



Impact of Nutrient Management on Wheat/Vegetable Yields and the Fate of ^{15}N -Labeled Fertilizer in the Yangtze River Basin

Sen Wang^{1,2}, Lin Sheng Yang³, Shi Peng Liao⁴, Bo Sun⁵, Xiao Jun Shi³, Jian Wei Lu⁴, Shi Wei Guo⁵, Jian Bo Shen¹, Fu Suo Zhang¹, Keith Goulding⁶ and Xue Jun Liu^{1*}

¹College of Resources and Environmental Sciences, China Agricultural University, Beijing, China, ²Huaneng Renewables Corporation Limited, Beijing, China, ³College of Resources and Environment, Southwest University, Chongqing, China, ⁴Department of Plant Nutrition, College of Resources and Environmental Sciences, Huazhong Agricultural University, Wuhan, China, ⁵College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, China, ⁶The Sustainable Soils and Grassland Systems Department, Rothamsted Research, Harpenden, United Kingdom

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*Correspondence:

Xue Jun Liu
liu310@cau.edu.cn

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The Yangtze River Basin (YRB) crosses three economic zones and major agricultural regions of eastern, central, and western China. Increasing non-point source pollution, caused by excessive nitrogen (N) inputs to farms, is one of the main causes of water contamination in the YRB. To improve N fertilizer use efficiency, we conducted a field experiment using ^{15}N -labeled urea at three sites located in upstream, midstream, and downstream regions of the YRB to evaluate the impacts of improved fertilizer management on crop yield, fertilizer N recovery, and losses in three crop rotations: rice–wheat (RW), rice–vegetable (RV) [tuber mustard at Jiangjin (Chongqing), cabbage at Shayang (Hubei), and savoy cabbage at Rugao (Jiangsu)] and maize–wheat (MW). Applying only 50% of the traditional application of N and P fertilizer maintained the wheat yield at Jiangjin and Shayang and savoy cabbage yield at Rugao. However, it caused a 27% reduction of the wheat yield at Rugao. The ^{15}N recovery showed that 27% of the fertilizer N was retained in the soil and that 25% less fertilizer N was lost to the environment compared to the traditional fertilizer application. Improved fertilizer management would reduce the environmental cost of farming in the YRB, but with some consequences to winter crop yields.

Keywords: wheat/vegetable, ^{15}N -labeled, yield, nitrogen fate, Yangtze River Basin

INTRODUCTION

The Yangtze River Basin (YRB) accounts for about one-fifth of the total land area of China, but its output of wheat (*Triticum aestivum* L.) and vegetables comprised 62% and 71%, respectively, of China's production in 2020 (National Bureau of Statistics of China, 2021; Ministry of Agriculture of the People's Republic of China, 2021). These high yields are due to the suitable weather, the fertile soil, and the typically-used double cropping systems such as rice–wheat, rice–vegetables, and maize–wheat (Timsina and Connor, 2001; Liu et al., 2005). From 2011 to 2020, the annual wheat grain production in China increased by 13%, from 118.57 to 134.25 million tons; the average wheat grain yield per unit area increased by 18%, from 4.89 to 5.74 t ha⁻¹; the annual vegetable production increased by 25%, from 597.67 to 749.12 million tons; and the average vegetable

yield per unit area increased by 15%, from 30.43 to 34.87 t ha⁻¹ (Ministry of Agriculture of the People's Republic of China, 2021). Nitrogen (N) fertilizer has played a vital role in these increases. However, the over-application of N fertilizer, while maintaining crop productivity, has greatly damaged the environment, causing eutrophication (Tilman et al., 2001; Fowler et al., 2013), air pollution from ammonia (NH₃) volatilization (Gu et al., 2014; Liu M. et al., 2017), global warming from emissions of nitrous oxide (N₂O) (Liu et al., 2004; Gao et al., 2014), and enhanced N deposition from the atmosphere (Liu et al., 2013) causing soil acidification (Matsuyama et al., 2005; Guo et al., 2010). The "Action plan for zero-growth fertilizer use by 2020", issued by the Ministry of Agriculture of the People's Republic of China in 2015, is aimed at improving fertilizer use efficiency by reducing unnecessarily large N inputs but still ensuring the effective supply of major agricultural products such as grain and so promoting sustainable agricultural development (Liu et al., 2016).

Food supplies in the south of China are dominated by rice and those in the north are dominated by wheat, of which 23% of the total is produced in the middle and lower YRB and in southwestern China (Wang et al., 2009). Jiangsu's agriculture is based on grain production with rice and wheat as the main crop, which comprises 15% of the wheat produced in the YRB. Hubei is also a major agricultural region based on grain production, with rice as the main crop. In Chongqing and Sichuan, N applications to winter wheat were 150–718 kg ha⁻¹ (Meng et al., 2013; Duan et al., 2014; Bouraima et al., 2016; Zhou et al., 2016) with applications as high as 240 kg N ha⁻¹ in Hubei province (Hu et al., 2015; Yi et al., 2015). In Jiangsu, 90–375 kg N ha⁻¹ were applied to wheat (Jiang et al., 2006; Ma et al., 2010; Zhao et al., 2016) and, generally, 234–870 kg N ha⁻¹ were applied to vegetables (including open field and greenhouse) because they are such an important part of the diet (Liang et al., 2013; Gai et al., 2016; Min and Shi, 2018).

According to the investigations and surveys in these regions, the traditional N fertilizer input (farmers' practice) is 300 kg N ha⁻¹. However, 150–180 kg N ha⁻¹ is the recommended N application rate for cereal crops (including wheat) to obtain the maximum profit (Zhu and Chen, 2002). An integrated study by Ju et al. (2009) supported the view that a reduction of 30%–50% of the fertilizer N application was possible without yield loss of wheat in the North China Plain and Taihu Lake Region of China. Min et al. (2012) reported that a 40% reduction of fertilizer N inputs can reduce N leaching and maintain the yield of intensive greenhouse vegetables. Goh and Vityakon (1983) found that an application of only 300 kg N ha⁻¹ can achieve high yields of spinach. We therefore conducted experiments to test whether a reduction in the N application rate from 300 and 600 kg N ha⁻¹ to 150 and 300 kg N ha⁻¹ would maintain wheat grain yields and vegetable yields in typical local crop rotations and reduce the environmental impact at three sites in the YRB.

