N Balance and Requirements Under Different N Management Practices in a No-Till Perennial Rice Cropping System

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N balance and requirements under different N management practices in a no-till perennial rice cropping system

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Abstract

Aims: In the absence of tillage, perennial rice is an innovation and supplement to rice production. Evaluating crop N uptake and N requirements and maintaining soil N balance are essential for informing decisions regarding optimal N management and the accessibility of the soil environment benefits of perennial rice cropping systems.

Methods: To assess the soil nitrogen cycle and balance, formulate optimal N fertilizer management for perennial rice, a field experiment with four nitrogen rates (N0, N1, N2 and N3 refer to 0, 120, 180 and 240 kg N ha^{-1}, respectively) integrated with three planting densities (D1, D2 and D3 refer to 100×10^3, 167×10^3 and 226×10^3 plants ha^{-1}, respectively) was conducted for two years over four seasons (2016-2017) in southern China.

Results: The results showed that N2D3 mode could sustainably produce higher dry matter accumulation (15.15 t ha^{-1}) and grain yields (7.67 t ha^{-1}) over four seasons, showed significantly higher N uptake (201 kg ha^{-1} each season) and less soil N loss (27.1%). Additionally, the N2D3 mode could reach the optimal N balance (-0.2 kg ha^{-1}) in perennial rice fields with low N requirements (23.9 kg N Mg^{-1} grain), resulting in higher N use efficiency (NAE: 26.5 kg N kg^{-1}, NRE: 64.9%).

Conclusion: In the perennial rice cropping system, 180 kg N ha^{-1} integrated with 226×10^3 plants ha^{-1} resulted in higher grain yields with lower N requirements, higher N use efficiencies, and lower soil N losses, thereby maintaining the soil N balance for sustainable perennial rice production.

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

1. Introduction

Due to the ongoing growth of the world's population, the demand for food is under great pressure (Godfray et al., 2010; Seck et al., 2012). Rice is a staple food for more than half of the world's population and faces more pressure than any other grain (Deng et al., 2019). In rice production, chemical fertilizer addition, increasing planting density, and improving cultivated area are proposed as effective ways to increase yields (Hayashi et al., 2006; Xu et al., 2017; Naylor et al., 2007). Due to the restricted amount of arable land available (Liu et al., 2005), fertilizer addition and increasing planting density are proposed as the primary ways to improve yield (Hou et al., 2019). Fertilizer addition is proposed as the main method due to farmers' desire to maximize grain yield (Lutes et al., 2016). Overfertilization has been a common phenomenon for farmers, but excessive or inappropriate fertilization does not always contribute to high grain yield and may result in low fertilizer use efficiency and cause a series of environmental problems (Li et al., 2018; Jing et al., 2007; Zhang et al., 2018). The prevailing rice production mainly relies on annual rice with plowing cropping systems, which is intensive work for farmers, especially in terraces and mountains (Huang et al., 2018). Annual plowing also intensifies soil erosion and degradation, which is not conducive to sustainable soil production (Aziz et al., 2013).

Perennial rice is bred by the clone characteristics of the rhizome of *Oryza longistaminata* and can survive and produce for several successive seasons or years (Huang et al., 2018; Zhang et al., 2021). With the release of perennial rice cultivar 23 (PR23) in 2018, the revolution of rice production caused by perennial crops began. From the second season or year, perennial rice could ratoon from the rhizome of the stubble of the last season and produce for the successive years (Zhang et al., 2017; Zhang et al., 2019; Huang et al., 2018; Zhang et al., 2021). Without tillage, seeding, and transplanting, perennial rice reduces labor and material input, resulting in considerable economic profit for farmers (Huang et al., 2018; Zhang et al., 2021). The absence of tillage always reduces soil erosion and enhances soil properties, achieves sustainable and environmentally beneficial rice production, and balances ecological and food security (Pimentel et al., 2012; Zhang et al., 2018).

Nitrogen (N) is an essential element for perennial rice production, and increasing the N fertilizer rate and planting density has been regarded as way to considerably improve rice yield (Hou et al., 2019; Wu et al., 2020). However, unreasonable N management could result in low crop yields along with serious environmental problems (Tilman et al., 2011). Proper N and planting density management formulated for perennial rice would help farmers produce rice environmentally and scientifically. Based on grain yield increases, evaluating the response of perennial rice to N rate and planting density, soil N balance and loss, and N requirements could help us formulate optimal N management and access the soil environment in perennial rice cropping systems. Due to the short-term perennial release of rice, the response of perennial rice to N and planting density management in perennial cropping systems is still unclear. Thus, a field experiment of four N application rates integrated with three planting densities was conducted to assess the dry matter accumulation and grain yield, plant nitrogen uptake and requirement, and 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 management, evaluate the productivity and soil nitrogen balance and loss in perennial rice fields, and formulate and provide proper N fertilizer management in a no-till 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 the town of Gasa (N 20°57′22″, E 100°45′43″, altitude 555 m), Jinghong, Yunnan Province, southern China, i.e., a typical double rice area that is characterized by a tropical monsoon climate. The average rainfall and temperature are 1136.6 mm and 23.3°C, respectively, and most rainfall occurs from April to October. Before 2016, prevailing annual rice plowing was conducted in the trial field. The soil was classified as a ferralitic soil with a soil pH of 5.05, a soil organic matter content of 34 kg kg^{-1}, and a total soil nitrogen content of 2.1 kg kg^{-1}, a total available soil nitrogen content of 156 mg kg^{-1}, a total available soil phosphorous content of 7.6 mg kg^{-1}, and a total available soil potassium content of 139 mg kg^{-1}.

