

RESEARCH ARTICLE

Nitrogen Use Efficiency as Affected by Phosphorus and Potassium in Long-Term Rice and Wheat Experiments

DUAN Ying-hua¹, SHI Xiao-jun², LI Shuang-lai³, SUN Xi-fa⁴ and HE Xin-hua^{1,5}

¹ Key Laboratory of Crop Nutrition and Fertilization, Ministry of Agriculture/Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China

² College of Resources and Environment, Southwest Agricultural University, Chongqing 400716, P.R.China

³ Institute of Plant Protection and Soil Science, Hubei Academy of Agricultural Sciences, Wuhan 430064, P.R.China

⁴ Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, P.R.China

⁵ School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia

Abstract

Improving nitrogen use efficiency (NUE) and decreasing N loss are critical to sustainable agriculture. The objective of this research was to investigate the effect of various fertilization regimes on yield, NUE, N agronomic efficiency (NAE) and N loss in long-term (16- or 24-yr) experiments carried out at three rice-wheat rotation sites (Chongqing, Suining and Wuchang) in subtropical China. Three treatments were examined: sole chemical N, N+phosphorus (NP), and NP+potassium (NPK) fertilizations. Grain yields at three sites were significantly increased by 9.3-81.6% (rice) and 54.5-93.8% (wheat) under NP compared with N alone, 1.7-9.8% (rice) and 0-17.6% (wheat) with NPK compared with NP. Compared to NP, NUE significantly increased for wheat at Chongqing (9.3%) and Wuchang (11.8%), but not at Suining, China. No changes in NUE were observed in rice between NP and NPK at all three sites. The rice-wheat rotation's NAE was 3.3 kg kg⁻¹ higher under NPK than under NP at Chongqing, while NAE was similar for NP and NPK at Suining and Wuchang. We estimated that an uptake increase of 1.0 kg N ha⁻¹ would increase 40 kg rice and 30 kg wheat ha⁻¹. Nitrogen loss/input ratios were ~60, ~40 or ~30% under N, NP or NPK at three sites, indicating significant decrease of N loss by P or PK additions. We attribute part of the increase in NUE soil N accumulation which significantly increased by 25-55 kg ha⁻¹ yr⁻¹ under NPK at three sites, whereas by 35 kg ha⁻¹ yr⁻¹ under NP at Chongqing only. This paper illustrates that apply P and K to wheat, and reduce K application to rice is an effective nutrient management strategy for both the NUE improvement and N losses reduction in China.

Key words: apparent nitrogen balance, grain yield, nitrogen agronomic efficiency, nitrogen loss, soil nitrogen accumulation

INTRODUCTION

To meet the food demand of a rapidly expanding human population, over 26 Mha rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) are grown on the same land in South and East Asia (Timsina *et al.* 2006). Approximately 50% of them are in subtropical

and tropical China and their grains contribute 72% of total cereal production and 56% of total calorie intake to China's 1.3 billion citizens (Timsina and Connor 2001).

High inputs of chemical nitrogen (N), phosphorus (P) and potassium (K) are common to increase crop yields in China. A total of 23.5 Mt N, 8.1 Mt P and 5.9 Mt K fertilizers were applied in 2010, accounting

Received 9 October, 2013 Accepted 18 December, 2013

Correspondence DUAN Ying-hua, Tel: +86-10-82108661, E-mail: duanyinghua@caas.cn; HE Xin-hua, Tel: +61-2-88431164, E-mail: xhhe@caas.ac.cn

for ~30% of total world's N, P and K application (National Bureau of Statistics of China 2011). However, N use efficiencies (NUE) are as low as 28% for rice and wheat (Miao *et al.* 2011), resulting in up to 44% applied N (~200 kg ha⁻¹ yr⁻¹) loss to the environment. Meanwhile, P or K input often exceeds plant need for good crop production, resulting in rapid accumulation of P and K in soil (Duan *et al.* 2011a). However, a ~50% increase of N, P and K application has only brought ~10% increases of crop yields from 1990 to 2010 in China (National Bureau of Statistics of China 2011). Over-fertilization has become a major concern for a sustainably intensive agriculture in China (Meng *et al.* 2013).

Three ways are used to describe NUE: N uptake by total plant biomass (straw, roots and grains), total aboveground biomass (straw and grains) or grain yield only (Good *et al.* 2004). In this study, unless stated, NUE and N agronomic efficiency (NAE) are the total aboveground biomass N (grain plus straw) or the grain yield between N and no-fertilization divided by the fertilized N to soil, respectively (Duan *et al.* 2011b). At present, most studies on NUE and NAE are based on short-term studies, and only a few have reported NUE on long-term fertilizations in the rice-wheat rotations (Yadvinder *et al.* 2009; Li *et al.* 2012). However, information about impacts of long-term chemical P and K fertilizations on top of sole N fertilization on NUE, N losses and soil N accumulation in a rice-wheat system is limited (Duan *et al.* 2011a).

The alternative soil wetting and drying with an anaerobic to aerobic cycle between rice and wheat is challenging of improving soil N conservation (Kundu and Ladha 1999). Flooding and puddling of paddy soils rapidly deplete soil O₂, resulting in NO₃⁻ leaching/denitrification and NH₃ volatilization (Duan *et al.* 2007). The N input minus N output or apparent N balance (ANB) is used to estimate N flow to the environment (Yadav *et al.* 2002). For example, ANB exceeds 200 kg ha⁻¹ yr⁻¹ in the rice and wheat-maize systems of China (Vitousek *et al.* 2009). A more optimal management of high yield with low N loss is therefore urgently needed for a sustainable agriculture in China.

