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RESEARCH ARTICLE

Postponed and reduced basal nitrogen application improves nitrogen use efficiency and plant growth of winter wheat

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Abstract

Excessive nitrogen (N) fertilization with a high basal N ratio in wheat can result in lower N use efficiency (NUE) and has led to environmental problems in the Yangtze River Basin, China. However, wheat requires less N fertilizer at seedling growth stage, and its basal N fertilizer utilization efficiency is relatively low; therefore, reducing the N application rate at the seedling stage and postponing the N fertilization period may be effective for reducing N application and increasing wheat yield and NUE. A 4-year field experiment was conducted with two cultivars under four N rates (240 kg N ha^{-1} (N240), 180 kg N ha^{-1} (N180), 150 kg N ha^{-1} (N150), and 0 kg N ha^{-1} (N0)) and three basal N application stages (seeding (L0), four-leaf stage (L4), and six-leaf stage (L6)) to investigate the effects of reducing the basal N application rate and postponing the basal N fertilization period on grain yield, NUE, and N balance in a soil-wheat system. There was no significant difference in grain yield between the N180L4 and N240L0 (control) treatments, and the maximum N recovery efficiency and N agronomy efficiency were observed in the N180L4 treatment. Grain yield and NUE were the highest in the L4 treatment. The leaf area index, flag leaf photosynthesis rate, flag leaf nitrate reductase and glutamine synthase activities, dry matter accumulation, and N uptake post-jointing under N180L4 did not differ significantly from those under N240L0. Reduced N application decreased the inorganic N content in the 0–60-cm soil layer, and the inorganic N content of the L6 treatment was higher than those of the L0 and L4 treatments at the same N level. Surplus N was low under the reduced N rates and delayed basal N application treatments. Therefore, postponing and reducing basal N fertilization could maintain a high yield and improve NUE by improving the photosynthetic production capacity, promoting N uptake and assimilation, and reducing surplus N in soil-wheat systems.

Keywords: basal nitrogen application stage, grain yield, nitrogen deficiency, nitrogen use efficiency, soil nitrogen balance, wheat

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1. Introduction

The balance between food supply and demand is a major concern as the global population continues to expand and arable land area decreases, and improving crop yield continuously to ensure global food security is the main goal of global agricultural production. Nitrogen (N) is a key limitation to plant growth, as evidenced by increases in crop yield after increasing the amount of available N. To address this limitation, large amounts of N fertilizer are applied to fields to improve crop yield (Gong *et al*. 2011). China has 10% of the world's arable land, but has consumed about 40% of global N fertilizer produced since 2006, resulting in N residues (loss) in fields or loss to the environment of more than 180 kg ha^{-1} yr^{-1}, the largest loss in the world (Gong *et al*. 2011; Miao *et al*. 2011). Even though N consumption in China almost doubled between 1990 and 2009, grain production increased by only 22% (Gong *et al*. 2011), mainly because of low N use efficiency (NUE). N fertilizer overuse and mismanagement results in higher tolerance to N fertilizers in cultivars, resulting in a decrease in the effect of N fertilizer on increasing yield and a decrease in NUE (Peng *et al*. 2006). Mismanagement of N fertilizer and low N uptake eficiencies have resulted in N loss *via* volatilization as ammonia (NH_3), leaching as nitrate (NO_3^-), nitrite (NO_2^-), and dissolved organic N, and nitrification/denitrification as N gas (N_2), nitrous oxide (N_2O), and nitric oxide (NO) emissions, causing many environmental problems (Miao *et al*. 2011). Therefore, the strategies to reduce the amount of N fertilizer while maintaining high crop yields are important for improving crop NUE and protecting the environment.

Wheat is an important food crop and a relatively high N-consuming crop, and wheat grain yield is closely related to the N fertilizer application rate. In recent years, many high-N fertilizer-resistant cultivars have been produced to meet the demand for high yields, and more N fertilizer is applied in the seeding stage to form soil environments high in N to meet the needs of wheat seedling growth. However, N uptake and use by plants depend not only on the supply but also on the interception and absorption ability of roots (Barbier-Brygoo *et al*. 2011; Oka *et al*. 2012). Root and shoot growth are slow at the seedling stage in wheat, and the root number, length, and interception area are low, resulting in weak N uptake ability. This, in turn, results in large amounts of N fertilizer residues in the soil and loss to the environment, leading to decreased NUE. Many studies have shown that wheat fertilizer NUE applied at the jointing and booting stages is 2–3 times greater than NUE when applied at the seedling growth stage, mainly because of increased N demand due to fast plant growth and a strong root system that can absorb more N at the jointing and booting stages (Wang 2011; Wu 2011). Therefore, reducing N fertilizer application at the wheat seedling stage may be an efficient management strategy to decrease N fertilization and improve its use efficiency.

Improving NUE is known to improve crop yields and prevent N losses to the environment (Fageria and Baligar 2005); however, increasing the NUE remains a challenge in practice (Ravier *et al*. 2017). NUE has been shown to increase when N fertilizer is applied under conditions of high crop demand and when N application is followed by rainfall (Ravier *et al*. 2017). In addition, NUE tends to increase with decreasing N application rates. Finally, NUE is generally lower for early applications (Recous and Machet 1999), and delaying N application could increase the NUE over the whole crop cycle. Moreover, some N fertilization strategies involving late N applications have yielded promising NUE and yield results (Zebarth *et al*. 2007; Ravier *et al*. 2017). Several studies have assessed the effects of low N on wheat seedling growth. Ravier *et al*. (2017) found that early N deficiencies had no impact on yield and grain protein content, but increased NUE; therefore, N nutrition index references should be revised to support more efficient N management. Low N impedes shoot growth, but induces root extension and increases the root/shoot ratio. Jiang *et al*. (2017) found that low N at the seedling stage inhibited wheat seedling growth and reduced the dry weight and N content, but promoted root expansion and the N uptake area of roots, increasing the N uptake rate. Under low-N conditions, wheat seedlings can increase N assimilation and use by increasing the photosynthetic capacity and N metabolism, thereby reducing the N content in root cells, providing a material basis and feedback signal for N uptake (Jiang *et al*. 2017). Low-N treatment enhanced N assimilation in wheat roots and accelerated N reuse and recycling in the source leaves of wheat. Moreover, new leaves with a better N status were more suitable for low N nutrition (Li *et al*. 2013). Peng *et al*. (2006) found that reducing the total N by 30% in the crop vegetative growth stage increased the NUE and increased the yield slightly. In addition, Liu *et al*. (2002) observed that growing winter wheat in high-fertility soil without basal N application did not affect the grain yield and had a minimal effect on limited crop N uptake. A soil inorganic N (SIN) content of more than 30 kg ha^{-1} in the 0–30 cm soil layer before wheat seeding is adequate to meet the demand for N in wheat from the seeding to greening growth period (Chen *et al*. 2014). Therefore, wheat has a strong low-N-tolerance at the seedling stage, and it might be the most suitable growth stage to reduce N application.

