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Effects of straw return and feed addition on the environment and nitrogen use efficiency in the rice–crayfish system

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Abstract

Purpose Understanding the direction of nitrogen flow in the rice–crayfish system (RC) and optimizing the rate of nitrogen fertilizer application (ORN) are of great significance for sustainable development of RC.

Methods To this end, a field experiment involving straw and feed treatment was conducted to delineate the flow of the nitrogen present in the straw (straw-N) and feed (feed-N). Second, under different feed and straw treatments, we carried out a mesocosm experiment with different rates of ¹⁵N-labeled nitrogen fertilizer application to explore the optimal rate under the effects of feed-N and straw-N supply.

Results The results showed that at 75 kg N ha⁻¹ (75N), 14% and 1.86% of feed-N and 4.3% and 8.78% of straw-N is absorbed by crayfish and rice, respectively. The percentage of feed-N and straw-N lost to the environment was the lowest at 75N, i.e., 84.48% and 86.92%,

respectively. Using straw return to the field and feeding as the basic management measures for RC, we observed that the rice yield was the highest under 75N, and the highest nitrogen utilization efficiency (NUE) was 25%, and the highest the percentage of soil storing nitrogen by the fertilizer-derived was 34% (lost to the environment, at least 41%).

Conclusions ORN could help in the effective utilization of feed-N and straw-N via a compensation effect, promote the absorption and transport of nitrogen, and ultimately lead to an improvement in NUE in RC.

Keywords: rice–crayfish model, nitrogen, ^{15}N isotope labeling, nitrogen use efficiency

Abbreviations

ORN optimizing the rate of nitrogen fertilizer application

SF Straw with feed

SN Straw with no feed

NF No straw with feed

NN No straw with no feed

0N 0 kg nitrogen ha^{-1}

75N 75 kg nitrogen ha^{-1}

150N 150 kg nitrogen ha^{-1}

225N 225 kg nitrogen ha^{-1}

$\text{N}_{\text{dff}}(\%)$ the proportion of rice N derived from the fertilizer

NUE(%) percentage of fertilizer-derived N that was used by the rice

$R_{\text{soil}}(\%)$ the percentage of fertilizer-derived N that was stored by the soil

$R_{\text{loss}}(\%)$ represents the percentage of fertilizer-derived N lost to the environment

Introduction

Nitrogen is essential for crop growth, and it can promote food production (Xu et al., 2012; Yi et al., 2019). Therefore, the use of nitrogen fertilizers is a very important measure in farmland management for intensive agricultural production (Matson et al., 1997). Studies have shown that between 1982 and 2017, grain production in China increased by 74%, while the use of chemical fertilizers tripled (Cui and Shoemaker, 2018). In China, the average nitrogen utilization efficiency (NUE) for crop production was estimated to be 0.25, indicating that large amounts of nitrogen are lost to the environment (China National Statistics Bureau, 2018).

During crop production, the residual nitrogen fertilizer that is not absorbed by crops damages both the ecosystem and human health (Erisman et al., 2013). High levels of nitrogen fertilizer can directly or indirectly drive soil acidification (Matsuyama et al., 2005). Further, it has been found that during intensive agricultural production in China over an almost 30-year period, large amounts of nitrogen fertilizer have been

69 added to a limited area of land. By the beginning of the 21st century, the
70 pH value of soil in China's main crop production areas had dropped
71 significantly, and the soil had become acidified (Guo et al., 2010).
72 Nitrogen fertilizers from rice fields and farmland water enter the surface
73 runoff and groundwater and cause the eutrophication of water bodies
74 (Chen et al., 2013). Residual nitrogen from fertilizers in rice fields also
75 enters the atmosphere in the form of N₂O after oxidation, increasing the
76 greenhouse effect (Davidson 2009). Therefore, the management of
77 nitrogen fertilizers in high-yield rice systems is critical.

78 The integrated rice cultivation model that was developed in China and
79 subsequently used for thousands of years allows the effective control of
80 nitrogen fertilizer use and can help improve nitrogen utilization in rice
81 fields (Hu et al., 2016). This model involves agricultural rice production
82 in combination with the breeding of fish, ducks, crayfish, turtles, or
83 loaches (Hu et al., 2016). Studies have shown that the rice–fish system
84 requires, on average, 19% less nitrogen fertilizer than the rice
85 monoculture system (Xie et al., 2011). In the rice–duck system, ducks are
86 active in the paddy field, and the intertillage effect is obvious. This effect
87 can lead to the loosening of paddy soil, promote gas exchange, improve
88 soil permeability, and improve the organic carbon, total nitrogen (TN),
89 and available nitrogen levels in the soil (Li et al., 2008). It has been found
90 that a duck can produce 10 kg of excrement during the rice–duck co-

cropping period, equivalent to 47 g of nitrogen, 70 g of phosphorous, and 31 g of potassium, thus fertilizing the rice field (Zhao et al., 2005).

The rice–crayfish (RC) model is an integrated rice cultivation model that has emerged in the middle and lower Yangtze river region in recent years (Yuan et al., 2020a). There are several differences in farmland management measures between traditional local rice fields and RC fields (Si et al., 2017). In RC fields, in the non-rice season, the flooding depth of the field is adjusted to 0.5–1.0 m in order to promote the growth and development of crayfish (Yuan et al., 2020b). In order to grow the crayfish to commercial standards, a large amount of feed is added to the paddy field. After the rice is harvested, rice straw is returned to the field and the field is flooded. This flooding is maintained until June of the following year, before rice crops are planted again. In the rice-growing season, the management and cultivation of rice is performed in a manner similar to that used in local traditional rice fields.

