



Interaction of Nitrogen and Phosphorus on Wheat Yield, N Use Efficiency and Soil Nitrate Nitrogen Distribution in the North China Plain

Yulu Chen^{1,2} · Panpan Zhang^{1,2} · Lifang Wang^{1,2,3} · Geng Ma^{1,2} · Zhuo Li^{1,3} · Chenyang Wang^{1,2,3}

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Abstract

To establish the optimal fertilization rate to maintain high wheat yield and low environmental risk in the North China Plain, a two-year field experiment (2016–2018) was conducted with six combinations of two N rates (N0; N1: 240 kg ha⁻¹) and three P rates (P0; P1: 150 kg ha⁻¹; P2: 240 kg ha⁻¹) to investigate the effects of these regimes on wheat grain yield, N assimilatory enzyme activity (nitrate reductase, NR and glutamine synthetase, GS), N use efficiency (NUE) and soil nitrate nitrogen distribution. Results showed that N application increased grain yield and grain protein concentration (GPC) significantly, in association with increased residual soil nitrate in both growing seasons, across the three P application rates. Only P application had no effect on these parameters. Significant interactions of N and P fertilizer were found on most of the tested parameters. Highest grain yield, GPC and NUE, and the lowest residual soil nitrate were observed under the treatment of N1P1. N accumulation in plant was highly correlated with grain yield and NUE, and N assimilatory enzyme activity at flowering was highly correlated with grain yield and GPC, which contributed to achieving high yield and NUE simultaneously. Regression analysis showed that the response of grain yield, NUE and residual soil nitrate to P rates fitted quadratic model, in which the maximum values of grain yield, NUE and the lowest residual soil nitrate were obtained at the extent of P rate 140~150 kg P ha⁻¹. Hence, we recommended fertilizer rate of N 240 kg ha⁻¹ and P 150 kg ha⁻¹ as the optimal nitrogen-phosphorus regime in the North China Plain, which can maintain higher crop yield while lower environmental risk.

Keywords Nitrogen · Phosphorus · Grain yield · Nitrogen use efficiency · Soil nitrate nitrogen

Introduction

Nitrogen (N) is an essential crop nutrient, affecting productivity and N use efficiency (NUE) in wheat (*Triticum aestivum* L.). Many studies have shown that N fertilizer has significant efficacy in increasing yield. In recent years,

nitrogen fertilizer is widely used in wheat production and contributes to increasing yield, even formed a concept that adding more N fertilizer can increase production (Grant et al. 2012; Liu et al. 2013; Duan et al. 2019). For instance, in the wheat planting area of the North China Plain, the N application rate of 325 kg ha⁻¹ exceeded the recommended optimum nitrogen application rate (120–180 kg ha⁻¹), resulting in a lower yield (5800 kg ha⁻¹) (Cui et al. 2008). Wang

Yulu Chen and Panpan Zhang contributed equally to this work.

✉ Chenyang Wang
xmzxwang@163.com

Yulu Chen
yuluchenlz@163.com

Panpan Zhang
zhangpan1602@163.com

Lifang Wang
wanglifang605@126.com

Geng Ma
nymg5135@163.com

Zhuo Li
lizhuo2018@126.com

¹ College of Agronomy, Henan Agricultural University, #15 Longzi Lake University District, Zhengdong New District, Zhengzhou, Henan 450046, People's Republic of China

² National Engineering Research Centre for Wheat, Henan Agricultural University, Zhengzhou 450046, People's Republic of China

³ National Key Laboratory of Wheat and Maize Crop Science, Zhengzhou 450046, People's Republic of China

et al. (2017) proposed a nitrogen fertilizer application rate of 185 kg ha^{-1} as the boundary for achieving high yields. Our previous study recommended that the application rate of 240 kg N ha^{-1} contribute to maintain high yield (Liu et al. 2018). But these studies are mainly focused on obtaining high yields. When soil nitrogen supply exceeds plant nitrogen requirements, which causes many environmental problems, such as surface water pollution, soil acidification, because of residual soil N leaching (Yang et al. 2017; Tedone et al. 2018), and an increase in N fertilizer input could significant increase crop yield but inevitably decrease NUE (Srivastava et al. 2018). The synergistic increase in both yield and NUE will be of economic benefit to farmers and reduce environmental contamination. In order to achieve both high yield, high NUE and ecological environment protection, agronomists proposed integrated soil-crop system management to coordinate nitrogen (N), phosphorous (P) and potassium (K) fertilizer applications for high yield and NUE (Chen et al. 2015). Therefore, the next study should focus more on optimizing fertilizer amounts and improving NUE to alleviate hazards to the environment on basis of achieving high yield.

In addition to N fertilizer, phosphorous (P) is an important source of nutrients for crop production, and is essential for the metabolism of carbohydrates (Quartin et al. 2007; Schlegel and Havlin 2017). Application of P fertilizer is beneficial to the glycolysis of photosynthetic products to synthesize phosphoenolpyruvate, enter the tricarboxylic acid cycle and synthesize ATP (Wang et al. 2009). High concentration of ATP promoted the absorption, assimilation and transport of nitrogen in wheat, ultimately promoted the accumulation of protein in the grain (May et al. 2008). P application can increase the activity of nitrate reductase (NR) and glutamine synthetase (GS) in wheat during anthesis, accelerate the movement of N from vegetative organs to reproductive organs, facilitating the redistribution and reuse of nutrients, resulting in improved grain protein concentration (GPC) (Schindler 1977; Stitt et al. 2002). P deficiency affects wheat tillering and reduces the number of ears per unit area, ultimately leading to a reduction in wheat productivity (Sweeney et al. 2008; Sandaña and Pinochet 2014). Studies have shown that P fertilizer can promote the uptake of fertilizer N and soil N by wheat, increase the utilization efficiency of fertilizer N in wheat (Kaiser et al. 2010). In crop production, combined application of fertilizer contributes to balance soil nutrients, improve soil structure and is more beneficial for environmental protection (Reganold 1995). Previous studies have shown that N is applied in combination with P increased nutrient uptake by stimulating root growth and reducing soil nitrate nitrogen accumulation (Hao et al. 2005; Wen et al. 2016). However, the physiological mechanism of N combined P fertilizer to high yield, high NUE and ecological environment protection of wheat have

seldom been studied. Therefore, optimize fertilizer management has been increasing attention.