We thus compared data of N input and output for

about two decades under chemical N, N plus P (NP) and NP plus K (NPK) fertilizations, to examine their long-term effects on grain yield, NUE, NAE, ANB, N loss, and soil N accumulation, all under a rice-wheat rotation at three Chinese subtropical sites. For an improved nutrient management strategy to the rice-wheat system under long-term N, NP, and NPK fertilizations, we aimed to address 1) grain yield; 2) plant N accumulation; 3) NUE and NAE; 4) soil N accumulation and 5) apparent N balance and loss. We hypothesized that grain yield, NUE, NAE, and soil N accumulation could be increased in the order of NPK>NP>N. Nitrogen losses would follow in an opposite pattern.

RESULTS

Grain yield of rice and wheat

Compared to no-fertilization, N, NP and NPK fertilizations significantly increased grain yield of rice and wheat at each site (Table 1). Annual total yield (rice+wheat) was significantly higher at all three sites under NP than under sole N, whilst only at Chongqing and Wuchang, but not at Suining, China, under NPK than under NP. Rice yield was similar under NP and NPK at all three sites, whilst wheat yield was significantly higher under NPK than under NP at Chongqing and Wuchang, but similar at Suining (Table 1).

Nitrogen accumulation in crops and relationships between N accumulation and grain yield

Nitrogen accumulation in both rice and wheat responded to fertilizations to a greater magnitude at both Chongqing and Suining than that at Wuchang (Fig. 1). Compared with the control, significant N accumulation for rice and wheat under sole N was increased by 56 and 33 kg ha⁻¹ at Chongqing and 27 and 20 kg ha⁻¹ at Suining, respectively, but not at Wuchang. Meanwhile, annual total N accumulation in the rice-wheat system (rice+wheat) at all three sites was significantly lower under sole N than under NP

Table 1 Annual grain yield and relative increment of rice and wheat under four fertilization treatments over the whole experimental period at the three long-term fertilization sites

Fertilizer treatment	Rice				Wheat				Annual total (rice+wheat)			
	Yield (t ha ⁻¹ yr ⁻¹)	Increment to control (%)	Increment to N (%)	Increment to NP (%)	Yield (t ha ⁻¹ yr ⁻¹)	Increment to control (%)	Increment to N (%)	Increment to NP (%)	Yield (t ha ⁻¹ yr ⁻¹)	Increment to control (%)	Increment to N (%)	Increment to NP (%)
Chongqing (1991-2006)												
Control	3.6±0.2 c	-	-	-	1.3±0.1 d	-	-	-	4.9±0.2 d	-	-	-
N	5.3±0.3 b	46.1±6.7 b	-	-	1.7±0.1 c	27.3±6.9 c	-	-	6.9±0.3 c	41.1±5.7 c	-	-
NP	6.1±0.3 a	69.1±8.4 a	15.8±4.0 b	-	2.6±0.1 b	96.7±11.7 b	54.5±7.7 b	-	8.7±0.3 b	76.4±9.9 b	25.0±4.4 b	-
NPK	6.7±0.2 a	85.1±8.4 a	26.7±4.3 a	9.8±0.6	3.0±0.1 a	130.8±14.1 a	80.4±8.3 a	15.4±0.2	9.7±0.3 a	96.8±8.8 a	39.5±5.2 a	11.5±0.1
Suining (1982-2005)												
Control	3.0±0.2 c	-	-	-	1.2±0.1 c	-	-	-	4.2±0.2 c	-	-	-
N	3.8±0.2 b	26.7±7.5 b	-	-	1.6±0.2 b	33.3±6.3 b	-	-	5.4±0.2 b	28.6±6.8 b	-	-
NP	6.9±0.1 a	130±17.4 a	81.6±12.0 a	-	3.1±0.2 a	158±27.9 a	93.8±13.9 a	-	10.0±0.2 a	138±13.1 a	85.2±11.0 a	-
NPK	7.1±0.2 a	136±17.3 a	86.8±11.8 a	2.9±0.4	3.1±0.2 a	158±29.5 a	93.8±12.0 a	0±0	10.3±0.2 a	145±13.1 a	90.7±11.5 a	3.0±0.4
Wuchang (1982-2005)												
Control	4.2±0.2 c	-	-	-	0.9±0.1 c	-	-	-	5.1±0.2 d	-	-	-
N	5.4±0.2 b	28.6±3.6 b	-	-	1.0±0.1 c	11.1±2.4 c	-	-	6.4±0.2 c	25.5±3.7 c	-	-
NP	5.9±0.2 a	40.5±4.7 a	9.3±2.2 a	-	1.7±0.1 b	88.9±9.0 b	70.0±8.7 b	-	7.6±0.2 b	49.0±4.5 b	18.8±2.8 b	-
NPK	6.0±0.2 a	42.9±5.1 a	11.1±2.6 a	1.7±0.2	2.0±0.1 a	122.2±7.9 a	100.0±12.3 a	17.6±0.2	8.0±0.2 a	56.9±4.9 a	25.0±3.3 a	5.3±0.4

Data are means±SE (n=16 at Chongqing and n=24 at Suining and Wuchang). Different letters within a column indicate significant differences of the means between treatments at $P<0.05$. The same as below.

-, no data.

and NPK, but similar under NP and NPK.

Mean annual yield significantly and positively correlate with mean annual N accumulation for both rice ($r^2=0.66$, $P<0.01$) and wheat ($r^2=0.92$, $P<0.01$) (Fig. 2). The slope of the linear relationship was 0.04 in rice and 0.03 in wheat, suggesting that a plant uptake increase of 1.0 kg N ha⁻¹ results in an increase of 40 kg rice ha⁻¹ and 30 kg wheat ha⁻¹.

