agronomy

Article

A Moderate Wetting and Drying Regime Combined with Appropriate Nitrogen Application Increases Grain Yield and Nitrogen Use Efficiency in Rice

Hanghang Huang [1,2], Rongyue Xu [1,2], Jixiang Yu [1,2], Weiyang Zhang [1,2], Junfei Gu [1,2], Kuanyu Zhu [1,2,*], Jianhua Zhang [3,4] and Jianchang Yang [1,2]

1 Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology/Agricultural College, Yangzhou University, Yangzhou 225009, China; hhh178357140330301@163.com (H.H.); 211702119@stu.yzu.edu.cn (R.X.); mz120221358@stu.yzu.edu.cn (J.Y.); wyz@yzu.edu.cn (W.Z.); gujf@yzu.edu.cn (J.G.); jcyang@yzu.edu.cn (J.Y.)
2 Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China
3 Department of Biology, Hong Kong Baptist University, Hong Kong 999077, China; jzhang@hkbu.edu.hk
4 The State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong 999077, China
* Correspondence: kyzhu@yzu.edu.cn; Tel.: +86-514-8797-9317

Abstract: This study investigated whether and how irrigation regimes interact with nitrogen (N) application rates to mediate the grain yield, N use efficiency (NUE) and water use efficiency (WUE) in rice and to understand the underlying mechanism. A field experiment was conducted with two irrigation regimes, continuously flooded (CF) and alternate wetting and moderate drying (AWMD), and three N application rates, 120 kg ha^{-1} N (a low N rate, LN), 240 kg ha^{-1} N (a medium N rate, MN) and 360 kg ha^{-1} N (a high N rate, HN) in 2021 and 2022. The results showed that the grain yield exhibited the lowest values at the LN, regardless of the irrigation regime, while it was the highest at the MN when the CF regime was adopted. The grain yield was comparable between the MN and HN regarding the AWMD regime. AWMD significantly increased the grain yield, NUE and WUE compared to CF at the same N rate, which was attributed to a higher photosynthetic rate, improved population quality, enhanced nonstructural carbohydrate remobilization from stems to grains during grain filling, and elevated activities of enzymes involved in N assimilation in the roots. The results suggest that an AWMD regime combined with MN treatment could pronounce a synergistic interaction on the grain yield, NUE and WUE in rice by improving root and shoot physiological performances.

Keywords: rice (*Oryza sativa* L.); grain yield; alternate wetting and moderate drying; nitrogen use efficiency; synergistic interaction

Citation: Huang, H.; Xu, R.; Yu, J.; Zhang, W.; Gu, J.; Zhu, K.; Zhang, J.; Yang, J. A Moderate Wetting and Drying Regime Combined with Appropriate Nitrogen Application Increases Grain Yield and Nitrogen Use Efficiency in Rice. *Agronomy* **2023**, *13*, 1729. https://doi.org/10.3390/agronomy13071729

Academic Editor: Min Huang

Received: 6 June 2023
Revised: 24 June 2023
Accepted: 26 June 2023
Published: 27 June 2023

1. Introduction

The global food security challenge is further exacerbated by the growing population, which necessitates sufficient and reliable food production [1]. It is reported that by 2050, there will be a 60% increase in rice (*Oryza sativa* L.) production to meet the escalating demand for food [2]. However, crops are struggling to cope with the diminishing water resources [3,4]. Rice, being a vital crop, is known for its high water requirements, with irrigation accounting for approximately 80% of the total water usage in Asian agriculture [5,6]. It conveys the idea that in order to address the urgent requirement for higher rice production and sustain a growing population in the face of limited water resources, various water-saving techniques have been put into practice [7–11]. It is reported that the AWD irrigation technique has been widely promoted and implemented on a large scale in China, with an annual coverage area reaching 12 million hectares [12,13]. This technique involves alternating periods of soil drying and flooding during the crop growth stages, effectively reducing irrigation water requirements and significantly enhancing water use efficiency [14,15]. However, there is a current controversy surrounding the potential of AWD irrigation technology to enhance crop productivity and resource use efficiency [13,16–18]. In light of this, it is crucial to investigate whether the AWD technique has a positive effect on the grain yield of rice.

In addition, nitrogen fertilizer, as a limiting factor in plant growth and development, plays a crucial role in crop yield formation [19–22]. China, as an important country of rice production, faces a critical challenge of high N fertilizer inputs and low N use efficiency (NUE) [23,24]. It is reported that the NUE in rice is relatively low, with over 60% of N being lost through processes like surface runoff, leaching in groundwater, ammonia volatilization, and microbial denitrification [25,26]. Improving grain yield and NUE while reducing nitrogen fertilizer application is a pressing concern in rice production [24,26]. Some studies have indicated that implementing an AWD regime may result in reduced total nitrogen uptake by plants, leading to lower NUE [27,28]. Conversely, other research has demonstrated that AWD can actually improve NUE by enhancing root and soil traits [29–31]. However, there is limited understanding of the potential interaction between irrigation regimes and N application rates in achieving higher yield and NUE in rice.

This study aimed to explore the interaction between AWD and N application rates and its impact on grain yield and NUE in rice. To investigate the underlying mechanism, various parameters were assessed, including tiller number, root and shoot biomass, root oxidation activity (ROA), leaf photosynthetic rate, dry matter weight, nonstructural carbohydrate (NSC) remobilization, and soil available N content. This research provides valuable insights into the biological processes involved in the interaction between water and N, and its influence on rice yield and NUE.

2. Materials and Methods

2.1. Cultivation Conditions and Materials

This study was carried out at Yangzhou University's research farm in Jiangsu Province, China (32°30′ N, 119°25′ E) from May to October in 2021 and repeated in 2022. The experimental field had a previous crop of wheat and featured a sandy soil texture (classified as Typic fluvaquents, Etisols (U.S. taxonomy)) with an average of 24.4 g kg^{-1} organic matter, 105 mg kg^{-1} alkali-hydrolysable N, 34.3 mg kg^{-1} Olsen-P, and 68.2 mg kg^{-1} exchangeable K. The monthly average air temperature, sunshine hours, and precipitation during the rice growing season in the two study years as recorded at a weather station near the experimental site are depicted in Figure S1.

An inbred *Japonica* cultivar Jinxiangyu 1 was used as the trial material. The seeds were sown on May 15th and transplanted after a 25-day period. The spacing between plants was set at 0.25 m × 0.16 m, with two seedlings per hill. The cultivar received a basal fertilizer application of 30 kg ha^{-1} phosphorus in the form of superphosphate and 40 kg ha^{-1} potassium in the form of potassium chloride, which were applied and incorporated into the soil one day before transplanting. The cultivar reached the heading stage (50% of plants) between 14 and 16 August, and harvesting took place on 17 October in both years. To ensure minimal yield losses, weeds, insects, and diseases were effectively controlled using a combination of chemical and manual methods.

