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# An innovated crop management scheme for perennial rice cropping system and its impacts on sustainable rice production

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ABSTRACT

Perennial grain has been proposed to provide an effective means of ensuring both food and ecological security. The newly released cultivar of perennial rice 23 (PR23) represents a new rice production system that is based on no-tillage. Nevertheless, how perennial rice responds to this new system remains unclear. Two field experiments were conducted over four successive seasons from 2016 to 2017 in Jinghong, Yunnan Province, Southern China. Field experiment 1 showed perennial rice is an economically viable and environmentally safe cropping system compared to annual rice, and could obtain a stable and sustainable grain yield economically for successive seasons across years. In the perennial rice cropping system, N fertilizer had some negative effects on the regrowth of perennial rice. Field experiment 2 with four N rates N0, N1, N2 and N3 with 0, 120, 180 and 240 kg N $ha^{-1}$, respectively and three planting densities D1, D2 and D3 with 10, 16.7 and 22.6 plants $m^{-2}$, respectively on perennial rice was conducted to assess and ameliorate these negative effects of N fertilizer on the regrowth of perennial rice. The results showed that: (1) the N2D3 treatment (180 kg N $ha^{-1}$ integrated with 22.6 plants $m^{-2}$) resulted in a stable and high grain yield across three successive regrowth seasons (6.93 t $ha^{-1}$) and optimized yield components (panicle no. $m^{-2}$, spikelet no. $panicle^{-1}$, grain weight) and root activity (10.81 g $h^{-1}$ $m^{-2}$); (2) the regrowth of perennial rice 23 was significantly limited by N fertilizer ($P<0.05$), and the N0D2 treatment had the best regrowth ability (97.8 %) across the three regrowth seasons; (3) additionally, the N2D3 treatment had the best N net productivity (27 kg N $kg^{-1}$), profit (79 CNY $kg^{-1}$) and sustainable production capacity (0.59), and could obtain more economic profit in successive perennial rice production. Perennial rice was able to be sustainably and economically produced for successive regrowth seasons across years, and the N2D3 treatment provided optimal conditions, which enhanced the regrowth rate, N productivity, economic benefit and yield potential. The use of less chemical N fertilizer and a higher planting density could enhance the sustainability of the grain yield and reduce fertilizer loss via a novel crop management scheme for perennial rice.

## 1. Introduction

Compared with the current global cropping acreage, a million more hectares of land need to be converted to crop production to meet the growing demand for food (Naylor et al., 2007). Annual crop production, which is responsible for 80 % of global food, leads to increased carbon emission, soil erosion, and water and environment pollution and requires a large amount of labour (Pimentel et al., 2012; Cox et al., 2010). Moreover, global climate change poses a high risk for annual crop yield losses (Wan, 2018). How to sustainably meet the food demand is a current hot topic of research (Cui et al., 2018; Glover et al., 2010; Tilman et al., 2011; Pimentel et al., 2012). Significant improvements in genetics or new crop species, such as transformation from annuals to perennials, potentially provide efficient strategies to increase crop production in an environmentally sustainable way (Wan, 2018; Glover et al., 2010; Hu et al., 2003).

After sowing and transplanting, perennial crops can survive and be harvested several times in successive years (Glover et al., 2010). Indeed,

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such crops can ratoon and regrow without sowing or planting after their first year. Once perennial crops become a feasible option, farmers can reduce some production efforts, decreasing the need for and intensity of labour and increasing economic profits (Huang et al., 2018). Moreover, without plowing every year or season, the continuous lack of tillage for perennial crops would reduce soil erosion and protect arable land (Cox et al., 2006; Zhang et al., 2011). In 2010, scientists from more than ten countries revealed that perennial crops would provide an effective means of protecting crop yields and the environment (Glover et al., 2010) and indicated that perennial crops would be economically viable within 20 years.

In 2018, the first cultivar of perennial rice 23, named PR23 (No. 2018033, http://www.ricedata.cn/variety/varis/618801.htm), which was bred via the clonal propagation characteristics of the rhizome of *Oryza longistaminata* and is capable of surviving for consecutive years, was released in China (Huang et al., 2018; Zhang et al., 2017, 2019c). PR23 was obtained from the cross between *Oryza sativa* cv. RD23 and *O. longistaminata*. RD23 is a popular *Indica* lowland rice cultivar from Thailand, and is grown widely across south-east Asia because of its broad adaptation, high yield potential, good disease resistance, and high grain quality. In contrast, *O. longistaminata* is a wild rhizomatous perennial species with poor agronomic characteristics which is original from Africa. The cross between the two species was made in 1997 to combine the perennial habit of *O. longistaminata* with the agronomic features, broad adaptation (Tao and Sripichitt, 2000), and yield potential of RD23 via iterative segregating populations from $F_2$ in 2003 to $F_{10}$ in 2010 (Huang et al., 2018).

The cultivar PR23 is a breakthrough as it represents a turning point from annual to perennial in crop domestication and improvement. Without the need for plowing, seeding, and transplanting during successive regrowth seasons, perennial rice can reduce soil erosion and the need for intensive labour and input in fields (Huang et al., 2018). To date, the perennial rice trail is on the way in China, Myanmar, Laos, Cambodia, Thailand, Vietnam, Indonesia, Uganda and Cote d'lvoire via International Perennial Rice Collaboration organized by Yunnan University. In China, perennial rice has been tested in more than 10 provinces (Yunnan, Guangxi, Guangdong, Fujian, Hunan, Hubei, Henan, Zhejiang, Jiangxi and Guizhou) and over an area greater than 5000 ha as of 2019.

Previous research on perennial rice has demonstrated that it can be widely planted in South China and Laos (Zhang et al., 2017; Huang et al., 2018), enhance the profits of farmers and reduce labour in fields (Huang et al., 2018). However, the management of perennial rice, as well as its response to N fertilizer, remains unclear. It still needs to be determined how to offset the negative effects of N fertilizer on the regrowth of perennial rice and to maintain an appropriate perennial rice population to obtain a stable and high grain yield as well as sustainable production over years. Therefore, two experiments were conducted in this study. Experiment 1 (comparison of perennial and annual rice cropping system) was to evaluate the sustainable production ability and economic benefit of perennial rice cropping system. A field experiment (Experiment 2) using different N fertilizer rates and planting densities of perennial rice was employed to optimize crop management and the response of perennial rice in Jinghong, Yunnan Province, China, to explore the response of perennial rice to N fertilizer and planting density. Our objectives were to select the appropriate N fertilizer rate and planting density for sustainable production of perennial rice, and the results provide a theoretical indication for the sustainable production of perennial rice. Lastly, our findings provide insight into the production of perennial grains.

## 2. Materials and methods

### 2.1. Experimental site

This experiment was conducted at the Perennial Rice Research Station of Yunnan University, Gasa town (N 20°57′22″, E 100°45′43″, altitude 555 m), Jinghong, Yunnan Province, China. The station is located in southern China, which has a tropical monsoon climate, and rice is harvested twice a year in this region. The average annual rainfall and temperature were 1136.6 mm and 23.3℃, respectively, and most rainfall was from April to October.

