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Intercropping of Rice and Water Mimosa (*Neptunia oleracea* Lour.): A Novel Model to Control Pests and Diseases and Improve Yield and Grain Quality while Reducing N Fertilizer Application

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Abstract: Cereal/legume intercropping is an effective agricultural practice for pest and disease control and crop production. However, global research on rice and aquatic legume intercropping is relatively rare. A field experiment during two seasons (2018 late season and 2019 early season) was conducted to explore the effects of rice and water mimosa intercropping on rice canopy microclimate, pest and disease, yield, grain quality, and economic income. Two cultivation patterns including rice/water mimosa intercropping and rice monocropping were employed, and three nitrogen (N) fertilizer application levels, including zero N (ZN, 0 kg ha^{-1} N), reduced N (RN, 140 kg ha^{-1} N), and conventional N (CN, 180 kg ha^{-1} N) levels, were applied for the above two cultivation patterns. The results showed that rice/water mimosa intercropping formed a canopy microclimate of rice with higher temperature and lower relative humidity and dew point temperature. In addition, there was a significant reduction in the occurrences of rice leaf blast by 15.05%~35.49%, leaf folders by 25.32%~43.40%, and sheath blight by 16.35%~41.91% in the intercropping treatments. Moreover, rice/water mimosa intercropping increased rice per unit yield by 43.00%~53.10% in the late season of 2018 and 21.40%~26.18% in the early season of 2019. Furthermore, rice grain quality was totally improved, among which brown and head rice rates increased but rice chalky rate and chalkiness degree decreased in the intercropping system. We suggest that combining rice/water mimosa intercropping and N fertilizer reduction can be used as an environmentally friendly eco-farming technique because it can decrease N fertilizer application by approximately 40 kg·ha^{-1}. This combination would not only mitigate nonpoint source pollution but also obtain advantages for controlling rice pests and diseases that would alleviate pesticide usage and improve rice yield and grain quality, which can be extended for green rice production to increase income for producers.

Keywords: rice; intercropping; water mimosa; pest and disease; microclimate; grain quality; yield

1. Introduction

Globally, rice (*Oryza sativa* L.) is one of the oldest and most important staple foods and is consumed by approximately 50% of the world's population and 60% of China's population [1–5]. In addition, modern, intensive agriculture has resulted in a series of problems, such as soil degradation, biodiversity loss, and nonpoint source pollution [6,7]. In many large, modern farms, crops are cultivated as monocultures that reduce the margins [8]. Therefore, achieving plant diversity is extremely important for the development of sustainable agroecological systems [9]. Intercropping is a vital, traditional agriculture practice. In intercropping systems, no less than two crops are simultaneously cultivated in the same field [9]. The intercropping system can efficiently utilize light, heat, water, nutrients, and other environmental resources [1]. Intercropping usually brings plenty of benefits, such as increasing crop yield, land equivalent ratio, and economic income, reducing soil degradation, and controlling weeds, pests, and diseases [10].

The influence of structural diversity of plants on microclimatic parameters has become increasingly clear [11]. In the intercropping system, the aboveground and belowground microclimates were changed through horizontal and vertical plant growth [12]. For instance, coffee (*Coffea arabica* L.) and macauba (*Acrocomia aculeata*. (Jacq.) Lood. ex Mart) intercropping decreased the air temperature of the coffee canopy because the coffee was shaded by macauba [13]. Another study showed that soybeans (*Glycine max* L.) had smaller roots and shoots than the 26-year-old hybrid poplar (*Populus deltoides* X nigra.), so the active radiation, soil water content, and ambient temperature decreased, but the relative humidity increased in their intercropping system [14].

Besides modifying the microclimate, intercropping is an environmentally friendly approach to control pests and disease. Rice blast (*Pyricularia oryzae* Cav.), sheath blight (*Rhizoctonia* solani), and leaf folder (*Cnaphalocrocis medinalis* Guenee) are common pests and diseases of rice. In addition, the disease incidence is related to the initial fungi amount, field microclimate, and plant traits. High temperature and humidity are the important causes of the disease, while aeration and permeability can reduce pest growth [1]. In China, rice blast, sheath blight, and leaf folders are the main diseases and pests that reduce rice yield [15]. The pesticide sales for rice were approximately 538 million US dollars in 2006 [16]. Using intercropping methods to control rice diseases and pests is an optimal choice in paddy fields. The mechanisms of intercropping to reduce the incidence of pests and diseases are possibly attributed to microclimate change, physical barrier, dilution effect, non-lodged effect, allelopathy, etc. [1]. Compared with rice monocultures, rice/water spinach (*Ipomoea aquatica* Forssk.) intercropping showed great control of rice sheath blight, leaf folders, and leaf blast [1,4]. In addition, rice/water chestnut (*Eleocharis dulcis* (Burm. f.) Trin.) intercropping suppressed rice sheath blight and blast [3]. Generally, intercropping rice and other crops would be a sustainable agricultural strategy to reduce the occurrence of pests and diseases in paddy fields.

In all kinds of intercropping systems, the cereal/legume intercropping system shows the advantages of increasing crop yield and improving grain quality. In a dry land rice/legume intercropping system, legumes fix N_2 from the atmosphere and transfer N to rice [17]. This biofertilizer supplied by legumes promoted crop growth and increased yield and grain quality. For instance, maize/lentil (*Lens culinaris* L.) intercropping promoted plant growth and increased the yield and grain protein [18]. In addition, compared with maize monoculture, maize/lablab (*Lablab purpureus* (Linn.) Sweet) intercropping increased the yield and grain quality of maize with higher crude protein, acid, and neutral detergent fiber contents [19].

In paddy fields, rice can be intercropped with some aquatic plants, such as water chestnut, water spinach, alligator flags (*Thalia dealbata* Fraser), and Azolla (*Azolla imbricate* (Roxb.) Nakai) [3–6]. However, few studies have focused on the intercropping of rice and aquatic leguminous plants in paddy fields. Water mimosa (*Neptunia oleracea* Lour.), an aquatic legume vegetable, is consumed by many Asian countries due to its high nutritional value [20,21]. Previous studies showed that rice intercropping with water mimosa increased the rice yield [22,23]. N fertilizer also promoted rice growth and increased the yield and grain quality of rice [24,25]. However, there are limited references studying the effect of rice/water mimosa intercropping and N fertilizer on rice pest and disease control, yield, grain quality, and economic income in paddy fields. In addition, previous studies on the microclimate in rice/water mimosa intercropping systems are relatively rare.