2.2 Experimental design

A split-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 application rates of 0, 120, 180 and 240 kg N ha^{-1}, abbreviated as N0, N1, N2 and N3, respectively, were used as the main plots, and three planting densities, 100×10^{3}, 167×10^{3} and 226×10^{3} plants ha^{-1}, abbreviated as D1, D2 and D3, respectively, were used as subplots (Fig. 1a). These four N application rates and three planting densities generated 12 combinations, namely, N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2 and N3D3 (Fig. 1a), each of which covered an area of 20 m^{2}.

The cultivar perennial rice 23 (PR23) was selected as the material, which was sown on 15 Dec 2015, to be transplanted in a plowing and level field on 30 Jan 2016, and harvested in late June and October of each year (Fig. 1c). After harvesting was completed in 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 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 as 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 heading stage and 10% at the filling stage, respectively. For different planting densities, the plant spacings for D1, D2 and D3 were 27, 20 and 17 cm, respectively, and the row spacings for these were 37, 30 and 26 cm, respectively.

2.3 Sampling and analytical methods

2.3.1 Grain yield

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

2.3.2 Soil and plant nitrogen

Soil nitrogen (N) and plant N were determined by using the Kjeldahl method. Soil samples were taken at five points forming an "S" shape and sieved through a 0.25 mm screen for soil nitrogen analysis. The plant samples were collected and divided into three grain, stem, and leaf sections at harvest time, crushed, and then 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:

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

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

$$\text{N balance (kg ha}^{-1}\text{)} = \Sigma N_{input} - \Sigma N_{uptake}$$

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

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

N agronomic efficiency (NAE) (kg N kg^{-1}) = grain yield (Ni-N0)/N application

N recovery efficiency (NRE) (kg N kg^{-1}) = plant N uptake (Ni-N0)/N application

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

2.4 Statistical analysis

Split-plot analysis with three-way ANOVA (N rate and planting density were set as two fixed factors, and season was set as a random factor) was used to assess differences between the significance of the main plot and subplots and the interactions of the treatments. 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. F-tests were conducted, and multiple comparisons were performed using the least significant difference test (LSD) ($P \leq 0.05$). Experimental data were analyzed 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.).

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). The statistics describing the season ($P<0.001$), nitrogen ($P<0.001$), density ($P<0.05$) and interactive effects of nitrogen with density ($P<0.05$) and season, nitrogen and density ($P<0.01$) all determined the grain yield of perennial rice. For the effects of N fertilizer, N1, N2 and N3 significantly increased the grain yield by 82.2%, 148% and 141%, respectively, compared with N0 (2.69 t ha^{-1}) ($P<0.05$). For the planting densities, D2 (5.34 t ha^{-1}) and D3 (5.64 t ha^{-1}) had significantly higher grain yields than D1 (4.59 t ha^{-1}) (P<0.05). In the four seasons, N2D3 resulted in a significantly higher average grain yield of 7.67 t ha^{-1}.

3.1.2 Dry matter accumulation

The dry matter accumulation of perennial rice in 2016-2017 is shown in Fig. 2. The dry matter of the regrowth seasons (2016S, 2017F and 2017S) remained stable with the transplanting season (2016F), was significantly affected by season (P<0.01), nitrogen (P<0.001) and density (P<0.01), and interacted with nitrogen and density (P<0.001) (Table 1). As the N rate and planting density increased, the dry matter of the leaves, stems, and panicles increased. N2D3 showed the highest aboveground dry matter accumulation (15.46 t ha^{-1}) in the four seasons (Table 1), and the leaf, stem, and panicle weights 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 the aboveground dry matter accumulation (leaf, stem and grain weight) compared with N0, and the increases were 43.2%, 77.5% and 77.1%, respectively (P<0.05). For the effect of planting density, D3 and D2 significantly increased the aboveground dry matter accumulation by 38.6% and 19%, respectively, compared to D1 (P<0.05).

In the first seasons of 2016 and 2017, the panicle accounted for a large proportion of dry matter, at 50.46% to 56.03%, and straw (leaf and stem) accounted for 43.97% to 49.54% (Fig. 2). In the second season (2016S, 2017S), straw accounted for a large proportion of dry matter (54.39% to 62.67%), and panicles accounted for 37.33% to 45.61%.

3.2 Plant N uptake

The N application rate and planting density significantly affected the N uptake of perennial rice (P<0.05) (Fig. 3), and the N uptake of perennial rice was stable in the first and second seasons. Compared to the second season (28.7 to 59.9%), perennial rice absorbs and transfers more N in grain in the first season (49.5% to 78.3%). The N uptake of grain accounted for 54.5% to 59.7% of that by dry matter, and those in 2016F, 2016S, 2017F and 2017S were 73.5%, 50.6%, 65.1% and 37.9%, respectively. As the N application rate and planting density increased, N uptake by stems, leaves, and grains increased (Fig. 3). 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 stems, N3 and D3 showed the highest values of 57 and 55 kg ha^{-1}, respectively. For the N uptake by leaves, N3 and D3 showed the highest values of 18 and 16 kg ha^{-1}, respectively. In the four seasons, N3D3 and N2D3 showed the highest N uptake values of 204 and 201 kg ha^{-1}, respectively.