Wheat is one of the world's three major food crops, and plays a pivotal role in food production and security (Menegat and Nilsson 2019). High yields and stable wheat production will be necessary to feed the growing population of China on the limited cropland in the coming decades, especially in the Huang-huai Plain, which is one of the most important food production areas in China (Ma et al. 2013; Zhang et al. 2013). In addition, GPC is one of the most important quality traits of wheat, especially in regions where dietary protein intake is primarily dependent on the protein content in the wheat grain (Ma et al. 2019a). Low availability of P in the soil, combined with the application of inappropriate rates of N fertilizer in crop production, leads to soil nutrient imbalance and limits the role of N and P in carbohydrate and nitrogen metabolism. In turn, this not only reduces wheat GY and GPC, but also causes serious environmental pollution (Asner et al. 2001; He et al. 2002). In the current study, therefore, we initiated a fixed-site experiment from 2010 using different combinations of N and P application rates to determine optimal N–P proportions in terms of optimizing GY, increasing NUE and reducing nitrate nitrogen accumulation in soils.

Materials and Methods

Experimental Site and Weather Conditions

A fixed-site field experiment was conducted at Kaifeng Experimental Farm ($34^{\circ} 41' \text{ N}$, $114^{\circ} 49' \text{ E}$) from 2010 with a wheat–maize rotation system in Henan Province, China. The fertilizer rate in each experimental plot was same across all years. Samples of soil and plant were collected in wheat growing seasons of 2016–2017 and 2017–2018. Figure 1 showed the climatic conditions during the 2016–2018 wheat growing seasons. The mean temperatures were 14.2° C and 12.2° C during the wheat growing seasons 2016–2017 and 2017–2018, respectively and the rainfall values were 194.5 mm and 151.8 mm , respectively. The soil type of Kaifeng is clay soil, and other properties of the plow layer soil are shown in Table 1.

'Yumai 49–198', a high-yielding winter wheat cultivar grown in the Huang-Huai wheat production region of China, was used in this study. Summer maize was grown prior to winter wheat, and all maize straw was returned to the soil before tillage in both years. The experimental design consisted of three completely randomized blocks with a split-plot design. The main plots were given to N rate with two levels: no nitrogen (N0) and $240 \text{ (N1) kg N ha}^{-1}$, and the subplots were assigned to P rate with three levels: 0 (P0), $150 \text{ (P1) kg P ha}^{-1}$, $240 \text{ (P2) kg P ha}^{-1}$. The area of each

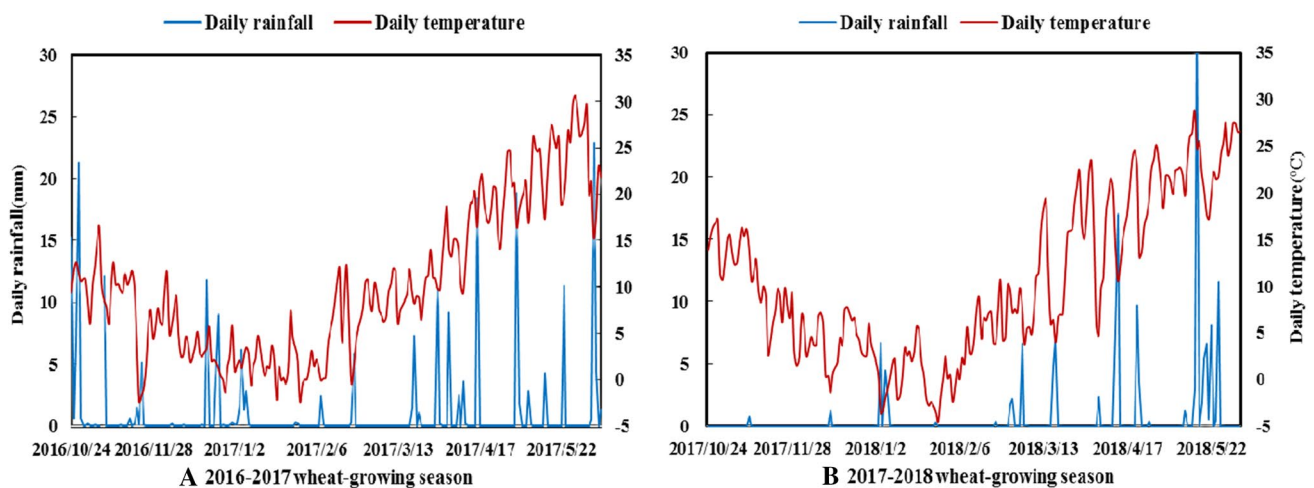


Fig. 1 Rainfall and temperature during wheat growing seasons in 2016–2017 and 2017–2018 at the experimental site

Table 1 Soil nutrient contents in 0–20 cm soil layer of experiment field before sowing

Growing season	PH	Total nitrogen (g/kg)	Alkali-hydrolyzable nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Organic matter (g/kg)
2016–2017	8.31	1.11	97.28	12.62	162.18	14.59
2017–2018	8.39	1.16	99.47	13.49	164.37	15.62

Experimental design and fertilizer treatment

subplot was 54 m² (6 m × 9 m). Nitrogen was applied as urea (46%) and phosphorus (P) as calcium superphosphate (15%, P₂O₅). Half of the total amount of N and all P fertilizer were spread before plowing at sowing. Additional half of N was applied at wheat jointing stage in plots of N240 treatment. Potassium (K) was applied as potassium chloride (60%) at rate of 120 kg ha⁻¹ (K₂O) in each plot. All plots were irrigated at jointing and flowing stages with 75 mm each time as measured with a water meter. The management practices for controlling pests, diseases, and weeds complied with local practices for high-yield wheat production.