Before the experiment, regular annual rice production had been conducted in the trial field. The soil conditions are shown in detail in Table 1.

### 2.2. Experimental design and performance

#### 2.2.1. Experiment 1: perennial Vs annual rice

A randomized complete blocks design with three replicates was performed over four successive seasons from 2016 to 2017. Two rice varieties were selected, perennial rice 23 (PR23) and annual rice HXR7. PR23 is a perennial rice cultivar released by Yunnan Crop Committee in 2018. HXR7 is a locally popular *Indica* lowland rice cultivar grown widely by farmers in Yunnan Province due to its high grain yield and its exceptional grain quality. The sowing and transplanting date of both HXR7 and PR23 in the first transplanting season were 30, Jan 2016 and 5, Mar 2016, respectively. In the regrowth seasons (2016S, 2017F, 2017S), the annual rice and perennial rice were managed according to the local rice production. After harvest, the crop management in annual rice consisted of plowing, reseeding and transplanting. For perennial rice, the new tillers that emerged from the rhizome of the straw were only maintained for successive regrowth seasons. After harvest, the straw 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. Meanwhile, no tilling was conducted across the three successive regrowth seasons. Other crop management was the same as local rice production.

#### 2.2.2. Experiment 2: N rate and planting density experiment of perennial rice

The experiment employed a spilt-plot design with three replicates and was performed over four successive seasons from 2016 to 2017. There were four N fertilizer rates (0 kg N ha$^{-1}$ (N0), 120 kg N ha$^{-1}$ (N1), 180 kg N ha$^{-1}$ (N2) and 240 kg N ha$^{-1}$ (N3)) used in the main plots and three planting densities (10 plants m$^{-2}$ (D1), 16.7 plants m$^{-2}$ (D2) and 22.6 plants m$^{-2}$ (D3)) among subplots 20 m$^2$ in size. These four N fertilizer rates and three planting densities generated 12 N fertilizer rates with planting density treatments: N0D1, N0D2, N0D3, N1D1, N1D2, N1D3, N2D1, N2D2, N2D3, N3D1, N3D2 and N3D3 (Tables 2 & 3 ).

The cultivar perennial rice 23 was sown on 15 Dec 2015 and was transplanted in a plowed and level field on 30 Jan 2016. For different rates of N application, N fertilizer (urea) was manually and evenly spread at a ratio of 5:2:2:1 during four stages corresponding to the transplanting time for 2016F or new tillers emerging, tilling, heading and filling, respectively. For different planting densities, the plant and row spacings for D1 were 27 cm and 37 cm, respectively. For D2, the plant and row spacings were 20 cm and 30 cm, respectively; for D3, the plant and row spacings were 17 cm and 26 cm. The crop management of perennial rice was the same with Experiment 1.

**Table 1**
Soil conditions prior to the initiation of experiments.

| Soil layer (cm) | pH | SOM (g kg$^{-1}$) | TN (g kg$^{-1}$) | AN (mg kg$^{-1}$) | AP (mg kg$^{-1}$) | AK (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 0–20 | 5.05 | 34 | 2.1 | 155.6 | 7.58 | 139.1 |

SOM: soil organic matter, TN: total nitrogen, AN: available nitrogen, AP: available phosphorus, AK: available potassium.

**Table 2**
Parameters of the different treatments.

| Treatment | Details N rates (kg $ha^{-1}$) | Plants ($m^{-2}$) |
|---|---|---|
| N0 | 0 | – |
| N1 | 120 | – |
| N2 | 180 | – |
| N3 | 240 | – |
| D1 | – | 10 |
| D2 | – | 16.7 |
| D3 | – | 22.6 |
| N0D1 | 0 | 10 |
| N0D2 | 0 | 16.7 |
| N0D3 | 0 | 22.6 |
| N1D1 | 120 | 10 |
| N1D2 | 120 | 16.7 |
| N1D3 | 120 | 22.6 |
| N2D1 | 180 | 10 |
| N2D2 | 180 | 16.7 |
| N2D3 | 180 | 22.6 |
| N3D1 | 240 | 10 |
| N3D2 | 240 | 16.7 |
| N3D3 | 240 | 22.6 |

**Table 3**
Dates of sowing, transplanting, cutting back and harvesting of perennial rice over four seasons during 2016–2017.

| Season | Date | | | |
|---|---|---|---|---|
| | Sowing | Transplanting | Cutting back | Harvest |
| First season (2016F) | 15, Dec 2015 | 30, Jan 2016 | | 8, Jun 2016 |
| Second season (2016S) | | | 10, Jun 2016 | 18, Oct 2016 |
| Third season (2017F) | | | 17, Feb 2017 | 18, Jun 2017 |
| Fourth season (2017S) | | | 19, Jun 2017 | 28, Oct 2017 |

### 2.3. Measurements

#### 2.3.1. Grain yield and sustainable yield index

In each plot, perennial rice was manually harvested from an area greater than 5 $m^2$ at harvest time and was adjusted to a 14 % water content (the international standard) to measure grain yield. The sustainable yield index (Muhammad et al., 2020) was used to evaluate the sustainable production capacity of perennial rice.

$$\text{Sustainable yield index (SYI)} = (\text{Ymean-}\sigma)/\text{Ymax} \quad (1)$$

Where Ymean is the mean grain yield of a treatment over four seasons, σis treatment standard deviation over four seasons, and Ymax is the maximum grain yield of a treatment over four seasons.

#### 2.3.2. Yield components

At harvest time, three replications with 10 uniform plants which could represent the rice population were selected to measure the yield components of perennial rice in each plot. Yield components of perennial rice including panicle no. $m^{-2}$, spikelet no. $panicle^{-1}$, grain weight and seed setting rate. The panicle no. was counted and calculated by the formula:

$$\text{Panicle no. } m^{-2} = \text{average panicle no. per plant} \times \text{planting density} \quad (2)$$

Thirty panicles were randomly selected and the number of spikelets was counted. Seed setting rate and grain weight was measured by floating selection and oven drying method. All the sample grains were watered for 3 min, floated to separate empty from filled grains, and these two fractions were then weighed. 3 g empty grains and 30 g filled grains were selected with three replications, and then dried to stable weight and counted the number to calculate the grain weight and seed setting rate by the formulae:

$$\text{Grain weight (mg)} = \text{filled grain weight/ filled grain no.} \quad (3)$$

$$\text{Seed setting rate (\%)} = \text{filled grain no. / (empty grain no.+ filled grain no.)} \times 100\ \% \quad (4)$$

#### 2.3.3. Regrowth rate

In the first season of 2016 (2016F: transplanting season), we set the regrowth rate to 100 %. In the following regrowth seasons, the second season in 2016 (2016S), first season in 2017 (2017F) and second season in 2017 (2017S), the regrowth rate was measured 7–10 days after cutting back perennial rice. In each subplot, all plants were used to calculate the regrowth rate during each regrowth season.

#### 2.3.4. Root activity

Root activity was measured by bleeding sap. At 19:00 after sunset, three uniform plants were selected and cut 7–10 cm above the ground (Song et al., 2011). These plants were then covered by weighted cotton wool, wrapped in plastic and bound by a rubber band in each plot. At 7:00 before sunrise the following day, cotton wool was weighted to calculate the amount of sap that had bled.

