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A Comparison of Nitrogen Transfer and Transformation in Traditional Farming and the Rice–Duck Farming System by ^{15}N Tracer Method

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Abstract: A field experiment was conducted in Ninghe, Tianjin, China, using the ^{15}N isotope method to determine the fate of N sources, application effect of organic fertilizer on the growth of rice plant organs, N uptake by rice, and N use efficiency. The experiment included eight treatments: CK-N (control + no-duck), CK-D (control + ducks), CF-N (chemical fertilizer + no-ducks), CF-D (chemical fertilizer + ducks), CM-N (chemical fertilizer + organic fertilizer + no-ducks), CM-D (chemical fertilizer + organic fertilizer + ducks), CD-N (chemical fertilizer 30% off + organic fertilizer + no-ducks), and CD-D (chemical fertilizer 30% off + organic fertilizer + ducks). The results showed that the application of organic fertilizer whether CM or CD in grain and leaf significantly increased N concentration; leaf and root P concentrations over control (CK) and chemical fertilizer (CF). In contrast, straw and root N concentrations, including grain and straw P concentrations did not show any difference between duck and no-duck treatment. Moreover, non-significant differences were found in ^{15}N fresh grain and husk concentration. Both organs ranged from 14.2–14.4 $g \cdot kg^{-1}$ and 6.2–6.3 $g \cdot kg^{-1}$, respectively. Likewise, N uptake and N use efficiency in fresh grain and husk were not significantly differed within duck and without duck treatment. However, N uptake in fresh grain and husk ranged at the rates of 54.90–93.69 and 6.43–11.04 kg ha^{-1} with duck and without duck treatment. N use efficiency in fresh grain and husk ranged from 21.55%–34.61% and 2.61%–4.24%, respectively. Overall organic fertilizer has a significant influence on rice growth and promotes crop productivity.

Keywords: nitrogen; transfer; transformation; N uptake; nitrogen use efficiency

1. Introduction

Rice is the most important staple food crop in the world [1] and contributes to more than 40% of the cereal yield [2]. In the last decades, rice yield in China rapidly increased due to the introduction of high yield varieties and increasing use of chemical N fertilizer [3]. Moreover, it has been mentioned that in order to meet the demand of the ever-increasing population, sustainable increases in rice yield by 1.21% annually is needed for food security in China [4,5]. As a result, the use of fertilizers, especially chemical fertilizer (CF) in China is more intensive and wide-spread than in any other country [6]. However, excessive use of N fertilizer has the consequence of severe environmental degradation with high potential for N loss in many pathways [7], decreased N use efficiency (NUE), decreased crop quality, and creation of environmental hazards in rice growing countries [8–11]. Therefore, an appropriate fertilizer input should be required and controlled to maintain rice yield. Adequate nitrogen (N) supply may enhance the rice growth and improve grain yield, and the application of appropriate levels of N fertilizer through improved management is key to increasing N use efficiency [12,13]. In addition, nitrogen is required to produce more food in agricultural systems. Therefore, the lack of N responds quickly to the addition of N fertilizers if applied in a timely manner and properly. Furthermore, nitrogen transformation in soil–plant systems involves the complex N cycling process, which increases the difficulty of N management. Basically, processes involving N in the soil–plant system are: mineralization, nitrification, immobilization, leaching, denitrification, and volatilization. In the present study, ducks were introduced to the field. Duck activities include walking, swimming, eating, grooming, paddling, and rubbing which can influence soil structure and fertility. Duck feces may be a good supply of organic fertilizer to the soil. Thus, it is estimated that the total excreted feces per duck can reach 10 kg, which contains 47 g N, 70 g P, and 31 g K [14].

Previous research has shown that duck stirring and intertillage can improve elements of the soil environment, such as soil air, texture, and structure [15]. Duck activities may enhance the decomposition of soil organic matter and nutrient transformation, which benefits the growth of rice plants. Moreover, we reduced the amount of inorganic fertilizer, maintained organic fertilizer application, and introduced ducks to the field in order to achieve the goal of clean rice production. Therefore, the amounts of fertilizers were strictly evaluated in order to avoid heavy N loss to the environment, environmental pollution, and to contribute to safe food production. Information about the combined application of organic and inorganic fertilizer and the use of ^{15}N labeled sources in traditional farming and the rice–duck farming system is limited. The aims of this research were to determine the fate of labelled N sources, including the effect of the application of organic fertilizer on the growth rice plant organs, N uptake by rice, and N use efficiency in duck and no-duck fields.

2. Materials and Methods

2.1. Study Site

The field experiment was conducted in 2017 during the rice-growing season at Ninghe district of Tianjin City, China (39°18–39°50′ N, 117°08–117°56′ E). Ninghe covers an area of 1414 km^2, with a typical humid continental climate with large seasonal temperature differences. The annual average temperature is 54.0 °F (12.2 °C) and the warmest month is July, with an average temperature of 79.2 °F (26.2 °C). The coolest month is in January with an average of 24.4 °F (−4.2 °C) and annual average precipitation of 591.8 mm.

