

## Article

# Reasonable Nitrogen Regime in the Main Crop Increased Grain Yields in Both Main and Ratoon Rice

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**Abstract:** Planting ratoon rice can realize one sowing and two harvests, which is of great significance for improving grain yield. However, the effects of nitrogen (N) regime in the main crop on the grain yield of ratoon rice and the associated physiological mechanisms are not clearly understood. The indica hybrid rice Liangyou 6326 was used, and three N fertilizer levels (100 kg ha<sup>-1</sup> (low N, LN), 250 kg ha<sup>-1</sup> (medium N, MN), and 400 kg ha<sup>-1</sup> (high N, HN)) and four different ratios of basal tillering fertilizer to panicle fertilizer (7:3, 6:4, 5:5, and 4:6) applied to the main crop were designed to investigate their effects on the grain yields of the main and ratoon crops. The results showed that excessive N application rate and panicle N application rate in the main crop was not conducive to the improvement of yield and agronomic nitrogen use efficiency (ANUE) in both seasons. The increased yield in the ratoon crop was attributed to the increase in the regeneration rate. Appropriate increasing of the panicle N application rate was beneficial for increasing the ROA and NSC concentration in the main crop, resulting in an increase in the number, length, and fresh weight of regenerated buds, which caused an improvement in the regeneration rate. However, when excessive panicle N was applied in the main crop, the excessive germination of regenerated buds decreased the length and fresh weight of the regenerated bud and resulted in a decrease in the regeneration rate. These results suggest that in the production of ratoon rice, reasonable N regime in the main crop could increase the yield and ANUE in both seasons.

**Keywords:** nitrogen rate; nitrogen ratio; yield; regenerative ability



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## 1. Introduction

The global population continues to grow and is expected to reach 8.5 billion by 2030 and 10.9 billion by 2100, according to UN's 2019 World Population Prospects. Global food production must be further increased to meet the needs of the growing population [1,2]. Rice (*Oryza sativa* L.) is an important food crop species worldwide, supporting more than half of the global population [3,4]. Given that global urbanization is increasing and water resources are becoming increasingly scarce, there are two main ways to increase global rice production: One is to increase the grain yield per unit area, and the other is to increase the multiple cropping index [5]. Because achieving major breakthroughs in rice yield per unit area is difficult, developing ratoon rice comprising one plant with two harvest periods is an effective way to increase rice yield [6,7]. Ratoon rice is rice planted in such a way that dormant buds in the stubble survive after the main crop is harvested, and subsequently, regenerate into panicles [8]. The use of ratoon rice has been adopted in many countries [9]. For example, in China, the planting area of ratoon rice reached nearly one million ha in 2019, and according to reports, there are more than 3.3 million ha of fields that are suitable

for planting ratoon rice in southern China [10]. Therefore, increasing research on ratoon rice and further improving its yield are of great significance to ensure global food security.

The yield of ratoon rice is affected by many factors, such as the height of the stubble [11], water management [12], variety [13], and nitrogen (N) fertilizer management [14], among which N fertilizer management has a critical influence on the yield of ratoon rice. N fertilizer management of ratoon rice mainly involves the application of N fertilizer to the main crop and the ratoon crop (bud fertilizer and seedling fertilizer). Many scholars have researched the effects of N fertilizer management in ratoon season on the growth and development of ratoon crops, and most agree that the application of bud and seedling fertilizers can promote the growth of regenerated buds and improve the regenerative capacity and yield of ratoon crops [15,16]. However, there are relatively few studies about the effects of N fertilizer management in the main crop on the growth and development of ratoon crops, and the results have been inconsistent or even contradictory. For example, Huang et al. (2022) reported that increasing N application rate in the late growth stage of the main crop could improve the effective tillering percentage, leaf area index, canopy light interception rates, and transport rate of stem and sheath in the main crop, leading to an increase in yields of both seasons [17]. Properly postponed N application in the main crop was beneficial to increasing the root activity and promoting the growth of rice, resulting in increasing the yields of both seasons [18]. Liu et al. (2014) found that increasing the amount of panicle N fertilizer in the main crop was beneficial for increasing the yields of the main and ratoon crops [19]. However, Chen et al. (2010) suggested that a moderate amount of delayed N fertilizer could increase the yield of the main crop but had little effect on the yield of the ratoon crop [20]. Wang et al. (2019) indicated that the N application rate in the main crop had little effect on the yield of the ratoon crop [14].

However, there is a lack of in-depth research on the effects of N regime in the main crop on the growth and development of regenerated buds and the regeneration rate. In addition, the N regime in the main crop on the yield of the ratoon crop under different N application rate have not been reported so far. Since the N application rates vary greatly in different areas in the field production of ratoon rice, it is necessary to study the effects of N regime in the main crop on the yield of ratoon rice under different N application rates. In this study, N was applied at three different levels in conjunction with four panicle N ratios to the main crops to investigate the effects of the N regime in the main crop on the yield of the main and ratoon crops and to determine rational N management practices for the high-yield cultivation of ratoon rice.

## 2. Materials and Methods

### 2.1. Experimental Site and Materials

The field experiment was conducted in a farmer's field at Xinyang (32°07' N, 114°05' E), Henan Province, North China, in 2018 and repeated in 2019. The soil in the experimental field was a clay loam with contents of organic carbon of 11.4 g kg<sup>-1</sup>, total N of 54.3 mg kg<sup>-1</sup>, Olsen-phosphorus (P) of 9.7 mg kg<sup>-1</sup>, and available potassium (K) of 79.8 mg kg<sup>-1</sup>. Two years' weather information during the rice-growing periods (from March to November) is shown in Figure S1.

The tested rice variety was Liangyou 6326, an indica two-line hybrid rice variety that is a result of the combination of Xuan 69S and Zhongxian WH26. This variety was released by the Xuancheng Agricultural Science Research Institute and passed the National Crop Variety Approval in 2007 [National Approved Rice 2007013].

### 2.2. Experimental Design

The experiments were arranged in accordance with a split-plot design, with four replicates (plot size: 4 × 5 m). The main plots were divided according to the rate of total N applied to the main crop, and the three N application rates were low N (LN), medium N (MN), and high N (HN), corresponding to 100 kg ha<sup>-1</sup>, 250 kg ha<sup>-1</sup>, and 400 kg ha<sup>-1</sup>, respectively. The N rates of MN and HN were based on the local standard

for ratoon rice cultivation “Technique rule for planting ratoon rice in the south of Henan Province” (DB41/T 1564-2018) and “Theory and technology of rice precise and quantitative cultivation” [21]. Subplots corresponded to different ratios of basal tillering fertilizer to panicle fertilizer application to the main crop. The ratios were 7:3 (PN30), 6:4 (PN40), 5:5 (PN50), and 4:6 (PN60). The details of the N treatments are summarized in Table 1. In addition, 0 kg ha<sup>-1</sup> N rate was set in both the main and ratoon crops to investigate the yield of N free. The seeds were sown in a greenhouse on March 4 in both 2018 and 2019. The seedlings were subsequently transplanted to the field on April 8 in both years, with a hill spacing of 0.33 × 0.15 m and 2 seedlings per hill. Before transplanting, all the plots were plowed and puddled. To prevent the flow of fertilizer between neighboring plots, the plots were separated by a 40 cm wide ridge created by a plastic film inserted into the soil to a depth of 30 cm. The technical drawing is shown in Figure S2.

**Table 1.** Nitrogen application rates (kg N ha<sup>-1</sup>) in the main and ratoon crops.

| Total N Rate | N Rates in Main Crop           |                    | Treatment Code | N Rates in Ratoon Crop |                |                     |
|--------------|--------------------------------|--------------------|----------------|------------------------|----------------|---------------------|
|              | Basal and Tillering Fertilizer | Panicle Fertilizer |                | Total N Rate           | Bud Fertilizer | Seedling Fertilizer |
| 100 (LN)     | 70 (70)                        | 30 (30)            | PN30           | 200                    | 50             | 150                 |
|              | 60 (60)                        | 40 (40)            | PN40           | 200                    | 50             | 150                 |
|              | 50 (50)                        | 50 (50)            | PN50           | 200                    | 50             | 150                 |
|              | 40 (40)                        | 60 (60)            | PN60           | 200                    | 50             | 150                 |
| 250 (MN)     | 175 (70)                       | 75 (30)            | PN30           | 200                    | 50             | 150                 |
|              | 150 (60)                       | 100 (40)           | PN40           | 200                    | 50             | 150                 |
|              | 125 (50)                       | 125 (50)           | PN50           | 200                    | 50             | 150                 |
|              | 100 (40)                       | 150 (60)           | PN60           | 200                    | 50             | 150                 |
| 400 (HN)     | 280 (70)                       | 120 (30)           | PN30           | 200                    | 50             | 150                 |
|              | 240 (60)                       | 160 (40)           | PN40           | 200                    | 50             | 150                 |
|              | 200 (50)                       | 200 (50)           | PN50           | 200                    | 50             | 150                 |
|              | 160 (40)                       | 240 (60)           | PN60           | 200                    | 50             | 150                 |

Note: LN, low N rate (the total amount of N applied was 100 kg ha<sup>-1</sup>); MN, medium N rate (the total amount of N applied was 250 kg ha<sup>-1</sup>); HN, high N rate (the total amount of N applied was 400 kg ha<sup>-1</sup>). PN30, PN40, PN50 and PN60, correspond to N application ratio of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5 and 4:6, respectively. The numbers in () indicate the percentage of N application to the total N application of the main crop.