Nitrogen use efficiency (NUE) and nitrogen agronomic efficiency (NAE)

Nitrogen use efficiency ranged from 18 to 61% for rice

and 1 to 48% for wheat, and was significantly higher under NP and NPK than under sole N at all three sites (Table 2). Wheat NUE was 9.3-11.8% higher under NPK than under NP at Chongqing and Wuchang, but similar at Suining. Rice NUE was similar between NP and NPK at all three sites.

Nitrogen agronomic efficiency (kg grain kg⁻¹ applied N) ranged 1.1-19.0 in wheat and 6.8-34.2 in rice, and was significantly higher under NP and NPK than under sole N at all three sites (Table 2). Wheat NAE was 31-33% higher under NPK than under NP at Chongqing and Wuchang, but similar at Suining. Rice NAE was similar between NP and NPK at Suining

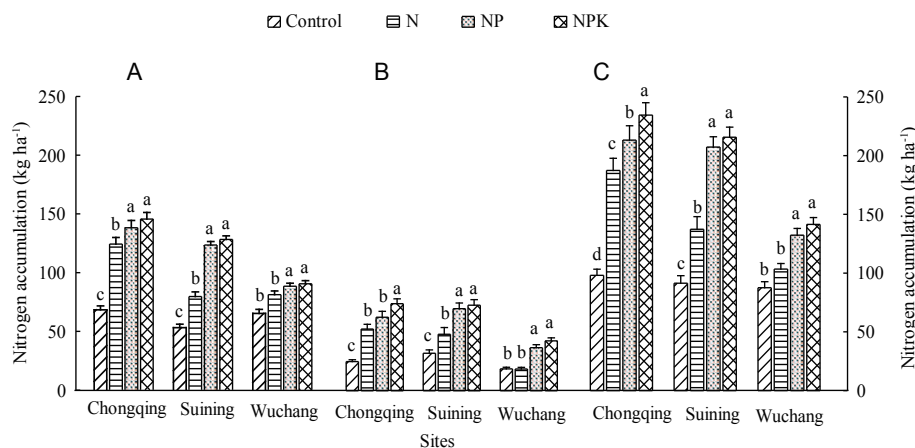


Fig. 1 Mean annual nitrogen accumulation by rice (A), wheat (B) and the rice-wheat system (C) over the long-term fertilization period at Chongqing (1991-2006), Suining (1982-2005) and Wuchang (1982-2005). Data are means±SE (n=16 at Chongqing, n=24 at Suining and Wuchang). Different letters above the bars indicate significant difference between treatments ($P<0.05$) at the same site. The same as below.

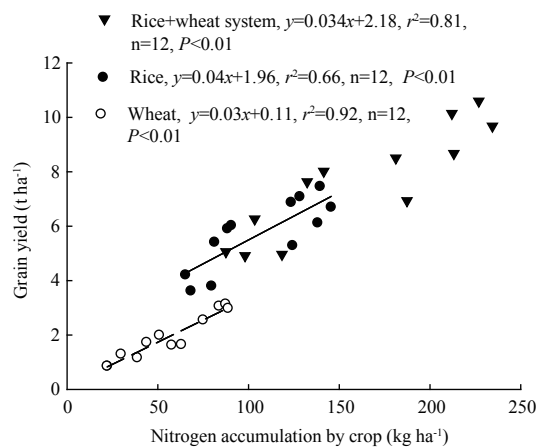


Fig. 2 Relationships between mean annual grain yield and mean annual nitrogen accumulation in rice, wheat and annual total (rice+wheat) over the long-term fertilization period at Chongqing (1991-2006), Suining (1982-2005) and Wuchang (1982-2005). Four treatments at each site comprised the 12 data points for each crop.

and Wuchang, but higher under NPK than under NP at Chongqing.

Nitrogen accumulation in soil

Soil total N amount based on the annual data collected over 16 or 24 yr from the various long-term fertilization treatments are given in Fig. 3. Soil total N was increased by 5-55 kg ha⁻¹ yr⁻¹ under all fertilizations at the three sites, and was significantly increased with the increase of experimental years under NPK ($r^2=0.58-0.78$, $P<0.05$) at all three sites but under NP ($r^2=0.45$, $P<0.05$) at Chongqing only. The increase rate of N accumulation in soil was the

highest under NPK, higher under NP and sole N, and the lowest under no-fertilization at all three sites. In addition, soil N amount under no-fertilization was stable at Chongqing and Wuchang, but in a decreasing trend at Suining.

Apparent N balances and N losses

Apparent N balances were positive (21.8-222.5 kg N ha⁻¹ yr⁻¹) under all three sites, except under no-fertilization at Suining, and were significantly ($P<0.05$) affected by fertilizations as N>NP>NPK>no-fertilization at Chongqing, but N>NP=NPK>no-fertilization at Suining and Wuchang (Fig. 4). Apparent N balances were the lowest (128 kg N ha⁻¹ yr⁻¹) under NPK at Wuchang while the highest (223 kg N ha⁻¹ yr⁻¹) under sole N at Suining and Wuchang.

Significantly lower N loss to the environment (kg ha⁻¹ yr⁻¹) among fertilizations ranked as NPK (98-117)>NP (125-150)>N (158-222), whilst significantly lower ratio of N loss to total N input (%) ranked as NPK (29-36)>NP (37-46)>N (51-62) at the three sites (Table 3). In addition, the N loss to the environment under no-fertilization was the lowest as a result of lowest N input, compared to the three fertilizations.

