

Article

Fates and Effects of the Input Nitrogen on N₂O Emissions in a Rice–Turtle Coculture System

Taojie Zhang ¹, Ranxin Dai ¹, Junlong Ye ¹, Liangliang Hu ¹ , Lei He ¹, Jianjun Tang ¹ , Lianghuan Wu ² and Xin Chen ^{1,*}

¹ College of Life Sciences, Zhejiang University, No. 866 Yuhangtang Road, Hangzhou 310058, China; 15968822310@163.com (T.Z.); 22007082@zju.edu.cn (R.D.); 0625d29@zju.edu.cn (J.Y.); zjuhull@126.com (L.H.); helei8705@126.com (L.H.); chandt@zju.edu.cn (J.T.)

² College of Environmental and Resource Sciences, Zhejiang University, No. 866 Yuhangtang Road, Hangzhou 310058, China; lhww@zju.edu.cn

* Correspondence: chen-tang@zju.edu.cn

Abstract

Rice–aquatic animal coculture systems receive both fertilizer-nitrogen (N) and feed-N inputs; however, the impacts of these inputs on N₂O emissions remain unclear. We conducted a field experiment in a rice–turtle coculture (RT) system using a randomized complete block design with seven treatments and three replications. The results of N applications were determined by the ¹⁵N-labeling method, and the N₂O emissions were measured by the static chamber–gas chromatography method. The results showed that 11.20% of fertilizer-N entered the rice, 0.39% entered the turtles, and 8.97% was retained by the soil. For feed-N, 29.9% was used by the rice, 10.13% was assimilated by turtles, and 49.68% remained in the soil (0–20 cm layer). In the subsequent year, fertilizer-N and feed-N retention rates were 6.17% and 37.61% respectively. In the RT system, N₂O emissions did not differ significantly from those in the rice monoculture (RM) system in the rice-growing season; however, they were significantly higher in the RT system than in the RM system in the winter fallow season. N₂O emissions from the winter fallow season accounted for 93.2% of the annual total, during which feed-N and fertilizer-N directly contributed 10.75% and 0.25% of emissions, and their priming effects contributed 48.4% and 5.1%. Our results suggested that assessing N₂O emissions from rice–aquatic animal coculture systems should incorporate year-round monitoring and that feed-N effects should be considered.

Keywords: rice–turtle coculture system; ¹⁵N stable isotope; nitrogen fate; feed nitrogen; fertilizer nitrogen; N₂O emissions



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1. Introduction

Rice is the staple food crop for over 3.5 billion people worldwide [1,2]. Rice yield has increased rapidly in recent decades; however, this increase in rice yield highly depends on nitrogen (N) supply [3]. The excessive application of chemical N fertilizer in conventional rice cultivation has resulted in a N use efficiency (NUE) typically below 40%, with large amounts of reactive N lost via ammonia volatilization, denitrification, leaching, and runoff, triggering a cascade of environmental problems such as water eutrophication, soil acidification, and increased greenhouse gas emissions [3,4]. Thus, avoiding excessive N use while maintaining yields is a top research priority [3,5].

Integrating rice cultivation with aquaculture (i.e., the coculture of rice with aquatic animals such as fish, shrimp, crab, and turtle) has been widely practiced as it enhances

production and farms' income, and reduces fertilizer and pesticide use [6–10]. Studies have shown that the integrated rice–aquaculture systems can reduce fertilizer-N input by 30–50% while maintaining or increasing rice yield and providing additional aquatic products [11–13]. Unlike rice monoculture systems, integrated rice–aquaculture systems often receive both fertilizer-N and feed-N. Experiments have indicated that these two types of exogenous N can be used in a complementary manner for both rice and aquatic animals [11,13,14]. However, whether and how these exogenous N sources affect the emissions of N-based greenhouse gases (e.g., nitrous oxide emissions) are not well known at present.

Nitrous oxide (N_2O) from paddy fields is a potent greenhouse gas with a global warming potential 273 times that of CO_2 [15]. N_2O is produced primarily during wetting–drying alternation [16,17], and the quantity of N_2O emissions is affected by the soil-N concentration [18]. N_2O emissions in integrated rice–aquaculture systems are a complex process. On the one hand, the introduction of aquatic animals alters the nitrogen cycling patterns in paddy fields. The input of high-protein feed significantly increases the total nitrogen input into the system. The excreta of aquatic animals (mainly ammonia nitrogen and organic nitrogen) are directly released into the water and soil, potentially providing more abundant substrates for nitrification and denitrification processes, and thereby increasing the production potential of N_2O [13,19]. On the other hand, the activities of aquatic animals (e.g., bioturbation, feeding behavior) may alter the soil's physical structure, redox status, and microbial community composition, thereby influencing the pathways of N_2O production and release [20,21]. Furthermore, long-term flooded conditions may inhibit nitrification and thus reduce N_2O emissions, but at the same time may promote complete denitrification to N_2 , or lead to N_2O emissions in specific microsites (such as the rhizosphere) or at the water–soil interface [22,23].

Some studies showed that rice–fish systems are beneficial for reducing N_2O emissions [24,25], while studies found that the rice–crayfish co-cropping systems and rice–duck systems increased N_2O emissions compared to rice monoculture [26,27]. The discrepancies among different studies may arise from variations in several factors, including aquaculture species, stocking density, feed management, water level management, and study time scales. Furthermore, existing research on N_2O emissions is often limited to the rice-cultivation period, with few studies focusing on emissions during the winter fallow season or on an annual whole-year basis.