In wheat production practice of China, about 60–70% of N fertilizer is used as basal fertilizer (i.e., applied before seeding), and the NUE is only 30–40%. Applying too much N fertilizer as basal fertilizer may be a major cause of the low NUE due to the asynchrony between N supply and N requirements. The nutrients needed to support the vegetative growth of wheat before the three-leaf stage are mainly derived from seed storage material. After the three-leaf stage, plant nutrition is mainly absorbed *via* root system. Therefore, postponing the basal N application time until after the three-leaf stage may effectively reduce

N fertilizer application rates and increase wheat grain yield and NUE, although the effects of postponing basal N fertilizer application under N deficiency on grain yield, N use efficiency, and N balance of winter wheat remain unclear.

In this study, field experiments using four N rates (240, 180, 150, and 0 kg N ha^{-1}) at three N application stages (seeding, four-leaf stage, and six-leaf stage) were conducted in four successive years (2012–2016) in Nanjing, China. The objectives of the study were: (1) to compare these treatments (reduced the basal N rates and postponed basal N application stage) with conventional N application methods to determine whether the wheat grain yield and NUE would be improved while reducing N loss; (2) if so, to determine the mechanism; and (3) to identify the best method to manage N fertilizer for high yield and NUE.

2. Materials and methods

2.1. Experimental design

The field experiments were conducted from 2012 to 2016 in Nanjing, China (32°02´N, 118°37.5´E), using the wheat cultivars Yangmai 13 in 2012–2014 and Yangmai 16 in 2014–2016. The soil in Nanjing is mainly paddy soil, the climate is humid and warm, and the annual rainfall is 800–1 400 mm. Fig. 1 presents the weather conditions at the experimental site during the wheat growing season. The soil was paddy soil, and Table 1 presents the soil properties of the fields at a depth of 0–25 cm before seeding. The plot size was 3 m×4 m. The basic seeding application was 1.8×10^{6} seeds ha^{-1} with a spacing of 0.25 m between rows. Four N rates (240 kg N ha^{-1} (N240), 180 kg N ha^{-1} (N180), 150 kg N ha^{-1} (N150), and 0 kg N ha^{-1} (N0)) at three topdressing stages (seeding (L0), four-leaf stage (L4), and six-leaf stage (L6)) were tested, together with 150 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1}. The N240L0 treatment was used as a control. Table 2 presents the details of the N application and topdressing stage in each treatment. The experiment was performed as a completely randomized block design with three replicates. Weeds were controlled by hand, and fungicides and pesticides were used at the jointing, booting, and grain-filling stages to prevent disease and pests. Because of the abundant rainfall during the study years, no irrigation was applied during the growing seasons.

2.2. Measurements

Grain yield, biomass, and yield components Four rows (1 m long) of plants in the middle of the plots (to avoid border effects) were marked with string at the heading stage to

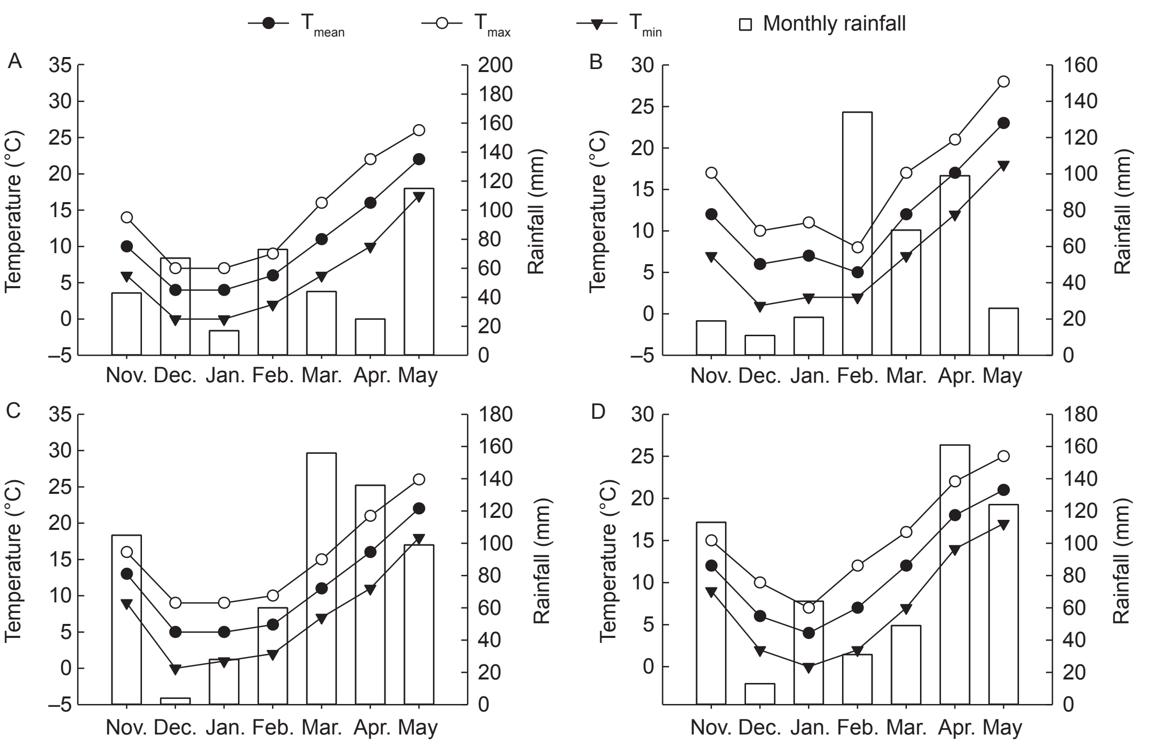

Fig. 1 Monthly mean rainfall and the mean, maximum and minimum temperatures during the wheat growing season during 2012–2013 (A), 2013–2014 (B), 2014–2015 (C), and 2015–2016 (D) in Nanjing, China. T_{mean} is the mean temperature, T_{max} is the maximum temperature, and T_{min} is the minimum temperature.