The N fertilizer used was urea, with an N content of 46%. The ratio of basal fertilizer to tiller fertilizer was 7:3. Basal fertilizer was applied one day before transplantation, and tiller fertilizer was applied 5 days after transplantation. Panicle fertilizer was applied twice at panicle initiation and at the beginning of spikelet differentiation, accounting for 60% and 40%, respectively. The same amounts of P (as calcium superphosphate, 12% P<sub>2</sub>O<sub>5</sub>) and K (as potassium chloride, 60% K<sub>2</sub>O) were applied in both years. All P and K were applied as basal fertilizer, and the amounts of P and K were 937.5 kg ha<sup>-1</sup> and 187.5 kg ha<sup>-1</sup>, respectively. At 15 days after heading of the main crop, 50 kg N ha<sup>-1</sup> was applied to each plot as bud fertilizer. After the main crop was harvested, 150 kg ha<sup>-1</sup> N was applied as a seedling fertilizer. Although the bud fertilizer was applied during the growth period of the main crop, it had a greater impact on the yield of the ratoon crop, so the bud fertilizer is usually included in the N management of ratoon season [22]. After transplanting, the field was kept flooded for 35 days, and then the water was drained. The field was reflooded at the jointing stage and drained again 7 days before the main crop was harvested. A water layer 1 to 3 cm above the soil surface was maintained during the entire ratoon crop growing season. Weeds were removed by hand. Diseases and insects were controlled by chemicals to avoid yield loss.

The main crop was harvested by hand on 12 August in both years with a stubble height of 0.35 m.

### 2.3. Sampling and Measurements

#### 2.3.1. Root Oxidation Activity (ROA)

At full heading, at the 15th day after heading, and at maturity of the main crop, after the mean stem number in each plot was recorded, representative plants from 10 hills were selected to measure the ROA in each plot. A block of soil (20 × 20 × 20 cm) around each

individual hill was removed, and after rinsing with running water (Figure S3), the root subsamples were taken to measure the ROA via oxidation of alpha-naphthylamine ( $\alpha$ -NA) according to the methods of Ramasamy et al. [23].

### 2.3.2. Nonstructural Carbohydrate (NSC) Accumulation in the Stem and Leaf

At the same time as the procedures described above, after the mean stem number in each plot was recorded, representative plants from 10 hills (0.495 m<sup>2</sup>) were selected in each plot and removed. The plants were separated into stems (culms + sheaths), leaves, and panicles. All the samples were first dried at 105 °C for 30 min and then dried to constant weight at 75 °C in an oven. The dried samples were subsequently crushed and passed through a 0.15 mm sieve to ultimately determine the NSC concentration [24]. Briefly, 100 mg dry sample was placed into 15 mL distilled water and boiled for 20 min. After filtration and constant volume, 1 mL of the content was taken, and 5 mL of anthrone reagent was added. Then spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan) was used to measure the soluble sugar and starch content. According to the above method, at harvest of the main crop, 10 representative stubbles were taken from each plot to determine the NSC concentration.

### 2.3.3. Regenerated Buds

On the 15th day after heading, the 25th day after heading, maturity, and the 7th day after the main crop was harvested, 5 hills were selected from each plot according to the average level of seedlings in the whole field. The fresh weight and length of living regenerated buds at each internode were measured, and the number of living and dead regenerated buds per stem was investigated (buds larger than 1 cm were included) as described by Xu et al. [25] and Zhang et al. [26] (Figure S4).

### 2.3.4. Yield and Its Components

Plants from 1 m<sup>2</sup> in each plot were harvested to measure the spikelet number per panicle and the panicles number per square meter. The filled grains were separated by submerging all spikelets in tap water after threshing. In addition, the grain yields of the main and ratoon crops were measured by hand-harvesting rice plants from 5 m<sup>2</sup> in each plot (the moisture was adjusted to 14%).

### 2.3.5. Statistical Analysis

The regeneration rate was calculated as the number of panicles per square meter in the ratoon crop/the number of panicles per square meter in the main crop.

Agronomic nitrogen use efficiency (ANUE) = (Grain yield – Grain yield of 0 N)/N application rate.

The minimum sample size of each indicator in this experiment is 4. Analysis of variance (ANOVA) was performed using SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA) to detect the effects of year and variety. The means were subjected to least significant difference (LSD) tests at  $p < 0.05$  (LSD<sub>0.05</sub>) and  $p < 0.01$  (LSD<sub>0.01</sub>). The number, length, and fresh weight of regenerated buds, regeneration rate, ROA and NSC content in stem are continuous variable variables. Therefore, this experiment uses Pearson's correlation and regression analysis to analyze their relationship.

## 3. Results

### 3.1. Differences in Experimental Factors

The yields of the main and ratoon crops and their components did not significantly differ between years but did significantly differ among N application rates and ratios (Table 2). The length, fresh weight, and number of regenerated buds per stem (unless otherwise specified, the following regenerated buds refer to living buds) at maturity of the main crop, ROA and NSC concentration in the stems and leaves on the 15th day after heading, NSC concentration in the stubble, and the regeneration rate of ratoon crop were

not significantly different between years but were significantly different among N rates and ratios (Table 3). Since year was not a significant factor in any experiment, this paper mainly used the mean values of the two years for analysis purposes.

**Table 2.** Analysis-of-variance of F-values of the grain yield and yield components of the main and ratoon crops between/among years, N regimes.

| Source of Variation |           | df | Panicle Number | Spikelet per Panicle | Filled Grain Rate | 1000-Grain Weight | Grain Yield |
|---------------------|-----------|----|----------------|----------------------|-------------------|-------------------|-------------|
| Main crop           | Y         | 1  | NS             | NS                   | NS                | NS                | NS          |
|                     | N         | 2  | 159.72 **      | 150.20 **            | 131.57 **         | NS                | 114.80 **   |
|                     | R         | 3  | 24.62 **       | 24.63 **             | NS                | NS                | 53.75 **    |
|                     | Y × N     | 2  | NS             | NS                   | NS                | NS                | NS          |
|                     | Y × R     | 3  | NS             | NS                   | NS                | NS                | NS          |
|                     | N × R     | 6  | NS             | NS                   | NS                | NS                | NS          |
| Ratoon crop         | Y × N × R | 6  | NS             | NS                   | NS                | NS                | NS          |
|                     | Y         | 1  | NS             | NS                   | NS                | NS                | NS          |
|                     | N         | 2  | 359.22 **      | 229.83 **            | NS                | NS                | 175.72 **   |
|                     | R         | 3  | 42.36 **       | NS                   | NS                | NS                | 7.10 **     |
|                     | Y × N     | 2  | NS             | NS                   | NS                | NS                | NS          |
|                     | Y × R     | 3  | NS             | NS                   | NS                | NS                | NS          |
|                     | N × R     | 6  | 50.04 **       | NS                   | NS                | NS                | 7.10 **     |
| Y × N × R           | 6         | NS | NS             | NS                   | NS                | NS                |             |

Note: Y, years. N, nitrogen rate. R, ratio of nitrogen application. \*\* represents a significant difference at the 1% level according to LSD tests, NS represents no significant difference at the 5% level according to LSD tests.

**Table 3.** Analysis-of-variance of F-values of key indices such as the growth and development of the main crop after full heading, growth of regenerative bud, and regenerative ability between/among years, N regime.