#### 2.3.5. N productivity and profit

The N productivity, net N productivity and N profit were calculated using the following formulae:

$$\text{N productivity (kg N kg}^{-1}) = \text{grain yield in } N_i / N_i \text{ application rate} \quad (5)$$

$$\text{N net productivity (kg N kg}^{-1}) = \text{grain yield } (N_i\text{-N0})/N_i \text{ application rate} \quad (6)$$

$$\text{N profit (CNY kg}^{-1}) = \text{profit } (N_i\text{-N0})/N_i \text{ application rate} \quad (7)$$

Note: $N_i$ is the N rate, where $i \geq 1$.

### 2.4. Statistical analysis

Split-plot analysis with three-way ANOVA (N rate and planting density were set as two fixed factors, and 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. When the three-factor or two-factor interaction effects were significant, single factor effects were analysed. If single factor effects were significant, one-way ANOVA was used to compare the eff ;ects of the diff ;erent seasons or N treatments or planting densities on the measured variables with three replications. F-tests were conducted, and multiple comparisons were performed using the least significant diff ;erence test (LSD) ($P \leq 0.05$). Experimental data was analysed 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. Perennial vs annual rice

In the experiment 1 of 2016–2017, perennial rice (6.46 t $ha^{-1}$) showed not significantly but consistently higher grain yield in 4 seasons (Fig. 1a), which was 3.8 % higher than annual rice (6.22 t $ha^{-1}$). Both annual and perennial rice had robust sustainable production ability (more than 0.80) (Fig. 1b), the SYI of perennial rice (0.80) was a bit less than annual rice (0.82).

For the economic benefit, perennial rice was significantly superior to annual rice (Fig. 2). In the transplanting season (2016F), the input of annual and perennial rice cropping system was on the same level

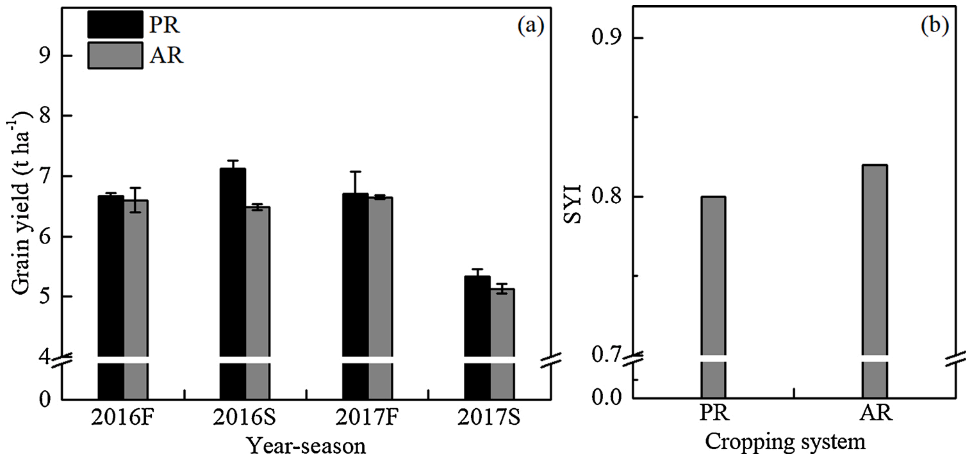


**Fig. 1.** The grain yield and sustainable yield index (SYI) of perennial rice (PR) and annual rice (AR) cropping system in the experiment 1. (a) Grain yield of perennial and annual rice. (b) Sustainable yield index of perennial and annual rice. Error bars are the standard errors (SE). Bars with different letters were significantly different. 2016F, first rice season of 2016. 2016S, second rice season of 2016. 2017F, first rice season of 2017. 2017S, second rice season of 2017.

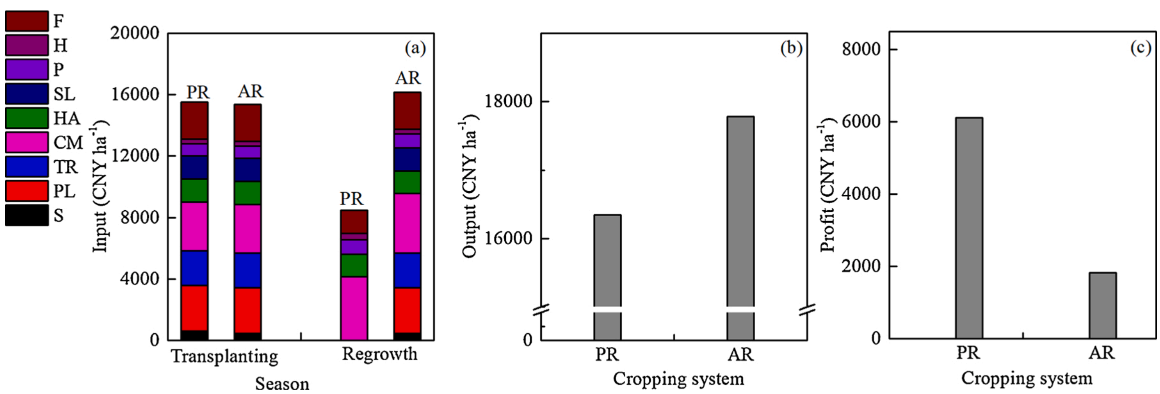


**Fig. 2.** The input, output and profit of perennial (PR) and annual rice (AR) cropping system in experiment 1. (a) Input of PR and AR cropping system. The total input including: F, fertilizer. H, herbicide. P, pesticide. CM, crop management. HA, harvest. TR, transplanting. PL, plowing. SL, seedling. S, seeds. (b) Output of PR and AR cropping system. (c) Profit of PR and AR cropping system.

(Fig. 2a), with 15,362 and 15,512 CNY ha$^{-1}$, respectively, while in the regrowth seasons, without seeds, seedling, plowing and transplanting, perennial rice economized 7684 CNY ha$^{-1}$ each season than annual rice cropping system. Although the output of annual rice cropping system was 8.8 % higher than perennial rice (Fig. 2b), the profit of perennial rice (6114 CNY ha$^{-1}$) was significantly higher (235 %) than annual rice (1827 CNY ha$^{-1}$) (Fig. 2c). The perennial rice cropping system has sustainable and stable production ability but would be more economic when compared to annual rice.

### 3.2. Grain yield and sustainable production capacity

The grain yield of perennial rice over four successive seasons from field experiment 2 is shown in Fig. 3. In the first season (2016F: transplanting season), the grain yield was high, and N2D3 resulted in the highest grain yield (9.93 t ha$^{-1}$). Despite the transplanting season (2016F), the grain yield remained stable across three successive regrowth seasons (Table 4). The grain yield was determined by the interaction of season and N rate with the planting density ($P < 0.01$) as well as either both of them and N rate ($P < 0.001$) over successive regrowth seasons (Table 4). For different N rates, N1, N2 and N3 significantly increased the grain yield compared with N0, with increases of 74 %, 159 % and 153 %, respectively. Among the different planting densities, D3 (4.96 t ha$^{-1}$) resulted in a significantly higher grain yield than D1 (3.91 t ha$^{-1}$). The N2D3 treatment significantly increased the grain yield (6.93 t ha$^{-1}$) relative to the other treatments in the three successive regrowth seasons.