Table 1 Soil properties at the experimental site at the beginning of the experiments in the four growing seasons

Growing season	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Olsen-P (mg kg^{-1})	Exchangeable K (mg kg^{-1})	Available N (mg kg^{-1})
2012–2013	11.40	1.20	14.36	78.65	16.64
2013–2014	10.95	1.23	9.85	72.30	16.97
2014–2015	11.71	1.25	9.75	70.39	15.78
2015–2016	10.95	1.33	9.85	74.30	16.25

Table 2 Nitrogen application rates and basal nitrogen application stage under different treatments

Treatment	N rate (kg N ha^{-1})	N application stage and rate (kg N ha^{-1})				
		Sowing (L0)	4th leaf stage (L4)	6th leaf stage (L6)	Jointing	Booting
N240L0	240	120			72	48
N240L4	240		120		72	48
N240L6	240			120	72	48
N180L0	180	60			72	48
N180L4	180		60		72	48
N180L6	180			60	72	48
N150L0	150	30			72	48
N150L4	150		30		72	48
N150L6	150			30	72	48

measure grain yield at maturity, and none of these marked plants were sampled prior to harvesting. Spike number per m^2 was recorded at physiological maturity in the marked areas, at which time the plants were cut with a sickle at soil level, hand-harvested, threshed, dried, and weighed to provide grain yield in kg ha^{-1} at 14% moisture. Fifty culms from the remaining four rows in the middle of each plot were sampled at soil level to measure kernel number, 1 000-kernel weight (TKW), and aboveground biomass.

Leaf area index (LAI) and net photosynthetic rate (P_n) Fifty culms were sampled at booting stage, leaf area was measured with an LI-3000 (Li-Cor Inc., NA, USA), and LAI was then calculated (LAI, leaf area per unit land).

P_n of flag leaves (five tagged leaves per plot) was measured on a sunny day between 09:30 and 11:00 at booting stage (flag leaf full expansion), anthesis stage, and 14 days after anthesis stage (14 DAA), using a portable photosynthesis device (LI-6400, Li-Cor Inc.). The chamber was equipped with a red/blue LED light source (LI6400-02B). The environmental conditions during measurement were according to Tian *et al*. (2011).

Dry matter and N accumulation Fifty culms of each plot were sampled at jointing, anthesis, and maturity stages. Plants were separated into leaves, stems, chaff, and grain (at maturity stage). The fresh samples were put into an oven at 105°C for 30 min and then dried at 80°C until they reached a constant weight for dry weight determination, and the dry samples were milled. Total N analyses using the micro-Kjeldhal method were performed following previously reported methods (Tian *et al*. 2016). Dates of each growth stage (i.e., jointing, anthesis, and maturity stages) were recorded.

In this study, the various parameters pertaining to N accumulation and translocation in wheat plants were calculated as follows (Wang *et al*. 2015):

$$\text{N accumulaliun amounl (kg ha}^{-1}\text{)=NC×DM} \quad (1)$$

Where, NC is N concentration (mg N g^{-1} DM), DM is dry matter (kg ha^{-1}).

NUE NUE includes fertilizer N recovery efficiency (NRE) and N agronomic efficiency (NAE). NRE was calculated using eq. (2) (Duan *et al*. 2014; Tian *et al*. 2016):

$$\text{NRE (\%)}=\frac{U_{WN}-U_{W0}}{A_{WN}}\times 100 \quad (2)$$

Where, U_{WN} (kg N ha^{-1}) is total shoot N uptake in N treatments, U_{W0} (kg N ha^{-1}) is total shoot N uptake in no N treatment, and A_{WN} (kg N ha^{-1}) is the amount of fertilizer applied.

The NAE is defined as the grain yield from N application over the total applied N and was calculated following previous studies (Chuan *et al*. 2013; Duan *et al*. 2014; Tian *et al*. 2016):

$$\text{NAE (kg kg}^{-1}\text{)}=\frac{Y_N-Y_0}{A_N} \quad (3)$$

Where, Y_N (kg grain ha^{-1}) is the grain yield in N treatments, Y_0 (kg grain ha^{-1}) is the grain yield in no N treatment, and A_N (kg N ha^{-1}) is the amount of applied fertilizer N (N in inorganic fertilizer and total N in manure).

Enzyme activity of nitrate reductase and glutamine synthetase Fully expanded fresh leaves in the booting, anthesis, and grain-filling (14 DAA) stages were sampled and used to analyze the changes in enzyme activities of N metabolism. Nitrate reductase (NR) activity was determined according to Abd-El-Baki *et al*. (2000), and glutamine synthetase (GS) activity was determined as described by

Lacuesta *et al*. (1990) with minor modifications, and the detail of the method was reported in Tian *et al*. (2016).

Soil NO_3^--N, NH_4^+-N content, and inorganic N balance Soil samples were taken with a soil auger (diameter: 3.2 cm) to a depth of 60 cm and separated into three layers: 0–20, 20–40, and 40–60 cm at seeding, overwintering, jointing, booting, anthesis, and maturity stages. Fresh soil samples were completely mixed and extracted instantly using 0.01 mol L^{-1} $CaCl_2$ solution (soil solution ratio: 1:5), then put on the shaker for 30 min (180 r min^{-1}), followed by filtration. The extracts were used for determination of content of NO_3^--N and NH_4^+-N by an automated continuous flow analyzer (AA3, Bran+Luebbe, Germany). Soil bulk density was measured by the cutting ring method and soil water content was determined by drying soil samples in an oven at 105°C until a constant weight. Twenty plants were sampled in each plot at overwintering, jointing, booting, anthesis, and maturity stages. Fresh plant samples were dried in an oven at 105°C for 30 min and then dried at 70°C to get the constant weight for determination of dry weight. The dry samples were milled for measurement of N content by semi-micro kjeldahl method. At anthesis, two 1-m long rows of plants were marked for harvest at maturity. Harvested plants were threshed carefully by hand, dried, and weighed to determine grain yield.