2.2. Treatment and Design

The experiment was conducted using a two-factorial complete randomized split-block design with six treatments. The main plot consisted of two irrigation regimes, while the split-plot included three N application rates. Each treatment was replicated three times. Each experimental plot covered an area of 30 m^2. There were three N application rates, including 120 kg ha^{-1} (a low rate, LN), 240 kg ha^{-1} (a medium rate, MN), and 360 kg ha^{-1} (a high rate, HN). Nitrogen fertilizer was applied in the form of urea, with a ratio of 4:2:2:2

for basal fertilizer, tillering stage, panicle initiation (PI), and pistil and stamen differentiation, respectively. The urea was applied using the broadcasting method, and mechanical deep plowing was conducted in the paddy field to mitigate ammonia volatilization.

The conventional flooded (CF) and alternate wetting and moderate soil drying (AWMD), were implemented from 10 days after transplanting until one week before harvest. In the CF regime, the field was continuously flooded with a water level of 3–5 cm, except for drainage at the end of tillering, until one week before final harvest. On the other hand, in the AWMD regime, irrigation was withheld until the soil water potential (SWP) reached −10 kilopascal (kPa) at a depth of 15–20 cm, which was based on previous findings [32]. A SWP monitoring system (ENVILog-100 Soil water potential monitoring system, Beijing Aozuo Ecological Instrument Co., Ltd., Beijing, China) was installed in the field to monitor SWP. Additionally, five tensiometers (Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China) were installed at a soil depth of 15−20 cm in each plot to monitor the accuracy of SWP measurements. Once the SWP reached the designed threshold, the plots were flooded with a water depth of 3.0−4.0 cm. To assess the degree of soil drying in the AWMD regime, an electric rain shelter was used to protect the entire paddy field from rain. The shelter was then removed once the rain ceased.

2.3. Sampling and Measurement

2.3.1. Leaf Water Potential, Photosynthetic Rate, and N Content

Leaf water potential (LWP) measurements were taken in a sunny mid-day (1130−1200 h) using the pressure chamber method described by Zhang et al. [33]. Measurements were conducted at two stages: mid-tillering (MT) and middle grain-filling (MG), corresponding to 20 and 90–93 days after transplanting, respectively, when the SWP in the AWMD treatment reached approximately −10 kPa. The measurements were also taken at 2 days after re-watering when adopting the AWMD regimes. Each measurement was repeated on six leaves. During the same period, the photosynthetic rate and nitrogen (N) content of the fully expanded top leaves were evaluated. The leaf photosynthetic rate was measured using a gas exchange analyzer (Li-Cor 6800, Li-Cor, Lincoln, NE, USA) between 0900 h and 1100 h, when the photosynthetically active radiation above the canopy ranged from 1300 to 1500 μmol m^{-2} s^{-1}. Six leaves were measured for each treatment. To determine the specific leaf N content, the method was referred to Wang et al. [32].

2.3.2. Population Traits and Non-Structural Carbohydrate (NSC) Remobilization

The observation of tiller number was conducted on 20 tagged hills of plants at jointing (JT), heading (HD), and maturity (MA), corresponding to 40–42, 60–62 and 125–128 days after transplanting, respectively. From each designated plot, plants were selectively collected from 5 central hills (excluding those at the borders) for assessing aboveground dry weight and leaf area index (LAI) at the JT, HD, and MA. The sampled plants were divided into leaves, stems (including culms and sheaths), and panicles (only at HD and MA). The measurement of leaf area was referred to Zhang et al. [33]. Subsequently, the plant samples were dried at 70 °C until a constant weight was achieved, and their dry matter weight was determined. The nonstructural carbohydrate (NSC) content in the stems and panicles was assessed at HD and MA stages using the method described by Yoshida et al. [34].

2.3.3. Root Traits and Soil N Content

Roots of six hills from each plot were sampled at MT, PI, HD and MG from each plot, and the roots were meticulously rinsed using a hydropneumatic elutriation device (Gillison's Variety Fabrications, Benzonia, MI, USA). After combining the roots from the six hills and recording their fresh weight, some root samples were allocated for physiological measurements, and some roots were used to determine dry weight. The ROA was measured following the method described by Wang et al. [32]. Some root samples were promptly frozen in liquid nitrogen and stored at −80 °C. Subsequently, the activities of glutamate synthase (GOGAT), glutamine synthetase (GS) and nitrate reductase (NR) were determined using the specific assay kits (Comin Biotechnology Co., Ltd., Suzhou, China).

Soil samples (0–20 cm depth) were collected during clear days at the mid-tillering (MT), mid-grain filling (MG) and before transplanting (BT) in two study years. The soil samples were analyzed for nitrate-N and ammonium-N contents using the method described by Zhang et al. [35].

2.3.4. Final Harvesting

Grain yield and its components were measured on 15−17 October in both years following the methods described in Yoshida et al. [33]. To minimize border effects, plants in two rows on each side of the plot were left unharvested. A hand-harvested area of 6 m^2 in center rows was selected to determine grain yield, and the harvested grains were adjusted to 14% moisture content. In both years, a random sample of 0.8 m^2 of plants (excluding marginal plants) was collected from each plot to determine aboveground biomass and yield components, including the total number of spikelets, the percentage of fully filled grains (with a specific gravity ≥1.06 g cm^{-3}), and the 1000-grain weight. The N content of the plant tissues was also determined by the Kjeldahl method (Foss 8400, Copenhagen, Denmark). The calculation of NUEs, NSC remobilization, photosynthetic NUE (PNUE) and leaf area duration (LAD), were referred to the method described by Zhang et al. [33].

2.4. Data Analysis

Analysis of variance was conducted using the SAS/STAT statistical analysis package (version 9.2, SAS Institute, Cary, NC, USA). The statistical model included factors such as replication, year (Y), irrigation regime (I), N rates (N), and their interactions (Y × I, Y × N, I × N, and Y × I × N). Mean values were compared using the least significant difference (LSD) test at a significance level of 5% (LSD 0.05). Due to the consistent trends observed in the experimental data over the two study years, the results section merged the data from both years for analysis.

3. Results

3.1. Grain Yield, NUE and WUE

Table S1 presents the computed F values for the differences in grain yield, NUE and WUE. Significant differences were observed among years (Y), irrigation regimes (I), nitrogen rates (N), as well as their interactions (Y × I, Y × N, and N × I) for grain yield, nitrogen uptake, internal nitrogen use efficiency (IE_N), partial factor productivity of applied nitrogen (PFP_N), nitrogen harvest index (HI_N), and WUE.