DISCUSSION

Crop yield and nitrogen use efficiency

Sustained crop productivity depends upon a continuous replenishment when required nutrients

Table 2 Annual nitrogen use efficiency (%) and agronomy efficiency (kg kg⁻¹) of rice and wheat under the long-term fertilization treatments at three study sites in subtropical China

Fertilization treatment	Rice		Wheat		Annual total (rice+wheat)	
	NUE (%) ¹⁾	NAE (kg kg ⁻¹) ²⁾	NUE (%)	NAE (kg kg ⁻¹)	NUE (%)	NAE (kg kg ⁻¹)
Chongqing (1991-2006)						
N	37.4±1.7 b	11.1±1.0 c	22.1±1.3 c	3.4±0.5 c	30.2±1.4 c	7.5±0.7 c
NP	46.6±2.1 a	16.7±1.2 b	30.1±1.4 b	8.4±0.6 b	38.8±1.7 b	12.8±0.9 b
NPK	51.5±2.0 a	20.5±1.2 a	39.4±1.7 a	11.2±0.9 a	45.8±1.8 a	16.1±1.1 a
Suining (1982-2005)						
N	20.6±1.8 b	6.8±0.8 b	14.5±2.2 b	3.9±0.6 b	17.6±2.0 b	5.4±0.7 b
NP	59.2±1.9 a	32.5±1.1 a	35.5±2.1 a	14.8±0.8 a	47.4±2.0 a	23.7±1.0 a
NPK	61.2±1.9 a	34.2±1.1 a	37.6±1.9 a	15.3±0.7 a	49.4±1.5 a	24.8±0.9 a
Wuchang (1982-2005)						
N	17.8±1.4 b	13.4±1.1 b	1.2±0.7 c	1.1±0.1 c	11.2±1.2 b	8.5±0.7 b
NP	25.7±1.4 a	18.9±1.1 a	36.0±2.8 b	14.5±1.2 b	29.8±2.0 a	17.1±1.1 a
NPK	28.1±1.7 a	20.2±1.3 a	47.8±2.7 a	19.0±1.1 a	36.0±2.1 a	19.7±1.3 a

¹⁾ Nitrogen use efficiency, kg N uptake by kg chemical N applied.

²⁾ Nitrogen agronomic efficiency, kg grain increased by per kg chemical N applied.

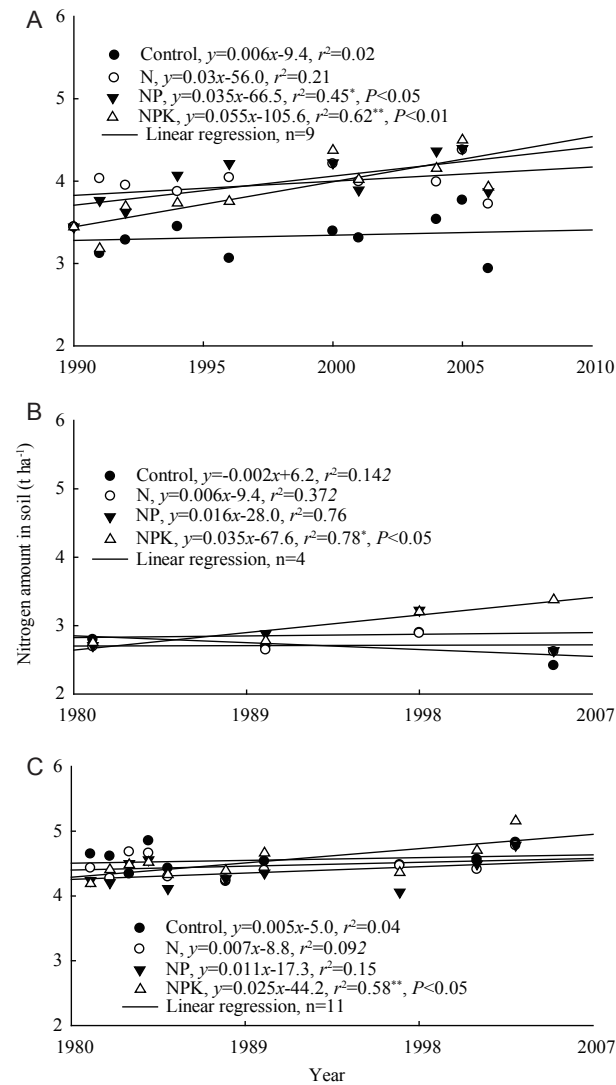


Fig. 3 Variations of soil nitrogen under different fertilizations over the long-term fertilization period at Chongqing (1991-2006, A), Suining (1982-2005, B) and Wuchang (1982-2005, C). * and ** indicate significance at $P=0.05$ and $P=0.01$, respectively.

become limiting to plant growth. Compared with N only fertilization, grain yields of both rice and wheat were significantly increased by NP, indicating the importance of P to improve crop productivity at all three long-term studies (Table 1). However, compared to grain yields in NP plots, those with NPK were not further increased by addition of 50 or 75 kg K ha⁻¹ at all three long-term sites for rice and at Suining for wheat. This weaker role of K compared with P in boosting yields may well be site dependent because of a comparatively low available P but high available K at Suining and Wuchang (Table 4). An increase of soil-exchangeable K during alternating wetting and

drying in rice-wheat rotations may also promote K availability (Timsina and Connor 2001).