2.2. Experimental Design and Operation

The seeds were sown on 7 June 2017, and then the rice seedlings of Japonica rice were transplanted directly to the plots during the tilling stage on 3 July and harvested on 18 October 2017. The application of ^{15}N-labelled fertilizer and ordinary fertilizers was performed during vegetative growth on 13 July 2017. Before applying isotope [$^{15}(NH_4)_2SO_4$] fertilizer and ordinary ammonium sulphate fertilizers to different treatments in two fields (duck and no-duck), the experiment field was drained and the soil was brought through the plots, irrigated, then the rice seedlings were transplanted. There were eight treatments replicated three times, arranged as a total of twenty-four boxes placed separately in each field (Figure 1). Eight treatments were used as follows: CK-N (control + no-duck), CK-D (control + ducks), CF-N (chemical fertilizer + no-ducks), CF-D (chemical fertilizer + ducks), CM-N (chemical fertilizer + organic fertilizer + no-ducks), CM-D (chemical fertilizer + organic fertilizer + ducks), CD-N (chemical fertilizer 30% off + organic fertilizer + no-ducks), and CD-D (chemical fertilizer

+ organic fertilizer + ducks). The plots covered an area of 21 m^2 (3 m × 7 m) and were prepared as follows: first, a hole (1.2 m length, 1.1 width) was dug down until the soil below the plough layer was reached. A white nylon box (1 m length, 1 m width; open at the top and bottom) was placed in the hole. Spacing of 50 cm × 50 cm was provided between rows. The boxes were distributed in eight plots across the open fields with and without ducks. The chosen boxes were given ^{15}N ammonium sulfate (20.20% atom enrichment, produced by the Shanghai Research Institute of Chemical Industry) instead of normal ammonium sulfate. Steel fencing was used to separate the duck and no-duck fields. Ducks were released to the farm at the vegetative stage. The fertilizer application rates of different treatments are shown in Table 1. The rates of fertilizers applied were the same in both fields. To avoid any diseases and yield loss, pests were controlled using recommended pesticides.