A significant interaction was observed among irrigation regimes and N rates on grain yield and NUE (Table S1). In all irrigation regimes, grain yield was the lowest at LN, while it was the highest at MN when adopting the CF regime (Table 1). There was no significant difference in the grain yield between MN and HN under the AWMD regime. The AWMD produced a higher grain yield compared to the CF regime with the same amount of nitrogen applied. The AWMD exhibited a greater spikelets number and percentage of filled grains when compared to CF at the same N rate (Table 1). It should be noted that the grain yield was much lower in 2022 than in 2021, due primarily to the very high temperature during the pollen grain enrichment and heading time in August of 2022 (Figure S1A), which resulted in much reduction in the spikelets number per panicle and the percentage of filled grains, and thereby a lower grain yield (Table 1).

AWMD exhibited a higher total N uptake compared to CF at LN and HN, while it showed a comparable value to CF at MN (Figure 1A,E). The N accumulation in the grain was the lowest at LN, regardless of irrigation regimes, but the highest at MN when adopt the CF regime. There was no significant difference in N accumulation in the grain between MN and HN when the AWMD regime was implemented (Figure 1A,E). When the same amount of N was applied, AWMD significantly increased the N accumulation in the grain compared to CF (Figure 1A,E). At the same N rate, AWMD significantly increased IE_N, PFP_N, and HI_N compared to CF (Figure 1B–D,F–H).

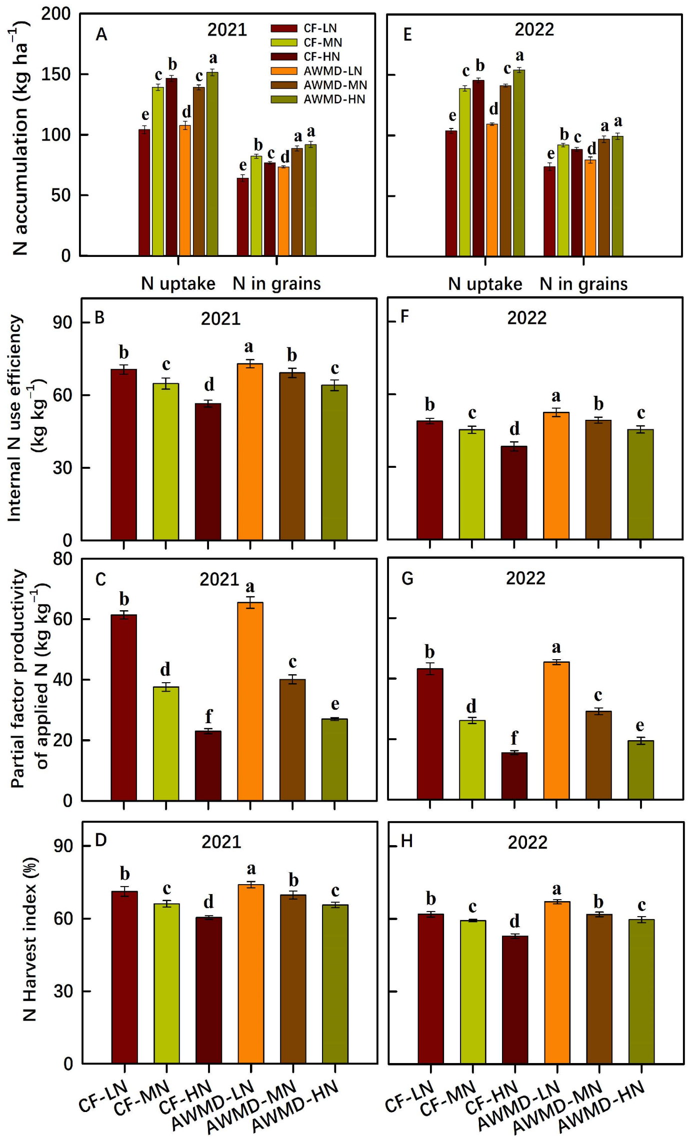

Figure 1. The N use efficiency of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes in 2021 (**A**–**D**) and 2022 (**E**–**H**). LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

AWMD showed lower irrigation water compared to CF at the same N rate, while no significant difference was observed in irrigation water among three N rates in both irrigation regimes (Figure 2A,C). The WUE in the CF regime was the highest at the NN rate, followed by the HN rate, and the lowest at the LN rate. In both irrigation regimes, WUE increased with increasing nitrogen rates. However, there was no significant difference in WUE between the NN and HN rates in the AWMD regime (Figure 2B,D).

Table 1. Effects of various nitrogen rates and irrigation regimes on grain yield, yield components and harvest index.

Year/Irrigation Regime	N Rate (kg ha^{-1})	Grain Yield (t ha^{-1})	Panicles per m^2	Spikelets per Panicle	1000-Grain Weight (g)	Filled Grains (%)	HI [a]
2021							
	LN	7.36 d	246 b	129 c	26.7 a	89.1 b	0.542 c
CF	MN	9.03 b	283 a	137 b	26.8 a	87.7 c	0.534 d
	HN	8.27 c	284 a	141 a	25.9 b	81.6 d	0.522 e
	LN	7.86 d	252 b	130 c	26.7 a	92.0 a	0.562 a
AWMD	MN	9.62 a	281 a	142 a	26.8 a	91.7 a	0.559 ab
	HN	9.71 a	283 a	143 a	26.8 a	91.2 b	0.550 b
2022							
	LN	4.93 d	223 b	122 c	27.4 a	67.1 b	0.372 c
CF	MN	6.19 b	278 a	132 b	27.5 a	62.3 c	0.361 c
	HN	5.72 c	279 a	135 a	26.7 b	57.6 d	0.338 d
	LN	5.48 d	229 b	123 c	27.6 a	70.8 a	0.426 a
AWMD	MN	7.01 a	284 a	136 a	27.6 a	69.6 a	0.418 ab
	HN	7.00 a	290 a	137 a	27.7 a	67.4 b	0.412 b

[a] HI, harvest index: total grain weight (dry weight)/total aboveground biomass (dry weight). LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