Food security is the prime consideration of the Chinese Government because of the increasing population, currently more than 1.4 billion, but, as noted above, pollution is an increasing concern. The Ministry of Agriculture and Rural Affairs of the People's Republic of China reported that the average fertilizer apparent recovery of rice, wheat, and maize

was only 35.2% in 2015. Therefore, research is needed to increase N use efficiency while at least maintaining yields. This paper reports research on the winter production of typical multiple cropping rotations of rice–wheat, rice–vegetables, and maize–wheat. ¹⁵N-labeling experiments at three sites in upstream, midstream, and downstream regions of the YRB region continued the same fertilizer treatments and management practices as in the summer season (Wang et al., 2019). Crops planted in winter (wheat and vegetables) have special characteristics (e.g., dry-farming in a rotation; higher fertilizer applications in the vegetable season; cold climatic conditions affecting N dynamics in soils and utilization by crops). The fate of N and its effects on the yield of summer crops have been reported previously (Wang et al., 2019). However, the fate of ¹⁵N-labeled fertilizer in winter season crops rotated with summer crops (e.g., rice and maize) has not been addressed to date. This paper reports the results for the winter season crops with the following objectives: 1) to research if crop yields can be maintained or even increased with reduced and more efficient fertilizer inputs; 2) to quantify fertilizer N recovery by the crops and soil and measure N utilization efficiency in the wheat and vegetable growing seasons.

MATERIALS AND METHODS

Experimental Site

The field experiments were conducted at three sites in the YRB in 2016–2017: the agronomic experimental station in Jiangjin County, Chongqing, China (106°11'E and 29°03'N) (upstream at 285 m altitude), the agronomic experimental station in Shayang County, Jingmen, Hubei, China (112°18'E and 30°43'N) (midstream at 87.3 m altitude), and the agricultural science research institute of Rugao County, Nantong, Jiangsu, China (120°29'E and 32°22'N) (downstream at 4 m altitude). Jiangjin, Shayang, and Rugao are typical agricultural counties in upstream, midstream, and downstream YRB. The Jiangjin site is a long-term experiment owned by Southwest University; Shayang belongs to Huazhong Agricultural University, while Rugao is the experimental site of Nanjing Agricultural University. The properties of the 0–40 cm soil layers at the three sites before transplanting are shown in **Table 1**. The so-named "Purple soil" is distributed widely in upstream YRB (Mo et al., 2005). The hydric anthrosol at Shayang is a special type of anthropogenically formed soil developed by long-term flooding, puddling, and rice planting according to the World Reference Base for Soil Resources (WRB). Fluvisol is fluvialite deposits with evident stratification and is widely distributed in downstream YRB. The greatly improved multiple cropping index (Xu et al., 2019) has shortened the alternating time of dry and wet cycles, which induces secondary gley formation and develops into the soil type corresponding to a Gleyic fluvisol in WRB.

Field Experiment Design

The field experiment compared three rotations during the wheat/vegetable production season: rice–wheat (RW), rice–vegetables (RV) (the vegetables were tuber mustard at Jiangjin, cabbage at

TABLE 1 | Average soil properties at the experimental sites at the beginning of the experiment.

Location	Soil type ^a	Organic matter (g·kg ⁻¹)	Total N (g·kg ⁻¹)	Olsen-P (mg·kg ⁻¹)	Available K (mg·kg ⁻¹)	pH (in H ₂ O)
Jiangjin	Hydragric Anthrosol (purple soil)	25.49	1.58	7.77	107.5	5.00
Shayang	Hydragric Anthrosol	18.05	1.04	4.44	117.4	7.52
Rugao	Gleyic Fluvisol	21.12	1.26	7.19	88.3	7.44

^aClassified according to WRB.

TABLE 2 | Fertilizer applications in the wheat/vegetable season.

Crops	Fertilizer treatments	N (kg N ha ⁻¹)		P (kg P ₂ O ₅ ha ⁻¹)	K (kg K ₂ O ha ⁻¹)
		Basal application	Topdressing	Basal application	Basal application
Wheat (RW, MW)	C	0	0	60	75
	R	60	90	60	75
	T	180	120	120	75
Vegetable (RV)	C	0	0	60	75
	R	120	180	120	180
	T	300	300	240	180

Notes: all the fertilizers were broadcast onto the soil and topdressings were applied avoiding foliage. C, control, no N fertilizer. R, reduced fertilizer. T, traditional fertilizer.

Shayang, and savoy cabbage at Rugao), and maize–wheat (MW). Each rotation comprised three treatments: no N fertilizer as the control (C), reduced N fertilizer (R), and traditional N fertilizer (T). Fertilizers for wheat and vegetables were applied as shown in **Table 2**, based on previous studies in Chongqing (Ju et al., 2009; Su et al., 2017; Hao et al., 2019). Plot sizes were 8 m × 5 m at Jiangjin, 5.5 m × 4.5 m at Shayang, and 10 m × 4 m at Rugao, arranged in randomized blocks in four adjacent fields at the three sites.

Microplots (0.5 m × 0.5 m) fenced with a stainless steel frame (0.3 m high, and driven 0.2 m deep into the soil to avoid surface runoff and lateral contamination) were installed at the side of each traditional fertilizer treatment and reduced fertilizer treatment plot, 1 m from the edge of the plots. ¹⁵N-labeled urea (abundance 5.14%; Shanghai Chem-Industry Institute) was applied to the microplots at the same N rate as the main plots and at the same time. Applications of P and K fertilizer and management practices on the microplots were the same as on the corresponding main plots. Each treatment had three replicates.

Crop varieties were Chuanmai 45, Zhengmai 9023, and Yangmai 16 wheat at Jiangjin, Shayang, and Rugao, respectively.