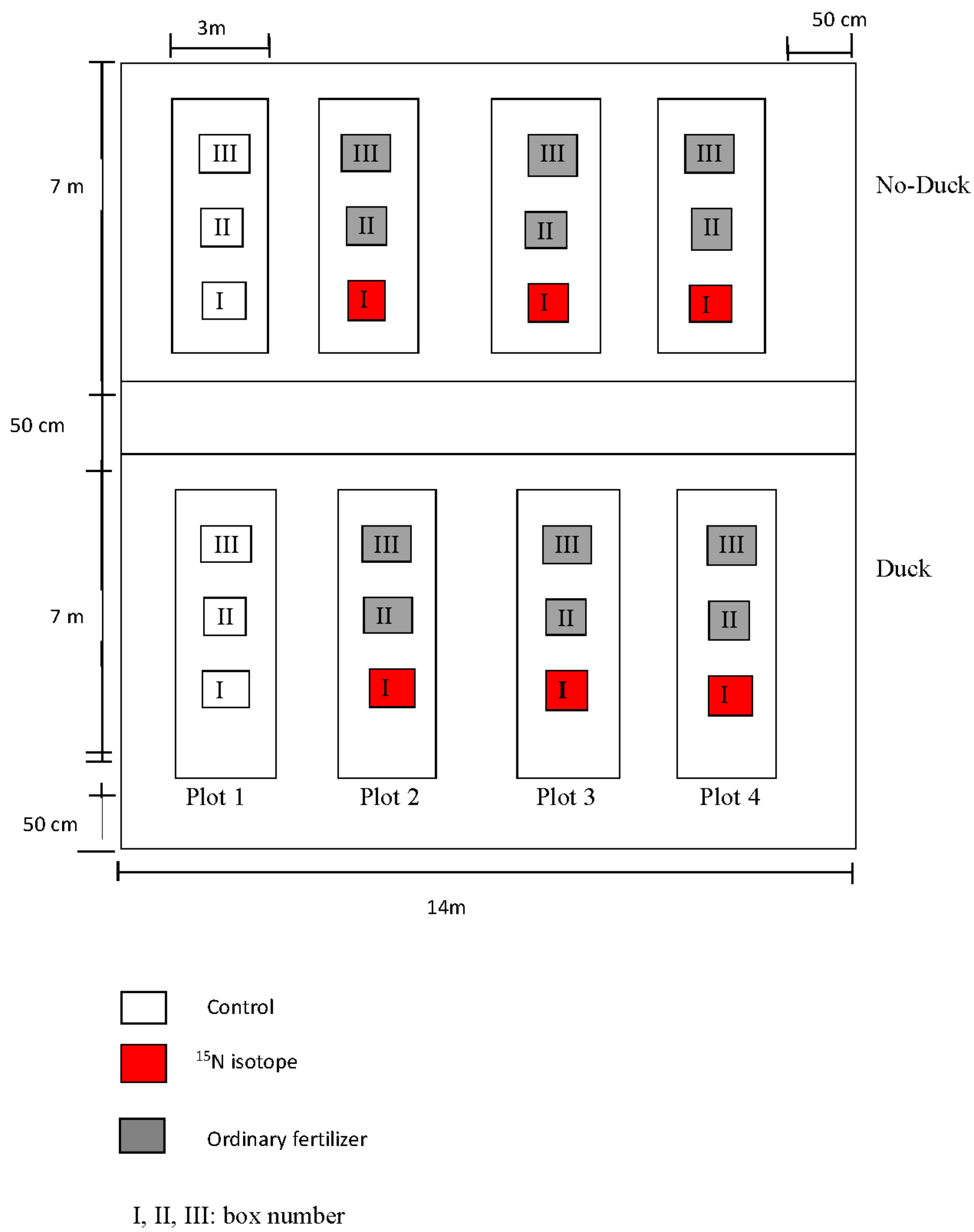

Figure 1. Schematic of the experimental set up. The N-15 ammonium sulphate fertilizer was applied only in the first box of each treatment from plot 2 to plot 4. Both duck and no-duck fields had a total of twenty-four boxes. Plot 1 refers to CK treatment; plot 2 = CF; plot 3 = CM; plot 4 = CD treatment. Duck and no-duck fields underwent identical treatment.

Table 1. Application rates of common fertilizers and ^{15}N isotope fertilizer in different treatments at the experimental farm.

Year	Site	Treatment	Fertilizers Applied per ha ($kg \cdot ha^{-1}$)	Fertilizers Applied ($g \cdot m^{-2}$)	^{15}N Applied ($g \cdot m^{-2}$)
2017	Ninghe	CK	Control with no fertilization	Control with no fertilization	Control with no fertilization
		CF	N: 200 P: 90 K: 120	Calcium superphosphate $Ca(H_2PO4)_2$: 124.8 Potassium sulfate (K_2SO_4): 186.584 Ammonium sulfate $(NH_4)_2SO_4$: 754.2844	$(^{15}NH_4)_2SO_4$ 35.73
		CM	CF: N: 163, P: 67, K: 105 OF: N: 37, P: 23, K: 15 Organic fertilizer: 1194	$Ca(H_2PO4)_2$: 92.74 K_2SO_4: 163.104 $(NH_4)_2SO_4$: 616.5111 Organic fertilizer: 2507.4	29.03
		CD	CF: N: 114, P: 47, K: 74 OF: N: 37, P: 23, K: 15 Organic fertilizer: 1194 (Rapeseed)	$Ca(H_2PO_4)_2$: 65.06 K_2SO_4: 115.064 $(NH_4)_2SO4$: 429.9333 Organic fertilizer: 2507.4	20.54

CK: Control; CF: Chemical fertilizer; CM: CF (Chemical fertilizer) + OF (Organic fertilizer); CD: CF (Chemical fertilizer 30% off) + OF (Organic fertilizer amount unchanged); Organic fertilizer it is estimated at TN: 15.96 g/kg, P_2O_5: 24.92 g/kg, K_2O: 18.1 g/kg.

2.3. Sampling and Measurement

Rice and soil samples were collected on 18 October 2017 at the end of the growing season. The soil samples were randomly collected with the auger from three points within each box at 0–20 cm and 20–40 cm depth. Two rice plant samples were randomly chosen inside each box at physiological maturity. During the harvest time, no noticeable crop damage was observed due to weeds, insects, or other diseases. After being harvested, plants were divided into grain, straw, leaf, and root. The last harvest was done on 4 November 2017 in the whole plots to determine rice yield (Tables A1 and A2). A small part of fresh Japonica rice was taken from the large bags and then separated into grain and husk to determine the content of nitrogen-15 isotope from both fresh organs. Soil and plant samples were brought to the laboratory for the analysis. The soil samples were air-dried and ground to pass through 100-mm mesh sieve for the determination of total N, P, NH_4-N, NO_3-N, soil organic matter (SOM), and ^{15}N analysis. Soil pH was measured through a 0.9-mm sieve with air-dried soil and 0.01 M of calcium chloride ($CaCl_2$), using a balance (METTLER TOLEDO). Soil total N, total P, NH_4-N, NO_3-N, and rice plant organs were measured by flow injection analysis (Automatic Analyzer AA3 type), and soil moisture content was measured by the oven-drying method (Table 2). To analyze NH_4-N and NO_3-N, a 5-g sample of fresh soil was extracted with 50 mL of 1 M KCl by shaking for half an hour, followed by centrifugation and filtering. Soil organic matter (SOM) was measured by the potassium dichromate oxidation method. Soil texture was determined by hydrometer. Rice plant organs, such as grains, leaves, straw, and roots, were oven-dried for three days at 75 °C and powdered in order to determine the content of nitrogen, phosphorus, and ^{15}N. The nitrogen content, both unlabeled and ^{15}N labeled, of rice plant organs, including grain, straw, leaf, and root, were measured. Isotope analysis was carried out with an elemental mass spectrometer.

