

Research Article

Effects of Chemical Fertilizer Application upon the Water Quality Parameters of a Rice–Eel (*Monopterus albus*) Coculture System

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Received 26 October 2022; Revised 20 December 2022; Accepted 7 January 2023; Published 7 February 2023

Academic Editor: Mohamed Abdelsalam

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The excessive use of chemical fertilizers causes many problems for which solutions are being sought in a variety of agricultural production systems. From the perspective of paying attention to the health requirements of aquatic animals in terms of water quality, this study investigated the impact of chemical fertilizer application on water quality in a rice (*Oryza sativa* L.; Cultivar “Qingxiangruangeng,” accession number: 2014004)–eel (*Monopterus albus*) coculture system in Shanghai, China. Chemical fertilizer was applied four times—as base fertilizer, rejuvenated fertilizer, tiller fertilizer, and ear granule fertilizer—during the production process of a rice–eel coculture system (June to October 2020). Changes in the water quality parameters of rice field’s surface water and ring ditch water in the regular chemical fertilizer group (RF) and no chemical fertilizer group (NF, the control) were compared before and at 24 h, 48 h, and 72 h after the chemical fertilizer application. The results for the analyzed physical and chemical indexes of each water area before and after four fertilizations revealed several consistent trends. First, the pH, dissolved oxygen (DO), water temperature (T), and chemical oxygen demand (COD) of either water area were similar between the NF and RF groups, whereas their total nitrogen (TN), total ammonia nitrogen (TAN), and total phosphorus (TP) levels differed significantly. After adding the above fertilizer containing nitrogen and phosphorus, 24 h later, the TN, TAN, and TP content had already increased significantly in comparison with the control. The maximum average content of TAN and nitrite nitrogen (NO_2^- -N) in the ring ditch water reached 12.30 mg/L and 0.37 mg/L, respectively, at 24 h after the chemical fertilizer application. Nonlinear regression analysis results showed that there was a significant positive relationship TN (δTN) and TAN (δTAN) vis-à-vis the nitrogen content of the fertilizer. The results of this study provide a timely empirical reference and data support for improving fertilizer management in rice–eel coculture systems.

1. Introduction

Since its implementation in the early 21st century, the rice–fish coculture model has come to be widely practiced in paddy fields of many Asian countries, especially in China [1, 2]. The products produced by rice–fish coculture systems are highly favored by consumers because of their safety and high quality.

The Asian swamp eel (*Monopterus albus*, Zuiew 1793) is one of the most economically important freshwater fish species in China and other Asian countries [3], namely Cambodia, Singapore, Thailand, and Vietnam [4]. The annual output of *M. albus* in China has reached 386 137 tonnes [5]. Currently, the main way to culture *M. albus* is in cages or

paddy fields. With mounting concern over prominent environmental and food safety problems associated with cage eel farming in ponds or rice fields, rice–eel (*M. albus*) coculture is becoming increasingly popular, and the price of *M. albus* produced by this model is significantly higher than that of pond cage culture products in China. Hubei, Jiangsu, and other provinces in China have promoted and demonstrated the ecological comprehensive model for joint planting and breeding of rice and eel, respectively. Yet, there are few research reports focused on the rice–eel (*M. albus*) coculture system. In practice, rice planting and *M. albus* breeding were carried out separately, without considering the interaction between them, especially the impact of fertilization on water quality. There is a lack of data support

to effectively build relevant industry standards, which is not conducive to the sustainable development of the industry.

Much research has been conducted on other rice–fish coculture models, especially on the reductions of its use of chemical fertilizers. For example, Hu et al. [6] found that relative to a rice monoculture, the use of nitrogen fertilizer and pesticides decreased, and the farmers' net income increased, in a rice–fish coculture system. Earlier, Xie et al. [1] showed that a rice–fish coculture system was able to reduce the amount of chemical fertilizer input by 24% when compared with a rice monoculture system. Although the rice–fish coculture model requires less chemical fertilizer applied than would a single rice cultivation model, it is impossible to not use any chemical fertilizer at all. A recent study suggests the application of chemical fertilizer may be related to the survival rate of loach juveniles [7]. When conventional chemical fertilizers are applied to rice fields, they will dissolve in the water area and then flow into the ring ditch water area, where they may induce the rapid increase of nutrients' content, such as ammonia nitrogen and nitrite nitrogen, which could pose a real threat to the health of aqua-cultured animals. Previous studies published in Chinese journals have paid more attention to altered water quality in the process of aquaculture, and a few papers published in international journals have paid attention to how the water quality of the field surface water area is changed during rice production (e.g., [8], leaving less known about the impact of fertilization in the rice–fish coculture system upon local water quality.

From the perspective of paying attention to the health requirements of aquatic animals in terms of water quality, this paper studied the effects of chemical fertilizer application on water quality in a rice–fish coculture system in China. To do this, we selected important and commonly used monitoring indicators in aquaculture [7], such as pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), and nitrite nitrogen (NO_2^- -N). TAN and NO_2^- -N are widely considered to be toxic to aquatic animals and capable of causing several adverse effects to them in the process of aquaculture [9, 10]. Identifying the ways in which these nitrogenous compounds are altered in rice–fish coculture system is particularly important to improve the technical level of this system's management. In this study, we elucidate the effects of chemical fertilizer on the physical and chemical environment of surface water and ring ditch water areas in rice fields under the rice–eel coculture system, so as to provide data support for the production and management of the rice–eel coculture model in a broader sense. In addition, this study could also serve as a practical reference for other rice–fish coculture models in Asia.

2. Materials and Methods

2.1. Study Site and Materials. The research was performed at the rice–fish coculture experimental base of the Zhuanghang Comprehensive Experimental Station of the Shanghai Academy of Agricultural Sciences, in China. The experimental plot area was newly excavated in 2016.

Six plots were used in the experiment; each plot is 20 m^2 , of which 10% is the ditch water area and 50% corresponds to the rice planting area (about 10 m^2), with the remaining ca. 40% of the plot consisting of the ridge area. A bird-proof net was arranged above the platform, a 40 mesh (aperture 0.425 mm) filter screen was set at the water inlet, and an antiescape net made of 40 mesh silk net was installed at the water outlet. The tested rice variety is “Qingxiang soft stem” (HNPS rice 2014 No. 004) bred by Shanghai Qingpu District Agricultural Technology Extension Service Center. The experimental *Monopterus albus* juveniles were collected from Changshu, Jiangsu Province. Two kinds of fertilizers were used in rice production: compound fertilizer and urea. The ratios of nitrogen, phosphorus, and potassium in these fertilizers are shown in Table 1.

2.2. Experimental Design and Treatments. Rice was planted on June 28, 2020. The spacing of rice plants and rows was 12 cm and 20 cm, respectively. Eight *M. albus* juveniles (mean \pm SD, $16.93 \pm 1.84 \text{ g}$) were released into each 20 m^2 plot on July 3, 2020. There was no water exchange and feed occurred during the whole production process.