Feed and straw input is closely related to the utilization efficiency of nitrogen fertilizers in the RC system. Owing to the shift from rice monoculture to the RC model, excess nutrients have accumulated in the soil and there have been negative effects on the environment (Hu et al., 2021). Research has shown that the amount of nitrogen fertilizer used by RC farmers in paddy fields is 150 kg N ha⁻¹ (Hu et al., 2021), but it is unclear if this value is optimal. While studies have explored the optimal

nitrogen fertilizer application rate in the rice–fish system under feed combinations that allow the best yield without causing environmental pollution (Hu et al., 2013), such studies in the RC system are lacking. Moreover, it is unclear whether rice production can be maintained while preventing environmental pollution in a large-scale commercial RC model.

Therefore, the overall aim of the present study was to identify the optimal nitrogen fertilizer application rate in RC fields after straw and feed addition. To this end, we conducted two experiments. In the field experiment, we compared the impact of the RC system on water bodies and environmental changes under straw and feed treatment. In the mesocosm experiment, we explored the utilization efficiency of the nitrogen fertilizer in RC fields under different rates of nitrogen fertilizer application.

2. Materials and methods

2.1. Study site

The field experiment was conducted at an experimental station in Houhu Village, Qianjiang City, Hubei Province (30° 39' N, 112° 71' E). The experimental site was located in the low-lying lake area of the Jiangnan plain. This area has a north subtropical humid monsoon climate with a mean long-term temperature of 16.1°C and had an annual

precipitation of 1100 mm in 2018 (Fig. 1). The studied area had been under continuous paddy RC cultivation for at least 3 years before the experiment was started in 2018. The soil was fluvo-aquic paddy soil arising from lake sediments and had a pH level of 7.12. The total nitrogen content was 2.41 g kg⁻¹ and the organic matter content was 26.43 g kg⁻¹.

2.2. Experimental design

In order to explore the impact of straw return and feed addition on the environment in RC fields, we set up a two-factor experiment. There were four test treatments — (i) straw with feed (SF), (ii) straw with no feed (SN), (iii) no straw with feed (NF), and (iv) no straw with no feed (NN) — and each treatment was repeated thrice, yielding a 12-test plot. The area of the rice plantation in each plot was 1000 m². A ring-shaped ditch (2.0 m wide and 2.0 m deep) was dug around the rice-planting region for crayfish to live in, and a 0.4-m high crayfish escape net was set outside the ring ditch.

In the non-rice season, straw was returned to the field using a harvester such that a low stubble remained. The straw was then crushed on the surface to cover the field. For the no-straw treatment, the straw was manually removed from the field after the rice harvest in 2017. In the cultivation system, we also used crayfish (*Procambarus clarkii*). Feed was added every afternoon from March 12 to June 1, but for the no-feed treatment, no additional feed was added. Crayfish were fed with

commercial formula feed containing 4.6% nitrogen per day at about 17:00 throughout the experiment. The daily amount of feed added was about 1.5% of the fresh body mass of the crayfish in each plot, and this amount increased as the crayfish grew. Crayfish were harvested daily between March and June; adult crayfish were picked, and the juveniles were returned to the original community. The amount of straw returned to the field and the amount of feed added are shown in Table 2.

In the rice season, we used the rice variety Taiyou 390 (*Oryza sativa* L). Three weeks after germination, rice seedlings were transferred to the test plots, with 25-cm gaps between rows and 26-cm gaps between the hills (four seedlings per hill) within each row. In the paddy field, there were three replicates for each treatment. One replicate for each treatment was randomly selected for the ^{15}N isotope tracer test to explore the optimal rate of nitrogen fertilizer application in the RC model. The following nitrogen application rates were used for each treatment: 0 kg N ha⁻¹ (0N), 75 kg N ha⁻¹ (75N), 150 kg N ha⁻¹ (150N), 225 kg N ha⁻¹ (225N). Each treatment was repeated thrice, yielding a total of 60 test micro areas wherein the ^{15}N isotope experiment was conducted. ^{15}N -labeled urea produced by Shanghai Research Institute of Chemical Industry was used as the nitrogen fertilizer, and the ^{15}N abundance was 10.28%. Base fertilizer and tiller fertilizer containing nitrogen were applied at a 1:1 ratio. After the surface water in the RC fields receded, but

before rice crops were planted, a round PVC pipe with a diameter of 0.4 m and height of 0.4 m was inserted 0.4 m underground. Further, 0.2 m of the pipe was kept above the ground to prevent the loss of ^{15}N and the entry of external N. A 0.5-cm hole was drilled in the PVC pipe 20 cm above the surface of the field. When the water level in the micro area dropped to the field surface, the field surface water flowed into the micro area as replenishment. While fertilizer was added, the hole was blocked with mud to prevent fertilizer loss in the micro area. During the growth period, other management measures such as pest control were implemented uniformly across treatment plots.

2.3 Plant and soil sampling and analysis

We collected 0–20 cm of the cultivated soil layer and stored it as fresh wet soil and natural air-dried dry soil. For the experiment, 1 g of 0.15 mm dry soil was obtained and concentrated sulfuric acid was added for digestion. The determination of soil bulk density was performed using the ring knife method. In the experiment, all the above-ground rice plants in the micro area were collected after rice growth was complete. The stems, leaves, and panicles were separated, heated at 105°C for 0.5 h, and then dried in an oven at 80°C until they reached constant weight. Rice plant samples that were ground and dried to a constant weight were 0.15 mm in diameter. The soil samples and dried plants were ground with a ball mill and wrapped separately into balls with tin foil. The isotopic abundance

was measured using a stable isotope mass spectrometer (IsoPrime 100 IRMS, Isoprime Ltd, Stockport, UK).

During the feeding period, 15 crayfish were randomly selected from among the crayfish caught from each plot and dried at 80°C until they reached constant weight. The TN content of the rice plants, crayfish feed, and straw were obtained separately using a C/N analyzer (Vario MAX-CN elemental analyzer, Germany).

