Coordinating Postanthesis Carbon and Nitrogen Metabolism of Hybrid Rice through Different Irrigation and Nitrogen Regimes

Yongjian Sun 1, Yuanyuan Sun 2, Fengjun Yan 1, Yue Li 1, Yuxia Wu 1, Changchun Guo 1, Peng Ma 1, Guotao Yang 1, Zhiyuan Yang 1 and Jun Ma 1,*

1 Rice Research Institute of Sichuan Agricultural University, Crop Ecophysiology and Cultivation Key Laboratory of Sichuan Province, Wenjiang, 611130, China; yongjians1980@sicau.edu.cn (Y.S.); yfjun1989@stu.sicau.edu.cn (F.Y.); liyuez@stu.sicau.edu.cn (Y.L.); Yuxia@stu.sicau.edu.cn (Y.W.); changchuns1991@stu.sicau.edu.cn (C.G.); mapeng1@stu.sicau.edu.cn (P.M.); yangguotao@stu.sicau.edu.cn (G.Y.); 14236@sicau.edu.cn (Z.Y.)
2 Institute of Plateau Meteorology, China Meteorological Administration, Chengdu, 610072, China; ytyy21@cma.cn
* Correspondence: majun@sicau.edu.cn

Received: 13 July 2020; Accepted: 12 August 2020; Published: 13 August 2020

Abstract: We sought to explore the role of postanthesis carbon and nitrogen (C-N) metabolism of hybrid rice in increasing yield and nitrogen utilization efficiency (NUE). We used the 13C and 15N dual-isotope tracer method and physiological/biochemical analysis and established different irrigation and nitrogen fertilization (W-N) regimes to investigate the relationship of C-N metabolism characteristics, yield, and NUE. The results showed that W-N regimes had significant effects on postanthesis absorption and translocation of N and photosynthate, yield and NUE. Aerobic irrigation combined with the N fertilization regime 30% base, 30% tillering, 40% booting was the best W-N coupling regime for rice yield and NUE increase. The regime enhanced flag leaf photosynthesis rate and the activities of ribulose 1,5-diphosphate carboxylase/oxygenase (RuBPCase), glutamine synthetase (GS), and other key enzymes of C-N metabolism, and improved the total accumulations of photoassimilates (0.97–21.57 mg 13C plant−1) and N (1.55–23.36 mg 15N plant−1), respectively. Correlation analysis showed that, under the W-N interaction, C-N metabolism enzymes promoted the positive synergistic effect between 13C and 15N accumulation in panicles (r = 0.825). In addition, the change in C/N ratio can be used as an indicator of the simultaneous improvement in yield and NUE in hybrid rice.

Keywords: isotope tracing; hybrid rice; irrigation regimes; N fertilization regimes; C-N metabolism

1. Introduction

Yield formation in rice mainly depends on the degree of coordination of carbon and nitrogen (C-N) metabolism during the filling stage [1,2]. It has been shown that 9–43% of grain yield comes from unstructured carbohydrates stored before flowering, while postanthesis photosynthetic products contribute 57–91% of yield [3], and 13CO2 tracing technology showed that the aboveground 13C assimilates fixed by photosynthesis accounted for 45.3–95% of such assimilates [4]. N metabolism, accumulation, and redistribution in rice vegetative and regenerative organs are also important factors in determining yield [5–7]. The previous work showed that a considerable portion of N is transported from vegetative organs to grains in rice, of which approximately 64% comes from leaves and 20% from stems [5]. Moreover, 15N tracing showed that 39% and 46%, respectively, of the 15N absorbed at the rice tillering and panicle differentiation stages, are transported to grains at the maturity stage [6].
The above studies focused on a single aspect of C or N metabolism. During the process of rice postanthesis yield formation, changes in C and N (C-N) metabolism in rice plants will directly affect the yield, transformation, and distribution of C and N substances [3,4,7]. The mechanisms of C-N metabolism regulation are coupled to each other and restrict each other in that not only is C metabolism affected by the regulation of N levels, but N-metabolic-pathway–related enzymes and metabolites are also subject to feedback from C-metabolism–related products [8]. Water and fertilizer are important parts of high-quality and high-yield rice cultivation techniques [9,10]. Previous studies by us and others showed that water and N have a coupling effect on rice yield formation and nitrogen use efficiency (NUE), and proper coordination of W-N regimes is conducive to improving grain yield and NUE [9–11]. However, many research on rice postanthesis C-N metabolism has mainly focused on one factor of the effects of water and fertilizer [12–15]. It is reported that 79–85% of $^{14}$CO$_2$-labeled photosynthetic products were transported from flag leaves to grains under severe water stress, while only 55–66% were transported from flag leaves to grains under traditional basin irrigation; the proportion of $^{14}$CO$_2$-labeled photosynthetic products transported from stems to grains also increased with the degree of water-stress [13]. Moreover, the proportions of $^{14}$CO$_2$-labeled photosynthetic products transported from stems to grains were, respectively, 19–22%, 45–46%, and 62–66% under flooded irrigation, mild water stress, and severe water stress at 33 d postanthesis [14]. $^{15}$N tracing showed that under different N regimes, the use efficiency of N applied at the booting and tillering stage reached 54–82% and 17–34%, respectively [15]. Cassman et al. [12] also showed that 53% of N was absorbed in rice within 10 d of N application during the booting stage, and the absorption rate was as high as 9–12 kg ha$^{-1}$ d$^{-1}$ within 4 days (d).

There are few reports on how W-N coupling regulates the synergistic process of postanthesis C-N metabolism in rice and on how this in turn improves yield and NUE. Specifically, it is rarely reported whether differences exist in the precise quantification of transport and distribution of postanthesis photoassimilates in rice between different irrigation regimes or different N regimes and whether the differences in postanthesis N transport from leaves and stems under W-N interaction have a synergistic effect on the transport and distribution efficiency of photoassimilates. To this end, based on the relevant research [3–5,12] and the results of our previous studies [2,7,9,10], using the $^{13}$C and $^{15}$N dual-isotope tracer and physiological/biochemical analysis methods, we studied the effects of different W-N regimes on postanthesis C-N metabolism, yield, and NUE of hybrid rice. We analyzed the common response mechanism of the postanthesis photoassimilate and N accumulation, transport, and distribution as well as their relationship with yield formation and with NUE in the context of W-N coupling. The findings of this study enrich our understanding of the W-N regulation mechanism in hybrid rice and provide a theoretical basis and practical guidance for the development of water-saving and high-yield rice production.

2. Materials and Methods

2.1. Experimental Design

Pot and field experiments were conducted on a research farm located at Chengdu, China (30°50' N, 103°56' E) in 2014 and 2015. The soil used for the pot experiment was from the same field as the field experiment each year. The soil texture of the plow layer was sandy loam, and the physical and chemical properties can be found in Table 1. The test cultivar was hybrid rice F-you 498.

| Year | Total N (g kg$^{-1}$) | Organic Matter (g kg$^{-1}$) | Available Nutrient (mg kg$^{-1}$) | pH | Bulk Density (g cm$^{-3}$) | Particle Composition |
|------|----------------------|-----------------------------|----------------------------------|----|--------------------------|---------------------|
|      |                      |                             | N | P | K                        |                     | Sand (%) | Clay (%) | Silt (%) |
| 2014 | 1.84                 | 20.9                        | 97.7 | 36.1 | 94.7 | 6.40 | 1.26 | 44 | 38 | 18 |
| 2015 | 1.90                 | 22.8                        | 102.9 | 35.9 | 100.8 | 6.42 | 1.27 | 47 | 33 | 20 |
2.1.1. Pot Experiment

The pot experiment was carried out in a mobile shelter, and the $^{13}$C and $^{15}$N dual-isotope tracer method was used to conduct a two-factor experiment of various W-N regimes with 12 treatment combinations (three irrigation regimes and four N regimes). Each treatment had 40 pots (15 pots were labeled by applying $^{15}$N fertilizer, 25 pots without labels were used for physiological indicator determination) as repetitions in a complete randomized block design. Three irrigation regimes with tap water (public drinking water quality, pH 7.12, Nitrate-N 9.6 mgL$^{-1}$, dissolved oxygen concentration 8.1 mgL$^{-1}$), a measuring cup was used to accurately record the water volume in each irrigation treatment and equitensiometer (Soil Research Institute, Nanjing, China) was installed in each pot to monitor soil water potential. When the equitensiometer readings dropped to the desired value, pots were re-watered. The three irrigation methods included the following:

1. Flooded irrigation ($W_1$). Maintain soil water potential of 0 kPa and keep 1–2 cm water layer;
2. Aerobic irrigation ($W_2$). The soil water potential reached $-25$ kPa and was re-watered with water layer 1–2 cm;
3. Deficit irrigation ($W_3$). The soil water potential reached $-40$ kPa and was re-watered with no water layer (soil water potential $-5$ kPa$-0$ kPa).

The levels of N application were determined according to our earlier research [2,7] that showed that the amount of N that needed to be applied to ensure high yield was 180 kg hm$^{-2}$ (equivalent to 1.0 g N plant$^{-1}$). Four N regimes in pot experiments including three different $^{15}$N regimes ($^{15}$N-labeled urea replace unlabeled urea, Shanghai Research Institute of Chemical Industry; abundance of 10.02%) were included, with the ratios for base, tillering, and booting fertilizer applied at the stages of 50:30:20, 30:30:40, and 30:10:60. No N application was also set as a control. These treatments were, respectively, named N$_1$, N$_2$, N$_3$, and N$_0$. The base fertilizer was applied 1 day before transplanting, tillering fertilizer was applied 7 d after transplanting, and the booting fertilizer was split into equal applications at the top 4th and 2nd leaf in the N$_1$, N$_2$, and N$_3$ treatments.