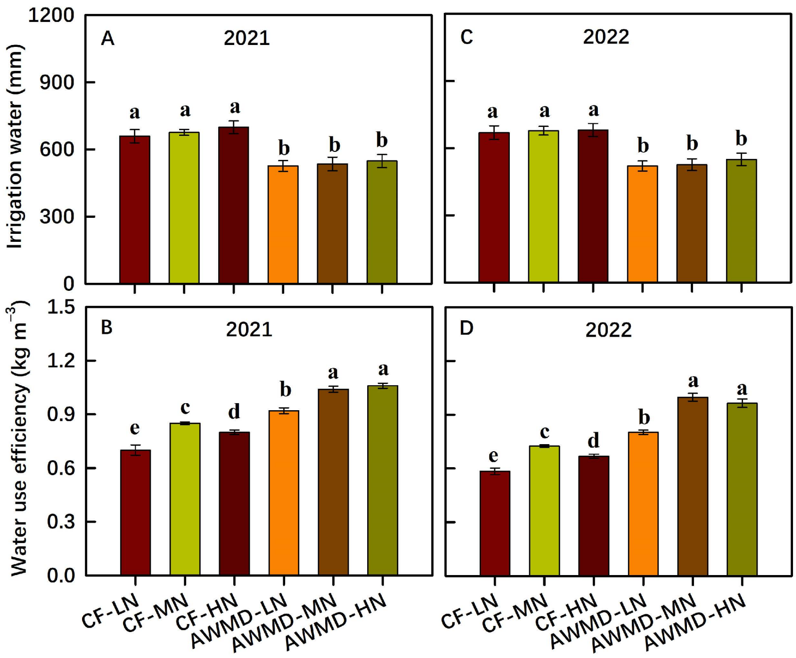

Figure 2. Irrigation water for the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes in 2021 (**A**,**B**) and 2022 (**C**,**D**). LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

3.2. Leaf Water Potential (LWP), Shoot Dry Weight, Tiller Number, and LAI

The AWMD significantly reduced LWP compared to CF during the soil drying period. However, after re-watering, similar values were observed in LWP between AWMD and CF. Under the same irrigation regime, there were comparable values in LWP among different nitrogen rates (Figure S2).

At JT and HD, the shoot dry weight increased with increasing N rates in both irrigation regimes, but showed similar values between MN and HN at MA (Figure 3A,D). When considering the same N rate, CF had a higher shoot dry weight than AWMD at HD, while it was comparable to AWMD at JT and MA (Figure 3A,D). The tiller number at JT and HD increased with increasing N rates within each irrigation regime, while it was lower in AWMD than in CF at JT and HD under the same N rate. At MA, the tiller number showed similar values between either CF and AWMD or between MN and HN (Figure 3B,E). The productive tillers percentage declined as N rates increased in both irrigation regimes, with AWMD exhibiting significantly higher percentages compared to CF at the same N rate (Figure 3C,F).

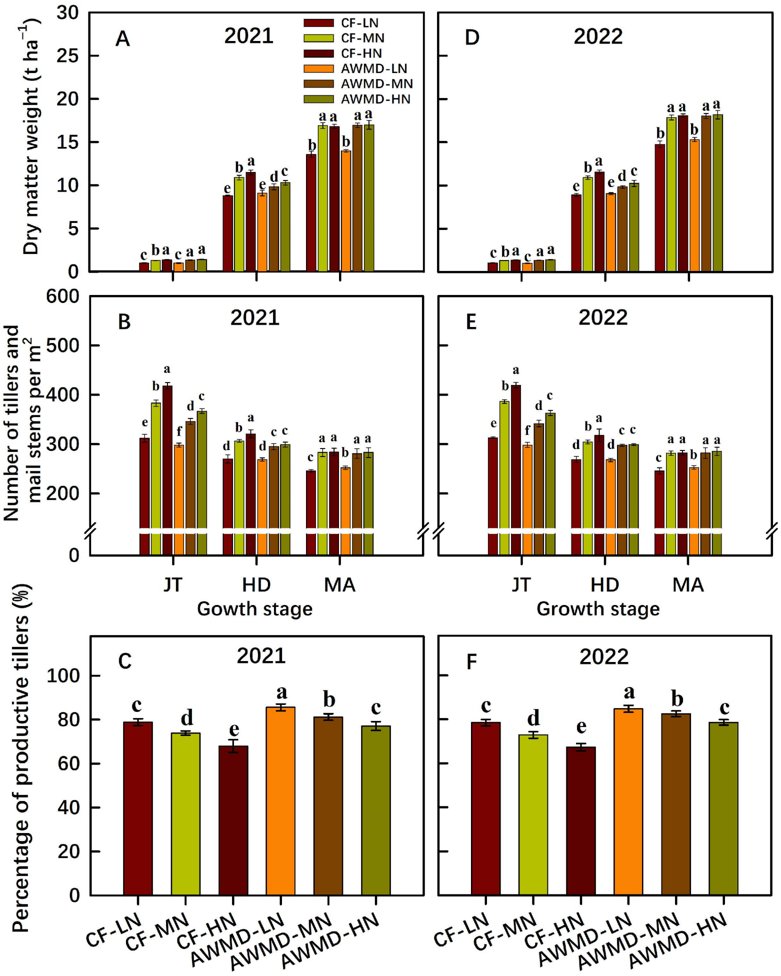

Figure 3. Effects of various nitrogen rates and irrigation regimes on dry matter accumulation (**A**,**D**), the number of tillers (**B**,**E**), and the percentage of productive tillers (**C**,**F**) of the *Japonica* rice cultivar Jinxiangyu 1. LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. JT, HD and MA represent jointing, heading and maturity stage, respectively. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

In both irrigation regimes, increasing N rates led to an increase in total leaf area index (LAI) at JT and HD, as well as in leaf area duration (LAD) from JT to HD (Figures S3A,B,E,F and 4A,D). There was similar values in total LAI between MN and HN at HD regarding the AWMD regime (Figure S3B,F). Within each N rate, there were no significant differences in LAI at JT and HD, as well as in LAD from JT to HD, between CF and AWMD regimes (Figures S3A,B,E,F and 4A,D).