2.4. Data analysis

2.4.1 Analysis of nitrogen balance

The balance of nitrogen output and input within the RC fields was calculated by subtracting the TN output (nitrogen present in harvested plants and crayfish) from the total input (nitrogen present in fertilizers, crayfish feed, and straw). A positive value indicated that some portion of the input nitrogen had not been used by rice and crayfish and remained in the field plot (as nitrogen in the soil or water) or had moved into the surrounding environment (as nitrogen in the atmosphere or drainage water).

2.4.2 Analysis of the fate of the nitrogen derived from crayfish feed and straw

In fields subjected to NN treatment (no crayfish feed or rice straw application), the nitrogen in crayfish (crayfish N) was derived from the environment only and the nitrogen in rice plants (rice N) was derived

from fertilizers and the environment. In fields subjected to NF treatment (crayfish feed and no straw application), crayfish N was derived from crayfish feed and the environment, and rice N was derived from fish feed, fertilizers, and the environment. In fields subjected to SN treatment, crayfish N was derived from straw and the environment, and rice N was derived from straw, fertilizers, and the environment. To understand the fate of the crayfish- and straw-derived nitrogen in the RC system, we performed eight calculations, which included the following variables:

- X_{SN} , nitrogen input through straw under the SN treatment;
- X_{NF} , nitrogen input through feed under the NF treatment;
- $crayfish_{SN-N}$, crayfish N under the SN treatment;
- $rice_{SN-N}$, rice N under the SN treatment;
- $crayfish_{NF-N}$, crayfish N under the NF treatment;
- $rice_{NF-N}$, rice N under the NF treatment;
- $fish_{NN-N}$, crayfish N under the NN treatment;
- $rice_{NN-N}$, rice N under the NN treatment;
- FX_{NF} , crayfish N derived from crayfish feed under the NF treatment;
- RX_{NF} , rice N derived from crayfish feed under the NF treatment;
- FX_{SN} , crayfish N derived from straw under the SN treatment;
- RX_{SN} , rice N derived from straw under the SN treatment;
- (%) FX_{NF} , percentage of total crayfish feed-derived nitrogen incorporated into the crayfish biomass under the NF treatment;

(%) RX_{NF} , percentage of total crayfish feed-derived nitrogen incorporated into the rice biomass under the NF treatment;

(%) environment NF, percentage of total crayfish feed-derived nitrogen remaining in the environment under the NF treatment;

(%) FX_{SN} , percentage of total straw-derived nitrogen incorporated into the crayfish biomass under the NF treatment;

(%) RX_{SN} , percentage of total straw-derived nitrogen incorporated into the rice biomass under the NF treatment;

(%) environment SN, percentage of total straw-derived nitrogen remaining in the environment under the NF treatment.

The calculations were performed as follows:

$$FX_{NF \text{ or } SN} = (\text{crayfish}_{NF \text{ or } SN} - N) - (\text{crayfish}_{NN} - N) \quad (1)$$

$$RX_{NF \text{ or } SN} = (\text{rice}_{NF \text{ or } SN} - N) - (\text{rice}_{NN} - N) \quad (2)$$

)

$$(\%)FX_{NF \text{ or } SN} = \left(\frac{FX_{NF \text{ or } SN}}{X_{NF \text{ or } SN}} \right) \times 100 \quad (3)$$

)

$$(\%)RX_{NF \text{ or } SN} = \left(\frac{RX_{NF \text{ or } SN}}{X_{NF \text{ or } SN}} \right) \times 100 \quad (4)$$

)

$$(\%) \text{environment NF or SN} = \frac{(X_{NF \text{ or } SN} - FX_{NF \text{ or } SN} - RX_{NF \text{ or } SN})}{X_{NF \text{ or } SN}} \times 100 \quad (5)$$

2.4.3 Stable isotope analysis

Stable isotope values were reported in δ notation:

$$\delta^{15}\text{N} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (6)$$

Where R is $^{15}\text{N}/^{14}\text{N}$, and the standard sample used for determining ^{15}N concentration is N_2 from the air ($^{15}\text{N} = 0.003676$).

The percentage of nitrogen from the fertilizer in the total rice biomass-N or total soil nitrogen was calculated as follows:

$$N_{\text{dff}}(\%) = \frac{(\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_0)}{(\delta^{15}\text{N}_{\text{fertilizer}} - \delta^{15}\text{N}_{\text{nature}})} \times 100 \quad (7),$$

where $N_{\text{dff}}(\%)$ represents the proportion of rice N derived from the fertilizer. $^{15}\text{N}_{\text{sample}}$, $^{15}\text{N}_0$, $^{15}\text{N}_{\text{fertilizer}}$, and $^{15}\text{N}_{\text{nature}}$ represent the plant sample, 0 kg N ha⁻¹ treatment sample, fertilizer, and natural abundance value in the mesocosms, respectively.

We estimated the percentage of fertilizer-derived nitrogen that was used by the rice as follows:

$$\text{NUE}(\%) = \frac{N_{\text{rice}} \times N_{\text{dff}}}{N_{\text{fertilizer}}} \quad (8),$$

where N_{rice} is the total rice biomass-N and $N_{\text{fertilizer}}$ is total fertilizer-N in the mesocosms experiment.

We estimated the percentage of fertilizer-derived nitrogen that was stored by the soil as follows:

$$R_{\text{soil}}(\%) = \frac{(N_{\text{dff}} \times H \times \text{BD} \times \text{TN}_{\text{soil}})}{N_{\text{fertilizer}}} \times 10 \quad (9),$$

where N_{dff} is the percentage of fertilizer-derived nitrogen in the soil. The H value is 20, representing the thickness of the soil layer (cm). BD is soil

bulk density (g cm^3), and TN_{soil} is the TN content of the soil (g kg^{-1}).
 $\text{N}_{\text{fertilizer}}$ is ^{15}N -labeled urea nitrogen content (kg ha^{-1}) and the 10 is for unit
conversion.

$$\text{R}_{\text{loss}}(\%) = 100 - \text{NUE}(\%) - \text{R}_{\text{soil}}(\%) \quad (10),$$

where $\text{R}_{\text{loss}}(\%)$ represents the percentage of fertilizer-derived nitrogen
lost to the environment.