Sampling and Analysis

At maturity, plants from a 9 m² area (3 m × 3 m) in the middle of each plot were harvested. The grain was weighed after air-drying. Grain nitrogen was measured using a nitrogen analyzer (Kjeltec 2300, FOSS, Visby, Sweden) according to the ICC Standard Method 105/2, and grain protein was calculated by multiplying the grain nitrogen by 5.7. Soil samples were collected over 0–100 cm soil depth at 20-cm intervals and stored in the 4 °C refrigerator before sowing and after harvest. For determination of soil nitrate content, fresh soil samples (5 g) were extracted by suspension in 50 ml 1 M KCl and shaken for 1 h, after filtration, the

extracts were immediately measured (Li et al. 2012), using a high-resolution continuous-flow analyzer AutoAnalyzer3 (AA3; SEAL Company, Norderstedt, Germany).

The soil nitrate accumulation (SNA; kg N ha⁻¹) in each soil layer was calculated as follows:

$$\text{SNA} = \text{CN} \times \text{BD} \times \text{SD} \times 0.1 \quad (1)$$

SD indicates the thickness of the soil layer (cm), BD is the soil bulk density (g cm⁻³), CN is the soil NO₃⁻-N concentration (mg kg⁻¹) of the corresponding layer, and 0.1 is the conversion coefficient.

NUE was defined as grain yield per unit of N available (from the soil and/or fertilizer) and divided it into two components: (i) N-uptake efficiency (UPE): total above-ground N (kg) at harvest per kilogram available N (from soil, initial soil N, plus fertilizer, N_{fer}). (ii) N-utilization efficiency (UTE): grain dry mass (kg) per kilogram (above-ground N) at harvest (Moll et al. 1982).

$$\text{UPE (kg kg}^{-1}\text{)} = \text{AGN}/(\text{N}_{\text{fer}} + \text{initial soil N}) \quad (2)$$

$$\text{UTE (kg ha}^{-1}\text{)} = \text{Grain yield}/\text{AGN} \quad (3)$$

here, initial soil N is SNA within the top 100 cm of the soil profile before sowing; AGN represents N accumulation in

the above-ground biomass at harvest; and N_{fer} is the N application rate in $kg\ ha^{-1}$.

The enzyme activities in the flag leaf were determined following extraction from sampled plants at 7-day intervals from 0 to 28 day after anthesis (DAA). Enzymes were extracted from leaves stored at $-80\ ^\circ C$ and these operations were performed at $4\ ^\circ C$. Nitrate reductase (NR) was expressed on a fresh weight (FW) basis, according to a protocol previously described (Ferrario-Méry et al. 1998), and determination of glutamine synthetase (GS) activity was expressed on a FW basis, using a protocol previously described by O'Neal and Joy (1973).

Statistical Analysis

Two-way analysis of variance (ANOVA) was carried out using SPSS 17.0 software (IBM, Armonk, NY, USA), with multiple pairwise comparisons using Duncan's multiple

range tests at $\alpha = 0.05$ to determine significant differences among treatments. All figures were generated with Origin 9.0 (OriginLab, Northampton, MA, USA), and the standard errors of the means were calculated and presented in the graphs as error bars. The relationships between Soil N_{end} , GY, GPC, NUE and P application rate were analyzed using regression or stepwise regression analyses to determine the best-fit equations. Pearson's correlation coefficients were used to analyze the relationships among GY, GPC, enzyme activity (NR and GS), Soil N_{end} and NUE.

Results

Grain Yield and Yield Components

Two-way ANOVA showed that N, P fertilizer and their interaction significantly influenced the grain yield and yield components ($P < 0.05$). (Table 2). Compared with the

Table 2 Effects of nitrogen and phosphorus regimes on grain yield and its components in each growing season

Growing season	Treatment	Ear no. ($10^4\ ha^{-1}$)	Kernel no. (no. ear $^{-1}$)	10^3 -grain weight (g)	Grain yield ($kg\ ha^{-1}$)
2016–2017	N0				
	P0	359.2 ^d	28.7 ^{ab}	46.1 ^a	3628.1 ^c
	P1	370.1 ^c	27.4 ^b	44.6 ^b	3603.8 ^c
	P2	365.4 ^{cd}	28.1 ^{ab}	44.2 ^b	3572.5 ^c
	N1				
	P0	604.5 ^c	29.3 ^a	42.1 ^b	6374.5 ^c
	P1	694.6 ^a	31.2 ^a	43.5 ^a	7441.3 ^a
	P2	653.3 ^b	30.5 ^a	43.1 ^a	6921.7 ^b
	2017–2018	N0			
P0		319.5 ^d	22.1 ^b	45.3 ^a	3113.1 ^c
P1		327.5 ^c	23.5 ^{ab}	44.4 ^b	3205.3 ^c
P2		323.1 ^c	21.3 ^b	43.9 ^b	2998.8 ^c
N1					
P0		529.9 ^c	24.9 ^a	41.7 ^b	5147.4 ^c
P1		631.3 ^a	26.6 ^a	43.0 ^a	6323.4 ^a
P2		598.8 ^b	23.1 ^{ab}	42.4 ^a	5830.1 ^b
Interactions					
	Nitrogen (N)	***	**	**	***
	Phosphorus (P)	**	*	*	*
	Year (Y)	**	***	ns	**
	N*P	**	*	**	*
	N*Y	*	ns	ns	**
	P*Y	ns	**	ns	ns
	N*P*Y	ns	ns	ns	ns

