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

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Abstract

A field experiment was conducted in Ninghe, Tianjin, China, using ^{15}N isotope method to evaluate the application of organic fertilizer on N distribution patterns of labelled and unlabeled N fertilizer, ammonium sulfate -^{15}N uptake by rice, N use efficiency (NUE), and the fate of $(^{15}NH_4)_2SO_4$ applied. 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 significantly increased N and P concentrations over control (CK) and chemical fertilizer (CF). Moreover, no-significant differences were found in ^{15}N fresh grain and husk concentration. Both organs ranged of 14.2-14.4 g kg^{-1} and 6.2-6.3 g kg^{-1}, respectively. N derived from the fertilizer and soil significantly affected fresh grain compared to fresh husk. However, N uptake and N use efficiency did not show any differences. We concluded that organic fertilizer has a significant influence on rice growth and promote 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 of chemical fertilizer N [3]. Moreover, it has been mentioned that in order to meet the demand of ever-increasing population, sustainable increases in rice yield by 1.21% annually is need 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 as a consequence of several environmental degradation with high potential of N loss in many pathways [7], decreases N use efficiency (NUE), decreases in crop quality, and creation of environmental hazards in rice growing countries [8,9,10,11]. 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 a key to increasing N use efficiency [12,13]. In addition, nitrogen is the desire to produce more food in agricultural system. 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 system involved different process, N cycling which is complex in the soil-plant system, increases the difficulty for N management. Basically, processes that N undergoes 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 can influence soil structure and fertility. Ducks feces may be a good supply of organic fertilizer

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deposited onto the soil. Thus, it is estimated that the total excreted feces per day 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 and kept organic fertilizer intact, and introduced also duck 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 contribute to the healthy food and safety. However, to the best of our knowledge the present study has been rarely reported. The information about the combined application of organic and inorganic fertilizer and the use of ^{15}N fertilizer on N soil-plant-animal cycle is limited. The specific objectives of this study are to: (1): Evaluate the application of organic fertilizer on N distribution patterns of labelled and unlabeled fertilizer under ducks and no-ducks field, N uptake and N use efficiency (NUE), (2): Access the fate of N fertilizer in the rice-soil plant system, we measured ^{15}N abundance in plant and soil.

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 1414.00 km^2, with a typical humid continental climate by large seasonal temperature differences. The annual average temperature of 54.0°F (12.2°C), the warmest on July with an average temperature of 79.2°F (26.2°C). The coolest on 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 June 7 in 2017, and then the rice seedlings of Japonica rice were transplanted directly to the plots during the tillering stage on July 3 and

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harvested on October 18 in 2017. The application of isotope nitrogen-15 and ordinary fertilizers was performed during the vegetative growth on 13th July 2017. Before applying isotope [$^{15}(NH_4)_2SO_4$] fertilizer and ordinary fertilizers to different treatments in two fields (duck and no-duck), the field experiment 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 for a total of twelve boxes placed separately in each field. Eight treatments were 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 cover an area of 21 m^2 (3 m x 7 m), were prepared as follows: first, a hole (1.2m length, 1.1 width) was dug down until the soil below the plough layer was reached. White nylon box (1m length, 1 m width; open at the top and bottom) was placed in the hole. The spacing of 50 cm x 50 cm between rows was provided. The boxes were distributed in eight plots across the open field of duck and without duck. 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 was used to fence and separate both field ducks and no-ducks. Ducks were released to the farm at 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.