The amount of inorganic N accumulation (N_{min}) and apparent N surplus amount (ANS) was calculated using eqs. (4) and (5) according to Shi *et al*. (2012) and Deng *et al*. (2014) as follows:

N_{min} (kg ha^{-1})=Soil layer thickness (cm)×Soil bulk density (g cm^{-3})×Soil inorganic N (including NO_3^--N and NH_4^+-N) content (mg kg^{-1})/10 (4)

ANS (kg ha^{-1})=(Initial soil N_{min} in the 0–60 cm soil layer+N fertilizer rate)–(Residual soil N_{min} in the 0–60 cm soil layer+Crop N uptake) (5)

2.3. Data analysis

All data were expressed as means over three replicates. A two-way analysis of variance (ANOVA) was performed on grain yield and its components, N efficiency, dry matter and N accumulation, LAI, NR and GS activity, and N balance of soil-wheat to compare differences among each N rates and N topdressing treatments for each year. The statistical analyses were conducted using the Statistical Product and Service Solutions Software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Grain yield, biomass, and nitrogen accumulation

There were no differences in the grain yield, biomass, and N accumulation amount (NAA) trends among the four experimental years (Fig. 2). Grain yield, biomass, and NAA decreased with decreasing N application rate, and the grain yield and biomass of the N180L4 treatment did not differ significantly from the N240L0 treatment (control). Under the N240, N180, and N150 treatments, grain yield, biomass, and NAA showed maximum with the L0, L4, and L6 treatments, respectively, indicating that the N application rate should be decreased in conjunction with a delay in N fertilization application stage to maintain high yield.

3.2. Spikes, kernels, and TKW

The numbers of spikes per area and kernels per spike decreased with decreasing N application rate, whereas the TKW increased (Table 3). Postponing basal N fertilizer application from L0 to L6 resulted in decreases in the number of spikes and kernels under the N240 and N150 treatments and showed an initial increase and subsequent decrease under the N180 treatment. The numbers of spikes and kernels under the N180L4 treatment did not differ significantly from the N240L0 treatment, whereas the TKW increased significantly (average over 4 yr: 3.6%). Therefore, the increase in grain yield in the N180L4 treatment was mainly attributed to an increase in TKW and stabilization of spikes and kernels.

3.3. Nitrogen recovery efficiency and nitrogen agronomic efficiency

There were no differences in N recovery efficiency (NRE) and N agronomic efficiency (NAE) among the four experimental years (Fig. 3). Postponing basal N fertilizer application from L0 to L6 resulted in a decrease in the NRE and NAE under N240, led to an initial increase and subsequent decrease under N180, and resulted in an increase under N150 (Fig. 3). These results suggest that the basal fertilizer application period should be postponed in conjunction with reduced N application rates for higher NUE. The maximum NRE and NAE were observed in the N180L4 treatment.

3.4. Dry matter accumulation during different growth periods

Dry matter accumulation (DMA) decreased with decreasing N application rate in each growth period (i.e., seeding to jointing (S–J), jointing to anthesis (J–A), and anthesis to maturity (A–M)). The maximum DMA of the whole growth period (seeding to maturity, S–M) was observed under the L4 treatment (Table 4; Fig. 2-E–H). DMA decreased with postponement of basal N fertilizer application from L0 to L6, before the jointing stage, and reached a maximum

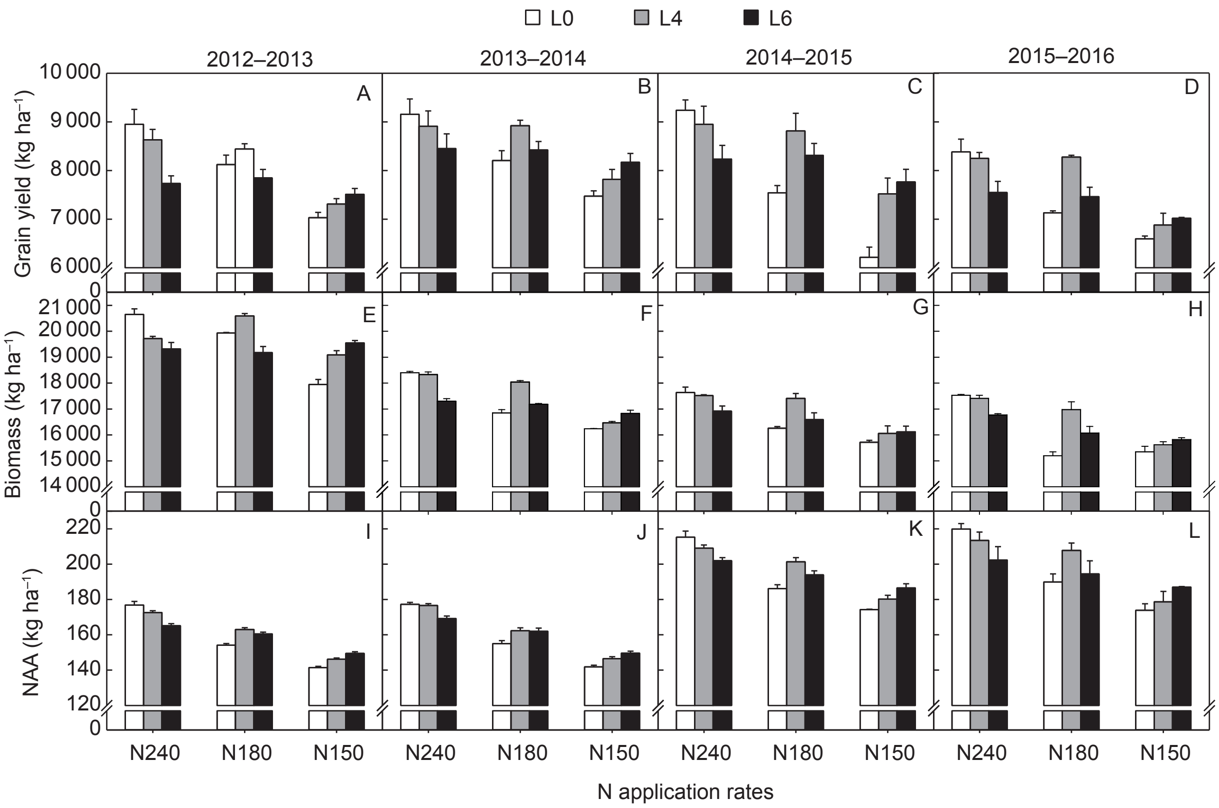

Fig. 2 Effects of nitrogen application rate and basal nitrogen application stage on grain yield, biomass at harvest, and nitrogen accumulation amount (NAA) at harvest in wheat during 2012–2013 (A, E, I), 2013–2014 (B, F, J), 2014–2015 (C, G, K), and 2015–2016 (D, H, L). L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. The error bars represent the standard error of the mean.