Therefore, the present study was conducted to explore whether the microclimate of the rice canopy would be changed by rice/water mimosa intercropping and whether this intercropping can control pests and diseases and increase rice yield and grain quality while reducing N fertilizer application. Our hypotheses were as follows: (1) Rice/water mimosa intercropping can modify the rice canopy microclimate; and (2) rice/water mimosa intercropping can control diseases and pests and then increase yield and grain quality while reducing N fertilizer application.

2. Materials and Methods

2.1. Study Site and Materials

A field experiment was performed at Zengcheng Teaching and Research Farm (23°14′ N, 113°38′ E), South China Agricultural University, Guangzhou, Guangdong Province, China. The area has a subtropical monsoon climate with a warm winter and hot summer (Figure S1). In addition, rice (*Oryza sativa* L., Huanghuazhan), a typical local crop that is widely cultivated in southern China for its good taste, grain quality, strong lodging resistance, and steady productivity, and water mimosa (*Neptunia oleracea* Lour.), an aquatic leguminous plant, were selected for the experiment. The basic physical and chemical properties of the soil were as follows: sandy loam, pH 4.88, containing 15.80 g kg^{-1} organic matter, 2.27 g kg^{-1} total N, 0.51 g kg^{-1} total phosphorous, 10.91 g kg^{-1} total potassium, 9.06 mg kg^{-1} ammonium nitrogen (N), 4.69 mg kg^{-1} nitrate N, 43.82 mg kg^{-1} available phosphorus, and 47.56 mg kg^{-1} available potassium.

2.2. Cultivating Experimental Design

A field experiment was conducted during 2018 and 2019 consisting of two rice growing seasons. The 2018 late growing season was from August to November, and the 2019 early growing season was from April to July. Six treatments with four replicates were applied to the experiment: rice monocropping (0, 140, and 180 kg·ha^{-1} N) and rice/water mimosa intercropping (0, 140, and 180 kg·ha^{-1} N). A completely randomized design was used in the field experiment. Each plot area was 35 m^2 (5 m $\times$ 7 m) and irrigated and drained independently. The seedlings' density of rice was 250,000 holes/ha and 166,667 holes/ha in monocropping and intercropping treatments. The density of water mimosa was 83,333 plants/ha in intercropping treatments. The cultivation standard per strip in the intercropping treatments was performed every four rows of rice and then connected to three rows of water mimosa. Within the rice monocropping treatments, rice row spacing was 0.2 m. In intercropping treatments, the intrarow spacing of rice and water mimosa was 0.2 m and 0.15 m, respectively, and the row distance between rice and water mimosa was 0.25 m (Figure 1). The area ratio of rice and water mimosa was 2:1 in the intercropping treatment. There were 2/3 and 1/3 areas of each plot occupied by rice and water mimosa in intercropping treatments, respectively. Urea ($CO(NH_2)_2$) was applied as N fertilizer before transplanting and at the rice tillering, heading, and filling stages with proportions of 40%, 20%, 30%, and 10%, respectively (Figure 1). In addition, the application method of phosphate and potassium fertilizer was the same in each treatment. Calcium superphosphate (P_2O_5 12%), as the phosphate fertilizer, was applied only as the base fertilizer at 45 kg ha^{-1}. Potassium chloride (KCl 60%) was used as potassium fertilizer at 135 kg ha^{-1}; one half was applied as the base, and the other half was applied as heading stage fertilizer. Seeds were soaked in water for 24 h at room temperature and then germinated under moisture conditions for seedling preparation. Germinated seeds were sown on 25 July (late growing season of 2018) and on 10 March (early growing season of 2019) for rising nurseries. In addition, 15 kg ha^{-1} urea fertilizer was applied during the rice seedling stage. Water mimosa (length of 0.3 m) and rice seedlings (three-leaf-and-one-leaflet stage) were then concurrently transplanted to the paddy field. Fertilization management methods were the same for the two seasons. The drainage and irrigation managements were to keep the water layer at 6~8 cm throughout the rice-growing season in the field because water mimosa grows better in water flooded condition, but irrigation was stopped 1 week before the rice harvest. We did not apply any pesticides, herbicides, or weed control practices for any treatments.

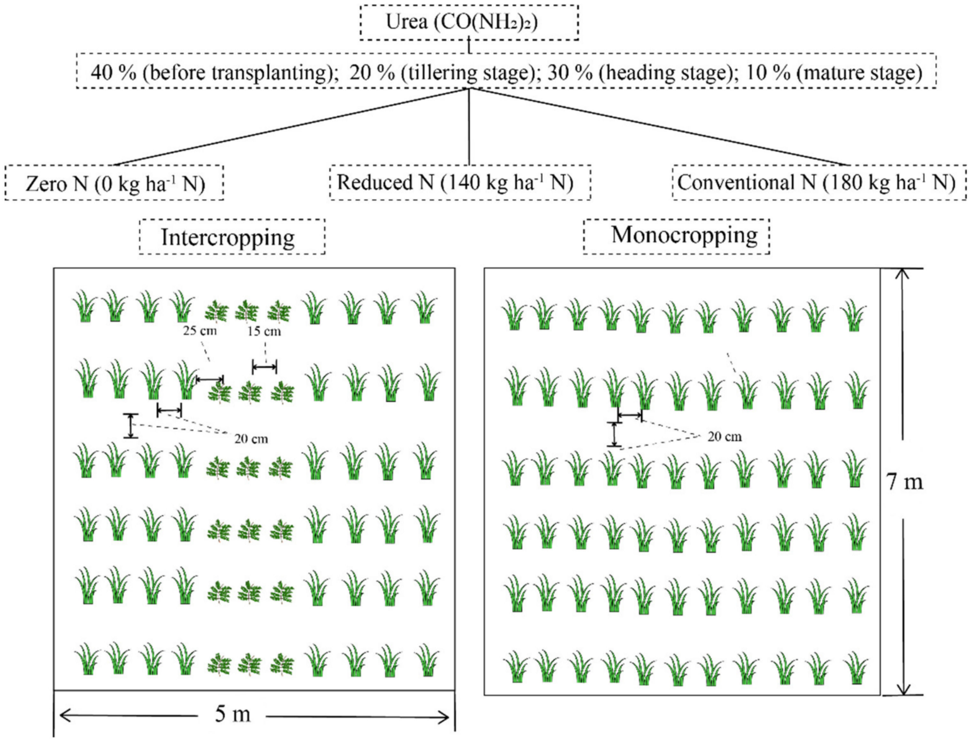

Figure 1. Experimental design of two cropping systems under three different levels of N fertilizer application treatments.