Although studies have shown the results of adding fertilizer-N and feed-N to paddy ecosystems [3,11,14], a quantitative understanding is still lacking regarding (i) the contrasting fates of these two exogenous N sources (fertilizer-N vs. feed-N) within plants, animals and soil; (ii) how their residual soil pools contribute to the winter fallow N_2O emissions, which have been largely overlooked in previous growing-season studies. In particular, the role of feed-derived organic nitrogen in priming native soil N mineralization and its relative importance compared to fertilizer-derived inorganic N remain unknown. We hypothesize the following: (1) feed-N retention in soil is much more than fertilizer-N, because the latter is rapidly lost via volatilization, denitrification, and leaching; (2) N_2O emissions in the winter fallow may be predominantly driven by the residual feed-N because of its high retention in soil; and (3) the winter fallow period may contribute the majority of annual N_2O emissions in this system. To address these hypotheses, we conducted a field experiment in a rice–turtle coculture system, in which Chinese soft-shelled turtle (*Pelodiscus sinensis*) was cultured along with rice during the rice-growing season. Using the ^{15}N -labeling method, we quantitatively compared the partitioning of the two N sources into rice plants, turtles, and soil layers, as well as their interannual residual retention rates. Through combined year-round continuous N_2O flux measurements and ^{15}N -source

apportionment, we elucidated the sources of N₂O (direct emissions, priming effects, and interactive effects), aiming to answer three scientific questions: (1) How do the fates of feed-N and fertilizer-N differ? (2) Does soil-N delivered from feed-N affect N₂O emissions? (3) What are the respective contributions of the two N sources to N₂O emissions? Addressing these questions will provide a scientific basis for sustainable nitrogen management and greenhouse gas mitigation in the integrated rice–aquaculture systems.

2. Materials and Methods

2.1. Ethics Statement

In this study, the aquatic animal (i.e., softshell turtles) was cultured and measured in accordance with the approved guidelines of the Zhejiang University Experimental Animal Management Committee (approval code: ZJU20250140).

2.2. Experimental Site and the Rice–Turtle Coculture System

A field experiment was conducted from 2023 to 2024 in Tongxiang City, Zhejiang Province, China. The region has a subtropical monsoon climate, with an annual mean temperature of 16–18 °C and annual precipitation of 1200–2000 mm. The experimental site is located in Lianfeng Village, Chongfu Town, Tongxiang City (120°25′4″ E, 30°33′51″ N). The terrain is flat, and the soil bulk density is approximately 1.12 g cm^{−3}, with a pH of about 6.8, an organic matter content of 22.13 g kg^{−1}, a total nitrogen content of 1.24 g kg^{−1}, an available phosphorus content of 51.36 mg kg^{−1}, and a readily available potassium content of 168.69 mg kg^{−1}.

The rice–turtle coculture system is one of the important types of integrated rice–aquaculture systems and is widely practiced in southeastern China [28]. Chinese soft-shelled turtle (*Pelodiscus sinensis*) is used in the rice–turtle coculture system. The turtles often live in rice fields for two years before being harvested. From November to the following May, when no rice is grown, the turtles stay in the refuge. High turtle yields require high-quality fish feed input.

2.3. Experimental Design

The experiment followed a randomized complete block design with seven treatments (Table 1) and three replications. Treatments 1–6 were used to analyze nitrogen fates and vertical distribution characteristics in the soil, as well as residual retention rates in the following year, while Treatments 1, 2, 6, and 7 were used to determine the interannual dynamics and source apportionment of N₂O emissions. Details of the basal fertilizer and feed application rates are presented in Table 1.

Table 1. Fertilizer and feed input.

System	Treatment	Fertilizer (g·Plot ^{−1})			Feed (g·Plot ^{−1})
		Urea	Ca ₃ (PO ₄) ₂	KCl	
RM ± U- ¹⁵ N	T1	0	49.12	35.7	/
	T2	143.97 (¹⁵ N)	49.12	35.7	/
RT ± U- ¹⁵ N	T3	0	49.12	35.7	876
	T4	143.97 (¹⁵ N)	49.12	35.7	876
RT ± F- ¹⁵ N	T5	143.97	49.12	35.7	876
	T6	143.97	49.12	35.7	876(¹⁵ N)
	T7	0	49.12	35.7	876(¹⁵ N)

Note: U-¹⁵N and F-¹⁵N are ¹⁵N-labeled urea and ¹⁵N-labeled feed.

Each plot measured 4 m² (2 m × 2 m), and an 80 cm isolation strip was established between adjacent plots to prevent lateral nitrogen movement. Each plot was equipped with

an independent inlet–outlet system for separate irrigation and drainage, and water flow rates were kept consistent across all plots. Seventy rice hills (Nanjing 46) were transplanted into each plot, with a hill spacing of 20 cm × 30 cm and two seedlings per hill. Urea was applied on the third day after transplanting, followed by a compound fertilizer topdressing on the tenth day; both were dissolved in deionized water and applied as a fine spray. Seven days after the compound fertilizer application, Chinese soft-shelled turtles (*Pelodiscus sinensis*) that were disinfected by immersion in 0.001% potassium permanganate solution for 15 min were introduced (450 g per turtle, two turtles per plot). A 200 cm high mesh net was installed around each plot to deter birds and animals. Feeding started on the third day after turtle introduction, with feed provided daily between 16:00 and 17:00 at a rate of 1% of body weight, adjusted every 10 days, and continued for 100 days. No additional topdressing or pesticides were applied during the experimental period.