The pots in the transparent plexiglass chambers (transmittance >85%, 1.5 $\times$ 1.5 $\times$ 1.8 m, Figure 1) were designed by randomized block. The pot used for this test was 27.6 cm high, 33.3 cm in the upper caliber, and 28.5 cm in the lower caliber; 15.5 kg of well-mixed and sifted dry soil was filled into each pot. Sowed and transplanted on April 3 and May 8 separately of each year, with two hills per pot, one plant per hill, and a spacing of 16.7 cm. The application of phosphate fertilizer (superphosphate) was equivalent to 90 kg hm$^{-2}$ of P$_2$O$_5$ (converted to 0.5 g P$_2$O$_5$ plant$^{-1}$), and the application of potassium fertilizer (potassium chloride) was equivalent to 180 kg hm$^{-2}$ of K$_2$O (converted to 1.0 g K$_2$O plant$^{-1}$); both were applied as base fertilizer. Before flowering, for each treatment (W-N regimes with 12 treatment combinations), eight pots were selected from 15 pots labeled with $^{15}$N fertilizer as well as the N$_0$ treatment with No N fertilizer application (control treatment) feed $^{13}$CO$_2$. After the isotope was labeled at the full-heading stage, four pots for each treatment were sampled immediately to analyze $\delta^{13}$C and $\delta^{15}$N values, and the other four pots for each treatment with isotope labeled were sampled to analyze $\delta^{13}$C and $\delta^{15}$N values at the maturity stage. Twenty pots that were not labeled were used for physiological indicator determination; eight pots (labeled or unlabeled pots could be used) were used for yield estimation and seed tests. The same experiment was run for two consecutive years.

The pulse labeling method [4] was slightly improved for use in $^{13}$C isotope tracing. At the full heading stage, at 9:00–15:00 on sunny days, 2 mol L$^{-1}$ H$_2$SO$_4$ was slowly injected with syringes through a dropper into four containers (uniform $^{13}$CO$_2$ release) that were hung on the top of the chamber (Figure 1) and were filled with NaH$^{13}$CO$_3$ (Cambridge Isotope Laboratories, USA, abundance of 99.0%), and the sample inlet hole was immediately sealed for uniform feeding of $^{13}$CO$_2$. The $^{13}$CO$_2$ labeling was performed in six transparent plexiglass chambers, with an indoor CO$_2$ concentration of 400 $\mu$L L$^{-1}$. Small fans were used to mix the air, and a CO$_2$ monitoring apparatus (GXH-3010E, Hua Yun Analytical Instrument Research
Institute Co. LTD, Beijing, China) was used for continuous measurement. Meanwhile, water circulation with tap water was used to cool the plexiglass chambers.

Figure 1. The transparent plexiglass chambers and four containers in chamber.

2.1.2. Field Experiment

The irrigation and N regimes with a complete randomized block design for the field experiments were the same as those for the pot experiments, except that no isotope labeling was done, and irrigation with underground water (pH 7.23, Nitrate-N 14.1 mg L⁻¹, ammonium-N 0.45 mg L⁻¹). Row and plant spacings were 33.3 × 16.7 cm, and the plot area was 25.6 m². Each treatment was repeated three times. A water meter was used to accurately record the volume of each irrigation treatment, ensuring that the irrigation volume for each plot was the same between the N treatments. Ridges (40 cm wide and 30 cm high) were constructed between plots, and they were wrapped with plastic film to prevent water and fertilizer from entering the surrounding plots.

2.2. Data Collection and Analysis

2.2.1. N Content, N Utilization Efficiency (NUE), and Water Use Efficiency (WUE)

For each W-N treatment combination in pot experiments, three pots of rice plants with no C or N labeling were selected at the full-heading stage and maturity stage separately, and the dry weights of aboveground leaves, stems, and panicles were determined. Upon digestion in concentrated H₂SO₄ with a fixed N catalyst, Kjeldahl Apparatus (FOSS-8400, Sweden) was used to measure the N content according to micro-Kjeldahl method [16], followed by the calculation method [7] of the total N uptake at harvest (MTNA), N grain production efficiency (NGPE), N dry matter production efficiency (NMPE), N agronomy efficiency (NAE), N recovery efficiency (NRE), and N physiological efficiency (NPE). NAE, NPE, and NRE were comprehensive indexes reflecting the NUE.

NGPE = grain yield/total N uptake;
NMPE = total dry matter production/total N uptake;
NAE = (grain yield in N supply - grain yield in zero N supply)/N supply rate;
NRE = (total N uptake in N supply - total N uptake in zero N supply)/N supply rate;
NPE = NAE/NRE;
WUE = grain yield/irrigation water rate.
2.2.2. Ribulose 1,5-Diphosphate Carboxylase/Oxygenase (RuBPCase) and Sucrose-Phosphate Synthase (SPS)

For each W-N treatment combination in pot experiments, flag leaves of the main stems from four pots of plants were selected without isotope labeling at full-heading, 15 d after full-heading and maturity stage under 9:00 a.m. on sunny days when the light intensity was 1100–1200 µmol m\(^{-2}\) s\(^{-1}\). Leaves were deveined, shredded, and mixed. The RuBPCase activity was measured by the spectrophotometric method used by Li and Li [17] and is expressed in the amount of µmol-fixed CO\(_2\) per min (µmol CO\(_2\) mg\(^{-1}\) protein min\(^{-1}\)). The SPS activity was measured by the methods used by Wang et al. [18] and Wang et al. [19] and is expressed in the amount of µmol sucrose produced per min (µmol mg\(^{-1}\) protein min\(^{-1}\)).

2.2.3. Nitrate Reductase (NR) and Glutamine Synthetase (GS)

Using the samples collected at the same time in Section 2.2.2, the NR activity was measured with the in vitro method used by Li [20] and is expressed in the number of micrograms of NaNO\(_2\) produced per hour (µmol mg\(^{-1}\) protein h\(^{-1}\)). The GS activity was measured by the methods of Lea et al. [21] and O’Neal and Joy [22] and is expressed in the amount of µmol γ-glutamylhydroxamate produced per hour (µmol mg\(^{-1}\) protein h\(^{-1}\)).

2.2.4. Net Photosynthesis Rate (\(P_n\))

For each W-N treatment combination in pot experiments, at full-heading, 15 d after full-heading and maturity stage, a Li-6400 photosynthesis apparatus (USA) was used to measure the \(P_n\) at 9:30–11:00 a.m. on sunny days when the photosynthetic active radiation reached 1200 µmol m\(^{-2}\) s\(^{-1}\). \(P_n\) was measured at 400 µmol mol\(^{-1}\) s\(^{-1}\) CO\(_2\), flow rate 0.5 L min\(^{-1}\), chamber relative humidity 70–75%, and chamber temperature 30 °C. Five pots of plants were selected, and the flag leaves of the main stems were used for measurement, with each leaf measured three times.

2.2.5. \(^{13}\text{C} and \(^{15}\text{N}\) Content

In pot experiments, eight pots were labeled with \(^{13}\text{C} and \(^{15}\text{N}\) and sampled for each W-N treatment combination at full-heading stage (four pots) and at maturity stage (four pots), respectively. Each of the treated plants was divided into four parts: leaves, stems, roots, and panicles. Samples were first fixed and oven-dried to constant weight and then were ground and sieved through an ultrafine sieve. An isotope ratio mass spectrometer (DELTA V Advantage) and an elemental analyzer (Flash EA1112 HT) manufactured by Thermo Fisher Scientific (USA) were used. Each sample was first subjected to high-temperature combustion in the elemental analyzer to generate CO\(_2\), and the \(^{13}\text{C}/^{12}\text{C}\) ratio in CO\(_2\) was determined by the mass spectrometer. By comparison to the international standard (Pee Dee Belnite), the δ\(^{13}\text{C}\) ratio of the sample was calculated. Upon high-temperature combustion, N\(_2\) was also generated, and the \(^{15}\text{N}/^{14}\text{N}\) in N\(_2\) was determined. By comparison to the international standard (atmospheric N\(_2\)), the δ\(^{15}\text{N}\) ratio of the sample was calculated. At the same time, the ratio of C/N was calculated based on the total C and total N contents.

2.2.6. Yield and Yield Components

Eight pots and twenty plants were randomly were selected in the pot and field experiments separately at the maturity stage. The effective panicle number (m\(^2\)), spikelets per panicle, total spikelets (product of effective panicles and spikelet number), grain filled percentage, and 1000-grain weight were measured and calculated. Grain yield was determined from per pot of pot experiments and a 10.5 m\(^2\) in the center of each plot of field experiments.
2.2.7. Climatic Conditions

The temperature, relative humidity, rainfall, and cumulative hours of sunshine over the 2 years from full-heading to maturity stage (30 d after full-heading) were provided by the Meteorological Observatory Station and averaged every 5 d. With only one exception, there were no significant differences in climate parameter values across the different stages of rice postanthesis and between the two years of the study (Figure 2). The exception was in 2015 when, 6–10 days after anthesis, a heavy rainfall of 102.9 mm occurred within a 12-h period (Figure 2E); there was almost no impact on the deficit irrigation treatment W3 due to timely drainage of the plots. Characterization of the sampling environment at the main stages postanthesis in both years of the study is shown in Table 2.

![Figure 2](image_url)

**Figure 2.** Natural climatic conditions during the experiments. Average temperature (A), maximum temperature (B), minimum temperature (C), rainfall (D), cumulative amount of sunshine hours (E) during days after full-heading (2014–2015). Vertical bars represent ± S.E. of the mean within 5 days. The S.E. was calculated across values within 5 days of different phases for each year.

**Table 2.** The main growth dates and sampling environment characterization in cropping calendar for different years in the pot experiments (2014–2015).

| Parameters                        | 2014 Year                      | 2015 Year                      |
|-----------------------------------|--------------------------------|--------------------------------|
| Cropping calendar                 | Full-Heading                  | 15 d after Full-Heading        | Maturity (30 d after Full-Heading) |
| Temperature (°C)                  | 4 August                      | 19 August                     | 4 September                     |
| Photosynthetic active radiation (μmol m⁻² s⁻¹) | 27.6                           | 25.3                          | 24.6                           |
| Soil water potential (-kPa)       | W₁ 14.4 ± 0.1                 | W₁ 10.2 ± 0.6                 | W₁ 19.8 ± 0.7                  |
|                                  | W₂ 26.1 ± 0.0                 | W₂ 22.2 ± 0.3                 | W₂ 33.9 ± 0.8                  |
|                                  | W₃ 0.0                        | W₃ 0.0                        | W₃ 9.8 ± 0.9                   |
|                                  |                                |                                | W₃ 20.1 ± 0.2                  |
|                                  |                                |                                | Maturity (30 d after Full-Heading) |
|                                  | 15 d after Full-Heading        | 11-15                         | 16-20                          |
|                                  | Full-Heading                  | 21-25                         | 26-30                          |
|                                  | Maturity (30 d after Full-Heading) | 26-30                       | 21-25                          |
|                                  | 21 August                     | 26.2                          | 24                             |
|                                  | 6 September                   | 6 September                   |                                |
|                                  | 11-15                         | 16-20                         | 21-25                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |
|                                  | 26-30                         | 21-25                         | 26-30                          |

2.3. Statistical Analysis

Date analysis and graphing functions were performed using SAS 8.1 (SAS Institute, Cary, NC, USA) and SigmaPlot 12.0, respectively. The randomized complete block model was used included sources of variation due to replication, year, irrigation method, N application ratio, and year × irrigation method, year × N application ratio, and irrigation method × N application ratio. Means were tested by
least significant difference (LSD) at the 0.05 level (LSD0.05). Correlation analysis deals with relationships among variables and find the correlation coefficients between a pair of variables in a dataset. The results of experiments in 2014 and 2015 were no significant differences between treatments and years (Table 3). Therefore, the average of the same indicator data was analyzed during two years in this study.