Fig. 4 shows the sustainable yield index of perennial rice in 4 seasons. Both high N rate (N2: 0.55 and N3: 0.57) and planting density (D3: 0.52) could significantly increase the sustainable production capacity of perennial rice. The N2D3 treatment (0.59) resulted in the highest sustainable production capacity in 4 seasons, followed by N3D2 (0.58).

### 3.3. Yield components

Panicle no. m$^{-2}$, spikelet no. panicle$^{-1}$, grain weight and seed-setting rate were the major determinants of the grain yield in the rice fields.

For the successive regrowth seasons of perennial rice, panicle no. m$^{-2}$ was significantly affected by the interaction of season and N rate with planting density ($P < 0.05$), N rate with season ($P < 0.05$), N rate ($P < 0.01$), planting density ($P < 0.01$) and season ($P < 0.05$) (Table 4). N2 (269), N3 (285) and D3 (286) showed a significantly higher panicle no. than the other N rates ($P < 0.05$) and planting densities ($P < 0.05$), and N2D3 led to the highest panicle no. m$^{-2}$ (333) (Table 4). Different from panicle no. m$^{-2}$, the interaction between season and N rate ($P < 0.01$), season and planting density ($P < 0.05$), N rate ($P < 0.001$) and planting density ($P < 0.05$) had significant effects on spikelet no. panicle$^{-1}$. N2 (132), N3 (131) and D1 (124) showed a significantly higher spikelet no. panicle$^{-1}$ than the other groups, and N3D1 (145) showed the highest spikelet no. panicle$^{-1}$ over successive regrowth seasons. The interaction between N rate and planting density with season significantly affected grain weight ($P < 0.001$). There was no significant difference among the

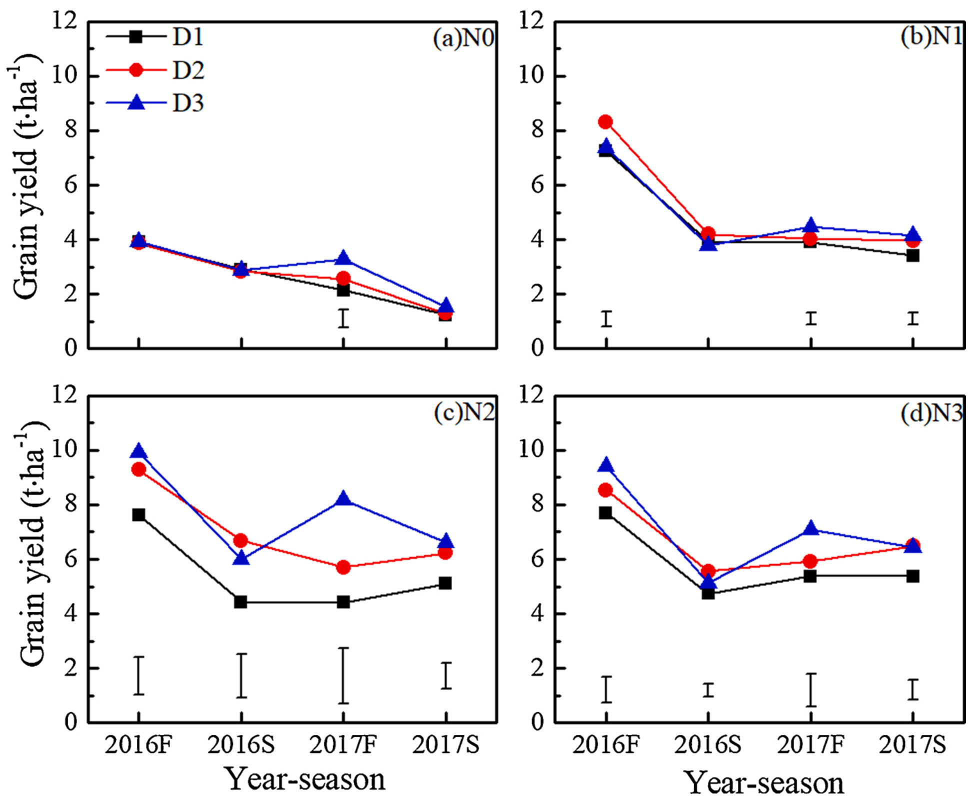


**Fig. 3.** Grain yield of perennial rice under different N rates and planting densities in 2016–2017. 2016F: first season in 2016 (transplanting season). 2016S: second season in 2016. 2017F: first season in 2017. 2017S: second season in 2017. Vertical bars represent the standard error for different treatments.

different N rates and planting densities ($P < 0.05$). Seed-setting rate was significantly affected by season ($P < 0.05$) and N rate ($P < 0.05$). N0 level (86.34 %) led to the highest seed-setting rate compared with the other N rates, but there was no significant difference among the different planting densities. N0D3 had the best seed-setting rate at 89.92 %. The N2D3 treatment resulted in a higher grain yield through its significant effects of panicle no. $m^{-2}$ and spikelet no. $m^{-2}$ on the grain yield.

### *3.4. Regrowth rate and root activity*

**(1) Regrowth rate**

A high regrowth rate is essential for enhancing rice populations and increasing the grain yield during successive regrowth seasons. The regrowth rate of perennial rice decreased significantly ($P < 0.05$) (Table 5), and the average regrowth rate was 90.24 % in the fourth season (2017S). In three successive regrowth seasons, the regrowth rate of perennial rice was determined by the interaction of N rate and season with planting density ($P < 0.001$) and season with planting density ($P < 0.01$) and N rate ($P < 0.001$) (Table 5). In two years, the regrowth rate declined as the N rates increased (Fig. 5), and N0 had the highest regrowth rate (97.34 % in the fourth season). The regrowth rate increased as planting density increased. D3 had the highest regrowth rate (94.11 % in the fourth season). By the fourth season, the N0D2 treatment had the highest regrowth rate (97.84 %). The N2D3 treatment also had a relatively high regrowth rate (92.18 %).

**(2) Root activity**

Roots are key for the absorption of soil water and nutrients; indeed, high levels of root activity provide the basis for the regrowth of perennial rice during successive regrowth seasons. Over four seasons, perennial rice showed stable root activity during regrowth seasons (Fig. 6). Root activity was significantly affected by the interactions of season and N rate with planting density ($P < 0.001$) and N rate with season ($P < 0.05$) and N rate ($P < 0.001$) and planting density ($P < 0.001$) during the regrowth seasons. N2 (9.49 g $h^{-1}$ $m^{-2}$) and D3 (8.68 g $h^{-1}$ $m^{-2}$) had the highest root activity among the different N rates and planting densities, and N2D3 (10.81 g $h^{-1}$ $m^{-2}$) had the highest root activity over the three regrowth seasons, followed by N3D3 (10.33 g $h^{-1}$ $m^{-2}$).