Annual NUE under N, NP or NPK was up to 37, 59 or 61% for rice and 22, 36 or 48% for wheat (Table 2), whereas NUE was up to 64% for rice (Chen *et al.* 2010). Based on the results from these three long-term studies, there is great potential for improving NUE in China by concurrent N plus P or NP plus K fertilization. The average NUE of rice-wheat rotations in China is about 28% (Miao *et al.* 2011). Compared with the sole N fertilization over 16 to 20 yr, P addition under NP significantly improved NUE in both rice and wheat at all three sites. Across Asia generally, long-term (15- to 20-yr) P fertilization improved rice yield and NUE while decreasing N loss (Dawe *et al.* 2003). In West Africa, maize NUE were also increased, and N losses were decreased, with the increase of P rate (Fofana *et al.* 2005). However, a further K addition under NPK only improved NUE in wheat at Chongqing and Wuchang, but not at Suining, and has no effect on NUE in rice at all three sites (Table 2). Similarly, a further K addition on top of NP also did not enhance crop yields further in Chinese maize-wheat systems (Wang *et al.* 2010; Duan *et al.* 2011b). Both our and other results do support that crop NUE by the long-term P addition on top of N under the same N rate is significantly improved at all sites and all tested crops. Furthermore, our results in this study did show that a further improvement on crop NUE under a further K addition on top of NP with the same N and P rate is not always true, but might be site and/or crop dependent (e.g., at Chongqing and Wuchang for wheat only).

Fate of fertilized nitrogen

We investigated the fate of fertilizer N by evaluating the apparent N balance (ANB) and N loss to the environment. At these three rice-wheat sites, ANB was 22-223 kg N ha⁻¹ yr⁻¹ (Fig. 4), which were comparable to 24-190 kg N ha⁻¹ yr⁻¹ in a rice-wheat system in Bangladesh (Timsina *et al.* 2006). At these three sites, N losses were 98-222 kg N ha⁻¹ yr⁻¹ and significantly greater decrease of N loss ranked by the order of NP-K>NP>N (Table 3) due to an enhanced plant growth and NUE under NP or NPK (Tables 1 and 2). These N losses to the environment accounted for 29-62% of

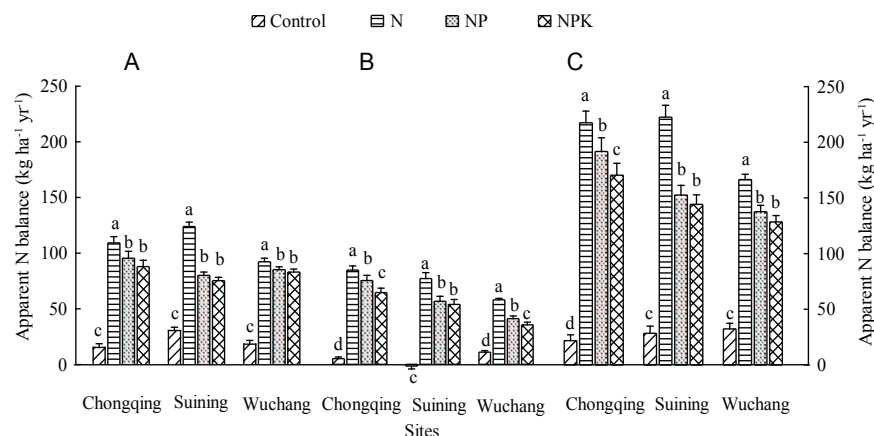


Fig. 4 Mean annual apparent N balance (ANB) over the long-term fertilization period at Chongqing (1991-2006, A), Suining (1982-2005, B) and Wuchang (1982-2005, C).

the total N input, or $\sim 1/3$ - $3/5$ of the total applied N. The major N losses are denitrification during wheat- (Xiong 2002) and ammonia volatilization during rice-growing seasons (Fillery and de Datta 1986; Xu *et al.* 2013).

Table 3 Annual total nitrogen input and nitrogen loss to the environment in the rice and wheat rotation over the long-term experimental period as affected by fertilizations at three sites in subtropical China

Location	Fertilizer treatments ¹⁾	Total N input (kg ha ⁻¹ yr ⁻¹)	N loss to the environment (kg ha ⁻¹ yr ⁻¹)	Ratio of N loss to total N input (%)
Chongqing (1991-2006)	Control (0)	120	10.0 \pm 4.3 d	8.3 \pm 3.6 d
	N (285)	405	204.5 \pm 7.4 a	50.5 \pm 1.8 a
	NP (285)	405	148.7 \pm 8.2 b	36.7 \pm 2.0 b
	NPK (285)	405	117.2 \pm 8.3 c	28.9 \pm 2.0 c
Suining (1982-2005)	Control (0)	120	40.2 \pm 4.2 d	33.5 \pm 3.5 c
	N (240)	360	222.3 \pm 6.3 a	61.8 \pm 1.8 a
	NP (240)	360	150.3 \pm 5.7 b	41.8 \pm 1.6 b
	NPK (240)	360	116.5 \pm 5.3 c	32.3 \pm 1.5 c
Wuchang (1982-2005)	Control (0)	120	25.7 \pm 3.6 d	21.4 \pm 3.0 d
	N (150)	270	158.4 \pm 3.2 a	58.7 \pm 1.2 a
	NP (150)	270	125.1 \pm 3.7 b	46.3 \pm 1.4 b
	NPK (150)	270	98.3 \pm 3.8 c	36.4 \pm 1.4 c

¹⁾ Amounts of annual fertilized N for the rice and wheat seasons are in brackets.

Nutrient management strategies

Best N management strategies are needed to optimize crop growth while concurrently protecting the environment. The apparent N balance and N losses were significantly decreased by the addition of P on top of N only, though with variations at these three sites, suggesting that supplement of P is essential for a sustainable rice-wheat system. Further determination of an optimal P rate is needed to different soils in rice-wheat systems.