Table 3 Effects of nitrogen application rate and basal nitrogen application stage on spikes per area, kernels per spike, and 1000-kernel weight (TKW) in wheat[1)]

Year		N240			N180			N150		
		L0	L4	L6	L0	L4	L6	L0	L4	L6
2012–2013	Spikes (m^{-2})	426 a	421 ab	414 b	403 c	404 c	393 d	346 f	393 d	367 e
	Kernels	49.7 a	49.1 a	47.8 abc	47.0 bcd	48.3 ab	45.9 cde	44.2 e	45.2 de	44.5 e
	TKW (g)	42.3 bc	41.7 c	40.1 d	42.8 abc	42.8 abc	43.5 a	42.7 abc	42.9 ab	43.5a
2013–2014	Spikes (m^{-2})	503 a	487 b	472 cd	470 d	496 ab	484 bc	442 f	454 ef	467 de
	Kernels	47.5 ab	48.0 a	48.0 a	44.6 cd	45.8 bc	44.5 cd	42.1 e	43.3 de	43.3 de
	TKW (g)	38.3 bc	38.1 bc	37.3 c	39.1 ab	39.3 ab	39.1 abc	40.2 a	39.8 ab	40.4 a
2014–2015	Spikes (m^{-2})	413 a	395 ab	359 c	359 c	385 b	354 c	322 d	314 d	312 d
	Kernels	55.8 a	54.3 a	53.7 ab	50.5 c	54.3 a	54.3 a	43.6 d	51.3 bc	53.4 abc
	TKW (g)	40.1 d	41.7 cd	42.7 bc	41.6 cd	42.2 bcd	43.2 bc	44.3 b	46.7 a	46.6 a
2015–2016	Spikes (m^{-2})	408 a	395 b	368 c	370 c	391 b	357 c	337 d	328 de	321 e
	Kernels	51.1 a	50.7 ab	48.4 c	46.5 d	50.0 b	48.2 c	44.1 e	46.4 d	47.7 c
	TKW (g)	40.2 g	41.3 f	42.4 e	41.4 f	42.4 e	43.3 d	44.3 c	45.2 b	45.8 a

[1)] N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The values are the average of three replications under the same treatment, the same letter in the same column means no significant difference according to the Tuckey's test (P=0.05).

under the L4 treatment from J–A growth period (Table 4, Fig. 2-E–H). L4 showed a 4.2% increase compared to L0 at the J–A growth period and a 7.7% increase at the A–M growth period. Compared to the N240L0 treatment, the DMA of the N180L4 treatment at the S–J growth period was significantly decreased, there was no significant difference at the J–A and A–M growth periods, and the DMA in each N150 topdressing treatment decreased significantly (Table 4). The DMA at S–M under the N180L4 treatment was the same as that under the N240L0 treatment, which showed the

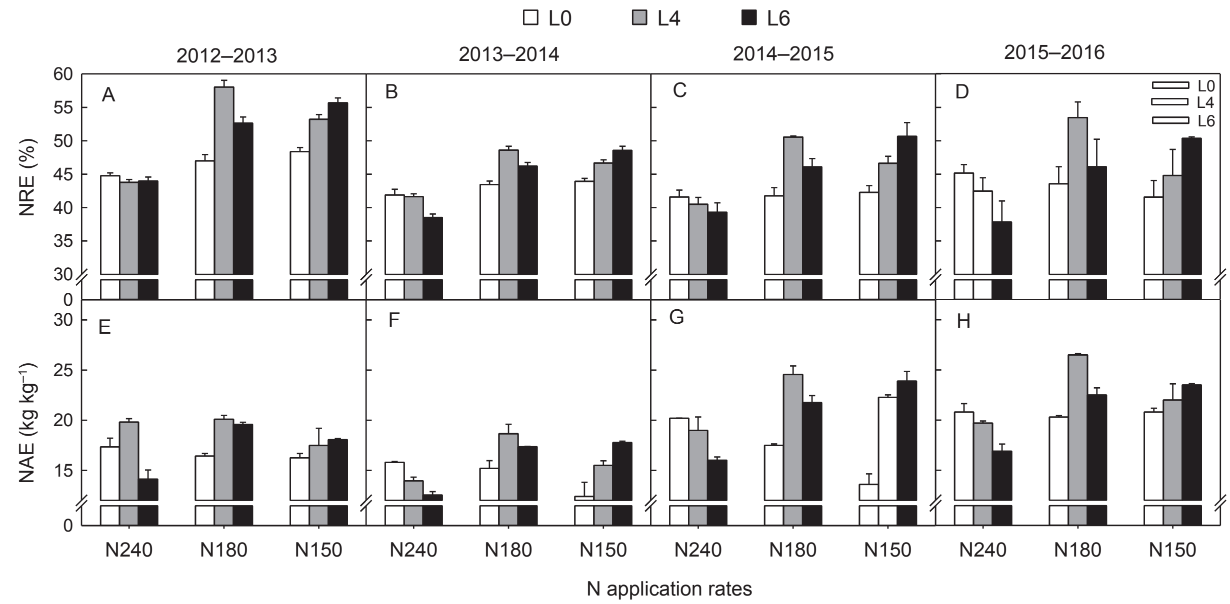

Fig. 3 Effects of nitrogen application rate and basal nitrogen application stage on nitrogen recovery efficiency (NRE) (A, B, C, D) and nitrogen agronomic efficiency (NAE) (E, F, G, H) in wheat during 2012–2013 (A and E), 2013–2014 (B and F), 2014–2015 (C and G), and 2015–2016 (D and H). L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. The error bars represent the standard error of the mean.