## 4. N productivity

The N productivity of perennial rice at different N rates and plant densities on perennial rice are shown in Fig. 7. In low N fertilizer rate (N1: 41 kg N $kg^{-1}$) and high planting density (D3: 38 kg N $kg^{-1}$), the N productivity was significantly higher than high N rate and low planting density (Fig. 7a). The N1D2 and N2D3 treatments resulted in the highest values, both were 43 kg N $kg^{-1}$. Based on N0 and D1, plants could use the N fertilizer more effectively in N2 and D2, D3, the N net productivity of N2D3 showed the highest value, was 27 kg N $kg^{-1}$ (Fig. 7b). The results illustrated N2D3 treatment was the optimal scheme for N productivity of perennial rice.

### *4.1. Relationship between grain yield and yield components, regrowth rate and root activity*

For perennial rice, N rate significantly affected grain yield, root activity and regrowth rate (Tables 4 & 5). The grain yield of perennial rice was significantly positively correlated to the root activity ($P < 0.01$) (Fig. 8a). Due to the high regrowth rate in 4 seasons (more than 90 %), the regrowth rate had a positive but not significant relationship with the grain yield ($P > 0.05$) (Fig. 8b). Despite the positive but not significant relationship of the regrowth rate with the grain yield, root activity was the main factor that affected the grain yield and other yield components of perennial rice. Indeed, the stability in root activity over four successive seasons reflects the high potential that perennial rice has to produce sustainable yields over several years.

**Table 4**
Yield of perennial rice over three regrowth seasons during 2016–2017.

| Treatment | Grain yield (t $ha^{-1}$) | Panicle no. $m^{-2}$ | Spikelet no. $panicle^{-1}$ | Grain weight (mg) | Seed setting rate (%) |
|---|---|---|---|---|---|
| Season | | | | | |
| 2016S | 4.42a | 217b | 115a | 23.80c | 81.12b |
| 2017F | 4.76a | 280a | 113a | 24.69b | 80.35b |
| 2017S | 4.32a | 216b | 102a | 25.74a | 88.23a |
| N rate | | | | | |
| N0 | 2.29c | 173c | 71c | 25.07a | 86.34a |
| N1 | 3.99b | 223b | 108b | 24.58a | 82.67bc |
| N2 | 5.93a | 269a | 132a | 24.82a | 84.32ab |
| N3 | 5.79a | 285a | 131a | 24.50a | 79.61c |
| Planting density | | | | | |
| D1 | 3.91b | 182c | 124a | 24.89a | 83.72a |
| D2 | 4.61ab | 244b | 109b | 24.49a | 83.80a |
| D3 | 4.96a | 286a | 98b | 24.85a | 82.19a |
| ANOVA | | | | | |
| S(df = 2) | 0.435(ns) | 6.815* | 1.075(ns) | 4.413(ns) | 14.582* |
| N(df = 3) | 31.283*** | 14.057** | 20.814*** | 1.118(ns) | 5.907* |
| D(df = 2) | 4.664(ns) | 27.999** | 7.954* | 3.612(ns) | 0.715(ns) |
| N × S(df = 6) | 6.513** | 4.129* | 5.495** | 0.792(ns) | 1.164(ns) |
| D × S(df = 4) | 5.677** | 2.920(ns) | 3.866* | 0.244(ns) | 1.302(ns) |
| N × D(df = 6) | 4.698* | 1.62(ns) | 0.559(ns) | 0.719(ns) | 2.501(ns) |
| N × D × S (df = 12) | 2.706** | 2.413* | 1.548(ns) | 3.373*** | 1.708(ns) |

2016S: second season in 2016. 2017F: first season in 2017. 2017S: second season in 2017.
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.

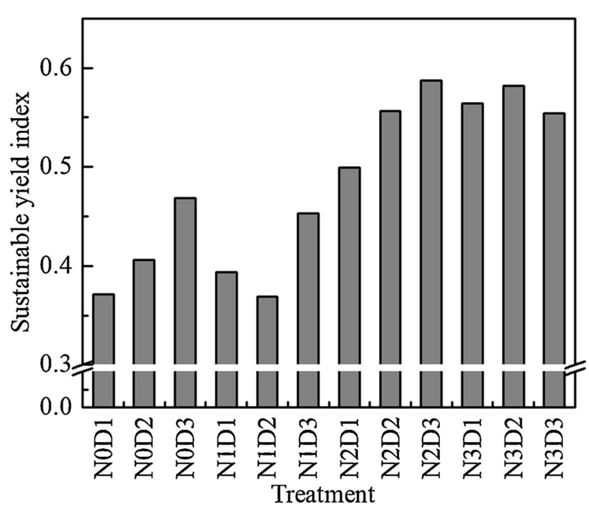


**Fig. 4.** The sustainable yield index of perennial rice over 4 seasons in 2016-2017.

### 4.2. Economic benefits and N profit

In perennial rice production, the input in transplanting season (2016F) mainly includes seeds, seedling, plowing, transplanting, crop management, pesticide and herbicide, fertilizer and harvest (Fig. 9). In the regrowth seasons (2016S, 2017F and 2017S), without seeds, seedling, plowing and transplanting, the new tillers ratooned from the rhizome the input decreased 7250 CNY $ha^{-1}$ each season when compared to 2016F (14,917 CNY $ha^{-1}$). The output of perennial rice mainly was the output of grain yield, and N2D3 produced significantly higher output (23,808 CNY $ha^{-1}$) and economic profit (14,223 CNY $ha^{-1}$) in 4 seasons. Additionally, N2D3 produced significantly higher N profit (79 CNY $kg^{-1}$) in 4 seasons (Fig. 7c). Perennial rice had significantly lower input in regrowth seasons, N2D3 treatment produced significant economic profit and N profit, and was the most economic scheme in perennial rice production.

**Table 5**
Regrowth rate and root activity during the regrowth seasons of 2016–2017.

| Treatment | Regrowth rate (%) | Root activity (g $h^{-1}$ $m^{-2}$) |
|---|---|---|
| Season | | |
| 2016S | 96.36a | 6.907b |
| 2017F | 92.25b | 8.565a |
| 2017S | 90.24c | 6.709b |
| N rate | | |
| N0 | 97.34a | 4.38d |
| N1 | 92.76b | 6.94c |
| N2 | 90.75b | 9.49a |
| N3 | 90.95b | 8.77b |
| Planting density | | |
| D1 | 90.86b | 5.84c |
| D2 | 93.88a | 7.65b |
| D3 | 94.11a | 8.68a |
| ANOVA | F-value | |
| S(df = 2) | 5.646(ns) | 6.469(ns) |
| N(df = 3) | 20.095*** | 21.053*** |
| D(df = 2) | 1.965(ns) | 62.985*** |
| N × S(df = 6) | 1.240(ns) | 3.230* |
| D × S(df = 4) | 8.601** | 0.573(ns) |
| N × D(df = 6) | 1.994(ns) | 0.784(ns) |
| N × D × S(df = 12) | 18.032*** | 3.950*** |

2016S: second season in 2016. 2017F: first season in 2017. 2017S: second season in 2017.
Different letters within a column represent significant differences at P < 0.05 (LSD).
S: season. N: N rate. D: planting density. N × S: interaction effect between N rate and season. D × S: interaction effect between planting density and season. N × D: interaction effect between N rate and planting density. N × D × S: interaction effect between N 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.