2.3. Sampling and Data Collection

2.3.1. Canopy Microclimate

The canopy microclimate of rice in the monocropping and intercropping treatments under the reduced N fertilizer level was measured by a small, portable climate instrument UA-002064 (Onset, Bourne, MA, USA). On the 50th, 55th, 60th, 65th, 70th, and 75th days after transplanting, daily changes of dew point temperature, air temperature, and relative humidity (RH) in the rice canopy were measured for the four plots in each treatment. A small, portable climate instrument was placed among the rows of rice. The instrument was tied on a pole at the height of the rice canopy, and its height was changed according to the height of the rice canopy. The data were recorded every 20 min, and then the dew point temperature, air temperature, and RH value were calculated to obtain an average value per hour.

2.3.2. Pest and Disease Survey

Rice leaf blast, leaf folders, and sheath blight were investigated on the 50th, 55th, 60th, 65th, 70th, and 75th days after transplanting in each season. Three holes of rice in different rows were selected randomly as the initiation points, and then five holes were extended in each row when using a parallel jumping sampling method. The calculation methods of rice leaf blast, leaf folders, and sheath blight were as follows.

The damaged leaf number caused by rice leaf blast and leaf folders and the total leaf number of the 15-hole rice were counted. The incidences of rice leaf blast and leaf folders were calculated by using the following Equation (1) [1]:

$$\text{The incidence of rice leaf blast (leaf folders) (\%)} = \text{damaged leaf number}/\text{total leaf number} \times 100\% \quad (1)$$

Rice sheath blight was surveyed, according to Wu et al. (2012) [26], and the disease categories of rice sheath blight were as follows:

0—No lesion;
1—Lesions on any leaf except the top three leaves;
2—Lesions up to the third topmost leaf;
3—Lesions up to the second topmost leaf;
4—Lesions up to the flag leaf or panicle.

The numbers of infected stems in different disease categories and total stems were counted. The disease index was calculated according to Equation (2) [1,26]:

$$\text{Disease index of rice sheath blight (\%)} = [\Sigma\ (\text{disease category} \times \text{infected stems number in this disease category}) / (\text{total stems number} \times \text{the highest disease category})] \times 100\% \quad (2)$$

2.3.3. Grain Yield, Yield Components, Grain Quality, and Economic Income

Rice and water mimosa yields were measured from four plots with an area of 1 m × 1 m for each replicate, and each sample contained insiders and outsiders of the plants. In addition, rice was threshed manually and sun-dried (adjusted to 14% moisture content) to obtain the grain yield, and the water mimosa yield was determined by harvesting the top tender part. Per unit yield indicated the rice yield which was calculated in the 3 × 1 m^2 plots, and the actual yield of rice and water mimosa represented the yield considering the area ratio of rice and water mimosa in the intercropping treatment. Yield components were measured according to Li et al. (2019) [27] and determined from five hills for each replicate. Effective panicle numbers per hill were counted and calculated per unit effective panicle numbers and actual panicle numbers. Then, the panicles were threshed manually and divided into filled grains and unfilled grains. Five subsamples of filled and unfilled grains were used to estimate the grain numbers per panicle and seed-setting rate. The 1000-grain weight was also calculated from the sampled grains.

Rice grain quality was measured according to Li et al. (2019) [27] and determined from five samples for each replicate. A 100-g sample of rice grain was passed through a dehusker for polishing and then divided into broken and unbroken grains. The brown, milled, and head rice rates were calculated as the percentages of the total (100 g) rice grains. Amylose content, soluble protein content, and alkali value were measured using an Infratec-1241 grain analyzer (FOSS-TECATOR, Hilleroed, Denmark). The chalky rice rate, chalkiness degree, and length/width were scanned with Plant Mirror Image Analysis (MICROTEK, Hsinchu, Taiwan, China), and then the resulting images were processed with SC-E software (HangzhouWanshen Detection Technology Co., Ltd., Hangzhou, Zhejiang, China).

Net income for each treatment was estimated using Equation (3) [28]:

$$\text{Net income} = \text{Gross income} - \text{Total cost of cultivation} \quad (3)$$

Here, the total cost of cultivation comprised the costs of inputs and labors. The costs of inputs (seeds and fertilizers) were based on the local market prices. Water mimosa is a perennial herb that can be reproduced in the field so that its plant prices are free [20,21]. The costs of labors were calculated by the cultural activities (land preparation, seedling, transplanting, applying fertilizer, and harvesting) and paid at the rate of 120 yuan (Chinese yuan) person-day^{-1} of 8 h. Gross income was calculated as the total value of economic yield (water mimosa and rice) per treatment. The market prices of rice and water mimosa were 4 yuan kg^{-1} and 7 yuan kg^{-1}, respectively.

2.4. Data Analysis

Data are expressed as the mean value ± standard error and subjected to two-way ANOVA in the two cultivation patterns with three different N fertilizer levels ($p < 0.05$). In addition, treatment differences in the same cropping system were statistically assessed by using Duncan's method of one-way ANOVA for multiple comparisons when the data met the normality and homoscedasticity hypotheses; otherwise, the data were evaluated through a Games–Howell method ($p < 0.05$). The differences between intercropping and monocropping were statistically evaluated by independent T-tests ($p < 0.05$). All statistical analyses were performed using SPSS 17.0. The data were tested by the Pearson correlation analysis using the "corrplot" package in R [29].

3. Results

3.1. Rice Canopy Microclimate

On the 50th, 55th, 60th, and 75th days in the late season of 2018 and on the 55th, 65th, and 75th days in the early season of 2019, the air temperatures were significantly higher under intercropping treatments than under the monocropping treatments, by 0.07~2.30 °C (Figure S2a,b). In contrast, in the late season of 2018, in the middle of the day, the dew point temperature and RH in the intercropping treatments were generally significantly lower, by 0.07~1.77 °C and 0.88~11.85% compared with those in the rice monocropping treatments, respectively (Figure S2c,e).