2.4. Measurement of the Fate of Nitrogen in the System

Before rice harvest, soil cores were collected from the plots of Treatments 1–6 using PVC tubes (5 cm diameter, 20 cm length). The cores were divided into three layers—0–5 cm, 5–10 cm and 10–20 cm—air-dried, ground to pass a 100-mesh sieve, and analyzed for $\delta^{15}\text{N}$ and total N. At rice maturity, three representative hills per experimental plot (along with the underlying 20 cm × 20 cm soil monolith) were selected. The plants were separated into roots, stems, leaves, and panicles, oven-dried at 105 °C for 30 min for enzyme deactivation, and then dried at 65 °C to constant weight. After weighing and grinding, total N and $\delta^{15}\text{N}$ were determined. Simultaneously, all turtles in each plot were harvested, kept in clean water for 48 h to evacuate gut contents, frozen at −20 °C, freeze-dried, ground, and analyzed for total N and $\delta^{15}\text{N}$. In the following year after harvest, soil samples were collected again using the same method to calculate the ^{15}N residual retention rate.

The $\delta^{15}\text{N}$ values and total N contents of all rice, soil, and turtle samples were determined using a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany).

The fate of fertilizer-N is determined by calculating the ratio (R) of fertilizer- ^{15}N to the N in rice, turtles, and soil using Equation (1):

$$R_{\text{fertilizer-N}}(\%) = \frac{(Y_{\text{labeled treatment}} - Y_{\text{control}})}{(Y_{\text{labeled urea}} - Y_{\text{control}})} \times 100 \quad (1)$$

where $Y_{\text{labeled treatment}}$ is the ^{15}N abundance in rice, turtles, and soil in the treatment with ^{15}N -labeled fertilizer (i.e., Treatment 4, Table 1); Y_{control} is the ^{15}N abundance in rice, turtles, and soil in the treatment without ^{15}N -labeling (i.e., Treatment 3, Table 1); and $Y_{\text{labeled urea}}$ is the ^{15}N abundance of the ^{15}N -labeled tracer (i.e., ^{15}N -labeled urea) [29].

The fate of feed-N is determined by calculating the ratio (R) of feed- ^{15}N to the N in rice, turtles, and soil using Equation (2).

$$R_{\text{feed-N}}(\%) = \frac{(Y_{\text{labeled treatment}} - Y_{\text{control}})}{(Y_{\text{labeled feed}} - Y_{\text{control}})} \times 100 \quad (2)$$

where $Y_{\text{labeled treatment}}$ is the ^{15}N abundance in rice, turtles, and soil in the treatment with ^{15}N -labeled feed (i.e., Treatment 6, Table 1); Y_{control} is the ^{15}N abundance in rice, turtles, and soil in the treatment without ^{15}N -labeling (i.e., Treatment 5, Table 1); and $Y_{\text{labeled feed}}$ is the ^{15}N abundance of the ^{15}N -labeled tracer (i.e., ^{15}N -labeled feed) [29].

2.5. Measurement of Interannual Dynamics and Source Apportionment of N₂O Emissions

System N₂O emissions were measured using the closed static chamber–gas chromatography method. Emissions were determined for T2 and T6 (corresponding to RM, rice monoculture, and RT, rice–turtle coculture, respectively). Each measurement was conducted between 8:00 a.m. and 12:00 p.m. Gas samples were collected using 50 mL syringes at 0 min, 15 min and 30 min after the chamber was placed over the rice plants, and the air temperature was recorded. The gas samples were stored in aluminum foil gas bags and transported to the laboratory, where N₂O concentrations were analyzed using a gas chromatograph Agilent GC 8860 (Agilent Technologies Inc., Santa Clara, CA, USA) (Agilent GC 8860). The ¹⁵N abundance of N₂O was measured using an IsoPrime 100 IRMS coupled to a Trace Gas preparation unit (IsoPrime Limited, Cheadle, UK). The N₂O emission flux was calculated according to Equation (3):

$$F = \rho \times h \times \frac{dC}{dt} \times \frac{273}{273 + T} \quad (3)$$

F : N₂O emission flux (μg·m^{−2}·h^{−1});

ρ : density of N₂O;

C : volume concentration of N₂O in the chamber;

t : time;

h : effective height of the chamber;

T : mean temperature inside the chamber during sampling.

For the first three weeks after fertilization, measurements were conducted once a week, after emissions stabilized. N₂O emissions were measured approximately every two weeks. After rice harvest, during the winter fallow period, measurements were taken every 1–2 months, with the final measurement conducted before plowing.

The static chambers for measuring inter-row soil N₂O emissions were constructed from white PVC pipes (15 cm diameter, 80 cm height), and measurements were conducted concurrently with the system N₂O emission measurements.

Source apportionment of soil N₂O emissions: inter-row soil gas samples were collected from T1, T2, T6, and T7, and the N₂O concentrations in T1, T2, T6, and T7, as well as the ¹⁵N abundance of N₂O in T1, T2, and T7 were determined.

The N₂O emission flux was calculated using the same Equation (3). The cumulative soil N₂O emission E (kg ha^{−1}) was calculated according to Equation (4):

$$E = \sum_{i=1}^n \left(\frac{F_i + F_{i+1}}{2} \right) (t_{i+1} - t_i) \cdot 24 \quad (4)$$

E : cumulative N₂O emission (kg ha^{−1});

n : number of gas sampling events;

F : N₂O emission flux (μg m^{−2} h^{−1});

$t_{i+1} - t_i$: interval in days between two consecutive samplings.