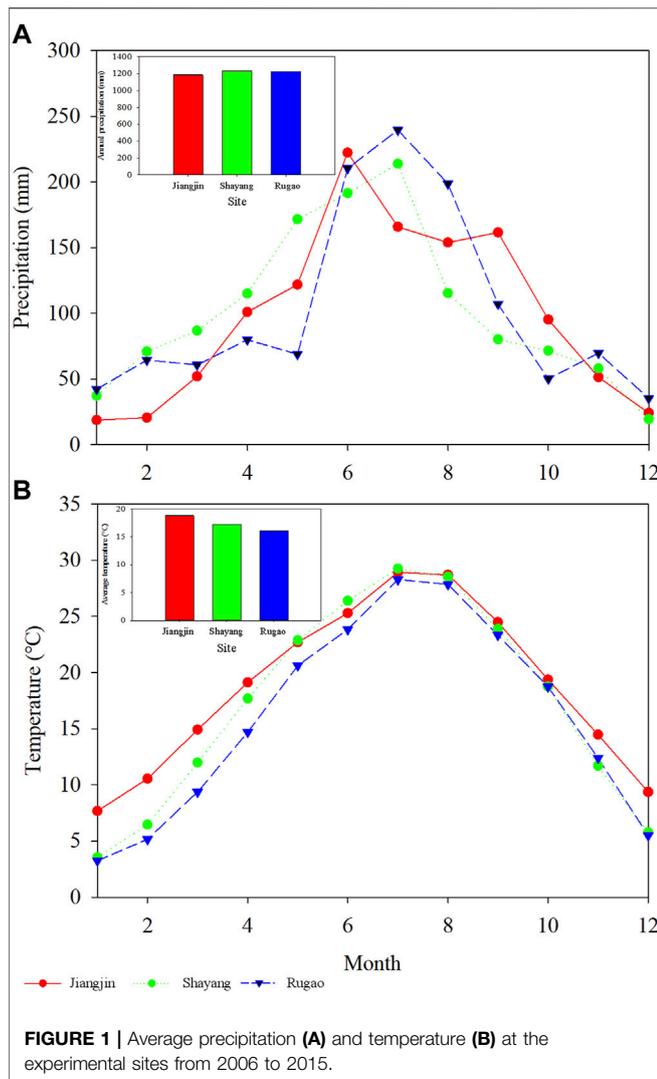
Plant and Soil Sampling and Analysis

Agricultural management practices for the wheat at the three sites, tuber mustard (*Brassica juncea* var. *tumida* L.) at Jiangjin, cabbage (*Brassica rapa pekinensis* L.) at Shayang, and savoy cabbage (*Brassica oleracea* L. var. *capitata* L.) at Rugao, are shown in **Table 3**. The wheat and vegetables were planted at 1.74–2.18 × 10⁶ plants ha⁻¹ and 9 × 10⁴ plants ha⁻¹, respectively. The recent (2006–2015) weather conditions (e.g., average precipitation and temperature during the wheat and vegetable growing period) in the three regions are shown in **Figure 1**.

The yields of wheat grain and vegetables were determined by harvesting all of each plot. Plant samples from microplots were carefully collected by hand using a sickle and a small shovel and separated into grain, straw, and roots. Aboveground parts were harvested first and then whole roots were dug out and washed with distilled water. All samples were dried at 65°C in a forced air

TABLE 3 | Agricultural management during the winter season at the three sites.

Crop rotation	Activity	Date		
		Jiangjin	Shayang	Rugao
2016–2017 Wheat (RW, MW)	Basal fertilization	3 November	26 October	30 November
	Sowing	4 November	27 October	31 November
	Topdressing	9 December	20 December	11 March
	Harvesting	3 May	15 May	30 May
2016–2017 Vegetable (RV)	Basal fertilization and transplanting	26 October	9 October	11 March
	First topdressing	2 November	20 December	28 March
	Second topdressing	2 December	27 January	12 April
	Third topdressing	27 December	17 February	5 May
	Harvesting	16 February	8 March	30 May



oven and ground to pass a 250- μm (65-mesh) screen. Samples from the microplots, including four hills of wheat and one hill of vegetables, were finely ground in a ball mill for ^{15}N analysis.

Soil samples were collected from 0–40 cm depths from all plots and microplots after harvesting the wheat and vegetables using a 3-cm inner diameter tube auger and divided into two layers (0–20 cm, 20–40 cm). All soil samples were air-dried and ground to pass a 150- μm (100-mesh) screen. Soil samples from microplots were finely ground in a ball mill. Grain, straw, roots, and soil samples were analyzed for total N content by the Kjeldahl method and ^{15}N abundance by mass spectrometry (Delta Plus XP, Thermo Finnigan, Pittsburg, PA). Tuber mustard has stems, leaves and roots. Usually, the stem is eaten but the leaves are discarded. All of the aboveground parts of cabbage and savoy cabbage can be eaten.

^{15}N Analysis

The percentages of fertilizer N recovered in wheat/vegetable grain/edible part, straw and roots and in the soil from the microplots at harvest were calculated using Eqs. 1–4, where

the ^{15}N atom% excesses were corrected for background abundance [0.3663% (Cabrera and Kissel, 1989)].

$$\begin{aligned} \text{N derived from fertilizer (Ndff) in the plant (kg N ha}^{-1}\text{)} \\ = \text{N uptake by the plant (kg ha}^{-1}\text{)} \times \text{atom\% excess in the plant} / \text{atom\% excess in fertilizer} \end{aligned} \quad (1)$$

$$\text{Ndff in soil (kg N ha}^{-1}\text{)} = \text{Total N in soil (kg ha}^{-1}\text{)} \times$$

$$\text{atom\% excess in soil} / \text{atom\% excess in fertilizer} \quad (2)$$

$$\text{Fertilizer N recovery (\%)} = \text{Ndff} / \text{N rate} \times 100 \quad (3)$$

$$\text{Loss of fertilizer N (\%)} = 100\%$$

– Fertilizer N recovery in the plant

– Fertilizer N recovery in the soil

(4)

Statistical Analysis

Statistical analyses were made using SPSS 23 (Statistical Product and Service Solutions Inc., Chicago, IL, United States). A two-way analysis of variance (ANOVA) was conducted to compare measured crop and soil data (biomass yield and fertilizer N recovery) by standard procedures on a randomized plot design for the rice/maize season within the following effects: crop rotation (RW, RV, and MW), fertilizer (C, R, and T), and crop rotation \times fertilizer. Different small letters mean statistically significant differences at $p < 0.05$ between the different rotations at the same site according to the Tukey HSD test. Microsoft Office Excel 2013 was used to process the data. OriginPro 2017 was used to draw the figures.

RESULTS

Crop Yields

Yields of wheat in the RW and MW rotations and of vegetables in the RV rotation are shown in **Figure 2**. The interactions of crop rotation and fertilizer rate had a significant effect on yield at Jiangjin and Rugao. At Jiangjin (Chongqing), the traditional fertilizer input resulted in a 90% increase (by 1.96 t ha⁻¹) and 31% decrease (by 1.67 t ha⁻¹) in the wheat yields in the RW and MW rotations compared with no fertilizer control, respectively. Reduced fertilizer caused a 116% and 3% increase in RW (2.23 t ha⁻¹) and MW (2.47 t ha⁻¹) wheat yields, respectively, compared to the zero N control. In the RV rotation, traditional fertilizer input and reduced fertilizer input produced 370% (13.99 t ha⁻¹) and 214% (9.34 t ha⁻¹) increases in tuber mustard yield relative to the control, respectively. However, although the traditional fertilizer input resulted in a bigger yield increase, it also increased the variability of yields.