3.2. Rice Pest and Disease

During the whole investigation, intercropping treatments reduced the occurrence of rice pests and diseases. For instance, the incidences of rice leaf blast in intercropping treatments were generally significantly lower than those in monocropping treatments except on the 50th day after transplanting in the late season of 2018 (Figure 2a,b, Table S1). Intercropping treatments also significantly reduced the occurrence of rice leaf folders compared with the monocropping treatments on the 60th~75th days after transplanting in both seasons (Figure 2c,d, Table S3). Moreover, there was a significant reduction in the incidence of rice sheath blight in the intercropping treatment on the 55th and 70th days after transplanting in the 2018 late season and on the 60th, 65th, and 75th days after transplanting in the 2019 early season (Figure 2e,f, Table S5). Additionally, the incidences of rice leaf blast, leaf folders, or sheath blight in 2018 late season generally were significantly higher than those in 2019 early season after the 70th day of transplanting (Tables S2, S4 and S6).

More importantly, the incidences of rice leaf blast, leaf folders, and sheath blight in the intercropping with reduced N treatments were significantly lower than those in the monocropping with zero, reduced, and conventional N treatments (Tables S1, S3 and S5). No significant difference was found between the N fertilizer treatments in pests and diseases.

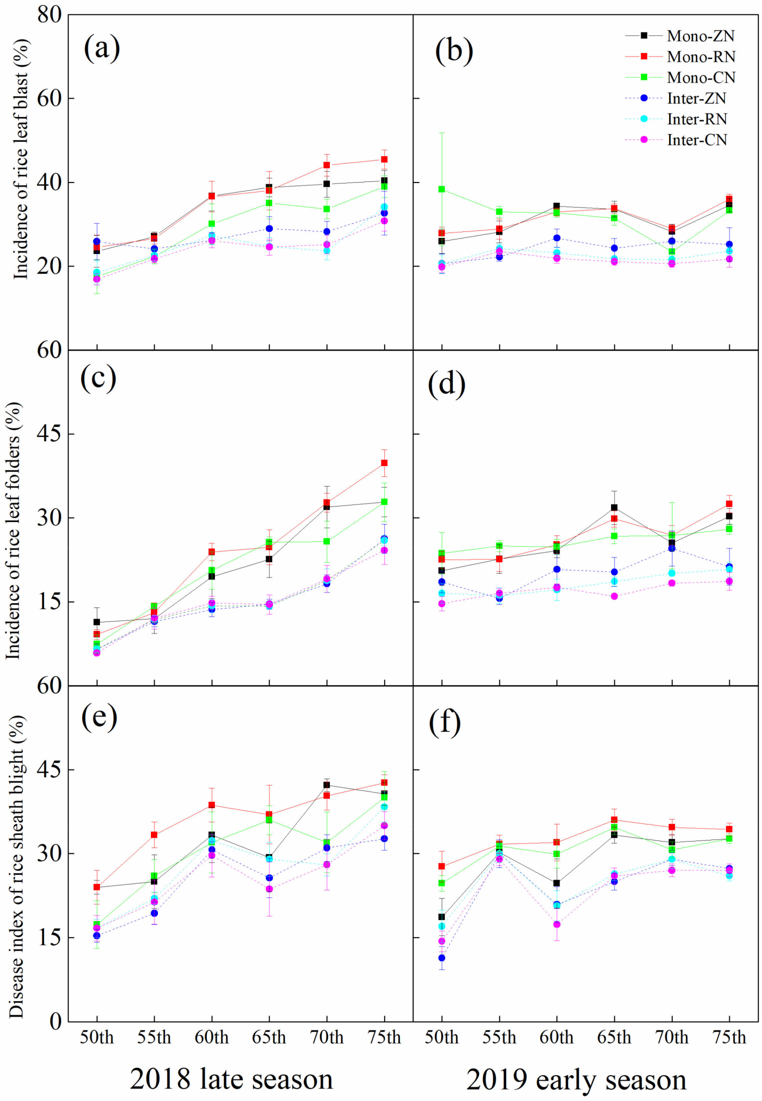

Figure 2. Pest and disease of rice on the 50th, 55th, 60th, 65th, 70th, and 75th days after transplanting. "Mono", monocropping; "Inter", Intercropping. ZN, RN, and CN indicate the 0, 140, and 180 kg ha^{-1} N fertilizer levels, respectively. (**a**,**c**,**e**) and (**b**,**d**,**f**) denotes 2018 late season and 2019 early season, respectively.

3.3. Rice Yield and Yield Components

There was a significant interactive effect between cultivation pattern and N fertilizer on rice yield and per unit effective panicle number (Table S7). The per unit yield, actual yield, and per unit effective and actual panicle number of rice and the yield of water mimosa in the CN and RN treatments were significantly higher than those in the ZN treatments. In addition, intercropping treatments significantly increased the rice's per unit yield and per unit effective panicle numbers by 43.00%~53.10% and 22.15%~41.41% in the late season of 2018 and 21.40%~26.18% and 15.72%~41.54% in the early season of 2019, respectively. However, no significant difference was found in grain numbers per panicle, seed-setting rate, or 1000-grain weight (Table 1). Additionally, rice yield and yield components in 2019 early season were generally significantly higher than those in 2018 late season except the grains per panicle (Table S8).

Furthermore, compared with rice monocropping with conventional N application treatments, intercropping with reduced N treatments significantly increased the rice's per unit yield by 35.64% in the 2018 late season and increased the per unit effective panicle numbers by 17.01% in the 2019 early season (Table 1).

3.4. Rice Grain Quality

The interactions between the two factors (cultivation pattern and N fertilizer) had significant effect on rice chalky rice rate and chalkiness degree (Table S9). Compared with the ZN treatments, the RN and CN treatments significantly increased the brown rice rate, protein content, length/width, and alkali value of rice in the early season of 2019 (Table 2). As our prediction, brown and head rice rates in intercropping treatments were slightly higher than those in monocropping treatments in the late season of 2018. There was a significant reduction in chalky rice rate and chalkiness degree in intercropping treatments compared with monocropping treatments during the two seasons (Table 2). No significant difference was found in the milled rice rate or amylose content (Table 2). Additionally, rice grain quality in 2019 early season was generally significantly higher than that in 2018 late season except the chalky rice rate, chalkiness degree, and length/width (Table S10).

The chalky rice rate in the intercropping with reduced N treatment was significantly lower than that in the monocropping with the conventional N treatment (Table 2). In addition, the length/width and alkali value in the intercropping with zero N treatments were significantly lower than those in the monocropping with the conventional N treatment in the early season of 2019 (Table 2).