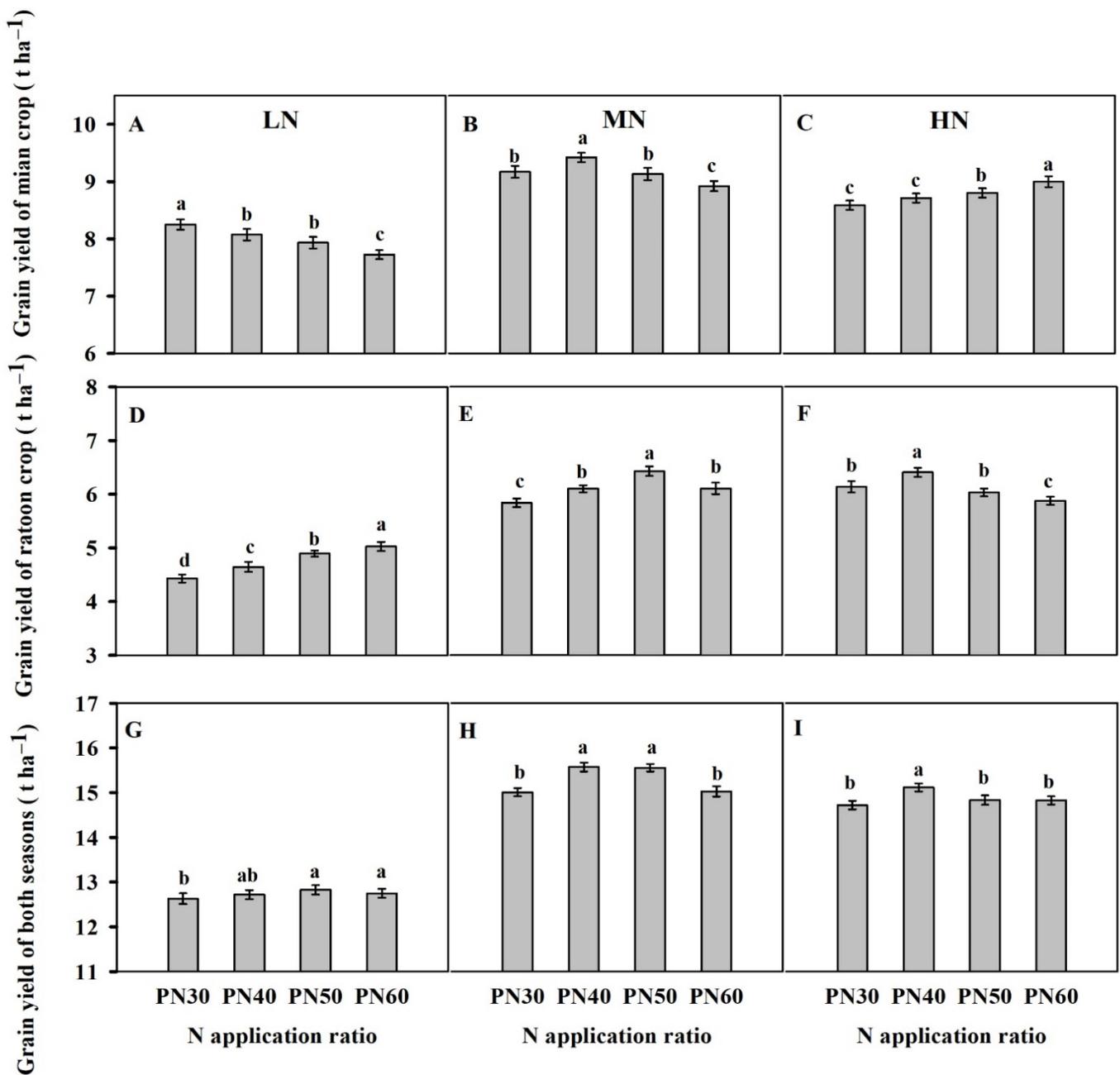
| Source of Variation | df | Indices of Regenerated Bud at MS |              |           | ROA at 15DAH | NSC Concentration in Stem and Leaf at 15DAH | NSC Concentration in Stubble | Regeneration Rate |
|---------------------|----|----------------------------------|--------------|-----------|--------------|---|------------------------------|-------------------|
|                     |    | Length                           | Fresh Weight | Number    |              |   |                              |                   |
| Y                   | 1  | NS                               | NS           | NS        | NS           | NS  | NS                           |                   |
| N                   | 2  | 118.4 **                         | 40.23 **     | 239.27 ** | 9.93 *       | 52.73 **                                    | 50.12 **                     | 13.68 **          |
| R                   | 3  | 59.29 **                         | 29.88 **     | 298.07 ** | 15.61 **     | 53.95 **                                    | 64.45 **                     | 70.91 **          |
| Y × N               | 2  | NS                               | NS           | NS        | NS           | NS  | NS                           | NS                |
| Y × R               | 3  | NS                               | NS           | NS        | NS           | NS  | NS                           | NS                |
| N × R               | 6  | 20.21 **                         | 14.33        | 6.44 **   | NS           | NS  | NS                           | 29.25 **          |
| Y × N × R           | 6  | NS                               | NS           | NS        | NS           | NS  | NS                           | NS                |

Note: Y, years. N, rate nitrogen application. R, ratio of nitrogen application. NSC, nonstructural carbohydrate. ROA, root oxidation activity. DAH, days after heading of the main crop. MS, maturity stage of the main crop. \*\* represents a significant difference at the 1% level according to LSD test, \* represents a significant difference at the 5% level according to LSD test, NS represents no significant difference at the 5% level according to LSD test.

### 3.2. Yield and Its Components in the Two Seasons

The yield of the main crop decreased in the order MN > HN > LN under different N levels. That is, a high N rate was not conducive to increasing the yield of the main crop. A significant decrease in the filled grain rate was the main reason for the decrease in yield in the main crop under HN (Figure 1A–C, and Table S1). Under the same N level, with increasing panicle N rate, the yield of the main crop decreased under LN, first increased and then decreased under MN (peaking at PN40) and increased under HN.

There was no significant difference in yield under MN and HN in the ratoon crops, but their yields were higher than that under LN. With the increasing proportion of panicle N fertilizer under the LN level, the yield increased. However, under MN and HN, the yield first increased and then decreased, and the highest yields were obtained at PN50 and PN40 (Figure 1D–F). According to the analysis of yield components of the ratoon crops, there were no significant differences in the number of spikes per panicle, filled grain rate, or 1000-grain weight among the different treatments, but there were significant differences in panicle numbers, which was the main reason for the changes in the yield of the ratoon crops (Table 4). Correlation analysis showed that there was a linear correlation between yield and panicle number of the ratoon crops and the coefficient of determination was high (Figure S5), indicating that an increased panicle number was the key to increasing the yield of the ratoon crops.



**Figure 1.** Effects of N regime in the main crop on the yield of the main crop (A–C), ratoon crop (D–F), and both seasons (G–I). Error bars are  $\pm$ SE. Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). PN30, PN40, PN50, and PN60 correspond to N application ratios of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. The different lowercase letters at the same N application rate indicate significant differences at the 5% probability level according to LSD tests.

In terms of the total yield of the main crop and ratoon crop, the highest yields under the LN level were obtained at PN50 and PN60, both of which were  $12.8 \text{ t ha}^{-1}$ . The highest yield under the MN level was obtained at PN40 and PN50 ( $15.6 \text{ t ha}^{-1}$ ), and that under the HN level was obtained at PN40 ( $15.1 \text{ t ha}^{-1}$ ) (Figure 1G–I). These results indicate that N application to the main crop at a reasonable rate and ratio had important effects on the yield of the main crop and ratoon crop in both seasons.

**Table 4.** Effects of N regime in the main crop on the yield components of the ratoon crop.

| Treatment |      | Panicles ( $10^4 \text{ ha}^{-1}$ ) | Spikelet per Panicle | Filled Grain Rate (%) | 1000-Grain Weight (g) |
|-----------|------|-------------------------------------|----------------------|-----------------------|-----------------------|
| LN        | PN30 | 296.8 g                             | 58.2 b               | 88.1 a                | 29.0 a                |
|           | PN40 | 311.2 f                             | 57.9 b               | 88.3 a                | 29.2 a                |
|           | PN50 | 328.6 e                             | 58.1 b               | 87.7 a                | 29.2 a                |
|           | PN60 | 338.9 d                             | 57.9 b               | 87.8 a                | 29.1 a                |
|           | Mean | 318.8 B                             | 58.0 B               | 88.0 A                | 29.1 A                |
| MN        | PN30 | 354.4 c                             | 64.1 a               | 88.1 a                | 29.2 a                |
|           | PN40 | 371.9 b                             | 64.1 a               | 88.2 a                | 29.2 a                |
|           | PN50 | 389.3 a                             | 64.2 a               | 88.2 a                | 29.2 a                |
|           | PN60 | 368.3 b                             | 64.3 a               | 88.1 a                | 29.3 a                |
|           | Mean | 371.0 A                             | 64.1 A               | 88.1 A                | 29.2 A                |
| HN        | PN30 | 371.0 b                             | 65.7 a               | 87.8 a                | 28.8 a                |
|           | PN40 | 386.2 a                             | 66.0 a               | 87.8 a                | 28.6 a                |
|           | PN50 | 365.9 b                             | 65.6 a               | 87.7 a                | 28.7 a                |
|           | PN60 | 350.1 c                             | 66.0 a               | 87.9 a                | 28.9 a                |
|           | Mean | 368.3 A                             | 65.8 A               | 87.8 A                | 28.7 A                |

Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). PN30, PN40, PN50, and PN60, correspond to N application ratio of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. Data in a column followed by different lower-case letters indicate significant differences at the 5% probability level according to the LSD test. Means followed by different upper-case letters indicate significant differences between the three nitrogen fertilizer levels at the 5% probability level according to the LSD test.

