agronomy

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Effects of Water and Nitrogen Management on Root Morphology, Nitrogen Metabolism Enzymes, and Yield of Rice under Drip Irrigation

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Abstract: This paper explores the effects of water and nitrogen management on drip irrigated rice root morphology, nitrogen metabolism and yield, clarifies the relationship between root characteristics and yield formation. Normal irrigation (W_1, 10,200 m^3/hm^2) and limited irrigation (W_2, 8670 m^3/hm^2, 85% of W_1) were set with nitrogen-efficient variety (T-43) and nitrogen-inefficient variety (LX-3) as the materials. Under the condition of a total nitrogen application rate of 300 kg/hm^2, three kinds of nitrogen management methods were applied, N_1: a seedling: tiller: panicle: grain ratio of 30%:50%:13%:7%; N_2: a ratio of 20%:40%:30%:10%; and N_3: 10%:30%:40%:20%. Their effects on root morphology, root architecture, and nitrogen metabolism enzyme activities were studied. The results showed, drip irrigated rice yields were highest under W_1N_2, reaching 9.0 t/hm^2 for T-43 and 7.3 t/hm^2 for LX-3. Compared with W_2, the root length density (RLD), surface area density (SAD), and root volume density (RVD) of finely branched roots, coarsely branched roots and adventitious roots increased by 49.5%, 44.6%, and 46.7%; the RLD, SAD, RVD, and root architecture RLD β values of the 0–30-cm soil layer increased significantly ($p < 0.05$); and the yield and nitrogen partial factor productivity increased by 20.7% and 23.3%, respectively, under W_1. Compared with N_1, RLD, SAD and RVD in 0–10 cm soil layer under N2 increased significantly by 24.8%, 35.6% and 31.4%, and RLDβ decreased significantly ($p < 0.05$); Leaf GS, GOGAT and GDH were increased by 37.9%, 17.0% and 40.9%; all indexes showed a downward trend under N_3. Compared with LX-3, T-43 RLD, SAD, RVD increased significantly ($p < 0.05$), nitrogen metabolism enzyme activity increased, and yield increased by 21.8%. Rational water and nitrogen management can optimize the root growth and distribution characteristics and achieve simultaneous improvement of rice yield, nitrogen absorption, and nitrogen utilization efficiency under drip irrigation.

Keywords: drip irrigation rice; water and nitrogen management; root morphology; nitrogen metabolism enzymes; yield; partial productivity of nitrogen fertilizer

Citation: Tang, Q.; Ma, Y.; Zhao, L.; Song, Z.; Yin, Y.; Wang, G.; Li, Y. Effects of Water and Nitrogen Management on Root Morphology, Nitrogen Metabolism Enzymes, and Yield of Rice under Drip Irrigation. *Agronomy* **2023**, *13*, 1118. https://doi.org/10.3390/agronomy13041118

Academic Editor: Wei Wu

Received: 12 March 2023
Revised: 2 April 2023
Accepted: 11 April 2023
Published: 14 April 2023

1. Introduction

China is one of the world's largest producers of rice (*Oryza sativa* L.), with a planting area of 29.9212 million hectares and a total output of 213 million tons in 2021, accounting for 25.44% and 31.17% of the country's grain sowing area and total output, respectively [1]. Increasing the output of rice is crucial to ensuring the stated goal of "basic self-sufficiency of grains, absolute security of rations, and the rice bowls of the Chinese people must be firmly in our own hands". Xinjiang has unique advantages in producing high-quality rice due to its abundant light and heat resources, with the highest yield reaching 12.5 t/hm^2, achieving high or even super-high yield levels [2]. However, traditional flooding irrigation requires a large amount of agricultural water, leading to low water utilization efficiency [3–5]. At the same time, it can cause a series of environmental problems, including methane (CH_4) emissions from rice fields and nitrogen loss [6,7]. Subsurface drip irrigation technology is an innovative cultivation mode developed in Xinjiang in recent years. This technology changes the traditional planting method of rice and delivers water directly to the roots of crops, slowly and evenly dripping into the root zone soil. This makes the crop demand and water and fertilizer synchronized in terms of quantity and time, effectively improving crop yield and the utilization efficiency of water and fertilizer. Compared with the traditional cultivation method, it saves water by 65% and fertilizer by more than 20% [8–11]. Therefore, it is of great strategic significance to continuously explore the cultivation technology of rice under mulched drip irrigation in arid and semi-arid areas to expand the scope of rice planting, increase rice yield and ensure national food security.

Nitrogen management is an important factor affecting rice growth and yield, and it is a hot issue in drip fertigation management research. At present, the excessive application of nitrogen fertilizer and unreasonable input ratio in rice production are widespread, resulting in low fertilizer utilization rate, excessive soil inorganic nitrogen residue, groundwater nitrate pollution and other problems and increasing ecological and environmental risks [12]. Ding et al. [13] proposed that the yield was the highest when the amount of base fertilizer and tiller fertilizer was reduced by 15%. Zhu et al. [14] found that the yield of rice could reach 9657.7 kg/hm^2 when the ratio of base fertilizer: tiller fertilizer: flower promoting fertilizer: flower preserving fertilizer was 15:30:40:15. Therefore, it is of great significance to further optimize the nitrogen fertilizer management under the drip irrigation cultivation mode and improve the accuracy of fertilizer use to promote the efficiency of rice production.

The root system is the primary organ that senses changes in environmental factors such as water and nutrients. By adjusting their own morphological characteristics, spatial architecture, plasticity, and metabolic enzyme activities, roots can promote the absorption and utilization of water and nutrients by plants and avoid or reduce adverse environmental damage to plants [15–18]. In addition, roots can promote crop growth and development through changes in plasticity, thereby reducing stress and maintaining high productivity. In particular, the distribution of roots can optimize their morphological characteristics according to environmental changes, thereby absorbing more water and nutrients [19,20]. Reasonable water management can increase rice root length and surface area by 17% and 25.2% [21], glutamine synthase (GS) glutamate synthase (GOGAT) activities by 15.2% and 17.1% [22], the yield by 33.0% [23], and the nitrogen partial factor productivity by 26.5% [24]. Yan et al. [25] found that the root growth was the most vigorous when the base: tiller: panicle fertilizer was 3:3:4, which improved the nitrogen accumulation and nitrogen use efficiency of rice, and significantly increased rice yield. Water and nitrogen have optimal coupling effects on rice growth and development in terms of quantity and time. As long as the supply of the two is reasonably matched, a mutual promotion mechanism will occur to achieve the synergistic improvement of crop yield and water and nitrogen use efficiency. Therefore, the water and nutrient absorption and utilization efficiency of crops can be improved by regulating rice roots, so as to achieve water-saving and efficient production and increase rice yield.

Many studies have focused on the effects of water and nitrogen management on the morphological characteristics of rice roots [17,23], the relationship between the rice root system and yield formation [15], the response of different nitrogen-efficiency varieties to nitrogen levels, etc. [26]. There are few reports on the regulatory effects of water and nitrogen management on the root characteristics and yield of rice under drip irrigation. To this end, we used nitrogen-efficient (T-43) and nitrogen-inefficient rice varieties (LX-3) as materials to study their root morphology and distribution, root architecture, nitrogen metabolism enzyme activity, crop yield, and its constituent factors, focusing on the relationship between the characteristics of the rice root system under drip irrigation and the yield and nitrogen partial factor productivity. Our aim was to clarify the regulatory effect of water and nitrogen management on the root growth and yield of rice under drip irrigation and to explore the differential response of nitrogen metabolism enzyme activities to water and nitrogen interactions and their relationship with yield formation. Our findings provide a theoretical basis for rice water-saving cultivation under drip irrigation in Xinjiang and rational application of nitrogen fertilizer.

2. Materials and Methods

2.1. Overview of the Study Area

This experiment was carried out at the Xinjiang Academy of Agricultural Sciences (Shihezi, Xinjiang, 44°18′ N, 86°03′ E) from 2020 to 2021. The study area has a typical arid and semi-arid continental climate, with sparse rainfall, concentrated light and heat, and dry air. The average annual temperature is 6.5–7.2 °C, the average annual rainfall is 115 mm and evaporation is 1942 mm. The soil tested was Calcaric Fluvisols, with pH of 8.37, organic matter content of 1.07%, total nitrogen of 0.68 g/kg, available phosphorus of 36 mg/kg, available potassium of 204 mg/kg, and medium fertility. In 2020 and 2021, the rainfall during the whole growth period of crops is 52.8 mm and 59.0 mm, respectively, with >5 mm effective rainfall for three times, and the average daily maximum temperature was 27.8 °C and 26.0 °C and minimum temperature was 8.2 °C and 7.2 °C, respectively (Figure 1).