3.5. Correlation Analysis

In the late season of 2018, the incidences of rice blast, leaf folders, and sheath blight generally had significant negative correlations with the air temperature, rice per unit yield, per unit effective panicle number, and head rice rate, but had significant positive correlations with the dew point temperature, RH, chalky rice rate, and chalkiness degree (Figure S3a). Although the incidences of pathogens did not have obvious correlations with microclimatic parameters, the incidences of pathogens had negative correlations with rice per unit yield, effective number, and head rice rate and had significant positive correlations with chalky rice rate and chalkiness degree in the early season of 2019 (Figure S3b).

3.6. Economic Analysis

In the two seasons, intercropping with reduced N treatments had the highest economic income and accounted for 18,234 and 18,830 yuan ha^{-1} in the 2018 late season and 2019 early season, respectively (Table 3). Thus, combining rice/water mimosa intercropping and N reduction could obtain the maximum benefit for farmers and producers.

The prices of rice and water mimosa are 4 and 7 yuan kg^{-1}, respectively. ZN, RN and CN indicate the 0, 140 and 180 kg ha^{-1} N fertilizer levels, respectively. "Mono"and "Inter" denote the mono-cropping and intercropping treatments.

Table 1. Yield and yield components in monocropping and intercropping treatments with the three different N fertilizer application levels.

Season	N Fertilizer	Pattern	Rice							Water Mimosa
			Per Unit Yield ($t{\cdot}ha^{-1}$)	Actual Yield ($t{\cdot}ha^{-1}$)	Per Unit Effective Panicle (10^4 ha^{-1})	Actual Effective Panicle (10^4 ha^{-1})	Grains per Panicle	Seed-Setting Rate (%)	1000-Grain Weight (g)	Yield ($t{\cdot}ha^{-1}$)
2018 late season	ZN	Mono	3.22 ± 0.16 Aa	-	252.75 ± 16.75 Bb	-	118.76 ± 4.93 Aa	54.15 ± 1.85 Aa	19.11 ± 0.31 Aa	-
		Inter	3.48 ± 0.08 Ab	2.32 ± 0.05 b	357.50 ± 12.75 Aa	238.33 ± 8.50 a	121.39 ± 5.03 Aa	55.72 ± 2.55 Aa	20.10 ± 0.06 Aa	1.64 ± 0.07 b
	RN	Mono	3.20 ± 0.26 Ba	-	331.50 ± 16.5 Ba	-	108.18 ± 2.66 Aa	56.77 ± 3.74 Aa	19.05 ± 0.32 Aa	-
		Inter	4.90 ± 0.16 Aa *	3.27 ± 0.10 a	405.00 ± 13.75 Aa	270.00 ± 9.17 a	116.95 ± 1.86 Aa	53.92 ± 3.99 Aa	20.14 ± 0.41 Aa	2.70 ± 0.19 a
	CN	Mono	3.61 ± 0.26 Ba *	-	382.75 ± 24.25 Aa	-	117.95 ± 7.94 Aa	54.66 ± 5.45 Aa	18.60 ± 0.63 Aa	-
		Inter	5.17 ± 0.12 Aa	3.45 ± 0.08 a	382.50 ± 16.50 Aa	255.00 ± 11.00 a	125.44 ± 11.32 Aa	57.01 ± 3.14 Aa	19.60 ± 0.18 Aa	2.36 ± 0.18 a
2019 early season	ZN	Mono	4.72 ± 0.15 Bb	-	325.00 ± 12.50 Ba	-	94.34 ± 2.56 Aa	79.09 ± 1.07 Aa	21.14 ± 0.15 Aa	-
		Inter	5.96 ± 0.21 Aa	3.97 ± 0.14 a	460.00 ± 15.25 Ab	306.67 ± 10.17 b	91.69 ± 2.83 Aa	77.85 ± 1.70 Aa	21.2 ± 0.26 Aa	1.38 ± 0.12 b
	RN	Mono	5.63 ± 0.11 Aa	-	397.50 ± 9.25 Ba	-	89.19 ± 4.04 Aa	76.36 ± 1.89 Aa	20.68 ± 0.17 Aa	-
		Inter	5.97 ± 0.19 Aa	3.98 ± 0.13 a	460.00 ± 10.75 Aa *	306.67 ± 7.17 b	97.96 ± 4.16 Aa	77.82 ± 2.49 Aa	20.81 ± 0.14 Aa	2.48 ± 0.15 a
	CN	Mono	5.34 ± 0.06 Ba	-	393.25 ± 12.25 Aa *	-	89.63 ± 3.57 Aa	76.59 ± 0.71 Aa	20.85 ± 0.23 Aa	-
		Inter	6.48 ± 0.23 Aa	4.32 ± 0.15 a	448.75 ± 16.75 Aa	299.17 ± 11.17 b	93.69 ± 6.05 Aa	78.20 ± 2.42 Aa	20.90 ± 0.30 Aa	2.31 ± 0.17 a

All the data presented are the means of four replicates ± standard errors. Different capital letters indicate significant differences between cultivation patterns under the same N fertilizer level ($p < 0.05$). Different lowercase letters indicate significant differences between N fertilizer levels in the same cultivation pattern ($p < 0.05$). Actual yield and effective panicle denote the indexes according to the area ratio of the two crops in the intercropping treatment. ZN, RN and CN indicate the 0, 140 and 180 kg ha^{-1} N fertilizer levels, respectively. "Mono"and "Inter" denote the monocrop-ping and intercropping treatments. The "*" represents a significant difference between mono-cropping with the conventional N treatments and intercropping with zero and reduced N treat-ments ($p < 0.05$).

Table 2. Grain quality of rice in monocropping and intercropping treatments with the three different N fertilizer application levels.