Two treatments were implemented: NF, the no fertilizer treatment (i.e., the control), and RF, the conventional regular fertilization treatment. The nitrogen application rate used was 300 kg/ha (pure N), according to the conventional dosage in Shanghai, and the phosphorus application rate was 117 kg/ha. Each treatment was replicated three times (the unit of replication is the 20 m^2 plot). Both treatments were randomly arranged in the experimental plots. The growth period of rice plants is from late June to late October. Fertilizer was applied four times during this period (June to October 2020); see Tables 1 and 2 for the experimental treatment and respective fertilizer amounts. Before each fertilization (BF) and at 24 h, 48 h, and 72 h after fertilization (respectively, AF24, AF48, and AF72), water samples were collected from the rice field's surface and the ring ditch of each experimental plot and were measured the physical and chemical indexes of both water areas (i.e., surface water area and ring ditch water area).

2.3. Physicochemical Measurements. A portable water quality analyzer (HACH HQ40d, USA) was used to measure the water temperature (T), dissolved oxygen (DO), and pH in situ, and 250 mL surface water samples from each of the middle of the paddy field and the ring ditch were collected with 250 mL wide-mouth bottle and taken to the laboratory. Total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), total ammonia nitrogen (TAN), and nitrite nitrogen (NO_2^- -N) were measured for each sample within 24 h of its collection. All the above response variables were tested by purchasing the HACH prefabricated reagent and following the instructions of the “Water Analysis Handbook” [11].

TABLE 1: Treatment groups and fertilizer dosage (kg/ha).

Fertilization stage	Base fertilizer	Rejuvenated fertilizer	Tiller fertilizer	Ear granule fertilizer		Actual nutrient consumption		
Fertilization time	2022.6.23	2020.7.6	2020.7.19	2020.8.9				
Fertilizer sources	Compound fertilizer	Compound fertilizer	Urea	Urea	Compound fertilizer	N	P ₂ O ₅	K ₂ O
No fertilization (NF)	0	0	0	0	0	0	0	0
Regular fertilization (RF)	449.8	337.3	73.3	81.4	187.4	266.1	116.9	155.9

Note. Urea contains 46% nitrogen; total nutrients of the compound fertilizer is 48%, in the form of N-P₂O₅-K₂O as 20%-12%-16%. The amount of fertilizer in the experimental plot is converted here according to each plot's area (10 m²).

TABLE 2: Nitrogen and phosphorus content of the chemical fertilizer applied each time (kg/ha). N: nitrogen, P₂O₅: phosphorus pentoxide.

Treatment groups	Base fertilizer		Rejuvenated fertilizer		Tiller fertilizer		Ear granule fertilizer	
	N	P ₂ O ₅	N	P ₂ O ₅	N	P ₂ O ₅	N	P ₂ O ₅
No fertilization (NF)	0	0	0	0	0	0	0	0
Regular fertilization (RF)	89.96	53.98	67.46	40.48	33.72	0	74.92	22.49

2.4. *Data Analysis.* Data processing was carried out in Microsoft Excel 2013 (Microsoft Co., Redmond, WA, USA), and the statistical analyses were conducted in SPSS 23.0 (IBM, Armonk, NY, USA). A two-way repeated measures ANOVA with water area (2 levels: surface vs ditch) and fertilizer (2 levels: NF vs RF) as the fixed factors, with the four time points as the repeated time factor, was conducted at $P < 0.05$. Origin 9.0 (Electronic Arts Inc, USA) was used for plotting the graphs. Nonlinear relationships between water parameters were tested using Pearson's correlation coefficient. The increase of TN (δ TN) and TAN (δ TAN) at 24 h postfertilization in water as a function of the nitrogen content of fertilizer was evaluated using nonlinear regression.

The relevant formulas were as follows:

$$\begin{aligned} \delta TN &= TN_{AF24} - TN_{BF}, \\ \delta TAN &= TAN_{AF24} - TAN_{BF}. \end{aligned} \tag{1}$$

3. Results

3.1. *Effects of Base Fertilizer on Water Quality.* The base fertilizer was a compound fertilizer, for which the contents of nitrogen and phosphorus were 89.96 kg/ha and 53.98 kg/ha, respectively (Table 2). The changes to water quality before and at 24 h, 48 h, and 72 h postfertilization are shown in Figure 1. Two-way repeated measures ANOVAs detected no significant differences at each time point in pH, dissolved oxygen (DO), or temperature (T) between the NF and RF treatments ($P > 0.05$). The water temperature of the field surface was $28.26 \pm 0.55^\circ\text{C}$ (mean \pm SD) (Figure 1(i)), and that of the ring ditch water area was $27.92 \pm 0.34^\circ\text{C}$ (mean \pm SD) (Figure 1(j)).

The pattern of variation in the content of total nitrogen (TN) and total ammonia nitrogen (TAN) in rice field's surface water area and ring ditch water area was consistent, with TN and TAN levels increased significantly after fertilization, following a trend of first rising and then falling over time, whereas the values of TN and TAN in the control

group (NF) did not fluctuate significantly. The two-way repeated measures ANOVAs revealed that at 24 h and 48 h postfertilization, the contents of TN and TAN in surface water and ring ditch water were significantly higher under the RF than NF treatment group ($P < 0.05$). After 24 h of fertilization, the mean content of TN in surface water and ring ditch water reached as high as 44.27 mg/L and 41.8 mg/L, respectively; their corresponding mean content of TAN peaked at 11.41 mg/L and 12.30 mg/L. There was no significant difference in nitrite nitrogen (NO₂⁻-N) content between RF and NF treatment group at any four time points during the experiment in either rice field surface water or ring ditch water (all P -values > 0.05). However, as evinced by Figure 1, the NO₂⁻-N content of either water area type showed an upward trend with prolonged fertilization, especially at 48 h postfertilization. The mean content of NO₂⁻-N in surface water and ring ditch water of rice fields was as high as 0.25 mg/L and 0.37 mg/L, respectively.

The total phosphorus (TP) content of surface water was significantly higher under the RF than NF treatment group at 24 h ($P < 0.05$) (Figure 1(o)), while the TP content of ring ditch water was significantly higher under the RF than NF treatment group at 48 h ($P < 0.05$) (Figure 1(p)). There was no significant difference between RF and NF treatment groups at the other monitoring times ($P > 0.05$). This showed that the increased TP content in the ring ditch water lagged behind that in surface water. At 24 h after this fertilization, the chemical oxygen demand (COD) content of rice field's surface water and ring ditch water was significantly higher under the RF than NF treatment group ($P < 0.05$), yet no significant differences were found at other monitoring times ($P > 0.05$).