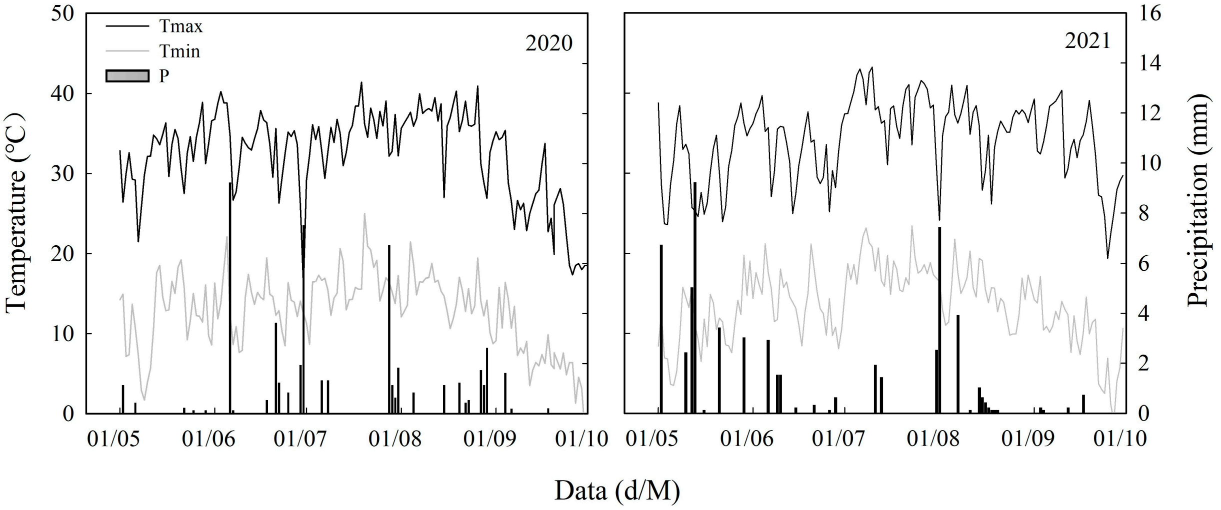

Figure 1. Maximum temperature (T max), minimum temperature (T min) and rainfall (P) during rice growth period (2020–2021).

2.2. Experimental Design

The rice varieties tested were the nitrogen-efficient T-43 and the nitrogen-inefficient LX-3. They were planted in random blocks with a plot area of 60 m^2 and three replicates. Two water treatments were set: normal irrigation (W_1, irrigation amount 10,200 m^3/hm^2) and limited irrigation (W_2, irrigation amount 8670 m^3/hm^2, 85% of W_1). Nitrogen application of 300 kg/hm^2 for high-yielding rice in the region as a standard, three nitrogen management modes were set, namely, N_1 (seedling: tiller: panicle: grain nitrogen = 30%:50%:13%:7%); N_2 (seedling: tiller: panicle: grain nitrogen = 20%:40%:30%:10%); N_3 (seedling: tiller: panicle: grain nitrogen = 10%:30%:40%:20%). Fertilizer application rate P_2O_5 was 150 kg/hm^2, K_2O was 90 kg/hm^2, fertilizer varieties were urea (N 46%), monoammonium phosphate (N 12%, P_2O_5 60%), potassium sulfate (K_2O 50%), all water and fertilizer integrated topdressing. Dissolve the fertilizer in a 25 L fertilization tank at one time, drip 2 h water before fertilization, open the fertilization device, and end fertilization 30 min before stopping water. In order to ensure the uniformity of irrigation, the inner patch drip irrigation belt was adopted, with nominal diameter of Φ16 mm, dripper spacing of 30 cm and dripper flow of

2.1 L/h. Irrigation water is well water drip irrigation, salinity 2.53 g/L, pH 7.2, chloride 363 mg/L, sulfide 909 mg/L, irrigation and fertilization are shown in Tables 1 and 2.

Table 1. Drip irrigation cycle and irrigation amount under membrane.

Growth Stage	**Date (D/M/Y)**	**Over Time/Days**	**Irrigation Frequency**	**Irrigation Times**	**Irrigation Quantity (m^3/hm^2)**	
					W1	**W2**
Seeding stage	28/04–27/05/2018 05/01–05/27/2019	30 27	One watering	1	450	450.0
Seeding—jointing stage	28/05–06/07	40	Every 3 days	14	164.4	195.0
Jointin—15 days before maturity	07/07–14/09	7	Every 3 days	35	164.4	195.0
5 days before maturation—Harvest	15/09–30/09	15				
Total/(m^3/hm^2)	28/04–30/09/2018 01/05–30/09/2019	155 152		50	8670.0	10,200.0

The planting mode of 1 film, 2 pipes and 8 rows were adopted, that is, 2 drip irrigation belts were laid, 8 rows of rice were planted, and the plant spacing was 10 cm. The sowing depth is 2.5~3 cm, the thickness of covering soil is 1~1.5 cm, and the number of grains per hole is 8~10. Pipe laying, film laying, sowing and soil covering are completed at one time; the cultivation mode was 10 cm + 26 cm + 10 cm (Figure 2). The water and pests were strictly monitored during the whole growth period, and the remaining management was consistent with field production. In 2020, it was sown on 28 April, and in 2021, it was sown on 1 May. Both years were harvested on 30 September.

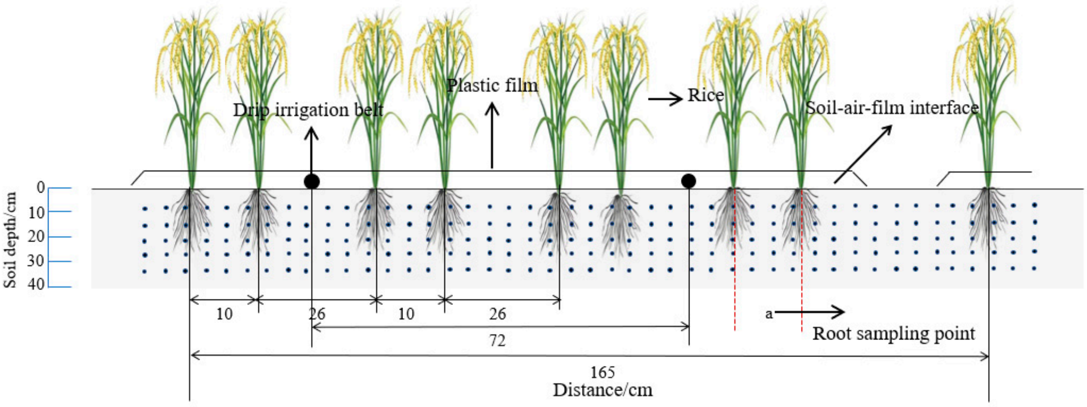

Figure 2. Rice planting pattern and root sampling schematic diagram under mulched drip irrigation.

Table 2. N rate for drip irrigation rice under plastic film mulching.

Treatments	Irrigation Amount (m^3/hm^2)	Nitrogen Fertilization Amount (kg/hm^2)	Seeding: Tiller: Spike: Grain Nitrogen	N Fertilization Rate (kg/hm^2)							
				Seeding Nitrogen		Tiller Nitrogen			Spike Nitrogen		Grain Nitrogen
				20 Days after Sowing	28 Days after Sowing	36 Days after Sowing	45 Days after Sowing	54 Days after Sowing	78 Days after Sowing	88 Days after Sowing	95 Days after Sowing
W_1N_1	10,200	300	30%:50%:13%:7%	45.0	45.0	50.0	50.0	50.0	19.5	19.5	21.0
W_1N_2			20%:40%:30%:10%	30.0	30.0	40.0	40.0	40.0	45.0	45.0	30.0
W_1N_3			10%:30%:40%:20%	15.0	15.0	30.0	30.0	30.0	60.0	60.0	60.0
W_2N_1	8670		30%:50%:13%:7%	45.0	45.0	50.0	50.0	50.0	19.5	19.5	21.0
W_2N_2			20%:40%:30%:10%	30.0	30.0	40.0	40.0	40.0	45.0	45.0	30.0
W_2N_3			10%:30%:40%:20%	15.0	15.0	30.0	30.0	30.0	60.0	60.0	60.0

2.3. Determination Indicators and Methods

2.3.1. Morphological Parameters of the Root System

At the heading stage and 20 days after heading, four representative rice plants were selected, and the rice root system was obtained in four layers (0–10, 10–20, 20–30, 30–40 cm) by the root drilling method (10 cm in height, 10 cm in diameter) in the middle position. The root system was flattened in a Plexiglas root box with a 2/3 water layer in the laboratory, the position of was adjusted using the tweezers to avoid overlapping, and the EpsonV800 scanner (Epson Expression, Nagano, Japan) scanned as an image file of 300 dpi. WinRHIZO Pro software was used (Regent, Vancouver, BC, Canada) to analyses the morphological parameters of rice adventitious roots, coarsely branched roots, and finely branched roots. Finely branched roots ($RD \leq 0.3$ mm), coarsely branched roots ($0.3 \text{ mm} < RD \leq 0.9$ mm), and adventitious roots ($RD > 0.9$ mm) were defined with reference to the method of Li Na [26]. The formulas for calculating root length density (RLD), surface area density (SAD), and root volume density (RVD) were as follows:

$$\text{RLD} = \text{RL}/\text{SV} \tag{1}$$

$$\text{SAD} = \text{RSA}/\text{SV} \tag{2}$$

$$\text{RVD} = \text{RV}/\text{SV} \tag{3}$$

In the formula: RLD: Root length density (cm/dm^3), RL: Root length (cm), SV: Soil volume (m^3), SAD: Root surface area density (cm^2/dm^3), RSA: Root surface area (cm^2), RVD: Root volume density (cm^3/dm^3), RV: Root volume (cm^3). Root length, root surface area and root volume were directly measured by software. The soil volume of each depth was 0.785 dm^3.

2.3.2. Root System Architectural Parameters

Using the asymptotic equation model proposed by M.R. Gale and D.F. Grigal [27], the vertical spatial distribution model of RLD, SAD, and RVD was established as:

$$Y = 1 - \beta^{D} \tag{4}$$

where D is the soil depth (cm); Y is the proportion of the morphological indices from the surface to the soil layer D in the total; and β is the depth coefficient, where the smaller β is, the closer the root distribution is to the soil surface, and the larger β is, the deeper the root distribution. The β value was calculated when D was 20 cm.