Accounted 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) (Fig. 4). The high N uptake of grain, straw yield, and dry matter in N2D3 and N3D3 resulted in a high dry matter and grain yield.

3.3 Soil N cycle

3.3.1 Soil N

Across all four seasons of perennial rice, the soil N was significantly driven by the interactional effect of season, N rate with planting density (P<0.001), N rate with planting density (P<0.05) and season (P<0.001) (Table 2). There was no significant difference among different N rates and planting densities (P<0.05) (Fig. 5). In the four seasons, soil N declined slowly as the experiment continued (P<0.05).

3.3.2 Soil N removal and loss

In the perennial rice cropping system, soil N was mainly taken up by plant removal (N uptake by plants) (Fig. 1b). There was a significant difference among the N removal of different treatments (Fig. 6 & Table 2). With the increment of N rate, the N removal by perennial rice significantly increased (P<0.05), 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 densities, as 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.

In contrast to the N removal by plants, the soil N loss and loss rate increased with the increase in the N rate and decrease in planting density (Fig. 6 & 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 significant difference 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. The soil N losses in D1, D2 and D3 were 146, 134 and 110 kg ha^{-1}, respectively. The soil N loss rates 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 were 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 (Fig. 6 & Fig. 7d).

3.3.3 Apparent N balance

The apparent soil N balance was calculated by the difference in 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 (Fig. 1b). The N input by rice stubble was mainly related to the N rate in straw and the biomass of straw. A high N rate and planting density would lead to a high N input for perennial rice (Fig. 7c). In the four seasons, the N inputs 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, the soil N balances 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 (Fig. 7e). Within the four seasons, N2D3 achieved the soil N balance among all treatments.

3.4 N effects and requirement

The N agronomic effect (NAE) and N recovery efficiency (NRE) are important indicators of the N fertilizer effect. With the increase in nitrogen, NAE and NRE increased, N2 resulted in better N effects (NRE: 46.5%, NAE: 22.2 kg N kg^{-1}) (Fig. 8). For planting density, D3 had a better N effect, 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 the NAE and NRE were 64.9 kg N kg^{-1} and 26.5%, respectively.

The N requirement refers to the amount of N required to produce 1 Mg of rice grain and is an important indicator to evaluate the N effect in perennial rice cropping systems. In the four seasons, the N requirement of perennial rice averaged 29.7 kg N Mg^{-1} grain, and the N requirements 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 (Fig. 7f).

4. Discussion

4.1 Dry matter accumulation

Increasing fertilizer and planting density have been proposed as effective ways to improve rice yield (Hou et al., 2019; Ahmed et al., 2016). As N fertilizer and planting density increase, grain yields increase to a certain extent (Zhang et al., 2018; Hayashi et al., 2006). In accordance with the annual rice, the grain yield and dry matter accumulation of perennial rice showed the same response to N fertilizer and planting density (Fig. 2 & Table 1). However, the addition of fertilizer does not always result in a high crop yield as it may result in low fertilizer use efficiency and more fertilizer erosion, thus causing a series of economic and environmental problems (Sharma et al., 2018). The higher N fertilizer in N3 did not result in a significantly higher grain yield but more soil N loss and low N use efficiency (NAE and NRE) in the perennial rice cropping system. The proper N fertilizer rate and planting density in N2D3 are conducive to soil nitrogen absorption and crop production. By the positive relationship of N uptake with straw and grain yield, the high N uptake in N2, N3 and D3 would lead to a high grain yield and dry matter accumulation. Although N3 resulted in high grain yield and dry matter in four seasons, similar to 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 high grain yield and dry matter accumulation, improve N use efficiency, and reduce soil N erosion (Chen et al., 2014). The optimal combination of the N rate with planting density in N2D3 resulted in the highest dry matter accumulation and grain yield in the perennial rice cropping system.

4.2 Plants N uptake and N use efficiency

N is an essential element for perennial rice production, and plant N uptake is closely related to dry matter accumulation and grain yield (Gardner and Drinkwater, 2009). Recent literature has 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 (Chen et al., 2011; Cui et al., 2018). A properly high N rate could help plants absorb more N for production (Ladha et al., 2005; Zhang et al., 2020). The N uptake of perennial rice significantly increased with increasing N application rate and planting density, and N3D3 and N2D3 showed significantly high N uptake. However, there was no significant difference between them. N3 did not increase the N uptake of perennial rice but showed more soil N loss than N2. Excessive N fertilizer input leads to luxury N absorption but enhances soil N loss and leaching (Jing et al., 2007; Li et al., 2018). In all four seasons, the N uptake of perennial rice remained stable. In the first season, N uptake by plants mainly transferred to grain yield and then led to a high grain yield of perennial rice. 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, increasing fertilizer loss and pollution have appeared in the field due to the desire for higher crop yields, which has caused more environmental problems (Zhang et al., 2018). In China, the fertilizer use efficiency is 30% to 35%, which is far below that of the rest of the world (Chen et al., 2014). Therefore, we need to improve the N fertilizer use efficiency while maintaining high crop yields. NAE and NRE were effective indicators to evaluate fertilizer use efficiency, and higher NAE and NRE values meant that fertilizer could produce more grain yield and result in higher fertilizer use efficiency (Xie et al., 2013). The high NAE and NRE in N2 and D3 revealed that at this nitrogen and planting density, perennial rice could utilize N fertilizer efficiently and resulted in the best N effect in the N2D3 mode.