2.4.4 Statistical analysis

All statistical analyses were performed using SPSS 22.0 (SPSS, Inc.,
USA). One-way analysis of variance (ANOVA) at $\alpha = 0.05$ probability
was conducted to test the significance of differences across different
treatments, and if significance was observed via ANOVA, an LSD test
was used to compare means. OriginPro 9.0 was used to prepare all
graphs.

3. Results

3.1 Rice aboveground biomass, yield, ^{15}N natural abundance, and nitrogen content of the rice fractions

During NF treatment, the rice stem biomass under 225N was
significantly higher than that under 0N ($p < 0.05$), but there was no
significant difference between the values at 75N and 150N (Table 2).
During NN, SF, and NF treatments, the rice yield under 75N and 150N
application was significantly higher than that under 225N and 0N

application ($p<0.05$) (Table 2). There was no significant difference in rice yield between 75N and 150N application (Table 2).

Under different nitrogen rates, the natural abundance of ^{15}N ($\delta^{15}\text{N}$) in the fractions of rice was significantly different ($p<0.05$); it was the highest under 225N, followed by 150N, 75N, and 0N (Table 3). During NN, SF, and SN treatment, there was no significant difference in nitrogen content in the fraction of rice at different nitrogen rates (Table 3). During NF treatment, at 225N, the nitrogen content in the leaves was significantly higher than that at 150N, 75N, and 0N ($p<0.05$). However, there was no significant difference in grain nitrogen content under different nitrogen rates (Table 3).

3.2 Crayfish yield and nitrogen content

The yield of crayfish under SF and NF treatments was significantly higher than that under NN and SN treatments (Table 4) ($p<0.05$). The yield of crayfish in SN was significantly higher than that in NN ($p<0.05$). Although there was no significant difference in crayfish production between SF and NF, the crayfish production with SF treatment was 9% higher than that with NF treatment (Table 4). The nitrogen accumulation in crayfish under SF and NF treatments was also significantly higher than that under NN and SN treatments ($p<0.05$) (Table 4). The crayfish yield was therefore mainly affected by feed input.

3.3 Soil nitrogen and ^{15}N content

After one year of RC co-culturing, the total nitrogen content of the soil did not differ significantly under different nitrogen rates in the NN, SF, SN, and NF treatments (Table 5). However, the ^{15}N content of the soil in each treatment increased significantly with an increase in nitrogen rate (225N>150N>75N) ($p<0.05$). Under 75N, 150N, and 225N nitrogen rates, the total nitrogen content of the soil during SF, SN, and NF treatments was significantly higher than that during NN treatment (Table 5) ($p<0.05$).

3.4 Nitrogen use efficiency in RC

3.4.1 Efficiency of straw- and feed-derived nitrogen use

We calculated the utilization rate of straw and feed nitrogen in the RC system using the subtraction method (Xie et al., 2011). Under the 0N, 150N, and 225N treatments, 13.66% of feed nitrogen was absorbed by the crayfish and the remaining 86.34% was lost to the environment (Fig. 2a, c, d). However, under a nitrogen application rate of 75N, 1.86% of the feed nitrogen was absorbed by the rice, and the loss of feed nitrogen to the environment was reduced to 84.48% (Fig. 2b).

Under different rates of nitrogen application, 4.3% of straw nitrogen was absorbed by crayfish (Fig. 2). However, under the 0N, 75N, 150N, and 225N treatment, 10.33%, 8.78%, 8.15%, and 0% of straw nitrogen was absorbed by the rice, respectively (Fig. 2a, b, c, d). With the increase in nitrogen rates, the amount of straw nitrogen lost to the environment

increased (Fig. 2).

3.4.2 Efficiency of fertilizer-derived nitrogen use

Under NN, SF, NF, and SN treatments, the percentage of fertilizer-derived nitrogen in rice stems, leaves, and grains (N_{dff}) increased significantly with the increase in nitrogen application rates ($p<0.05$) (Table 6). As shown by the nitrogen distribution pattern (Table 6), the nitrogen uptake by grains was significantly higher than that by rice stems and leaves. Under SF treatment, panicles accounted for 65.1%, 66.8%, and 62.4% of the total nitrogen uptake at application rates of 75N, 150N, and 225N, respectively.

There was no significant difference in the NUE of rice stems and leaves across nitrogen levels under each treatment (Table 6). Under NN and SF treatments, the NUE of rice panicles at application rates of 75N and 150N was significantly higher than that at 225N ($p<0.05$) (Table 6). However, there was no significant difference in NUE between the 75N and 150N rates. Although the NUE of rice panicles at 150N was significantly higher than that at 75N and 225N under the SN treatment ($p<0.05$), there was no significant difference between the values at 75N and 150N.

The application of chemical nitrogen fertilizers significantly increases food production. However, not all the nitrogen fertilizer added to agricultural fields remains available for crop nourishment — some is

absorbed by the crop, some is stored in the soil, some is discharged into the atmosphere in gaseous form, and some is lost to water bodies due to rainfall and irrigation. Nitrogen gas is discharged into the atmosphere, intensifying the greenhouse effect and accelerating global warming. The addition of nitrogen to surface water bodies, especially those receiving return flow from the agricultural farmland in the irrigation region, can lead to eutrophication.