### 3. Results and Analysis

#### 3.1. Grain Yield and Yield Components

It can be seen from Tables 4 and 5 that the effects of W-N regimes on the yield and its components reached a significant level, and the interaction effects of the two factors on yield, the effective panicle number, spikelet number, total spikelets, and 1000-grain weight all were significant. Under each W-N treatment (Table 4), the effects of irrigation regime was significantly stronger than the effect of N regimes. Under W2, the N2 treatment exhibited the highest yield, and the combination of W2 and N2 formed the best W-N regime in this study. If the increase in the percentage of postponing N topdressing (from N2 to N3), the yield significantly decreased. Under W1, the N2 treatment showed the highest yield out of all the N regimes. Here, when the increase in the percentage of postponing N topdressing, the yield decreased, but did not decrease significantly. Under W3, the N1 treatment showed the highest yield; N regimes that exceeded N2 would cause a significant decrease in yield. The yield results in the field trials across the years were consistent with the results in the pot experiments (Table 5).

### Table 3. Analysis of variance for yield and its components and nitrogen utilization deficiency (NUE) of rice between years and irrigation methods and N application ratios in the pot experiments (F values) (2014–2015).

| Analysis of Variance | Grain Yield | Effective Panicles | Spikelets Per Panicle | Filled Grains | 1000-Grain Weight | MTNA | NAE | NRE |
|----------------------|-------------|--------------------|-----------------------|--------------|------------------|------|-----|-----|
| Year (Y)             | 1.18 **     | 1.94 **            | 2.07 **               | 2.69 **      | 0.27 **          | 1.99 ** | 1.91 ** |
| Y X Irrigation method| 0.46 **     | 1.70 **            | 2.40 **               | 1.76 **      | 0.62 **          | 1.08 ** | 1.57 ** |
| Y X N application ratio 0.43 ** | 2.17 ** | 2.22 **       | 1.98 **               | 0.76 **      | 1.92 **          | 1.93 ** | 1.61 ** |

MTNA: Total N accumulation at maturity; NAE: N agronomy efficiency; NRE: N recovery efficiency. *: denote non-significance at p > 0.05.

### Table 4. Effects of different irrigation methods and N application ratios on yield and its components in the pot experiments (2014–2015).

| Irrigation Method | Nitrogen Application Ratio | Effective Panicles (plant−1) | Spikelets (No. Panicle−1) | Total Spikelets (Plant−1) | Filled Grains (%) | 1000-Grain Weight (g) | Grain Yield (g pot−1) |
|------------------|---------------------------|------------------------------|---------------------------|---------------------------|------------------|-----------------------|------------------------|
| W1               | N0                        | 9.04 ef                      | 160.03 e                  | 1446.7 b                  | 83.93 a          | 29.84 b               | 70.87 e                |
|                  | N1                        | 11.89 a                      | 181.73 bc                 | 2149.9 d                  | 79.42 d          | 29.05 ab              | 100.41 c               |
|                  | N2                        | 11.80 ab                     | 195.71 a                  | 2309.4 ab                 | 79.06 de         | 30.84 ab              | 106.33 b               |
|                  | N3                        | 11.34 bc                     | 200.40 a                  | 2272.5 bc                 | 78.17 f          | 29.31 cd              | 104.00 bc              |
| Average           | 11.02 a                   | 184.22 a                     | 2044.4 a                  | 80.15 a                   | 29.76 a          | 95.40 a               | 95.26 a                |
| W2               | N0                        | 9.38 e                       | 166.96 d                  | 1566.1 g                  | 84.60 a          | 29.96 ab              | 72.61 e                |
|                  | N1                        | 12.03 a                      | 183.10 b                  | 2197.2 cd                 | 81.24 b          | 30.03 ab              | 106.56 b               |
|                  | N2                        | 12.07 a                      | 197.46 a                  | 2383.3 a                  | 79.82 cd         | 30.34 a               | 110.38 a               |
|                  | N3                        | 11.24 c                      | 200.86 a                  | 2257.7 bc                 | 78.41 ef         | 29.55 bc              | 103.06 bc              |
| Average           | 11.17 a                   | 187.10 a                     | 2101.3 a                  | 81.02 a                   | 29.97 a          | 98.40 a               | 99.91 a                |

**Values within a column followed by different letters are significantly different at p < 0.05; * Significant at p < 0.01, ** denote non-significance at p > 0.05, respectively. Average yield and its components for each irrigation method across all four N application and followed different letters are significantly different at p < 0.05. Data of yield components are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; W x N: Water–nitrogen interaction.**


Table 5. Effects of different irrigation methods and N application ratios on yield and its components in the field experiments (2014–2015).

| Irrigation Method | Nitrogen Application Ratio | Effective Panicles (×10^6 m⁻²) | Spikelets (No. panicle⁻¹) | Total Spikelets (×10⁶ m⁻²) | Filled Grains (%) | 10⁻³ Grain weight (g) | Grain Yield (kg hm⁻²) | 2014 | 2015 |
|-------------------|----------------------------|----------------------------------|--------------------------|---------------------------|------------------|----------------------|------------------------|-------|-------|
|                   | N₀                         | 162.5 fg                         | 161.9 de                  | 262.9 h                   | 82.9 a           | 29.83 a              | 6389.7 e               | 6355.5 f |       |
|                   | N₁                         | 213.2 ab                         | 184.0 b                   | 392.3 d                   | 78.2 de          | 29.75 b              | 9125.0 e               | 9061.2 c |       |
|                   | N₂                         | 211.8 abc                        | 201.5 a                   | 426.7 b                   | 78.9 cd          | 29.86 ab             | 9805.2 ab              | 9725.0 b |       |
|                   | N₃                         | 203.6 bcd                        | 203.3 a                   | 413.8 bc                  | 77.7 e           | 29.65 b              | 9467.0 bc              | 9428.6 bc |       |
| Average           |                            | 197.8 a                          | 187.7 a                   | 374.0 a                   | 79.4 a           | 29.77 a              | 8666.7 a               | 8642.5 a |       |
| W₁                | N₀                         | 168.0 f                          | 168.7 cd                  | 283.5 g                   | 83.7 a           | 29.98 b              | 6623.5 e               | 6743.8 f |       |
|                   | N₁                         | 215.9 a                          | 186.6 b                   | 402.8 cd                  | 80.3 b           | 30.01 ab             | 9601.9 b               | 9588.3 b |       |
|                   | N₂                         | 216.6 a                          | 203.7 a                   | 441.0 a                   | 79.7 d           | 30.18 a              | 10086.0 a              | 10286.2 a |       |
|                   | N₃                         | 202.5 cd                         | 202.1 a                   | 409.2 c                   | 78.3 de          | 29.82 ab             | 9406.7 bc              | 9500.7 b |       |
| Average           |                            | 200.7 a                          | 190.3 a                   | 384.1 a                   | 80.2 a           | 30.00 a              | 8925.0 a               | 9097.9 a |       |
| W₂                | N₀                         | 154.2 g                          | 154.9 e                   | 238.8 f                   | 79.7 bc          | 28.86 cd             | 5280.5 f               | 5368.5 g |       |
|                   | N₁                         | 195.1 d                          | 181.3 b                   | 353.7 e                   | 75.2 f           | 29.13 c              | 7599.2 d               | 7643.0 d |       |
|                   | N₂                         | 193.8 d                          | 178.9 bc                  | 346.5 e                   | 74.9 f           | 28.69 d              | 7202.6 d               | 7179.0 e |       |
|                   | N₃                         | 180.3 e                          | 180.9 b                   | 326.2 f                   | 73.2 g           | 28.12 e              | 6648.5 e               | 6523.5 f |       |
| Average           |                            | 180.8 b                          | 174.0 b                   | 316.3 b                   | 78.7 b           | 28.70 b              | 6642.7 b               | 6478.9 b |       |
| W₃                | N₀                         | 14.65 **                         | 10.76 **                  | 12.15 **                  | 7.52 **          | 16.60 **             | 77.86 **               | 110.44 ** |       |
|                   | N₁                         | 55.45 **                         | 22.38 **                  | 38.24 **                  | 4.50 *           | 4.01 *               | 66.42 **               | 86.22 ** |       |
| Average           |                            | 2.99 **                          | 2.91 *                    | 2.73 *                    | 0.18 **          | 2.62 *               | 3.14 *                 | 4.45 **   |       |

Values within a column followed by different letters are significantly different at p < 0.05; * Significant at p < 0.05; ** Significant at p < 0.01, nm denote non-significance at p > 0.05, respectively. * Average yield and its components for each irrigation method across all four N application and followed different letters are significantly different at p < 0.05. Data of yield components are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; WxN: Water–nitrogen interaction.

It can also be seen from Tables 4 and 5 that the number of effective panicles, spikelet number, and total spikelets were more affected by N regimes than irrigation treatments, while grain filling percentage and 1000-grain weight were more affected by irrigation treatments than N regimes, which indicated that suitable irrigation and N regimes could regulate yield components to achieve the purpose of greater yield. Among the various irrigation regimes, the averaged values of yield components were in the order W₂ > W₁ > W₃; the difference between W₂ and W₁ treatments was not significant, but the yield components in W₃ treatment were significantly lower than those of W₂ and W₁ treatments. Under various N regimes, when the increase in the proportion of N application was postponed, the effective panicles number under each irrigation regime showed a trend of increasing first and then decreasing, spikelet number increased, and grain filling percentage decreased. Excessive N application shifted to postpone or the W₃ treatment caused a significant decrease in the 1000-grain weight.