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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 ($kg.ha^{-1}$)	Fertilizers applied ($g.m^{-2}$)	^{15}N applied ($g.m^{-2}$)
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)

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2.3. Sampling and measurement

Rice and soil samples were collected on 18th 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 0-20cm and 20-40cm 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 4th November 2017 in the whole plots to determine rice yield. A small part of fresh Japonica rice was taken from the large bags 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 through a 0.9-mm sieve with air-dried soil was measured at 0.01 M of calcium chloride ($CaCl_2$), using a balance METTLER TOLEDO. Soil total N, total P, NH_4-N, and NO_3-N and rice plant organs were measured by flow injection analysis (Automatic Analyzer AA3 type), and soil moisture content was measured by oven dried method (Table 2). To analyze NH_4-N and NO_3-N, a 5-g sample of fresh soil were extracted with 50 mL of 1 M KCl by shaking half an hour and then centrifuged and filtered, and soil organic matter (SOM) by potassium dichromate oxidation method. Soil texture was determined by hydrometer method. Rice plant organs such as grains, leaves, straw and roots were oven-dried for three (3) days at 75ºC, all ground powdered in order to determine the content of nitrogen, phosphorus, and ^{15}N content. Crop measurements, nitrogen content of both unlabeled and ^{15}N labeled of rice plant organs such as grain, straw, leaf, and root were measured. The isotope analysis was carried out by using Elemental Mass Spectrometer.

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Table 2 Physicochemical proprieties of soil at the experimental farm

Year (Location)	Duck levels	pH	Total N ($g.kg^{-1}$)		Total P ($g.kg^{-1}$)	NH_4-N ($mg.kg^{-1}$)		NO_3-N ($mg.kg^{-1}$)		SMC (%)		SOM ($g.kg^{-1}$)	Distribution of soil particles (%)		
			0-20cm	20-40cm		0-20cm	20-40cm	0-20cm	20-40cm	0-20cm	20-40cm		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

2.4. Calculation methods

N derived from the fertilizer (Ndff) and from the soil (Ndfs) was calculated according to van Cleemput et al. [16].

%Ndff= atom%15N excess plant⁄atom%15N excess fertilizer⁄x100

%Ndfs=100-%Ndff

N use efficiency was calculated according to Zhu et al. [17].

NUE (Isotopic method) = 15N uptake⁄15N input⁄x100

2.5.Statistical analysis

Statistical analyses were executed by using SPSS version 20 statistical software (IBM, Chicago, Illinois, USA). A one-way analysis of variance (ANOVA) was undertaken to assess differences among duck and without duck treatments. Differences at the 0.05 level of probability were considered statistically significant. The graph was performed by using Origin 8.5 (Origin Lab) software and MS word was used to generate tables.

3. Results and Discussion

3.1. Effect of duck presence and without duck presence on rice plant growth

The results indicated that grain and leaf of N and P concentrations were significantly higher in the presence of duck than that in the absence of duck in the field (Fig.1a, 1b, and Fig.3e, 3f) whereas, straw N and P concentrations were not significantly affected whether ducks were present in the field or not (Fig.2c and 2d). Likewise, root N concentration was not significantly affected by the presence and the absence of duck in the field (Fig. 4g). By contrast, P concentration was affected when ducks were present and absent in the field (Fig.4h), (Table 3).

Overall, most of the results indicated that N and P concentrations were higher when ducks were present in the field. Our results strong supported the findings of Zhang et al.[18] that the presence of duck might be stimulate rice growth, cause changes of shape, height, stalk thickness, and effective tillering. Duck activities not only stimulate rice growth but can also increase its lodging resistance [19]. Comparable

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results are found by other researchers despite they did not evaluate the effect of duck and without duck on the concentration of rice plant organs such as grain, straw, leaf, and root, but they found that the presence of duck on rice land caused increase in rice height, grain number per panicle, and grain yield [20]. Moreover, duck's movement and feeding activity in rice plots cause the soil distribution and therefore it results in improving soil physical properties which improve the root system of rice [21]. Rice-duck mutualism organic farming takes advantage of controlling plant diseases, insect pests and increase rice production income [22].