The N requirement denotes the N productivity ability and N production efficiency. The N requirement is also an effective indicator used to evaluate the N fertilizer use efficiency and productivity and refers to the N requirement to produce 1 Mg grain (Yin et al., 2019). The low N requirement revealed that producing the same grain yield requires less N fertilizer. The low N requirement in N2D3 indicated that N would produce more grain yield and have a higher use efficiency with less N loss or pollution in this mode. The highest AE and PEP and proper N requirement in N2D3 also illustrated that in this mode, the N fertilization effect was the best, and that perennial rice would produce more grain yield, less fertilizer loss and pollution, and obtain more economic profit.

4.3 N cycle and balance

Soil N is the main soil nutrient used for enhancing crop production, and the soil N supply and balance immediately determines crop productivity (Cassman et al., 2002). The majority of crop N came from the soil; if the 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 (Quan et al., 2020). In the perennial rice cropping system, the source of soil N included soil base N, N fertilizer application, and N from some stubble decomposition, while the removal of soil N, including N, was taken up by dry matter and soil N loss. Maintaining the soil N balance is the premise for sustainable rice production. In this study, the N2D3 mode maintained the apparent soil N balance in the field, and the negative N balance in N0 would lead to soil degradation and reduced crop yields. If exogenous N replenishment was lower than soil N consumption, the soil N supply capacity would hardly be sustained after long-term soil N mining, which will eventually lead to soil fertility degradation and crop yield reductions (Mulvaney et al., 2009; Ju and Christie, 2011). The high N balance in N3 would lead to surplus N and more soil N loss, which would result in serious environmental problems.

5. Conclusion

Studying the N utilization and N cycle in perennial rice cropping systems helps us evaluate the N effects and soil N loss and to formulate optimal N management for sustainable perennial rice production. In this study, the N2D3 mode resulted in higher and sustainable grain yield and dry matter accumulation with better N effects (NAE and NRE). Additionally, the N2D3 mode took up more N nutrients from the soil and resulted in less soil N loss, maintaining the apparent N balance in the soil. In perennial rice cropping systems, the N2D3 (180 kg N ha^{-1} integrated with 226×10^3 plants ha^{-1}) mode would be optimal for sustainable production and soil N balance, which would result in less soil N loss and pollution.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

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Figures

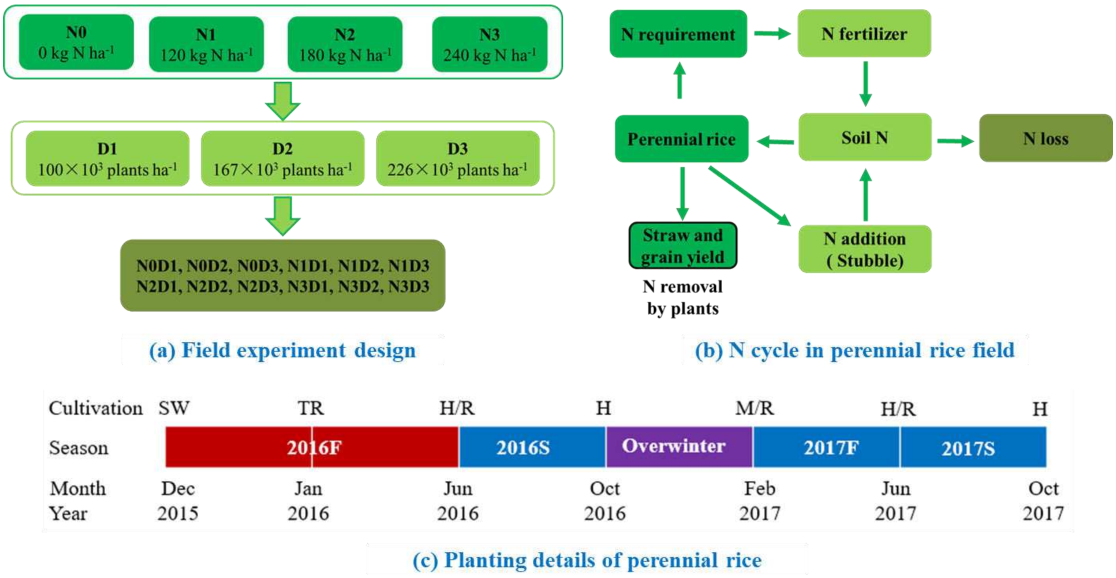

Fig. 1 Field experimental design, planting details, and N cycle in a perennial rice field. (a) Field experimental design with different N application rates and planting densities. (b) N cycle in perennial rice cropping system. (c) Planting details of perennial rice. SW, sowing. TR, transplanting. H, harvest. R, regrowth. M, stubble management (cutting back); 2016F, first season (red color) from sowing to first harvest; 2016S, 2017F and 2017S, three regrowth seasons (blue color) from regrowth to harvest in each season. Overwinter, from the last harvest in the first year in winter to the first regrowth in the second year.

