no significance between N1 and N2 ($p < 0.05$). The soil N loss rates of N0, N1, N2 and N3 were 16.8%, 41.8%, 40.3% and 50.6%, respectively, while soil N loss in D1, D2 and D3 were 146, 134 and 110 kg ha^{-1}, respectively. The soil N loss rate of D1, D2 and D3 were 57.3%, 50%.9 and 41.3%, respectively. High planting density significantly reduced soil N loss ($p < 0.05$). N3D1 resulted in the highest soil N loss and loss rate, which was 191 kg ha^{-1} and 59.1%, respectively, and N0D2 resulted in the lowest value, which was 12 kg ha^{-1} and 13.3%, respectively (Figures 7 and 8d).

3.3.3. Apparent N Balance

Soil apparent N balance was calculated by the difference between soil N input and soil N removal. In the perennial rice cropping system, soil N input includes N fertilizer application and decomposition of rice stubble (Figure 2b). The N input by rice stubble is mainly related to the N rate in straw and the biomass of straw. A high N rate and planting density would lead to high N input for perennial rice (Figure 8c). In the four seasons, N input by stubble of N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2 and N3D3 were 8.4, 9.2, 10.8, 12.9, 13.2, 16.9, 13.1, 17.3, 20.8, 14.9, 19.7 and 22.2 kg ha^{-1}, respectively. According to the soil N input and soil N removal, soil N balance of N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2 and N3D3 were −66.3, −71.6, −73.5, 8.1, 14.7, −10.6, 63.4, 37.0, −0.2, 122.3, 85.9 and 57.9 kg ha^{-1}, respectively (Figure 8e). In the four seasons, N2D3 achieved the soil N balance among all treatments.

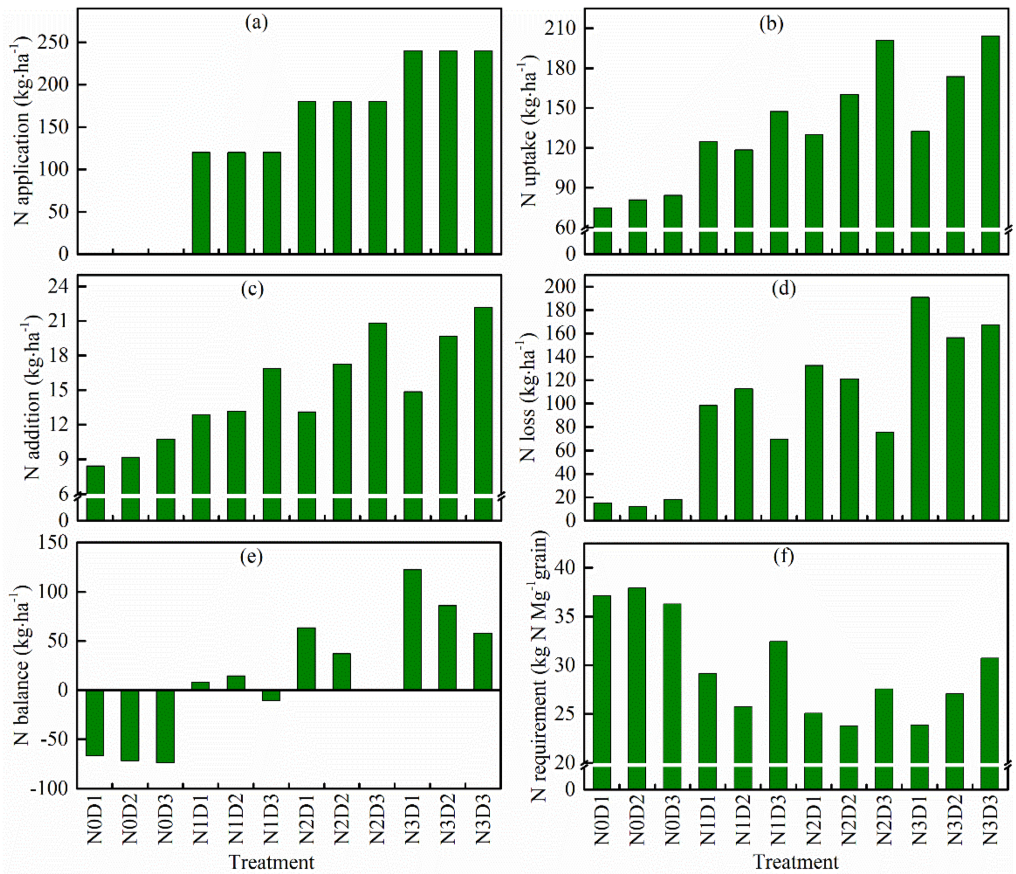

Figure 8. Soil N balance and N requirement under different N rates and plating densities in the perennial rice cropping system. (**a**) N application. (**b**) N uptake. (**c**) N addition. (**d**) N loss. (**e**) N balance. (**f**) N requirement.