The flow of nitrogen at different nitrogen fertilizer application rates under different straw and feed treatments in RC fields is shown in Figure 3. The storage of fertilizer nitrogen in the soil ($R_{soil}(\%)$) was calculated using formula 9 and the soil bulk density value (Table 7) (Fig. 3). In the SF and NF treatments, the $R_{soil}(\%)$ value under the 75N nitrogen rate was significantly higher than that under the 150N and 225N treatments ($p < 0.05$). Under SF treatment, the amount of nitrogen fertilizer stored in the soil and rice plants at application rates of 75N, 150N, and 225N was 44 kg ha⁻¹, 72 kg ha⁻¹, and 88 kg ha⁻¹, respectively. The nitrogen not accounted for in the rice plant and soil was presumably lost; therefore, under SF treatment, the rates at which ¹⁵N was lost were 41%, 52%, and 61% at application rates of 75N, 150N, and 225N, respectively. The amount of nitrogen recovered increased and the rate of loss increased when more nitrogen fertilizer was added to the fields. When fertilizer application was reduced to 75N, nitrogen uptake by rice plants and soil

reduced to 43 kg ha⁻¹, 44 kg ha⁻¹, and 40 kg ha⁻¹ under NN, SF, and NF treatments, respectively, but the loss rate (Rloss(%)) significantly reduced too, reaching 43%, 41%, and 46% ($p<0.05$), respectively. Although, Rloss (%) at application rates of 75N, 150N, and 225N under the SN treatment was 66%, 66%, and 62%, respectively, there was no significant difference in the nitrogen levels across micro areas with these application rates.

3.4.3 Nitrogen storage in the RC system

With the increase in the nitrogen rate, the nitrogen storage in rice grains increased slightly under the NN, SF, SN, and NF treatments, but the differences were not significant (Fig. 4). Under different nitrogen rates, the nitrogen accumulation in crayfish was significantly higher under SF and NF treatments than under NN and SN treatments ($p<0.05$). Under 0N, the rice and crayfish in the RC system could absorb 2 and 1.5 kg N ha⁻¹ from the environment NN and SN treatment, respectively (Fig. 4a). However, under SF and NF treatment, 60 kg ha⁻¹ and 30 kg ha⁻¹ of nitrogen was released into the environment, respectively. With the increase in the amount of nitrogen fertilizer, the nitrogen released into the environment in the RC system under treatments of returning straw to the field and feeding increased. Under different nitrogen fertilizer rates, the storage capacity for nitrogen was significantly higher in the SF and NF treatments than in the NN and SN treatments (Fig. 4) ($p<0.04$).

4. Discussion

Our results showed that the yield of crayfish under feeding conditions (SF and NF) was significantly higher than that under no feeding conditions (SN and NN). As the direct food source for crayfish, feed can effectively promote their growth (Mukherjee et al., 2010; Naseem et al., 2020). Interestingly, our research found that the yield of crayfish in the SF condition was 13% higher than that in the NF condition, and the value in the SN condition was 2.3% higher than that in the NN condition (Table 4). Returning straw to the field increases the production of crayfish. During the non-rice season, crayfish can feed on rotten straw and microorganisms in order to obtain nutrients (Chien and Avault, 1983; Brown et al., 1990). Crayfish feed on straw, which accelerates the decomposition of straw owing to the over-abdominal effect, promotes the accumulation of organic matter in the paddy soil, and improves the fertility of the paddy soil (Yuan et al., 2020).

Under SF and NF conditions, 75N and 150N treatments provided the highest rice yield, and there was no significant difference in rice yield between 75N and 150N treatment (Table 2). However, in the SN condition, the $R_{loss}(\%)$ under 150N and 75N nitrogen application rates was 41% and 52%, respectively, with a significant difference between the values (Fig. 3). Although under the NF condition, the $R_{loss}(\%)$ was not significantly different between 75N and 150N, the $R_{loss}(\%)$ at 150N was

15% higher than that at 75N (Fig. 3). Under the SF condition, although the dry matter accumulation in rice stems and leaves was the highest at an application rate of 225N, the dry matter in the grains was the lowest (Table 2). Further, the total nitrogen accumulation in the stems, leaves, and grains was the lowest under SF treatment (Fig. 4). The excessive use of nitrogen fertilizers can make rice stems and leaves flourish but reduce the overall efficiency, resulting in insufficient grains and poor maturity of rice plants (Yang et al., 2018). The application of high-nitrogen fertilizers results in an increase in pests and diseases in rice fields and reduces rice yields (Bowling, 1963; Prabhu et al., 1996; Slaton et al., 2003). In the paddy field, rice plants can use the feed nitrogen that is not consumed by animals, as well as the ammonia that is excreted by fish and prawns (Mirhaj et al., 2014). In the RC system, feed nitrogen is a supplementary nitrogen source that can be used by rice, and therefore, the application of nitrogen fertilizer can be reduced. Our research shows that rice plants can use 13.66% and 4.3% of feed-N and straw-N in the RC system, respectively (Fig. 2). In the rice-fish system, rice plants can absorb 31.8% of feed-N, and the rice yield increases (Xie et al., 2011). Therefore, rice yield can be maintained with the application rate of 75N, and we suggest that nitrogen fertilizer application should be reduced in the RC system.

Aquaculture provides protein for human nutrition (Nissa et al., 2021), but the eutrophication and environmental pollution caused by intensive

aquaculture production are widespread (Herbeck et al., 2013; Bian et al., 2012). The eutrophication of water bodies during intensive aquaculture is mainly caused by the nitrogen and phosphorus from unconsumed feed (Cantas and Yildirim., 2019). Combining the breeding of aquatic animals with the cultivation of rice can effectively promote the recycling and utilization of materials in the paddy field (Xie et al., 2011; Zhang et al., 2016). In the RC system, feed-N and straw-N can not only be absorbed by crayfish but can also be absorbed and utilized by rice, effectively promoting the nitrogen use efficiency.