3.2. *Effects of Rejuvenated Fertilizer on Water Quality.* Rejuvenated fertilizer consisted of urea and compound fertilizer, whose nitrogen and phosphorus contents were 67.46 kg/ha and 40.48 kg/ha, respectively (Table 2). Figure 2 shows water quality changes before and at 24 h, 48 h, and 72 h postfertilization. The two-way repeated measures

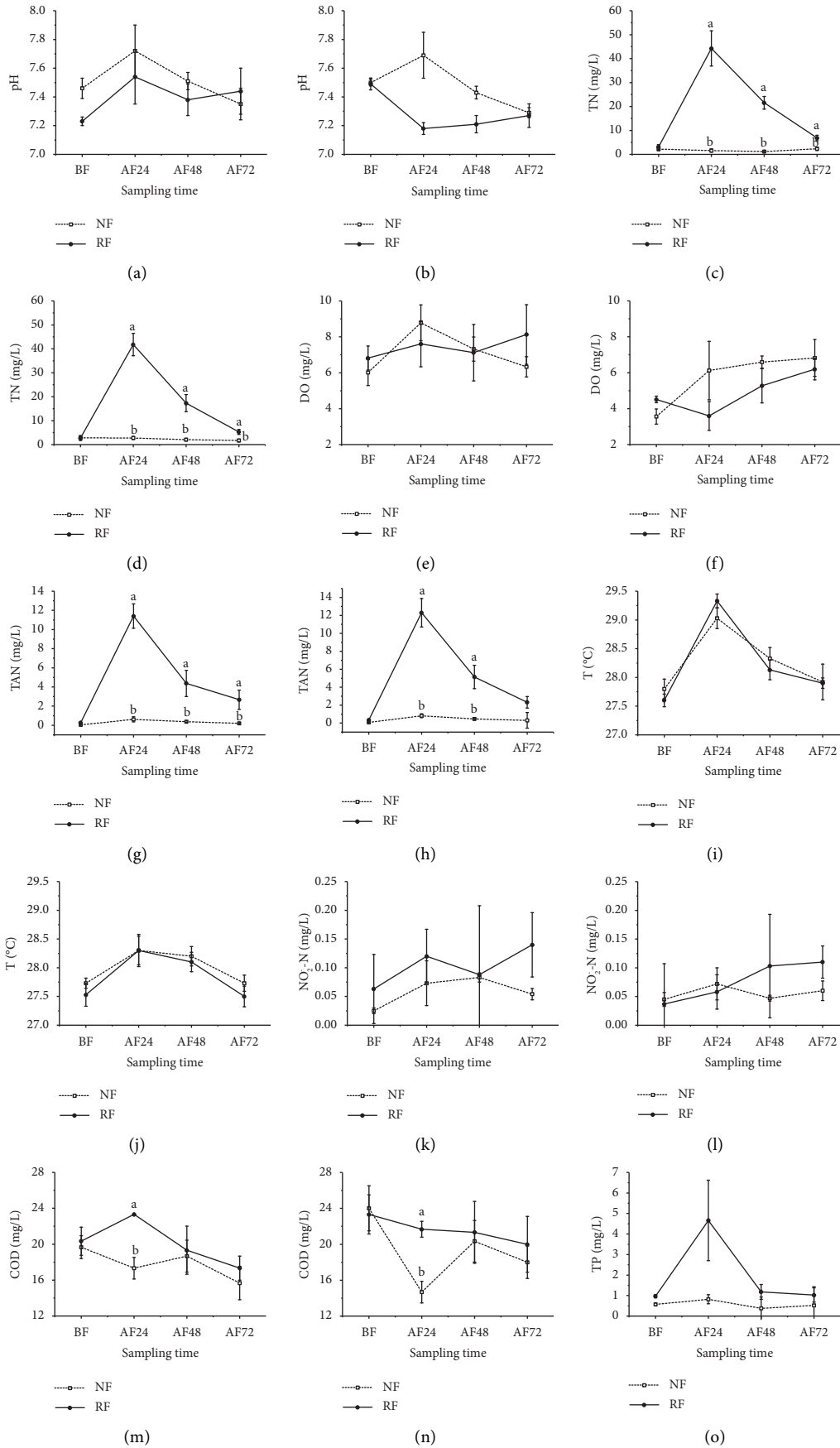


FIGURE 1: Continued.

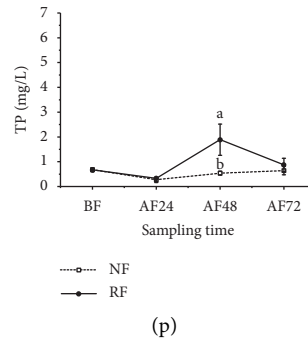


FIGURE 1: Changes in the water quality parameters of rice field's surface water and ring ditch water in the fertilization (RF) and non-fertilization (NF) treatment groups before (BF) and at 24 h (AF24), 48 h (AF48), and 72 h (AF72) after the base fertilizer application (mean \pm SE, $n = 3$). DO: dissolved oxygen, (T) temperature, TN: total nitrogen, TP: total phosphorus, TAN: total ammonia nitrogen, NO_2^- -N: nitrite nitrogen, COD: chemical oxygen demand. (a), (c), (e), (g), (i), (k), (m), (o) Rice field. (b), (d), (f), (h), (j), (l), (n), (p) Ring ditch.

ANOVAs revealed no significant differences in pH, DO, T, or COD between the NF and RF treatment groups (all P -values >0.05).

Before fertilization, the levels of TN, TP, and TAN were similar between the RF and NF treatment groups (all P -values >0.05). At 24 h postfertilization, the content of TN, TP, and TAN in rice field's surface water and ring ditch water in the RF treatment group significantly surpassed those in the NF treatment group ($P < 0.05$). The TN, TP, TAN values in surface water and ring ditch water, respectively, were 14.62 and 16.35 mg/L, 2.91 and 5.50 mg/L, 5.05 and 1.80 mg/L. At 48 h and 72 h postfertilization, the differences in the TN and TAN content between RF and NF treatment groups were negligible ($P > 0.05$). The two-way repeated measures ANOVAs showed that the TP content of surface water was still significantly greater under the RF than NF treatment group at both 48 h and 72 h postfertilization (Figure 2(o), $P < 0.05$), while that of ring ditch water was similar between the RF and NF treatment group at 72 h (Figure 2(o), $P > 0.05$). After fertilization, there was no significant difference in NO_2^- -N content between the two treatments although the NO_2^- -N content in both the RF and NF groups showed an upward trend.

3.3. Effects of Tiller Fertilizer on Water Quality. The tiller fertilizer consisted of urea, and this contains 33.72 kg/ha and 0 kg/ha of nitrogen and phosphorus, respectively (Table 2). The changed water quality before and at 24 h, 48 h, and 72 h since fertilization is depicted in Figure 3. Because this fertilization did not input any phosphorus, the trend for the TP content in both surface water and ring ditch water in either the RF or NF group was similar over time, with no significant difference between the two groups before and after fertilization (Figures 3(o) and 3(p)). The statistical analysis indicated no significant differences in pH, DO, T, or COD between the NF and RF treatment groups (all P -values >0.05).

After fertilization, when compared with NF, the TN and TAN contents under the RF treatment group increased significantly. At 24 h postfertilization, the TN content of

surface water under RF significantly exceeded that under NF ($P < 0.05$), and the mean content of TN was 12.13 mg/L and 7.70 mg/L in surface water and ring ditch water, respectively. At 24 h after this fertilization, the TAN content of ring ditch water was significantly higher under the RF than NF treatment group ($P < 0.05$), and the mean TAN content in surface water and ring ditch water was, respectively, 4.93 mg/L and 3.40 mg/L. The NO_2^- -N content did not differ significantly between the RF and NF treatment groups before or any time after fertilization, but the NO_2^- -N content of the RF group did show an upward trend.