### 3.3. Regeneration Rate and Its Relationship with Panicle Number and Yield in the Ratoon Crop

The regeneration rate reflects the regeneration ability of ratoon rice, and this factor and the number of mother stems together determine the panicle number of the ratoon crop. The regeneration rate showed a trend of first increasing and then decreasing with increasing N fertilizer levels (Figure 2). As the panicle N application rate increased, the regeneration rate increased under the LN level, while under MN and HN, it first increased and then decreased. The change trend was similar to that of panicle number in the ratoon crop (Table 4), and the correlation analysis showed that compared with the number of mother stems, the regeneration rate had a greater correlation with the panicle number and yield of the ratoon crop (Table 5), indicating that the change in regeneration rate was the main reason for the changes in the panicle number and yield of the ratoon crop.

**Table 5.** Effects of N regime in the main crop on the yield components of the ratoon crop.

| Parameters            | N Rate | Panicle Number | Yield      |
|-----------------------|--------|----------------|------------|
| Number of mother stem | LN     | −0.9986 **     | −0.9927 ** |
|                       | MN     | −0.4694        | −0.5729    |
|                       | HN     | 0.6810         | 0.5474     |
| Regeneration rate     | LN     | 0.9998 **      | 0.9947 **  |
|                       | MN     | 0.8707         | 0.9220 *   |
|                       | HN     | 0.7989         | 0.8374     |

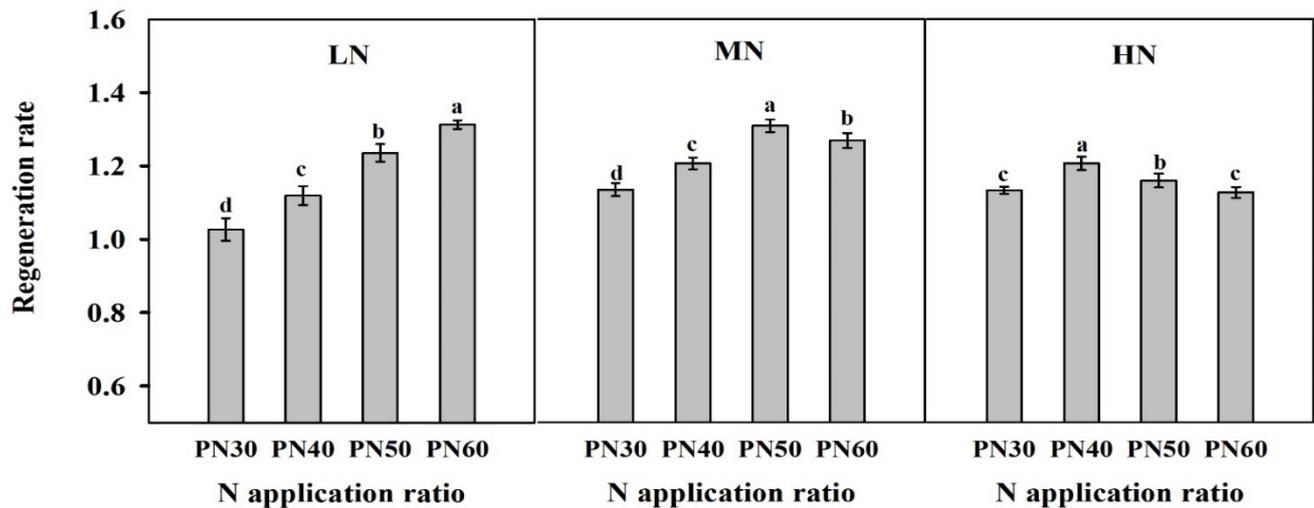
Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). \*\* represents the significant difference at the 1% level according to LSD test, \* represents a significant difference at the 5% level according to LSD test.

### 3.4. Regenerated Buds and Its Relationship with Regeneration Rate

#### 3.4.1. Number, Length, and Fresh Weight of Regenerated Buds

The total number of regenerated buds per unit area (including living and dead regenerated buds) increased from the 15th day after heading to the 7th day after harvesting of the main crop, but due to the increase in the number of dead regenerated buds, the number of living regenerated buds per unit area began to decrease after the main crop was harvested (Figure S6). During the growth of regenerative buds, due to the different growth rates of regenerative buds in different internodes, the regenerative buds that grow slowly

are likely to die [9,27]. Compared with the dead bud, the living bud can better reflect the regeneration ability, so this paper mainly focuses on the growth of the living buds. Before the main crop was harvested, the number of regenerated buds per stem increased with increasing N application rate and proportion of panicle N fertilizer (Figure 3A–C). On the 7th day after harvesting, there was no significant difference in the number of regenerated buds per stem under MN and HN levels, and both of them were higher than that under LN level. With the increasing proportion of panicle N fertilizer, the number of regenerated buds per stem on the 7th day after harvest increased under the LN level but first increased and then decreased under the MN and HN levels, with the greatest values occurring at PN50 and PN40, respectively.

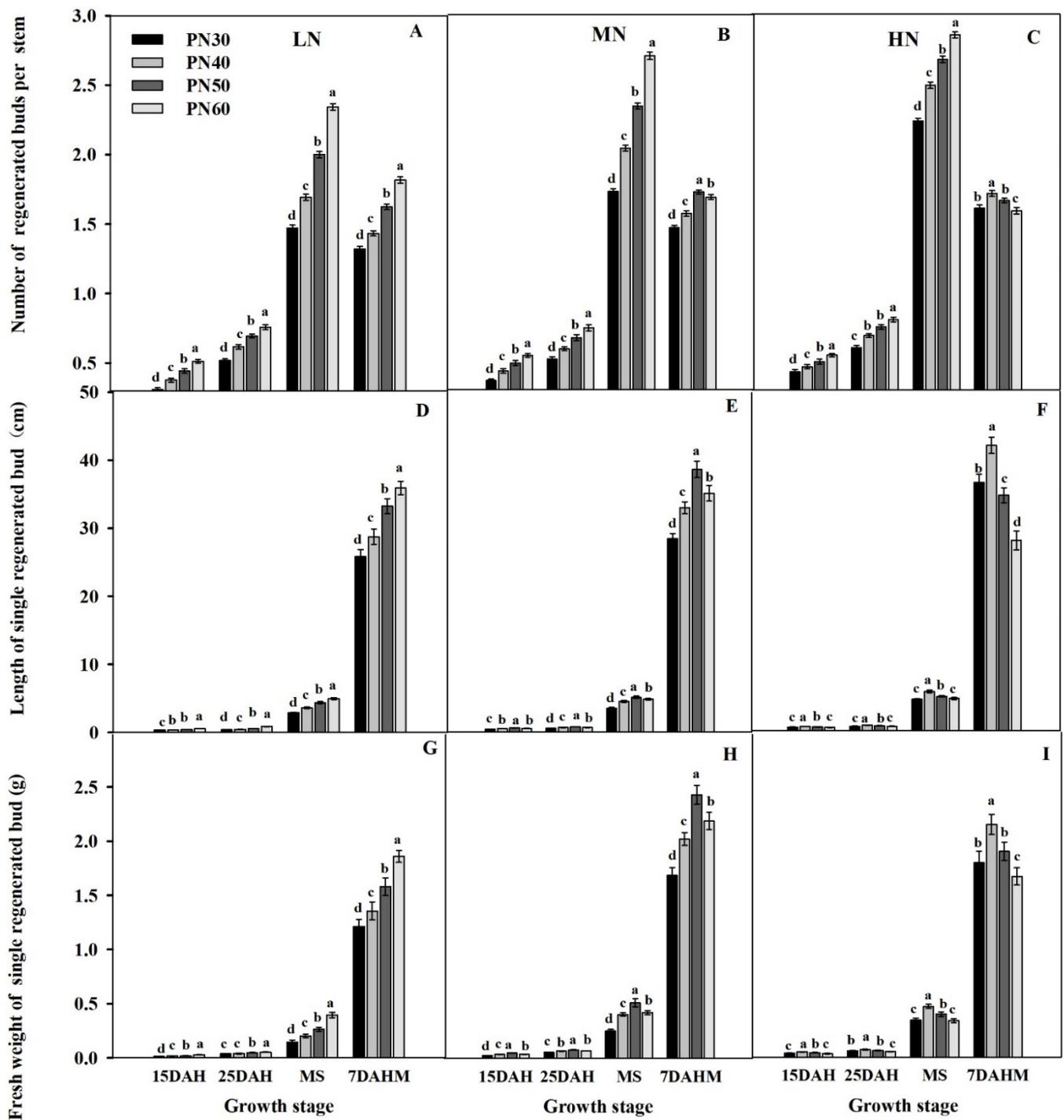


**Figure 2.** Effects of N rate and the ratio of the main crop on the regeneration rate. Error bars are  $\pm$  SE. Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). PN30, PN40, PN50, and PN60 correspond to N application ratios of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. The different lowercase letters at the same growth stage indicate significant differences at the 5% probability level according to the LSD test.