Means with similar letters have not significant different at 5% probability based on LSD test within each growing season

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

***Significant at the 0.001 probability level

N0, N1 increased grain yield by 98.2% and 83.1%, across the three P treatments in 2016–2017 and 2017–2018, respectively. Correspondingly, it increased ear number by 78.4% and 81.4%, respectively. In the absence of N fertilizer (N0), any P application had no significant effect on grain yield, while under N1 treatment, the highest grain yield of 7441.3 kg ha⁻¹ and 6323.4 kg ha⁻¹ were obtained in P1 treatment in the growing seasons of 2016–2017 and 2017–2018, respectively. The highest ear number was obtained under N1P1 treatment, which was 694.6 and 631.3 in 2016–2017 and 2017–2018, respectively. In the absence of N application, P fertilizer significantly reduced kernel weight, however it increased kernel weight significantly under N1 treatment. The result also showed that year had significant effect on grain yield and its components except the kernel weight ($P < 0.01$). The grain yield in 2016–2017 was 15.6% higher than that in 2017–2018. The yield gap between two years was attributed to weather conditions (Fig. 1). In 2017–2018 wheat growing season, less rainfall and uneven distribution pattern led to relatively lower grain yield, mainly caused by the decrease of ear number (11.7%).

Nitrate Reductase and Glutamine Synthetase Activities

Changes in NR and GS activities in the flag leaves after anthesis were shown in Fig. 2. The results showed that the activity of NR and GS reached the maximum at 7 days after anthesis and flowering, respectively, and then gradually decreased in both growing seasons. N application caused an increase in NR and GS activities from 0 to 28 days after anthesis in both growing seasons. Significant interaction of N and P was found in NR and GS activities ($P < 0.01$) (Table 3). Under the N0 treatment, increased P application decreased NR and GS activities, with a significant difference between P0 and P1 treatments, but no significant difference between P1 and P2 treatments. In the N1 treatment, P application significantly increased NR and GS activities, but the increase was not significant when the application exceeded 150 kg ha⁻¹. In 2017–2018 wheat growing season, the NR and GS activities were lower than those in 2016–2017, with an increase by 15.4% and 10.9%, respectively.

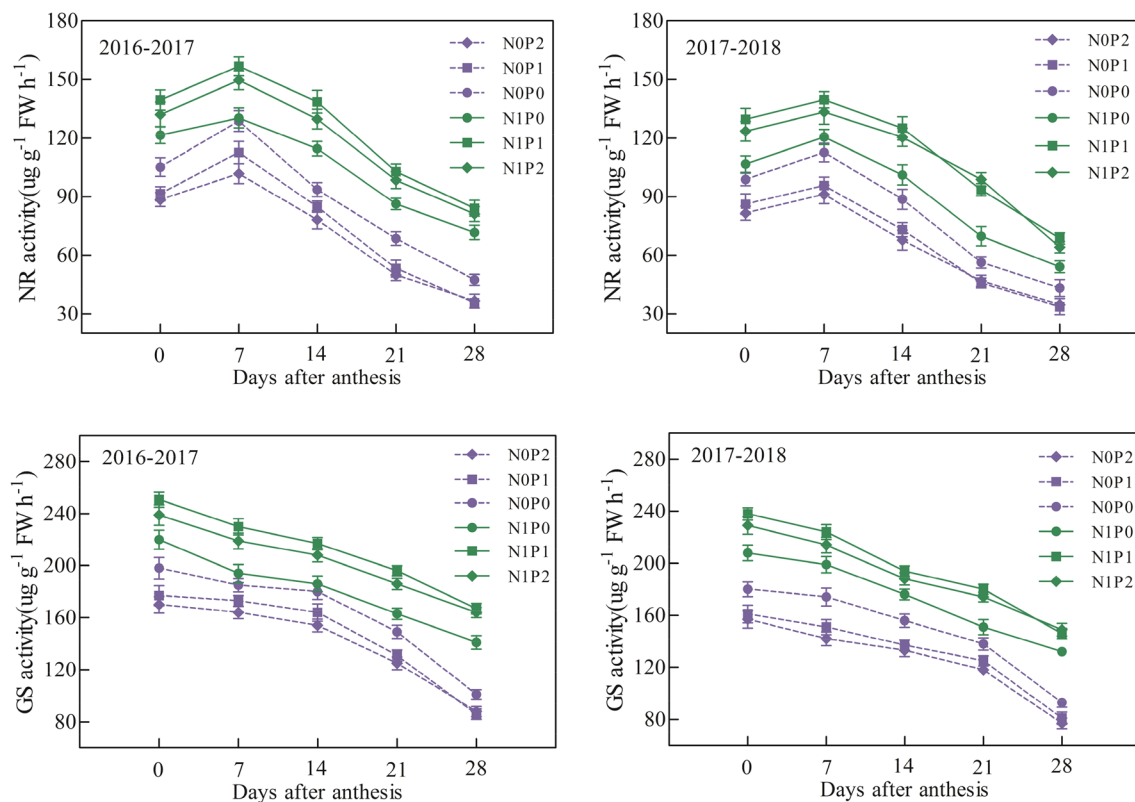


Fig. 2 Effects of nitrogen and phosphorus regimes on nitrate reductase (NR) and glutamine synthetase (GS) activities of flag leaves in each growing season. Data are means \pm SD. Different lowercase letters represent significant differences between different treatments ($P < 0.05$)