3.2. WUE and NUE

WUE was more affected by irrigation treatments than N regimes (Table 6). Compared to WUE under the W₁ treatments, the W₂ and W₃ treatments were higher by 65.6–179.5% and 263.5–312.0%, respectively. At the maturity stage, the average total N accumulation, NGPE, and NUE (NAE and NPE) were in the order W₂ > W₁ > W₃. The difference between the W₂ and W₁ treatments was not significant; these parameters of the W₃ treatment were significantly lower than those of the W₂ and W₁ treatments. With the increase in the percentage of postponing N topdressing, all the irrigation treatments showed a trend of increasing first and then decreasing; shifting 40% of the N application to postpone was suitable for the W₂ and W₁ treatments, and 20% was suitable for the W₃ treatment. Further increasing in the percentage of postponing N topdressing led to N accumulation and a reduced NUE under each irrigation regime, and the reduction level reached significance, especially for W₂ and W₃, and the condition of N application shifted to postpone, NMP showed an increasing trend, while NGPE exhibited a trend of increasing first and then decreasing. This showed that although shifting an excessive proportion of N application to postpone was conducive to dry matter production, it was not conducive to N transport or utilization or to NGPE improvement, so it ultimately led to a significant decrease in NUE. The effects of irrigation and N regimes on WUE, MTNA, and NUE
indicators reached significance, and the interaction effects of the two factors had a significant effect on WUE and NUE.

Table 6. Effects of different irrigation methods and N application ratios on water use efficiency and N use efficiency in rice in the pot experiments (2014–2015).

| Irrigation Method | Nitrogen Application Ratio | WUE (g L\(^{-1}\)) | MTNA (g pot\(^{-1}\)) | NGPE (kg kg\(^{-1}\)) | NMPE (kg kg\(^{-1}\)) | NRE (kg kg\(^{-1}\)) | NAE (kg kg\(^{-1}\)) | NPE (%) |
|-------------------|---------------------------|---------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------|
| W1                | N\(_0\)                   | 0.69 f               | 1.02 e                 | 46.50 c                | 16.45 d               | 31.51 c                |                        |         |
|                   | N\(_1\)                   | 0.97 ef              | 1.95 b                 | 31.23 cde              | 106.73 de             | 48.98 b                | 18.09 b               | 36.93 a  |
|                   | N\(_2\)                   | 1.04 e               | 2.00 b                 | 33.39 b               | 107.31                |                        |                        |         |
|                   | N\(_3\)                   | 1.01 e               | 1.98 b                 | 52.21 cde             | 107.76 cd             | 48.11 bc               | 16.44 c               | 34.19 b  |
|                   | Average                    | 0.93 c               | 1.74 a                 | 56.50a                | 115.38a               | 47.86 a                | 16.39 a               | 34.21 a  |
| W2                | N\(_0\)                   | 1.16 e               | 1.06 e                 | 69.57 a               | 144.02 b              |                        |                        |         |
|                   | N\(_1\)                   | 1.67 d               | 2.01 b                 | 52.93 bc              | 104.77 e              | 47.34 bc               | 16.24 c               | 34.30 b  |
|                   | N\(_2\)                   | 1.77 d               | 2.12 a                 | 53.01 bc              | 106.30 de             | 52.95 a               | 19.28 a               | 36.42 a  |
|                   | N\(_3\)                   | 1.64 d               | 1.99 b                 | 52.30 cde             | 109.56                | 46.68 bc               | 15.26 cd              | 32.69 bc  |
|                   | Average                    | 1.56 b               | 1.79 a                 | 56.95a                | 116.16a               | 48.99 a                | 16.93 a               | 34.47 a  |
| W3                | N\(_0\)                   | 2.08 c               | 0.86 f                 | 68.61 a               | 155.76 a              | -                      | -                     |         |
|                   | N\(_1\)                   | 2.94 a               | 1.65 c                 | 50.70 d               | 111.51 cd             | 39.51 d                | 12.30 e               | 31.13 c  |
|                   | N\(_2\)                   | 2.79 ab              | 1.65 c                 | 48.30 e               | 112.66 c              | 39.15 d                | 10.15 f               | 25.91 d  |
|                   | N\(_3\)                   | 2.55 b               | 1.51 d                 | 48.23 e               | 112.81 c              | 32.28 e                | 6.77 g                | 20.96 e  |
|                   | Average                    | 2.59 a               | 1.42 b                 | 53.96a                | 123.18a               | 36.98 b                | 9.74b                 | 26.00 b  |

F value

| W x N | 6.21 ** | 2.63 * | 0.72 ** | 0.71 * | 5.00 ** | 27.93 ** | 17.85 ** |

Values within a column followed by different letters are significantly different at p < 0.05; * Significant at p < 0.05; ** Significant at p < 0.01, ns denote non-significance at p > 0.05, respectively. * Average water use efficiency and N use efficiency for each irrigation method across all N application and followed different letters are significantly different at p < 0.05. WUE: water use efficiency; MTNA: Total N uptake at maturity; NMPE: N dry matter production efficiency; NGPE: N grain production efficiency; NRE: N recovery efficiency; NAE: N agronomy efficiency; NPE: N physiological efficiency; Data are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; W x N: Water-nitrogen interaction.

3.3. Net Photosynthesis Rate and Enzymes of C Metabolism in Flag Leaves

The irrigation and N regimes significantly affected the net photosynthesis rate (\(P_n\)) (Figure 3A), RuBPCase and SPS activity (Figure 3B,C) in flag leaves. With the progression of growth and development, the \(P_n\) and enzyme activities involved in C metabolism in different irrigation regimes showed decreasing trends, and the physiological indicators under the W\(_2\) treatment were higher than those under W\(_1\) or W\(_3\). However, the responses of different irrigation regimes to the proportion of N application shifted to postpone were not consistent: under the W\(_1\) treatment, when the proportion of N application shifted to postpone reached 40–60%, postanthesis flag leaf \(P_n\) and C metabolism enzyme activities were not significantly different from when it was not shifted. Under the W\(_2\) treatment, as the proportion of N application shifted to postpone increased, the \(P_n\) and activities of C metabolism enzymes in flag leaves increased first and then decreased, and they were highest in the N\(_2\) treatment; when the proportion of N application shifted to postpone reached 60%, the \(P_n\) and activities of C metabolism enzymes in flag leaves decreased significantly with the progression of growth stage. Under the W\(_3\) treatment, 20–40% of N application shifted to postpone was a suitable range. These data also indirectly show that W\(_2\) was more sensitive than W\(_1\) to the later-shifting of N application during the seed-setting stage and had higher physiological metabolic activities.
It can be seen from Figure 4 that the irrigation and N regimes significantly affected the enzyme activities of GS and NR (Figure 4A,B) in flag leaves. Each irrigation regime and N regime affected the activities of various enzymes of N metabolism in almost the same way as seen for C metabolism (Figure 3B,C). However, the GS activity in leaves had a slowly decreasing trend with the progression of growth, and the NR activity decreased significantly at 15–30 d after the full-heading stage.

### 3.4. Enzymes of N Metabolism in Flag Leaves

It can be seen from Figure 4 that the irrigation and N regimes significantly affected the enzyme activities of GS and NR (Figure 4A,B) in flag leaves. Each irrigation regime and N regime affected the activities of various enzymes of N metabolism in almost the same way as seen for C metabolism (Figure 3B,C). However, the GS activity in leaves had a slowly decreasing trend with the progression of growth, and the NR activity decreased significantly at 15–30 d after the full-heading stage.
in panicles under the average of W to varying degrees, than those under W
the full-heading stage, although the
13
of total
13
by 38.25–44.23 mg
13
under the W
13
under the W

3.5. Postanthesis Photoassimilates, N Distribution and C/N Ratio

Figure 4. Effects of different water regimes and N application ratios on GS (A) and NR (B) enzymes activity of flag leaves at various growth stages in hybrid rice in the pot experiments (2014–2015). Vertical bars represent ± S.E. of the mean. The S.E. was calculated across 3 replicates for each year and average across 2 years. Different letters from top the column indicate statistical significance at the p = 0.05 level among different N application ratios under the same water regimes within the same period.

It can be seen from Table 7 that from the full-heading to maturity stage, the W2 treatment facilitated the accumulation of photoassimilates, which was 0.97–6.07 mg 13C plant−1 and 5.44–21.54 mg 13C plant−1 higher than under the W1 and W3 treatments, respectively. W2 also facilitated the transport of 13C assimilates from leaves and stems to grains (13C translocation was calculated as 13C accumulation in leaves or stems at full-heading stage minus 13C remaining in leaves or stems at maturity stage), and the amount from stems was significantly higher than that from leaves. Compared to those under the W1 and W3 treatments, the transport from leaves under the W2 treatment was higher by 0.55–0.67 mg 13C plant−1 and 1.14–2.92 mg 13C plant−1, respectively, and the transport from stems under the W2 treatment was higher by 1.18–3.87 mg 13C plant−1 and 5.58–11.71 mg 13C plant−1. The 13C assimilates in panicles under the W2, W1, and W3 treatments were, respectively, increased by 38.25–44.23 mg 13C plant−1 (accounting for 41.98–43.74% of total 13C), 34.24–39.97 mg 13C plant−1 (accounting for 40.24–42.36% of total 13C), and 27.73–32.28 mg 13C plant−1 (accounting for 36.45–38.87% of total 13C). However, the differences in the 13C assimilates in vegetative organs during different postanthesis stages under the three irrigation regimes did not have the same level of significance: at the full-heading stage, although the 13C assimilates in roots were not significantly different between the irrigation regimes, the 13C assimilates in leaves, stems, and panicles under W2 were all higher, to varying degrees, than those under W1, and those under the W1 and W2 treatments were significantly higher than those under W3. In contrast, at the maturity stage, the difference in 13C assimilates in leaves between the average of irrigation treatments was not significant, but the amount of 13C assimilates in panicles under the average of W2 treatment was significantly higher than that under the W1 or
W3. These findings indirectly indicate that the W2 treatment facilitated the postanthesis transport of assimilates from “source” to “sink”.