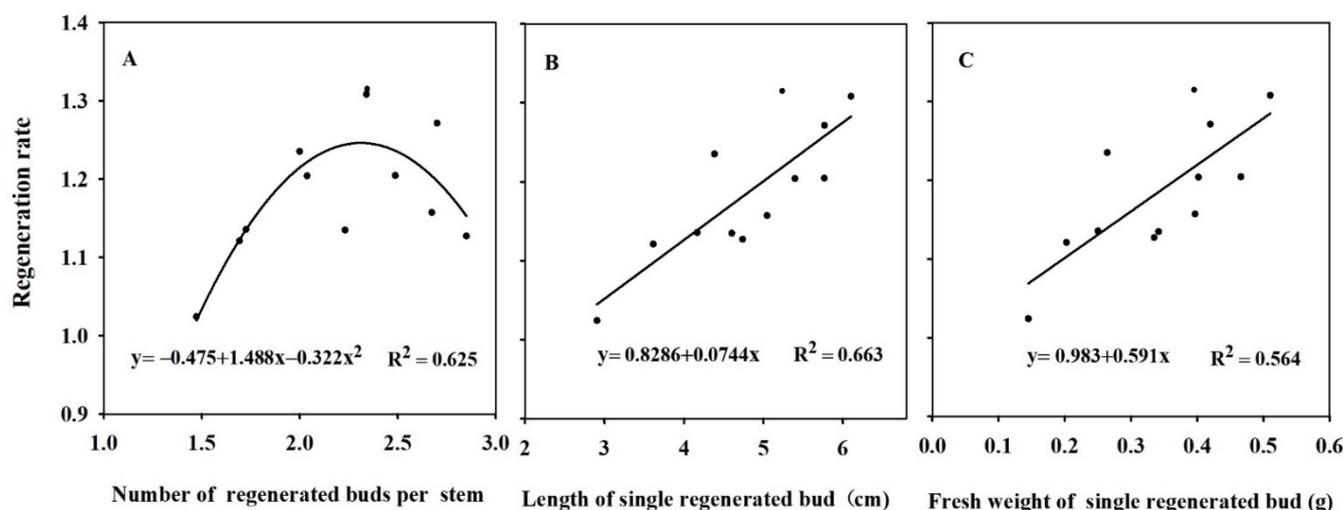
Under different N levels, the length and fresh weight of regenerated buds followed the order  $\text{MN} > \text{HN} > \text{LN}$  (Figure 3D–I). With the increasing proportion of panicle N fertilizer, the length and fresh weight of regenerated buds increased under the LN level and first increased and then decreased under the MN and HN levels, with the greatest values occurring at PN50 and PN40, respectively. At maturity of the main crop, the number of regenerated buds per stem was nonlinearly positively correlated with the length and fresh weight of regenerated buds (Figure S7). The development level of regenerated buds gradually improved with an increasing number of regenerated buds per stem and decreased with excess germination of the regenerated buds.

### 3.4.2. Correlations between the Regeneration Rate and the Length, Fresh Weight, and Number of Regenerated Buds

The regeneration rate was linearly correlated with the length and fresh weight of regenerated buds (Figure 4B,C), but was nonlinearly correlated with the number of regenerated buds (Figure 4A). The regeneration rate first increases and then decreases with an increase in the number of regenerated buds per stem.



**Figure 3.** Effects of N rate and the ratio of the main crop on the number (A–C), length (D–F), and fresh weight (G–I) of regenerated buds. Error bars are  $\pm$  SE. Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). PN30, PN40, PN50, and PN60 correspond to N application ratios of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. DAH, days after heading of the main crop. MS, maturity stage of the main crop. DAHM, days after harvest of the main crop. The different lowercase letters at the same growth stage indicate significant differences at the 5% probability level according to the LSD test.



**Figure 4.** Relationships between the number (A), length (B), and fresh weight (C) of regenerated buds at maturity of the main crop and the regeneration rate.

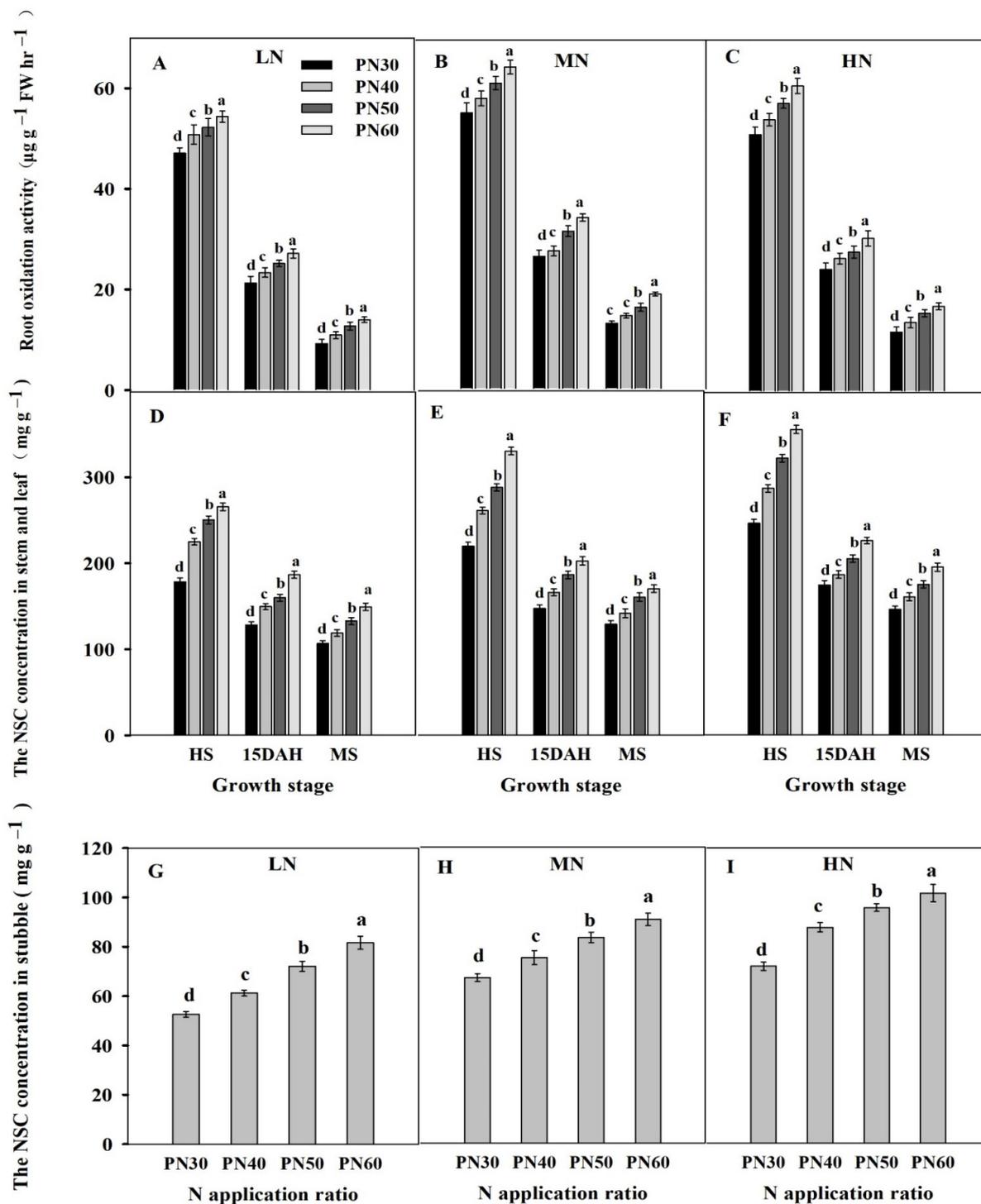
### 3.5. Effects of N Regime on the ROA and NSC Concentration in the Stem and Leaf of the Main Crop

Under different N levels, the ROA at different growth stages decreased in the order  $MN > HN > LN$  (Figure 5A–C). Under the same N level, ROA at different growth stages increased with the increasing proportion of panicle N fertilizer, and this trend was most obvious at the maturity stage. At the maturity stage in the main crop, compared with that at PN30, the ROA at PN60 increased by 51.4%, 44.4%, and 43.1% under the LN, MN, and HN levels, respectively.