Season	N Fertilizer	Pattern	Brown Rice Rate (%)	Milled Rice Rate (%)	Head Rice Rate (%)	Length/ Width	Chalky Rice Rate (%)	Chalkiness Degree (%)	Amylose Content (%)	Protein Content (%)	Alkali Value
2018 late season	ZN	Mono	75.88 ± 0.31 Ba	62.13 ± 0.64 Aa	51.68 ± 1.77 Aa	3.42 ± 0.03 Aa	3.14 ± 0.41 Aa	0.75 ± 0.17 Aa	17.50 ± 0.16 Aa	12.68 ± 1.36 Aa	6.75 ± 0.06 Aa
		Inter	76.83 ± 0.10Ab	63.00 ± 0.63 Aa	52.93 ± 1.04 Aa	3.43 ± 0.06 Aa	2.28 ± 0.35 Aa	0.29 ± 0.05 Ba	17.30 ± 0.10 Aa	14.13 ± 0.03 Aa	6.75 ± 0.05 Aa *
	RN	Mono	77.15 ± 0.60 Aa	62.03 ± 0.69 Aa	50.15 ± 0.42 Ba	3.47 ± 0.03 Aa	3.25 ± 0.23 Aa	0.61 ± 0.07 Aa	17.83 ± 0.23 Aa	13.18 ± 0.83 Aa	7.03 ± 0.05 Aa
		Inter	76.13 ± 0.59 Aab	61.23 ± 1.06 Aa	52.20 ± 0.30 Aa	3.42 ± 0.05 Aa	1.72 ± 0.25 Ba *	0.24 ± 0.06 Ba	17.63 ± 0.11 Aa	13.15 ± 0.93 Aa	7.05 ± 0.05 Aa
	CN	Mono	76.65 ± 0.85 Aa	62.18 ± 1.14 Aa	50.08 ± 1.53 Aa	3.49 ± 0.05 Aa	2.77 ± 0.35 Aa *	0.44 ± 0.07 Aa	17.75 ± 0.09 Aa	13.05 ± 0.85 Aa	7.08 ± 0.09 Aa *
		Inter	78.05 ± 0.22 Aa	63.20 ± 0.16 Aa	51.40 ± 0.36 Aa	3.48 ± 0.06 Aa	1.59 ± 0.31 Aa	0.43 ± 0.07 Aa	17.48 ± 0.11 Aa	13.23 ± 0.81 Aa	7.08 ± 0.05 Aa
2019 early season	ZN	Mono	73.38 ± 0.33 Ab	58.36 ± 1.07 Aa	38.35 ± 2.32 Aa	3.37 ± 0.02 Ab	2.88 ± 0.43 Aa	0.46 ± 0.04 Aa	17.28 ± 0.23 Aa	8.38 ± 0.31 Ab	6.65 ± 0.02 Ab
		Inter	73.45 ± 0.31 Aa	57.43 ± 1.81 Aa	39.35 ± 0.65 Aa	3.38 ± 0.02 Aa *	1.98 ± 0.41 Aa	0.28 ± 0.038 Ba	17.30 ± 0.39 Aa	8.50 ± 0.31 Ab *	6.73 ± 0.06 Aa *
	RN	Mono	74.13 ± 0.25 Aab	57.74 ± 0.23 Aa	39.12 ± 1.69 Aa	3.42 ± 0.02 Aab	2.98 ± 0.26 Aa	0.35 ± 0.02 Aa	16.90 ± 0.15 Aa	9.95 ± 0.12 Aa	6.88 ± 0.03 Aa
		Inter	73.73 ± 0.37 Aa	60.66 ± 1.58 Aa	41.01 ± 1.28 Aa	3.42 ± 0.01 Aa	1.53 ± 0.17 Ba *	0.25 ± 0.02 Ba	17.08 ± 0.29 Aa	9.83 ± 0.23 Aa	6.88 ± 0.03 Aa
	CN	Mono	74.55 ± 0.23 Aa	60.05 ± 1.31 Aa	39.43 ± 0.65 Aa	3.46 ± 0.01 Aa *	2.47 ± 0.27 Aa *	0.34 ± 0.07 Aa	16.85 ± 0.13 Aa	10.00 ± 0.26 Aa *	6.98 ± 0.05 Aa *
		Inter	74.20 ± 0.42 Aa	59.20 ± 0.51 Aa	37.48 ± 1.86 Aa	3.43 ± 0.01 Aa	1.59 ± 0.12 Ba	0.30 ± 0.02 Aa	17.15 ± 0.44 Aa	9.83 ± 0.28 Aa	6.88 ± 0.85 Aa

All the data presented are the means of four replicates ± standard errors. Different capital letters indicate significant differences between cultivation patterns under the same N fertilizer level ($p < 0.05$). Different lowercase letters indicate significant differences between N fertilizer levels in the same cultivation pattern ($p < 0.05$). ZN, RN and CN indicate the 0, 140 and 180 kg ha^{-1} N fertilizer levels, respectively. "Mono"and "Inter" denote the monocropping and intercropping treatments. The "*" represents a significant difference between monocropping with the conventional N treatments and intercropping with zero and reduced N treatments ($p < 0.05$).

Table 3. Economic analysis (yuan ha^{-1}) of monocropping and intercropping treatments with the three different N fertilizer application levels.

Season	N Fertilizer	Pattern	Rice Seed	Water Mimosa	Fertilizer	Labor	Rice Value	Water Mimosa Value	Net Income
2018 late season	ZN	Mono	600	-	731	7500	12,880	-	4049
		Inter	300	0	731	8250	6960	11,495	9174
	RN	Mono	600	-	1949	7500	12,800	-	2751
		Inter	300	0	1949	8250	9800	18,933	18,234
	CN	Mono	600	-	2291	7500	14,440	-	4049
		Inter	300	0	2291	8250	10,340	16,522	16,021
2019 early season	ZN	Mono	600	-	731	7500	18,880	-	10,049
		Inter	300	0	731	8250	11,920	9694	12,333
	RN	Mono	600	-	1949	7500	22,520	-	12,471
		Inter	300	0	1949	8250	11,940	17,389	18,830
	CN	Mono	600	-	2291	7500	21,360	-	10,969
		Inter	300	0	2291	8250	12,960	16,183	18,302

4. Discussion

4.1. Canopy Microclimate Modifying

Intercropping is a simple and effective way to modify the microclimate of rice canopies [30–32]. In our two-season field experiments, intercropping treatments increased the canopy air temperature compared with the rice monocropping treatments (Figure S2). These results were similar to those of a previous study that showed that canopy temperature increased in maize/cowpea intercropping systems relative to sole maize cropping [33,34]. Additionally, rice/water mimosa intercropping reduced the dew point temperature and relative humidity of rice canopy (Figure S2). Similarly, many studies have demonstrated that intercropping reduces the canopy relative humidity and dew point temperature [30,31]. In the rice/water mimosa intercropping system, water mimosa prostrated growth, rice erected growth, and the height of rice was much higher than that of water mimosa [21]. It is known that higher plants have stronger competition for light in intercropping systems, and high irradiation is usually combined with high temperature and low humidity [14,32]. In addition, the height difference between the two crops exhibited better air circulation. Moreover, this intercropping system had a higher air temperature and lower relative humidity and dew point temperature and, thus, exhibited a special canopy microclimate of rice as a result (Figures 3 and S2).