Table 7. Effects of different irrigation methods and N application ratios on postanthesis accumulation and translocation of 13C of hybrid rice (mg 13C plant−1) in the pot experiments (2014–2015).

| Irrigation Method | Nitrogen Application Ratio | Labeling Full-Heading Stage | Maturity Stage |
|-------------------|---------------------------|-----------------------------|---------------|
|                   |                           | Leaf | Stem | Panicle | Root | Leaf | Stem | Panicle | Root |
| W1                | N0                        | 13.51 gh | 36.13 f | 8.73 ef | 5.23 e | 6.49 d | 15.06 e | 36.72 e | 5.34 de |
|                   | N1                        | 23.91 de | 44.74 c | 9.77 de | 6.68 cd | 12.26 c | 22.12 cd | 44.01 c | 6.71 a  |
|                   | N2                        | 25.87 ab | 50.00 b | 11.38 b | 7.78 b  | 13.54 a | 23.32 b  | 51.35 b | 6.83 a  |
|                   | N3                        | 25.94 ab | 48.79 b | 10.38   | bcd     | 8.67 a  | 13.94 a  | 23.39 b | 50.10 b | 6.34 abc|
| Average           |                           | 22.31 a | 44.92 a | 10.07 a | 7.09 a  | 11.56 a | 20.97 a  | 45.55 b | 6.31 a  |
| W2                | N0                        | 14.06 g | 38.67 ef | 9.57 de | 5.05 e  | 6.09 d  | 14.98 e  | 41.40 cd | 4.88 ef |
|                   | N1                        | 24.79 cd | 47.77 b | 10.17 cd | 6.24 d  | 12.47 bc | 22.04 cd | 48.42 b | 6.04 bc |
|                   | N2                        | 26.77 a  | 53.98 a | 13.04 a | 7.32 bc | 13.89 a | 23.43 ab | 57.27 a | 6.52 ab |
|                   | N3                        | 25.76 bc | 48.66 b | 11.20 bc | 9.12 a  | 13.14 ab | 24.44 a  | 50.97 b | 6.19 bc |
| Average           |                           | 22.85 a | 47.27 a | 11.00 a | 6.93 a  | 11.40 a | 21.22 a  | 49.52 a | 5.91 a  |
| W3                | N0                        | 12.98 h | 32.58 g | 7.73 f  | 5.41 e  | 6.86 d  | 14.95 e  | 32.42 f | 4.46 fg |
|                   | N1                        | 23.81 de | 42.58 ed | 10.08 ed | 7.07 c  | 12.63 bc | 22.70 bc | 42.36 ed | 5.84 ed |
|                   | N2                        | 22.62 ef | 40.32 de | 9.26 de | 7.34 bc | 12.02 c | 21.48 d  | 40.18 d | 5.86 c  |
|                   | N3                        | 23.01 ef | 36.25 f | 8.86 e  | 7.94 b  | 13.31 ab | 22.03 cd | 36.59 c | 4.15 g  |
| Average           |                           | 20.61 b | 37.93 b | 8.98 b  | 6.94 a  | 11.21 a | 20.29 a  | 37.89 c | 5.08 b  |
| F value           | W                         | 12.97 ** | 57.16 ** | 47.70 ** | 2.37 ** | 2.50 ** | 3.37 ** | 82.48 ** | 68.65 **|
|                   | N                         | 120.34 | 52.02 ** | 38.51 ** | 98.64 ** | 63.47 ** | 50.75 ** | 61.06 ** |
|                   | W × N                     | 2.04 ** | 3.64 ** | 7.19 ** | 2.29 ** | 3.55 ** | 1.67 ** | 4.80 ** | 8.38 **|

Values within a column followed by different letters are significantly different at *p < 0.05; ** Significant at *p < 0.01; *** denote non-significance at *p > 0.05, respectively. + Average 13C accumulation for each irrigation method across all N application and followed different letters are significantly different at *p < 0.05. Data are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; W × N: Water–nitrogen interaction.

It can also be seen from Table 7 that the effects of N regime on the postanthesis accumulation and transport of 13C assimilates in various nutrient organs under different irrigation regimes all reached significance. Under the same irrigation regime, when the increase in the percentage of postponing N topdressing, the amounts of 13C assimilates in stem and panicle at the full-heading stage increased first and then decreased, and the total amount of assimilates in each nutrient organ was the highest under W2N2 treatment. This indicated a moderate later-shift of N application was conducive to the transport of 13C assimilates from leaves and stems to grains, while too much of a later-shift (to the N3 treatment level) led to a decrease in the amount and proportion of the transport from leaves and stems, which is not conducive to the increase in 13C assimilates in grains.

It can be seen from Table 8 that the effects of irrigation and N regimes on the accumulation and distribution of 15N in various postanthesis vegetative organs reached significant level, and the interaction effect of the two factors had a significant impact on panicles and roots during the full-heading stage and on various vegetative organs during maturity, as well as on the total cumulative 15N in rice plants. Under different N treatments, postanthesis N accumulation under W2 was 1.55–3.57 and 15.18–23.36 mg 15N plant−1 higher than those under W1 and W3, respectively. N transport from leaves (15N translocation was calculated as 15N accumulation in leaves at full-heading stage minus 15N remaining in leaves at maturity stage) under W2 was 0.11–0.39 mg 15N plant−1 and 0.09–4.82 mg 15N plant−1 higher than those under W1 and W3, respectively, and N transport from stems (15N translocation was calculated as 15N accumulation in stems at full-heading stage minus 15N remaining in stems at maturity stage) under W2 was 0.01–0.65 mg 15N plant−1, 0.07–0.58 mg 15N plant−1 higher than those under W1 and W3. The 15N accumulation in panicles under W2, W1, and W3 was increased by 35.91–48.23 mg 15N plant−1 (accounting for 45.34–56.61% of 15N total), 35.20–46.11 mg 15N plant−1 (accounting for 45.33–55.81% of 15N total), and 27.09–31.25 mg 15N plant−1 (accounting for 47.49–48.13% of 15N total), respectively. The pattern of the total 15N accumulation in
various vegetative organs and at different growth stages between different irrigation treatments was basically consistent with the effects on $^{13}$C assimilate accumulation and transport, with the average values having the order $W_2 > W_1 > W_3$. However, the $^{15}$N transport amount in leaves was significantly higher than in stems. Under each irrigation regime, with the increase in the percentage of postponing N topdressing, the total $^{15}$N accumulation in leaves, stems, and panicles (not roots) showed varying degrees of decreasing trends; the N1 and N2 treatments had a significantly higher $^{15}$N accumulation than the N3 treatment. In contrast, at the maturity stage, under W1 and W2, the N2 treatment had a higher total $^{15}$N accumulation in plants than the N1 treatment, which also indirectly showed that N2 facilitated N absorption during the maturity stage. When the proportion of N application shifted to postpone was exceedingly large (N3 treatment), though it facilitated the accumulation of N during the seed-setting period, the total amount of $^{15}$N accumulation was significantly lower than that under N2 treatment.

### Table 8. Effects different irrigation method and N application ratios on postanthesis accumulation and translocation of $^{15}$N of hybrid rice (mg $^{15}$N plant$^{-1}$) in the pot experiments (2014–2015).

| Irrigation Method | Nitrogen Application RAtio | Full-Heading Stage | Maturity Stage |
|------------------|----------------------------|--------------------|----------------|
|                  | Leaf | Stem | Panicle | Root | Total $^{15}$N | Leaf | Stem | Panicle | Root | Total $^{15}$N |
| W1               | W1_1 | 27.45 | 17.09 | 10.81 | 2.32 | 2.32 | 57.68 | 13.05 | 14.32 | 47.58 | 2.18 | 77.13 |
|                  | N1   | 26.81 | 15.91 | 9.22  | 2.95 | 2.95 | 54.90 | 11.30 | 13.14 | 55.33 | 2.86 | 82.63 |
|                  | N2   | 22.24 | 14.17 | 8.61  | 3.16 | 3.16 | 48.19 | 16.64 | 12.39 | 43.92 | 4.80 | 77.65 |
| Average          | 25.50 | 15.72 | 9.55  | 2.81 | 2.81 | 53.59 | 13.66 | 13.25 | 48.91 | 3.28 | 79.14 |
| W2               | W1_1 | 28.41 | 18.86 | 11.03 | 2.37 | 2.37 | 60.67 | 16.09 | 48.53 | 2.23 | 80.47 |
|                  | N1   | 27.35 | 17.13 | 9.53  | 3.01 | 3.01 | 57.01 | 11.53 | 13.70 | 57.76 | 3.21 | 86.20 |
|                  | N2   | 22.69 | 14.45 | 8.79  | 3.23 | 3.23 | 49.15 | 16.97 | 12.63 | 44.69 | 4.80 | 79.20 |
| Average          | 26.15 | 16.91 | 9.79  | 2.87 | 2.87 | 55.61 | 14.04 | 14.14 | 50.33 | 3.45 | 81.96 |
| W3               | W1_1 | 23.12 | 15.15 | 9.23  | 2.63 | 2.63 | 50.13 | 11.28 | 11.60 | 40.48 | 1.94 | 65.29 |
|                  | N1   | 22.47 | 13.48 | 8.54  | 2.71 | 2.71 | 47.00 | 11.47 | 10.63 | 38.58 | 2.15 | 62.84 |
|                  | N2   | 16.12 | 11.24 | 7.72  | 2.95 | 2.95 | 37.53 | 9.91  | 14.10 | 34.31 | 2.34 | 57.05 |
| Average          | 20.57 | 13.29 | 8.26  | 2.76 | 2.76 | 44.61 | 11.08 | 10.71 | 37.79 | 2.14 | 61.73 |

Values within a column followed by different letters are significantly different at $p < 0.05$; * Significant at $p < 0.05$; ** Significant at $p < 0.01$, ns denote non-significance at $p > 0.05$, respectively. * Average $^{15}$N accumulation for each irrigation method across all N application and followed different letters are significantly different at $p < 0.05$. Data are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; $W \times N$: Water–nitrogen interaction.