2.4. Calculation

N use efficiency was calculated according to Zhu et al. [16].

$$\text{NUE(Isotopic method)} = \frac{\text{15N uptake}}{\text{15N input}} \times 100 \tag{1}$$

2.5. Statistical Analysis

Statistical analyses were executed by SPSS version 20 statistical software (IBM, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was undertaken to assess differences between duck and no-duck treatments. The means of different treatments were compared based on the least significant difference test (LSD) with multiple comparisons. Significant differences at $p < 0.05$ between treatments are indicated by different letters. Graphing was performed with Origin 8.5 (Origin Lab) software and MS word was used to generate tables.

Table 2. Physicochemical proprieties of soil at the experimental farm.

Year (Location)	Duck Levels	pH	Total N ($g \cdot kg^{-1}$)		Total P ($g \cdot kg^{-1}$)	NH_4-N ($mg \cdot kg^{-1}$)		NO_3-N ($mg \cdot kg^{-1}$)		SMC (%)		SOM ($g \cdot kg^{-1}$)	Distribution of Soil Particles (%)		
			0–20 cm	20–40 cm		0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm		Clay	Silt	Sand
2017 (Ninghe)	Duck	7.42	1.03	0.89	0.89	3.92	1.56	25.98	32.94	36.6	34.69	22.75	31.5	62.75	5.75
	No-duck	7.47	0.92	0.91	0.90	2.23	2.01	35.65	26.15	36.9	34.96	19.59	35.25	55.75	9

SMC: soil moisture content; SOM: soil organic matter.

3. Results and Discussion

3.1. Effect of Duck Presence on Rice Plant Growth

The results indicated that grain N concentration significantly differed among CK, CF, and CM treatment when comparing the presence and absence of ducks in the field (Figure 2A), whereas CD did not differ significantly between conditions. Likewise, straw N concentration did not respond significantly between treatments whether ducks were present in rice field or not (Figure 2B). Moreover, significant differences between treatments were observed in leaf N concentration (Figure 2C). In contrast, root N concentration was not affected by the presence or absence of ducks in the field (Figure 2D), (Table 3). Grain and straw P concentrations were not significantly affected by the presence and absence of ducks in rice field (Figure 3A,B). In contrast, leaf and root were significantly affected by the presence of ducks (Figure 3C,D). In most cases, the presence of ducks alone did not show differences between treatments. There were, however, some significant differences when comparing duck to no-duck conditions.

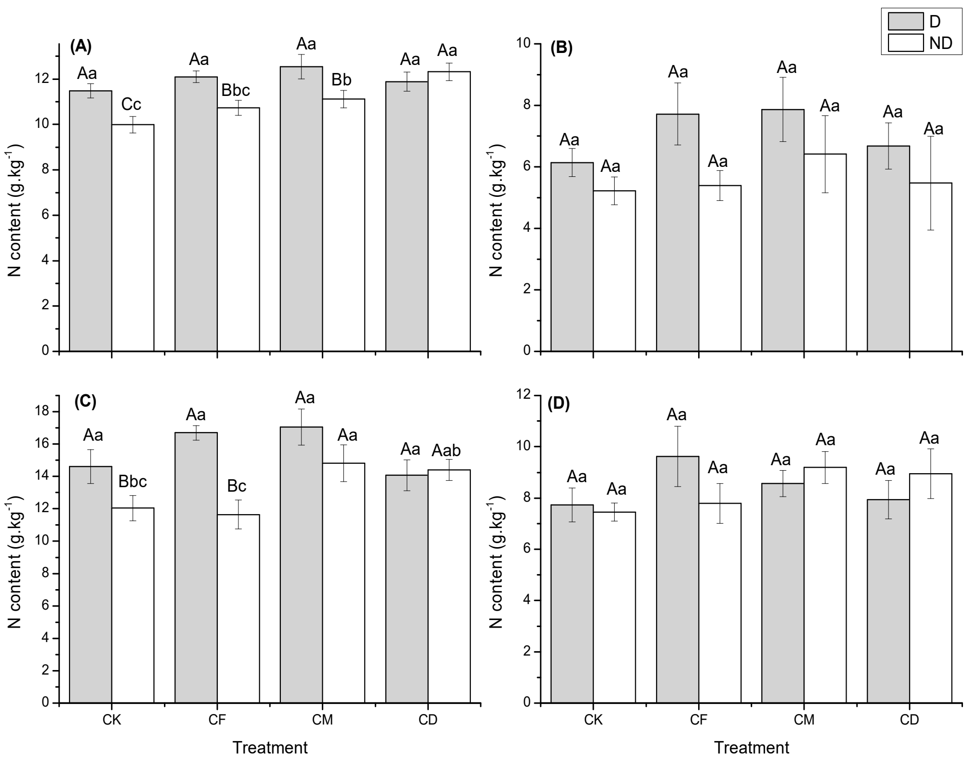

Figure 2. Effects of organic fertilizer with duck presence (D) and without duck presence (ND) on grain N content (**A**), straw N Content (**B**), leaf N content (**C**), and root N content (**D**). Values are mean ±SE (n = 3). Different upper-case letters indicate significant differences at the 5% level among treatments. Different lower-case letters indicate significant differences at the 5% level between D and ND at each field. CK: control, CF: chemical fertilizer, CM: chemical fertilizer + organic fertilizer, CD: chemical fertilizer 30 off + organic fertilizer.