2.3.3. Content of Nitrogen Metabolism Enzymes

While collecting rice roots at the heading stage and 20 d after heading, another root and flag leaf sample was collected and immediately frozen in liquid nitrogen for the determination of related enzyme activities. Glutamine synthetase (GS) and Glutamate synthase (GOGAT) were measured by the spectrophotometric method with a Solarbio kit. A change of 0.01 in the absorbance at 540 nm/min/g tissue in 1 mL was defined as one unit of enzyme activity (U·g) of GS. One unit of activity of GOGAT (U·g) was that which produced 1 nmol of NADH/g tissue/min. Glutamate dehydrogenase (GDH) activity was determined by UV spectrophotometry, and one unit of enzyme activity (nmol/min/g) was that which consumed 1 nmol NADH/min/g tissue.

2.3.4. Seed Test and Yield

At the mature stage, nine holes of rice were taken from each plot to investigate the panicle numbers, spikelet per panicle, filled grain rate, and 1000-grain weight, and the yield was calculated.

2.4. Data Processing and Statistical Methods

In order to clarify the effects of water and nitrogen management on root morphology, nitrogen metabolism enzyme activity and yield of drip irrigation rice. Microsoft Excel 2016 was used for data collation and calculation, all results were expressed as the mean ± standard error of three repeated measurements. Two-factor randomized block analysis (ANOVA) was performed using SPSS 26.0 (SPSS Inc., Armonk, CA, USA) software [28]. and the mean value was compared by the minimum significant difference of $p < 0.05$ (LSD 0.05). Sigma Plot12.5 (SYSTAT, San Jose, CA, USA) software was used to draw charts [29], and redundancy analysis (RDA) was performed using Canoco 5.0 software [30].

3. Results

3.1. Effects of Water and Nitrogen Management on Rice Yield, Composition Factors and Nitrogen Partial Factor Productivity under Drip Irrigation

Compared with LX-3, the average yield of T-43 increased by 21.1% under W_1 and 22.5% under W_2 in the two years together (Table 3). Between the water treatments, yield and nitrogen partial factor productivity under the W_2 decreased by 20.7% and 23.3%, respectively ($p < 0.05$), compared with those of W_1. During nitrogen management, compared with N_1, yields of W_1 T-43 under N_2 and N_3 increased by 49.2% and 17.3%, and those of LX-3 increased by 40.6% and 18.6% ($p < 0.05$). The yields of W_2 T-43 under N_2 and N_3 increased by 49.5% and 19.9% ($p < 0.05$), and those of LX-3 increased by 19.1% and 13.2%. Both T-43 and LX-3 showed their highest yields under W_1 N_2, followed by W_1 N_3. The panicle number, spikelet per panicle, and 1000-grain weight of T-43 decreased with decreasing water content by 5.5%, 6.3%, and 8.9%, respectively; the trend of LX-3 was the same. Compared with N_1, the panicle number of N_2 increased by 20.7% ($p < 0.05$), though the filled grain rate and 1000-grain weight were not significantly different. Those of N_3 significantly decreased. ANOVA showed that water and nitrogen management and their interaction had significant or extremely significant effects on panicle number, yield, and nitrogen partial factor productivity, but their interaction had no significant effect on spikelet per panicle, filled grain rate, or 1000-grain weight.

Table 3. Effects of water and nitrogen management on rice yield and its components under drip irrigation.

Year	Cultivars	Treatmens	Panicle Numbers × 10^4/hm^2	Spikelet per Panicle Particle/Spike	Filled Grain Rate %	1000-Grain Weight g	Yield t/hm^2	PFP kg/kg
2020	T-43	W_1N_1	430.5 ± 16.7^{c}	88.6 ± 4.2^{bc}	79.8 ± 2.0^{a}	23.3 ± 1.0^{ab}	6.1 ± 0.4^{c}	20.2 ± 1.2^{bc}
		W_1N_2	543.3 ± 8.4^{a}	101.5 ± 4.6^{a}	81.9 ± 2.3^{a}	23.1 ± 1.3^{ab}	8.9 ± 0.9^{a}	29.8 ± 3.2^{a}
		W_1N_3	501.1 ± 6.4^{b}	83.8 ± 3.4^{c}	81.1 ± 1.3^{a}	24.4 ± 0.3^{a}	7.1 ± 0.2^{b}	23.6 ± 0.7^{b}
		W_2N_1	423.7 ± 19.3^{c}	83.1 ± 0.5^{c}	77.0 ± 5.6^{a}	21.6 ± 0.7^{bc}	5.0 ± 0.4^{c}	16.7 ± 1.2^{c}
		W_2N_2	514.8 ± 12.9^{b}	91.0 ± 2.9^{b}	79.2 ± 1.5^{a}	21.4 ± 0.1^{c}	7.5 ± 0.2^{b}	23.2 ± 1.0^{b}
		W_2N_3	493.2 ± 11.1^{b}	75.8 ± 4.5^{d}	80.0 ± 1.0^{a}	20.8 ± 0.7^{c}	5.7 ± 0.2^{c}	17.4 ± 1.9^{c}
	LX-3	W_1N_1	437.3 ± 3.8^{d}	69.0 ± 2.9^{bc}	72.5 ± 1.0^{bc}	26.4 ± 0.1^{ab}	4.9 ± 0.1^{d}	16.4 ± 0.3^{de}
		W_1N_2	563.3 ± 4.7^{a}	69.6 ± 2.5^{bc}	78.2 ± 3.4^{a}	26.8 ± 0.7^{a}	7.0 ± 0.2^{a}	23.4 ± 0.8^{a}
		W_1N_3	486.7 ± 18.9^{c}	66.6 ± 1.6^{c}	75.9 ± 2.6^{ab}	26.1 ± 0.2^{ab}	5.5 ± 0.2^{c}	18.3 ± 0.6^{c}
		W_2N_1	453.3 ± 18.9^{d}	72.4 ± 1.8^{ab}	69.9 ± 0.6^{c}	24.7 ± 0.0^{c}	4.8 ± 0.0^{d}	16.2 ± 0.2 e
		W_2N_2	523.3 ± 4.7^{b}	72.6 ± 1.7^{ab}	71.2 ± 0.2^{bc}	25.4 ± 0.5^{bc}	5.9 ± 0.2^{b}	19.6 ± 0.6^{b}
		W_2N_3	484.4 ± 6.3^{c}	69.5 ± 3.7^{a}	68.8 ± 3.1^{c}	24.7 ± 0.5^{c}	4.9 ± 0.1^{c}	16.3 ± 0.4^{cd}
2021	T-43	W_1N_1	384.7 ± 19.6^{cd}	94.8 ± 2.0^{c}	82.5 ± 5.5^{ab}	23.1 ± 0.2^{a}	5.9 ± 0.2^{cd}	19.8 ± 0.7^{cd}
		W_1N_2	479.6 ± 16.9^{a}	105.0 ± 0.7^{b}	90.8 ± 1.5^{a}	22.9 ± 0.5^{a}	9.0 ± 0.3^{a}	29.9 ± 0.9^{a}
		W_1N_3	455.8 ± 25.6^{ab}	102.1 ± 4.2^{b}	81.1 ± 2.1^{ab}	21.5 ± 0.4^{bc}	7.7 ± 0.8^{b}	26.1 ± 1.1^{b}
		W_2N_1	369.0 ± 17.7^{d}	86.8 ± 1.2^{d}	82.5 ± 6.3^{ab}	22.6 ± 0.9^{ab}	5.1 ± 0.7^{d}	17.0 ± 2.3^{d}
		W_2N_2	427.1 ± 20.0^{b}	112.4 ± 0.0^{a}	87.0 ± 0.0^{ab}	21.2 ± 0.0^{c}	7.6 ± 0.4^{b}	25.2 ± 1.2^{b}
		W_2N_3	423.0 ± 14.0^{bc}	92.8 ± 3.5^{c}	85.1 ± 2.9^{ab}	22.5 ± 0.5^{ab}	6.4 ± 0.2^{c}	21.4 ± 0.7^{c}
	LX-3	W_1N_1	424.4 ± 5.0^{cd}	70.8 ± 1.5^{ab}	79.6 ± 1.5^{ab}	26.9 ± 0.8^{a}	5.5 ± 0.4^{bc}	19.5 ± 1.3^{bc}
		W_1N_2	508.8 ± 10.6^{a}	76.1 ± 5.0^{a}	82.1 ± 0.8^{a}	27.9 ± 0.9^{a}	7.6 ± 0.5^{a}	25.5 ± 0.6^{a}
		W_1N_3	471.5 ± 30.2^{ab}	71.4 ± 4^{ab}	79.3 ± 2.3^{ab}	27.9 ± 0.9^{a}	6.4 ± 0.5^{b}	21.4 ± 1.6^{b}
		W_2N_1	397.7 ± 20.9^{d}	65.9 ± 1.3^{b}	79.6 ± 4.4^{ab}	26.1 ± 0.7^{a}	4.6 ± 0.4^{c}	15.5 ± 1.4^{d}

Table 3. *Cont.*

Year	Cultivars	Treatmens	Panicle Numbers × 10^4/hm^2	Spikelet per Panicle Particle/Spike	Filled Grain Rate %	1000-Grain Weight g	Yield t/hm^2	PFP kg/kg
		W_2N_2	450.3 ± 5.9 ^{bc}	65.3 ± 2.4 ^{b}	81.2 ± 4.0 ^{ab}	25.9 ± 1.4 ^{a}	5.3 ± 0.6 ^{bc}	17.6 ± 2.0 ^{cd}
		W_2N_3	433.9 ± 16.0 ^{bcd}	65.4 ± 7.4 ^{b}	75.4 ± 1.1 ^{b}	26.3 ± 0.8 ^{a}	4.8 ± 0.3 ^{c}	16.0 ± 1.0 ^{d}
				F-value				
		W	23.49 **	105.64 **	18.09 **	49.75 **	103.53 **	103.41 **
		N	99.51 **	241.26 **	5.59 *	0.002 ^{ns}	89.73 **	89.41 **
		W × N	4.57 *	0.09 ^{ns}	0.87 ^{ns}	1.11 ^{ns}	5.08 *	5.07 *

The same column data (mean ± standard deviation) followed by the same letter indicates no significant difference at the 5% level. * and ** indicated significant differences at 0.05 and 0.01 levels, respectively, and ^{ns} indicated no significant difference at 0.05 level. T-43: nitrogen-efficient variety, LX-3: nitrogen-inefficient variety; W and N represent water and nitrogen management, respectively. W_1: 10,200 m^3/hm^2, W_2: 8670 m^3/hm^2; N_1 (seedling: tiller: panicle: grain fertilizer 30%:50%:13%:7%), N_2 (seedling: tiller: panicle: grain fertilizer 20%:40%:30%:10%), N_3 (seedling: tiller: panicle: grain fertilizer 10%:30%:40%:20%); HS: heading stage, 20 DAH: 20 days after heading; PFP: Nitrogen partial factor productivity.