In the RC system, due to the replenishment of feed-N and straw-N, the threshold of fertilizer nitrogen application during rice planting should be lower than the conventional application rate used by local farmers. Compared with the nitrogen application levels of 150N and 225N, the nitrogen content of rice plants and crayfish under the 75N treatment was higher. With the increase in the nitrogen application rate, the nitrogen content in rice plants and crayfish did not increase significantly, but the nitrogen levels in the environment did (Fig. 4). The unreasonable application of nitrogen fertilizer in the RC system causes economic losses and also increases the risk of non-point source pollution in the rice field (Hu et al., 2021). A reasonable amount of nitrogen fertilizer should be used in conjunction with straw return to the field and feeding treatment to ensure a high yield of rice, while improving the nitrogen use efficiency in

the RC system, effectively reducing environmental pollution. This can provide a win-win situation in RC culture during the production process.

5. Conclusion

Integrating intensive crayfish culture with rice culture can allow for the procurement of substantial crayfish yields and stable rice yields. Straw and feed addition increase the nitrogen input in the RC system. The part of the feed-derived nitrogen that is not consumed by the crayfish can be absorbed by rice plants, and part of the straw-derived nitrogen can also be absorbed by rice plants and crayfish. Our research shows that under the RC model, with field management measures such as straw return to the field and feeding, the amount of nitrogen fertilizer used in the rice season should be controlled and maintained under 75 kg ha⁻¹. This will not only ensure a good rice yield but also reduce the accumulation of feed-N, straw-N, and fertilizer-N in the environment, thus reducing the potential for RC culture-induced environmental pollution during the production process.

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Author Contributions: Pengli Yuan: Conceptualization, design, data collection, Writing, Revision. Mingli Ni: Material preparation. Cougui Cao: Supervision. Ligeng Jiang: Writing – review. Anas Iqbal: Editing. Jinping Wang: Experiment design.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1. Amount of straw and feed used in different treatments

Year		Straw amounts (t ha ⁻¹)	Feed amounts (t ha ⁻¹)
2017-2018	NN	1.81	
	SF	7.60	2.80
	SN	7.40	
	NF	1.73	2.80

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Table 2. Dry weight of rice plants

Treatment	N-rate (kg N ha ⁻¹)	Stem (t ha ⁻¹)	Leaf (t ha ⁻¹)	Grain (t ha ⁻¹)
NN	0	4.86±1.86aA	3.15±1.27aA	8.58±0.30cA
	75	4.37±0.29aA	3.00±0.31aA	10.21±0.34abA
	150	4.41±0.69aA	3.19±0.05aA	10.61±0.16aA
	225	4.34±0.23aA	3.46±0.19aA	9.95±0.47bA
SF	0	3.69±0.52aA	2.66±0.32aA	7.16±0.19cB
	75	3.67±0.59aA	2.75±0.24aA	9.68±0.66abA
	150	4.15±0.16aA	3.05±0.11aAB	10.16±0.22aB
	225	4.22±0.36aA	3.08±0.19aA	9.16±0.25bB
NF	0	3.64±0.20bA	2.75±0.14aA	8.80±0.37cA
	75	4.11±0.33abA	2.78±0.21aA	9.45±0.31aA
	150	4.52±0.38abA	2.92±0.21aB	9.60±0.12aC
	225	4.07±0.24aA	2.82±0.51aA	8.80±0.30bB
SN	0	4.22±0.40aA	2.87±0.64aA	7.22±0.40cB
	75	3.78±0.71aA	2.79±0.49aA	8.42±0.34bB
	150	4.47±0.39aA	3.09±0.123aAB	9.60±0.29aC
	225	4.48±0.65aA	3.16±0.50aA	8.55±0.46bB

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Note: Different capital letters represent significant differences at $p<0.05$ between different treatments

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under the same nitrogen fertilizer level; different lowercase letters represent significant differences at

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 $p<0.05$ under the same treatment and different nitrogen fertilizer levels. NN, no straw with no feed; SF,

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straw with feed; SN, straw with no feed; NF, no straw with feed. All values are indicated as

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means ± standard deviation.

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Table 3. ^{15}N Abundance and total nitrogen content in the rice plants in the mesocosm experiment

N-rate (kg N ha ⁻¹)	Rice parts	$\delta^{15}\text{N}$				Total nitrogen content(g/kg)			
		NN	SF	NF	SN	NN	SF	NF	SN
0	Stem	65±5aB	72±9bB	104±10bA	116±6aA	6.42±0.58aA	5.27±1.14aAB	4.85±0.25aB	6.42±0.50aA
	Leaf	74±6aC	142±11aB	228±38aA	79±2bC	11.87±1.40aB	10.54±1.56aB	9.83±0.31bB	14.92±1.41aA
	Grain	74±7aB	121±2aA	110±9bA	59±3bC	12.52±0.71aAB	13.40±1.69aA	11.12±0.70aB	12.50±0.76aAB
75	Stem	2281±83cA	2976±64cA	2275±584bA	2578±111cA	5.81±0.25aAB	6.00±0.55aAB	5.23±0.47aB	6.11±0.50aA
	Leaf	3096±248cAB	3425±680cA	2049±182cA	2162±413cB	10.50±0.73aB	11.49±0.32aB	10.61±0.56bB	14.62±2.21aA
	Grain	2664±326cA	2800±200cA	1502±223cB	1574±140cB	12.19±0.33aA	11.96±0.96aA	10.71±1.53aA	12.18±0.91aA
150	Stem	3803±594bAB	4768±529bA	3029±690bB	4273±196bA	5.91±0.49aA	5.87±0.38aA	5.00±0.33aB	5.64±0.23aAB
	Leaf	5086±191bB	6141±611bA	3550±801bC	4405±230bBC	10.12±0.94aA	11.21±0.01aA	10.71±1.07bA	11.56±3.89aA
	Grain	4712±120bA	4826±376bA	2881±213bC	4089±220bB	12.08±1.36aA	13.40±1.51aA	12.08±0.49aA	11.87±0.64aA
225	Stem	6667±777aA	6155±539aAB	5273±423aB	5772±40aAB	6.38±0.49aA	5.39±0.98aA	5.33±0.68aA	6.31±0.56aA
	Leaf	6683±330aAB	7652±720aA	5707±948aB	6100±340aB	11.04±1.11aA	10.21±1.14aA	13.58±1.36aA	13.18±3.12aA
	Grain	6174±71aA	6031±576aAB	4288±12.42aC	5493±265aB	11.91±1.35aA	11.64±0.51aA	12.7±2.01aA	11.82±0.61aA