Overall, most of the results indicated that N and P concentrations were higher when ducks were present in the field. Our results strongly supported the findings of Zhang et al. [17] who showed that the presence of ducks might stimulate rice growth and cause changes of shape, height, stalk thickness, and effective tilling. Duck activities not only stimulate rice growth but can also increase its lodging resistance [18]. Comparable results were found by other researchers, although they did not evaluate the effect of ducks on the concentration of rice plant organs such as grain, straw, leaf, and root, but they found that the presence of ducks on rice land caused increases in rice height, grain number per panicle, and grain yield [19]. Moreover, ducks' movement and feeding activity in rice plots cause variations in soil distribution, thus resulting in improved soil physical properties which subsequently improve the root systems of rice plants [20]. Mutual rice–duck organic farming takes advantage of controlling plant diseases, insect pests, and increases in rice production [21].

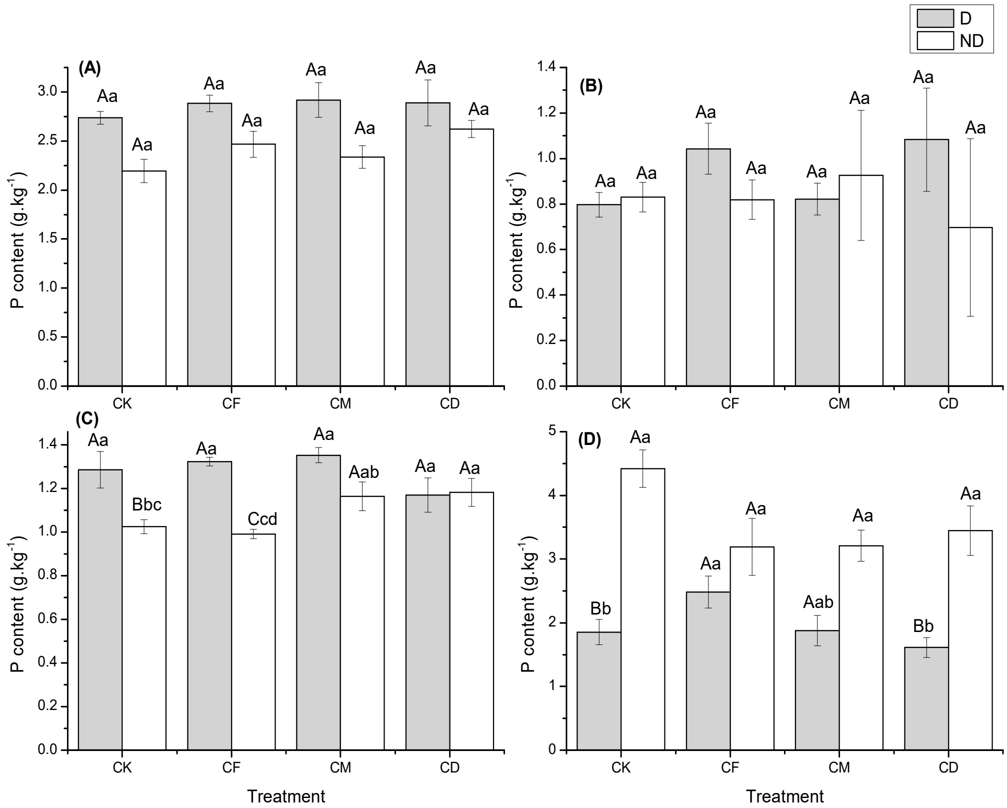

Figure 3. Effects of the application of organic fertilizer with duck presence (D) and without duck presence (ND) on grain P content (**A**), straw P Content (**B**), leaf P content (**C**), and root P content (**D**). Values are mean ±SE (n = 3). Different upper-case letters indicate significant differences at 5% level among treatments. Different lower-case letters indicate significant differences at 5% level between D and ND at each field.

3.2. Effects of Organic Fertilizer in the Field

The highest grain N concentration was observed in CM when ducks were present in the field (Figure 2A). The order of grain N concentration was CK < CD < CF < CM in the presence of ducks and gradually increased from lower to higher grain N concentration (CK < CF < CM < CD) in the absence of ducks. Grain N concentrations ranged from 11.47 g·kg^{-1} to 12.54 g·kg^{-1} with duck presence and 9.99–12.32 g·kg^{-1} in the absence of ducks. A similar trend was observed in straw and leaf, with the highest N concentration occurring in CM when ducks were present in the field (Figure 2B,C). Root N concentration was higher in CF when ducks were present in rice field (Figure 2D).