3.2. Effects of organic fertilizer in the field

The results indicated that the combination of chemical and organic fertilizer of grain N concentration did not differ significantly between treatments. However, grain N concentration was higher in CM than that in other treatments in the presence of duck in the field (Fig.1a). Grain P concentration showed similar trend in CM significantly higher compared to other treatments. The combination of chemical and organic fertilizer (CM) increased N and P concentrations when compared to control (CK), chemical fertilizer (CF), and chemical fertilizer reduced the amount and organic fertilizer intact (CD). The highest grain N concentration was observed in CM and the lowest in CK with duck presence and absence in the field. Grain N concentration ranged from 11.47 g kg^{-1} to 12.54 g kg^{-1} in duck presence and 9.99-12.32 g kg^{-1} in duck absence in the field, respectively (Table 3). CM of grain N concentration causes an increase of 8.53% compared to CK with the presence of duck. Whereas, CM causes an increase of 10.08% without duck presence in comparison to CK. Straw N concentration was higher in CM than that other treatments when ducks were present and absent in the field while P concentration was higher in CM when ducks were absent in the field. Moreover, except CD without duck of P concentration, the highest leaf N and P concentrations were observed in CM when ducks were present in the field. Leaf N concentration varied from 14.08 to 17.05 g kg^{-1} in the duck presence and 8.94-14.81 g kg^{-1} in duck absence, respectively (Table 3). CM of leaf N concentration causes an increase of 17.41% in comparison to the lowest concentration (CD) in duck

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presence while CM of leaf N concentration causes an increase of 39.63% in duck absence compared to the lowest (CD), respectively. Root N concentration did not significantly differ within duck and no-duck treatment. Whereas, the highest root N concentration was observed in CF with duck presence and in CM without duck presence. The lowest root N concentration was observed in CK for both duck and no-duck in the field (fig.4g). Unlike, P concentration was differed significantly among treatments (Fig.4f). CF of P concentration was higher in duck presence than that other treatment.

Moreover, the results of this study demonstrated that the combined application of chemical and organic fertilizer (CM) may be benefit for the growth of rice plants and also maintained 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 that the combined application of organic and inorganic fertilizers is advantageous making full use of the on-farm organic fertilizers, which is beneficial to the increase of crop and the maintenance of soil fertility [23]. Therefore, an important strategy to sustain and enhance soil fertility and improve fertilizer utilization efficiency is to combine application of chemical and organic fertilizers [24]. However, to avoid heavy nitrogen loss and environmental pollution, the nitrogen application should be strictly controlled. Our trial field experiment may help farmers how to use the amount of fertilizers might be applied by combining chemical and organic fertilizer, and reducing the amount of chemical fertilizer that needs to be applied, also to avoid over application. Over application could be considered as a result of N loss and environmental risk. It has been demonstrated by the previous research that inappropriate fertilization patterns and excessive use of N fertilizer have a resulted in considerable N losses through ammonia (NH_3) volatilization and leaching [25, 26]. It has been also demonstrated that overuse of chemical fertilizer N may promote soil acidification in the long term [27]. Therefore, decreasing the amount of inorganic fertilizer may solve environmental issues. Other studies showed that decreasing N rates from 0.74 g pot^{-1}

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(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 rate [28].

Furthermore, the findings of Siavoshi et al. [29] 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 requires for green production, healthy food and may be low cost to the environment over chemical fertilizer.