Note: Different capital letters represent significant differences at $p<0.05$ between different treatments under the same nitrogen fertilizer level; different lowercase letters represent significant differences at $p<0.05$ under the same treatment and different nitrogen fertilizer levels. All values are indicated as means ± standard deviation.

Table 4. N accumulation in crayfish.

	NN		SF		SN		NF	
Wet weight(g)	54.1	± 26.4a	78.9	± 14.5a	80.0	± 33.1a	73.7	± 35.6a
Dry weight(g)	13.9	± 7.6a	21.7	± 5.7a	20.0	± 9.9a	21.1	± 12.0a
water content(%)	0.7	± 0.0a	0.7	± 0.0a	0.8	± 0.0a	0.7	± 0.0a
TN(g kg ⁻¹)	6.9	± 0.4a	6.6	± 0.4a	6.4	± 0.7a	6.4	± 0.9a
Crayfish yield (t ha ⁻¹)	0.6	± 0.02c	1.2	± 0.03a	0.7	± 0.01b	1.1	± 0.05a
Crayfish-N (kg ha ⁻¹)	4.4	± 0.2c	7.9	± 0.6a	4.5	± 0.3c	7.0	± 0.8b

Note: Different lowercase letters represent significant differences at $p<0.05$ between different treatments. All values are indicated as means ± standard deviation.

Table 5. Total nitrogen and ^{15}N (g kg⁻¹) content in soil

N rate (kg ha ⁻¹)	TN				^{15}N			
	NN	SF	SN	NF	NN	SF	SN	NF
75	2.78±0.04aB	3.00±0.12aA	2.97±0.10aA	2.97±0.23aA	0.97±0.10cA	1.08±0.11cA	0.73±0.17cB	1.20±0.12bA
150	2.82±0.15aB	3.07±0.10aA	3.00±0.02aA	2.96±0.08aA	2.11±0.19bA	1.47±0.13bB	1.38±0.19bB	1.18±0.12bC
225	2.83±0.13aB	3.01±0.02aA	3.24±0.37aA	3.20±0.19aA	2.36±0.09aA	2.09±0.13aA	2.40±0.30aA	2.27±0.15aA

Different capital letters represent significant differences at $p<0.05$ between different treatments under the same nitrogen fertilizer level; different lowercase letters represent significant differences at $p<0.05$ under the same treatment and different nitrogen fertilizer levels. All values are indicated

as means $\pm$ standard deviation.

Table 6. Nitrogen use efficiency under different rates of nitrogen application

Plant part	N rate (kg N ha ⁻¹)	N _{diff}				NUE(%)			
		NN	SF	NF	SN	NN	SF	NF	SN
Stem	75	8.8 $\pm$ 0.3Ac	11.5 $\pm$ 0.3Ac	8.5 $\pm$ 2.3Ab	9.8 $\pm$ 4.4Ac	3.0 $\pm$ 0.3Aa	3.4 $\pm$ 0.5Aa	2.5 $\pm$ 0.8Aa	2.9 $\pm$ 0.9Aa
	150	14.8 $\pm$ 2.4ABb	18.6 $\pm$ 2.1Ab	11.5 $\pm$ 2.7Bb	16.5 $\pm$ 0.8ABb	2.6 $\pm$ 0.5ABa	3.0 $\pm$ 0.4Aa	1.8 $\pm$ 0.6Ba	2.8 $\pm$ 0.4Aa
	225	26.1 $\pm$ 3.1Aa	24.0 $\pm$ 2.1ABa	20.4 $\pm$ 1.7Ba	22.4 $\pm$ 0.2ABa	3.2 $\pm$ 0.3Aa	2.5 $\pm$ 0.8ABa	2.0 $\pm$ 0.3Ba	2.8 $\pm$ 0.6ABa
Leaf	75	12.0 $\pm$ 1.0Ac	13.0 $\pm$ 2.7Ac	7.3 $\pm$ 0.7Bc	8.2 $\pm$ 1.6Bc	5.1 $\pm$ 1.1Aa	5.5 $\pm$ 1.5Aa	2.9 $\pm$ 0.5Ba	4.4 $\pm$ 1.0ABa
	150	20.0 $\pm$ 0.8Bb	23.8 $\pm$ 2.4Ab	13.2 $\pm$ 3.2Cb	17.1 $\pm$ 0.9Bb	4.3 $\pm$ 0.5ABa	5.5 $\pm$ 0.7Aa	2.8 $\pm$ 0.9Ba	4.1 $\pm$ 1.5ABa
	225	26.0 $\pm$ 1.3ABa	30.0 $\pm$ 2.9Aa	21.8 $\pm$ 3.8Ba	23.9 $\pm$ 1.3Ba	4.4 $\pm$ 0.4Aa	4.1 $\pm$ 0.5Aa	3.6 $\pm$ 0.4Aa	4.3 $\pm$ 0.6Aa
Grain	75	10.0 $\pm$ 1.3Ac	10.6 $\pm$ 0.8Ac	5.5 $\pm$ 0.9Bc	6.0 $\pm$ 0.6Bc	17.0 $\pm$ 2.0Aa	16.4 $\pm$ 1.3Aa	7.5 $\pm$ 2.1Ba	8.2 $\pm$ 1.2Bb
	150	18.1 $\pm$ 0.5Ab	18.7 $\pm$ 1.5Ab	11.0 $\pm$ 0.8Bb	15.9 $\pm$ 0.9Cb	15.7 $\pm$ 2.2Aab	16.9 $\pm$ 1.3Aa	8.5 $\pm$ 0.7Ca	12.1 $\pm$ 0.4Ba
	225	24.2 $\pm$ 0.3Aa	23.4 $\pm$ 2.3ABa	16.6 $\pm$ 1.1Ca	21.5 $\pm$ 1.0Ba	12.7 $\pm$ 1.7Ab	11.1 $\pm$ 1.6ABb	7.8 $\pm$ 0.7Ca	9.6 $\pm$ 0.5Bb
Total	75	31.2 $\pm$ 0.5Ac	35.2 $\pm$ 3.6Ac	21.3 $\pm$ 3.7Bc	24.0 $\pm$ 5.2Bc	25.0 $\pm$ 0.8Aa	25.2 $\pm$ 2.6Aa	12.9 $\pm$ 3.4Ba	15.5 $\pm$ 1.1Bb
	150	53.0 $\pm$ 1.7ABb	61.1 $\pm$ 5.5Ab	35.8 $\pm$ 6.7Cb	49.6 $\pm$ 0.7Bb	22.6 $\pm$ 1.6ABab	25.3 $\pm$ 2.1Aa	13.1 $\pm$ 2.1Ca	19.0 $\pm$ 1.9Ba
	225	76.2 $\pm$ 4.6Aa	77.4 $\pm$ 4.9Aa	58.8 $\pm$ 6.1Ca	67.8 $\pm$ 0.9Ba	20.4 $\pm$ 1.5Ab	17.8 $\pm$ 2.3ABb	13.4 $\pm$ 0.5Ca	16.8 $\pm$ 1.6Bab