3.4. N Effects and Requirement

N agronomic efficiency (NAE) and N recovery efficiency (NRE) are important indicators of the N fertilizer effect. With the increment in nitrogen, NAE and NRE increased and N2 resulted in better N effects (NRE: 46.5%, NAE: 22.2 kg N kg^{-1}) (Figure 9). D3 performed a better N effect for planting density, NRE was 55.9%, and NAE was 20.3 kg N kg^{-1}. In the four seasons, N2D3 resulted in the best N effect and NAE and NRE were 64.9 kg N kg^{-1} and 26.5%, respectively.

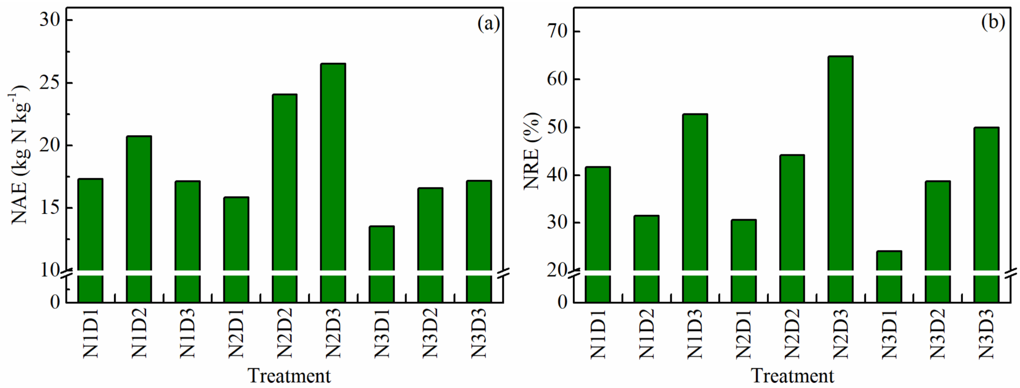

Figure 9. N agronomic efficiency and requirement under different N rates and plating densities in the perennial rice cropping system. (**a**) N agronomic efficiency (NAE) of other further additional treatments. (**b**) N recovery efficiency (NRE) of different treatments.

The N requirement refers to the amount of N required to produce 1 Mg of the rice grain, which is an important indicator to evaluate the N effect in perennial rice cropping system. In four seasons, the N requirement of perennial rice averaged 29.7 kg N Mg^{-1} grain, and the N requirement of N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2, and N3D3 were 37.1, 37.9, 36.3, 29.2, 25.7, 32.4, 25.1, 23.8, 27.6, 23.9, 27.1 and 30.7 kg N Mg^{-1} grain, respectively (Figure 8f).

4. Discussion

4.1. Dry Matter Accumulation

Despite the high yield potential in the transplanting season, the sustainable dry matter and grain yield of perennial rice over regrowth seasons illustrated that perennial rice has a high and sustainable yield potential over the years (Table 1). Increasing fertilizer and planting density have been proposed as effective ways to improve rice yields [8,26]. When the N fertilizer and planting density increased, the grain yields increased to a certain extent [4,12]. In accordance with the annual rice, grain yield and dry matter accumulation of perennial rice showed the same response to N fertilizer and planting density (Figure 3 and Table 1). However, the improvement of fertilizer did not always result in a high crop yield, but sometimes low fertilizer use efficiency and more fertilizer runoff, thus causing a series of economic and environmental problems [27]. The more N fertilizer in N3 did not result in a significantly higher grain yield but it did result in more soil N loss and low N use efficiency (NAE and NRE) in the perennial rice cropping system. Additionally, N fertilizer and planting density often have an interaction effect on rice yield [15]; dry matter and grain yield of perennial rice are significantly affected by the interactional effect of N rate, planting density with the season, season with N rate and season with planting density. The proper N fertilizer rate and planting density in N2D3 are conducive to soil nitrogen absorption and crop production. The high N uptake in N2, N3, and D3 would lead to a high grain yield and dry matter accumulation by the positive relationship of N uptake with straw and grain yield (Figure 5). Although the N3 also resulted in a high grain yield and dry matter in four seasons as with N2, the high soil N loss and low N use efficiency would lead to high N erosion risk and less economic profit. Proper N application and planting density could help to obtain a high grain yield and dry matter accumulation, improve N use efficiency and reduce soil N erosion [28]. The optimal combination of the N rate with planting density in N2D3 resulted from the highest dry matter accumulation and grain yield in a perennial rice cropping system.