3.4. Effects of Ear Granule Fertilizer on Water Quality. Ear granule fertilizer consisted of urea and compound fertilizer, whose nitrogen and phosphorus contents were 74.92 kg/ha and 22.49 kg/ha, respectively (Table 2). The changes to water quality before and at 24 h, 48 h, and 72 h postfertilization are presented in Figure 4. The statistical analysis uncovered no significant differences in pH, COD, or NO_2^- -N between the NF and RF treatment groups (all P -values >0.05). At 48 h and 72 h after fertilization, the DO content of surface water was significantly lower under the RF than NF treatment group, but no significant differences were found at the other monitoring time points.

As seen in Figure 4, the trends for TN, TP, and TAN in the water areas of the RF vis-à-vis the NF treatment group are the same. After fertilization, the content of TN, TP, and TAN in the RF group increased significantly but then decreased with more elapsed time. The two-way repeated measures ANOVAs showed that at 24 h after fertilization, the contents of TN, TP, and TAN were significantly greater under the RF than NF treatment (all P -values <0.05); their corresponding mean values in surface water and ring ditch water were 20.27 mg/L and 19.97 mg/L, 5.11 mg/L and 5.50 mg/L, and 7.27 mg/L and 7.60 mg/L.

3.5. Correlation Analysis between Water Quality Indexes. Pearson correlation analysis results are summarized in Table 3. Significant positive correlations were found between

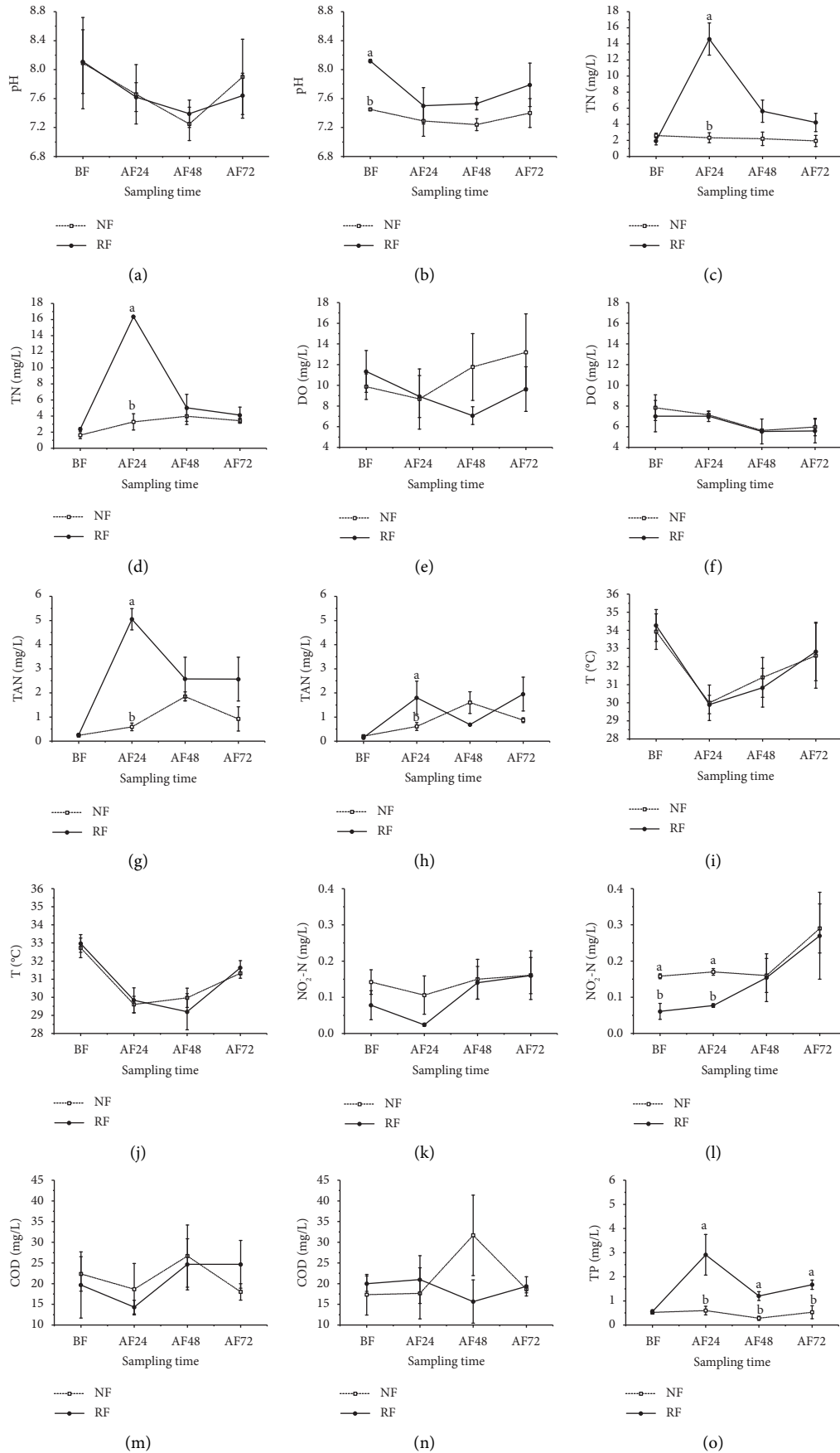


FIGURE 2: CONTINUED.

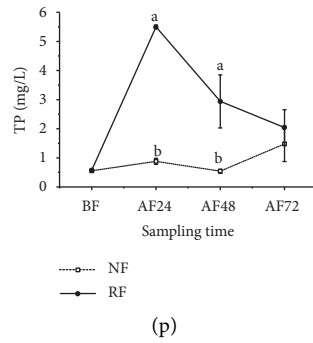


FIGURE 2: Changes in the water quality parameters of rice field's surface water and ring ditch water in the fertilization (RF) and non-fertilization (NF) treatment groups before (BF) and at 24 h (AF24), 48 h (AF48), and 72 h (AF72) after the rejuvenated fertilizer application (mean \pm SE, $n = 3$). DO: dissolved oxygen, (T) temperature, TN: total nitrogen, TP: total phosphorus, TAN: total ammonia nitrogen, NO_2^- -N: nitrite nitrogen, COD: chemical oxygen demand. (a), (c), (e), (g), (i), (k), (m), (o) Rice field. (b), (d), (f), (h), (j), (l), (n), (p) Ring ditch.