## 5. Discussion

### 5.1. Perennial vs annual rice

SYI is an important factor to evaluate whether a crop could produce sustainable and stable grain yields in consecutive years (Muhammad et al., 2020). The perennial rice 23 performed the same yield potential as annual rice and high SYI illustrated that perennial rice had sustainable and stable production ability and yield potential over the years. Without seeds, seedling, plowing and transplanting in the successive regrowth seasons, perennial rice is capable of surviving for many consecutive years (Zhang et al., 2017) which reduced the large materials and labour input in annual field (Huang et al., 2018), and could economize approximately half of the input of annual rice and obtain more profit in rice production (Fig. 2). Moreover, with the absence of tillage, perennial rice cropping system reduced soil disturbance, would control soil erosion effectively and benefit for soil amelioration (Denardin et al., 2019). Perennial rice is an economic and environmental safely cropping system, and is able to produce a stable and sustainable grain yield over successive seasons across years.

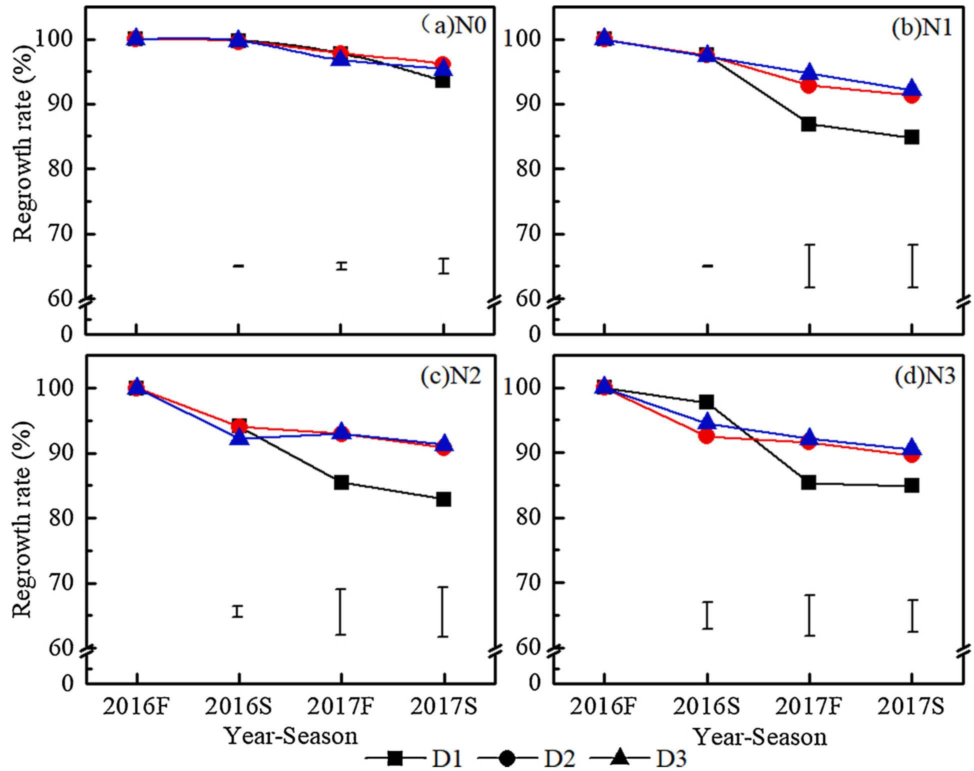


**Fig. 5.** Regrowth rate of perennial rice under different N rates and planting densities in 2016–2017. 2016F: first season in 2016 (transplanting season). 2016S: second season in 2016. 2017F: first season in 2017. 2017S: second season in 2017. Vertical bars represent the standard error for different treatments.

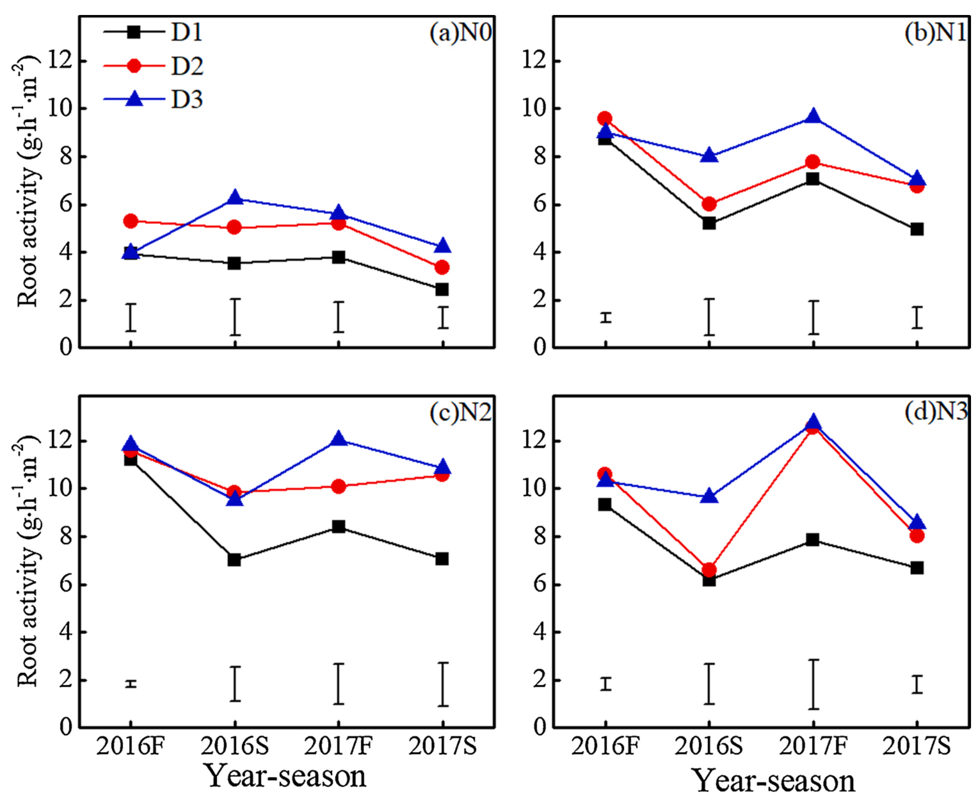


**Fig. 6.** Root activity of perennial rice under different N rates and planting densities in 2016–2017. 2016F: first season in 2016 (transplanting season). 2016S: second season in 2016. 2017F: first season in 2017. 2017S: second season in 2017. Vertical bars represent the standard error for different treatments.