At Shayang (Hubei), the traditional fertilizer input increased ($p < 0.05$) wheat grain yield in the RW and MW rotations by 541% (3.42 t ha⁻¹) and 459% (4.19 t ha⁻¹), respectively, over the control. Reduced fertilizer input significantly increased yields over the control by 453% (2.95 t ha⁻¹) for RW and 420%

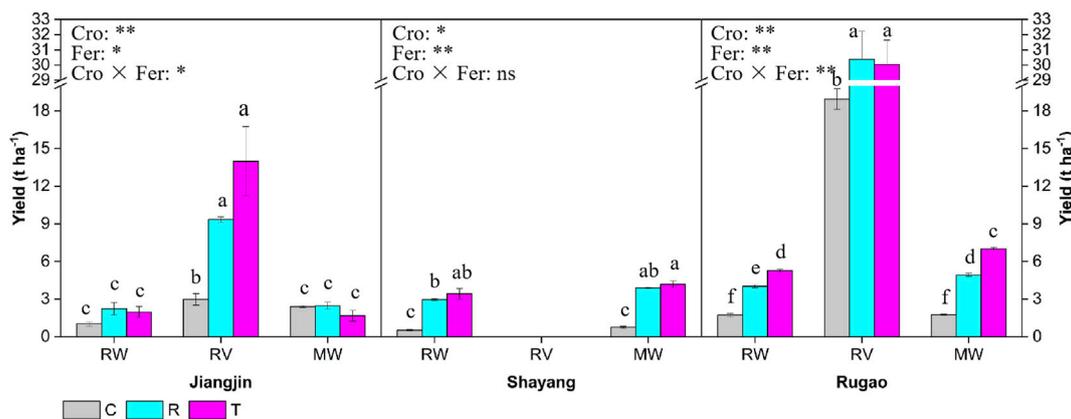


FIGURE 2 | Wheat and vegetable yields at Jiangjin, Shayang, and Rugao. Note: RW = Rice–Wheat rotation. RV = Rice–Vegetable rotation. MW = Maize–Wheat rotation. Error bars present the standard error of the measurements from three replicates. C = no N fertilizer input. R = reduced fertilizer input. T = traditional fertilizer input. Different small letters above the bars denote significance at the 5% level. Cro = crop system. Fer = fertilization. Cro × Fer = the interactions of crop rotation and fertilization. ns, * and ** mean no significance, $p < 0.05$ and $p < 0.01$.

(3.90 t ha⁻¹) for MW, but there were no significant differences between the traditional and reduced treatments. Because of an abnormal amount of precipitation at transplanting, which could not be handled by the drainage system in the concrete frame, flooding of the RV cabbage at Shayang resulted in no yield.

At Rugao (Jiangsu), the traditional fertilizer application produced 207% (by 5.28 t ha⁻¹) and 300% (by 7.02 t ha⁻¹) increases ($p < 0.05$) in RW and MW wheat yields, respectively, compared to no N control. Reduced fertilizer input resulted in 134% (by 4.02 t ha⁻¹) and 182% (by 4.94 t ha⁻¹) increases in grain yield for RW and MW, respectively, compared to the control. The traditional fertilizer input also increased the yield in the RW and MW rotations by 31% and 42%, respectively, over the R treatment. Savoy cabbage receiving the traditional fertilizer rate (yield = 30.04 t ha⁻¹) and reduced fertilizer rate (yield = 30.38 t ha⁻¹) in the RV rotation yielded 59% and 60% more, respectively, than the controls, but there was no significant difference between the T and R treatments.

The Recovery of ¹⁵N-Labeled Fertilizer in the Crops

The atom % ¹⁵N recovery in wheat (grain, straw and roots) in the RW and MW rotations and in the vegetables in the RV rotation (leaves, stem, and roots) are shown in **Figure 3**. There were significant interactions between the crop rotation and fertilizer in determining the ¹⁵N recovery in grain, straw, and roots at Jiangjin, with the average recovery in the grain, straw, and roots of wheat from the T treatment at Jiangjin being only 7%, 8%, and 1%, respectively; i.e., very low. Recoveries in the R treatment were 11%, 12%, and 1% in the grain, straw, and roots, respectively. ¹⁵N recoveries from the R treatment in the stem, leaves, and roots of tuber mustard were 25%, 11%, and 2%, respectively, and those from the T treatment were 16%, 5%, and 1%, respectively. Recoveries in the grain and stem from the R treatment at Jiangjin were significantly higher (by 34, 75 and

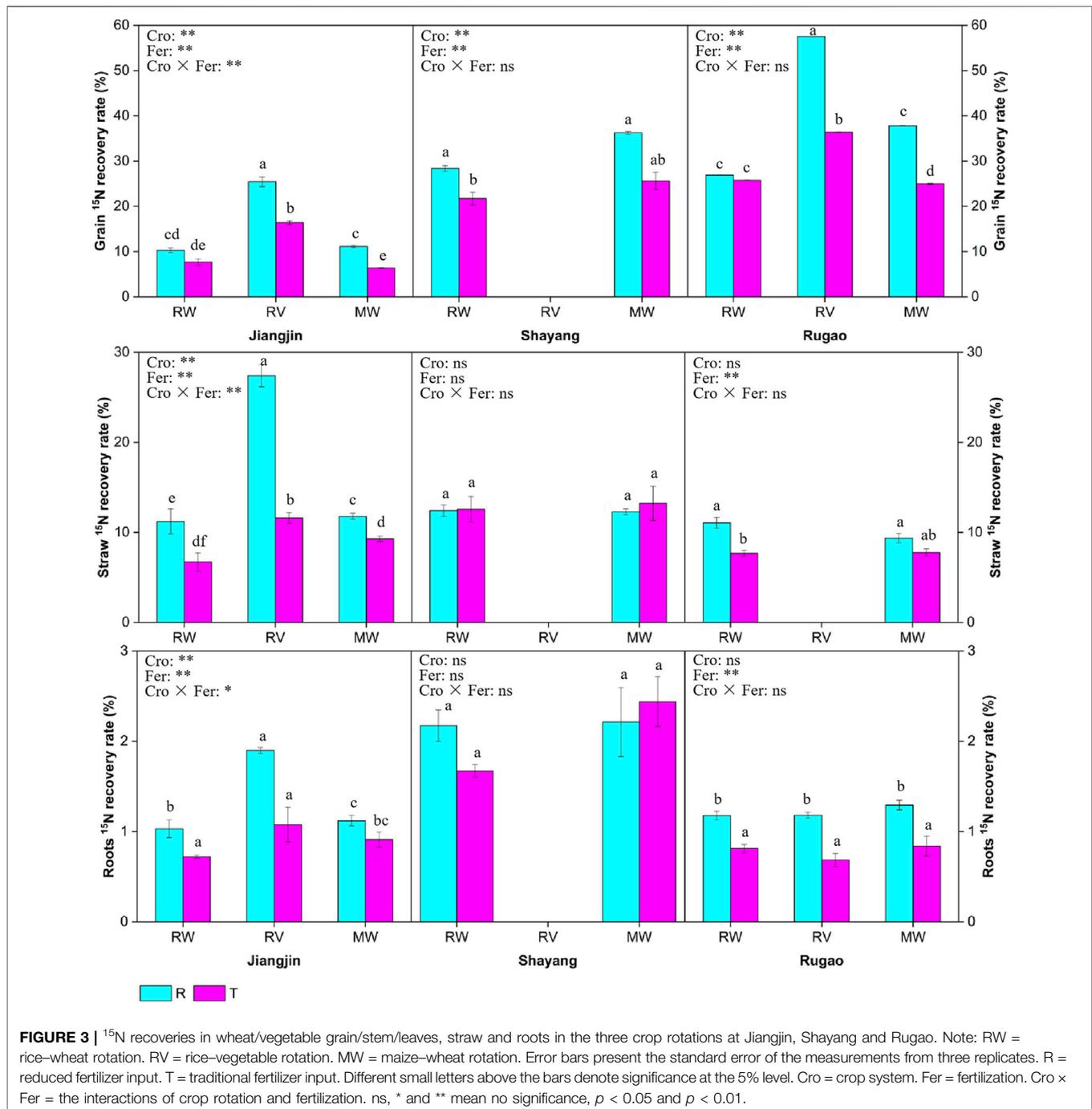
55%) than those from the T treatment in the RW, MW (grain), and RV (stem) rotations, respectively. The recoveries from the R treatment in the straw and leaves of wheat and cabbage at Rugao were higher ($p < 0.05$) than those from the T treatments in the RW (by 67%), MW (by 27%), and RV (by 136%). The ¹⁵N recoveries in the roots of crops receiving reduced fertilizer inputs were 43% and 76% higher than those receiving the traditional fertilizer input in the RW and RV rotations, respectively.