Table 3 Variance analysis of N assimilatory enzyme activity (NR and GS), above-ground N accumulation (AGN), grain protein concentration (GPC), residual soil N (Soil N_{end}), nitrogen-uptake efficiency

(UPE) and nitrogen-utilization efficiency (UTE) in winter wheat according to growing season, nitrogen and phosphorus application

Factors	NR	GS	AGN	GPC	Soil N _{end}	UPE	UTE
Nitrogen (N)	**	**	***	***	***		
Phosphorus (P)	*	*	*	**	*	**	*
Year (Y)	**	*	**	*	**	**	**
N*P	**	***	**	**	**		
N*Y	ns	ns	ns	**	**		
P*Y	ns	ns	ns	*	ns	*	ns
N*P*Y	*	ns	ns	*	*		

*Significant at the 0.05 probability level

**Significant at the 0.01 probability level

***Significant at the 0.001 probability level

Nitrogen Accumulation and Nitrogen Use Efficiency (UPE and UTE)

Analysis of variance showed that above-ground N accumulation (AGN) and grain protein concentration (GPC) were significantly affected by N, P and their interaction ($P < 0.05$) (Table 3). Compared to N0, N1 increased AGN and GPC by an average of 144.8% and 29.9%, respectively, in 2016–2017, and by 147.9% and 33.6%, respectively in 2017–2018 (Table 4). In the absence of N fertilizer, P application had no significant effect on AGN and GPC in both growing seasons except for effect on AGN in 2016–2017. While under N1 treatment, P application significantly increased AGN and GPC. The maximum values of GPC were obtained in the presence of the P1 application in both growing seasons, with an increase of 8.1% and 21.8% in 2016–2017 and 2017–2018, respectively, compared with P0. Meanwhile, the highest AGN were obtained under the P1 treatment, with an increase in of 11.7% and 13.7%, compared with P0, in 2016–2017 and 2017–2018 growing seasons, respectively, but there is no significant difference when the P rate was increased from 150 to 240 kg ha⁻¹. Under N1 treatment, compared with the P0, P1 application resulted in a significant increase in UPE of 16.9% and 22.2% in 2016–2017 and 2017–2018, respectively, with a corresponding increase in UTE of 10.4% and 8.5%, respectively. When P application rate exceeds 150 to reach 240 kg ha⁻¹, both UPE and UTE showed a decreasing trend, but UPE did not reach a significant level ($P < 0.05$).

Accumulation and Distribution of Soil Nitrate Nitrogen (NO₃⁻-N) in Soil Profiles

The residual soil N (Soil N_{end}) was significantly affected by N, P and their interaction ($P < 0.05$) (Table 3). N1 treatment

Table 4 Effects of nitrogen and phosphorus regimes on above-ground N accumulation (AGN), grain protein concentration (GPC), nitrogen-uptake efficiency (UPE) and nitrogen-utilization efficiency (UTE) in each growing season

Growing season	Treatment	AGN (kg ha ⁻¹)	GPC (%)	UPE (kg kg ⁻¹)	UTE (kg kg ⁻¹)	
2016–2017	N0					
	P0	103.9 ^c	11.4 ^c	/	/	
	P1	99.5 ^c	10.7 ^c	/	/	
	P2	100.1 ^c	10.5 ^c	/	/	
	N1					
	P0	236.6 ^b	13.6 ^b	0.53 ^c	26.9 ^c	
	P1	250.9 ^a	14.7 ^a	0.62 ^a	29.7 ^a	
	P2	255.2 ^a	14.2 ^{ab}	0.59 ^a	27.1 ^b	
	2017–2018	N0				
		P0	92.8 ^c	11.6 ^{bc}	/	/
P1		91.3 ^c	10.9 ^c	/	/	
P2		85.8 ^d	11.0 ^c	/	/	
N1						
P0		207.9 ^c	13.3 ^c	0.45 ^c	24.8 ^c	
P1		234.9 ^a	16.2 ^a	0.55 ^a	26.9 ^a	
P2		226.6 ^b	15.4 ^b	0.51 ^a	25.7 ^b	

Means with similar letters have not significant different at 5% probability based on LSD test within each growing season. UPE and UTE have no effective values without nitrogen treatment

significant increased Soil N_{end}, the average value in two years was 4.5 times higher than that of N0, across the three P treatments (Fig. 3). Under N0 treatment, P application had no significant effect on Soil N_{end}. When applied P fertilizer was 0, 150 and 240 kg ha⁻¹, the corresponding Soil N_{end} was 30.1, 31.4 and 31.6 kg ha⁻¹ respectively, across all experimental seasons. However, under N1 treatment, P application caused a significant decrease in Soil N_{end}, the application of P was 0, 150 and 240 kg ha⁻¹, the corresponding Soil

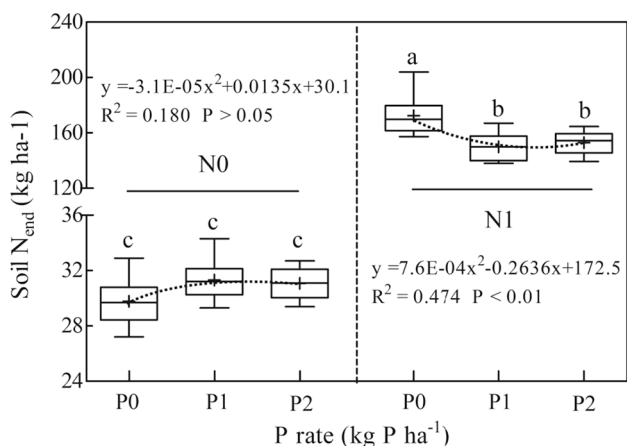


Fig. 3 Box plots for the effect of P regime on residual soil nitrate (Soil N_{end}) after harvest under N0, N1 treatments, and the correlation between P application rate and Soil N_{end}. The “+” inside of the boxes represent the mean values of all data