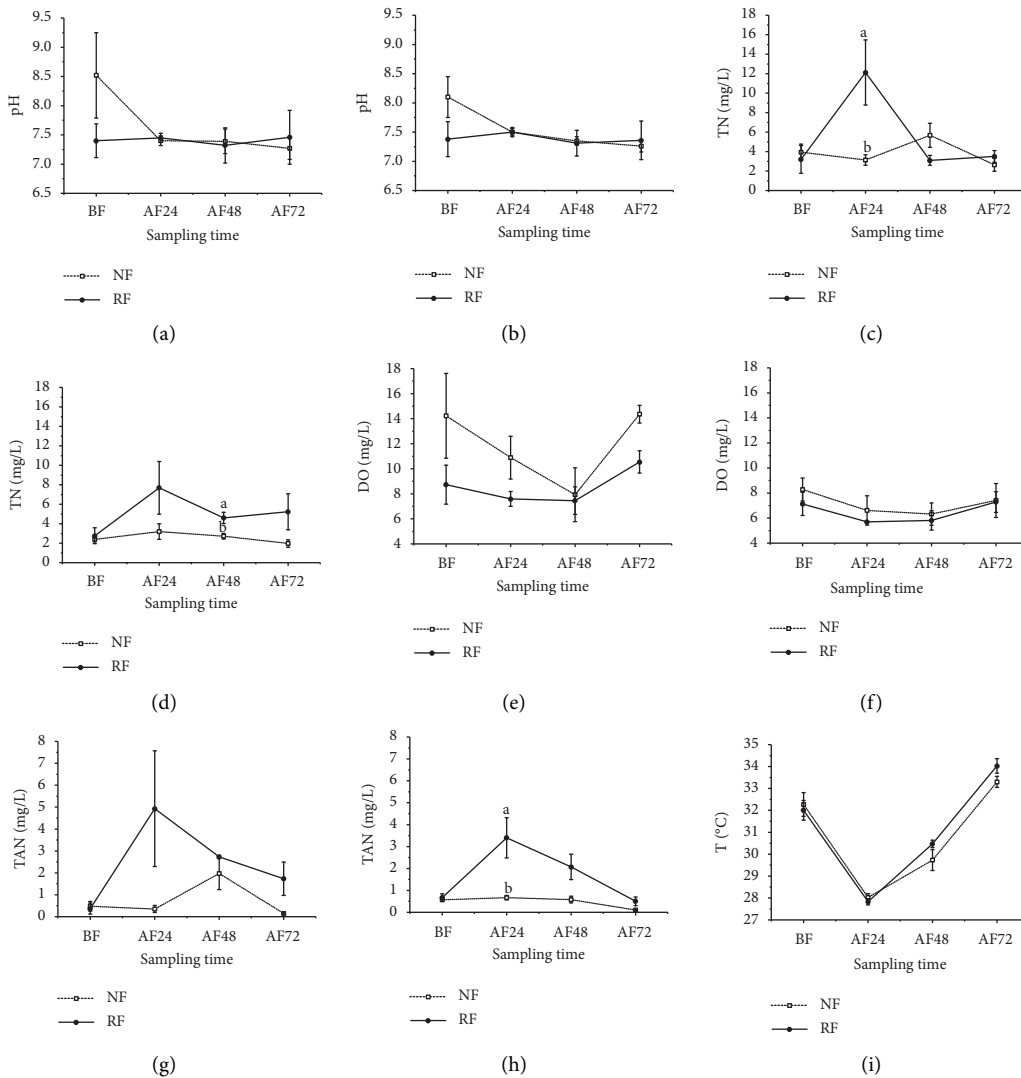


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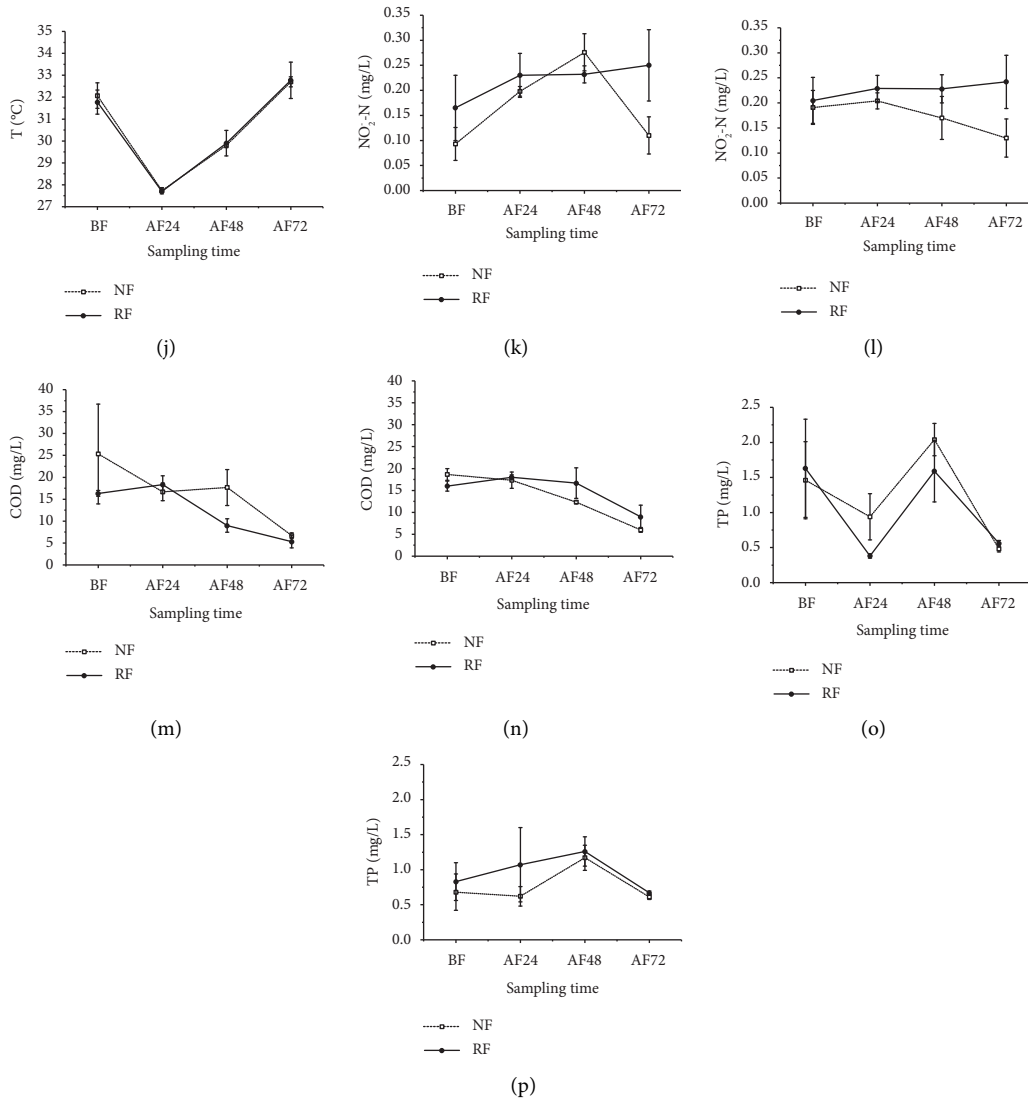


FIGURE 3: Changes of water quality parameters of rice field's surface water and ring ditch water in the fertilization (RF) and non-fertilization (NF) treatment groups before (BF) and at 24 h (AF24), 48 h (AF48), and 72 h (AF72) after the tiller fertilizer application (mean \pm SE, $n = 3$). DO: dissolved oxygen, (T) temperature, TN: total nitrogen, TP: total phosphorus, TAN: total ammonia nitrogen, NO₂⁻-N: nitrite nitrogen, COD: chemical oxygen demand. (a), (c), (e), (g), (i), (k), (m), (o) Rice field. (b), (d), (f), (h), (j), (l), (n), (p) Ring ditch.