At Shayang, the average recoveries in the grain, straw, and roots of wheat receiving the traditional fertilizer input were 20%, 10%, and 2%, respectively, and the average recoveries in those receiving reduced fertilizer input were 25%, 112%, and 2%, respectively. The total ¹⁵N recoveries in the grain from the R treatment on the RW and MW rotations were significantly higher (by 31% and 19%, respectively) than those receiving traditional fertilizer input. There were no significant differences between the 2 N rates in the recoveries in the straw and roots.

At Rugao, the average recoveries in grain, straw, and roots of wheat receiving the traditional fertilizer rate were 25%, 8%, and 1%, respectively; in the R treatment, they were 32%, 10%, and 1%, respectively. There were no significant differences between the 2 N treatments in the recoveries in the grain in the RW rotation, but more ¹⁵N was recovered in the straw and roots in RW. Total ¹⁵N recoveries in the grain, straw, and roots of the R treatment were significantly higher (by 51%, 21%, and 54%, respectively) than those grown with the traditional fertilizer input in the MW rotation. The percentage ¹⁵N recoveries in leaves and roots of savoy cabbage were 58% and 1%. The percentage recovery from the T treatment was 36% and 1% and that from the R treatment 58% and 73%, i.e., higher ($p < 0.05$) compared to the T treatment.

Distribution of ¹⁵N-Labeled Fertilizer N in Soil

The recoveries of ¹⁵N fertilizer in the soil are shown in **Figure 4**. There were significant interactions of the crop rotation with



fertilizer rate in the 0–20 and 0–40 cm soil layers at Jiangjin and Rugao. At Jiangjin, the percentage recoveries of ^{15}N in the RW, MW, and RV rotations were 17%, 15%, and 36% in the 0–20 cm soil layer and 7%, 7%, and 9% in the 20–40 cm layer in the T treatment over the whole wheat/vegetable season. The percentages of the total soil recoveries in the shallow (0–20 cm) soil layer were 70%, 69%, and 80% in the RW, MW, and RV rotations, respectively. In the R treatment, the recoveries in the RW, MW, and RV rotations were 19%, 20%, and 19%, respectively, in the 0–20 cm soil and 10%, 7%, and 9%,

respectively, in the 20–40 cm layer. The percentages of total recovery in the shallow soil layer were therefore 67%, 68%, and 73%, respectively, in the R treatment. The recovered ^{15}N residues in the soil from the R treatments were 20% and 26% higher, respectively, in the RW and MW rotations but 38% lower in RV rotation compared with the T treatment.

At Shayang, 18%, 21%, 16%, and 19% of the residual ^{15}N was found in the 0–20 cm soil layer in the RWT (traditional fertilizer rate), RWR (reduced fertilizer rate), MWT, and MWR treatments, respectively. ^{15}N recovery in the surface layer was

increased significantly by reducing the fertilizer application. Recovery in the 20–40 cm soil layer in the RW and MW rotations was 6% and 4% for the T treatment and 7% and 7% for the R treatment. The percentages of the total recovery of ¹⁵N in the surface soil layer were 75% and 75% in the RW rotation receiving the traditional and reduced fertilizer rates, respectively, and 79% and 73% in the MW rotation receiving the traditional and reduced fertilizer input, respectively. Reducing the fertilizer

rate increased ¹⁵N recovery in the soil by 17% and 29% in the RW and MW rotations, respectively, compared to the traditional fertilizer rates used by farmers.

At Rugao, the rate of N applied had no significant effect on the amount of ¹⁵N recovered in the soil in the wheat/vegetable season. Of the total ¹⁵N recovered, 94%, 91%, 85%, 83%, 86%, and 75% were in the surface 0–20 cm of soil in the RW (T), RW (R), MW (T), MW (R), RV (T), and RV (R) treatments, respectively.