The N₂O emission flux derived from fertilizer or feed, F_f was calculated according to Equation (5):

$$F_f = \frac{a_{N_2O} - a_1}{a_f - a_1} \times F \quad (5)$$

F_f : N₂O emission flux derived from fertilizer or feed (μg m^{−2} h^{−1});

a_{N_2O} : ¹⁵N abundance of N₂O emitted from soil in the ¹⁵N-labeled treatment (atom%);

a_1 : natural ¹⁵N abundance of N₂O from unfertilized control soil (0.362695 atom%);

a_f : ¹⁵N abundance of the applied fertilizer or feed (atom%).

F : N_2O emission flux ($\mu\text{g m}^{-2} \text{h}^{-1}$).

$a_{\text{N}_2\text{O}}$ (atom%) was calculated according to Equation (6):

$$a_{\text{N}_2\text{O}} = \frac{a_{30} \times c_{30} - a_0 \times c_0}{c_{30} - c_0} \quad (6)$$

$a_{\text{N}_2\text{O}}$: ^{15}N abundance of N_2O emitted from ^{15}N -labeled soil (atom%);

a_{30} : ^{15}N abundance of N_2O collected at 30 min; c_{30} : N_2O concentration collected at 30 min;

a_0 : ^{15}N abundance of N_2O collected at 0 min; c_0 : N_2O concentration collected at 0 min.

The cumulative soil N_2O emission primed by fertilizer or feed input, E_p (kg ha^{-1}), was calculated according to Equation (7):

$$E_p = E - E_f - E_0 \quad (7)$$

E_p : cumulative soil N_2O emissions primed by fertilizer or feed input (kg ha^{-1});

E : cumulative N_2O emissions (kg ha^{-1});

E_f : cumulative N_2O emissions derived from fertilizer or feed (kg ha^{-1});

E_0 : cumulative N_2O emissions from the unfertilized treatment (kg ha^{-1}).

2.6. Statistical Analysis

Statistical analysis was conducted using the GLM in SPSS (V.20.0). The test of homogeneity of variances was conducted before analysis. One-way analysis of variance (ANOVA) was performed on retention of labeled fertilizer-N and feed-N in soil, and N_2O emission between treatments. Two-way ANOVA was performed on soil $\delta^{15}\text{N}$ with treatment and soil layer as factors. Two-way repeated measures ANOVA was performed on N_2O emission rate between treatments.

3. Results

3.1. Fate of Fertilizer-N and Feed-N in Paddy Fields

Isotopic tracing for fertilizer- ^{15}N demonstrated that 10.21% of the input fertilizer-N entered the rice plants, including roots, stems, leaves and grains, and 11.39% remained in the 0–20 cm soil layer in the rice monoculture (RM) system (Table 2). The remaining 78.41% was unaccounted for, presumably lost to the environment via gaseous emissions and leaching/runoff. In the rice–turtle (RT) coculture system, 11.2% of the input fertilizer-N entered the rice plants, including roots, stems, leaves and grains, 0.39% entered the turtles, and 8.97% remained in the 0–20 cm soil layer (Table 2). Like the RM system, the remaining 79.44% was unaccounted for, presumably lost to the environment via gaseous emissions and leaching/runoff. Isotopic tracing for feed- ^{15}N also showed that in the RT system, 10.13% of the input feed-N was utilized by the turtles, 29.21% was utilized by the rice plants, including roots, stems, leaves and grains, and 49.68% remained in the 0–20 cm soil layer. The remaining 10.27% was unaccounted for, presumably lost to the environment via gaseous emissions and leaching/runoff (Table 2).

Our experiment also showed that the $\delta^{15}\text{N}$ values had a vertical distribution in the soil profiles of both rice monoculture and rice–turtle coculture systems with fertilizer- ^{15}N and feed- ^{15}N (Figure 1A). The $\delta^{15}\text{N}$ values in the surface layer (0–5 cm) were significantly higher than in other soil layers ($p < 0.05$, Figure 1A). In the subsequent year, the $\delta^{15}\text{N}$ values in the surface layer decreased significantly ($p < 0.05$, Figure 1A).

Table 2. Fate of fertilizer-N and feed-N based on ^{15}N -tracing method.

Pool	Fate of Fertilizer-N (%)		
	RM + U- ^{15}N	RT + U- ^{15}N	RT + F- ^{15}N
Rice root	1.32 ± 0.06	0.92 ± 0.05	1.37 ± 0.12
Rice stalks	2.68 ± 0.15	3.66 ± 0.09	8.75 ± 0.55
Rice leaf	1.69 ± 0.07	2.93 ± 0.18	5.04 ± 0.2
Rice grain	4.53 ± 0.27	3.69 ± 0.11	14.74 ± 0.6
Turtle	-	0.39 ± 0.03	10.13 ± 0.6
0–20 cm soil	11.39 ± 0.75	8.97 ± 0.68	49.68 ± 2.25

Note: RM: rice monoculture; RT: rice–turtle coculture; U- ^{15}N : labeled urea- ^{15}N ; F- ^{15}N : labeled feed- ^{15}N ; values are mean ± Sem.