Note: Different capital letters represent significant differences ($p<0.05$) between different treatments under the same nitrogen fertilizer application rates; different lowercase letters represent significant differences ($p<0.05$) under the same treatment but different nitrogen fertilizer application rates. All values are indicated as means $\pm$ standard deviation.

Table 7. Soil bulk density (g cm⁻³) in the mesocosm experiment

N rate (kg N ha ⁻¹)	NN	SF	SN	NF
75	1.17 $\pm$ 0.09aA	1.05 $\pm$ 0.06aA	1.14 $\pm$ 0.07aA	1.13 $\pm$ 0.12aA
150	1.11 $\pm$ 0.02aA	1.07 $\pm$ 0.03aA	1.09 $\pm$ 0.10aA	1.03 $\pm$ 0.10aA
225	1.14 $\pm$ 0.10aA	1.17 $\pm$ 0.07aA	1.17 $\pm$ 0.06A	1.21 $\pm$ 0.08aA

Note: Different capital letters represent significant differences at $p<0.05$ between different treatments under the same nitrogen fertilizer level; different lowercase letters represent significant differences at $p<0.05$ under the same treatment and different nitrogen fertilizer levels. All values are indicated as means $\pm$ standard deviation.

Figure captions

Fig. 1. Changes in daily temperature and rainfall at the experimental sites in 2018. Red, green, and blue represent the maximum temperature, minimum temperature, and rainfall, respectively.

Fig. 2. Pie charts showing the proportion of nitrogen derived from crayfish feed in harvested rice, harvested crayfish, and the environment (e.g., 13.6% of the N supplied by crayfish feed was estimated to be present in the RC system) and the proportion of nitrogen derived from straw in harvested rice, harvested crayfish, and the environment in the RC system (e.g., 4.3% of the nitrogen supplied by straw was estimated to be contained in the RC system) under different nitrogen application conditions. (a) 0 kg N ha⁻¹ level, (b) 75 kg N ha⁻¹ level, (c) 150 kg N ha⁻¹ level, and (d) 225 kg N ha⁻¹ level. The calculations for the balance of nitrogen output and input within the RC system are described in the Materials and methods section 2.4.1. The calculations used to determine the fate of feed-N and straw-N are described in the Materials and methods section 2.4.2.

Fig. 3. Flow direction of fertilizer nitrogen. Different capital letters represent significant differences ($p < 0.05$) between different treatments under the same nitrogen fertilizer application rates; different lowercase letters represent significant differences ($p < 0.05$) under the same treatment but different nitrogen fertilizer application rates. The labels 75, 150, and 225 on the X-axis represent the nitrogen application rates of 75 kg ha⁻¹, 150 kg ha⁻¹, and 225 kg ha⁻¹, respectively. NN, no straw with no feed; SF, straw with feed; SN, straw with no feed; NF, no straw with feed. Error bars represent the standard deviation. NUE (%), the percentage of the nitrogen in the fertilizer absorbed and utilized by rice plants; R_{soil} (%), the percentage of the nitrogen in the fertilizer stored in the soil; R_{loss} (%), the percentage of the nitrogen in the fertilizer lost to the environment.

Fig. 4. Nitrogen storage in rice grains, leaves, and stems; crayfish; and the environment in a field experiment in which the rice-crayfish (RC) system was subjected to different nitrogen application conditions. (a) 0 kg N ha⁻¹ level, (b) 75 kg N ha⁻¹ level, (c) 150 kg N ha⁻¹ level, and (d) 225 kg N ha⁻¹ level. Values are means $\pm$ SD. A negative mean for environmental nitrogen indicates that rice and/or crayfish, in addition to obtaining N from feed, obtained N from environmental sources. A positive value for environmental N indicates that N was lost to the environment. NN, no straw with no feed; SF, straw with feed; SN, straw with no feed; NF, no straw with feed.

■ Crayfish
 ■ Rice
 ■ Environment