N_{end} was 172.5, 150.1 and 154.3 kg ha⁻¹, when P fertilizer applied was increased from 150 to 240 kg ha⁻¹, the Soil N_{end} showed a slight increase, but did not reach a significant level (P < 0.05). Significant interaction of N and year was also found on Soil N_{end} (P < 0.01) (Table 3). Under N0 treatment, the Soil N_{end} (0–100 cm) in 2016–2017 was 9.0% higher than that in 2017–2018, however, under N1 treatment, higher Soil N_{end} was obtained in 2017–2018, with an increase by 12.6% compared to 2016–2017 (Fig. 4). In a view of N distribution, under N0 treatment, the peak value of Soil N_{end} was observed in the surface soil layer (0–20 cm) in both seasons, while under N1 treatment, it was observed

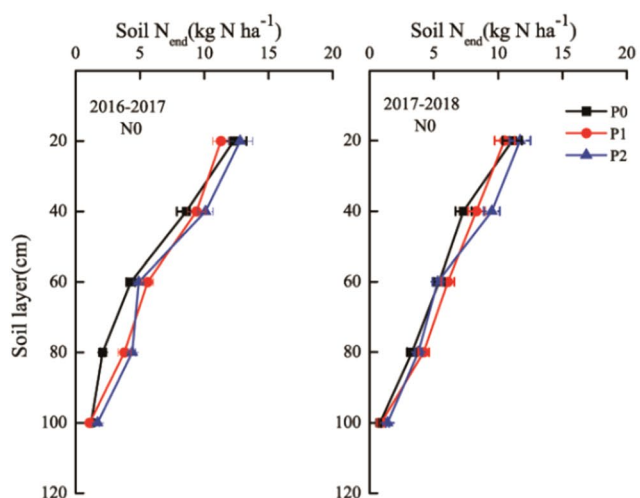


Fig. 4 Effects of nitrogen and phosphorus regimes on nitrate distribution in the 0–100 cm soil layers at maturity. Asterisk represents a significant difference for one nitrogen treatment at a particular sampling

in the layer of 60–80 cm and 80–100 cm in 2016–2017 and 2017–2018 growing seasons, respectively.

Correlation Analysis and Relationships Between P Rate and GY, GPC, Soil N_{end} and N Use Efficiency (UPE and UTE)

Correlation analysis (Table 5) showed that AGN was positively correlated with GY (r = 0.93**, P < 0.01), UPE (r = 0.89**, P < 0.01) and UTE (r = 0.88**, P < 0.01). N metabolism enzyme activities (NR and GS) were positively correlated with GPC (r = 0.80**, P < 0.01; and r = 0.83**, P < 0.01, respectively). In addition, the Soil N_{end} was negatively correlated with UPE (r = -0.71**, P < 0.01) and UTE (r = -0.69**, P < 0.01).

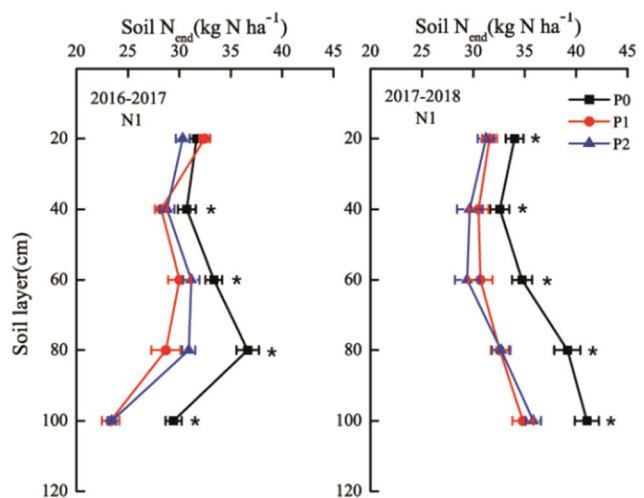
GY and GPC showed a quadratic curve relationship with the P application rate (p < 0.01), both first increased and then slowly reached saturation, around P rate of 150 kg ha⁻¹ (Fig. 5). The UPE and NUE also showed a quadratic curve

Table 5 Correlation coefficients among above-ground N accumulation (AGN), N assimilatory enzyme activity (NR, GS), residual soil nitrate (Soil N_{end}) and grain yield (GY), grain protein concentration (GPC), N-uptake efficiency (UPE) and N-utilization efficiency (UTE)

Variable	AGN	NR	GS	Soil N _{end}
GY	0.93**	0.61*	0.73**	0.51 ns
GPC	0.61*	0.80**	0.83**	0.33 ns
UPE	0.89**	0.65**	0.69**	-0.71**
UTE	0.88**	0.71**	0.76**	-0.69**