4.2. Pest and Disease Control

It is believed that intercropping usually reduces the occurrence of harmful organisms due to the high biodiversity and stability of the ecosystem [1]. In the present study, rice/water mimosa intercropping reduced the incidences of rice leaf blast, leaf folders, and sheath blight (Figure 2, Tables S1, S3 and S5). A similar study on rice and water spinach intercropping in paddy fields also showed that pests and diseases were substantially lower in intercropping systems [4]. In addition, our study also showed that the occurrence of pathogens had a positive correlation with dew point temperature and relative humidity and a negative correlation with air temperature in the late season of 2018 (Figure S3). High air temperature inhibited rice leaf roller egg hatching and reduced the longevity of this insect [1]. Hence, the high air temperature in this intercropping system might contribute to reducing the breeding of rice folders. Universally, dew is formed at night, and relative humidity can control fungal spores to germinate the conidial sporulation and dispersal process of pathogens [31,33]. Thus, the high temperature and low dew point and humidity probably had some functions to control pathogens in the present study.

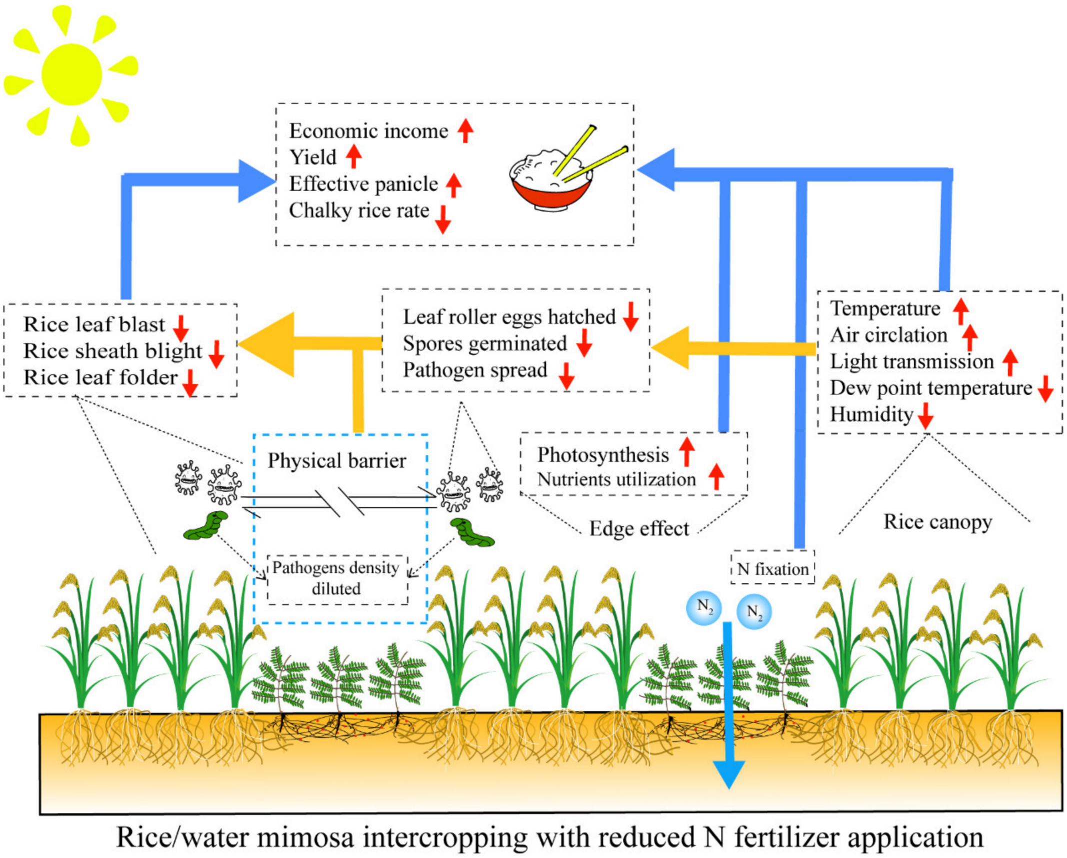

Figure 3. Mechanisms of rice/water mimosa intercropping with reduced N application to modify canopy microclimate of rice, control pest and disease, and improve rice yield, grain quality, and economic income.

According to the characteristics of the rice pest and disease, we speculated and summarized that the causes of rice/water mimosa intercropping reduced the incidences of pathogens would be as follows. (1) The strip distribution in the intercropping system established a physical barrier that blocked the horizontal spread of the pathogens [1]. (2) Microclimatic factors such as high temperature, low relative humidity and dew point temperature, stronger solar radiation, and better air circulation in the intercropping system had some functions in resisting pests' and diseases' activity, such as insect migration and reproduction [4,34]. (3) Water mimosa was neither the food nor the host of the pathogens; hence, the density of pathogens was relatively diluted. Thus, the incidences of pests and diseases reduced in the rice/water mimosa intercropping system (Figure 3).

Moreover, previous studies revealed that N fertilizer usually increases the disease susceptibility of rice [35–37]. However, in our study, there were no significant differences between N fertilizer levels in the incidences of rice leaf blast, sheath blight, and leaf folders. The reason for this phenomenon was probably that the application of N fertilizer was insufficient to increase the disease susceptibility of rice in the present study because 180 kg ha^{-1} N was the optimal N fertilizer for rice growth in paddy fields [1,4,35–37]. We also found that the pests and diseases in the monocropping with conventional N treatments were significantly higher than those in the intercropping with zero, reduced, and conventional N treatments (Figure 2, Tables S1, S3 and S5). Therefore, the reduction in rice pest and disease rates in rice/water mimosa intercropping could mainly be attributed to the border effect.

4.3. Yield and Yield Components Advantages

Environmental conditions and management methods play a crucial role in yield and yield components [2]. The increase in rice per unit yield in the early season of 2019 might be attributed to the higher rainfall compared with that in the late season of 2018 (Figure S1 and Table S8). This is consistent with a previous study that showed that sufficient irrigation could increase crop yield [38]. In the present study, we observed that the interaction between cultivation pattern and N fertilizer significantly affected the rice per unit yield and effective panicle numbers in the two seasons (Table S7). These results are in line with previous research that showed that the interactive effect between the intercropping pattern and N fertilization significantly affected plant growth in a groundnut (*Arachis hypogaea* Linn.) and sesame (*Sesamum indicum* Linn.) intercropping system [39]. In the present research, compared with zero N treatment, the reduced and conventional N treatments substantially increased the yield of rice and water mimosa (Table 1). As a comparison, previous research found that crop yield increased with N fertilizer levels [40,41]. It is acknowledged that higher grain yield was obtained due to higher panicle numbers [40]. Similar to rice yield, reduced and conventional N treatments also had higher rice effective panicle numbers than zero N treatments (Table 1). Ju et al. (2019) [24] also reported that N fertilizer application increased the panicle numbers of rice. Consequently, both reduced and conventional N treatments were beneficial to rice yield and effective panicle numbers.