The irrigation and N regimes significantly affected the C/N ratio in various postanthesis vegetative organs, and the interaction effect of the two factors on the C/N ratio reached a significant significant level (Table 9). After N fertilizer application for the same irrigation regime, the C/N ratios in vegetative organs showed increasing trends to different extents (N3 > N2 > N1) with the increase in the percentage of postponing N topdressing at the full-heading stage. However, the C/N ratios in aboveground vegetative organs showed decreasing trends to different extents (N1 > N2 > N3) with the increase in the postponement of N topdressing at the maturity stage. As for the change in C/N ratio in various postanthesis organs, from the full-heading to maturity stage, for each irrigation regime under a given N application treatment, the average C/N ratios in leaves and panicles showed a significant increasing trend, while those in stems and roots showed decreasing trends to varying degrees. For the optimal treatment combinations under each irrigation regime ($W_1N_2$, $W_2N_2$, and $W_3N_1$) that resulted in high yield, from the full-heading to maturity stage, the C/N ratios were approximately twice those under other regimes in leaves and in panicles and roughly half in stems and in roots. These changes in C/N were the most suitable in postanthesis vegetative organs and can be used as a high-yield identification indicator.
Table 9. Effects of different irrigation methods and N application ratios on postanthesis total carbon nitrogen ratio of different organs in hybrid rice in the pot experiments (2014–2015).

| Irrigation Method | Nitrogen Application Ratio | Full-Heading Stage | Maturity Stage |
|-------------------|---------------------------|--------------------|---------------|
|                   | Leaf | Stem | Panicle | Root | Leaf | Stem | Panicle | Root |
| $W_1$             | N$_0$ | 20.28 b | 94.67 a | 19.56 f | 28.27 f | 56.09 b | 66.96 a | 80.18 a | 60.46 b |
|                   | N$_1$ | 15.81 d | 48.95 h | 20.05 ef | 29.64 ef | 47.32 c | 31.59 b | 61.00 cd | 34.27 d |
|                   | N$_2$ | 17.84 ef | 58.00 ef | 23.64 d | 30.43 e | 36.02 e | 28.59 bc | 46.99 e | 15.07 f |
|                   | N$_3$ | 20.05 bc | 75.11 c | 23.74 d | 41.12 b | 30.65 g | 24.58 cd | 35.25 g | 13.73 f |
| Average           |      | 18.50 b | 69.19 a | 21.75 b | 32.36 b | 42.52 b | 37.93 a | 55.85 a | 30.88 b |
| $W_2$             | N$_0$ | 25.11 a | 93.60 a | 25.32 bc | 30.45 e | 60.51 a | 70.80 a | 86.32 a | 66.59 a |
|                   | N$_1$ | 20.42 b | 52.02 gh | 25.00 c | 35.26 d | 54.44 b | 32.42 b | 69.64 b | 36.82 c |
|                   | N$_2$ | 20.76 b | 61.78 de | 27.35 a | 35.94 d | 41.73 d | 30.33 b | 55.53 d | 18.09 e |
|                   | N$_3$ | 24.71 a | 82.82 b | 27.89 a | 48.03 a | 36.04 e | 28.52 cd | 37.24 fg | 20.36 e |
| Average           |      | 22.75 a | 72.56 a | 26.39 a | 37.42 a | 48.18 a | 40.52 a | 62.18 a | 35.46 a |
| $W_3$             | N$_0$ | 19.91 bc | 82.22 b | 19.67 f | 30.45 e | 60.51 a | 70.80 a | 86.32 a | 66.59 a |
|                   | N$_1$ | 17.58 f | 49.15 h | 21.00 e | 38.54 c | 34.03 ef | 25.28 ed | 43.61 ef | 19.80 e |
|                   | N$_2$ | 18.78 de | 56.35 fg | 23.85 d | 39.28 bc | 32.82 f | 21.49 d | 41.37 ef | 20.06 e |
|                   | N$_3$ | 19.02 ed | 65.60 d | 26.17 b | 41.00 b | 30.79 g | 20.98 d | 39.96 fg | 19.78 e |
| Average           |      | 18.82 b | 63.33 b | 22.67 b | 37.14 a | 38.27 c | 33.96 b | 47.56 b | 30.45 b |

$F$ value

| $W$ | 65.93 ** | 21.21 ** | 51.30 ** | 29.71 ** | 60.48 ** | 30.05 ** | 77.69 ** | 26.47 ** |
| N   | 28.60 ** | 98.26 ** | 29.96 ** | 91.40 ** | 119.66 | 139.47 | 151.76 | 179.20 ** |
| $W$×$N$ | 2.76 * | 3.70 ** | 2.62 ** | 7.78 ** | 9.35 ** | 2.69 * | 16.12 ** | 13.74 ** |

Values within a column followed by different letters are significantly different at $p < 0.05$; ** Significant at $p < 0.01$, respectively. * Average C/N ratio for each irrigation method across all N application and followed different letters are significantly different at $p < 0.05$. Data are 3 replicates for each year and average across 2 years. W: Irrigation method; N: N application ratio; W×N: Water–nitrogen interaction.

3.6. Relationship of Postanthesis C and N Metabolism under the W-N Interaction

Under various irrigation and N regimes, the enzyme activities of N metabolism (GS and NR) each had significant positive correlations with $P_n$ and the activities of enzymes (RuBPCase and SPS) in C metabolism in flag leaves during filling stages (Table 10), with different correlation coefficients at each growth stage. The GS and NR activities in flag leaves had the highest correlation coefficient with C metabolism-related indicators at 15 d after the full-heading and full-heading stage, respectively, and the correlation coefficient between GS and RuBPCase and that between NR and SPS were high. The correlations between N-metabolism-related indicators and the $^{13}$C accumulation in panicles ($r = 0.865^{**}–0.914^{**}$) were significantly higher than those between C-metabolism-related indicators and $^{15}$N accumulation in panicles ($r = 0.577^{**}–0.785^{**}$). This also indirectly showed the relationship between postanthesis C and N in rice under the W-N interaction: N metabolism promoted C accumulation, and C metabolism regulated N accumulation, thereby achieving a significant positive correlation ($r = 0.825^{**}$) between $^{13}$C and $^{15}$N accumulation in panicles.

3.7. Relationships of Postanthesis C-N Metabolism Indexes to Yield and NUE under the W-N Interaction

It can be seen from Table 11 that under various irrigation and N regimes, although the $P_n$ of the flag leaf at the maturity stage did not have a significant correlation with NPE, the $P_n$ of the flag leaf and the enzyme activities of C-N metabolism at various postanthesis growth stages of rice had significant positive correlations with yield and NUE, and the highest correlation coefficients occurred at 15 d after the full-heading stage. Increasing the $P_n$ of the flag leaf and enhancing the activities of RuBPCase and GS in the flag leaf at 0–15 d after the full-heading stage and enhancing the SPS activity in the flag leaf at 15–30 d after the full-heading stage had a stronger effect in ensuring the simultaneous improvement in rice NUE and yield.
Table 10. Coefficients of correlation between some carbon physiological indexes of flag leaves and $^{13}$C accumulation in panicle and N metabolism physiological index of flag leaves and $^{15}$N accumulation in panicle at postanthesis various growth stages (2014–2015).

| Physiological Index | Days after Full-Heading (d) | Glutamine Synthetase | Nitrate Reductase | Panicle $^{13}$C Accumulation |
|---------------------|-----------------------------|----------------------|-------------------|------------------------------|
|                      | 0              | 15                  | 30                | 0   | 15  | 30  | 30 (Maturity) |
| Photosynthetic rate  | 0.835 **        | 0.875 **            | 0.875 **          | 0.805 **        | 0.805 **        | 0.778 *          | 0.648 *          |
| Ribulose             | 0.900 **        | 0.923 **            | 0.883 **          | 0.865 **        | 0.881 **        | 0.698 *          |
| 1,5-bisphosphate carbonylase | 0.846 ** | 0.846 **            | 0.846 **          | 0.846 **        | 0.846 **        | 0.698 *          |
| Sucrose phosphate synthase | 0.890 ** | 0.906 **            | 0.927 **          | 0.885 **        | 0.919 **        | 0.745 *          |
| Panicle $^{13}$C accumulation | 0.905 ** | 0.914 **            | 0.841 **          | 0.889 **        | 0.865 **        | 0.825 **          |

* Significant at $p < 0.05$; ** Significant at $p < 0.01$, respectively.

Table 11. Coefficient of correlation between some physiological indexes of flag leaves and grain yield and N uptake and utilization at postanthesis various growth stages (2014–2015).

| Physiological Index | Days after Full-Heading (d) | Grain Yield | MTNA | NRE | NAE | NPE |
|---------------------|-----------------------------|-------------|------|-----|-----|-----|
| Photosynthetic rate | 0              | 0.854 **    | 0.899 ** | 0.713 * | 0.707 * | 0.635 * |
|                      | 15             | 0.861 **    | 0.928 ** | 0.724 * | 0.741 * | 0.678 * |
|                      | 30             | 0.841 **    | 0.854 ** | 0.686 * | 0.688 * | 0.566 ** |
| Ribulose             | 0              | 0.918 **    | 0.874 ** | 0.833 ** | 0.837 ** | 0.780 ** |
| 1,5-bisphosphate carbonylase | 0.931 ** | 0.931 **    | 0.928 ** | 0.884 ** | 0.872 ** | 0.818 ** |
|                      | 15             | 0.931 **    | 0.928 ** | 0.884 ** | 0.872 ** | 0.818 ** |
|                      | 30             | 0.886 **    | 0.721 *  | 0.819 ** | 0.814 ** | 0.712 *  |
| Sucrose phosphate synthase | 0.872 ** | 0.872 **    | 0.849 ** | 0.798 ** | 0.805 ** | 0.726 *  |
|                      | 15             | 0.911 **    | 0.921 ** | 0.863 ** | 0.877 ** | 0.829 ** |
|                      | 30             | 0.925 **    | 0.905 ** | 0.882 ** | 0.863 ** | 0.790 ** |
| Glutamine synthetase | 0              | 0.938 **    | 0.893 ** | 0.944 ** | 0.924 ** | 0.872 ** |
|                      | 15             | 0.949 **    | 0.901 ** | 0.949 ** | 0.937 ** | 0.889 ** |
|                      | 30             | 0.923 **    | 0.835 ** | 0.892 ** | 0.904 ** | 0.878 ** |
| Nitrate reductase    | 0              | 0.911 **    | 0.830 ** | 0.845 ** | 0.835 ** | 0.772 ** |
|                      | 15             | 0.915 **    | 0.866 ** | 0.896 ** | 0.892 ** | 0.835 ** |
|                      | 30             | 0.896 **    | 0.795 ** | 0.871 ** | 0.883 ** | 0.793 ** |

MTNA, NRE, NAE and NPE are for total N accumulation at maturity, N recovery efficiency, N agronomy efficiency, and N physiological efficiency, respectively. * Significant at $p < 0.05$; ** Significant at $p < 0.01$; ns denote non-significance at $p > 0.05$, respectively.