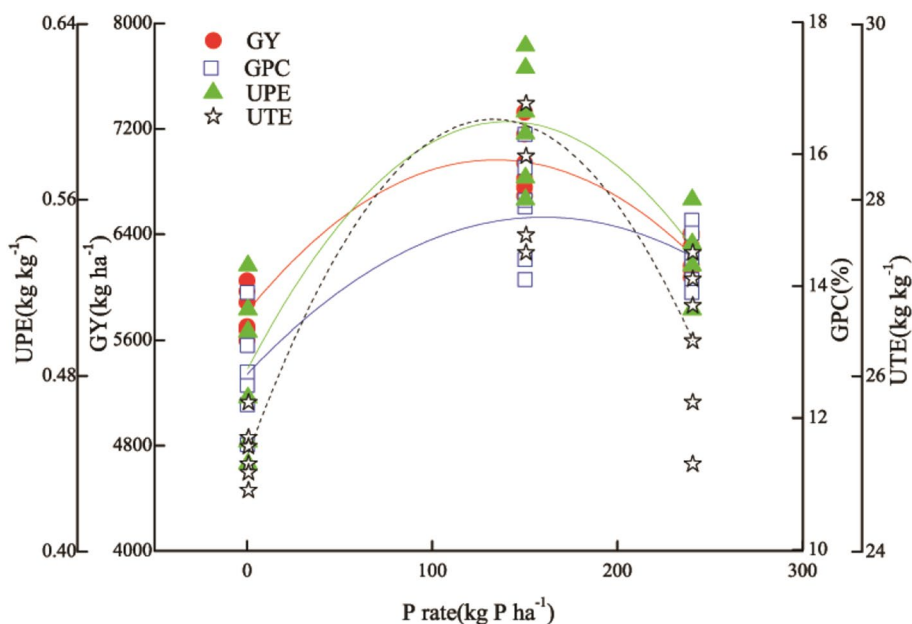
*Significant at the 0.05 probability level

**Significant at the 0.01 probability level



date within each growing season at P 0.05 using Duncan’s multiple range tests. Data are means ± SD

Fig. 5 Relationship between P application rate and grain yield (GY), grain protein concentration (GPC), N-uptake efficiency (UPE) and N-utilization efficiency (UTE). Fitted quadratic curves for GY, GPC, UPE and UTE were $y = -0.0443x^2 + 12.7813x + 5977.62$, $R^2 = 0.862$, $P < 0.01$; $y = -6.2E-05x^2 + 0.0181x + 11.92$, $R^2 = 0.918$, $P < 0.01$; $y = -5.2E-06x^2 + 0.0014x + 0.51$, $R^2 = 0.879$, $P < 0.01$ and $y = -1.9E-04x^2 + 0.0517x + 25.43$, $R^2 = 0.940$, $P < 0.01$



relationship with the P application rate ($P < 0.01$), and maximum values were 0.60 and 28.74 kg kg^{-1} at P application rates of 134.6 and 136.1 kg P ha^{-1} , respectively. In the presence of the N1 treatment, increasing the P application could improve GY, GPC and N use efficiency (UPE, UTE), but excessive P application will inevitably lead to a decrease in these indexes.

Discussion

Grain Yield and Yield Components

Appropriate application of N and P is a key factor in achieving sustained high yields of wheat. (Habbib et al. 2017; Irfan et al. 2019). In the current study, we found that applying N fertilizer regardless of P fertilizer level resulted in higher grain yield (GY) (Table 2). Large yield gaps between N0 and N1 treatments suggest that considerable potential for increasing yield, particularly in areas with insufficient soil fertilization. This is confirmed by previous studies, in which the use of N fertilizer can effectively increase GY (Grant et al. 2016; Mehrabi and Sepaskhah 2018; Wang et al. 2019). The increased GY with N application resulted mainly from greater ear number per hectare in both years. Therefore, increasing ear number in nutrient deficient soil is a key measure to achieve high yield of wheat. In our study, the interaction of N and P significantly affected wheat yield and its components (Table 2). Only applied P fertilizer significantly increased the ear number, but resulted in a decrease in kernel weight, which ultimately had no effect on GY in both growing seasons. P fertilizer promoted tiller in wheat, while

N fertilizer deficiency reduced the translocation of photosynthates to grains and destroyed coordination of source-sink relationship, thereby leading to incomplete grain filling and lower kernel weight. (Sylvester-Bradley and Kindred 2009; Liang et al. 2017). N application of 240 kg ha^{-1} combined with 150 kg P ha^{-1} showed the most significant effect on increasing yield, indicating that the combination can better meet the crop demand for nutrition, coordinate crop vegetative and reproductive growth, thereby increasing yield (Wollenweber et al. 2003). In this experiment, the GY of 2017 was significantly higher than that of 2018, which was mainly related to the rainfall difference (Fig. 1). The rainfall in 2017 was 28.1% higher than that in 2018, the corresponding increases in ear number and GY were 11.7% and 16.7%, respectively (Table 2). This was constant with the previous study of Ma et al. (2019b), who indicated that rainfall during the critical period contributes to the formation of high yield.

N Assimilatory Enzyme activity

Nitrate reductase (NR) has long been known to catalyze the rate-limiting step of nitrate assimilation in higher plants, and glutamine synthetase (GS) is a key enzyme for NH_4^+ assimilation (Zhang et al. 2017; Bashri et al. 2018). Generally, it is considered that the activities of flag leaf NR and GS determine the rate of nitrogen assimilation (Habash et al. 2007). Our results showed that positive correlations existed between grain protein concentration (GPC) and activities of NR and GS (Table 5). Our results also showed that NR and GS activities followed a similar trend in all treatments, with the activities of both enzymes in the N1 treatment being significantly higher than that in the N0 treatment (Fig. 2).

This finding was consistent with previous results showing that N deficiency caused a decrease in the NR and GS activities (Balotf et al. 2016). Furthermore, previous studies indicated that P application increased the NR and GS activities in the wheat flag leaf in the early and middle stages of grain filling, resulting in an increase in the GPC (Wang et al. 2009). Our results showed that the interaction of N and P had a significant effect on NR and GS activities (Table 3). P application in the absence of added N caused a significant decrease in NR and GS activities, whereas, in the N1 treatment, the highest enzyme activities were observed at the rate of 150 kg P ha⁻¹, this N/P combination being shown to achieve the highest GPC, with increasing P levels reducing the activities of these enzymes. Wang et al. (2009) also found that appropriate P fertilizer applying (135 ~ 150 kg P ha⁻¹) with N rate of 225 kg ha⁻¹ promoted N metabolism, whereas, with increase of P application, the promotion was reduced. In the absence of N fertilizer, P application might inhibit N metabolism by decreasing the expression of GS gene (Zhang et al. 2017; Valentine et al. 2017).