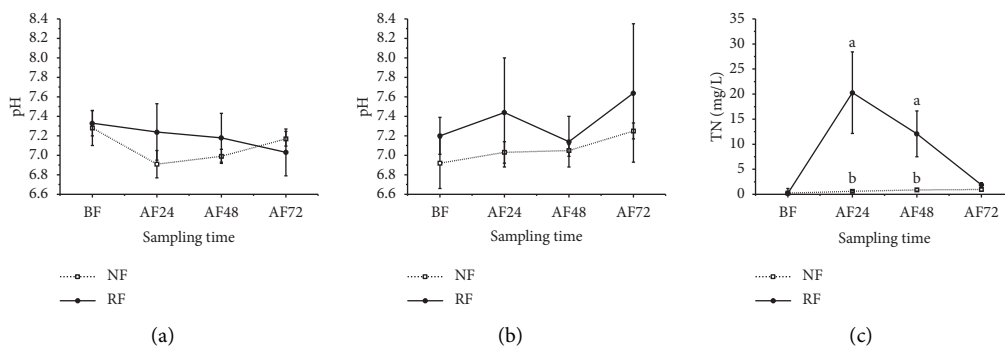


FIGURE 4: Continued.

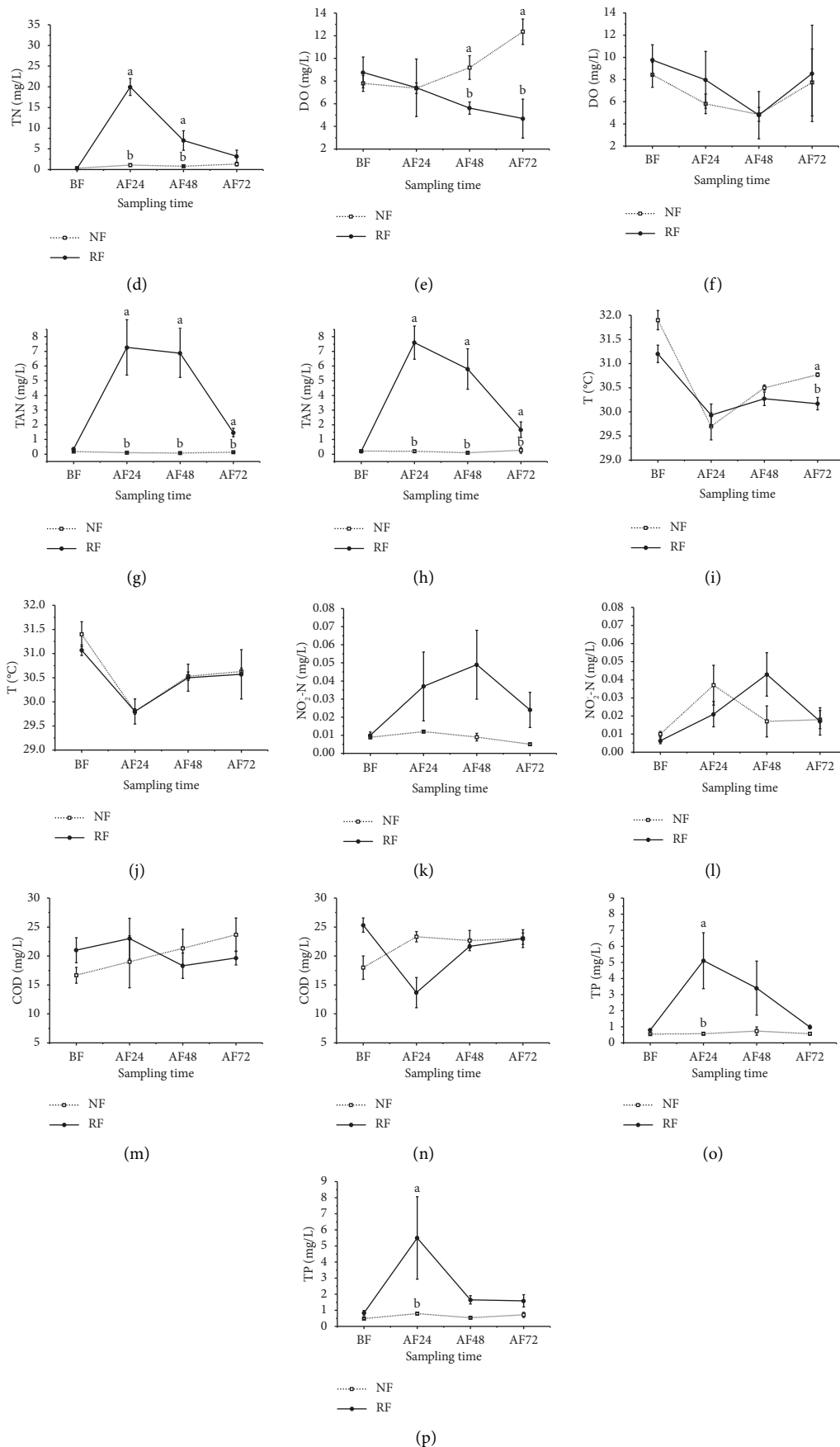


FIGURE 4: Changes of water quality parameters of rice field's surface water and ring ditch water in the fertilization (RF) and non-fertilization (NF) treatment groups before (BF) and at 24 h (AF24), 48 h (AF48), and 72 h (AF72) after the ear granule fertilizer application (mean \pm SE, $n = 3$). DO: dissolved oxygen, (T) temperature, TN: total nitrogen, TP: total phosphorus, TAN: total ammonia nitrogen, NO₂⁻-N: nitrite nitrogen, COD: chemical oxygen demand. (a), (c), (e), (g), (i), (k), (m), (o) Rice field. (b), (d), (f), (h), (j), (l), (n), (p) Ring ditch.

TABLE 3: Pearson correlations between the physical-chemical properties of rice field's surface water and ring ditch water. DO: dissolved oxygen, T: temperature, TN: total nitrogen, TP: total phosphorus, TAN: total ammonia nitrogen, NO₂⁻-N: nitrite nitrogen, COD: chemical oxygen demand.

		DO	T	TN	TP	TAN	NO ₂ ⁻ -N	COD
pH	r	0.540**	0.344**	-0.052	0.079	-0.162*	0.030	0.053
	p	0.000	0.000	0.530	0.337	0.048	0.719	0.519
DO	r		0.501**	-0.149	0.054	-0.243**	0.031	-0.028
	p		0.000	0.070	0.511	0.003	0.704	0.739
T	r			-0.213**	-0.095	-0.194*	0.130	-0.169*
	p			0.009	0.246	0.018	0.113	0.040
TN	r				0.532**	0.882**	-0.102	0.096
	p				0.000	0.000	0.214	0.244
TP	r					0.503**	-0.142	-0.026
	p					0.000	0.082	0.755
TAN	r						-0.092	0.089
	p						0.264	0.282
NO ₂ ⁻ -N	r							-0.256**
	p							0.002

Note. Coefficients marked in bold type are significant; * denotes $P < 0.05$; ** denotes $P < 0.01$. Bold values indicate a significant correlation between the two indicators, negative values indicate a significant negative correlation, and positive values indicate a significant positive correlation.