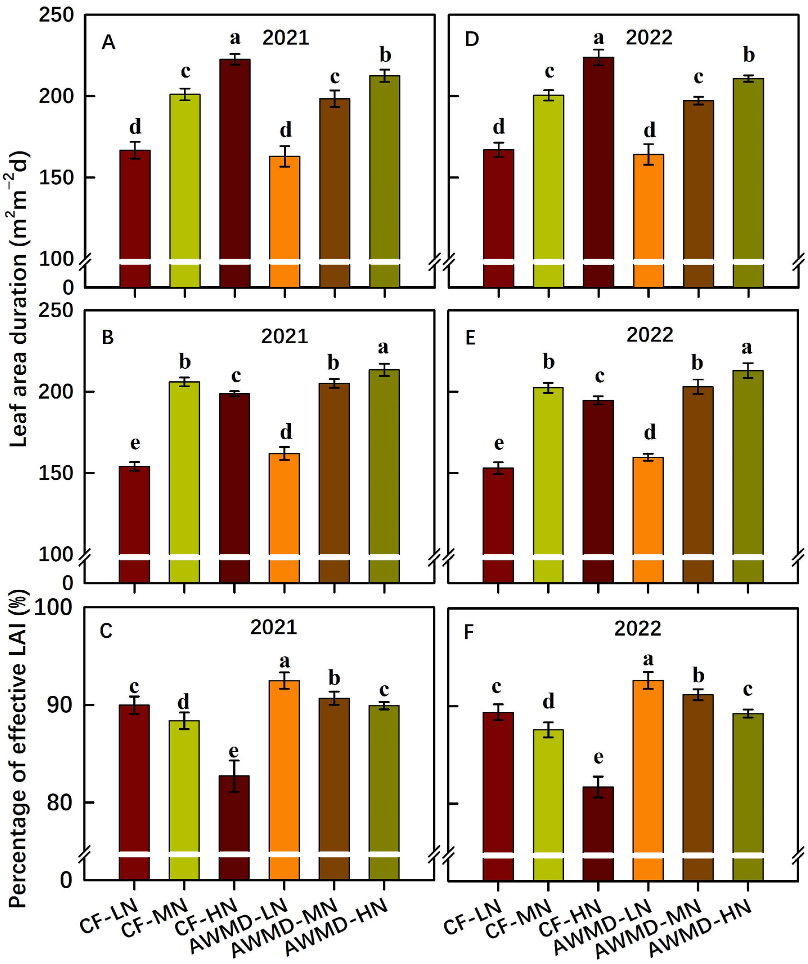

Figure 4. Effects of various nitrogen rates and irrigation regimes on leaf area duration (LAD) from JT to HD (**A**,**D**) and from HD to MA (**B**,**E**), and the percentage of effective LAI (**C**,**F**) of the *Japonica* rice cultivar Jinxiangyu 1. The percentage of effective LAI was defined as the percentage of effective LAI to total LAI at the heading stage. LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

Similar values were observed in effective LAI between the two irrigation regimes or between HN and MN at HD (Figure S3C,G). However, AWMD significantly increased the percentage of effective LAI compared to CF (Figure 4C,F). Additionally, AWMD also significantly increased LAI at MA and LAD during grain filling compared to CF (Figures S3D,H and 4B,E).

3.3. Non-Structural Carbohydrate (NSC) Remobilization

The NSC accumulation in stems increased with higher N input, regardless of irrigation regimes, at HD and MA (Figure 5A,C). When the same amount of N was applied, NSC accumulation in stems was marginally higher at HD, but significantly lower at MA in AWMD compared to CF (Figure 5A,C). The increase in N rates decreased the NSC remobilization within the two irrigation regimes. At the same N rate, AWMD significantly enhanced the NSC remobilization compared to CF (Figure 5B,D).

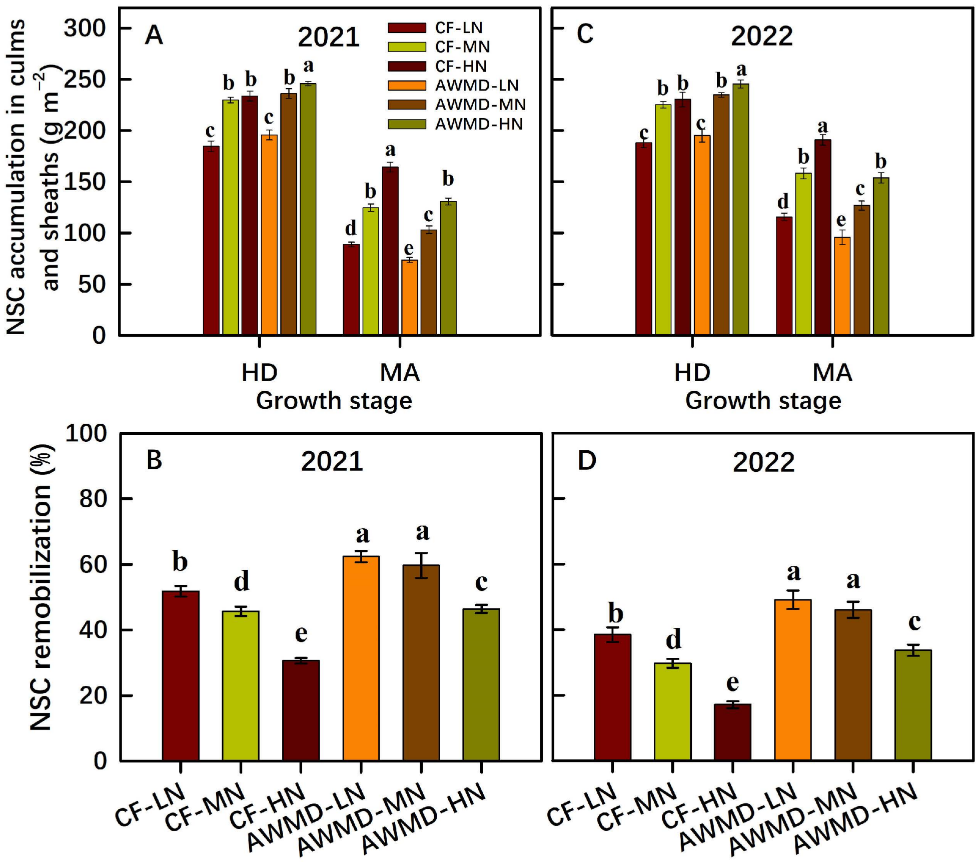

Figure 5. Effects of various nitrogen rates and irrigation regimes on nonstructural carbohydrate (NSC) accumulation in the stems at HD and MA stage (**A**,**C**) and NSC remobilization from the stem to grain during grain filling (**B**,**D**) of the *Japonica* rice cultivar Jinxiangyu 1. LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

3.4. Leaf N Content, Photosynthesis and PNUE

At the same N rate, there was no significant difference in leaf photosynthetic rate during the soil drying period (i.e., MT-D, MG-D) between the CF and AWMD regimes (Figure 6A,D). However, there was a significant increase in leaf photosynthetic rate under HN at the later growth stage (MG-D) in the AWMD regimes compared to the CF regimes. After re-watering (i.e., MT-W, MG-W), AWMD significantly enhanced the photosynthetic rate compared to CF. The photosynthetic rate significantly increased with higher N input regarding the AWMD regime (Figure 6A,D).

The SLN content showed an increase with higher N input in both the soil drying and re-watering stages within the same irrigation regime (Figure 6B,E). Under the same N rate, no significant difference was observed in the SLN content among the irrigation regimes (Figure 6B,E).

Leaf photosynthetic N use efficiency (PNUE) was significantly decreased with higher N input in the same irrigation regime (Figure 6C,F). At the same N rate, AWMD showed no significant difference with CF in the PNUE during the soil drying period. However, after re-watering, AWMD significantly increased the PNUE compared to CF (Figure 6C,F).