The further K addition on top of NP affected rice and wheat yield differently. Both yield and NUE of rice were similar between NP and NPK at all three sites, but yield and NUE of wheat were significantly improved under NPK than under NP at Chongqing and Wuchang. Thus K should be fertilized to wheat, but not to rice. Moreover, N losses were decreased by $\sim 21\%$ under NPK than under NP at all three sites, suggesting a positively environmental benefit of K fertilization on decreasing N losses.

Table 4 Site geography and climate at the three long-term experimental sites in subtropical China

Item	Chongqing	Suining	Wuchang
Latitude, longitude	30°26'N, 106°26'E	30°42'N, 104°6'E	30°24'N, 106°24'E
Altitude (m, above the sea level)	266	225	20
Mean annual accumulated temperature (°C) ¹⁾	5869	5600	5189
Mean annual temperature (°C) ¹⁾	18.3	17.4	16.6
Mean annual frost-free days ¹⁾	330	305	268
Mean annual evaporation (mm) ¹⁾	990	991	1500
Mean annual precipitation (mm) ¹⁾	1105	1000	1269
Mean annual sunshine hours ¹⁾	1250	1300	2080

¹⁾ Averaged data over the whole fertilization period at Chongqing (1991-2006), Suining (1982-2005) and Wuchang (1982-2005).

Nutrient management strategies should also consider geography, climate and/or soil conditions. The further K addition improved the NUE by wheat at Chongqing and Wuchang, but not at Suining, or not by rice at all three sites. We attributed this finding to the lower soil exchangeable K content at Chongqing (85–88 mg kg⁻¹) and Wuchang (99–100 mg kg⁻¹), and higher soil exchangeable K content at Suining (121–131 mg kg⁻¹) during the fertilization period (data not shown). The purple paddy soil at Suining is also rich in K-containing smectite and hydromica minerals (Zhang *et al.* 2010). Therefore, this study does provide valuable information for the nutrient management recommendations in China's rice-wheat system or in other regions for achieving high grain yields and low potential environmental risks.

CONCLUSION

Our study showed that about 60, 40 and 30% fertilized N was lost under the current N, NP and NPK fertilizations, which could have potential risks to increase nitrate leaching and/or ammonia volatilization. The addition of chemical P on top of N only fertilization played an important role in improving both yield and NUE of rice and wheat. A further addition of chemical K on top of NP did enhance the yield and NUE in wheat at Chongqing and Wuchang, but not at Suining or not in rice at any of the three sites. While K fertilizer may not be required for rice at these sites, K may be necessary to wheat, depending on soil conditions. For developing effective nutrient management, strategies these findings may apply to other regions with similar cropping systems, soil and climate conditions.

MATERIALS AND METHODS

Site description

Three long-term fertilization experiments were conducted to investigate how chemical fertilizations could promote yields and NUE of rice and wheat, and soil N accumulation over 16 yr (1991–2006) at Chongqing and 24 yr (1982–2005) at Suining and Wuchang of subtropical China. Their geography and climate during these periods are in Table 4.

Cropping systems

The annual crop rotation was rice followed by wheat at all three sites. The sowing and harvest time was in late May and late August for rice, and in early November and early May for wheat. The rice cultivar was II-You-868 at Chongqing and Suining, and Shanyou 63 at Wuchang. The wheat cultivar was Xinongmai 1 at all three sites. Approximately 10 seeds of wheat or a single rice seedling with 2–3 tillers were planted in a single spot within a row. The density of rice and wheat was about 250 000 seedlings ha⁻¹ with a 24-cm row space and 17 cm between seedlings in the row at three sites.

Fertilizer treatments

The experiment had four fertilization treatments or plots (four replicates each) in a randomized complete block design: 1) no-fertilization (control); 2) sole chemical N (N); 3) N plus P (NP) and 4) NP plus K (NPK) (Table 5). An equivalent amount of chemical N was applied at each site. The respective N, P and K fertilizers were urea, calcium superphosphate and potassium chloride. For N application, 40% was as basal fertilizers prior to seeding, 40% at tillering and 20% at earing for rice, while 50% was at seeding, 25% at returning green, and 25% at jointing stage for wheat. Both P and K were applied before rice transplanting and wheat sowing. The plots, with 80-cm-deep cement baffle separations, were 12 m² (4 m×3 m) at Chongqing and Suining, and 40 m² (8 m×5 m) at Wuchang.

Soil and plant sampling and analysis

Ten random soil cores (5 cm diameter) from each plot (0–20 cm) were annually collected after ~15 d of wheat harvest, air-dried and mixed as one composite sample (0.25 mm sieved for total or 1.0 mm sieved for available nutrient analysis). Grains and straws, not roots, from each plot were oven-dried at 65°C to constant weight.

Analyses of soil organic C were from methods by Walkley and Black (1934), soil total N and available N by Black (1965), total P by Murphy and Riley (1962), available P by Olsen *et al.* (1954), and total K and available K by Kundsén *et al.* (1982). Grain or straw N in H₂SO₄-H₂O₂ digested solution was measured using the micro-Kjeldahl method (Page *et al.* 1982).