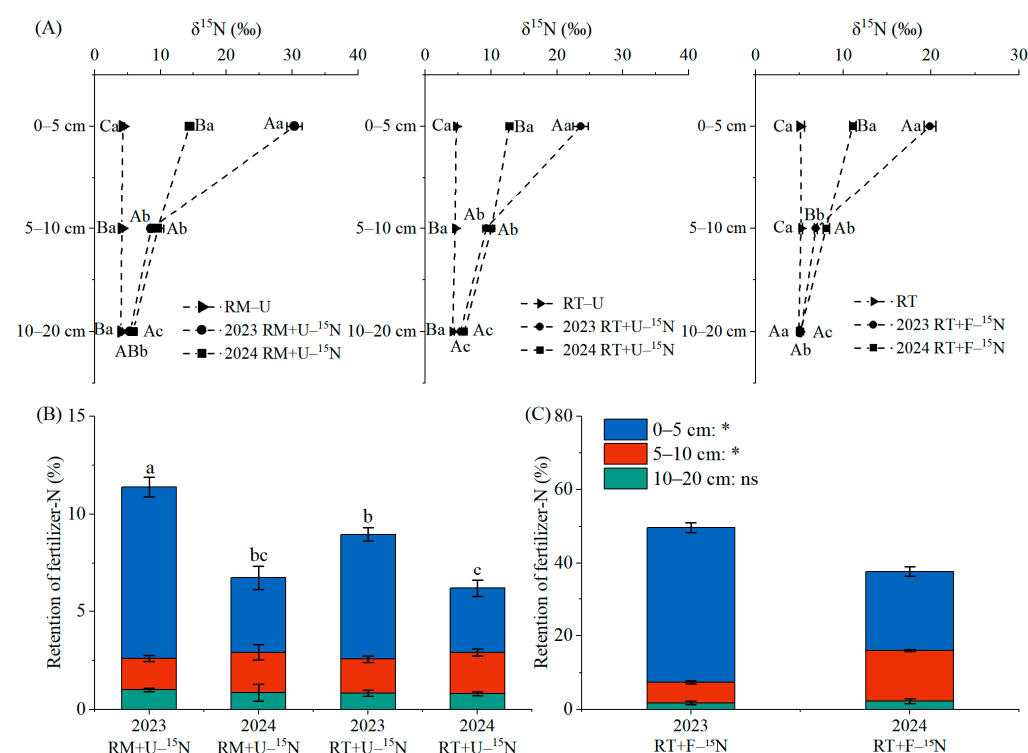


Figure 1. Labeled fertilizer-N and feed-N remain in soil in the current and following year. (A): $\delta^{15}\text{N}$ values in soil profile. (B). Retention rates of fertilizer-N in soil profile in RM and RT. (C). Retention rates of feed-N in soil profile in RT. RM: rice monoculture; RT: rice–turtle coculture. U- ^{15}N : labeled urea- ^{15}N . F- ^{15}N : labeled feed- ^{15}N . Values are mean ± SE. *: $p < 0.05$. Different letters indicate statistically significant differences ($p < 0.05$). Uppercases indicate significant differences at the same soil layer; lowercases indicate significant differences between the different soil layers.

Fertilizer-N showed low retention in the soil (8.97% in the current year, 6.17% in the following year, Figure 1B). In contrast, feed-N achieved a retention rate as high as 49.68% in the current year and retained 37.61% in the following year (Figure 1C).

3.2. N_2O Emissions in the Rice-Growing Season and the Winter Fallow Period

In the rice-growing season, N_2O emissions between the rice–turtle coculture system and the rice monoculture were not significant (Figure 2A, $p = 0.10$). In the winter fallow period, however, N_2O emissions in the rice–turtle coculture system were significantly higher than those in the rice monoculture (Figure 2B, $p < 0.001$). Annually, 93.19% of the total N_2O emissions in the rice–turtle coculture system were from the winter fallow season, whereas this proportion was only 52.85% in the rice monoculture system (Figure 2C).

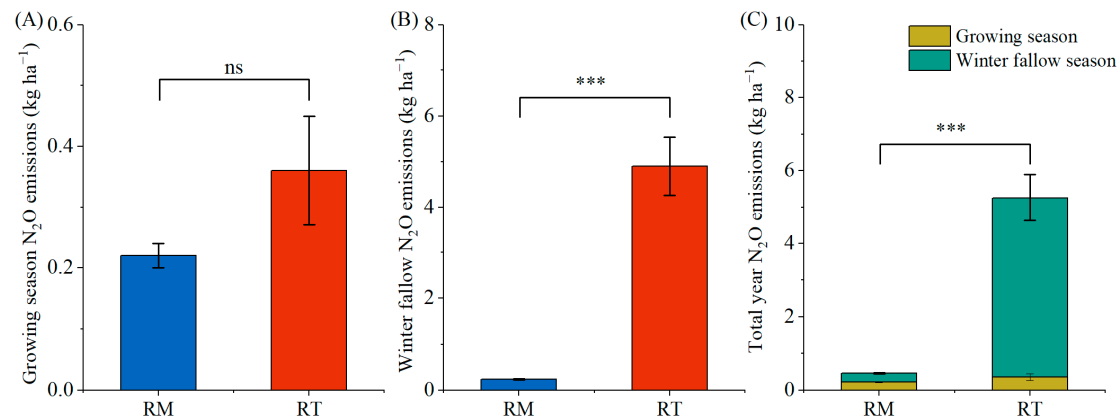


Figure 2. N₂O emission in the growing season (A), in the winter (B) and in a whole year (C). RM: rice monoculture; RT: rice–turtle coculture; Values are mean $\pm$ SE. ns: no significant difference; ***: $p < 0.001$.