Table 4 Effects of nitrogen application rates and basal nitrogen application stage on dry matter accumulation (g m^{-2}) during the seeding to jointing (S–J), jointing to anthesis (J–A), and anthesis to maturity (A–M) growth periods in wheat[1)]

Year	Growth period	N240			N180			N150		
		L0	L4	L6	L0	L4	L6	L0	L4	L6
2012–2013	S–J	6079 a	5943 a	4676 cd	5148 b	4313 e	4766 cd	4522 de	4897 bc	4657 cd
	J–A	9610 bc	8665 e	9507 cd	10250 bc	12040 a	9802 bc	9782 bc	9792 bc	10320 b
	A–M	4964 bc	5111 b	5136 b	4535 cd	5737 a	4607cd	3645 e	4400 d	4574 cd
2013–2014	S–J	3481 a	3428 a	3452 a	3288 b	3278 b	3251 bc	3207 bc	3164 c	3180 c
	J–A	9548 a	9610 a	8929 b	8771 bcd	9519 a	8927 b	8602 d	8645 cd	8822 bc
	A–M	5382 a	5274 a	4916 bc	4765 cd	5236 a	4991 b	4434 e	4656 d	4833 bcd
2014–2015	S–J	3563 a	3538 a	3561 a	3225 b	3207 bc	3218 b	3114 cd	3163 bcd	3098 d
	J–A	9567 ab	9472 b	9215 c	9232 c	9608 a	9307 c	8882 e	8897 e	9023 d
	A–M	4513 ab	4507 ab	4148 abc	3806 c	4596 a	4069 bc	3722 c	3999 c	4003 c
2015–2016	S–J	3385 a	3364 a	3259 a	2956 bc	3048 b	2959 bc	2850 bc	2912 bc	2753 c
	J–A	9810 a	9728 a	9415 b	8333 f	9691 a	9028 cd	8779 e	8835 de	9060 c
	A–M	4335 a	4319 a	4093 b	3906 cd	4239 a	4080 b	3723 e	3871 d	4003 bc

[1)] N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The values are the average of three replications under the same treatment, the same letter in the same column means no significant difference according to the Tuckey's test (P=0.05).

maximum N reduction among the treatments. This indicated that postponing basal N fertilizer application while reducing N fertilizer application rates could improve DMA after the jointing stage, especially during the post-anthesis period, and could increase the DMA of the whole growth period.

3.5. Nitrogen uptake in growth period

The largest N uptake occurred during J–A under all treatments (Table 5). During S–J, reducing the N rate from 240 to 180 kg N ha^{-1} did not significantly reduce N uptake, and N uptake showed no significant difference among the topdressing stage treatments. During J–A and A–M, N uptake decreased with decreasing N application rate; however, it did not differ significantly between the N240L0 and N180L4 treatments (Table 5). During S–M, the total N uptake decreased with decreasing N rate and increased with a delay in the topdressing application (Table 5; Fig. 2-I–L). Among the treatments, the N uptake during S–M under the N180L4 treatment was significantly lower than that under

Table 5 Effects of nitrogen application rate and basal N application stage on the nitrogen accumulation amount (g m^{-2}) during the sowing to jointing (S–J), jointing to anthesis (J–A), and anthesis to maturity (A–M) growth periods in wheat[1)]

Year	Growth period	N240			N180			N150		
		L0	L4	L6	L0	L4	L6	L0	L4	L6
2012–2013	S–J	80.5 a	78.3 a	74.1 b	68.0 cd	69.3 c	70.2 c	65.1 d	65.7 d	66.0 d
	J–A	79.8 a	79.2 ab	76.5 b	72.2 c	77.7 ab	76.3 b	65.1 e	68.7 d	70.6 cd
	A–M	16.5 a	15.1 ab	14.5 abc	13.8 abc	15.9 ab	13.9 abc	11.2 c	11.7 c	12.8 bc
2013–2014	S–J	76.2 a	76.3 a	73.4 b	63.6 de	64.9 d	68.1 c	61.2 f	62.6 ef	63.7 de
	J–A	83.1 a	82.4 a	81.9 a	77.7 b	81.6 a	78.3 b	71.7 c	72.4 c	73.0 c
	A–M	18.0 a	18.0 a	13.9 b	13.6 b	17.7 a	13.5 b	8.8 d	11.5 c	12.9 bc
2014–2015	S–J	79.1 a	77.7 ab	76.5 b	72.0 c	72.8 c	73.8 c	67.0 d	68.4 d	71.2 c
	J–A	105.8 a	104.5 ab	102.9 b	98.8 c	103.4 ab	99.9 c	96.1 d	97.6 cd	99.1 c
	A–M	25.8 a	25.9 a	25.9 a	15.3 c	25.8 a	20.2 b	11.3 d	14.9 c	16.7 c
2015–2016	S–J	82.5 a	80.0 b	74.4 d	74.1 d	76.9 c	71.1 e	67.3 g	68.0 fg	70.2 ef
	J–A	108.9 a	106.5 b	103.2 c	101.9 cd	101.0 d	98.1 e	96.8 e	94.3 f	90.8 g
	A–M	28.6 a	27.0 a	26.0 a	19.1 b	27.7 a	24.2 a	15.8 b	16.7 b	18.8 b

[1)] N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The values are the average of three replications under the same treatment, the same letter in the same column means no significant difference according to the Tuckey's test (P=0.05).

N240L0, but N180L4 showed the maximum N uptake among the reduced-N treatments. This indicated that postponing basal N fertilizer application while reducing the N fertilizer application rate could improve N accumulation after the jointing stage, especially during J–A.

3.6. Leaf area index

The LAI at the booting stage decreased with decreasing N application rate, but showed no significant difference between the N180 and N240 treatments (Fig. 4). Among the topdressing treatments, the LAI of L4 was larger than those of L0 and L6. The largest LAI values among the N topdressing treatments were observed in L0, L4, and L6 under the N240, N180, and N150 treatments, respectively. The LAI under the N180L4 treatment did not differ significantly from that with N240L0 treatment, whereas the LAI of each N topdressing treatment was significantly reduced under the N150 treatment compared with the control. This indicated that postponing basal N application while reducing the N application rate was conducive to increasing LAI, thereby increasing the canopy light use efficiency.