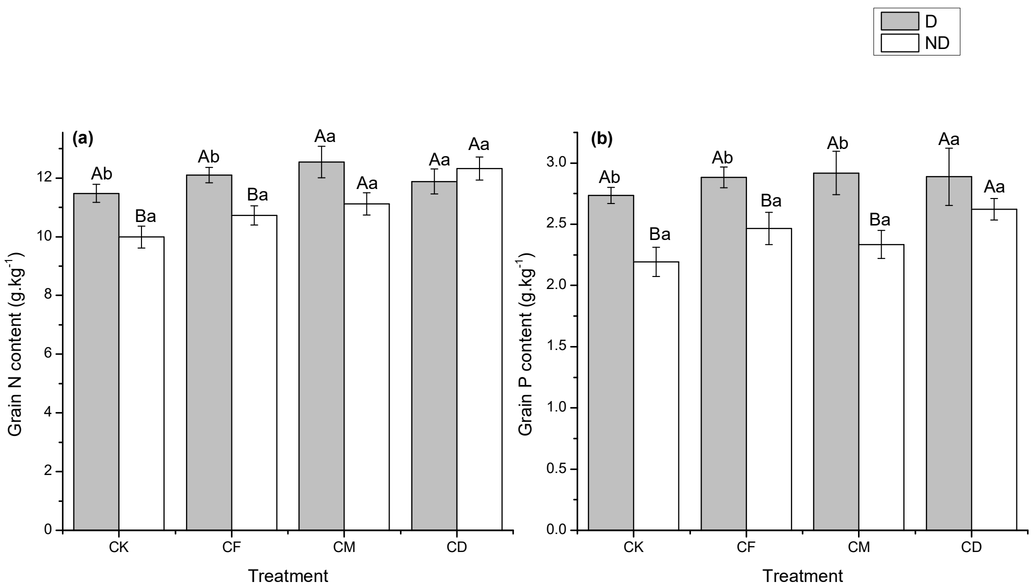

Figure 1 Effects of organic fertilizer under duck presence (D) and without duck presence (ND) on grain N content (a) and P content (b). Values are mean ± SE (n=3). Different lowercase and uppercase letters indicate significant difference at the 5% level among treatments. CK: control with no fertilization, CF: chemical fertilizer, CM: chemical and organic fertilizer, CD: chemical fertilizer reduced and organic fertilizer intact.

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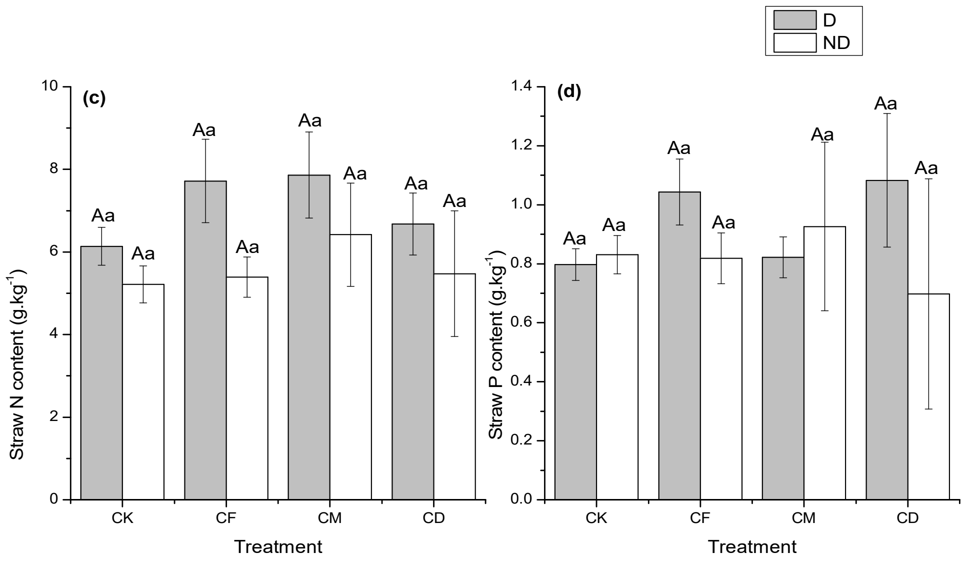

Figure 2 Effects of the application of organic fertilizer under duck presence (D) and without duck presence (ND) on straw N content (c) and P content (d).