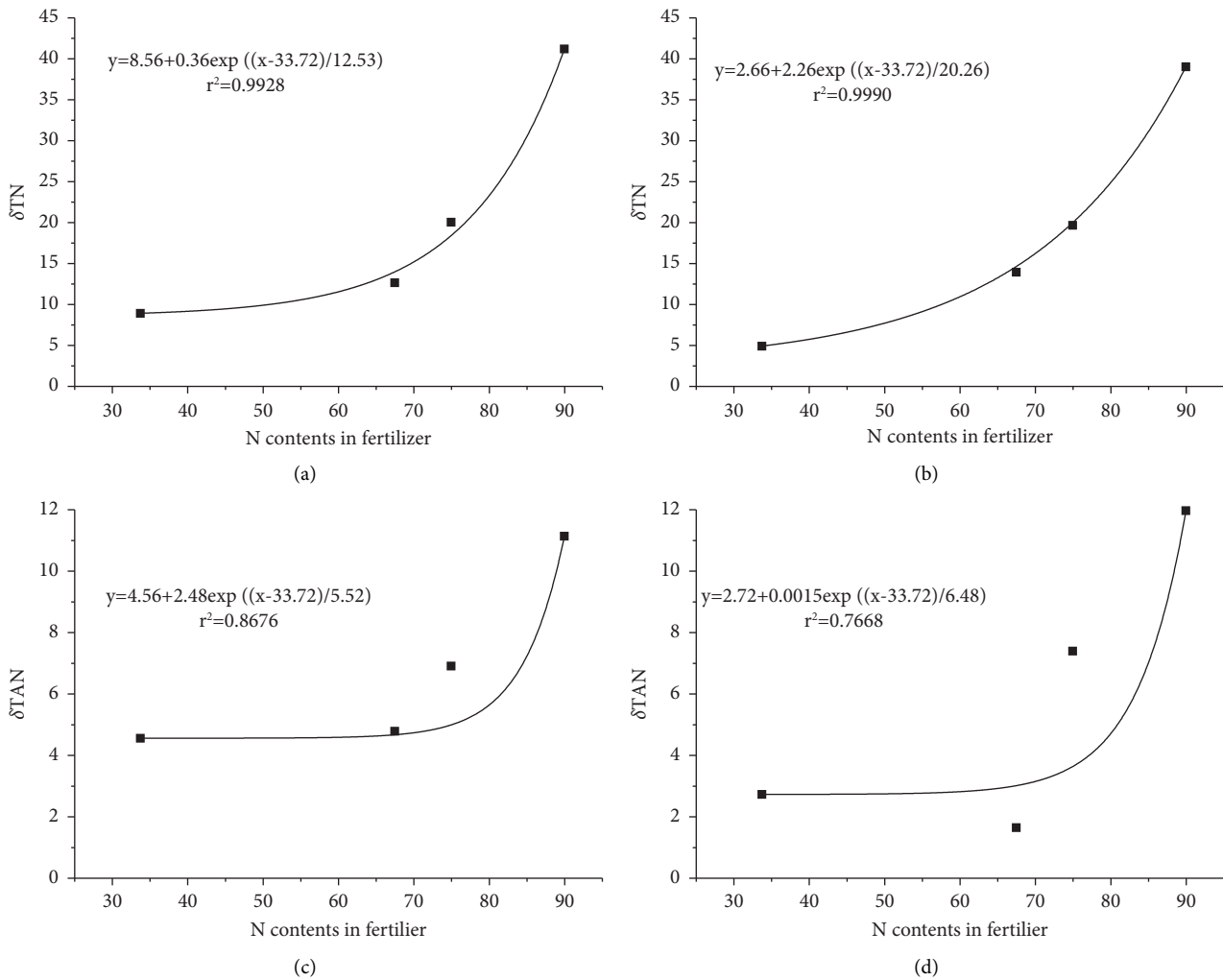


FIGURE 5: Regressions for total nitrogen (TN) and total ammonia nitrogen (TAN) increment after 24 h of fertilization (δTN and δTAN) versus the nitrogen (N) content in the applied chemical fertilizer. (a), (c) Rice field. (b), (d) Ring ditch.

DO, pH, and T ($P < 0.05$), and also among TN, TP, and TAN (Table 3). Conversely, TAN and DO were negatively correlated, as were pH and T (Table 3). Significant negative correlations were also found between TN and T, and between COD and T or NO_2^- -N (Table 3).

3.6. Regressions between the Nitrogen Increase and Fertilization Amount. It can be seen from Figures 1 and 4 that the TN and TAN contents in both water areas increased significantly at 24 h after fertilization. The increase of TN (δTN) and TAN (δTAN) in water after 24 h of fertilization was then regressed against the nitrogen content in fertilizer (Figure 5). This uncovered significant positive relationships of δTN or δTAN in water with the fertilizer's nitrogen content. Accordingly, with more fertilizer application, the increase of TN and TAN in rice field's surface water and ring ditch water also increased 24 h later.

4. Discussion and Conclusion

Through the analysis of test results of the physical and chemical indexes of two water areas (surface and ditch) before and after fertilization at four time points, a consistent pattern emerged. Applying the chemical fertilizer negligibly affected the pH, dissolved oxygen (DO), temperature (T), and chemical oxygen demand (COD) of either water area, but it exerted significant effects on total nitrogen (TN), total ammonia nitrogen (TAN), and total phosphorus (TP) contents. The pH is mainly affected by carbon dioxide (CO_2), which is also affected by the photosynthesis of algae, respiration of aquatic organisms, oxidation and decomposition of organic matter, as CO_2 is the product of organic decomposition [12]. As it is well known that aquatic plants will consume CO_2 and produce oxygen during photosynthesis, which will lead to an increase in DO content and a decrease in pH. The present results showed that the pH and DO in both NF group and RF group have a consistent change trend before and after fertilization, indicating that the application of chemical fertilizer has no obvious impact on the plankton amount in a short period of time, so the changes of pH and DO instantaneous monitoring values in NF group and RF group were similar. However, 72 h after the fourth fertilization, the DO and T of surface water in the NF group were significantly higher than those in the RF group. The increase in temperature will strengthen the photosynthesis of the water area, which may be the reason why the DO content in the NF group was significantly higher than that in the RF group.