3.3. Contributions of Fertilizer-N and Feed-N to N₂O Emissions

During the rice-growing season, both RM and RT fertilized treatments exhibited significant N₂O emission peaks one week after fertilization, whereas the unfertilized treatment showed very low emissions (Figure 3). During the winter fallow period, emission peaks occurred only in RT treatments (Treatment 4–Treatment 7) regardless of chemical fertilizer application (Figure 3).

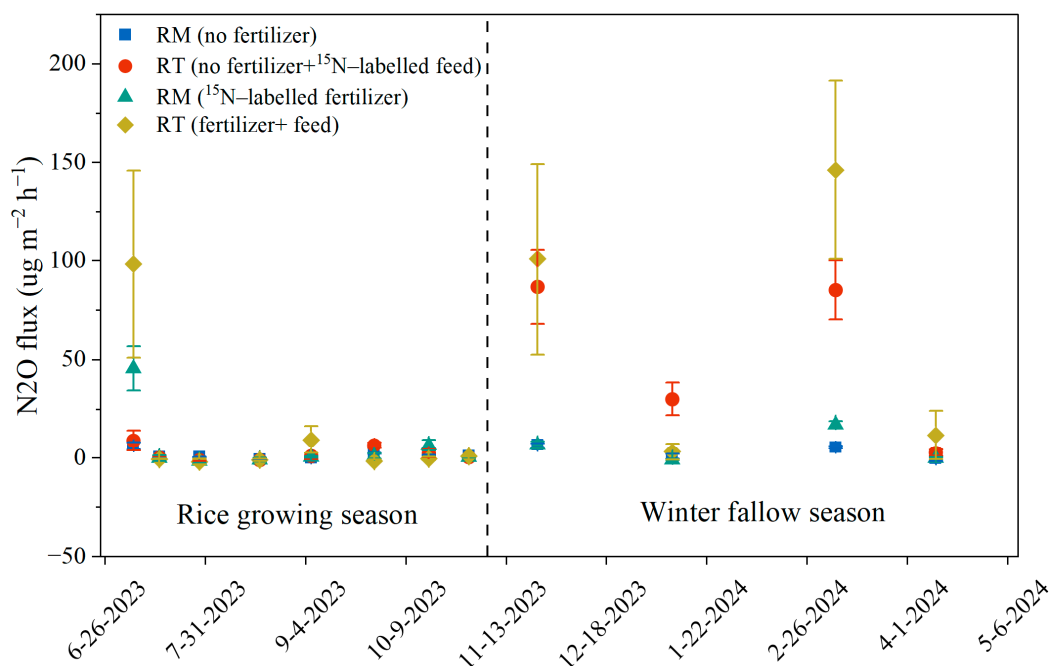


Figure 3. Interannual dynamics of emission N₂O rate. RM: rice monoculture; RT: rice–turtle coculture; values are mean $\pm$ SE.

In the rice-growing season, fertilizer-N directly contributed to 16.77% to 29.67% of N₂O emission in RM (Figure 4A), For RT, the contributions of fertilizer-N and feed-N and their interactions with N₂O emissions were 11.5–13.22%, 6.76–7.77% and 20.4%, respectively (Figure 4A). Both the application of fertilizer-N and feed-N increased N₂O emissions (Figure 4B). The fertilizer-primed soil N₂O emissions were 2.3 times the direct emissions, while the feed-primed N₂O emissions were 1.7 times the direct emissions (Figure 4B).

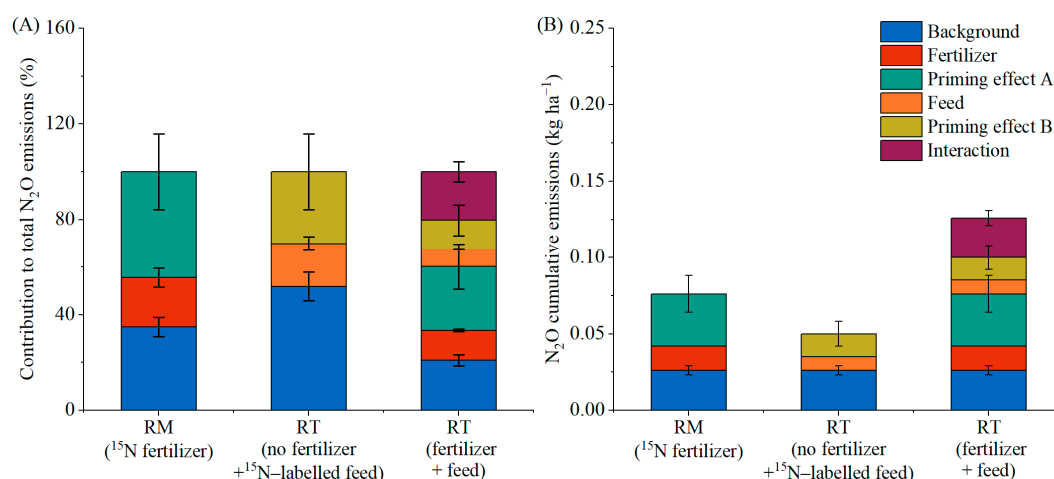


Figure 4. Contribution of fertilizer-N and feed-N to N₂O emissions in the rice-growing season. (A): Contribution rate of different parts; (B): N₂O emissions from various parts. RM: rice monoculture; RT: rice–turtle coculture; Background: N₂O emissions from unfertilized controls; Fertilizer: N₂O emissions from fertilizers; Priming effect (A): N₂O emissions from soil excitation by fertilizers; Feed: N₂O emissions from feeds; Priming effect (B): N₂O emissions from soil excitation by feeds; Interaction: N₂O emissions from soil excitation through the interactive effects of two measures. Values are mean ± SE.