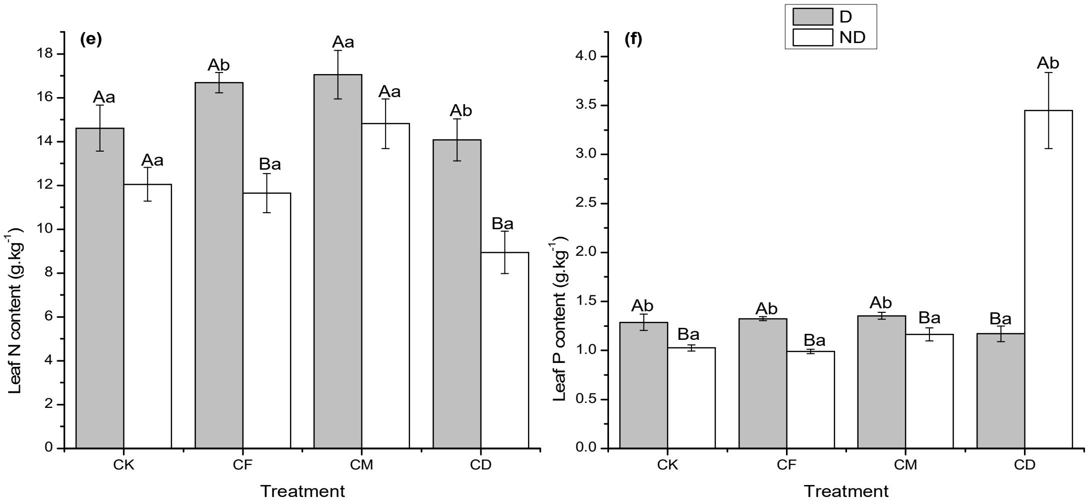

Figure 3 Effects of organic fertilizer application under duck presence (D) and absence of duck (ND) on leaf N content (e) and P content (f).

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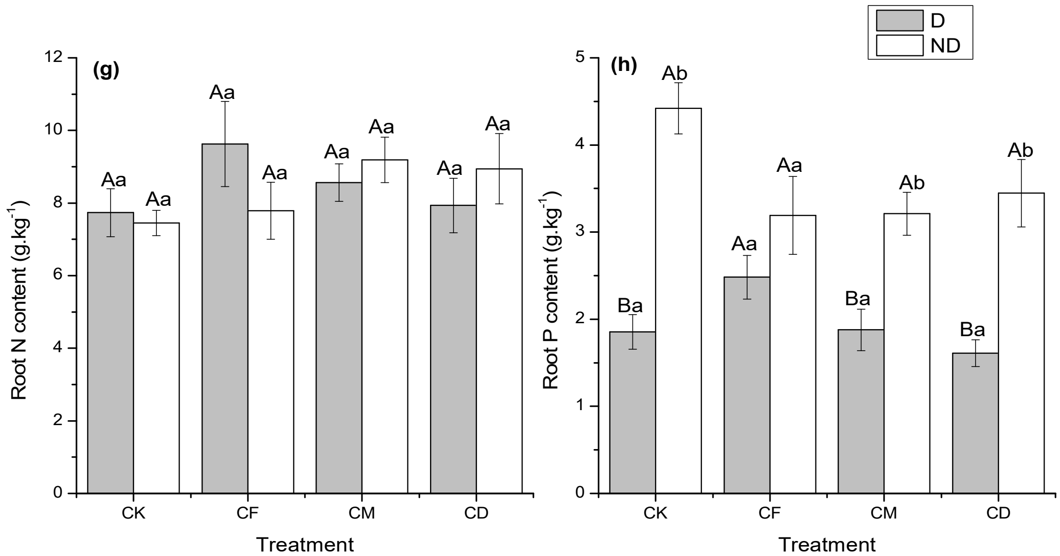

Figure 4 Effects of organic fertilizer application under duck presence (D) and absence of duck (ND) on root N content (g) and P content (h).

Table 3 Effects of organic fertilizer within duck and without duck treatments on rice plant organs