Moreover, grain P concentration was higher in CM when ducks were present in comparison to other treatments (Figure 3A,C). By contrast, straw P concentration was higher in CD (Figure 3B) with duck presence and higher in CM without ducks. P concentration with duck presence ranged from 2.73–2.91 g·kg^{-1}, 0.79–1.08 g·kg^{-1}, 1.17–1.35 g·kg^{-1}, and 1.61–2.48 g·kg^{-1} in grain, straw, leaf, and root, respectively. In most of our findings, CK showed lower N and P concentrations. Leaf P concentration was higher in CM with duck presence and in CD without duck presence, while root P concentration was higher in CF than that in other treatments when ducks were present in the field. Root P concentration showed the highest concentration in CK when ducks were absent (Figure 3D). P concentration without ducks ranged from 2.19–2.62 g·kg^{-1}, 0.69–0.92 g·kg^{-1}, 0.99–1.18 g·kg^{-1}, and 3.19–4.41 g·kg^{-1} in grain, straw, leaf, and root, respectively (Table 3).

Moreover, the results of this study demonstrated that the combined application of chemical and organic fertilizer (CM) may be beneficial for the growth of rice plants and for maintaining grain N content with higher quantity compared to CF (chemical fertilizer) when applied alone. In addition, a large amount of N content was observed when chemical fertilizer was reduced (CD) when compared to no fertilization treatment. The results were similar to those reported by other researchers, showing that the combined application of organic and inorganic fertilizers is advantageous, making full use of the on-farm organic fertilizers, which is beneficial for increasing crop yield and the maintenance of soil fertility [22]. Therefore, an important strategy to sustain and enhance soil fertility and improve fertilizer utilization efficiency is to combine the application of chemical and organic fertilizers [23]. However, to avoid heavy nitrogen loss and environmental pollution, the nitrogen application should be strictly controlled. Our trial field experiment may help farmers use the appropriate amount and combination of fertilizers. Over-application could be considered a result of N loss and a source of environmental risk. It has been demonstrated in previous research that inappropriate fertilization patterns and excessive use of N fertilizer result in considerable N losses through ammonia (NH_3) volatilization and leaching [24,25]. It has also been demonstrated that overuse of chemical N fertilizer may promote soil acidification in the long term [26]. Therefore, decreasing inorganic fertilizer use may solve environmental issues. Other studies showed that decreasing N rates from 0.74 g pot^{-1} (equivalent to the recommended field rate of 150 kg ha^{-1}) to 0.44 g pot^{-1} (equivalent to 60% of the recommended rate) resulted in lower fertilizer N loss rates [27].

Furthermore, the findings of Siavoshi et al. [28] proved that organic fertilizer has a significant influence on growth and productivity in rice.

Based on the results, the present study indicated that organic fertilizer strongly influenced rice plant growth compared to chemical fertilizer applied alone. In most cases, organic fertilizer is more suitable for green production of healthy food and may be of lower cost to the environment than chemical fertilizer.

3.3. Total ^{15}N Content

Total ^{15}N significantly differed between treatment conditions in the 0–20 cm soil layer when ducks were present in the field (Table 4). However, the same soil layer did not respond significantly in the absence of ducks. At the 20–40 cm soil depth, total ^{15}N was not affected by duck presence or absence. In addition, grain ^{15}N concentration was not significantly affected by the presence or absence of ducks. However, the highest grain ^{15}N content was observed in CF (14.30 g·kg^{-1}), followed by CM (13.75 g·kg^{-1}) and CD (13.50 g·kg^{-1}) when ducks were present in the field. In contrast, when ducks were absent, the highest grain ^{15}N concentration was observed in CM (13.90 g·kg^{-1}), followed by CF (12.85 g·kg^{-1}) and CD (12.25 g·kg^{-1}), respectively. The grain ^{15}N concentration order was CF > CM > CD with duck presence and CM > CF > CD without ducks. On the other hand, straw ^{15}N content significantly differed with and without ducks. The highest straw N content was observed in CM (12.70 g·kg^{-1}) with duck presence. A similar trend was observed in CM with no ducks (9.85 g·kg^{-1}) when compared to CF and CD. The order for straw ^{15}N concentration was CM > CF > CD in duck presence and CM > CD > CF without ducks. Moreover, leaf ^{15}N content significantly differed with and without ducks, while leaf ^{15}N content did not respond significantly to duck presence. The highest leaf ^{15}N content was observed in CM with and without duck presence. Our results suggested that the addition of organic fertilizer is a good supply for enhancing and maintaining rice productivity. Moreover, root ^{15}N content significantly differed among the no-duck conditions and the highest root ^{15}N content was observed in CF without duck presence. In contrast, root ^{15}N content did not show any difference with duck presence (Table 4). From the above discussion, it is clear that replacing chemical fertilizer with organic fertilizer significantly influenced the growth of rice plant organs. Furthermore, our results are in agreement with the findings of Chen et al. [29], showing that N content in grain was higher when compared with straw N content.

Table 3. Results from an ANOVA analysis evaluating the effects of organic fertilizer with and without duck presence on rice plant organs.