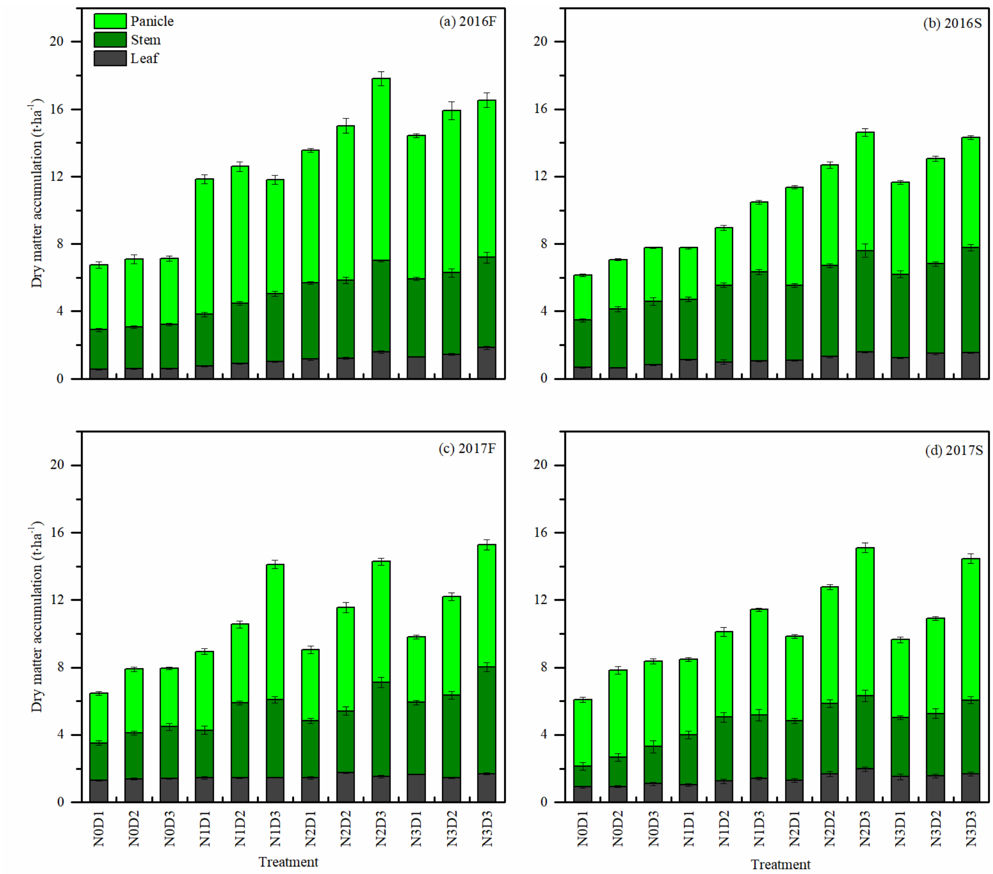

Fig. 2 Dry matter accumulation of different treatments. Dry matter accumulation includes the dry matter of stems, leaves, and grains; 2016F, first season of 2016 (transplanting season); 2016S, second season of 2016 (regrowth season); 2017F, first season of 2017 (regrowth season); 2017S, second season of 2017 (regrowth season).