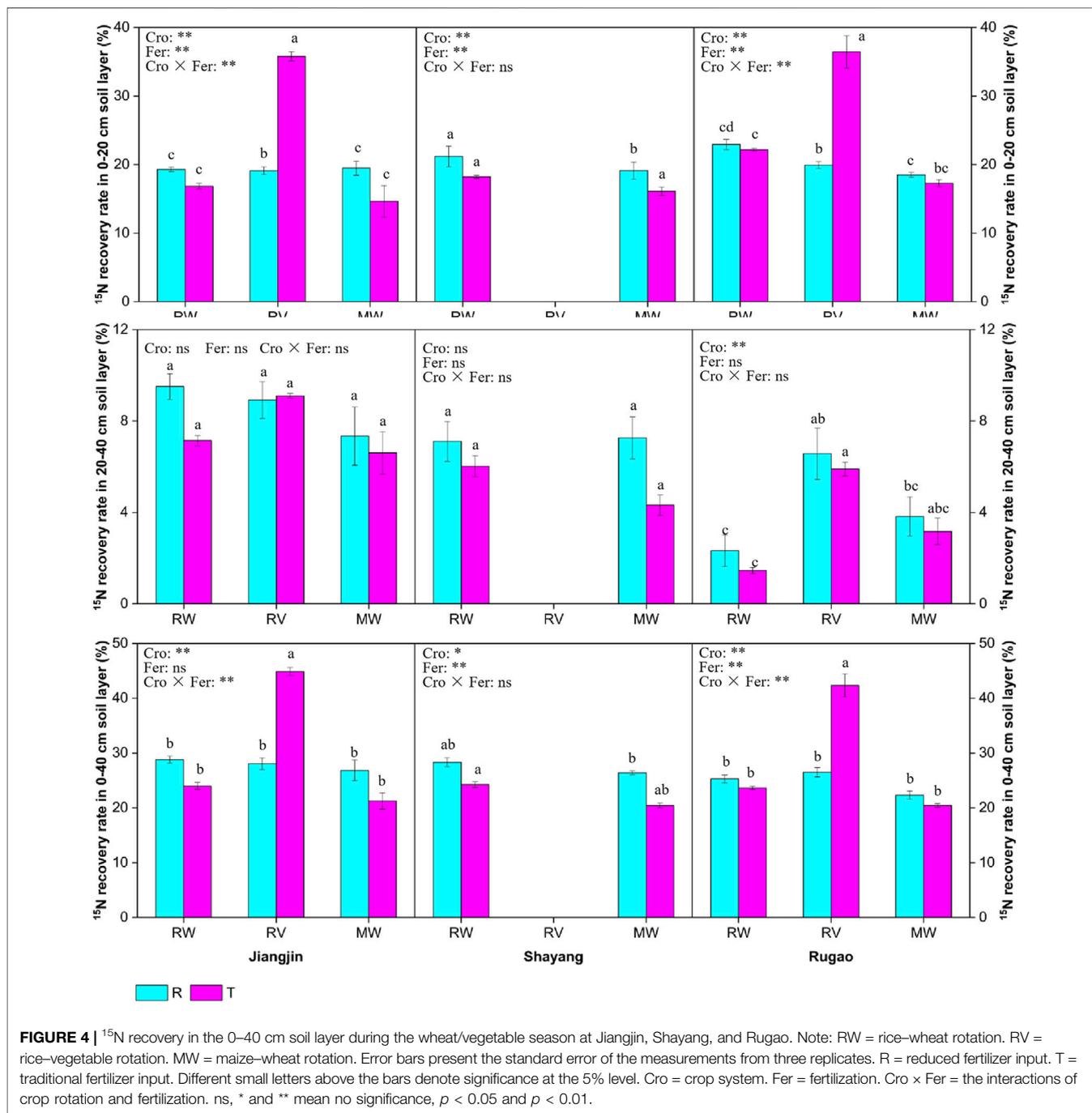


TABLE 4 | Average N losses as percentages and amounts from the crop rotations in the YRB (mean \pm SD).

Site	Rotation system	Treatment	^{15}N loss rate (%)	Total N loss (kg N ha $^{-1}$)
Jiangjin	RW	R	48.7 \pm 0.7b	73.0 \pm 1.3c
		T	60.9 \pm 2.1a	182.8 \pm 6.2a
	RV	R	17.2 \pm 5.3c	51.7 \pm 15.8c
		T	26.0 \pm 1.3c	156.2 \pm 7.8b
	MW	R	49.1 \pm 3.9b	73.7 \pm 5.9c
		T	62.2 \pm 5.6a	186.5 \pm 16.8a
Shayang	RW	R	28.7 \pm 5.0a	43.1 \pm 7.4b
		T	39.8 \pm 5.2a	119.4 \pm 15.5a
	RV	R	0	0
		T	0	0
	MW	R	23.9 \pm 4.2a	35.9 \pm 6.3b
		T	39.0 \pm 6.4a	116.9 \pm 19.1a
Rugao	RW	R	35.6 \pm 3.4ab	53.3 \pm 5.2b
		T	42.1 \pm 3.8a	126.3 \pm 11.3a
	RV	R	14.8 \pm 3.2d	44.4 \pm 9.6b
		T	20.6 \pm 5.1cd	123.4 \pm 30.6a
	MW	R	29.2 \pm 4.3bc	43.8 \pm 6.4b
		T	45.9 \pm 4.0a	137.7 \pm 12.0a

Note: RW, rice–wheat rotation; RV, rice–vegetable rotation; MW, maize–wheat rotation. R, reduced fertilizer input. T, traditional fertilizer input. Means followed by different small letters indicate significant differences according to Tukey HSD, test ($p \leq 0.05$), when comparing with the R and T treatments.

TABLE 5 | ANOVA results (p values) for the individual effects of interactions of fertilizer (R and T) and crop rotation (RW, RV, and MW) on ^{15}N loss and total N loss.

		Fertilizer	Crop rotation	Fertilizer \times crop rotation
Jiangjin	^{15}N loss rate	<0.0001	<0.0001	0.5710
	Total N loss	0.0017	<0.0001	0.7868
Shayang	^{15}N loss rate	0.3779	0.0025	0.5295
	Total N loss	0.5416	<0.0001	0.7676
Rugao	^{15}N loss rate	<0.0001	0.0003	0.0640
	Total N loss	0.7021	<0.0001	0.4838

Note: Statistically significant effects ($p < 0.05$) are marked in bold.

Losses of ^{15}N -Labeled Urea During the Wheat/Vegetable Season

The loss of ^{15}N was calculated as the difference between the ^{15}N applied and its measured uptake by wheat (grain, straw, and roots), tuber mustard (stem, leaves, and roots), savoy cabbage (leaves and roots), and the residue in the soil (Table 4). In our experiment, a significant decrease in total N loss (i.e., the N unaccounted for) was observed in improved fertilizer treatments relative to traditional fertilizer management of the three cropping systems in winter, with total N losses of winter-season crops (wheat and vegetables) reduced by 60%–67%, 58%–68%, and 64%–69% at Jiangjin, Shayang, and Rugao, respectively. Across all three sites in the YRB, improved (i.e., reduced) fertilizer rates decreased N losses to the environment by 63% and 66% from wheat and vegetable rotations. According to the two-way ANOVA (Table 5), N loss was mainly determined by crop rotation ($p < 0.05$) at the three sites, but fertilizer rate had a significant effect at Jiangjin.