In the winter fallow season, the direct contribution of fertilizer-N was 2.09–2.79% in RM, while the contributions of fertilizer-N and feed-N and their interaction with N₂O emissions were 11.5–13.22%, 0.17–0.25% and 32.7% respectively in RT (Figure 5A). The fertilizer-N priming effect was 24.3 times the direct emissions, while the feed-N priming effect was 4.6 times the direct emissions (Figure 5B).

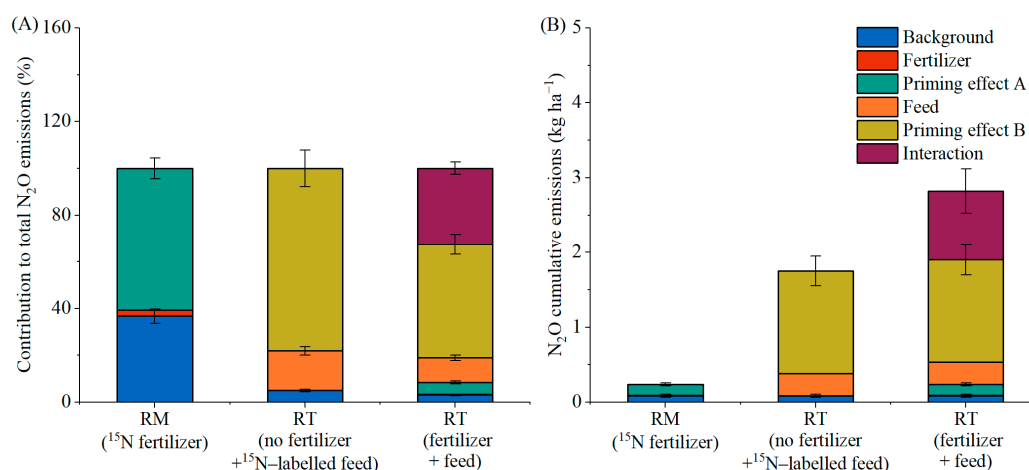


Figure 5. Contribution of fertilizer-N and feed-N to N₂O emissions in the winter fallow period. (A): Contribution rate of different parts; (B) N₂O emissions from various parts. RM: rice monoculture; RT: rice–turtle coculture; Background: N₂O emissions from unfertilized controls; Fertilizer: N₂O emissions from fertilizers; Priming effect (A): N₂O emissions from soil excitation by fertilizers; Feed: N₂O emissions from feeds; Priming effect (B): N₂O emissions from soil excitation by feeds; Interaction: N₂O emissions from soil excitation through the interactive effects of two measures. Values are mean ± SE.

4. Discussion

4.1. Differences in the Fates of Feed-N and Fertilizer-N in the Rice–Turtle System

By using the ¹⁵N-tracing method, our results provided quantitative evidence of the distinctly different partitioning patterns of fertilizer-N and feed-N in the rice–turtle cocul-

ture system. After application, only 20.56% of fertilizer-N was recovered by the system, with as much as 79.44% lost to the environment; in contrast, the recovery rate of feed-N reached 89.73%, with a loss rate of only 10.27% (Table 2). The fundamental reason for this difference lies in the contrasting chemical forms and transformation kinetics of the two N sources.

In paddy ecosystems, once urea fertilizer is applied to paddy fields, it is rapidly hydrolyzed to NH_4^+ , which can be quickly taken up by rice roots, lost via ammonia volatilization, or oxidized to NO_3^- by nitrifying bacteria under aerobic conditions [30,31]. In flooded soils, NO_3^- is highly susceptible to denitrification to N_2 or N_2O , or to leaching loss with percolating water [30,32]. In the present study, the seasonal loss rate of fertilizer-N was as high as 79.44% (Table 2), consistent with previous reports showing that the N-use efficiency of paddy fertilizer is typically below 30% and loss rates exceed 60% [33,34].

In contrast, feed-N enters the system in organic form: approximately 10.13% is directly assimilated by turtles and converted into animal biomass, while the remainder is deposited on the field surface as uneaten feed and feces. The organic N in feed must be mineralized to NH_4^+ by microorganisms before it becomes available for rice uptake. This process is slow and sustained, making the release of feed-N highly synchronized with the N-demand rhythm of rice, especially during the mid-to-late booting and grain-filling stages [35,36]. In this study, the contribution rate of feed-N to rice panicles (14.74%) was four times that of fertilizer-N (3.69%) (Table 2), directly demonstrating that slow-release N sources are more conducive to grain-N accumulation. This finding is consistent with the results of Xie et al. [11], who observed efficient utilization of feed-N by rice in a rice–fish system, corroborating the ecological principle of complementary animal–crop utilization.