3.2. Effects of Water and Nitrogen Management on Morphological Parameters of Rice Roots under Drip Irrigation

ANOVA showed that water and nitrogen management and their interaction had significant or extremely significant effects on RLD of each branch root of rice, but water had no significant effect on RVD of coarse branch roots, and nitrogen management had no significant effect on SAD of coarse branch roots and adventitious roots (Table 4).

Table 4. Analysis of variance for the morphology of the rice root system under drip irrigation in response to water and nitrogen management.

	Fine Branch Root (D ≤ 0.3 mm)			Coarse Branch Root (0.3 mm < D ≤ 0.9 mm)			Adventitious Root (D > 0.9 mm)		
	RLD	SAD	RVD	RLD	SAD	RVD	RLD	SAD	RVD
W	145.3 **	19..5 **	4.3 *	93.1 **	7.2 *	1.3 ^{ns}	144.5 **	41.6 **	38.6 **
N	38.2 **	11.8 **	6.35 *	23.1 **	2.6 ^{ns}	10.1 **	46.7 **	60.7 **	3.4 ^{ns}
W × N	36.9 **	17.35 *	18.05 **	7.7 *	28.2 **	7.9 *	22.8 **	15.1 **	6.3 *

* and ** indicated significant differences at 0.05 and 0.01 levels, respectively, and ^{ns} indicated no significant difference at 0.05 level; RLD: Root length density, SAD: Surface area density, RVD: Root Volume density; W: water treatment, N: nitrogen management, W × N: Interaction of water and nitrogen transport.

The RLD of finely branched roots was the highest, ranging from 48.15~59.62%, followed by that of coarsely branched roots, ranging from 33.9~43.26%, and that of the adventitious root was less than 10%. The ratio of SAD to RVD was the highest in coarsely branched roots, ranging from 50.47~72.34%; the ratio in finely branched roots was less than 20% (Figure 3). The effects of water and nitrogen management on the RLD, SAD, and RVD of rice roots at all levels were significantly different ($p < 0.05$). Comparing water treatments, the RLD, SAD, and RVD of finely branched roots, coarsely branched roots, and adventitious roots under W_2 were 49.5%, 44.6%, and 46.7% lower than those under W_1, respectively (all $p < 0.05$). Compared with N_1, the RLD, SAD, and RVD of coarsely branched roots and adventitious roots under N_2 were 28.8%, 33.2%, and 51.3% higher, respectively ($p < 0.05$), while those of N_3 were significantly lower. Compared with T-43, the SAD, RVD, and RVD of the adventitious roots of LX-3 increased by 18.4%, 22.0%, and 26.7%, respectively, while the RLD, SAD, and RVD of the coarsely branched roots decreased by 19.6%, 23.0%, and 30.2%, and the SAD of adventitious root decreased by 19.3%.

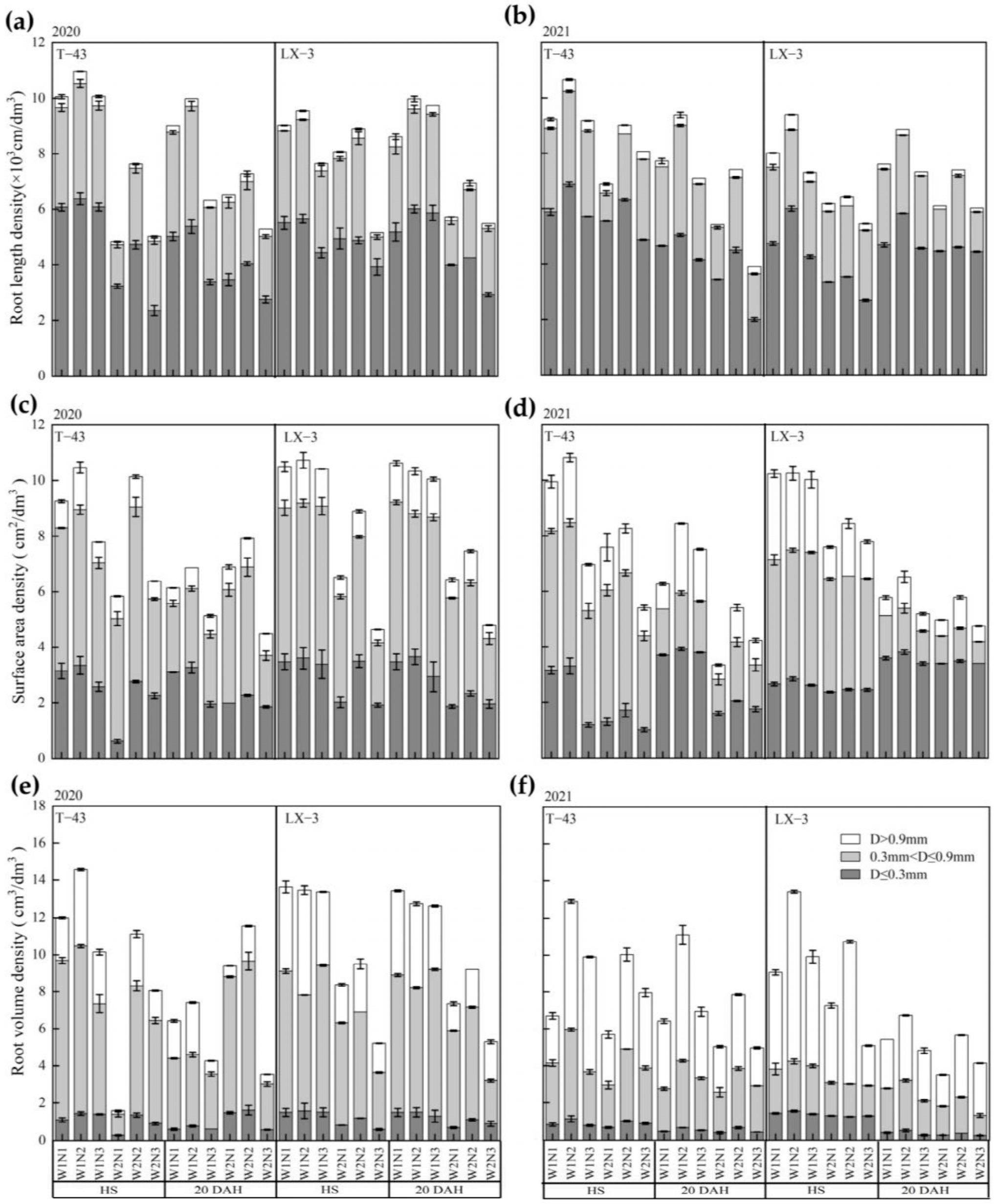

Figure 3. Effects of Water and Nitrogen Application on Root Morphological Indexes of Rice under Drip Irrigation. T-43: nitrogen-efficient variety, LX-3: nitrogen-inefficient variety; W and N represent water and nitrogen management, respectively, W_1: 10,200 m^3/hm^2, W_2: 8670 m^3/hm^2; N_1 (seedling: tiller: panicle: grain fertilizer 30%:50%:13%:7%), N_2 (seedling: tiller: panicle: grain fertilizer 20%:40%:30%:10%), N_3 (seedling: tiller: panicle: grain fertilizer 10%:30%:40%:20%); HS: heading stage, 20 DAH: 20 days after heading.

3.3. Influence of Water and Nitrogen Management on the Root Architecture Index of Rice under Drip Irrigation

3.3.1. Effects of Water and Nitrogen Management on the Vertical Distribution of Rice Roots under Drip Irrigation

The indicators of the rice root system were mainly distributed in the 0–10-cm soil layer and showed a decreasing trend in its vertical distribution, and the indicators in the soil layer below 10 cm decreased rapidly (Figure 4). The RLD, SAD, and RVD of the 0–10-cm soil layer accounted for 47.4~60.4%, 50.3~61.4%, and 18.2~23.9% of the total root system, respectively. Compared with W_1, the RLD, SAD, and RVD under W_2 were significantly decreased (except in T-43 in 2021), with average decreases of 40.3%, 50.3%, and 46.2%, respectively ($p < 0.05$). In the 10–20-cm soil layer, compared with W_1 in 2021, RLD and RVD under W_2 increased by 38.6% and 40.0% ($p < 0.05$), but all indicators decreased significantly in 2022. In the 20–30-cm soil layer, T-43 RLD, SAD, and RVD significantly increased, and in LX-3 they decreased significantly. In the 30–40-cm soil layer, RLD, SAD, and RVD accounted for 6.4~9.1%, 9.1~9.6%, and 8.0~9.1% of the total root system, respectively, and under W_2 they were 54.5%, 45.4%, and 70.6% greater than they were under W_1 ($p < 0.05$). Compared with N_1, the RLD, SAD, and RVD of the 0–10-cm soil layer under N_2 increased by 24.8%, 35.6%, and 31.4%, respectively ($p < 0.05$). Those of the 10–20-cm and 20–30-cm soil layers under N_2 were significantly lower than N_1; each index of T-43 decreased significantly under W_1 and increased significantly under W_2; each index of LX-3 under N_2 increased significantly over N_1; and each index of the 30–40-cm soil layer increased significantly. All the indices of each soil layer under N_3 decreased significantly.