4. Discussion

C and N metabolism are the two most basic metabolic processes in plants [8]. How to adjust the relationship between the two through water and fertilizer treatments such that assimilation is coordinately distributed between the two is key to the balance of C-N metabolism and the improvement in yield and NUE in rice. The previous work showed that water-saving irrigation can not only enhance the N metabolism and improve the photosynthetic productivity of leaves but also help increase the activities of ADP-glucose pyrophosphorylase, starch synthase, starch branching enzyme, and other key enzymes of C metabolism during filling stage [23–25]. Honjyo et al. showed that increasing the application of N can increase the chlorophyll content and keep the activities of GS, SPS, and phosphoenolpyruvate carboxylase at high levels, which are conducive to promoting the formation of photosynthetic products and the accumulation of grain starch and protein, thereby achieving synergistic increases in yield and protein content [26]. However, it has rarely been reported which factor between water and N treatment has a more significant effect on the regulation of C-N metabolism. It was also not known, under W-N coupling, what the postanthesis C-N metabolism characteristics are in rice, or what the accumulation and distribution characteristics of C and N substances are.
This study showed that the regulatory effects of irrigation regimes on WUE, NUE, and the accumulation of C-assimilates in panicles were significantly higher than the regulatory effects of N regimes; the regulatory effects of N regimes, on the other hand, had a significant regulatory effect on the N accumulation at the maturity stage, C/N, NGPE, NMPE, and yield. A proper water-saving regime and a proper proportion of N application shifted to postpone can coordinate the enzyme activities of C-N metabolism (Figures 2 and 3) and promote the coordinated transport of postanthesis C assimilates and N (Tables 7 and 8). Such optimization can also achieve simultaneous increases in rice yield and NUE and can further realize the advantages of W-N coupling (Tables 4–6), which further confirms and supplements the results of previous studies, including ours [7,13,14]. This study also showed that when the amount of N application shifted to postpone reached 60% (N3 treatment), compared to W1, the inadequate irrigation (W2 and W3 treatments) caused a decrease in the enzyme activities of C-N metabolism of postanthesis flag leaves, a significant decrease in N accumulation, an increased retention of C assimilates in leaves and stems, and a significant decrease in C assimilates in panicles, leading to a significant reduction in NAE and NPE. This may be because N cycling would deplete the metabolism/assimilation capacity of inorganic N in the organism and form organic C-N compounds [8] and cause C-N metabolism to be dysregulated. The greater the application of base fertilizer and fertilizer at the tillering stage, the greater the loss, and the lower the overall NUE [15]. This study further showed that when a large proportion of N application was shifted to postpone, the amount of N loss was decreased with regard to N accumulation, yet the accumulation and coordinated transport of N and photosynthetic C were low, which also led to a significant reduction in the overall NUE. This result further strengthens the conclusions of a previous study [15,24].

This study also found that the increase in water stress intensity under irrigation regimes increased the proportion of 13C photosynthetic products transported from leaves to grains, which is basically consistent with the findings of Yang et al. [13]; however, in this study, it was shown that severe water stress was not conducive to the transport of 13C photosynthetic products from stem/sheath to grains, which is contrary to the result of Yang et al. [14] that severe water stress promoted the proportion of photosynthetic products transported from stems (up to 62–66%). This may be because although severe water deficit could increase the proportion of 13C photosynthetic products transported from leaves, the significant decrease in the amount of stem/sheath transport from leaves is the main stage for the decrease of 13C assimilates in grains. Under each irrigation regime, with the proportion of 15N-labeled N application shifted to postpone, the difference in 15N accumulation between various W and N regimes at the full heading stage was significant. This is not quite consistent with the results in Ye et al. [27], in which the absorption of total 15N in rice was similar between various treatments until the full heading stage, when 15N-labeled N fertilizer was applied twice, as the base fertilizer and before the full heading stage. In this study, the accumulation of 15N in different organs of rice plants at the full heading stage decreased as the proportion of N application shifted to postpone increased (Table 8), and it was especially significantly reduced under the N3 treatment. From the full heading to maturity stage, the absorption of 15N was further improved under the N3 treatment over the N2 treatment. These results show that shifting N application to postpone could promote the absorption of N in postanthesis rice, but the total 15N absorption under the N3 treatment was still significantly lower than that under N2. This result may indicate that under the N3 treatment, a gap of denitrification existed between the application of base fertilizer (40% N) and topdressing (60%), and postanthesis N retention occurred (Table 8)—rice plants absorbed N in the soil before topdressing, while the later-dressed 15N-labeled fertilizer may cause the N absorbed at the postanthesis stage to not be transported to grains in a timely manner but rather be retained in leaves and roots. This study only focused on the whole plant level in full-heading and maturity stages, and the dynamic accumulation of 13C and 15N during grain filling, and their relationship needs to be further studied.

Some studies argue that the level of rice yield is closely related to the absorption and utilization of N. C-N metabolism, as the most basic metabolic pathway in crops, provides a material basis for crop growth and development, which largely determines grain yield [28,29]. Matt et al. showed that under
sufficient supply of N, the decrease in Rubisco activity can lead to decreases in NR activity, nitrate accumulation, and amino acid level [30]. Therefore, the inhibition of leaf photosynthesis would reduce leaf sugar levels and thus lead to the inhibition of N metabolism. Moreover, the accumulation of photosynthetic products under low-light or basin irrigation conditions decreased the leaf photosynthesis rate and affected N metabolism. Under too much N accumulation, N assimilation would compete with and consume too much C, so the unstructured carbohydrates required for the development of young panicles would then be reduced, thereby affecting C metabolism [31–33]. However, all the above studies studied a single vegetative organ, such as leaves or grains, and few studies have reported the relationship between C metabolism and N metabolism in various vegetative organs or whether N metabolism or C metabolism has a more significant effect on nutrient accumulation in grains. This study showed that during the filling stage, under appropriate W-N treatments, the activities of enzymes of C-N metabolism in leaves can also directly regulate C and N accumulation in grains. Specifically, the activities of key enzymes of N metabolism in leaves had a more obvious effect in promoting the accumulation of \(^{13}\text{C}\) in panicles, as evidenced by their high correlations (Table 10). Thus, C metabolism in leaves plays a positive role in regulating N accumulation in grains to achieve the synergy of C-N metabolism between various vegetative organs. This also further showed that under the W-N interaction, the stimulatory effect of the enzyme activities of postanthesis N metabolism on C accumulation in panicles and the regulatory effect of the enzyme activities of C metabolism on N accumulation in panicles are important reasons that C-N metabolism coupling can help achieve high yield and high NUE in rice.

Selecting indicators that can evaluate NUE and yield is key to the evaluation of crop NUE and high yield [34]. The endopeptidase activity in rice leaves during filling stage can be used as an indicator of rice filling characteristics, yield composition, and quality, and GS activity and soluble protein content also have important reference value for evaluating the NUE in rice [35,36]. Our previous research results also showed that the GS activity in functional leaves can be used as an indicator to accurately quantify the N accumulation at various growth stages of rice plants, and the enzyme activities of NR and GS in the flag leaf at the heading stage can be used as a comprehensive indicator of the simultaneous increase in rice yield and NUE [9]. According to this study, under different irrigation and N regimes, the photosynthesis rate of the flag leaf and the enzyme activities of C-N metabolism, such as RuBPCase and GS, at various postanthesis growth stages of rice plants can accurately reflect the NUE and yield levels of rice (Table 11), which further confirms the results of previous studies [9,36].

At the same time, this study also showed by \(^{13}\text{C}\) labeling that the postanthesis accumulation and transport of photosynthetic C assimilates had significant consistency and synergy with the photosynthesis rate of the flag leaf and the activities of key enzymes of C-N metabolism. It further clarified that C-N metabolism can succinctly and clearly reflect rice yield and NUE as well as the accumulation and transport of photoassimilates. It showed that the improvement in N metabolism capacity can simultaneously improve the level of C metabolism and that the activity of key enzymes of C metabolism can be used as physiological and biochemical indicators for screening and identifying high-NUE rice genotypes. Weigelt et al. showed that the normal growth of crops does not require high N and C contents but can be accomplished when the C/N ratio reaches a certain balance [37]. However, few studies have reported how the balance of photosynthetic C assimilate accumulation in rice plants changes with water and N absorption dynamics in roots, or how the C/N ratio changes under W-N coupling. This study found that under high-yield conditions, from the full heading to maturity stage, the C/N ratio was approximately twice that under other conditions in leaves and in panicles and approximately half in stems and in roots (Table 9). Hence, the C/N change value of postanthesis vegetative organs in rice can be used as an indicator and a theoretical basis for the simultaneous improvement in yield and NUE. The optimization of the C/N ratios of various rice vegetative organs from the full heading to maturity stage serves as another important approach to achieve high yield, high WUE, and high NUE; this approach is more accurate than the determination of rice yield and NUE through measuring the activity of a specific enzyme of N or C metabolism at a specific growth stage.
5. Conclusions

Irrigation and N regimes had significant effects on postanthesis NUE, photoassimilate distribution, physiological characteristics, and yield. The aerobic irrigation combined with the N regime 30% base, 30% tillering, 40% topdressing equally divided into two portions and applied at top 4th and 2nd leaf can promote the postanthesis accumulation of N, increase the activities of C-N metabolism enzymes in flag leaves, and promote the accumulation and transport of photoassimilates and N in vegetative organs such as leaves, stems, roots, and panicles. Thus, such strategies can become the main regulatory approach to achieving simultaneous increases in yield and NUE. Under flooded irrigation, on the basis of 60% base tiller N fertilizer, booting N-fertilizer should account for 40% applied equally at top 4th and 2nd leaf. Under deficit irrigation, however, booting N-fertilizer should account for 20% applied equally at top 4th and 2nd leaf. Under the W-N interaction, the stimulatory effect of the enzyme activities of postanthesis N metabolism on the C accumulation in panicles and the regulatory effect of the enzyme activities of C metabolism on the N accumulation in panicles are important reasons that C-N metabolic coupling promotes high yield and NUE in rice plants. From the full-heading to maturity stage, the C/N ratio was multiplied by approximately 2 in leaves and in panicles and by roughly half in stems and in roots under the best regimes. The change in C/N ratio can be used as an evaluation indicator for the simultaneous improvement in yield and NUE in rice and can serve as an alternative important regulatory approach to achieving high yield, high WUE, and high NUE at the same time.