Calculation of N input, output, apparent N balance and N accumulation

Total N inputs and outputs were calculated as follows (Timsina *et al.* 2006):

$$\text{Total N inputs (kg ha}^{-1}\text{)} = \text{Fertilizer N} + \text{N}_2\text{-Fixation} + \text{N from rain} + \text{N from irrigation} + \text{N from seeds} \quad (1)$$

Table 5 Chemical fertilizer rates (N-P-K, kg ha⁻¹) over the long-term experiments at Chongqing (1991-2006), Suining (1982-2005) and Wuchang (1982-2005)

Treatment	Chongqing rice/Wheat	Suining rice/Wheat	Wuchang rice/Wheat
No-fertilization (control)	0-0-0/0-0-0	0-0-0/0-0-0	0-0-0/0-0-0
Chemical N (N)	150-0-0/135-0-0	120-0-0/120-0-0	90-0-0/60-0-0
Chemical N and P (NP)	150-60-0/135-60-0	120-26-0/120-26-0	90-20-0/60-13-0
Chemical N, P and K (NPK)	150-60-60/135-60-60	120-26-50/120-26-50	90-20-75/60-13-50

Where, the amounts of fertilizer N were given in Table 5; N₂-fixation was 57.5 and 15.0 kg ha⁻¹ during the rice and wheat season, respectively (Zhu 1992; Shi 2003); Nitrogen from rain, irrigation and seeds was 15.6, 6.0 and 4.5 kg ha⁻¹ during the rice season, and 13.4, 0 (no irrigation) and 7.9 kg ha⁻¹ during the wheat season (Fan *et al.* 2007). The total N from the environment (NFE) was thus 120 kg N ha⁻¹ yr⁻¹ in these three rice-wheat sites.

Total N outputs (kg ha⁻¹)=N in grains+N in straw (2)

The amount of N input minus N output or apparent N balance (ANB) signifies the amount of N that is not used by crops (Yadav *et al.* 2002):

ANB (kg ha⁻¹)=Total N input-Total N output (3)

Nitrogen loss (kg ha⁻¹)
=ANB-N increased in the 0-20 cm depth soil (4)

The annual N increased (kg ha⁻¹ yr⁻¹) was determined using the linear regression:

$y=ax+b$ (5)

Where, y is the N amount in soil; x is the year; a is the slope (N increased in the 0-20-cm depth soil).

The annual N amount in the 0-20 cm depth soil (kg ha⁻¹ yr⁻¹)=Bulk density (kg m⁻³)× Total soil N (g kg⁻¹ yr⁻¹)×0.2 m×10⁴ m² ha⁻¹× 1.0 kg 1000 g⁻¹ (6)

Soil bulk density varied between 1090 and 1390 kg m⁻³ with treatments, sites and years.

Nitrogen use efficiency and agronomy efficiency

Nitrogen use efficiency (NUE) is the increased N in aboveground biomass (grain plus straw) between N and no-fertilization divided by total N applied (Duan *et al.* 2011b):

$NUE=(U_N-U_0)/A_N \times 100\%$ (7)

Where, U_N or U_0 is total N accumulation in aboveground biomass (grain and straw) under a specific N or no-fertilization, and A_N is the total applied N from the N fertilization. By definition, hereafter in this study NUE represents an approximate estimation of the ability of a plant to take up fertilized N from the soil.

Nitrogen agronomic efficiency (NAE) is the increased grain yield between N and no-fertilization divided by the total applied N (Duan *et al.* 2011b):

$NAE(\text{kg grain kg}^{-1} \text{N})=(Y_N-Y_0)/A_N \times 100\%$ (8)

Where, Y_N or Y_0 is the grain yield under a specific N or no-fertilization, and A_N is the total N from the N fertilization. By definition, hereafter in this study NAE

represents an approximate estimation of the ability of a plant to use fertilized N to produce grain yield.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Treatment means of grain yield, N accumulation, NUE and NAE were compared by Duncan's least significant difference (LSD) test at $P=0.05$. Regressions were also performed for the relationships between grain yields and N accumulations by crops or annual change rate of total N concentration in soil. All statistical analyses were performed with SPSS for Windows 11.0.1 (SPSS 2001, Chicago, USA).

Acknowledgements

We acknowledge all colleagues at the three long-term fertilization sites for their unremitting assistances. We thank Prof. Warren Dick of the Ohio State University, USA for his critical review and English editing to this manuscript. Financial supports are from the National Natural Science Foundation of China (41001175), the National Basic Research Program of China (2011CB100501) and the Special Basic Research Fund for Public Institutes in China (202-2).

References

- Black C A. 1965. *Methods of Soil Analysis. Part 2.* American Statistical Association, Madison, WI.
- Chen Y, Tang X, Yang S M, Wu C Y, Wang J Y. 2010. Contributions of different N sources to crop N nutrition in a Chinese rice field. *Pedosphere*, **20**, 198-208.
- Dawe D, Dobermann A, Ladha J K, Yadav R L, Bao L, Gupta R K, Lal P, Panauallah G, Sariam O, Singh Y, *et al.* 2003. Do organic amendments improve yield trends and profitability in intensive rice systems? *Field Crops Research*, **83**, 191-213.
- Duan Y H, Xu M G, He X H, Li S L, Sun X F. 2011a. Long-term pig manure application reduces the requirement for chemical phosphorus and potassium in two rice-wheat sites in subtropical China. *Soil Use and Management*, **27**, 427-436.
- Duan Y H, Xu M G, Yang X Y, Huang S M, Wang B R, Gao S D. 2011b. Long-term evaluation of manure application on maize yield and nitrogen use efficiency in China. *Soil Science Society of America Journal*, **75**, 1562-1573.
- Duan Y H, Zhang Y L, Ye L T, Fan X R, Xu G H, Shen