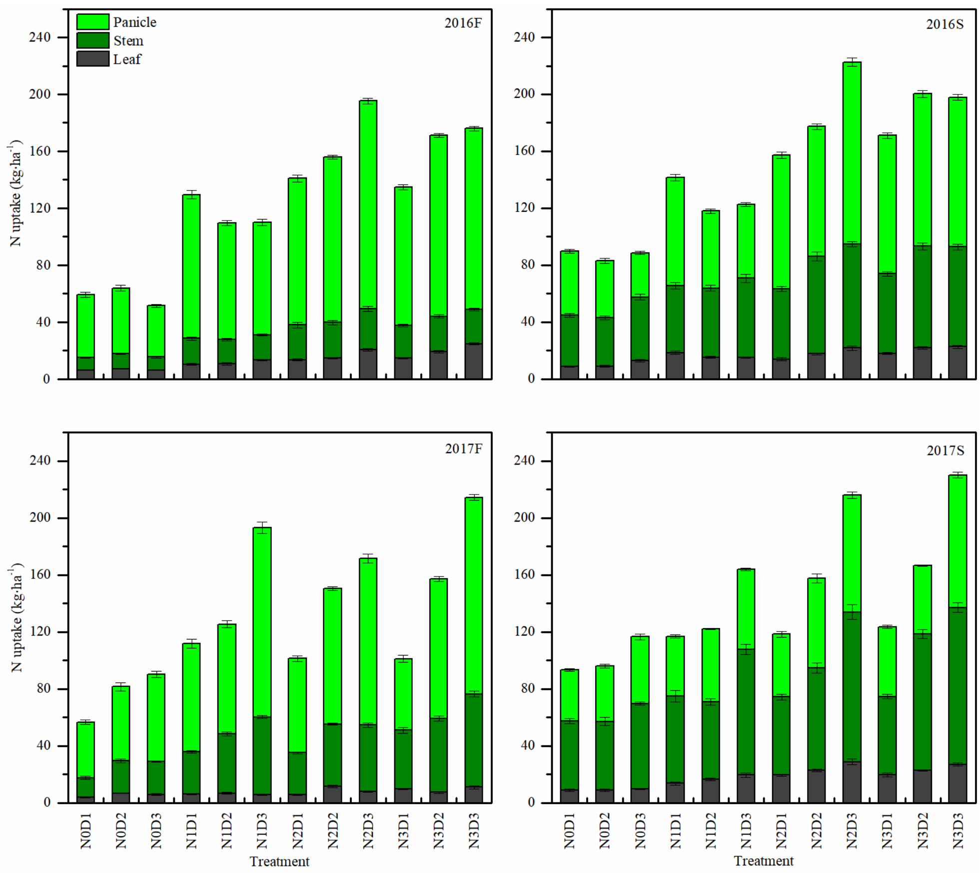

Fig. 3 N uptake of aboveground dry matter. Plant N uptake includes the N uptake of stems, leaves, and grains; 2016F, first season of 2016 (transplanting season); 2016S, second season of 2016 (regrowth season); 2017F, first season of 2017 (regrowth season); 2017S, second season of 2017 (regrowth season).

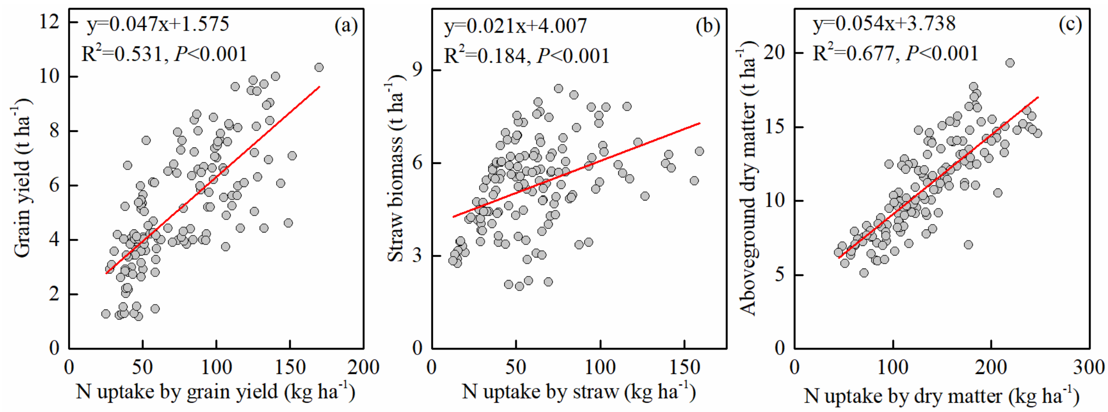

Fig. 4 The relationship of N uptake with grain yield, straw biomass, and aboveground dry matter. (a) Relationship of N uptake by the grain and the corresponding grain yield. (b) Relationship of N uptake by straw (stem and leaf) and straw biomass. (c) Relationship of N uptake by dry matter and aboveground dry matter.