Intercropping generally increased the crop yield. In the present study, intercropping treatments increased rice per unit yield by 43.00%~53.10% in the late season of 2018 and 21.40%~26.18% in the early season of 2019. Meanwhile, per unit effective panicle numbers were higher in rice/water mimosa intercropping than in rice monocropping (Table 1). Our results were consistent with previous studies. Ning et al. (2017) [1] and Liang et al. (2016) [4] both found that rice/water spinach intercropping increased the rice yield and effective panicle numbers. In addition, intercropping treatments significantly increased the yield, while there was no significant difference in grain components between cultivation patterns during most of the time (Table 1). The reason probably was that rice had higher per unit effective panicle numbers among the intercropping treatments, and the effective panicle number was one of the yield components. The increase in rice per unit yield in intercropping treatments could also be attributed to the promoting effect of water mimosa N fixation [23,42]. Moreover, the rice per unit yield and effective panicle numbers in the intercropping with reduced N treatments were higher than those in the monocropping with conventional N treatments (Table 1). These findings suggested that rice/water mimosa intercropping combined with reduced N fertilizer application would be a good practice choice to increase rice yield in paddy fields.

4.4. Grain Quality and Economic Incomes Improvement

Grain quality comprises grain appearance, milling, cooking, eating, and nutritional qualities and depends on genetic, environmental, and crop management factors [27,43]. Our results indicated that N fertilizer improved rice grain quality. For instance, the brown rice rate, protein content, length/width, and alkali value in the reduced and conventional N treatments were higher than those in the zero N treatments (Table 2). These results are consistent with previous studies which found that crop quality indexes improved with N fertilizer rates [25,44,45].

Moreover, intercropping had a positive and significant effect on brown and head rice rates (Table 2). An increase in crop quality in intercropping systems was also reported by Yusef et al. (2014) [37]. Low chalkiness is often associated with more translucent rice grain and represents a higher value and price of rice in markets [46,47]. In the present study, rice/water mimosa intercropping resulted in a significant reduction in the chalky rice rate and chalkiness degree (Table 2). The lower chalky rate and chalkiness degree of rice probably bring more profit to farmers and producers. In the rice/water mimosa intercropping system, the reason that led to better grain quality could be attributed to the following aspects: (1) Organic fertilizer and biofertilizer supplied by water mimosa could improve grain quality by improving photosynthesis and nutrient uptake by cereals, and these nutrients were eventually transported to the seed and contributed to improving grain quality (Figure 3) [48]. (2) Rice grain quality was associated with microclimatic parameters (Figure S3). Similarly, intercropping had higher temperature and good ventilation and light transmission, which could lead to less inhomogeneous substances, such as white opacity and chalkiness in grains [49]. (3) Our study found that the incidences of pathogens had negative correlations with the head rice rate and had positive correlations with the chalky rice rate and chalkiness degree (Figure S3). These results are consistent with a previous study which showed that lower pathogens in rice resulted in better grain quality (Figure 3) [50,51].

Notably, compared with monocropping with conventional N treatments, intercropping with zero N treatments resulted in lower length/width and alkali values. However, intercropping with reduced N treatment decreased the chalky rice rate relative to monocropping with conventional N treatments (Table 2). Therefore, we could infer that intercropping with reduced N treatment is an optimal choice for rice grain quality.

For economic analysis, we found that the intercropping system with reduced N treatments had the highest economic income (Table 3). Higher net income has also been reported under potato/legume and sorghum/legume intercropping systems [28,52]. Rice/water mimosa intercropping with reduced N treatments achieved the maximum economic income which would be beneficial for farmers and producers. However, there were only two seasons of performance assessment in this study, and further long-term experiments are needed in the future.

5. Conclusions

Combining rice/water mimosa intercropping and N fertilizer reduction could not only decrease N fertilizer application by approximately 40 kg ha^{-1}, which would mitigate nonpoint source pollution, but also form a canopy microclimate of rice with higher temperature, lower relative humidity and dew point temperature, and better control of rice pests and diseases to a certain extent, which would alleviate pesticide usage, finally increasing rice productivity, grain quality, and economic income, and that can be extended for green rice production to increase income for farmers and producers. In a word, our study provided an environmentally friendly eco-farming technique in rice production.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture12010013/s1, Figure S1: Rainfall (RF) and average temperature (AT) per month during the two seasons in Zengcheng, Guangzhou, Guangdong, China. Figure S2: Air temperature, relative humidity (RH) and dew point temperature of rice canopy in the monocropping and intercropping under the reduced N fertilizer application level. Figure S3: Pearson correlations among daily air temperature (AT), dew point temperature (DPT) and relative humidity (RH) of rice canopy at 50th ~ 75th after transplanting, rice leaf blast (RLB), rice leaf folders (RLF) and rice sheath blight (RSB) at 75th day after transplanting, rice yield (RY), per unit effective panicle number (EPN), head rice rate (HRR), chalky rice rate (CRR) and chalkiness degree (CD) at maturity stage. Table S1: Incidence (%) of rice leaf blast in monocropping and intercropping treatments with the three different N fertilizer application levels. Table S2: Variance in incidence of rice leaf blast between the 2018 late season and 2019 early season. Table S3: Incidence (%) of rice leaf folders in monocropping and intercropping treatments with the three different N fertilizer application levels. Table S4: Variance in incidence of rice leaf folders between the 2018 late season and 2019 early season. Table S5: Disease index (%) of rice sheath blight in monocropping and intercropping treatments with the three different N fertilizer application levels. Table S6: Variance in incidence of rice sheath blight between the 2018 late season and 2019 early season. Table S7: Interaction effects between the two factors (cultivation pattern and N fertilizer) on rice yield and yield components by two-way ANOVA. Table S8: Variance in rice yield and yield components between the 2018 late season and 2019 early season. Table S9: Interaction effects between the two factors (cultivation pattern and N fertilizer) on rice grain quality determined by two-way ANOVA. Table S10: Variance in rice grain quality between the 2018 late season and 2019 early season.

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