3.7. Leaf net photosynthetic rate

Under all treatments, the photosynthetic rate (P_n) of flag leaves increased from booting to anthesis growth period, but decreased from anthesis to 14 DAA. The flag leaf P_n decreased with decreasing N application rate, but there was no significant difference in the flag leaf P_n between the N180 and N240 treatments. Among the N topdressing treatments, the P_n of L4 was higher than those of L0 and L6. The P_n of flag leaves under the N180L4 treatment did not differ significantly from that under N240L0, but the values under N150 were significantly lower (Fig. 5). This indicated that postponing basal N fertilizer application while reducing the N fertilizer application rate could improve the flag leaf P_n and increase photosynthates accumulation to provide sufficient material sources for grain production.

3.8. Nitrate reductase and glutamine synthetase activity

The activities of nitrate reductase (NR) and glutamine synthase (GS) in the flag leaves across the treatments were similar among the four experimental years (Fig. 6). Under all treatments, NR and GS activities increased from booting to anthesis and decreased from anthesis to 14 DAA. NR and GS activities in flag leaves decreased with decreasing N application rate, but there was no significant difference between the N180 and N240 treatments. Among the topdressing treatments, the highest activities were observed with the L4 treatment, and the L0, L4, and L6 treatments showed maximum activities under the N240, N180, and N150 treatments, respectively. In the N180L4 treatment, NR activity in the booting and anthesis growth stages and GS activity from booting to 14 DAA did not differ significantly from those under N240L0, but decreased significantly under the N150 treatment. This indicated that postponing the basal N fertilizer application time under reduced N fertilizer application could promote the N assimilation efficiency and improve N uptake and accumulation.

3.9. Soil inorganic nitrogen content and apparent nitrogen surplus in growth period

The soil inorganic N (SIN) content in the 0–60-cm soil layer decreased with decreasing N rate, but increased with

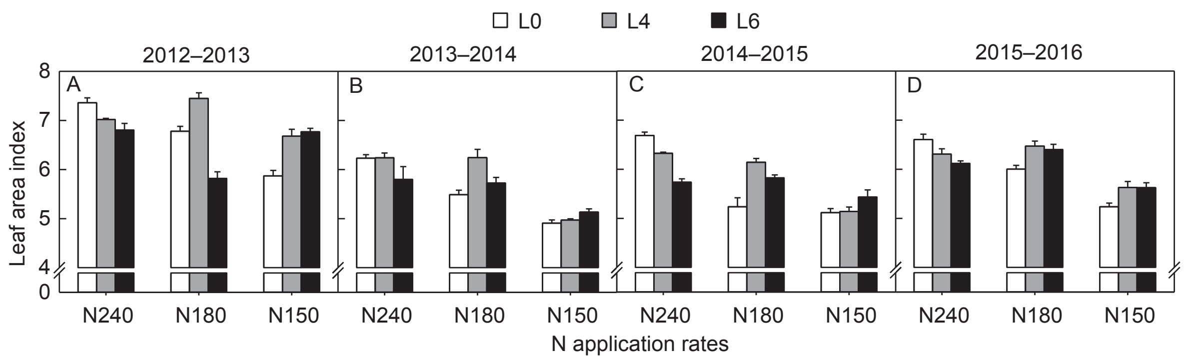

Fig. 4 Effects of nitrogen application rates and basal nitrogen application stage on leaf area index (LAI) at anthesis stage in wheat grown during 2012–2013 (A), 2013–2014 (B), 2014–2015 (C), and 2015–2016 (D). L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. The error bars represent the standard error of the mean.