Fate of ^{15}N -Labeled Urea During the Wheat/Vegetable Season

The fate of ^{15}N -labeled urea in wheat/vegetable season under the different rotations at the three sites is shown in Figure 5. At the conclusion of the experiment at Jiangjin, the average percentage of recovery of ^{15}N in wheat was 16% and 24% with the traditional and reduced fertilizer inputs, respectively, i.e., very low. The average recovery from the soil was 23% and 28% with the traditional and reduced fertilizer input, respectively. Thus, fertilizer ^{15}N loss to water and air (62%) under the traditional fertilizer input was reduced by 21% by reducing the fertilizer input (actual loss = 49%), and by 34% (from 26 to 17%) under the wheat rice–vegetable rotation.

At Shayang, the average N uptakes by the wheat were 32% (T) and 39% (R). The average ^{15}N recoveries from the soil under wheat were 22% and 27% in the T treatment and R treatment, respectively, so the N loss to the environment was reduced by 26%.

At Rugao, the average fertilizer ^{15}N recovered in the wheat was 34% with the traditional fertilizer input and 44% with the reduced fertilizer input, with average recoveries from the soil of 22% (T) and 24% (R). Thus, fertilizer N loss was reduced by 27% by reducing the fertilizer input. Savoy cabbage utilized 36% and 58% of the N applied and left 22% and 24% in the soil at the traditional and reduced fertilizer rates, respectively; a 28% reduction in loss to the environment.

DISCUSSION

The average wheat yield at Jiangjin obtained by reducing the fertilizer application ($2.35 \pm 0.76 \text{ t ha}^{-1}$) was higher but not significantly different from that using the traditional fertilizer

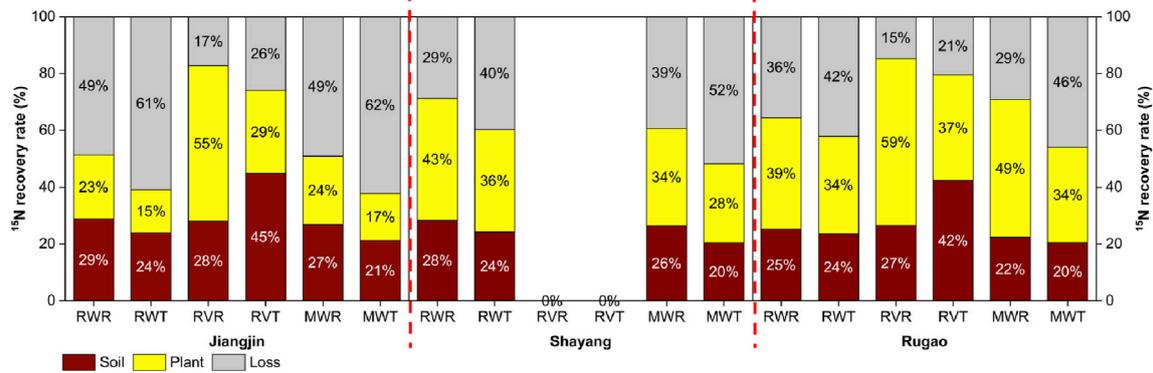


FIGURE 5 | ^{15}N recoveries in the crops at the end of the rotations, soil, and losses. Note: RW = rice–wheat rotation. RV = rice–vegetable rotation. MW = maize–wheat rotation. RWR = reduced fertilizer input in RW rotation, RWT = traditional fertilizer input in RW rotation, the same as RV and MW rotations in Jiangjin, Shayang, and Rugao.

input ($1.81 \pm 0.82 \text{ t ha}^{-1}$). All treatments at Jiangjin yielded less than in previous experiments in the Sichuan Basin ($2.74 \pm 0.93 \text{ t ha}^{-1}$) (Duan et al., 2014; Zhou et al., 2016) due to fusarium head blight (FHB). The disease mainly occurs in warm, humid climates (such as Jiangjin, which has no useful tools or sufficient experience for FHB control) and can lead to a loss of yield and quality (significant yield losses on more than 5 million ha per year in China are caused by FHB) (Goswami and Kistler, 2004; Cowger et al., 2016; Liu Y. Y. et al., 2017; Chen et al., 2019). In Shayang county, the grain yield was $3.80 \pm 0.69 \text{ t ha}^{-1}$ with the traditional fertilizer rate and $3.42 \pm 0.53 \text{ t ha}^{-1}$ with the reduced rate; higher than those reported in the literature ($2.85 \pm 0.81 \text{ t ha}^{-1}$) (Hu et al., 2015; Yi et al., 2015). In contrast, the yield of wheat at Rugao was $6.15 \pm 0.97 \text{ t ha}^{-1}$ with the traditional fertilizer rate and $4.48 \pm 0.54 \text{ t ha}^{-1}$ with the reduced rate. The average of these (5.32 t ha^{-1}) was comparable with that reported by Jiang et al. (2006) and Ma et al. (2010). However, this is less than that recommended for food security (7.12 t ha^{-1}) by Chen et al. (2014). It might therefore be necessary to decrease the area of wheat planted in Jiangjin and Shayang and focus on other more productive crops (e.g., a green manure crop in Jiangjin and oilseed rape in Shayang), based on our results and predicted climate change. Statistics show that the cultivated area and yield of wheat in Chongqing has decreased: from 2011 to 2020, the cultivated wheat in Chongqing decreased by 80%, from 90,530 to 18,520 ha, and the annual wheat grain production decreased by 78%, from 0.27 to 0.06 million tons (Ministry of Agriculture of the People's Republic of China, 2021). In the rice–vegetable rotation at Jiangjin, the traditional fertilizer input produced a lower output ($13.99 \pm 2.77 \text{ t ha}^{-1}$) than that measured in a previous study ($29.90 \pm 6.56 \text{ t ha}^{-1}$) (Bai et al., 2009), and yields were much more variable. Reducing the fertilizer input reduced the yield ($9.34 \pm 0.21 \text{ t ha}^{-1}$) but not significantly and yields were more stable. The average yield of savoy cabbage in the RV rotation at Rugao ($30.21 \pm 2.70 \text{ t ha}^{-1}$) was also less than that in earlier experiments ($48.69 \pm 17.45 \text{ t ha}^{-1}$) (Franczuk et al., 2010; Gai et al., 2016; Maucieri et al., 2017) and there was no significant difference between the two fertilizer levels ($30.04 \pm$

2.00 t ha^{-1} with traditional fertilizer and $30.38 \pm 2.30 \text{ t ha}^{-1}$ with reduced fertilizer).