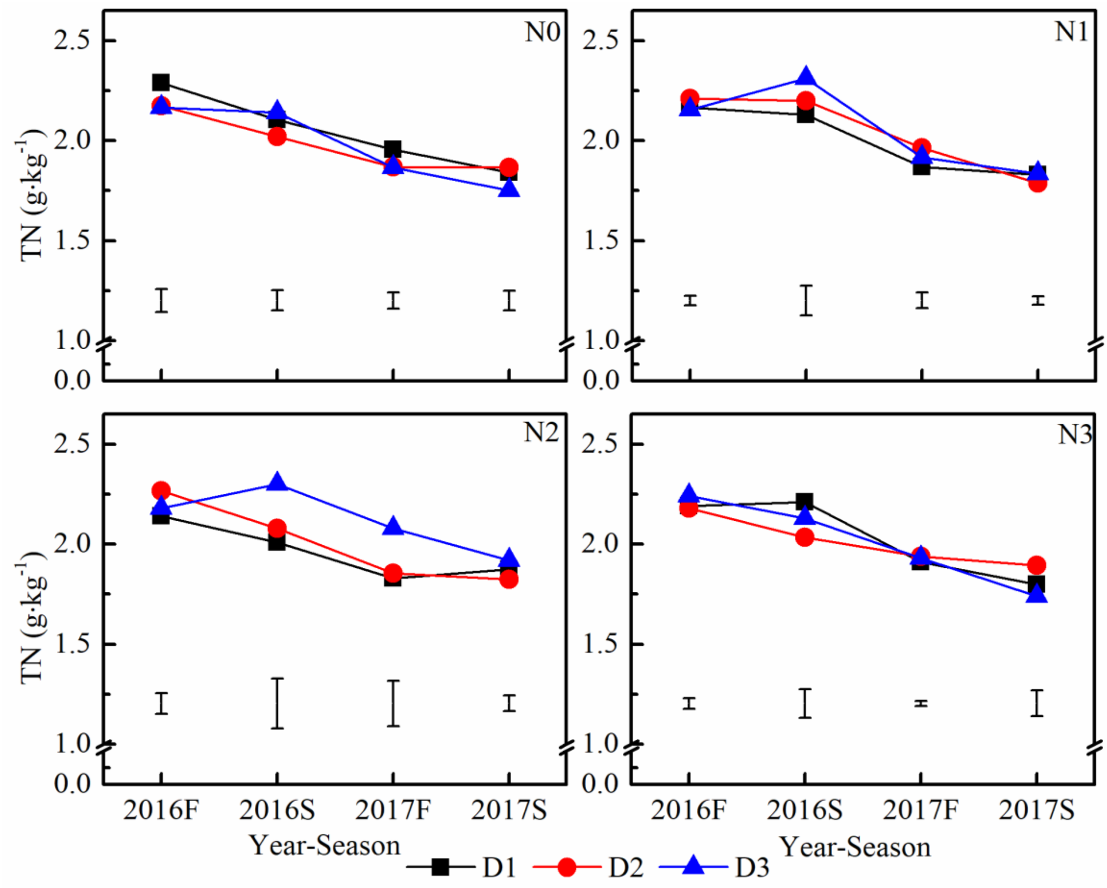

Fig. 5 Soil total nitrogen (TN) of different treatments; 2016F, first season of 2016 (transplanting season). 2016S, second season of 2016 (regrowth season); 2017F, first season of 2017 (regrowth season); 2017S, second season of 2017 (regrowth season). Vertical bars represent the standard error for different treatments.

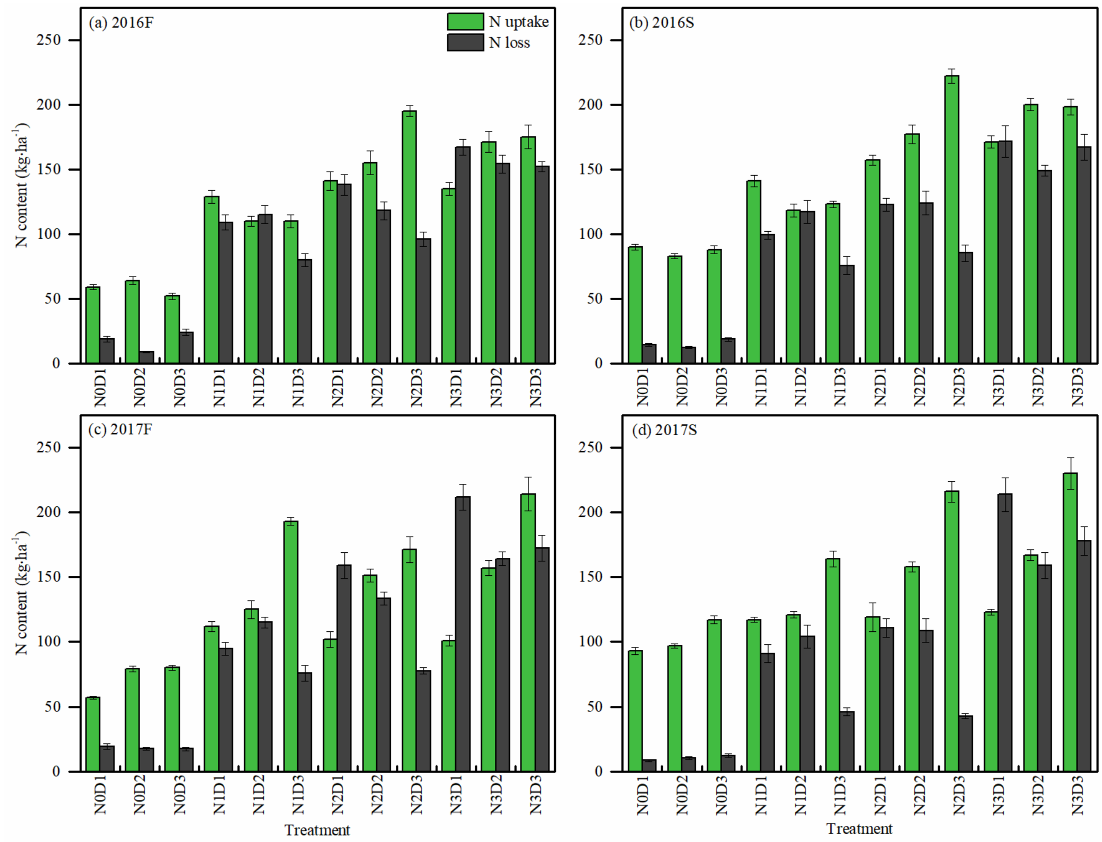

Fig. 6 N removal by plants and soil N loss in the perennial rice cropping system.