Plant Nitrogen Accumulation and Soil Nitrate Nitrogen Distribution

Promoting N absorption in crops and increasing above-ground N accumulation (AGN) are effective strategies to improve yield and reduce nitrate residues (Ying et al. 2017). Our research, based on fixed-site experiments, showed that the N1 treatment caused a significant increase in AGN, compared to N0 treatment (Table 4). This result was consistent with those of Xu et al. (2018a), who found that applying N fertilizer could promote N uptake and increase AGN in crops. Data from the current study also showed that the interaction of N and P had a significant effect on AGN (Table 3). Applying P fertilizer without N had no significant effect on AGN. The maximum AGN was observed in the treatment of N1P1 in both seasons (Table 4). Our previous studies demonstrated that nitrogen 240 kg ha⁻¹ combined with phosphorus 150 kg ha⁻¹ improved root weight density (RWD) and root length density (RLD), and optimized root distribution in the soil which resulted in higher nitrate absorption and GY (Wang et al. 2014; Liu et al. 2018). In our study, AGN in 2016–2017 was 12.2% higher than that in 2017–2018 (Table 4), mainly caused by appropriate rainfall during jointing and boot stages (3/14–4/25) in 2016–2017 (Fig. 1). Xu et al. (2018b) found that irrigation at jointing stage could optimize the canopy structure of wheat which was beneficial to plant physiological water demand and nitrogen absorption.

Soil surplus N can be lost by NO₃⁻-N leaching, which pollutes the environment (Cui et al. 2010; Shi et al. 2012). The soil NO₃⁻-N accumulation after harvest proved to be a better predictor of NO₃⁻-N leaching in the 0–100 cm soil

level (Kätterer et al. 1993). In our study, the application rate of 240 kg N ha⁻¹ caused a significant increase of residual nitrate (Soil N_{end}) in the 0–100 cm soil layer, compared with the plots without N fertilizer (N0) in both growing seasons (Fig. 3). This finding agreed with the results of Dai et al. (2016) and Wang et al. (2010), who reported that the Soil N_{end} increased sharply with N application rate in the Loess Plateau of China. In addition, we found that the interaction of N and P significantly affected Soil N_{end} (Table 3). N and P combined application significantly reduced Soil N_{end} compared with N fertilizer alone (Fig. 3). These results implied that the combination of N and P stimulated root growth of wheat and increased nitrate absorption in the soil, which resulted in higher accumulation of above-ground nitrogen in plant. This result was consistent with that of Jing et al. (2012), who found that N plus P promoted nutrient acquisition by increasing root surface available. Furthermore, in the absence of N fertilizer, difference of soil N_{end} was observed between the two years, 9.6% higher in 2016–2017 than that in 2017–2018 (Fig. 4). This was mainly due to higher rainfall in 2016–2017 (Fig. 1), which resulted in increasing of P availability in the soil, and then enhanced microbial community and N mineralization (Mori et al. 2010; Mehnaz et al. 2019).

Optimizing Fertilizer Management for High Yield and N Use Efficiency

Wheat crops with increased NUE will be of economic benefit to farmers and will also help to reduce environmental pollution (Plaza-Bonilla et al. 2017). N-uptake efficiency and N-utilization efficiency are important indicators of NUE that can be estimated reasonably (Moll et al. 1982; Hu and Zhang 2016). Application of N and P fertilizer is an important measure to regulate NUE. Previous studies showed that, at N rate of 180 kg ha⁻¹, NUE of wheat increased to the peak value at P rate of 135 kg ha⁻¹ and then decreased (Zhang et al. 2014). Similar result was also obtained in maize by Wei et al. (2017). As shown in the present work, under N application (240 kg ha⁻¹), the grain yield, UPE and UTE showed parabolic relationship with P rates (Fig. 5), by which the maximum values of yield, UPE and UTE were obtained at P rate of 140~150 kg ha⁻¹. With the increase in the amount of P applied, both yield and NUE showed a downward trend. Excessive P fertilizer might inhibit the growth of root system, weaken absorption of soil nitrogen (Yuan et al. 2009), or damage plant photosynthetic system (Tsvetkova and Georgiev 2003). Therefore, the combination of N 240 kg ha⁻¹ with P 150 kg ha⁻¹ is recommend for high yield and NUE wheat production in the North China Plain.

Conclusion

Single N fertilizer without P application improved wheat grain yield and protein concentration, but also increased the residual of nitrate nitrogen in the soil. The combination of N and P increased activities of NR and GS in flag leaf, promoted above-ground N accumulation of wheat, reduced the residual soil nitrate, all of which contribute to achieve high yield and nitrogen use efficiency.

Interactions of N and P were found on parameters such as the activity of N assimilatory enzymes, N accumulation in plants, residual soil nitrate and wheat grain yield, indicating that appropriate combinations of N and P are beneficial to sustainable production of wheat. We concluded that fertilizer rate of 240 kg N ha⁻¹ plus 150 kg P ha⁻¹ should be recommend for winter wheat production in the North China Plain.

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Author contributions CW and LW conceived of and designed the study. YC and PZ analyzed the data and wrote the manuscript. YC, PZ, and ZL carried out the field measurements and soil analysis. GM assisted with manuscript writing and editing. All authors approved the final version of the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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