Author Contributions: J.M.: Conceptualization, Funding acquisition, Supervision, Validation; Y.S. (Yongjian Sun): Investigation, Methodology, Writing—original draft; Y.S. (Yuanyuan Sun): Methodology, Software, Writing—original draft; F.Y.: Software, Investigation; Y.L.: Data curation; Y.W.: Formal analysis; C.G.: Investigation; P.M.: Investigation; G.Y.: Supervision; Z.Y.: Software. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Sichuan Provincial Science and technology support project (2020YJ0411), the National Key Research and Development Program Foundation of Ministry of Science and Technology of China (2018YFD0301202), the Funding of Academic and Technical Leaders Cultivation Foundation of Sichuan Provincial Human Resources and Social Security Department (2016–183).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Haefele, S.M.; Jabbar, S.M.A.; Siopongco, J.D.L.C.; Tirol-Padre, A.; Amarante, S.T.; Sta-Cruz, P.C.; Cosico, W.C. Nitrogen use efficiency in selected rice (Oryza sativa L.) genotypes under different water regimes and nitrogen levels. Field Crops Res. 2008, 107, 137–146. [CrossRef]

2. Sun, Y.; Yan, F.; Sun, Y.; Xu, H.; Guo, X.; Yang, Z.; Yin, Y.; Guo, C.; Ma, J. Effects of different water regimes and nitrogen application strategies on grain filling characteristics and grain yield in hybrid rice. Arch. Agron. Soil Sci. 2018, 64, 1152–1171. [CrossRef]

3. Kumar, R.; Sarawgi, A.K.; Ramos, C.; Amarante, S.T.; Ismail, A.M.; Wade, L.J. Partitioning of dry matter during drought stress in rainfed lowland rice. Field Crops Res. 2006, 96, 455–465. [CrossRef]

4. Lu, Y.; Watanabe, A.; Kimura, M. Input and distribution of photosynthesized carbon in a flooded soil. Glob. Biogeochem. Cycles 2002, 16, 321–328. [CrossRef]

5. Mae, T.; Ohira, K. The remobilization of nitrogen related to leaf growth and senescence in rice plants (Oryza sativa L.). Plant Cell Physiol. 1981, 22, 1067–1074.

6. Huang, J.; Zou, Y.; Peng, S.; Buresh, R.J. Nitrogen uptake, distribution by rice and its losses from plant tissues during. Plant Nutr. Fertil. Sci. 2004, 10, 579–583. (In Chinese with English Abstract).

7. Sun, Y.; Ma, J.; Sun, Y.; Xu, H.; Yang, Z.; Liu, S.; Jia, X.; Zheng, H. The effects of different water and nitrogen managements on yield and nitrogen use efficiency in hybrid rice of China. Field Crops Res. 2012, 127, 85–98. [CrossRef]

8. Krapp, A.; Aliba-Colombani, S.V.; Daniel-Vedeile, F. Analysis of C and N metabolisms and of C/N interactions using quantitative genetics. Photosynth. Res. 2005, 83, 251–263. [CrossRef]
9. Sun, Y.; Sun, Y.; Li, X.; Guo, X.; Ma, J. Relationship of activities of key enzymes involved in nitrogen metabolism with nitrogen utilization in rice under water-nitrogen interaction. *Acta Agron. Sin.* 2009, 35, 2055–2063. (In Chinese with English Abstract). [CrossRef]

10. Yang, Z.; Li, N.; Ma, P.; Li, Y.; Zhang, R.; Song, Q.; Guo, X.; Sun, Y.; Xu, H.; Ma, J. Improving nitrogen and water use efficiencies of hybrid rice through methodical nitrogen-water distribution management. *Field Crops Res.* 2020, 246, 107698. [CrossRef]

11. Wang, S.; Cao, W.; Ding, Y.; Tian, Y.; Jiang, D. Interactions of water management and nitrogen fertilizer on nitrogen absorption and utilization in rice. *Sci. Agric. Sin.* 2004, 37, 497–501, (In Chinese with English Abstract).

12. Cassman, K.G.; Peng, S.; Olk, D.C.; Ladha, J.K.; Reichardt, W.; Dobermann, A.; Singh, U. Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Res.* 1998, 56, 7–39. [CrossRef]

13. Yang, J.; Zhang, J.; Wang, Z.; Zhu, Q.; Wang, W. Hormonal changes in the grains of rice subjected to water stress during grain filling. *Plant Physiol. Commun.* 2001, 127, 315–323. [CrossRef] [PubMed]

14. Yang, J.; Zhang, Z.; Wang, Z.; Liu, L.; Zhu, Q. Postanthesis water deficits enhance grain filling in two-line hybrid rice. *Crop Sci.* 2003, 43, 2099–2108. [CrossRef]

15. Lin, J.; Li, G.; Xue, L.; Zhang, W.; Xu, H.; Wang, S.; Yang, L.; Ding, Y. Subdivision of nitrogen use efficiency of rice based on 15N tracer. *Acta Agron. Sin.* 2014, 40, 1424–1434, (In Chinese with English Abstract). [CrossRef]

16. Yoshida, S.; Forno, D.A.; Cock, D.H.; Gomez, K.A. *Laboratory Manual for Physiological Studies of Rice*, 3rd ed.; International Rice Research Institute: Los Baños, Philippines, 1976; p. 83.

17. Li, C.; Li, L. Comparison between the spectrophotometric method and 14C-labelled method for measuring RuBP Case activity. *Plant Physiol. Commun.* 1989, 1, 49–50, (In Chinese with English Abstract).

18. Wang, H.; Lee, P.; Chen, W.; Huang, D.; Su, J. Osmotic stress induced changes of sucrose metabolism in cultured sweet potato cells. *Exp. Bot.* 2000, 51, 1991–1999. [CrossRef]

19. Wang, W.; Cai, Y.; Cai, K.; Zhang, J.; Yang, J.; Zhu, Q. Regulation of soil water deficits on stem stored carbohydrate remobilization to grain of rice. *Acta Phytoecol. Sin.* 2005, 29, 819–828, (In Chinese with English Abstract).

20. Li, H. *Experimental Principle and Technique for Plant Physiology and Biochemistry*; Higher Education Press: Beijing, China, 2000; pp. 125–127.

21. Lea, P.J.; Blackwell, R.D.; Chen, F. Enzymes of primary metabolism. In *Methods in Plant Biochemistry*; Harborne, J.B., Ed.; Academic Press: Cambridge, MA, USA, 1990; Volume 3, pp. 260–273.

22. O’Neal, D.; Joy, K.W. Glutamine synthetase of pea leaves. 1. Purification stabilization and pH optima. *Arch. Biochem. Biophys.* 1973, 113, 113–122. [CrossRef]

23. Qin, J.; Hu, F.; Zhang, B.; Wei, Z.; Li, H. Role of straw mulching in non-continuously flooded rice cultivation. *Agric. Water Manag.* 2006, 283, 252–260. [CrossRef]

24. Tao, H.; Brueck, H.; Dittert, K.; Kreye, C.; Lin, S.; Sattelmacher, B. Growth and yield formation of rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Res.* 2006, 95, 1–12. [CrossRef]

25. He, H.; Yang, R.; Jia, B.; Chen, L.; Fan, H.; Cui, J.; Yang, D.; Li, M.; Ma, F. Rice photosynthetic productivity and PSII photochemistry under non-flooded irrigation. *Sci. World J.* 2014, 1, 171–192.

26. Honjo, K. Studies on protein content in rice grain: II. Effects of the fertilization on protein content and protein production in paddy grain. *Jpn. J. Crop Sci.* 1971, 40, 190–196. (In Japanese with English Abstract). [CrossRef]

27. Ye, L.; Song, W.; Lyu, H.; Li, Y.; Shen, Q.; Zhang, Y. Accumulation and translocation of nitrogen at late-growth stage in rices different in cultivar nitrogen use efficiency. *Acta Pedol. Sin.* 2010, 47, 303–310, (In Chinese with English Abstract).

28. Camargo, F.A.O.; Gianello, C.; Tedesco, M. Soil nitrogen availability evaluated by kinetic mineralization parameters. *Commun. Soil Sci. Plant.* 2004, 35, 1293–1307. [CrossRef]

29. Li, C.; Xue, L.; Gu, W.; Yang, C.; Wang, S.; Ling, Q.; Ding, Y. Comparison of yield components and plant type characteristics of high-yield rice between Taoyuan, a ‘special eco-site’and Nanjing, China. *Field Crops Res.* 2009, 112, 214–221. [CrossRef]

30. Matt, P.; Krapp, A.; Haake, V.; Mock, H.; Stitt, M. Decreased rubisco activity leads to dramatic changes of nitrate metabolism, amino acid metabolism and the levels of phenylpropanoids and nicotine in tobacco antisense RBCS transformants. *Plant J.* 2002, 30, 663–677. [CrossRef]
31. Poorter, H.; Nagel, O. The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: A quantitative review. *Aust. J. Plant Physiol.* **2000**, *27*, 595–607.

32. Ahmadi, A.; Baker, D.A. The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Regul.* **2001**, *35*, 81–91. [CrossRef]

33. Zhang, H.; Zhang, S.; Zhang, J.; Yang, J.; Wang, Z. Postanthesis moderate wetting drying improves both quality and quantity of rice yield. *Agron. J.* **2008**, *100*, 726–734. [CrossRef]

34. Peng, S.; Huang, J.; Zhong, X.; Yang, J.; Wang, G.; Zou, Y.; Zhang, F.; Zhu, Q.; Buressh, R.; Witt, C. Challenge and opportunity in improving fertilizer-nitrogen use efficiency of irrigated rice in China. *Agric. Sci. China* **2002**, *1*, 776–785.

35. Zeng, J.; Cui, K.; Huang, J.; He, F.; Peng, S. Responses of physio- biochemical properties to N-fertilizer application and its relationship with nitrogen use efficiency in rice (*Oryza sativa* L.). *Acta Agron. Sin.* **2007**, *33*, 1168–1176, (In Chinese with English Abstract)

36. Hu, J.; Yang, L.; Zhou, J.; Wang, Y.; Zhu, J. Effect of free air CO₂ enrichment (FACE) and nitrogen level on endopeptidase activities in rice leaves during grain filling stage. *Chin. J. Rice Sci.* **2008**, *22*, 155–160, (In Chinese with English Abstract)

37. Weigelt, K.; Kuster, H.; Rutten, T.; Fait, A.; Fernie, A.R.; Miersch, O.; Wasternack, C. ADP-glucose pyrophosphorylase-deficient pea embryos reveal specific transcriptional and metabolic changes of carbon-nitrogen metabolism and stress responses. *Sci. Plant Physiol.* **2009**, *149*, 395–411. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).