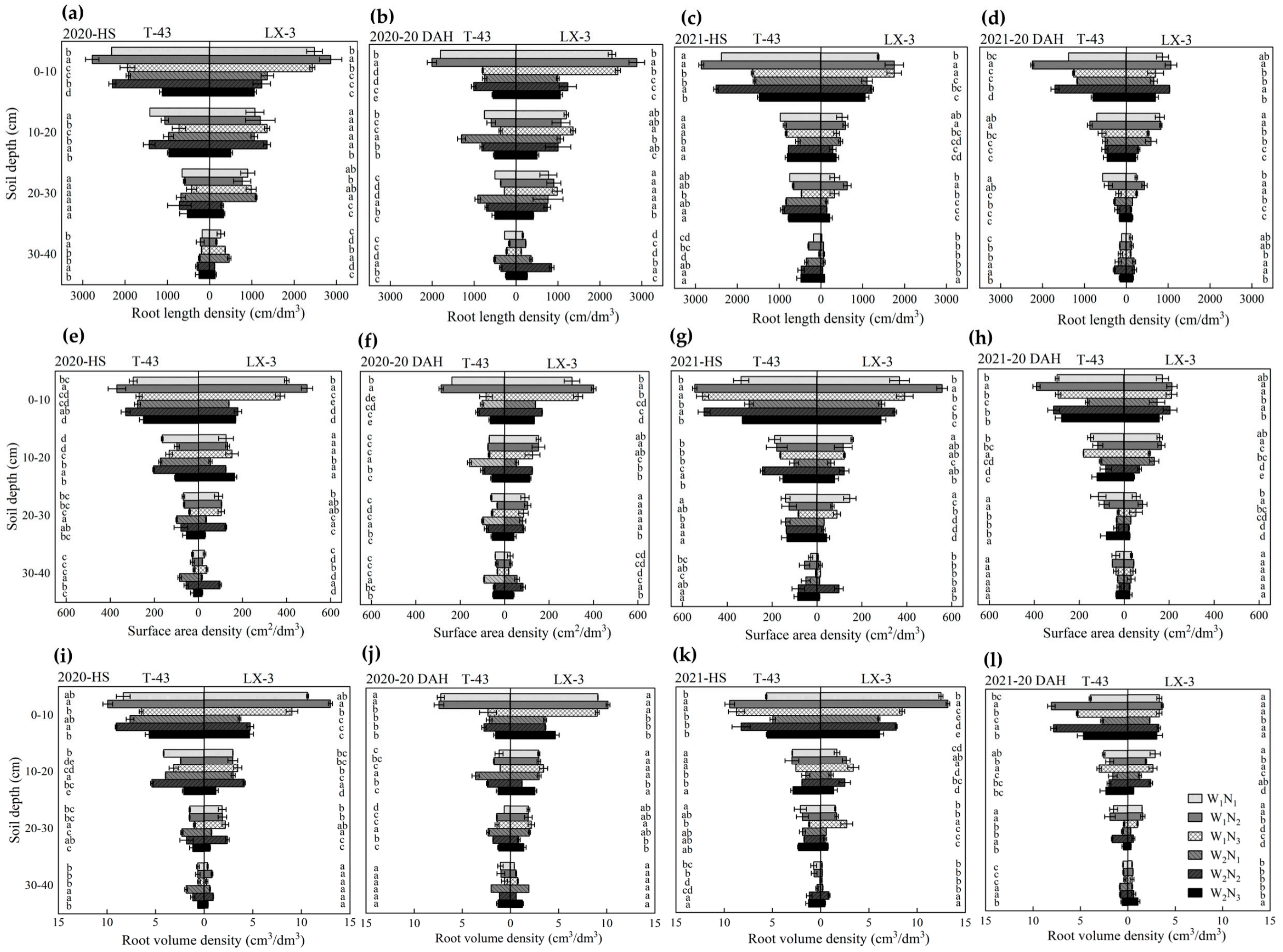

Figure 4. Effects of water and nitrogen management on spatial distribution characteristics of rice roots under drip irrigation. T-43: nitrogen-efficient variety, LX-3: nitrogen-inefficient variety; W and N represent water and nitrogen management, respectively. W_1: 10,200m^3/hm^2, W_2: 8670 m^3/hm^2; N_1 (seedling: tiller: panicle: grain fertilizer 30%:50%:13%:7%), N_2 (seedling: tiller: panicle: grain fertilizer 20%:40%:30%:10%), N_3 (seedling: tiller: panicle: grain fertilizer 10%:30%:40%:20%); HS: heading stage, 20 DAH: 20 days after heading.

3.3.2. Effects of Water and Nitrogen Management on the β Value of the Rice Root Architecture Parameter under Drip Irrigation

Water and nitrogen management had significant effects on the rice architecture parameter β under drip irrigation ($p < 0.05$). Compared with W1, the RLD β value of T-43 under W2 decreased by 0.6%; the RVD β value of HS decreased by 1.8%, and that at 20 DAH increased by 2.1% (Table 5). There was no significant difference in SAD β value ($p < 0.05$). Compared with W1, LX-3 under W2 showed a 2.3% decrease in RLD β value (except at 20 DAH in 2021) and a 2.5% increase in RVD. Comparing RLD between nitrogen management, N2 yielded a lower RLD than N1 and N3 by 2.1% and 1.9%, respectively ($p < 0.05$). The change rules of SAD β, RVD β, and RLD β were the same. The rice roots were distributed close to the soil surface when nitrogen application was postponed, and if the nitrogen application was postponed too long, nitrogen tended to be distributed in the deep layer. ANOVA showed that water had a significant effect on SAD β of HS rice roots ($p < 0.01$); nitrogen management had a significant effect on RLD β, SAD β, and RVD β ($p < 0.05$); and their interaction had a significant effect on RLD β and RVD β ($p < 0.05$). There was no significant difference in SAD β.

Table 5. Effects of water and nitrogen management on root architecture parameter β of rice under drip irrigation.

Year	**Cultivars**	**Treatments**	**Root Length Density β Value**		**Root Surface Area Density β Value**		**Root Volume Density β Value**	
			HS	**20 DAH**	**HS**	**20 DAH**	**HS**	**20 DAH**
2020	T-43	W_1N_1	0.923 ± 0.005^{bc}	0.947 ± 0.001^{b}	0.963 ± 0.001^{a}	0.960 ± 0.000^{b}	0.910 ± 0.006^{bc}	0.946 ± 0.003^{abc}
		W_1N_2	0.922 ± 0.000^{bc}	0.932 ± 0.000 e	0.963 ± 0.002^{a}	0.961 ± 0.000^{b}	0.909 ± 0.006^{bc}	0.950 ± 0.002^{ab}
		W_1N_3	0.932 ± 0.005^{ab}	0.941 ± 0.003^{c}	0.963 ± 0.001^{a}	0.961 ± 0.001^{b}	0.910 ± 0.017^{bc}	0.960 ± 0.006^{a}
		W_2N_1	0.921 ± 0.003^{bc}	0.937 ± 0.003^{d}	0.963 ± 0.002^{a}	0.962 ± 0.000^{b}	0.933 ± 0.002^{a}	0.939 ± 0.003^{bc}
		W_2N_2	0.912 ± 0.014^{c}	0.944 ± 0.001^{bc}	0.963 ± 0.000^{a}	0.962 ± 0.000^{ab}	0.904 ± 0.007^{c}	0.933 ± 0.017^{bc}
		W_2N_3	0.943 ± 0.003^{a}	0.956 ± 0.003^{a}	0.964 ± 0.000^{a}	0.965 ± 0.002^{ab}	0.924 ± 0.014^{ab}	0.953 ± 0.007^{a}
	LX-3	W_1N_1	0.932 ± 0.006^{b}	0.926 ± 0.011^{c}	0.961 ± 0.002^{a}	0.961 ± 0.002^{b}	0.910 ± 0.009^{bc}	0.911 ± 0.005^{b}
		W_1N_2	0.919 ± 0.003^{c}	0.926 ± 0.007^{c}	0.964 ± 0.001^{a}	0.964 ± 0.001^{ab}	0.901 ± 0.014^{cd}	0.911 ± 0.010^{b}
		W_1N_3	0.935 ± 0.004^{b}	0.935 ± 0.002^{bc}	0.961 ± 0.000^{a}	0.961 ± 0.000^{b}	0.921 ± 0.009^{b}	0.921 ± 0.010^{b}
		W_2N_1	0.929 ± 0.003^{b}	0.952 ± 0.015^{a}	0.963 ± 0.004^{a}	0.963 ± 0.003^{ab}	0.942 ± 0.002^{a}	0.951 ± 0.003^{a}
		W_2N_2	0.954 ± 0.000^{a}	0.945 ± 0.005^{ab}	0.964 ± 0.001^{a}	0.963 ± 0.002^{ab}	0.886 ± 0.015^{d}	0.929 ± 0.003^{ab}
		W_2N_3	0.906 ± 0.008^{d}	0.941 ± 0.002^{abc}	0.962 ± 0.002^{a}	0.965 ± 0.000^{ab}	0.919 ± 0.003^{b}	0.919 ± 0.037^{b}
2021	T-43	W_1N_1	0.938 ± 0.012^{ab}	0.935 ± 0.003^{a}	0.920 ± 0.004^{c}	0.926 ± 0.003^{ab}	0.912 ± 0.008^{b}	0.939 ± 0.008^{a}
		W_1N_2	0.904 ± 0.005^{c}	0.895 ± 0.004^{c}	0.897 ± 0.003^{d}	0.896 ± 0.003^{c}	0.890 ± 0.001^{c}	0.919 ± 0.006^{b}
		W_1N_3	0.927 ± 0.000^{b}	0.937 ± 0.010^{a}	0.934 ± 0.010^{ab}	0.933 ± 0.006^{a}	0.929 ± 0.013^{a}	0.894 ± 0.003^{c}
		W_2N_1	0.949 ± 0.008^{a}	0.919 ± 0.001^{b}	0.939 ± 0.006^{ab}	0.925 ± 0.014^{ab}	0.941 ± 0.003^{a}	0.927 ± 0.008^{b}
		W_2N_2	0.934 ± 0.009^{ab}	0.896 ± 0.006^{c}	0.928 ± 0.006^{bc}	0.900 ± 0.004^{c}	0.926 ± 0.000^{ab}	0.873 ± 0.003^{d}
		W_2N_3	0.946 ± 0.012^{a}	0.921 ± 0.004^{b}	0.943 ± 0.004^{a}	0.918 ± 0.000^{b}	0.933 ± 0.011^{a}	0.919 ± 0.004^{b}
	LX-3	W_1N_1	0.949 ± 0.002^{a}	0.923 ± 0.000^{bc}	0.943 ± 0.009^{a}	0.923 ± 0.014^{ab}	0.895 ± 0.001^{b}	0.931 ± 0.004^{a}
		W_1N_2	0.907 ± 0.013^{b}	0.931 ± 0.000^{ab}	0.896 ± 0.001^{c}	0.932 ± 0.002^{a}	0.896 ± 0.005^{c}	0.945 ± 0.006^{b}
		W_1N_3	0.912 ± 0.006^{b}	0.935 ± 0.011^{a}	0.915 ± 0.009^{b}	0.924 ± 0.004^{ab}	0.936 ± 0.013^{a}	0.937 ± 0.004^{c}
		W_2N_1	0.902 ± 0.003^{b}	0.918 ± 0.003^{c}	0.895 ± 0.000^{c}	0.907 ± 0.002^{c}	0.894 ± 0.002^{a}	0.907 ± 0.000^{b}
		W_2N_2	0.883 ± 0.007^{c}	0.906 ± 0.004^{d}	0.872 ± 0.008^{d}	0.916 ± 0.009^{bc}	0.864 ± 0.006^{ab}	0.912 ± 0.003^{d}
		W_2N_3	0.911 ± 0.011^{b}	0.925 ± 0.008^{abc}	0.900 ± 0.011^{c}	0.922 ± 0.010^{abc}	0.889 ± 0.003^{a}	0.913 ± 0.006^{b}
					F-value			
		W	1.12^{ns}	0.08^{ns}	24.92 **	0.01^{ns}	1.77^{ns}	7.00 *
		N	57.22 **	52.89 **	11.62 **	44.51 **	44.94 **	4.25 *
		W × N	27.31 **	8.48 *	1.83^{ns}	2.47^{ns}	16.39 **	4.27 *