Year (Location)	Duck Presence	Treatment	N Content (g·kg^{-1})				P Content (g·kg^{-1})			
			Grain	Straw	Leaf	Root	Grain	Straw	Leaf	Root
2017 (Ninghe)	Duck	CK	11.47 ± 0.31^{Aa}	6.13 ± 0.45^{Aa}	14.60 ± 1.04^{Aa}	7.73 ± 0.66^{Aa}	2.73 ± 0.06^{Aa}	0.79 ± 0.05^{Aa}	1.28 ± 0.08^{Aa}	1.85 ± 0.19^{Bb}
		CF	12.10 ± 0.26^{Aa}	7.71 ± 1.00^{Aa}	16.68 ± 0.45^{Aa}	9.62 ± 1.16^{Aa}	2.88 ± 0.08^{Aa}	1.04 ± 0.11^{Aa}	1.32 ± 0.01^{Aa}	2.48 ± 0.25^{Aa}
		CM	12.54 ± 0.53^{Aa}	7.86 ± 1.04^{Aa}	17.05 ± 1.11^{Aa}	8.56 ± 0.51^{Aa}	2.91 ± 0.17^{Aa}	0.82 ± 0.06^{Aa}	1.35 ± 0.03^{Aa}	1.87 ± 0.23^{Aab}
		CD	11.88 ± 0.42^{Aa}	6.67 ± 0.75^{Aa}	14.08 ± 0.95^{Aa}	7.93 ± 0.74^{Aa}	2.88 ± 0.23^{Aa}	1.08 ± 0.22^{Aa}	1.17 ± 0.07^{Aa}	1.61 ± 0.15^{Bb}
	No-Duck	CK	9.99 ± 0.37^{Cc}	5.21 ± 0.44^{Aa}	12.05 ± 0.77^{Bbc}	7.45 ± 0.34^{Aa}	2.19 ± 0.11^{Aa}	0.83 ± 0.06^{Aa}	1.02 ± 0.03^{Bbc}	4.41 ± 0.29^{Aa}
		CF	10.72 ± 0.33^{Bbc}	5.39 ± 0.48^{Aa}	11.64 ± 0.89^{Bc}	7.78 ± 0.78^{Aa}	2.46 ± 0.13^{Aa}	0.81 ± 0.08^{Aa}	0.99 ± 0.02^{Ccd}	3.19 ± 0.44^{Aa}
		CM	11.11 ± 0.38^{Bb}	6.41 ± 1.25^{Aa}	14.81 ± 1.13^{Aa}	9.19 ± 0.62^{Aa}	2.33 ± 0.11^{Aa}	0.92 ± 0.28^{Aa}	1.16 ± 0.06^{Aab}	3.21 ± 0.24^{Aa}
		CD	12.32 ± 0.38^{Aa}	5.47 ± 1.52^{Aa}	14.39 ± 0.64^{Aab}	8.94 ± 0.96^{Aa}	2.62 ± 0.08^{Aa}	0.69 ± 0.39^{Aa}	1.18 ± 0.06^{Aa}	3.44 ± 0.38^{Aa}

CK: control, CF: chemical fertilizer, CM: chemical fertilizer + organic fertilizer, CD: chemical fertilizer 30 off + organic fertilizer. Organic fertilizer unchanged amount. Mean ± SE ($n = 3$), different small letters within column and capital letters with the same row for treatment indicate significant differences at $p < 0.05$, according to LSD tests.

Table 4. Mean comparison of total ^{15}N with and without duck presence in the field.

Year (Location)	Duck Presence	Treatment	Total ^{15}N (g·kg^{-1})					
			0–20 cm	20–40 cm	Grain	Straw	Leaf	Root
2017 (Ninghe)	Duck	CF	1.97 ± 0.006^{Aa}	1.34 ± 0.03^{Aa}	14.30 ± 0.00^{Aa}	12.60 ± 0.10^{Aab}	18.00 ± 0.05^{Bb}	14.05 ± 0.20^{Aa}
		CM	0.98 ± 0.0005^{Bbc}	0.78 ± 0.006^{Aa}	13.75 ± 0.17^{Aa}	12.70 ± 0.03^{Aa}	20.15 ± 0.02^{Aa}	12.10 ± 0.14^{Aa}
		CD	1.06 ± 0.015^{Bb}	0.77 ± 0.001^{Aa}	13.50 ± 0.00^{Aa}	7.52 ± 0.04^{Cc}	14.40 ± 0.03^{Cc}	8.90 ± 0.04^{Aa}
	No-duck	CF	0.94 ± 0.08^{Aa}	0.86 ± 0.002^{Aa}	12.85 ± 0.01^{Aa}	6.85 ± 0.03^{Aa}	13.25 ± 0.10^{Aa}	9.59 ± 0.02^{Aa}
		CM	1.09 ± 0.006^{Aa}	1.47 ± 0.054^{Aa}	13.90 ± 1.11^{Aa}	9.85 ± 0.13^{Aa}	17.55 ± 0.11^{Aa}	8.97 ± 0.004^{Aab}
		CD	1.4 ± 0.024^{Aa}	1.19 ± 0.038^{Aa}	12.25 ± 0.005^{Aa}	7.03 ± 0.02^{Aa}	13.85 ± 0.03^{Aa}	6.04 ± 0.031^{Cc}