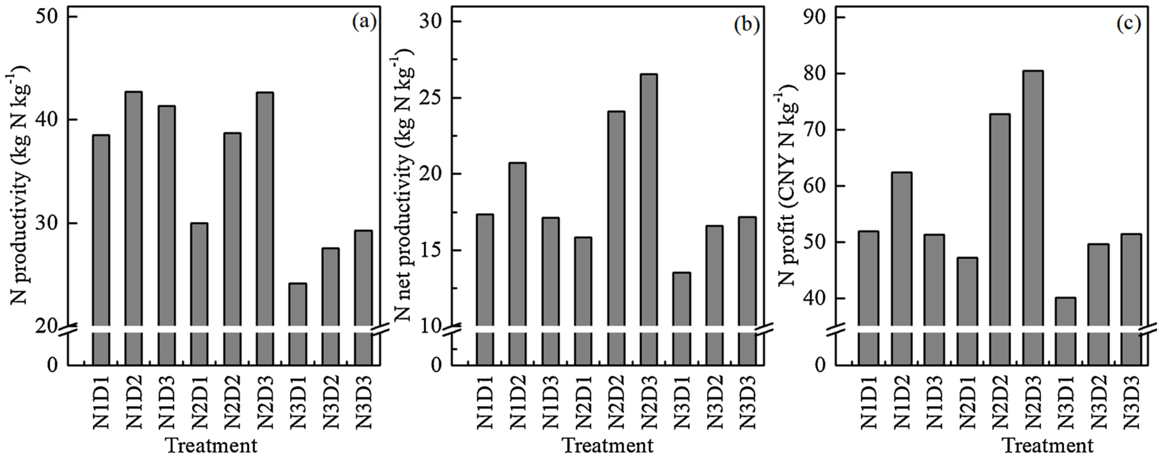


**Fig. 7.** The N productivity and profit of perennial rice. (a) The N productivity of each treatment. (b) The net productivity of each treatment. (c) The N profit of each treatment.

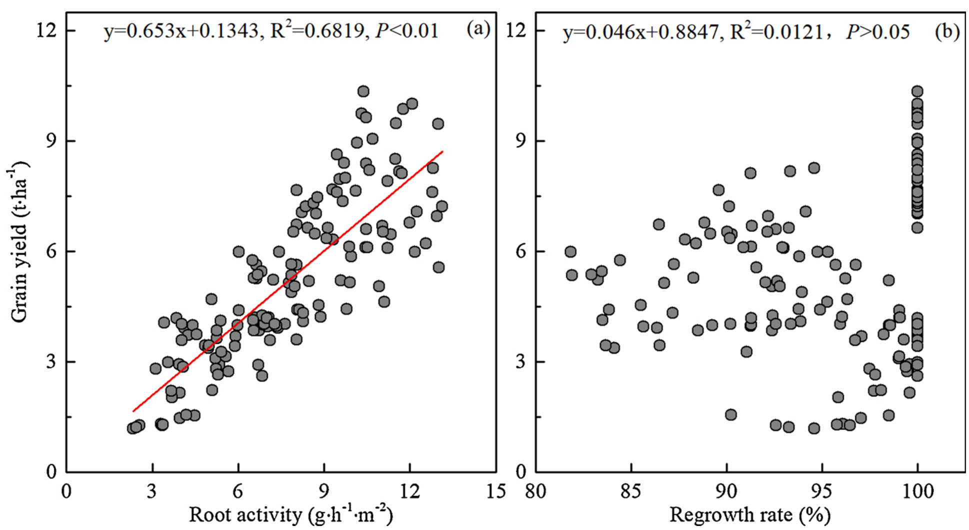


**Fig. 8.** The relationship of grain yield with root activity and regrowth rate of perennial rice. (a) The relationship of root activity with grain yield. (b) The relationship of regrowth rate with grain yield.

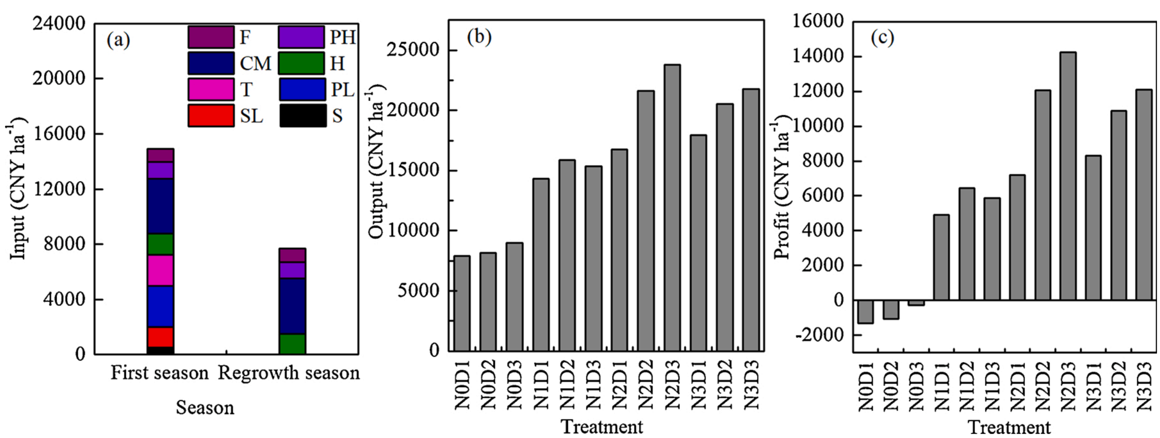


**Fig. 9.** The average input, output and profit of perennial rice in 2016-2017. (a) The input of transplanting and regrowth season. The total input including: F, fertilizer. PH, pesticide and herbicide. CM, crop management. H, harvest. T, transplanting. PL, plowing. SL, seedling. S, seeds. (b) The average output of each treatment. (c) The average profit of each treatment.

### 5.2. Regrowth rate and root activity

An optimal rice population and robust plants often have great potential to obtain high grain yield (Shen et al., 2013). For perennial rice, a high regrowth rate and root activity are critical for maintaining a robust rice population over successive regrowth seasons. In this experiment, the regrowth rate of perennial rice significantly decreased across seasons, but the grain yield remained stable. This pattern is likely benefit from the strong regrowth ability and self-regulation via new tillers from rhizome of perennial rice. Perennial rice had great regrowth ability that derived from the parent of *O. longistaminata* (Zhang et al., 2017, 2019c; Huang et al., 2018). Additionally, rice, including perennial rice, has a robust ability to self-regulate to maintain a healthy crop community (in terms of panicle no. $m^{-2}$) (Yang et al., 2014). Till the fourth season, the regrowth rate of perennial rice was still above on 90 %. Based on the high regrowth rate of perennial rice, the ability of perennial rice to self-regulate would compensate for the minor decrease that occurs in the rice population, which also likely explains why the regrowth rate showed a decreasing trend and why the panicle no. and grain yield of perennial rice remained at high and stable levels during the regrowth seasons (Tables 4 & 5).

The regrowth rate decreased as N rate increased, suggesting that N fertilizer limits the regrowth of perennial rice. This may be some physiological or genetic mechanism of perennial rice response to N fertilizer which is studying by our team now. Similar N effect was observed on sugarcane that high N fertilizer would limit the emergence rate and shooting rate in ratooned years (Zeng et al., 2020). As perennial rice is the first released perennial grain over the world in 2018, similar phenomenon on other perennial grains is not yet found now. However, as planting density increased, the regrowth rate of perennial rice increased, suggesting that controlling plant densities can contribute to sustainable regrowth. The result was consistent with the previous research on sugarcane that rational high planting density would lead to higher shooting rate and tillers in ratoon years (Qiu et al., 2019). These observations also explain why the N2D3 treatment produced the highest grain yield across years. Specifically, the positive effect of a high planting density in D3 on the regrowth and self-regulation ability of perennial rice compensated for the negative effect of N2 on regrowth. To sustainably produce perennial rice, chemical N fertilizer inputs need to be reduced within an appropriate range to ensure an adequate grain yield while high planting densities are maintained. Additionally, dense planting and less N fertilizer are the most effective and environmentally friendly ways for increasing yield and reducing N loss (Zhu et al., 2016).