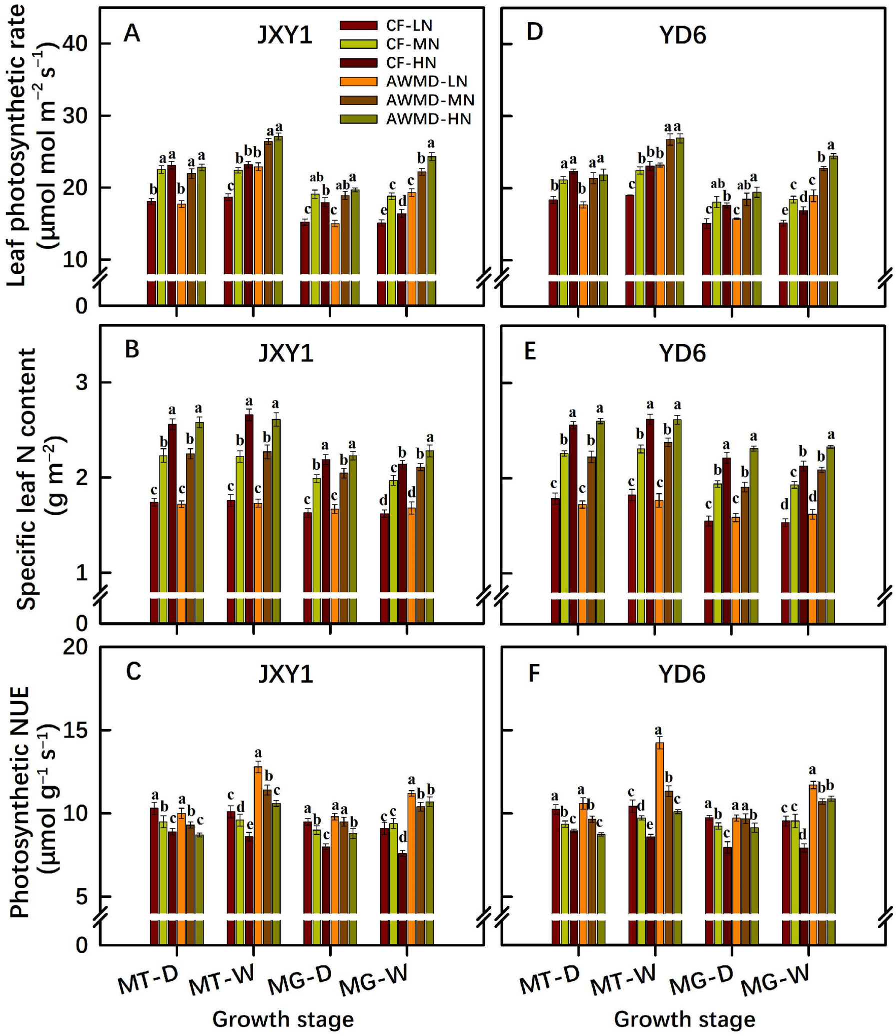

Figure 6. Leaf photosynthetic rate (**A**,**D**), specific leaf N content (**B**,**E**) and photosynthetic NUE (**C**,**F**) of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes. LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. MT, mid-tillering; MG, mid-grain filling; MT-W and MG-W indicate 2 days after the plant was re-watered in AWMD, respectively. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

3.5. Root Dry Weight and ROA

When the same irrigation regime was adopt, the root dry weight at HD showed similar values between MN and HN, but it was lower in LN than in MN or in HN (Figure S4A,B). The root–shoot ratio was decreased significantly with higher N input. When the same amount of N was applied, the irrigation regime had no significant impact on the root dry weight at MN and HN. However, AWMD significantly increased the root dry weight at LN (Figure S4A,B).

Compared to LN, both MN and HN significantly increased root oxidation activity (ROA) in both irrigation regimes (Figure 7A–D). During the soil drying period, similar values of ROA were observed between AWMD and CF regimes at LN and MN. However, AWMD exhibited higher ROA than CF under the same N rate during the re-watering period (Figure 7A–D).

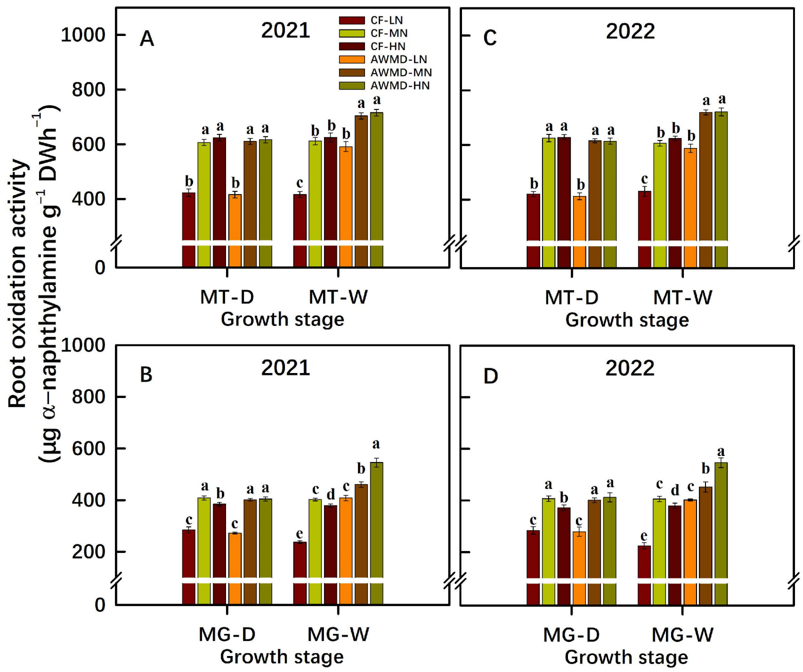

Figure 7. Root oxidation activity (ROA) of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes in 2021 (**A**,**B**) and 2022 (**C**,**D**). LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. MT, mid-tillering; MG, mid-grain filling; MT-W and MG-W indicate 2 days after the plant was re-watered in AWMD, respectively. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

3.6. Activities of the Enzymes Related to N Assimilation in Roots

The activity of glutamate synthase (GOGAT) was elevated significantly with higher N input in both irrigation regimes (Figure S5A,B). At the same N rate, there was no significant difference in GOGAT activity between AWMD and CF during the soil drying period. However, after re-watering, AWMD showed a significant increase in GOGAT activity compared to CF. Similar results were observed for the activities of glutamine synthetase (GS) and nitrate reductase (NR) (Figures S6A,B and S7A,B).