The same column data (mean ± standard deviation) followed by the same letter indicates no significant difference at the 5% level. * and ** indicated significant differences at 0.05 and 0.01 levels, respectively, and ^{ns} indicated no significant difference at 0.05 level. T-43: nitrogen-efficient variety, LX-3: nitrogen-inefficient variety; W and N represent water and nitrogen management, respectively. W_1: 10,200 m^3/hm^2, W2: 8670 m^3/hm^2; N_1 (seedling: tiller: panicle: grain fertilizer 30%:50%:13%:7%), N_2 (seedling: tiller: panicle: grain fertilizer 20%:40%:30%:10%), N_3 (seedling: tiller: panicle: grain fertilizer 10%:30%:40%:20%); HS: heading stage, 20 DAH: 20 days after heading.

3.4. Effects of water and Nitrogen Management on the Activities of Nitrogen Metabolism Enzymes in Rice under Drip Irrigation

3.4.1. Effects of Water and Nitrogen Management on Nitrogen Metabolism Activity of Rice Leaves under Drip Irrigation

GS, GOGAT, and GDH in rice leaves under drip irrigation showed a downward trend with the growth process, and the performance of each treatment was consistent. Compared with W_1, W_2 significantly increased the activities of GS and GOGAT ($p < 0.05$). The GS activity of N_1, N_2, and N_3 increased by 10.37%, 26.9%, and 29.12% on average, while that of GOGAT increased 9.38%, 17.27%, and 19.5% (Table 6). Compared with N_1, the GS, GOGAT, and GDH activities of N_2 were increased by 37.9%, 17.0%, and 40.9%, respectively, but the GS, GOGAT, and GDH activities of N_3 were decreased by 50.5%, 40.1%, and 35.2%, respectively ($p < 0.05$). ANOVA showed that water and the interaction of water with nitrogen had a significant effect on leaf GOGAT and GDH ($p < 0.05$) but had no significant effect on GS; nitrogen management had a significant effect on leaf GS ($p < 0.05$) but had no significant effect on GOGAT.

Table 6. Effects of water and nitrogen management on nitrogen metabolism enzyme activities in rice leaves under drip irrigation.

Year	Cultivars	Treatments	GS Activity/U·g		GOGAT Activity/U·g		GDH Activity/nmol/min/g	
			HS	20 DAH	HS	20 DAH	HS	20 DAH
2020	T-43	W_1N_1	79.3 ± 5.6^{b}	53.6 ± 0.0^{c}	471.3 ± 30.1^{d}	361.2 ± 20.1^{c}	370.3 ± 51.6^{b}	283.3 ± 30.1^{d}
		W_1N_2	95.8 ± 9.2^{ab}	74.5 ± 6.8^{b}	614.1 ± 3.0^{a}	411.0 ± 10.0^{b}	417.7 ± 13.0^{b}	422.0 ± 0.0^{c}
		W_1N_3	56.0 ± 3.0^{b}	49.0 ± 1.1^{d}	360.2 ± 0.0^{e}	354.2 ± 0.1^{e}	361.0 ± 33.7^{b}	220.9 ± 12.0^{b}
		W_2N_1	85.8 ± 4.5^{b}	62.5 ± 4.4^{b}	536.5 ± 6.0^{b}	375.5 ± 35.1^{d}	458.2 ± 24.1^{c}	163.6 ± 8.9^{c}
		W_2N_2	96.0 ± 16.3^{a}	77.3 ± 2.2^{a}	648.1 ± 21.0^{a}	467.2 ± 15.0^{a}	619.6 ± 86.1^{a}	524.9 ± 0.0^{a}
		W_2N_3	85.9 ± 9.9^{b}	64.4 ± 0.5^{b}	429.1 ± 12.0^{abc}	387.8 ± 25.1^{b}	373.2 ± 4.3^{d}	342.7 ± 48.2^{cd}
	LX-3	W_1N_1	42.6 ± 2.3^{d}	31.1 ± 2.2^{b}	475.3 ± 5.0^{e}	460.2 ± 0.0^{d}	465.0 ± 86.1^{bc}	487.2 ± 36.1^{c}
		W_1N_2	61.5 ± 2.4^{b}	69.2 ± 1.1^{a}	602.8 ± 18.8^{a}	481.8 ± 21.3^{b}	904.3 ± 21.0^{a}	816.8 ± 6.0^{b}
		W_1N_3	45.4 ± 1.4^{c}	39.2 ± 9.3^{a}	425.5 ± 12.0^{c}	377.5 ± 38.4^{d}	674.6 ± 12.0^{cd}	507.2 ± 16.0^{a}
		W_2N_1	44.9 ± 8.0^{c}	36.8 ± 7.8^{b}	580.8 ± 60.2^{bc}	496.4 ± 5.0^{c}	680.8 ± 10.0^{cd}	645.0 ± 40.4^{c}
		W_2N_2	92.4 ± 1.4^{a}	82.0 ± 8.0^{a}	678.2 ± 65.3^{a}	572.7 ± 40.1^{a}	775.1 ± 25.8^{ab}	409.9 ± 24.1^{d}
		W_2N_3	68.2 ± 1.2^{a}	45.8 ± 2.3^{b}	549.8 ± 19.1^{ab}	482.3 ± 20.2^{ab}	543.0 ± 8.6^{d}	409.9 ± 72.3^{d}
2021	T-43	W_1N_1	62.3 ± 2.6^{c}	54.4 ± 1.6^{b}	545.4 ± 90.4^{c}	512.0 ± 24.2^{cd}	391.8 ± 30.1^{b}	361.7 ± 60.2^{c}
		W_1N_2	73.1 ± 3.8^{a}	68.8 ± 2.4^{a}	583.7 ± 60.2^{b}	528.9 ± 3.0^{a}	753.6 ± 30.1^{a}	361.7 ± 60.2^{c}
		W_1N_3	39.0 ± 6.0^{c}	35.2 ± 2.2^{b}	471.3 ± 30.1^{d}	422.0 ± 41.1^{d}	663.1 ± 12.5^{a}	150.7 ± 30.1^{d}
		W_2N_1	62.8 ± 5.8^{c}	58.1 ± 2.7^{b}	615.3 ± 30.1^{a}	517.4 ± 30.1^{bc}	560.2 ± 0.0^{c}	572.7 ± 30.1^{b}
		W_2N_2	88.4 ± 2.4^{b}	71.5 ± 7.5^{a}	723.4 ± 41.3^{b}	696.3 ± 15.0^{a}	633.0 ± 30.1^{a}	844.0 ± 60.2^{a}
		W_2N_3	52.4 ± 9.5^{b}	44.8 ± 0.0^{b}	635.9 ± 30.1^{a}	521.9 ± 57.2^{b}	602.8 ± 24.1^{ab}	753.6 ± 90.4^{a}
	LX-3	W_1N_1	67.4 ± 8.4^{bc}	41.1 ± 2.1^{b}	548.0 ± 9.0^{d}	459.4 ± 6.0^{e}	660.2 ± 0.0^{d}	572.7 ± 90.4^{a}
		W_1N_2	95.0 ± 3.0^{a}	56.2 ± 3.7^{b}	568.2 ± 1.5^{c}	556.6 ± 33.1^{b}	693.3 ± 30.1^{a}	602.9 ± 0.0^{a}
		W_1N_3	57.0 ± 4.0^{c}	36.9 ± 0.9^{b}	465.4 ± 4.8 f	426.7 ± 12.0^{c}	452.9 ± 90.5^{b}	241.1 ± 60.2^{b}
		W_2N_1	78.0 ± 1.0^{ab}	47.0 ± 2.0^{b}	584.2 ± 15.0^{b}	493.3 ± 6.0^{b}	471.7 ± 30.1^{c}	433.0 ± 33.5^{a}
		W_2N_2	95.5 ± 3.4^{a}	64.0 ± 3.6^{a}	656.5 ± 22.6^{a}	649.5 ± 45.2^{a}	564.2 ± 60.3^{a}	413.9 ± 34.4^{a}
		W_2N_3	77.9 ± 2.2^{ab}	43.0 ± 1.9^{b}	510.4 ± 0.0^{e}	439.5 ± 15.3^{c}	481.1 ± 12.7^{cd}	413.9 ± 15.7^{a}
					F-value			
		W	1.9^{ns}	4.6^{ns}	23.6^{**}	21.3^{**}	20.4^{**}	11.9^{**}
		N	8.5^{*}	15.0^{**}	0.4^{ns}	418.9^{**}	0.4^{ns}	0.4^{ns}
		W × N	2.4 ns	8.1^{*}	16.0^{**}	13.4^{**}	33.9^{**}	9.6^{*}