CF: chemical fertilizer, CM: chemical fertilizer + organic fertilizer, CD: chemical fertilizer 30 off + organic fertilizer. Organic fertilizer unchanged amount. Mean ± SE, different small letters within column and capital letters with the same row for treatment indicate significant differences at $p < 0.05$, according to LSD tests. CK was not considered in the analysis for the ^{15}N isotope determination. It is known as natural abundance (0.3663 at.% ^{15}N).

3.4. Fresh Grain ^{15}N, Husk ^{15}N Content, N Uptake, and N Use Efficiency

There was no significant effect on fresh grain and husk ^{15}N content resulting from duck presence (Table 5). However, fresh grain and husk ^{15}N ranged from 14.2 to 14.4 $g \cdot kg^{-1}$ and 6.2–6.3 $g \cdot kg^{-1}$, respectively. Likewise, fresh grain and husk ^{15}N uptake and ^{15}N use efficiency were not significantly affected by the presence of ducks. ^{15}N uptake ranged from 54.90–93.69 kg ha^{-1} in grain and 6.43–11.04 kg ha^{-1} in husk, respectively. ^{15}N use efficiency ranged from 21.55%–34.61% in fresh grain and 2.61%–4.24% in fresh husk, respectively.

We examined the effects of duck presence on fresh grain and husk by using the ^{15}N tracer technique. Our results demonstrated that fresh grain ^{15}N concentration was higher than fresh husk ^{15}N concentration. A trend of stalks > leaves > grains > husks was reported elsewhere [30].

Table 5. Mean comparison of fresh grain and husk with duck presence or absence.

Year	Site	Treatment	^{15}N Content ($g \cdot kg^{-1}$)		^{15}N Uptake ($kg \cdot ha^{-1}$)		NUE (Isotopic Method)	
			Grain	Husk	Grain	Husk	Grain	Husk
2017	Ninghe	Duck	14.2 ± 0.02^a	6.3 ± 0.04^a	54.90 ± 13.41^a	6.43 ± 1.96^a	21.55^a	2.61^a
		No-Duck	14.4 ± 0.02^a	6.2 ± 0.05^a	93.69 ± 5.97^a	11.04 ± 1.36^a	34.61^a	4.24^a

Means in each column followed by the same letter are not significantly different at the 5% probability level. Data are means ± SE (n = 3). NUE: N use efficiency. According to the yield data CK, CF, CM, and CD were simplified to duck and no-duck treatments, respectively.

4. Conclusions

N is an essential nutrient for improving crop productivity and is also the most widely applied fertilizer because it is usually considered the main limiting factor in most agricultural systems. However, excessive application of fertilizers may be harmful to the environment and cause N loss to the environment, environmental risks, environmental pollution, and non-point pollution. To ensure high-quality rice, practical and safe production methods and measures should be adopted. Therefore, organic fertilizer is preferred for clean rice production. The results showed that the application of organic fertilizer is the key to maintaining productivity in the soil–rice plant system instead of inorganic fertilizer applied alone. Thus, it is important to consider organic fertilizer when estimating N transfer and transformation in traditional farming and rice–duck farming systems. Moreover, there was no difference between fresh grain and husk N uptake and N use efficiency. However, the ducks' feces were not examined in our study. Therefore, further studies may require an appropriate technique for examining duck feces.

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Abbreviations

^{15}N	Nitrogen-15
P	Phosphorus
NH_4-N	ammonium nitrogen
NO_3-N	nitrate nitrogen

Appendix A

Table A1. The weight measurement of whole rice plants harvested in all plots at the Ninghe experimental farm in 2017.

Duck Presence	Treatment	Plot No	Grain (Small Bag) (g)	Husk (Small Bag) (g)	Rice (Large Bag) (kg)
Duck	CK	1	78.62	19.32	7.75
	CF	2	63.60	15.10	5.00
	CM	3	85.45	21.07	12.80
	CD	4	86.74	24.63	12.95
No-duck	CK	1	77.15	19.72	14.45
	CF	2	71.68	17.96	15.50
	CM	3	75.91	20.52	20.25
	CD	4	73.41	21.66	16.55

Table A2. Yield and yield components at Ninghe in 2017.

Duck Presence	Treatment	Yield Components		
		Grain (kg/ha)	Husk (kg/ha)	Rice (kg/ha)
Duck	CK	2961.963	728.037	3690
	CF	1923.278	456.484	2380
	CM	4888.7995	1205.591	6095
	CD	4802.08	1363.30	6166
No-duck	CK	5479.232	1400.08	6880
	CF	5901.048	1478.214	7380
	CM	7590.1824	2050.8534	9642
	CD	6084.148	1795.064	7880

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