Year (Location)	Duck levels	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±0.31Ab	6.13±0.45Aa	14.60±1.04Aa	7.73±0.66Aa	2.73±0.06Ab	0.79±0.05Aa	1.28±0.08Ab	1.85±0.19Ba
		CF	12.10±0.26Ab	7.71±1.00Aa	16.68±0.45Ab	9.62±1.16Aa	2.88±0.08Ab	1.04±0.11Aa	1.32±0.01Ab	2.48±0.25Aa
		CM	12.54±0.53Aa	7.86±1.04Aa	17.05±1.11Aa	8.56±0.51Aa	2.91±0.17Ab	0.82±0.06Aa	1.35±0.03Ab	1.87±0.23Ba
		CD	11.88±0.42Aa	6.67±0.75Aa	14.08±0.95Ab	7.93±0.74Aa	2.88±0.23Aa	1.08±0.22Aa	1.17±0.07Ba	1.61±0.15Ba
	No-Duck	CK	9.99±0.37Ba	5.21±0.44Aa	12.05±0.77Aa	7.45±0.34Aa	2.19±0.11Ba	0.83±0.06Aa	1.02±0.03Ba	4.41±0.29Ab
		CF	10.72±	5.39±0	11.64±	7.78±0	2.46±0	0.81±0	0.99±0	3.19±0

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			0.33Ba	.48Aa	0.89Ba	.78Aa	.13Ba	.08Aa	.02Ba	.44Aa
		CM	11.11±0 .38Aa	6.41±1 .25Aa	14.81± 1.13Aa	9.19±0 .62Aa	2.33±0 .11Ba	0.92±0 .28Aa	1.16±0 .06Ba	3.21±0 .24Ab
		CD	12.32± 0.38Aa	5.47±1 .52Aa	8.94±0. 96Ba	8.94±0 .96Aa	2.62±0 .08Aa	0.69±0 .39Aa	3.44±0 .38Ab	3.44±0 .38Ab

CK: control with no fertilization, CF, chemical fertilizer; CM, chemical and organic fertilizer; CD, chemical fertilizer decreased and organic fertilizer intact. Different lower-case and upper-case letters indicate significant differences at $P<0.05$ between treatments. Data are means ± SE (n=3).

3.3. Total ^{15}N content, N derived from the fertilizer and from the soil

Total ^{15}N concentration significantly differed among duck and no-duck treatment at 0-20cm soil layer while total ^{15}N did not significantly differ between duck and no-duck treatment at 20-40cm soil layer (Table 4). However, CM treatment was the lowest at 0-20cm soil depth in duck presence in the field. In contrast, CM was higher at 20-40cm soil depth without duck in the field than other treatments. In addition, grain and straw ^{15}N concentrations were significantly affected by the presence and absence of duck in the field. 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}) in the presence of duck. The order was CF>CM>CD when ducks were present in the field. On the other hand the highest straw ^{15}N content was observed in CM (12.70 g kg^{-1}) in duck presence, similar trend to CM of no-duck (9.85 g kg^{-1}) when compared to CF and CD. The order of straw N concentration was CM>CF>CD in duck presence and CM>CD>CF in the absence of duck in the field. In contrast, leaf ^{15}N content was not differed between duck and without duck treatment. The highest leaf ^{15}N content was observed in CM with and without duck presence. Our results suggested that organic fertilizer is a good supply for enhancing and maintaining rice productivity. Unlike root ^{15}N content significantly differed among treatment and the highest root ^{15}N content was observed in CF with and without duck presence. Moreover, N derived from the fertilizer did not significantly differ between treatment at both depths (0-20cm and 20-40cm soil depth) whether ducks were present or not in the field. However, grain, straw, leaf, and root N derived from the fertilizer and from the soil significantly differed among treatments.

CD of N derived from the fertilizer was higher in all rice plant organs in duck presence. From the above discussion it is clear that reducing the amount of chemical fertilizer by adding the amount of organic fertilizer significantly influenced the growth of rice plant organs. The percentage of N derived from the fertilizer ranged from 1.36 to 5.49 %, 1.52-5.67 %, 1.30-5.67 %, and 1.74-7.21 % (in grain, straw, leaf, and root, respectively) in the presence of duck. On the other hand, N derived from the fertilizer ranged 2.49 to 6.27%, 2.92-6.9%, 2.67-6.88% and 3.31-6.85% without duck, respectively.