4.2. Plants N Uptake and N Use Efficiency

N is the essential element for perennial rice production. Plant N uptake is closely related to dry matter accumulation and grain yield [29]. Recent literature reported that increasing crop nutrient uptake has emphasized the need for greater synchrony between crop nutrient demand and the nutrient supply from all sources throughout the growing season [30,31]. A proper high N rate could help plants absorb more N for production [32,33] and perennial rice should also show the same result. The N uptake of perennial rice significantly increased with the increase in N rate and planting density. N3D3 and N2D3 showed exceptionally high N uptake, but there was no significant difference between them. The N3 did not increase the N uptake of perennial rice but led to more soil N loss when compared with N2. Excessive N fertilizer input leads to luxury N absorption but also enhances soil N loss and leaching [10,11]. In four seasons, N uptake of perennial rice remained stable. In the first season, N uptake by plants was mainly transferred into grain yield, leading to a high grain yield of perennial rice. However, in the second season, more N was absorbed by straw, and then the grain yield was lower than that in the first season. The lower N uptake in the second season was one of the main reasons for the low yield of perennial rice.

In recent years, more and more fertilizer loss and pollution have appeared in the field by the desire for a higher crop yield, causing more environmental problems [12]. In China, the fertilizer use efficiency was 30–35%, which was far below that in the world [28]. So, we need to improve the N fertilizer use efficiency when pursuing a high crop yield. NAE and NRE were effective indicators in order to evaluate fertilizer use efficiency. Higher NAE and NRE values meant the fertilizer could produce higher grain yields and increase fertilizer use efficiency [34]. The high NAE and NRE in N2 and D3 stated that perennial rice could utilize N fertilizer efficiently in this nitrogen and planting density, resulting in the best N effect in the N2D3 mode.

The N requirement is also an effective indicator to evaluate the N fertilizer use efficiency and productivity, which refers to the N requirement to produce 1 Mg grain [23]. The low N requirement stated that working to the exact grain yield requires less fertilizer. The common N requirement in N2D3 indicated that N would produce a higher grain yield and have high and efficient use efficiency with less N loss and pollution in this mode. The highest NAE and NRE and proper N requirement in N2D3 also illustrated that the N fertilizer effect was the best in this mode. The perennial rice would produce a higher grain yield and less fertilizer loss and pollution and obtain more economic profit.

4.3. N Cycle and Balance

Soil N is the main soil nutrient for crop production. Soil N supply and balance immediately decided the crop productivity [35]. The soil N decreased as the experiment continued in the perennial rice field; this may be attributed to two reasons. First, the continuous high crop yield of perennial rice brought excessive nitrogen from the field, but the applied nitrogen could not compensate for this. Second, the no-till system with frequent irrigation in the perennial rice cropping system carried more nitrogen leaching and decomposition of soil organic matter. The N2D3 treatment reached the N balance of N input and output but decreased soil nitrogen in the field. This may be the increased N leaching carried by no-tillage with frequent irrigation. The majority of crop N came from the soil. If soil N was balanced in terms of inputs and outputs, the gaps between soil N consumption and fertilizer N replenishment would imply that other forms of exogenous N compensated for the soil N deficits, such as N deposition and biotic N fixation [36]. In the perennial rice cropping system, the source of soil N includes soil base N, N fertilizer application, and N from some stubble decomposition, while the output of soil N includes N taken by plants and soil N loss. Maintaining soil N balance is the premise for sustainable rice production. In this study, the N2D3 mode resulted in almost soil N balance in the field. The minus N balance in N0 would lead to soil degradation and a reduction in crop yields. If exogenous N replenishment was lower than soil N consumption, it would hardly sustain the soil N supply capacity, eventually leading to soil fertility degradation and crop yield reductions [37,38]. In contrast, the high N balance in N3 would lead to surplus N and more soil N loss, which would result in serious environmental problems.

5. Conclusions

Studying the N utilization and N cycle in the perennial rice cropping system helps us to evaluate the N effects and soil N loss and formulate optimal N management for sustainable perennial rice production. In this study, the N2D3 mode resulted in a higher and more sustainable grain yield and dry matter accumulation with better N effects (NAE and NRE). Additionally, perennial rice under N2D3 mode uptake more N nutrients from the soil and this resulted in less soil N loss that could maintain the apparent N balance. In the perennial rice cropping system, N2D3 (180 kg N ha^{-1} integrated with 226 × 10^3 plants ha^{-1}) mode was the optimal N dependent planting density for sustainable production and soil N balance with less soil N loss and pollution.

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