GS: Glutamate synthase, GOGAT: Glutamate synthase, GDH: Glutamate dehydrogenase; The same column data (mean ± standard deviation) followed by the same letter indicates no significant difference at the 5% level. * and ** indicated significant differences at 0.05 and 0.01 levels, respectively, and ^{ns} indicated no significant difference at 0.05 level. T-43: nitrogen-efficient variety, LX-3: nitrogen-inefficient variety; W and N represent water and nitrogen management, respectively. W_1: 10,200 m^3/hm^2, W2: 8670 m^3/hm^2; N_1 (seedling: tiller: panicle: grain fertilizer 30%:50%:13%:7%), N_2 (seedling: tiller: panicle: grain fertilizer 20%:40%:30%:10%), N_3 (seedling: tiller: panicle: grain fertilizer 10%:30%:40%:20%); HS: heading stage, 20 DAH: 20 days after heading.

3.4.2. Effects of Water and Nitrogen Management on Nitrogen Metabolism Activity of Rice Roots under Drip Irrigation

Compared with W_1, the GS, GOGAT, and GDH of the W_2 group increased significantly at 0–10 cm and 10–20 cm in rice roots, and HS increased by 35.7%, 24.1%, and 27.6% on average (Table S1). At 20 DAH, they increased by 60.8%, 26.5%, and 26.7% on average. Comparing N_1 with other nitrogen regimes, the root GS, GOGAT, and GDH of N_2 increased by 48%, 83.5%, and 194.8% on average, and those of N_3 increased by 41.5%, 35.8%, and 115.4%. Compared with T-43, the LX-3 plants had 32.9% lower GS, 34.2% greater GOGAT of HS 0–10 cm, and 25.5% higher GDH of HS 0–10 cm and 10–20 cm. GS, GOGAT, and GDH all increased in each growth stage with the postponing of nitrogen application, but the increase was slowed if the nitrogen application was postponed too long. ANOVA showed that water had no significant effect on GS or GOGAT in HS roots, but GDH had a significant difference

($p < 0.01$), and 20 DAH had the opposite findings; nitrogen management had a significant effect on nitrogen metabolism enzymes ($p < 0.01$); interactions of water and nitrogen had significant or extremely significant effects on GS, GDH, and GOGAT (10–20 cm) but had no significant effect on 20 DAH GDH.

3.5. Correlation Analysis of Root Morphological Characteristics and Nitrogen Metabolism Enzymes and Yield

RDA showed that the eigenvalues of the 1st and 2nd axes of T-43 were 0.7047 and 0.1692, and those of LX-3 were 0.6724 and 0.2093, respectively (Figure 5). which had both biological and statistical significance. RLD, SAD, GS, GOGAT, GDH, of T-43 in the 0–10-cm soil layer and the SAD, GOGAT, GDH, SAD and RVD β values of the 10–20-cm soil layer were positively correlated with yield and nitrogen partial factor productivity. The RVD of the 20–30-cm soil layer and the RLD, SAD, and RVD of the 30–40-cm soil layer were negatively correlated with leaf GDH. The SAD and RVD of LX-3 in the 0–10-cm soil layer; the root GS, GOGAT, GDH, RLD, GS, GOGAT, and GDH of the 10–20-cm soil layer; the RVD of the 20–30-cm soil layer; and the RLD, SAD, RVD, RVD β, and leaf GOGAT of the 30–40-cm soil layer were positively correlated with yield and nitrogen partial factor productivity. SAD in the 30–40-cm soil layer and leaf GOGAT were negatively correlated.

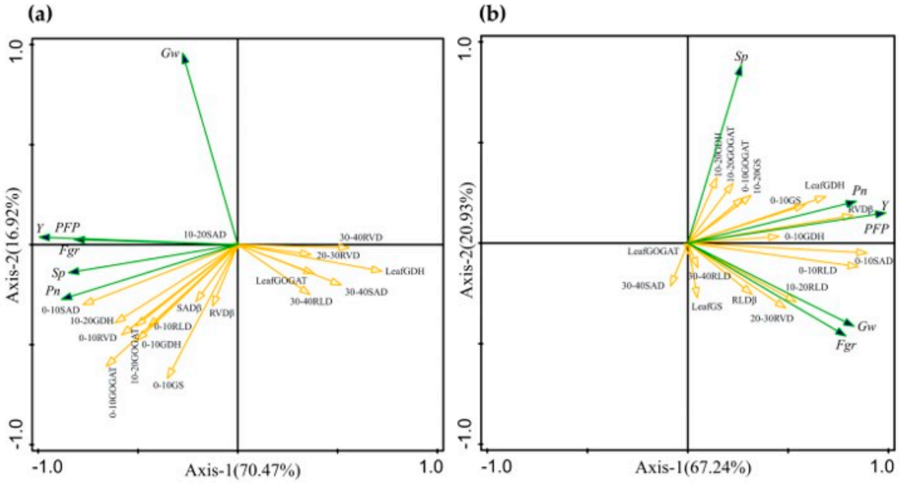

Figure 5. RDA analysis of root growth, physiological indexes and yield of rice. (**a**): T-43, (**b**): LX-3. 0–10 RLD: Root length density of 0–10 cm soil layer, 0–10 SAD: Surface area density of 0–10 cm soil layer, 0–10 RVD: Root volume density of 0–10 cm soil layer, 0–10 GS and 10–20 GS: Glutamine synthetase of 0–10 cm soil layer and 10–20 cm soil layer, 0–10 GOGAT and 10–20 GOGAT: Glutamate synthetase of 0–10 cm and 10–20 cm soil layer, 0–10 GDH: Glutamate dehydrogenase of 0–10 cm soil layer; RLD β:Root length density β value, RVD β:Root volume density β value; Pn: Panicle numbers, Sp: Spikelet per panicle, Fgr: Filled grain rate, GW: 1000-Grain weight, Y:Yield, PFP: Nitrogen partial factor productivity.

4. Discussion

4.1. Effects of Water and Nitrogen Fertilizer Management on the Root Morphology and Growth Characteristics of Rice under Drip Irrigation

The root system is an important organ by which crops absorb water and nutrients, and its competitiveness is closely related to the root system length, expansion area, root system architecture, and plasticity [17,18,22,23]. Changes in environmental factors such as soil moisture and nutrients affect root morphogenesis [31]. Different diameter classes of rice root system have different root functions. The main function of finely branched roots (diameter < 0.1 mm) and coarsely branched roots (0.1 mm ≤ diameter < 0.3 mm) is to absorb water and nutrients, while adventitious roots (≥0.3 mm) play bigger roles in fixation and transduction [26]. Gu et al. [32] showed that the effect of water and nitrogen management on root morphological characteristics was the largest in the root length distribution of coarse branch roots and fine branch roots. In this study, finely branched roots of rice under drip irrigation had the largest proportion of RLD (48.15~59.62%), while that of adventitious roots was <10%. Coarsely branched roots had the largest ratio of SAD to RVD (50.47~72.34%), while finely branched roots had <20%, It shows that under drip irrigation conditions, rice roots can better adapt to the soil environment and improve the absorption capacity of water and nutrients; this ratio in finely branched roots and coarsely branched roots decreased with limited irrigation. It may be that compared with traditional flooding irrigation, soil moisture is not enough to meet the maximum demand for rice root growth, and roots will compete for water, resulting in a decrease in the proportion of branching roots. Ji et al. [33] believed that the development of rice coarsely branched roots had a direct impact on nitrogen uptake. In this study, when nitrogen application was appropriately postponed (N_2), the RLD, SAD, and RVD of rice finely branched roots and coarsely branched roots increased, it may be that roots can respond to changes in soil nutrient environment by adjusting their own morphology, so rice root morphology can have a greater impact on nitrogen absorption, thereby promoting the growth and development of rice roots.