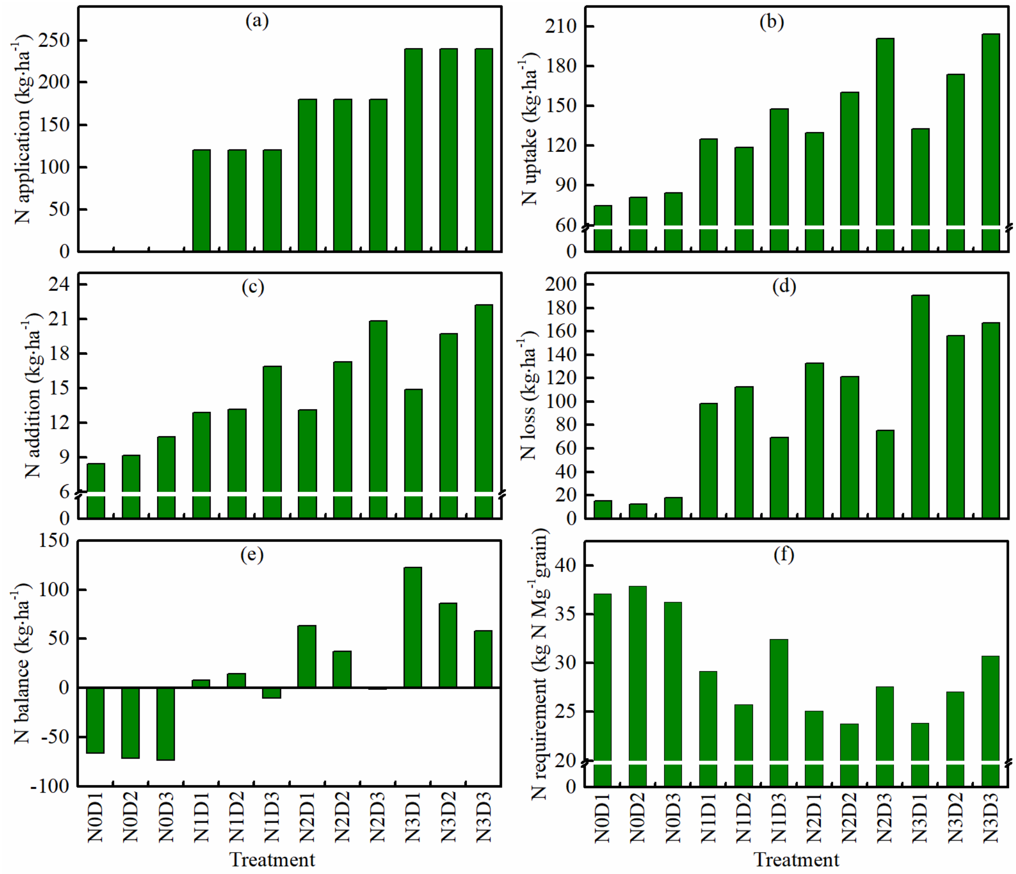

Fig. 7 Soil N balance and N requirement under different N rates and plating densities in a perennial rice cropping system. (a) N application. (b) N uptake. (c) N addition. (d) N loss. (e) N balance. (f) N requirement.

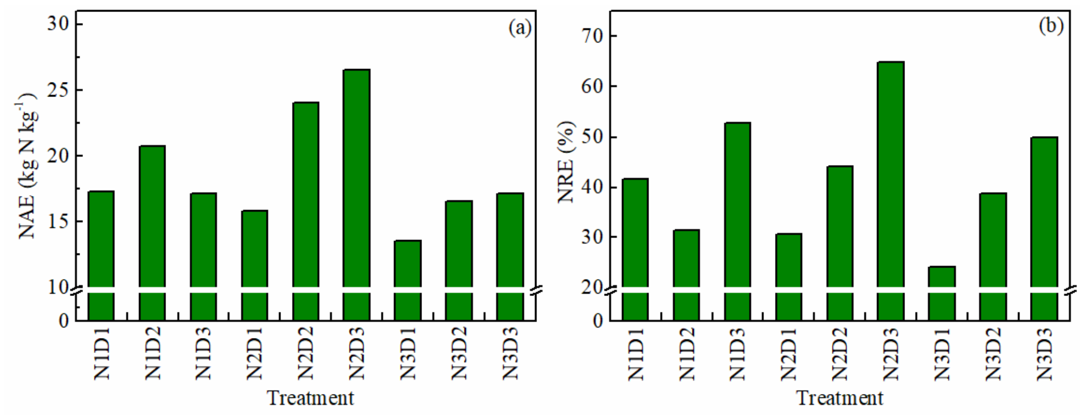

Fig. 8 N agronomic efficiency and requirements under different N rates and plating densities in a perennial rice cropping system. (a) N agronomic efficiency (NAE) of different treatments. (b) N recovery efficiency (NRE) of different treatments.

Tables

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 ($t\ ha^{-1}$)	Biomass ($t\ ha^{-1}$)	Grain ($t\ ha^{-1}$)
Season					
2016F	1.08b	3.96b	7.50a	12.54a	7.26a
2016S	1.13b	4.65a	4.71c	10.49b	4.42b
2017F	1.50a	4.00b	5.17bc	10.68b	4.76b
2017S	1.36a	3.28c	5.79b	10.43b	4.32b
N rates					
N0	0.91c	2.55d	3.75c	7.22c	2.69d
N1	1.16b	3.88c	5.56b	10.59b	4.90c
N2	1.47a	4.59b	7.07a	13.14a	6.68a
N3	1.54a	4.87a	6.78a	13.19a	6.48b
Planting density					
D1	1.16c	3.40c	4.94c	9.49c	4.59c
D2	1.26b	3.96b	5.80b	11.02b	5.34b
D3	1.40a	4.56a	6.63a	12.59a	5.64a
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.

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	124.67a	98.42a	2.20a
2016S	147.33a	96.46a	2.14b
2017F	128.50a	104.81a	1.92c
2017S	143.50a	90.40a	1.83d
N rate			
N0	79.92d	12.29d	2.00a
N1	130.25c	93.52c	2.03a
N2	163.67b	109.67b	2.03a
N3	170.17a	171.60a	2.02a
Planting density			
D1	115.44c	109.31a	2.01a
D2	133.31b	100.67b	2.01a
D3	159.25a	82.58c	2.04a
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.