Vegetables had even greater yield variability than wheat across the three sites, suggesting significant risks if wheat was replaced by vegetables in winter. Specifically, the Jiangjin site is not suitable for wheat while the Shayang site is not suitable for vegetables if there is no drainage system (no harvested yield) in winter. Rugao achieved the highest wheat and vegetable yields of all the sites, reflecting the influence of soil fertility (Rugao > Shayang > Jiangjin), climate (sunshine in winter: Rugao > Shayang > Jiangjin; precipitation in winter: Rugao < Shayang < Jiangjin), and good field management [Rugao (hired/professional farmers) > Shayang (half-professional farmers) > Jiangjin (smallholder farmers)].

Figures 3, 4 show clearly how the ^{15}N recovered in the crops (wheat and vegetables) was influenced by fertilizer input. Reducing the fertilizer input resulted in higher fertilizer N uptake in the grain and smaller losses, except for the recoveries in the straw and roots of the MW rotation at Shayang. At the same time, N recovery was very low in wheat at Jiangjin due to the low yield caused by disease. Previous research had also demonstrated that reducing N inputs can increase N efficiency and decrease environmental impacts (Chen et al., 2014; Yi et al., 2015). Also, returning wheat straw to the soil can increase SOC stock (Hao et al., 2013; Guan et al., 2015), water capture and retention, microbial C and microbial N, the activities of cellulase and catalase (Li et al., 2018), and microbial and invertebrate activity (in particular earthworms) (van Gestel et al., 2003) and decrease soil pH (Walter et al., 1996). A wheat–straw mulch on upland crops can ameliorate salinization, prevent water and wind erosion of soil (Dong and Qian, 2002), and reduce N uptake by weeds (Liu et al., 2005). We found that ^{15}N recovery in wheat/vegetable grain, straw, and roots in the YRB was increased by 41%, 48%, and 43% by reducing the fertilizer rate compared with the traditional fertilizer input used by farmers. We therefore recommend the reduction of the amount of fertilizer applied and returning the stubble to

the field to improve soil quality and increase the recovery of fertilizer N.

The average fertilizer ^{15}N recoveries in the 0–20 cm soil layer were 16%, 17%, and 20% at the traditional fertilizer rate and 19%, 20%, and 21% with reduced fertilizer at Jiangjin, Shayang, and Rugao, respectively. The ^{15}N recovered in topsoil under the vegetable crops receiving traditional and reduced fertilizer applications was 36% and 19%, respectively, at Jiangjin and 36% and 20%, respectively, at Rugao. Overall, the percentage recoveries of residual ^{15}N in the topsoil (0–20 cm) out of the total recoveries in the 0–40 cm layer of soil under crops receiving the traditional and reduced fertilizer rates were 73% and 69%, respectively, at Jiangjin, 75% and 73%, respectively, at Shayang, and 88% and 83%, respectively, at Rugao. This was caused by runoff and leaching resulting from the abnormal amounts of precipitation during the application of basal fertilizer and topdressing. The higher soil residues observed at Jiangjin were because rainfall leaches N deep into the soil where soil colloids adsorb NH_4^{4+} (Xu et al., 2021). Also, soil pH values at Shayang and Rugao were 7.52 and 7.44, respectively, which resulted in more ammonia volatilization than at Jiangjin (Harrison and Webb, 2001; Huang et al., 2017).

Yan et al. (2014) pointed out that N residues in soil following high N inputs can be taken up by subsequent crops and so should be considered as part of the available N. However, we found that fertilizer ^{15}N recovery in the soil of a wheat crop was significantly increased by 23% and 23% by reducing the fertilizer rate at Jiangjin and Shayang; the increase was 8% at Rugao, which was not significant. Thus, reducing N applications is still advisable. In contrast, the very high demand for N by vegetable crops resulted in less N remaining in the soil under the R treatment compared to the traditional fertilizer rate.

Overall, reducing the application of synthetic N fertilizer to a wheat/vegetable rotation in the YRB will reduce N losses to the environment (including runoff, leaching, and gaseous loss) (Tian et al., 2007; Wang et al., 2007; Qiao et al., 2012; Xu et al., 2012; Xue et al., 2014; Zhong et al., 2016; Zhang et al., 2017). We observed the maximum fertilizer N loss from the traditional fertilizer application to the MW rotation and the minimum loss from the reduced fertilizer application to the RW rotation (MW at Rugao). The large ^{15}N loss at Jiangjin resulted from the heavier rainfall over the growing period generating more N losses *via* surface runoff and leaching (Xie et al., 2019; Liu et al., 2020). Also, the higher temperature at Jiangjin triggers more NH_3 volatilization (Xu et al., 2021). More fertilizer N remained in the vegetable plots than in the wheat plots after harvest of both crops in Jiangjin and Rugao. This can be explained by the much earlier harvest date of vegetables at Jiangjin and/or two additional topdressings at both sites (see **Table 3**), providing more opportunities for ^{15}N labeled fertilizer to be retained in the soils growing vegetables.

CONCLUSION

Research in the winter growing season at three sites in the YRB supports our conclusion that reducing the application of N

fertilizer is an effective way of balancing sustainable crop yields for a secure food supply and environmental benefit. A 50% reduction of the N applied to wheat and vegetables maintained the wheat yields at Jiangjin and Shayang and vegetable yields at Jiangjin and Rugao. However, the yields of wheat at Rugao were reduced. More (27%) fertilizer N was retained in the soil (0–40 cm) and recycled to the next rice/maize crop, and there was a 25% reduction of fertilizer N loss compared with traditional N use. This confirms the importance of reducing N inputs to crop rotations in the YRB. Moreover, the yield gap of three sites proved that the agricultural infrastructure (e.g., drainage system) and management (e.g., hired/professional farmers) have substantial effects on yield in the YRB. More research is needed to optimize crop rotations across the whole crop production season for better whole system productivity, N use efficiency, and less environmental impact. Such integrated analyses are vital if the YRB is to adapt to predicted global climate change and regional green development within the Yangtze Economic Zone. In the future, we aim to quantify all N inputs (including atmospheric deposition and manures as well as fertilizer) and outputs (including crop uptake, losses to surface and groundwater, and gaseous loss) and, synthesizing the relevant data (including yield and future food demands), to recommend optimal rotations for the different regions of the YRB.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SW, FSZ, and XJL contributed to the conception of the study; SW, LSY, SPL, and BS performed the experiment; SW, KG, and XJL contributed significantly to analysis and manuscript preparation; SW performed the data analyses and wrote the manuscript; SW, XJS, JWJ, SWG, JBS, KG, and XJL helped perform the analysis with constructive discussions.

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Conflict of Interest: SW was employed by the company Huaneng Renewable Corporation Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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