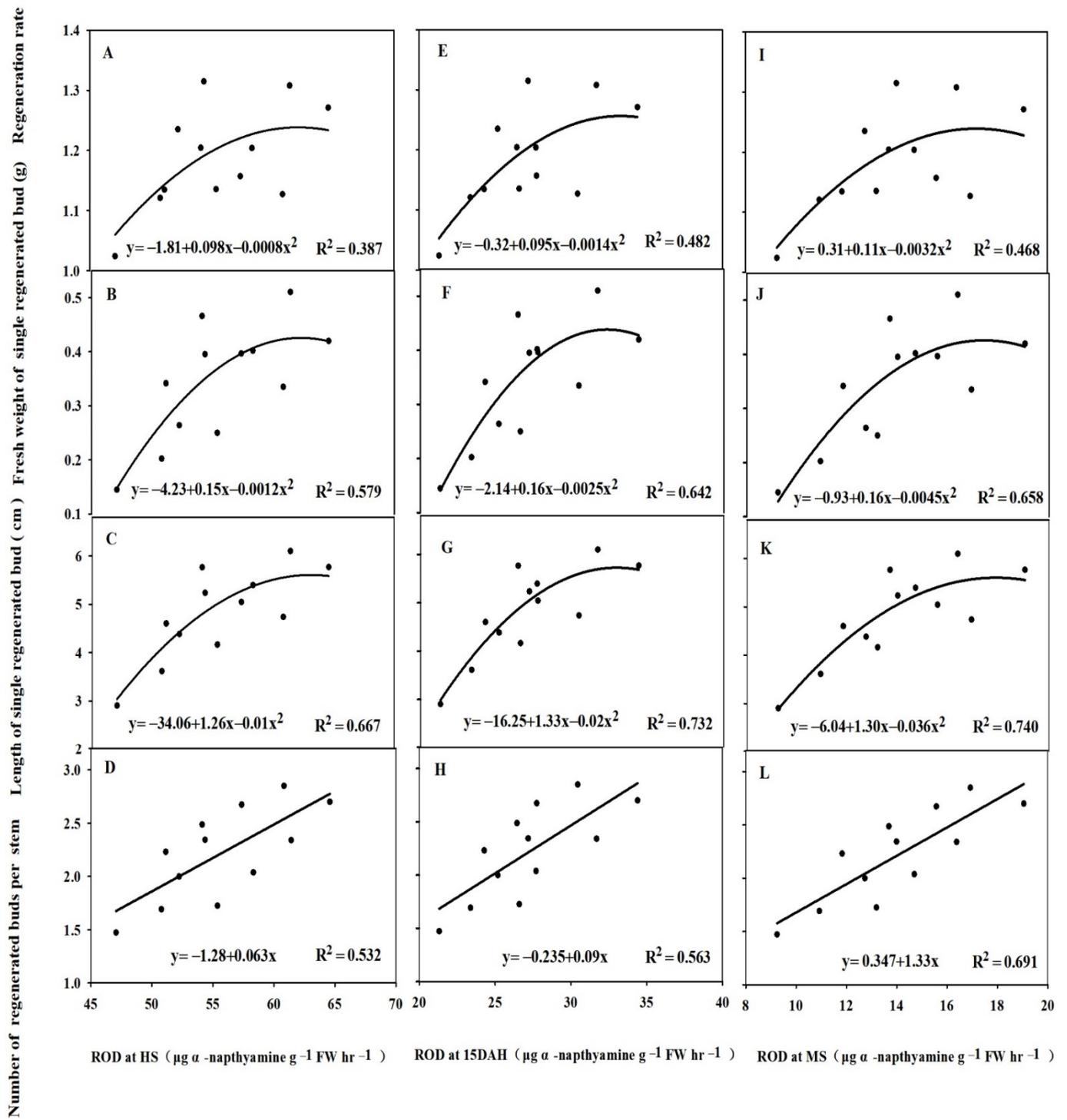
After full heading of the main crop, the NSC concentration in the stems and leaves decreased. The NSC concentration in stems and leaves and in stubble decreased in the order  $HN > MN > LN$  (Figure 5D–I). However, under the same N level, the concentration improved with an increasing proportion of panicle N fertilizer. At the maturity stage, compared with those at PN30, the stems and leaves NSC concentration at PN60 increased by 40.1%, 32.2%, and 33.8% under the LN, MN, and HN levels, respectively, and the stubble NSC concentrations increased by 55.1%, 35.2%, and 41.3%.

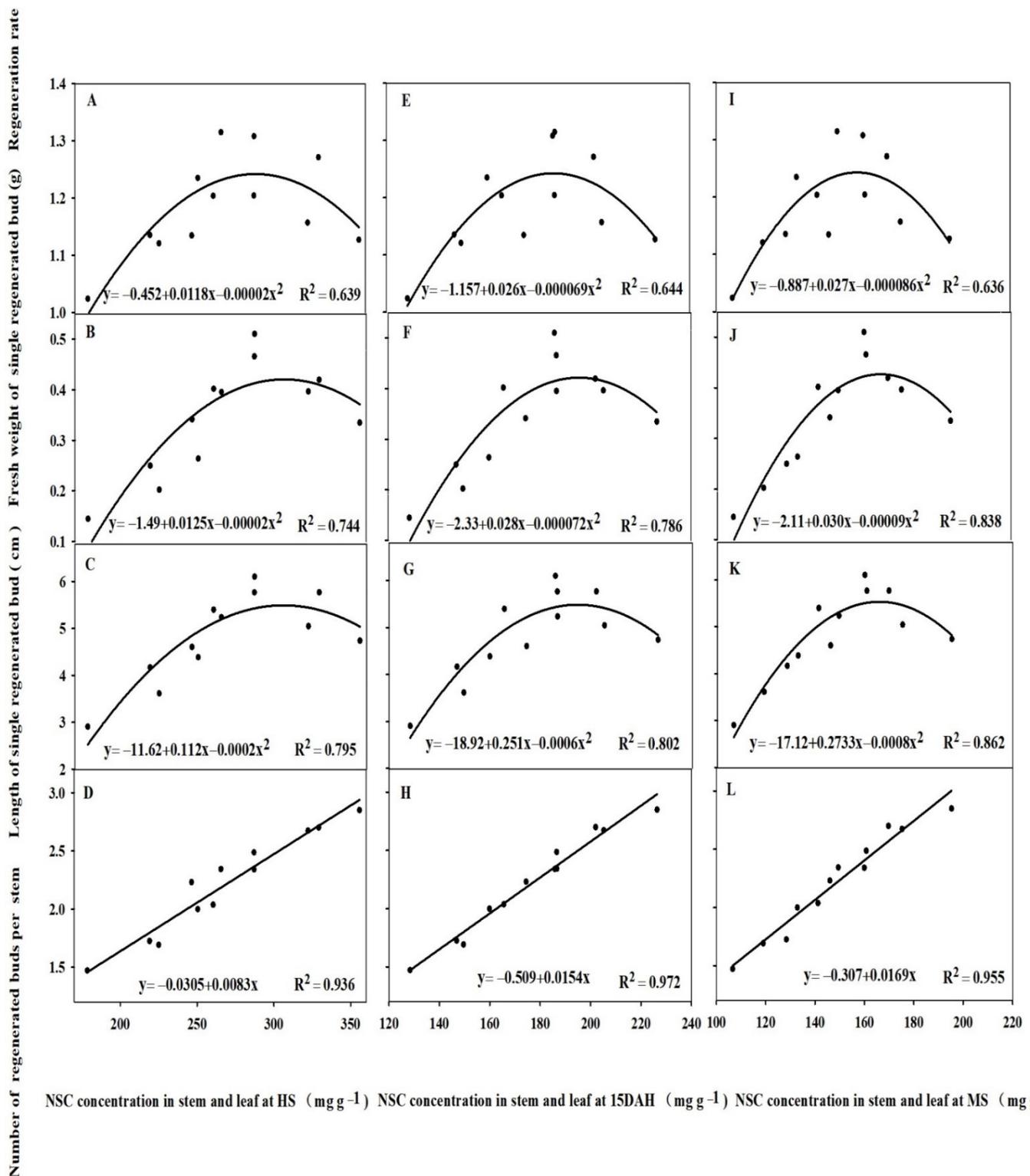
### 3.6. Relationships between the ROA and NSC Concentration in Stem and Leaf of the Main Crop and Regeneration Rate of the Ratoon Crop

The NSC concentration in the stems and leaves and the ROA in rice plants after heading of the main crop were linearly correlated with the maximum number of regenerated buds per stem but nonlinearly correlated with the length and fresh weight of regenerated buds and the regeneration rate (Figures 6 and 7). These results indicated that the number, length, fresh weight, and regeneration rate of regenerated buds increased with increasing NSC concentration in stems and leaves and increasing ROA. However, when the NSC concentration in stems and leaves and the ROA were too high, though the regenerated buds continued to increase, the development level of regenerated buds and regeneration rate decreased. Compared with ROA, the NSC concentration in stems and leaves had a higher coefficient of determination with the number, length, fresh weight, and regeneration rate of regenerated buds. Compared with the values at the full heading stage, the NSC concentration in stems and leaves and ROA on the 15th day after heading and at maturity were more strongly correlated with the number, length, fresh weight, and regeneration rate of regenerated buds.