3.7. Soil Available N Content

At the mid-tillering stage, the contents of available N, ammonium-N, and nitrate-N showed a similar changing pattern at LN and MN, they showed the highest values at HN in the CF regime (Figure 8A–F). The contents of available N, ammonium-N, and nitrate-N were comparable between the MN and HN treatments, but were the lowest at LN in the AWMD regimes. Compared to CF, AWMD significantly increased the availability of N (including available N, ammonium-N, and nitrate-N) in the soil at all N rates, particularly at MN (Figure 8A–F).

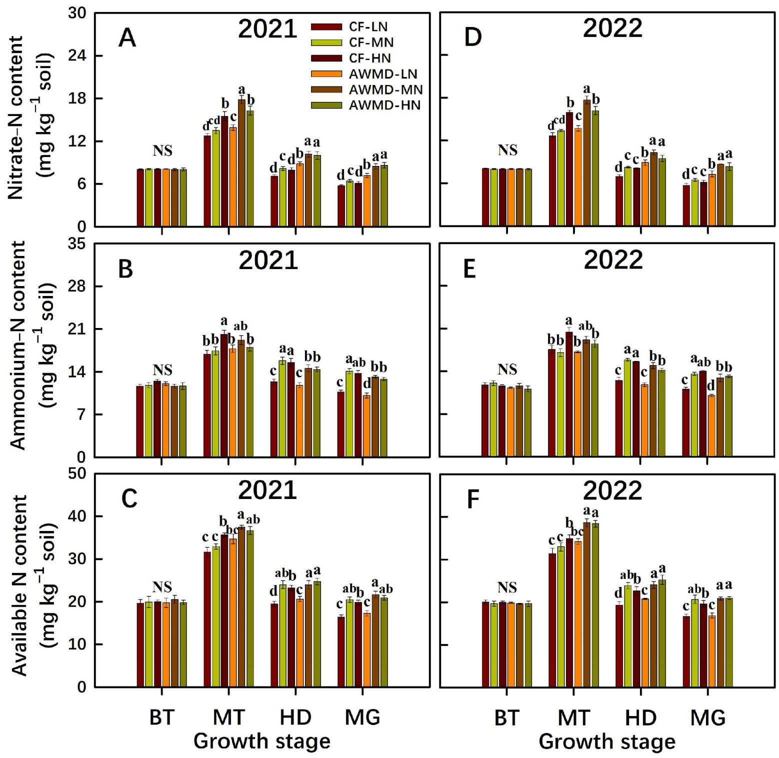

Figure 8. Nitrogen content in the soil (0−20 cm) of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes in 2021 (**A**–**C**) and 2022 (**D**–**F**). LN, MN and HN denote lower amount (120 kg ha^{-1} N), medium amount (240 kg ha^{-1} N) and high amount (360 kg ha^{-1} N) of nitrogen application, respectively, during the growing season. BT, before transplanting; MT, mid-tillering; HD, heading; MG, mid-grain filling. Different letters indicate statistical significance at the p = 0.05 level within the same column and the same year.

4. Discussion

Although there are reports on the effects of AWD and N application management on rice yield and NUE, the interaction effect of irrigation regimes and N rates is still a subject of ongoing debate [13,36,37]. While some studies suggest no significant interaction, implying that irrigation regimes and N rates independently affect these parameters, other observations demonstrate that adopting an AWD regime can have a positive impact on grain yield and NUE, with the degree of improvement depending on the N application rates [32,33]. In this study, we found that the combination of the AWMD regime with a MN rate resulted in the highest grain yield, NUE and WUE among all treatment combinations (Table 1, Figures 1 and 2). These findings indicate a synergistic interaction between the AWMD regime and a MN rate in enhancing grain yield, NUE and WUE.

It is reported that the soil hydrological conditions are a key factor determining the interaction effect of irrigation and N application [33,38]. Our results herein showed that the LWP in rice under the soil drying condition (i.e., SWP = −10 kPa) was greater than −1.2 MPa (Figure S2). It is believed that a mid-day LWP greater than −1.2 MPa in rice would not have a significant impact on the reduction of leaf photosynthetic rate, and consequently, it would not have adverse effects on shoot growth and development [14]. Notably, the leaf photosynthetic rate was enhanced when the plants were re-watered in the AWMD regime (Figure 4A,D). The results indicate that the degree of soil drying at −10 kPa is the safe threshold for AWD, which could simultaneously satisfy plant water demand and stimulate plant growth. Hence, adopting an AWMD regime coupled with a MN rate could achieve the goals of increasing grain yield, NUE and WUE.

What factors contribute to the AWMD regime's ability to achieve higher grain yield, NUE and WUE, particularly at the MN rate? Herein, we summarized two key points as explanations. The first is the "re-watering effect" [32,33]. In the present study, AWMD significantly enhanced the ROA, activities of enzymes related to N assimilation in roots, and photosynthetic rate during the soil drying period when compared with CF. These improved parameters contribute to a greater development of shoots and roots (e.g., shoot and root dry weight, LAI, LAD) (Figures 6, 7 and S2–S5), resulting in higher grain yield and NUE (Table 1 and Figure 1). The second is known as the "compensatory effect" [32,33]. While AWMD resulted in a decrease in the maximum number of tillers and total LAI, it significantly increased the percentage of productive tillers and effective LAI at JT and HD compared to CF (Figures 3 and 4), suggesting that the AWMD regime can reduce canopy redundancy growth, leading to a healthier canopy structure compared to the CF regime. This process is beneficial to canopy photosynthesis [39,40], particularly at the jointing and grain-filling stages, resulting in higher production of photo-assimilates, thereby resulting in a greater number of spikelets per panicle and a higher percentage of filled grains [39,41] (Table 1). Additionally, when compared with CF, AWMD enhanced the NSC remobilization from stems to grains (Figure 6), which could lead to a higher HI (Table 1). It is generally believed that a higher HI was closely related to a higher efficiency of internal N utilization (i.e., IE_N) [42,43]. Meanwhile, the key to enhance crop water use efficiency (WUE) lies in harnessing the biological potential, thereby improving crop yields with limited water inputs [44]. We observed that AWMD significantly reduced irrigation water and improved WUE (Figure 2), indicating that AWMD unleashes the maximum biological potential of rice plants. However, the underlying reasons for this phenomenon remain unclear. Previous research suggests that a substantial reduction in transpiration can be achieved without compromising photosynthesis by appropriately reducing stomatal conductance [44]. It is also widely believed that AWMD can moderately increase the abscisic acid (ABA) level in rice plants, thereby properly decreasing stomatal conductance [45,46]. In summary, these processes can reduce transpirational water loss while promoting matter production and remobilization. Interestingly, in the present study, AWMD exhibited higher photosynthesis and NSC remobilization compared to CF, indicating a greater potential for efficient matter production enabled by AWMD, possibly through the ABA pathway, and ultimately leading to an improved WUE. Further investigation involving ABA is warranted in the future.