Optimizing the spatial distribution of roots can improve the ability of plants to obtain water and nutrients [34]. There are many models for the distribution of crop roots in soil, such as normalized RLD [35], artificial neural network [36], and root length distribution function [37]. This paper adopts an asymptotic equation β model proposed by M.R. Gale and D.F. Grigal [27] in 1987 to describe the distribution of rice roots in soil under drip irrigation. In this study, the roots of rice under drip irrigation were mainly distributed in the surface soil (0–10 cm); compared with W_1, the RLD, SAD, and RVD of each soil layer under W_2 decreased significantly, and the RLD β value significantly increased 20 DAH; RLD, SAD, and RVD under N_2 increased significantly, while the values of RLD β, SAD β, and RVD β decreased significantly. Under normal irrigation, nitrogen shifts back appropriately to promote root growth. For drip-irrigated rice, mild water stress caused by limited irrigation induces rice roots to root down, expanding the area and volume of the root system in the soil, which is conducive to enhancing water and nutrient uptake by the rice root system [32]. There are significant genotypic differences in root trait genes between cultivars. Zhu [38] and Ji et al. [33] found that the root length, surface area, and volume of each branch of nitrogen-efficient varieties were significantly larger than those of nitrogen-inefficient varieties. In this study, nitrogen-efficient (T-43) rice had better RLD, SAD, and RVD of adventitious roots and branched roots, and in each soil layer T-43 had better values than nitrogen-inefficient LX-3. The reason for this difference may be that nitrogen-efficient rice needs to absorb more nutrients to maintain its own function. The increase in morphological and architecture indices further expands the absorption space and increases the supply of soil nutrients. Thus, the root system can make adaptive adjustments to environmental factors such as water and nutrient changes, which gives it the ability to regulate its root morphogenesis and architecture of rice under drip irrigation through water and fertilizer management.

4.2. Effects of Water and Nitrogen Fertilizer Management on Physiological Characteristics of Nitrogen Metabolism in Rice under Drip Irrigation

Nitrogen absorbed by rice roots must be assimilated by nitrogen metabolism enzymes before it can be absorbed and utilized. The enzymes involved in nitrogen metabolism mainly include GS, GOGAT, and GDH [22]. The GS–GOGAT metabolic pathway is the nitrogen metabolism centre responsible for the assimilation of NH_4^+ into amide nitrogen in crops, GDH is responsible for the synthesis of α-ketoglutarate and the release of ammonium from glutamate oxidation [39]. It is generally believed that mild water stress will increase the activities of GS, GOGAT, and GDH, while severe water stress will significantly reduce the enzyme activities [23]. In this study, compared with W_1, W_2 increased GS by 10.37%, 26.9%, and 29.12% and GOGAT by 9.38%, 17.27%, and 19.5% under the N_1, N_2, and N_3 treatments, respectively, it shows that the soil aeration environment is better under limited irrigation, which is conducive to maintaining higher nitrogen metabolism enzyme activity in roots, so as to maintain the balance of nitrogen metabolism in cells and adapt to the response of arid environment. The activities of GS, GOGAT, GDH, and other nitrogen metabolizing enzymes in the leaves and roots of rice under drip irrigation were the highest in N_2, indicating that increasing panicle fertilizer was conducive to maintaining higher nitrogen assimilation enzyme activities in rice under drip irrigation. RDA showed that there were significant or extremely significant correlations between leaf and root nitrogen metabolism enzyme activities and rice yield and nitrogen partial factor productivity. Optimizing rice water and nitrogen management under drip irrigation had a significant effect of adjusting fertilizer with water and promoting water with fertilizer. The proper postponing nitrogen application (here, treatment N_2) increases the activity of nitrogen metabolism enzymes, which is beneficial to the formation of yield. In addition, rice can absorb and utilize nitrate nitrogen. The rice under drip irrigation keeps the soil water content around 85% throughout the growth period. This aerobic environment causes a large proportion of the applied nitrogen to be absorbed and utilized by the rice in the form of NO_3^-. Therefore, attention should be given to nitrate reductase and nitrite reductase related to NO_3^- in future research.

4.3. The Relationship between Water and Nitrogen Management on High Yield and High Efficiency of Rice under Drip Irrigation and Root Morphology and Physiological Characteristics

Reasonable water and fertilizer management is an important basis for improving rice yield and achieving efficient utilization of water and fertilizer resources. A lot of studies have been done on the effects of different water and nitrogen management on rice population growth and yield formation [3,16,26]. Sun et al. [40] based on the study of rice yield and nitrogen use efficiency under different water and fertilizer conditions, think that different nitrogen management regulation combined with appropriate irrigation measures can significantly improve rice yield. In this study, under drip irrigation cultivation mode, normal irrigation (W_1) helped to improve the panicle number, filled grain rate, and 1000-grain weight of rice, and it increased yield by 20.7% over W_2. With the longer postponement of nitrogen application, the panicle number, filled grain rate, and yield showed a trend of rising and then falling, peaking under N_2. The results showed that compared with limited irrigation, rice yield components (panicle number, filled grain rate, 1000-grain weight) performed better under normal irrigation, which compensated for the negative effect of too few spikelet's per panicle on yield. Based on ensuring a higher panicle number, appropriate reduction of nitrogen fertilizer application in the early stage and increasing the spikelet number and grain fertilizer can promote the later growth and grain filling of rice, increase the filled grain rate and 1000-grain weight, and lay the foundation for high rice yield. Compared with N_1, the yields of T-43 treated with N_2 and N_3 were increased by 49.4% and 18.6%, respectively, and the yield of LX-3 was increased by 29.9% and 15.9%. Overall, W_1 N_2 had a higher yield (8.1 t/hm^2) and nitrogen partial factor productivity (27.1 kg/kg).

Crop high yield and root morphology, physiological characteristics have been the focus of academic research, but also the focus of debate [15,17,23,26]. Zhao [41] showed that root morphological indexes were closely related to spike number, grain number per spike, 1000-grain weight and yield. Root nitrogen metabolism enzymes were also significantly positively correlated with seed setting rate and 1000-grain weight. Some studies [37,42] have also shown that rice yield is closely related to the spatial distribution of roots. The deep and more longitudinal roots are beneficial to improve the ventilation and light transmission of the population and increase the photosynthesis of the population. The upper roots significantly increase the seed setting rate and 1000-grain weight, and the lower roots are beneficial to the early tillering and large panicles. The results of this study also showed that the nitrogen efficient varieties (T-43) 0–10 cm and 10–20 cm SAD, 10–20 cm GOGAT, 10–20 cm GDH and spike number, grain number per spike, seed setting rate and yield were significantly positively correlated; the 0–10 cm and 10–20 cm RLD of low nitrogen inefficient varieties (LX-3) were significantly positively correlated with seed setting rate and 1000-grain weight. RVDβ and 10–20 cm GDH were significantly positively correlated with grain number per spike and yield, which was different from the results of Cai et al. This may be that drip irrigation infiltration causes water and nutrients to concentrate in the upper part of the soil. Under drip irrigation cultivation mode, on the one hand, through the different effects on root morphology and physiological activity, to affect the growth of rice, on the other hand, by affecting the growth environment of rice to affect its water and nutrient supply and absorption.

5. Conclusions

There was an obvious interaction effect between water and nitrogen management. The rice yield of W_1 increased by 26.8% on average, and the yield and nitrogen partial factor productivity of W_1 and N_2 were the highest, under which T-43 reached 8.9 t/hm^2 and 29.8 kg/kg, and LX-3 reached 7.3 t/hm^2 and 24.4 kg/kg. W_1N_2 was the best water-nitrogen coupling operation mode in this experiment. Water and nitrogen management had significant effects on root morphology and nitrogen metabolism enzyme activities in rice under drip irrigation. Normal irrigation (W_1) promoted the growth of finely branched roots, coarsely branched roots, and adventitious roots in the root system, and the roots gathered in the surface soil (0–10 cm). Limited irrigation (W_2) had higher nitrogen metabolism and promoted rooting. The GS, GOGAT, and GDH of finely branched roots, coarsely branched roots, and adventitious roots were significantly greater under N_2, and the root architecture RLD β, SAD β, and RVD β were significantly lower. There were significant correlations between root morphology, nitrogen metabolism enzyme activity and yield and nitrogen partial factor productivity in rice under drip irrigation. SAD, GS, GOGAT, and GDH in the 0–10-cm soil layer; RLD, GS, and RVD β; and the leaf GS of the 10–20-cm soil layer were all positively correlated with yield and nitrogen partial factor productivity. The 20–30 cm RVD, 30–40 cm SAD, yield, and nitrogen partial factor productivity were negatively correlated.

This study can provide a theoretical basis for understanding the differences in the effects of water and nitrogen management on root morphological characteristics and nitrogen metabolism enzyme activities and can guide the rational cultivation of rice under drip irrigation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13041118/s1, Table S1: Effects of water and nitrogen application on nitrogen metabolism enzyme activities in rice roots under drip irrigation.

Author Contributions: Conceptualization, Q.T., Y.M., L.Z., Z.S., Y.Y., G.W. and Y.L.; validation, Q.T., Y.M., L.Z. and Z.S., Y.Y.; formal analysis, Q.T., L.Z.; investigation, Y.M., L.Z., Z.S. and Y.Y.; writing—Original draft preparation, Q.T.; writing—Review and editing, Q.T., G.W. and Y.L.; visualization, Q.T. and L.Z.; supervision, G.W. and Y.L.; project administration, G.W. and Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (31860345, 31460541) and Youth Innovative Top Talents Project of Shihezi University (CXBJ202003).

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

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