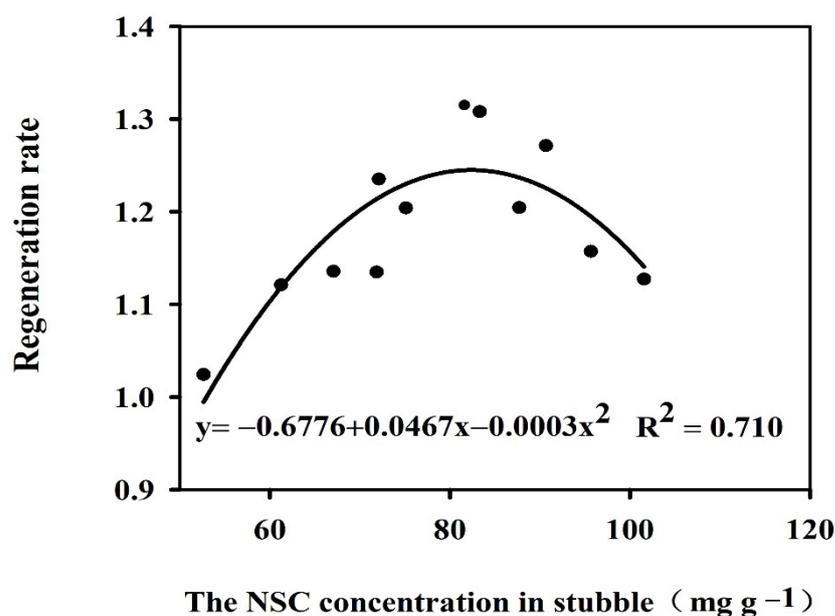
**Figure 5.** Effects of N rate and the ratio of the main crop on root oxidation activity (A–C), NSC concentration in the stem and leaf (D–F) after heading of the main crop and in the stubble (G–I). Error bars are  $\pm$  SE. Note: LN, low N rate (the total amount of N applied was  $100 \text{ kg ha}^{-1}$ ); MN, medium N rate (the total amount of N applied was  $250 \text{ kg ha}^{-1}$ ); HN, high N rate (the total amount of N applied was  $400 \text{ kg ha}^{-1}$ ). PN30, PN40, PN50, and PN60 correspond to N application ratios of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. HS, full heading stage of the main crop. DAH, days after heading of the main crop. MS, maturity stage of the main crop. The different lowercase letters at the same growth stage indicate significant differences at the 5% probability level according to the LSD test.





**Figure 7.** Relationships between NSC concentration of stem and leaf in the main crop and number (D,H,L), length (C,G,K), and fresh weight of regenerated buds (B,F,J) at maturity of main crop and regeneration rate (A,E,I). Note: HS, full heading stage of the main crop. DAH, days after heading of the main crop, MS, maturity stage of the main crop. ROA, root oxidation activity.

The stubble NSC concentration was nonlinearly correlated with the regeneration rate. With increasing stubble NSC concentration, the regeneration rate first increased and then decreased (Figure 8).



**Figure 8.** Relationships between stubble NSC concentration and regeneration rate.

### 3.7. Effects of N Regime in the Main Crop on the Nitrogen Use Efficiency in Main and Ratoon Crop

The ANUE in the main crop decreased with the N application rate in the main crop increased. As the panicle N application rate increased in the main crop, the ANUE in the main crop decreased under LN, increased under HN, first increased and then decreased under MN. In the ratoon crop, there was no difference in ANUE between HN and MN, but they were all higher than LN. And the ANUE increased under LN, first increased and then decreased under MN and HN with the panicle N application rate increased in the main crop. In terms of the ANUE in both seasons, the ANUE showed the order MN > LN > HN. And as the panicle N application rate increased in the main crop, the ANUE first increased and then decreased under LN, MN, and HN. The results showed that excessive N application rate and panicle N application rate in the main crop were not conducive to the increase of ANUE in both seasons.

## 4. Discussion

### 4.1. Reasonable N Regime in the Main Crop Improved the Growth and Development of Regenerated Buds

There are few reports on the effect of N regime in the main crop on the growth and development of regenerated bud. The growth and development of regenerated buds are usually reflected by their number, length, and fresh weight. The number, length, and fresh weight were closely related to the NSC content in stems and ROA in plants after full heading in the main crop [28–30]. Before the main crop was harvested, the growth of regenerated buds depended on the nutrients stored in the stems and leaves of the main crop [31]. Therefore, under a high stubble cutting height, the NSC concentration in stems and leaves after heading in the main crop was positively correlated with the number and length of regenerated buds [30]. Zhang et al. (2005) reported that increasing the ROA in plants after full heading in the main crop could promote the growth of regenerated buds [27]. In this paper, the NSC concentration in the stems and leaves and ROA in the main crop were positively correlated with the number, length, and fresh weight of regenerated buds (Figures 6 and 7).

Previous studies reported that appropriately increasing the panicle N application rate reduced the number of ineffective tillers and increased the percentage of productive tillers, leading to an increase in the dry matter weight per stem [32–34]. The canopy transmittance and root activity were improved by applying more N in the late stage of rice [17,35]. Our

result is consistent with those of our predecessors. Increasing the panicle N application rate increased the NSC concentration in stems and leaves and ROA after heading of the main crop (Figure 5). Therefore, increasing panicle N application rate and N application rate could improve the number, length, and fresh weight of regenerated bud by improving NSC concentration in stems and leaves and ROA (Figures 6 and 7). However, our study also showed that the length and fresh weight of regenerated buds were nonlinearly correlated with the ROA and NSC in the main crop (Figures 6 and 7). With the increase of panicle N application rate and N application rate, the length and fresh weight first increased and then decreased. The decrease in length and fresh weight of regenerated buds was related to the number of regenerated buds. The length and fresh weight of regenerated buds first increased and then decreased with the increasing number of regenerated buds (Figure S7). This may have occurred because nutrient competition among regenerated buds intensified after excessive germination, leading to low development in regenerated buds.

#### 4.2. Reasonable N Regime in the Main Crop Improved the Regeneration Rate

The regeneration rate is the ratio of the panicle number in the ratoon crop to that in the main crop and can directly reflect the regeneration ability of individual plants. It was closely related to the growth and development of regenerated buds. Xu et al. (2021) found that the fresh weight and length of regenerated buds were significantly and positively correlated with the regeneration rate [31]. Increasing the length and fresh weight of regenerated buds could reduce the mortality of regenerated buds and increase the regeneration rate [36]. Our result also showed that the regeneration rate was linearly correlated with the length and fresh weight of regenerated buds (Figure 4B,C). Since the ROA and NSC in the stem and leaf of the main crop were nonlinearly correlated with the length and fresh weight of regenerated buds, the regeneration rate had a similar trend with ROA and NSC in the stem and leaf of the main crop (Figures 6 and 7). However, there are few studies on the relationship between the number of regenerated buds and regeneration rate. This research showed that the regeneration rate was nonlinearly correlated with the number of regenerated buds (Figure 4A). This may be due to the nonlinear correlation between the number of regenerated buds and the length and fresh weight of regenerated buds (Figure S7). The length and fresh weight of regenerated buds decreased after regenerated bud excessive germination, and the regenerated buds with slow growth tended to die at the later stage of the ratoon crop. Therefore, although excessive N application rate and panicle N application rate were beneficial to increase the number of regenerated buds, it would reduce the length and fresh weight of regenerated buds, leading to a decrease in regeneration rate. We can conclude that a reasonable N regime in the main crop could simultaneously increase the number of regenerated buds and the development of regenerated buds, improving the regeneration rate and the yield of the ratoon crop.

#### 4.3. Effects of N Regime in the Main Crop on the Yield of the Main and Ratoon Crops

The N regime strongly affected rice yields. Zhu et al. (2017) and Liang et al. (2021) revealed that rice yield first increased and then decreased with increasing N application rate [37,38], and we reached a similar conclusion (Figure 1A–C). Ye et al. (2019) indicated that N application at a later stage of rice reduced the panicle number and was not conducive to increasing grain yields [39]. However, Li et al. (2018) reported that increasing the proportion of panicle N fertilizer improved N use efficiency and increased grain yield [40]. Our results are different from those of previous studies. With increased panicle N application, we found that the yield of the main crop decreased under the LN level, increased under the HN level, and first increased and then decreased under the MN level (Figure 1A–C). These results indicated that the yield was not only related to the N application rate but also closely related to the application time and N ratio.