The content of TN, TAN, and TP increased significantly at 24 h after the chemical fertilizer application. Of course, the premise is that the applied chemical fertilizer contains the P element. If only urea is applied, it will not cause significant changes to the water's TP content. Nitrite nitrogen (NO_2^- -N) showed a slow upward trend, and its detection did not peak within 72 h. Our results are consistent with those of Das et al. [13], who studied the impact of applying organic and inorganic fertilizers on water quality: they found that compared with applying an organic fertilizer, inorganic

fertilizer application can quickly yield a peak value of TAN in water; the time of NO_2^- -N reaching its peak value in water lags behind that for TAN. This may explain why there was no significant difference in the NO_2^- -N content between the NF and RF groups, and the content of TAN is generally augmented. The most commonly used N-fertilizer is converted into ammonia (NH_3) and CO_2 very quickly in moist soil [14]. The faster rise in TAN levels in response to chemical fertilizer applications is attributed to the rapid hydrolysis of urea to ammonia in the presence of higher oxygen levels [15]. Furthermore, with more N in chemical fertilizer, the increase of TN and TAN in water 24 h since fertilization is also significantly enhanced. These results are consistent with those of Bhakta et al [16]. They reported that applying increasing doses of fertilizers-poultry droppings, cattle manure, single super phosphate, and urea was procured and mixed in different proportions to create a fixed carbon (C), nitrogen (N), and phosphorus (P) ratio of 88.6:7.5:1, resulting in a gradual rise in the concentrations of ammonium-N and nitrite-N.

Water quality is one of the most important contributors to fish health and stress levels. Poor water quality can cause diseases in the fish species and further increase their mortality rate [17, 18]. In terms of water quality evaluation indicators, DO, ammonia nitrogen (NH_3), and NO_2^- -N are the most common limiting factors in aquaculture. More NH_3 in the water environment will inhibit the excretion of ammonia nitrogen in the fish and increase the concentration of ammonia in their blood and tissues, rendering the bloodless capable of carrying oxygen which disrupts normal metabolism [19]. Our study showed that the mean TAN content in the ring ditch water reached was 12.3, 1.8, 3.4, and 7.6 mg/L, respectively, after 24 h of four chemical fertilizer applications. Three of them exceeded the international aquaculture water quality standard (i.e., TAN < 3.0 mg/L) [20]. The Asian swamp eel (*Monopterus albus*) is normally considered an air-breathing fish capable of lowering the toxicity of ammonia in its environment and body via unique strategies of ammonia detoxification [21–23]; hence, it is extremely tolerant of high levels of ammonia. In the laboratory, *M. albus* exhibited a very high tolerance of environmental ammonia; at pH 7.0 and 28°C, the 48, 72, and 96 h median lethal concentrations of total ammonia were 209.9, 198.7, and 193.2 mmol/L, respectively [21]. It may also encounter high concentrations of environmental ammonia (about 90 mmol/L) in rice fields that undergo agricultural fertilization [24]. Therefore, the adverse effects of chemical fertilizer on *M. albus* may in fact be small. Nevertheless, the high concentration of ammonia in the water areas (aquatic environment) will pose a grave threat to the fertilized eggs or juveniles of *M. albus*. The hatching rate of oosperms of *M. albus* declines with an increasing ammonia content [25]. Rice field-based ecological breeding is currently the chief way of artificial breeding of *M. albus* in China, which has a large scale in Hubei Province. Therefore, it is necessary to pay attention to the effect of chemical fertilizers on water quality in the process of raising juveniles of *M. albus* in paddy fields. In addition, most fish species have a low tolerance to ammonia nitrogen, and many of them would

succumb to levels of <5 mmol/L NH_4Cl [23]. From our study's results, evidently, the total nitrogen and ammonia nitrogen rise first and then fall after fertilizer application. The peak value observed at 24 h is not necessarily the highest attainable value, however. Accordingly, we should pay special attention to the water quality changes and aquatic animal activities within 48 h window after the application of chemical fertilizer. Besides the water quality indexes related to nitrogen and phosphorus nutrients, the other water quality parameters were deemed tolerable to the fish in the rice–fish ecosystem.

As demonstrated in this study, chemical fertilizer applications cause a sharp rise in nitrogen and phosphorus nutrients in water in just a short time, with the peak values of total nitrogen and ammonia nitrogen increasing in more chemical fertilizer applied, which may pose a potential threat to aquatic animals. More than 85% of 1122 rice-producing farm households in China relied on complex fertilizers in their rice production activities, and these complex fertilizers accounted for more than half of the total amount of chemical fertilizers reportedly used [26]. Though there have been many reports that rice–fish coculture can reduce the application rate or amounts of fertilizer, the observed weight loss effect was only 20%–30% [6], and chemical fertilizer remains a commonly used fertilizer in rice–fish coculture systems. How to further reduce the impact of chemical fertilizer application on water quality in the rice–fish coculture system is therefore a pressing problem that we should think about and try to address. After consulting the available literature, we suggest the following two directions are the most promising. (1) Develop fertilizers for enhanced nitrogen-use efficiency. Nutrient losses from N-fertilizers, at around 50%, contribute significantly to low fertilizer-use efficiency [27]. Enhanced-efficiency fertilizers (EEFs) are continuously being developed to regulate the slow release of N from fertilizers, enabling the improved uptake and utilization by plants, thereby lowering losses and increasing crop productivity per unit of fertilizer. Presently, considerable efforts and advances have been made in this respect [28]. (2) Improve the management of rice–fish coculture systems. Management options with the greatest mitigation potential for rice (or rice-based cropping systems) are replacing urea with ammonium sulfate, using a nitrogen (N) inhibitor application, reducing the N-fertilizer application, and including a biochar application [29]. Furthermore, the rice–fish coculture system itself has the effect of reducing chemical fertilizer input and persistence when compared with the rice monoculture system, and the coupling of rice with fish production can achieve synergistic outcomes in food production systems that reduce environmental impacts per unit area of production [30]. Different fertilization treatments will undoubtedly have a significant impact on rice cultivation and yield [31]. Studies have shown that the rice–fish urea treatment was the most profitable, in that it leads to the highest gross margin for farmers [32]. This implies we may not be able to remove entirely the use of chemical fertilizers in the coupled rice and fishery system, but we could aim to adjust the application ratio of fertilizer to feed or consider applying an organic fertilizer. For

example, one experimental study indicated that adjusting the ratio of N added as fertilizer vs. the N added as feed to 37% fertilizer-N and 63% fish feed-N was able to increase the fish yield without reducing either the rice yield or N-use efficiency and releasing more N into the aquatic environment [33]. Another study showed that the growth of fish varied significantly under different fertilizer treatments, with that of chicken manure providing the best growth at 17.7 ± 5.97 g [31].

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors' Contributions

Quan Yuan and Wenzong Zhou conceived and designed the study; Quan Yuan and Weiwei LV conducted the experiment; Xiaolin Sun helped in the rice field management; Weiwei Huang provided language help; Wenzong Zhou agrees to be accountable for all aspects of the published work and to resolve any problems involved in the accuracy and integrity of any part of the work. Quan Yuan and Weiwei LV contributed equally to this work.

Acknowledgments

This work was supported by the China Agriculture Research System of MOF and MARA (grant number CARS-46), the Shanghai Sailing Program (grant number 21YF1441200), and the National Agricultural Experimental Station for Agricultural Environment, Fengxian (grant number NAES035AE03).

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