Plants exchange substances and energy with the surrounding environment primarily via their roots (Yang et al., 2004; Zhang et al., 2019a). As a consequence, higher root activity reflects more water, nutrient and energy exchange with the environment (Liu et al., 2020; Zhang et al., 2019b) and results in a higher grain yield. Root activity was significantly related to the grain yield of perennial rice (Fig. 8a), high levels of root activity are essential to ensure a higher crop yield (Zhang et al., 2019b) and regrowth rate in the regrowth seasons. The root activity of perennial rice remained stable over successive regrowth seasons suggests that perennial rice had developed a root system that could absorb enough soil nutrients and water during the regrowth season (Pimentel et al., 2012), as the roots did not degenerate over successive regrowth seasons. The root activity of N2 was significantly higher than the other N rates, demonstrating that modest quantities of N fertilizer are capable of promoting the growth and activity of the root system. Indeed, low or excessive application of chemical N fertilizer limited the root growth and activity of rice (Zhang et al., 2019b). Moreover, the high planting density in D3 promoted higher root activity in the unit area in the field and then resulted in high root activity in N2D3. Thus, crop management that combines an appropriate N fertilizer rate and plant density coupled with the no-tillage can promote high root activity and support the sustainable production of perennial rice for several years.

### 5.3. Grain yield and its components

Perennial rice is a new cultivar of rice. Grain yield and yield components are the main metrics for assessing the success of the perennial rice 23 cultivar (Zhang et al., 2017, 2019c). In this experiment, the successful and stable grain yield of perennial rice over several seasons showed that this novel rice production system, which does not require tillage, can facilitate the sustainable production of perennial rice in an environmentally feasible manner (Huang et al., 2018). In this experiment, the N2D3 treatment produced the most stable and highest grain yield over four successive seasons across two years. This finding is likely attributed to the high root activity of tillers from rhizome and the greater ability of perennial rice to self-regulate documented in N2D3, which supports a robust rice population and an adequate supply of nutrients needed for perennial rice growth. Proper fertilizer and field management could help farmers obtain a high- and good-quality grain yield, reduce labour inputs and increase economic profits (Huang et al., 2018; Cui et al., 2018; Tilman et al., 2011). Based on the significantly positive correlation between root activity and panicle no. $m^{-2}$, spikelet no. $panicle^{-1}$ and grain weight, the high root activity in the N2D3 treatment enhanced the yield components and grain yield over successive regrowth seasons of perennial rice (Table 4).

The grain yield and yield components of N2 and N3 were not significantly different; however, these components were significantly higher for N2 and N3 than those for N0 and N1. This finding is illustrated by the fact that the N2 rate had a stronger positive effect on perennial rice production and the fact that the use of additional N fertilizer had no significant positive effect on the grain yield and yield components (Guo et al., 2010). In contrast, the use of more fertilizer in N3 resulted in a low seed-setting rate (Table 4). Excessive application of N fertilizer had no positive effect on the crop yield but instead resulted in a yield loss (Guo et al., 2010; Peng et al., 2006). Indeed, over-fertilization has been shown to reduce fertilizer use efficiency and increase fertilizer loss and pollution (Peng et al., 2006; Hossain et al., 2005). Planting density also had a significant effect on the grain yield. Both D2 and D3 had high yield potential, but D3 had higher regrowth potential and root activity (Table 5).

### 5.4. N productivity and economic benefits

Nitrogen (N) is an essential element of proteins, nucleic acids, enzymes, hormones and chlorophyll and plays an important role in maintaining the nutrient cycle and determining plant growth (Xu et al., 2020; Fowler et al., 2013). In this experiment, N fertilizer had a significantly positive relationship with panicle no. $m^{-2}$, spikelet no. $panicle^{-1}$, root activity and grain yield. These patterns illustrated that the addition of modest levels of N fertilizer can enhance both crop growth and crop yield (Ren et al., 2017). However, in recent years, increases in fertilizer loss and pollution have become more widespread as N fertilizer has often been excessively applied to increase crop yield, resulting in increased soil degeneration and environmental problems (Cai et al., 2020; Tian et al., 2016; Zhao et al., 2016; Zhang et al., 2018). In China, fertilizer use efficiency has been estimated to be 25 %, which is far below the worldwide average of 42 % (Chen et al., 2014). However, fertilization does not always increase crop yield; indeed, the excessive use of fertilizer can result in low fertilizer use efficiency, decrease economic profits and create environmental problems (Peng et al., 2006; Tian et al., 2016). N productivity was effective indicator for evaluating N fertilizer productivity (Wang et al., 2018). The N3 treatment in this experiment did not result in significantly higher grain yields compared with the N2 treatment; however, the N net productivity (Fig. 7) were significantly lower. Moreover, for perennial rice, high N fertilizer also limited its regrowth across successive seasons. Therefore, N fertilizer productivity needs to be improved to obtain a high crop yield.

Compared with annual rice, the absence of seeds, seedling, plowing and transplanting of perennial rice in regrowth seasons reduced the intensive works in field and huge investment in materials that could obtain more profit in rice production (Huang et al., 2018). The fact that the highest values of N net productivity and N profit were observed in the N2D3 treatment demonstrated that the N fertilizer effect and productivity was the most optimal in this treatment, maximizing the grain yield, reducing fertilizer loss and pollution and enhancing regrowth.

## 6. Conclusion

(1) Perennial rice is an economic and environmental cropping system, and was able to produce a stable and sustainable grain yield over successive seasons across years.
(2) The N2D3 (180 kg $ha^{-1}$ nitrogen integrated with 22.7 plants $m^{-2}$) treatment resulted in a high and stable grain yield with high root activity and regrowth rate of perennial rice for successive regrowth seasons across years, and resulted in more economic benefit and less N loss and pollution. Thus, the N2D3 treatment provided optimal conditions and economic profit for sustainable perennial rice production.
(3) Generally, sustainable production of perennial rice at a certain grain yield requires a modest application of N chemical fertilizer coupled with a high planting density to maintain a high regrowth rate and reduce fertilizer loss.
(4) The optimal conditions for the management of perennial rice have been poorly explored. Thus, our findings demonstrate the benefits of perennial rice production and suggest that the potential benefits of producing other perennial grains should be further examined.

## CRediT authorship contribution statement

**Yujiao Zhang:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft. **Guangfu Huang:** Investigation, Data curation, Supervision. **Shilai Zhang:** Investigation, Supervision. **Jing Zhang:** Investigation, Supervision. **Shuxian Gan:** Investigation, Supervision. **Mao Cheng:** Investigation, Supervision. **Jian Hu:** Investigation, Supervision. **Liyu Huang:** Supervision. **Fengyi Hu:** Conceptualization, Supervision, Writing - review & editing.

## Declaration of Competing Interest

The authors report no declarations of interest.

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