There have been few studies on the effects of the N regime in the main crop on the yield of ratoon crops, and the results have been inconsistent. Zhang et al. (2019) revealed that N application rate and N application method in the main crop had a great effect on

the yield of the ratoon crop [12]. Some scholars reported that increasing N application rate in the late growth stage of the main crop was beneficial to promoting the growth and development of the aboveground and underground organs of rice, which could improve the yield of the main and ratoon crops [17–19]. Nakano et al. (2009) indicated that N application rate of  $22.5 \text{ g N m}^{-2}$  in the main crop had a higher dry matter yield for the ratoon crop than with  $15.0 \text{ g N m}^{-2}$ , and more N was applied early in the main crop could obtain higher dry matter yield [41]. However, Chen et al. (2010) and Wang et al. (2019) showed that an increase in total N application or panicle N fertilizer in the main crop had little effect on the yield of the ratoon crops [14,20]. Our research showed that, the effect of increasing panicle N application on the yield of the ratoon crops was different under different N application rates. With increased panicle N application, the yield in the ratoon crop increased under LN level, while it first increased and then decreased under MN and HN levels, with the greatest values occurring at PN50 and PN40, respectively (Figure 1D–F). High N application rate in the main crop was not conducive to increasing yield in the main and ratoon crops (Figure 1). Further analysis showed that the N regime in the main crop mainly affected the yield of the ratoon crop by changing the regeneration rate in the ratoon crop (Table 5). In this experiment, the yield of the ratoon crop was mainly affected by the panicle number in the ratoon crop (Figure S5). The panicle number in the ratoon crop is usually determined by the number of mother stem and regeneration rate. Although the number of mother stems and the regeneration rate were both changed by different N regime, the correlation analysis revealed that compared with the number of mother stems, the regeneration rate had a greater correlation with panicle number in the ratoon crop (Table 5). Especially under the LN and MN levels, when the number of mother stems was reduced by increased panicle N application, the improvement in regeneration rate still led to a higher yield in PN60 and PN50 (Figure 1 and Table 4). Therefore, the appropriate way to obtain a high yield in the ratoon crop should be to continuously improve the regeneration rate through breeding methods and cultivation techniques.

We can conclude that an appropriate proportion of basal tillering fertilizer to panicle fertilizer under different N rates could improve the regeneration rate and yield of the ratoon crop and simultaneously increase the yield of the main crop, achieving the purpose of high yield in both seasons.

#### *4.4. Effects of the N Regime in the Main Crop on the N Use Efficiency of the Main and Ratoon Crops*

N use efficiency (NUE) is also an important factor in determining whether the application of N fertilization is reasonable. N uptake rate and NUE are closely related to the N application rate. Xu et al. (2015) reported that appropriately increasing N application rate was beneficial to improving N uptake and NUE, leading to an increase in rice yield [42]. However, under high N application rate, the N recovery efficiency decreased, the ANUE first increased and then decreased, and rice yield did not increase. Zhang et al. (2019). revealed that with the increase of N application rate, the N uptake rate and ANUE first increased and then decreased [43]. Our result showed that with the N application rate increased, ANUE decreased in the main crop, there was no increasing trend in HN in the ratoon crop, and it first increased then decreased in both seasons (Table 6). Excessive N application reduces the number and physiological activity of roots [44] and reduces the absorption of inorganic N in paddy soil [43], resulting in less N absorbed by plants and a lower N uptake rate and NUE.

The N application ratio also has a great influence on the ANUE. Because the seedlings had less root system and less N was absorbed in the early stage of rice growth, appropriately reducing basal N and appropriately increasing panicle and tiller N could improve the NUE [45]. Our result is consistent with that (Table 6). In addition, Xu et al. (2011) reported that N uptake and N use efficiency were not only related to N regime, but also to soil characteristics [46]. Under high N supply of soil, reducing the N application rate in basal was beneficial to improve NUE and rice yield. However, under low N supply of soil,

reducing the N application rate in basal would result in decrease in NUE and rice yield [47]. Liu et al. (2005) indicated that compared with low N supply of soil, the effect of N rate on rice yield was reduced, and NUE was also decreased under high N supply of soil [48]. In China, the yield of rice is usually 5–6 t ha<sup>-1</sup> in N-free areas of paddy soils [49]. In this experiment, the yield of N free area was 5.28 t ha<sup>-1</sup> in the main crop and 8.43 t ha<sup>-1</sup> in the total yield of two seasons, belonging to the area with medium and upper fertility. Therefore, during the ratoon rice production of the area, appropriately reducing the application rate of base tillering fertilizer and increasing the application rate of panicle fertilizer is beneficial to improve the NUE and the yield of both seasons.

**Table 6.** Effects of N regime in the main crop on the nitrogen use efficiency in the main and ratoon crop.

| Treatment | ANUE in Main Crop | ANUE in Ratoon Crop | ANUE in Both Seasons |        |
|-----------|-------------------|---------------------|----------------------|--------|
| LN        | PN30              | 29.7 a              | 6.3 f                | 14.1 b |
|           | PN40              | 27.9 b              | 7.4 e                | 14.2 b |
|           | PN50              | 26.6 c              | 8.6 d                | 14.6 b |
|           | PN60              | 24.5 d              | 9.3 d                | 14.4 b |
|           | Mean              | 27.2 A              | 7.9 B                | 14.3 B |
| MN        | PN30              | 15.5 f              | 13.4 c               | 14.6 b |
|           | PN40              | 16.6 e              | 14.9 b               | 15.8 a |
|           | PN50              | 15.3 f              | 16.4 a               | 15.8 a |
|           | PN60              | 14.6 g              | 14.7                 | 14.7 b |
|           | Mean              | 15.5 B              | 14.8 A               | 15.2 A |
| HN        | PN30              | 8.3 i               | 15.0 b               | 10.5 d |
|           | PN40              | 8.6 i               | 16.2 a               | 11.1 c |
|           | PN50              | 8.8 i               | 14.5 b               | 10.7 d |
|           | PN60              | 9.3 h               | 13.6 c               | 10.7 d |
|           | Mean              | 8.7 C               | 14.8 A               | 10.8 C |

Note: ANUE, agronomic nitrogen use efficiency. LN, low N rate (the total amount of N applied was 100 kg ha<sup>-1</sup>); MN, medium N rate (the total amount of N applied was 250 kg ha<sup>-1</sup>); HN, high N rate (the total amount of N applied was 400 kg ha<sup>-1</sup>). PN30, PN40, PN50, and PN60, correspond to N application ratio of basal tillering fertilizer to panicle fertilizer of 7:3, 6:4, 5:5, and 4:6, respectively. Data in a column followed by different lower-case letters indicate significant differences at the 5% probability level according to the LSD test. Means followed by different upper-case letters indicate significant differences between the three nitrogen fertilizer levels at the 5% probability level according to the LSD test. ANUE in the ratoon crop = (Grain yield of ratoon crop – Grain yield of 0 N in ratoon crop)/N rate in ratoon crop. ANUE in both seasons = (Grain yield of both seasons – Grain yield of 0 N in both seasons)/N rate in both seasons. The grain yield of 0 N in the main and ratoon crops was 5.28 t ha<sup>-1</sup> and 3.15 t ha<sup>-1</sup>, respectively.

## 5. Conclusions

The rate and ratio of N application in the main crop had an important influence on the rice yield and ANUE of the main and ratoon crops. Excessive total and panicle N application rate in the main crop were not conducive to improving the grain yield and ANUE in both seasons. Under different N application rate, appropriate increasing the panicle N application rate was beneficial for increasing the ROA and NSC concentration in stem and leaf in the main crop, resulting in improving the growth and development of regenerated buds, which caused an increase in the regeneration rate and yield of the ratoon crop. A total N rate of 250 kg ha<sup>-1</sup> and a ratio of basal tillering fertilizer to panicle fertilizer of 5:5 in the main crop could increase grain yields and ANUE in both seasons.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12040527/s1>, Figure S1: Mean temperature (A), sunshine hours (B) and rainfall (C) monthly during rice growth in 2018 and 2019. Figure S2: Technical drawings for the experimental program. Figure S3: The process of obtaining root samples from the field. Figure S4: Field investigation on the growth and development of regenerated buds. Figure S5: Relationships between the yield and panicle number in the ratoon crop. Figure S6: Effects of N regime in the main crop on the number of living (A–C) and dead (D–F) regenerated buds per square meter. Figure S7: Relationships between number, fresh weight, and length of regenerated buds at maturity of the main crop. Table S1: Effects of N regime in the main crop on the yield components of the main crop.

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