The high retention of feed-N in the soil, indicating $\delta^{15}\text{N}$ values and retention rate, is another key finding in this study (Figure 1). Organic N binds with soil organic matter to form stable organo–mineral complexes, which are physically protected by soil aggregates and chemically protected by humic substances [37,38]. Simultaneously, the co-input of carbon and N from feed stimulates microbial growth, and the microbial biomass N formed through the microbial assimilation of inorganic N is subsequently converted into stable soil-organic N upon cell death [39,40]. This process explains why 37.61% of feed-N was retained in the following year (Figure 1), whereas only 6.17% of fertilizer-N remained (Figure 1). From the perspective of soil-N pool management, feed-N essentially functions as a “slow-release organic N fertilizer”, with its long-term effect far exceeding that of fast-release chemical fertilizers.

4.2. High N_2O Emissions in the Winter Fallow Period Driven by Feed-N

Our results showed that the N_2O emission peak in the rice–turtle system occurred not during the flooded rice-growing season but during the dry winter fallow period, which accounted for 93.2% (Figure 2) of the annual total and was predominantly driven by feed-N (58.9%, Figure 5A). This result challenges the conventional practice of assessing the environmental impacts of rice–aquaculture systems solely during the rice-growing season. Prolonged flooding (20 cm water depth) during the growing season maintained the soil under strictly anaerobic conditions, in which nitrification was suppressed. Although denitrification occurred, complete denitrification ($\text{NO}_3^- \rightarrow \text{N}_2$) dominated, making it difficult for N_2O to accumulate as an intermediate product [16,17]. Moreover, the diffusion rate of N_2O through the water layer was extremely low, and most of the N_2O was reduced to N_2 during upward transport [23,41]. Consequently, N_2O emissions from both TM and RT systems remained at very low levels during the growing season, with no significant differences among treatments (Figure 2).

Drainage and field-drying during the winter fallow period triggered a pronounced wetting–drying alternation effect. The soil shifted from anaerobic to alternating aerobic–anaerobic conditions, activating both faunal and microbial activities and accelerating organic nitrogen mineralization. In this study, the retention rate of feed N in the 0–5 cm surface soil layer was as high as 42.35% (Figure 1B). This accumulated organic N was extensively mineralized after the field was drained, releasing NH_4^+ and NO_3^- and thereby providing abundant substrates for nitrification and denitrification. More importantly, feed-N exerted a strong priming effect—the N_2O that was added through the priming effect (48.4%) was 4.6 times that from direct emissions (8.81–12.68%, Figure 5A). The mechanism underlying the priming effect is as follows: the input of fresh organic matter (uneaten feed and feces) provides heterotrophic microorganisms with readily available carbon and N sources, which in turn accelerates the microbial decomposition of native soil organic matter to acquire energy, leading to additional N_2O release [42,43]. This cascading amplification effect represents the core mechanism by which feed-N drives the winter fallow N_2O surge.

Unlike feed-N, the direct contribution of fertilizer-N during the winter fallow period was less than 0.25%, and its priming effect accounted for only 5.1% (Figure 5A). This is because most of the fertilizer-N had already been lost during the growing season (Table 2, 79.44%), and the residual portion was primarily leached as NO_3^- to soil layers below 20 cm, far from the active surface layer (0–10 cm) where N_2O production occurs. This finding clearly indicates that in the rice–turtle system, feed-N is the regulatory target for N_2O emission mitigation during the winter fallow period, rather than chemical fertilizer-N.

4.3. Implication for the Assessment Framework

The findings of this study help to reconcile the controversies in previous N_2O emission studies on integrated rice–aquaculture systems. Some studies reported that rice–fish systems reduce N_2O emissions [25,44], whereas other studies reported increased N_2O emissions from rice–duck and rice–crayfish systems [26,27,45]. This discrepancy likely arises from differences in aquatic animal species and the corresponding feed input intensity. Fish are typically stocked at low densities and supplied with feed of relatively low protein content, leading to limited accumulation of feed-N [4,11]. In contrast, turtles stocked at high densities and receiving feed with high protein content (the turtle feed in this study contained 9.39% N) lead to the substantial accumulation of feed-N in the soil [28]. Furthermore, the intensity of bioturbation varies among animal species, which also influences soil aeration and N distribution [46]. Therefore, environmental assessments of rice–aquaculture systems must adopt a species-specific approach, taking into account stocking density and feed management [47,48].

The current assessment frameworks for paddy greenhouse gas emissions mainly considered the growing season and non-growing season emissions are sometimes negligible. However, recent studies have indicated that N_2O emissions from paddy fields during the non-growing season cannot be ignored [49,50]. Our study demonstrates that neglecting the winter fallow period would underestimate annual N_2O emissions from the rice–turtle system by over 90%, leading to completely erroneous conclusions. Measuring only the growing season would suggest that the rice–turtle system does not differ from rice monoculture systems. Therefore, it is recommended that when monitoring greenhouse gas emissions from paddy ecosystems, complete wetting–drying cycles should be included, particularly after drainage and field-drying and during the early winter fallow period.

5. Conclusions

This study elucidates the contrasting fates of fertilizer-N and feed-N in rice–turtle coculture systems and their distinct effects on N_2O emissions. We demonstrate that feed-N,

in contrast to fertilizer-N, exhibits high soil retention and long-term residual persistence. We reveal that the winter fallow period—rather than the rice-growing season—is the dominant source of annual N₂O emissions in this system, a peak entirely driven by feed-N through both direct emissions and a strong priming effect. These findings underscore the importance of the winter fallow season and feed-N in N₂O emissions in rice–aquaculture systems. Our study provides a scientific basis for sustainable N management in integrated rice–aquaculture farming.

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