It is noteworthy that there is a hypothesis that adopting an AWD irrigation regime may lead to a reduction in total nitrogen uptake in plants, resulting from more N losses through the processes in ammonia volatilization, nitrification, and denitrification [27,28]. In this study, we observed that the AWMD regime, compared to the CF regime, resulted in varying degrees of increased nitrogen accumulation at the maturity stage (Figure 1). This finding indicates that AWMD does not reduce nitrogen uptake but instead has a promoting effect. The underlying reason was the significant improvements in the root morphology of rice plants under the AWMD regime, including ROA, root dry weight, and activities of enzymes related to nitrogen assimilation (Figures 7, S3 and S5–S7). Additionally, the AWMD regime enhanced nitrogen availability in the soil, particularly during the early growth stages (Figure 8C,F). These findings support the notion of increased nitrogen uptake by rice plants and, in conjunction with the influence of AWMD, facilitate higher root and shoot activities, thus further enhancing the utilization of absorbed nitrogen.

In addition, in the present study, the interaction between water and nitrogen led to both positive and negative effects on plant traits, particularly when comparing CF and AWMD. There are reports indicating that ammonium-N tends to be more readily retained when the paddy field is supplemented with CF [47,48]. Such an irrigation regime greatly facilitates the efficient and rapid uptake of nitrogen by the rice roots, which, traditionally, is highly advantageous for rice growth and development, such as more tiller numbers and higher total LAI [49,50]. In contrast, these parameters were all decreased in the AWMD regime (Figures 3 and 4). Fundamentally, it can be attributed to the fact that plant nutrients rely on water as a vehicle for transportation [51]. However, the implementation of AWMD, which significantly reduces water irrigation, consequently limits the vegetative growth [32,33,52]. On the other hand, although AWMD showed a disadvantage in quantitative changes of rice development, it induces qualitative changes within the plants compared to CF, especially the activities of root and shoot (Figures 6 and 7). There are observations indicating an increase in nitrate-N content under the AWD regime, while reports suggest that nitrate-N induces the synthesis of root-derived cytokinins [33,53]. Moreover, our previous work has demonstrated that root-derived cytokinins can improve both root and shoot activities, such as ROA and photosynthesis [54]. Therefore, we speculate that the qualitative changes within rice plants may be activated by root-derived cytokinins through the increase in nitrate-N content in the AWMD regime. However, further investigation is also needed to confirm whether AWMD regulates root-derived cytokinins and promotes plant physiological performance.

Although this study has elucidated the interactive effects of water and nitrogen on grain yield, NUE and WUE in rice, it has been reported that the appropriate nitrogen application rates for attaining a desired grain yield may vary depending on soil moisture conditions, while the optimal soil moisture conditions may differ with different nitrogen application rates [55]. The irrigation regimes and nitrogen rates employed in the present study were relatively limited, thus precluding the determination of the optimal water-nitrogen combination that synergistically enhances grain yield and resource use efficiency. Therefore, further investigations are needed to explore the optimal water–nitrogen coupling model for improving rice grain yield and resource-use efficiency by incorporating a broader range of water and nitrogen treatments.

5. Conclusions

The irrigation regimes strongly interacted with the N rates. The combination of AWMD with a moderate N rate exhibited a synergistic interaction, leading to increases in grain yield, NUE and WUE in Jinxiangyu 1. Greater ROA, higher PNUE, enhanced activities of enzymes related to N assimilation in roots, improved population quality (e.g., higher effective LAI and more productive tillers), more NSC remobilization from stems to grains during grain filling and an increase in available N content contributed to the synergistic interaction.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071729/s1, Figure S1: Temperatures of mean, maximum and minimum (A), and sunshine hours (B) during the rice growing season in Yangzhou in 2021 and 2022; Figure S2: Leaf water potentials of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes in 2021 (A) and 2022 (B); Figure S3: Leaf area index (LAI) of the *Japonica* rice cultivar Jinxiangyu 1 under various N rates and irrigation regimes on jointing leaf area index (LAI) (A,E), heading LAI (B,F), effective LAI (C,G) and maturity LAI (D,H), respectively; Figure S4: Root dry weight (RTW) and root-shoot ratio (RSR) of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes at the heading stage in 2021 (A) and 2022 (B); Figure S5: Activities of glutamate synthase (GOGAT) in the root of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes; Figure S6: Activities of glutamine synthase (GS) in the root of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes; Figure S7: Activities of nitrate reductase (NR) in the root of the *Japonica* rice cultivar Jinxiangyu 1 under various nitrogen rates and irrigation regimes; Table S1: Analysis of variance of grain yield, NUE and WUE.

Author Contributions: Conceptualization, W.Z., J.G., K.Z., J.Z. and J.Y. (Jianchang Yang); validation, J.Y. (Jixiang Yu); investigation, R.X.; data curation, H.H.; writing—original draft preparation, H.H.; writing—review and editing, W.Z., K.Z. and J.Y. (Jianchang Yang); supervision, J.Z. and J.Y. (Jianchang Yang); project administration, J.Y. (Jianchang Yang); funding acquisition, K.Z., J.Z. and J.Y. (Jianchang Yang). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Jiangsu Funding Program for Excellent Postdoctoral Talent (2022ZB618), the Ministry of Agriculture and Rural Affairs of China (FSNK202218080316), the National Natural Science Foundation of China (32071943, 32272198, 31461143015), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD-2020-01), and the Government Funding to the Chinese University of Hong Kong State Key Laboratory of Agrobiotechnology via Innovation and Technology Commission (2022/23–2023/24), National Key Research and Development Program of China (2022YFD23003004).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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

Abbreviations

AWD: alternate wetting and drying; AWMD: alternate wetting and moderate drying; BT: before transplanting; CF: continuously flooded; DAT: days after transplanting; DW: dry weight; GOGAT: glutamate synthase; GS: glutamine synthetase; HD: heading; HI: harvest index; HI_N: N harvest index; HN: high N rate, 360 kg ha−1 N; I: irrigation; IE_N: internal N use efficiency; JT: jointing; LAI: leaf area index; LAD: leaf area duration; LN: low N rate, 120 kg ha−1 N; LWP: leaf water potential; MA: maturity; MG: middle grain-filling stage; MN: medium N rate, 240 kg ha−1 N; MT: mid-tillering; NR: nitrate reductase; NSC: nonstructural carbohydrate; NUE: nitrogen use efficiency; PFP_N: partial factor productivity N; PI: panicle initiation; PNUE: photosynthetic nitrogen use efficiency; ROA: root oxidation activity; RTW: root dry weight; RSR: root-shoot ratio; SLN: specific leaf nitrogen SWP: soil water potential; WUE: water use efficiency.

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