The percentage of grain N derived from the soil was significantly higher in CM with and without duck in the field. The order of grain N derived from the soil of duck treatment was CM>CF>CD. In contrast, the highest amount of straw N derived from the soil was observe only in CM without duck in the field. Likewise, the highest amount of leaf N derived from the soil was observed in CM in the absence of duck when compared to CF and CD. Similar trend to root N derived from the soil. Our results are in agreement with the findings of Chen et al. [30] that N derived from soil was higher in grain and straw when compared to N derived from the fertilizer.

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Table 4 Total ^{15}N, N derived from fertilizer and soil

Year (Location)	Duck levels	Treatment	Total ^{15}N (g.kg^{-1})						Percentage of N derived from fertilizer (%)						Percentage of N derived from soil (%)			
			0-20 cm	20-40 cm	Grain	Straw	Leaf	Root	Soil 0-20 cm	Soil 20-40 cm	Grain	Straw	Leaf	Root	Grain	Straw	Leaf	Root
2017 (Ninghe)	Duck	CF	1.97Ab (±0.06)	1.34Aa (±0.39)	14.30Ab (±0.00)	12.60Ab (±1.00)	18.00Aa (±0.50)	14.05Aa (±2.05)	0.50Aa (±0.18)	0.55Aa (±0.08)	1.72Ba (±0.56)	1.52Ba (±0.78)	1.30Ba (±0.14)	1.74Ba (±0.55)	98.27Ab (±0.56)	98.47Ab (±0.78)	98.69Ab (±0.14)	98.25Ab (±0.55)
		CM	0.98Aa (±0.00)	0.78Aa (±0.06)	13.75Aa (±1.75)	12.70Aa (±0.30)	20.15Aa (±0.25)	12.10Aa (±1.40)	0.34Aa (±0.07)	0.29Aa (±0.10)	1.36Aa (±0.87)	3.52Aa (±0.50)	3.58Aa (±0.40)	3.50Aa (±0.47)	98.63Aa (±0.87)	96.47Aa (±0.50)	96.41Aa (±0.40)	96.49Aa (±0.47)
		CD	1.06Aa (±0.15)	0.77Aa (±0.01)	13.50Ab (±0.00)	7.52Aa (±0.44)	14.40Aa (±0.30)	8.90Ab (0.42)	0.45Aa (±0.04)	0.35Aa (±0.04)	5.49Ab (±0.20)	5.67Aa (±0.12)	5.67Ab (±0.32)	7.21Ab (±0.25)	94.50Ba (±0.20)	94.32Aa (±0.12)	94.32Ba (±0.32)	92.78Ba (±0.25)
	No-duck	CF	0.94Ba (±0.07)	0.86Aa (±0.02)	12.85Ba (±0.15)	6.85Ba (±0.32)	13.25Aa (±1.05)	9.59Aa (±0.20)	2.25Aa (±1.62)	0.44Aa (±0.02)	6.27Ab (±0.17)	6.9Ab (±0.12)	6.88Ab (±0.42)	6.85Ab (±0.85)	93.72Ba (±0.17)	93.06Ba (±0.12)	93.11Ba (±0.42)	93.14Ba (±0.85)
		CM	1.09Aa (±0.06)	1.47Aa (±0.54)	13.90Aa (1.10)	9.85Aa (±1.34)	17.55Aa (±1.15)	8.97Aa (±0.04)	0.28Aa (0.07)	0.33Aa (±0.05)	2.49Aa (±0.88)	2.92Aa (±1.25)	2.67Aa (±0.85)	3.31Aa (±0.87)	97.50Aa (±0.88)	97.07Aa (±1.25)	97.32Aa (±0.85)	96.68Aa (±0.87)
		CD	1.4Aa (±0.24)	1.19Aa (±0.38)	12.25Ba (±0.05)	7.03Aa (±0.29)	13.85Aa (±0.35)	6.04Ba (±0.31)	0.54Aa (±0.13)	0.35Aa (±0.05)	3.31Ba (±0.32)	4.18Aa (±0.40)	3.50Ba (±0.07)	5.24Ba (±0.00)	96.68Ab (0.32)	95.81Aa (±0.40)	96.49Ab (0.07)	94.75Ab (±0.00)

CF, chemical fertilizer; CM, chemical and organic fertilizer; CD, chemical fertilizer decreased and organic fertilizer intact. Different lower-case and upper-case letters indicate significant differences at $P<0.05$ between treatments. Data are means ± SE (n=3).