- Q R. 2007. Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. *Annals of Botany*, **99**, 1153-1160.
- Fan M S, Lu S H, Jiang R F, Liu X J, Zeng X Z, Goulding K W T, Zhang F S. 2007. Nitrogen input, ¹⁵N balance and mineral N dynamics in a rice-wheat rotation in southwest China. *Nutrient Cycling in Agroecosystems*, **79**, 255-265.
- Fillery I R P, de Datta S K. 1986. Ammonia volatilization from nitrogen sources applied to rice fields. I. Methodology, ammonia fluxes, and ¹⁵N loss. *Soil Science Society of America Journal*, **50**, 80-86.
- Fofana B, Tamélokpo A, Wopereis M C S, Breman H, Dzotsi K, Carsky R J. 2005. Nitrogen use efficiency by maize as affected by a mucuna short fallow and P application in the coastal savanna of West Africa. *Nutrient Cycling in Agroecosystems*, **71**, 227-237.
- Good A G, Shrawat A K, Muench D G. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Advances in Soil Sciences*, **9**, 597-605.
- Kundsen D, Peterson G A, Pratt P F, Page A L. 1982. Lithium, sodium, and potassium. In: *Methods of Soil Analysis. Part 2*. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI, USA.
- Kundu D K, Ladha J K. 1999. Sustaining productivity of lowland rice soils: Issues and options related to N availability. *Nutrient Cycling in Agroecosystems*, **53**, 19-33.
- Li D Q, Tang Q Y, Zhang Y B, Qin J Q, Li H, Chen L J, Yang S H, Zou Y B, Peng S B. 2012. Effect of nitrogen regimes on grain yield, nitrogen utilization, radiation use efficiency, and sheath blight disease intensity in super hybrid rice. *Journal of Integrative Agriculture*, **11**, 134-143.
- Miao Y X, Stewart B A, Zhang F S. 2011. Long-term experiments for sustainable nutrient management in China. A review. *Agronomy for Sustainable Development*, **31**, 397-414.
- Meng H Q, Xu M G, Lü J N, He X H, Li J W, Shi X J, Peng C, Wang B R, Zhang H M. 2013. Soil pH dynamics and nitrogen transformations under long-term chemical fertilization in four typical Chinese croplands. *Journal of Integrative Agriculture*, **12**, 2092-2102.
- Murphy J, Riley J P. 1962. A modified single solution method for the determination of phosphate in nature water. *Analytica Chimica Acta*, **27**, 31-36.
- National Bureau of Statistics of China. 2011. *China Statistical Yearbook*. China Statistics Press, Beijing, China. (in Chinese)
- Olsen S R, Cole C V, Watanabe F S, Dean A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. In: *United States Department of Agriculture Circ.* 939. U.S. Government. Print Office, Washington, DC.
- Page A L, Millar R H, Keeney D R. 1982. *Methods of Soil Analysis, Part 2*. ASA and SSSA, Madison, WI, USA. pp. 149-157.
- Shi X J. 2003. Nutrient cycling in rice-upland crop rotation systems. Ph D thesis, China Agricultural University, China. (in Chinese)
- Timsina J, Connor D J. 2001. Productivity and management of rice-wheat cropping systems: Issues and challenges. *Field Crops Research*, **69**, 93-132.
- Timsina J, Panaullah G M, Saleque M A, Ishaque M, Pathan A B M B U, Quayyum M A, Connor D J, Sana P K, Humphreys E, Meisner C A. 2006. Nutrient uptake and apparent balances for rice-wheat sequences. I. Nitrogen. *Journal of Plant Nutrition*, **29**, 137-155.
- Vitousek P M, Naylor R, Crews T, David M B, Drinkwater L E, Holland E, Johnes P J, Katzenberger J, Martinelli L A, Nziguheba F, *et al.* 2009. Nutrient imbalances in agricultural development. *Science*, **324**, 1519-1560.
- Walkley A, Black I A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, **37**, 29-38.
- Wang Y C, Wang E L, Wang D L, Huang S M, Ma Y B, Smith C J, Wang L G. 2010. Crop productivity and nutrient use efficiency as affected by long-term fertilisation in North China Plain. *Nutrient Cycling in Agroecosystems*, **86**, 105-119.
- Xiong Z Q. 2002. The fate of nitrogen fertilizer and effect on environment in rice-wheat rotation. PhD thesis, Institute of Soil Science, Chinese Academy of Sciences, China. (in Chinese)
- Xu M G, Li D C, Li J M, Hosen Y, Cong R H, He X H. 2013. Polyolefin-coated urea decreases ammonia volatilization in a double rice system of southern China. *Agronomy Journal*, **105**, 1-8.
- Yadav R L, Tomar S S, Sharma U C. 2002. Output: input ratios and apparent balances of N, P and K inputs in a rice-wheat system in North-West India. *Experimental Agriculture*, **38**, 457-468.
- Yadvinder S, Gupta R K, Gurpreet S, Jagmohan S, Sidhu H S, Bijay S. 2009. Nitrogen and residue management effects on agronomic productivity and nitrogen use efficiency in rice-wheat system in Indian Punjab. *Nutrient Cycling in Agroecosystems*, **84**, 141-154.
- Zhang H M, Xu M G, Shi X J, Li Z Z, Huang Q H, Wang X J. 2010. Rice yield, potassium uptake and apparent balance under long-term fertilization in rice-based cropping systems in southern China. *Nutrient Cycling in Agroecosystems*, **88**, 341-349.
- Zhu Z L. 1992. Management and fate of fertilizer nitrogen in agroecosystem. In: Zhu Z L, Wen Q X, eds., *Soil Nitrogen of China*. Nanjing Scientific Press, China. pp. 213-219. (in Chinese)

(Managing editor SUN Lu-juan)