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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 between duck and without duck treatment (Table 5). However, there was significant effect on fresh grain N derived from the fertilizer among duck and without duck treatment. Whereas fresh husk N derived from the fertilizer did not significantly differ between duck and no-duck treatment. Likewise, the percentage of N derived from the soil significantly affected fresh grain N content within treatments while fresh husk was not differed within treatment. Both N uptake and N use efficiency of fresh grain and husk were not significantly differed within duck and no-duck treatment. The percentages of fresh grain and husk N derived from the fertilizer within duck and no-duck ranged at the rates of 0.20-0.36% and 0.11-0.21%, respectively (Table 5). N derived from the soil ranged of 99.63-99.79% and 99.78-99.88%, respectively. N uptake and NUE in fresh grain and husk were ranged of 54.90-93.69%, 6.43-11.04% and 21.55-34.61, 2.61-4.24%, respectively.

Moreover, our study seems to be scarcely reported by the previous work. We examined the effects of duck and without duck on fresh grain and husk by using ^{15}N tracer technique. Our results proved that a large portion of N is found and accumulated in grain than husk. Husk represents a very small portion with low efficiency. A trend of stalks>leaves>grains>husks was reported elsewhere [31]. The results of our study demonstrated that a large concentration of N was derived from the soil than that of fertilizer (Table 5).

Table 5 Effects of duck and without duck and N-15 on fresh grain and husk

Year	Site	Treatment	^{15}N content (g.kg^{-1})		Percentage of N derived from fertilizer (%)		Percentage of N derived from soil (%)		^{15}N uptake (kg.ha^{-1})		NUE (Isotopic method)	
			Grain	Husk	Grain	Husk	Grain	Husk	Grain	Husk	Grain	Husk
2017	Ninghe	Duck	14.2±0.02a	6.3±0.04a	0.36±0.01a	0.21±0.10a	99.63±0.01b	99.78±0.10a	54.90±13.41a	6.43±1.96a	21.55a	2.61a
		No-Duck	14.4±0.02a	6.2±0.05a	0.20±0.02b	0.11±0.03a	99.79±0.02a	99.88±0.03a	93.69±5.97a	11.04±1.36a	34.61a	4.24a

NUE: N use efficiency

4. Conclusion

N fertilizer is an essential nutrient for improving crop productivity and is also the most widely applied fertilizer because it is usually considered the main nutrient limiting factor in most agricultural systems compared to P. However, excessive application of fertilizers may be harmful to the environment, cause N loss to the environment, environmental risks, environmental pollution, and non-point pollution. To ensure high-quality rice, a practical safety production methods and measures should be adopted. Therefore, organic fertilizer seems to be preferred to produce clean rice production, food healthy and food safety. The results showed that the application of organic fertilizer is the key to maintaining productivity in 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 system. Moreover, N derived from the soil was significantly higher than N derived from the fertilizer; whereas there was no difference in fresh grain N uptake and N use efficiency.

However, the study was conducted under field conditions; the results may be not necessarily applicable to pot experiment. Therefore, further studies are needed to collect the field soil samples replicate the study under greenhouse conditions.

Author contributions

Xiangqun Zheng contributed to the conceptualization of this project. Bo Yang conceived the study design, methodology, and also revised the manuscript. Yuanqing Guan, Bingchang Tan, Peizhen Chen and Lili Wang participated in formal analysis. All authors reviewed and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

N^{15} Nitrogen-15

P Phosphorus

NH_4-N ammonium nitrogen

NO_3-N nitrate nitrogen

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Appendix

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

Duck levels	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

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Table A2 Yield and yield components at Ninghe in 2017

Duck levels	Treatment	Yield components		
Duck		Grain (kg/ha)	Husk (kg/ha)	Rice (kg/ha)
	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