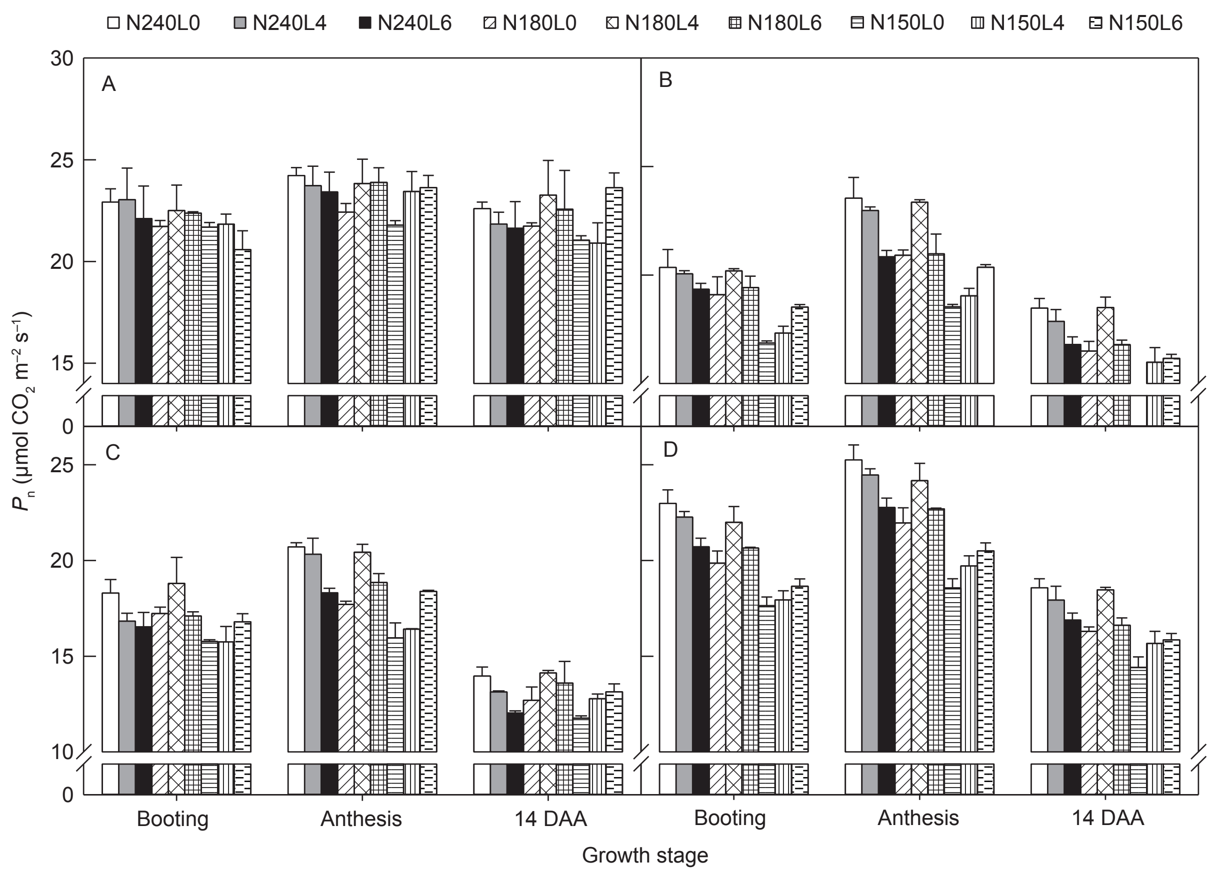

Fig. 5 Effects of nitrogen application rate and basal N application stage on the leaf net photosynthetic rate (P_n) of wheat at the booting, anthesis, and 14 days after anthesis (14 DAA) growth stages during 2012–2013 (A), 2013–2014 (B), 2014–2015 (C), and 2015–2016 (D). N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The error bars represent the standard error of the mean.

postponement of basal fertilizer application (Fig. 7-A–C). Under all treatments, the greatest SIN concentration was observed in the overwintering growth stage, followed by the anthesis, jointing, and maturity stages. The trends in SIN content among the treatments differed among the growth stages. At the overwintering stage, the SIN content in the 0–60-cm soil layer decreased with decreasing N rate and increased with postponement of basal fertilizer

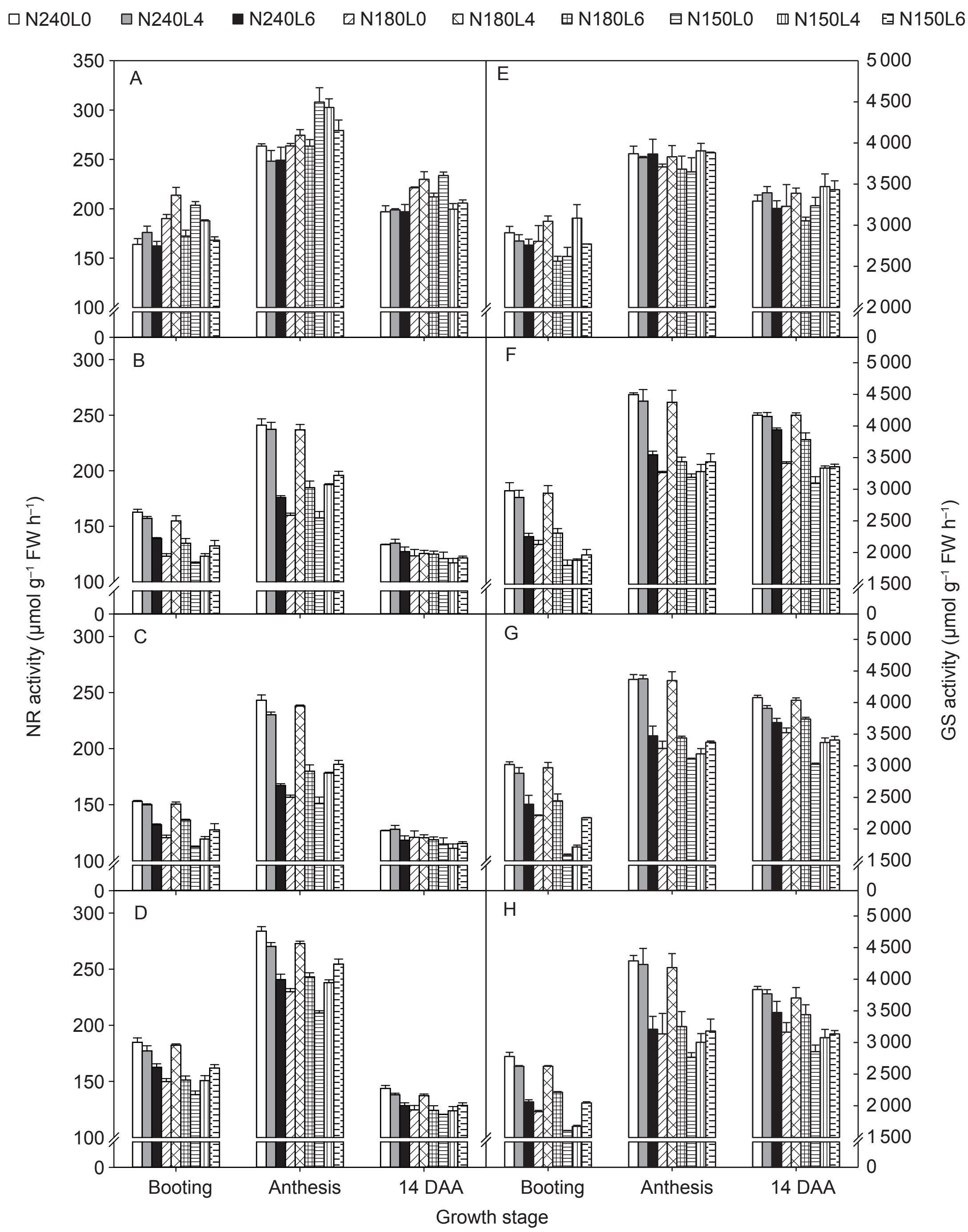

Fig. 6 Effects of nitrogen application rate and basal N application stage on the enzyme activity of nitrate reductase (NR) (A–D) and glutamine synthetase (GS) (E–H) in wheat at the booting, anthesis, and 14 days after anthesis (14 DAA) growth stages during 2012–2013 (A and E), 2013–2014 (B and F), 2014–2015 (C and G), and 2015–2016 (D and H). N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The error bars represent the standard error of the mean.

application. At the jointing stage, the SIN of all treatments decreased, and the differences among the treatments were smaller because this stage represented the peak period of N uptake. At anthesis, the SIN content of all treatments increased owing to fertilizer application at booting stage, and the SIN contents under the L4 treatments were lower than those under the L6 treatments. At maturity stage, the treatments showed the same trends as in the other stages.

The N balance was calculated during different growth

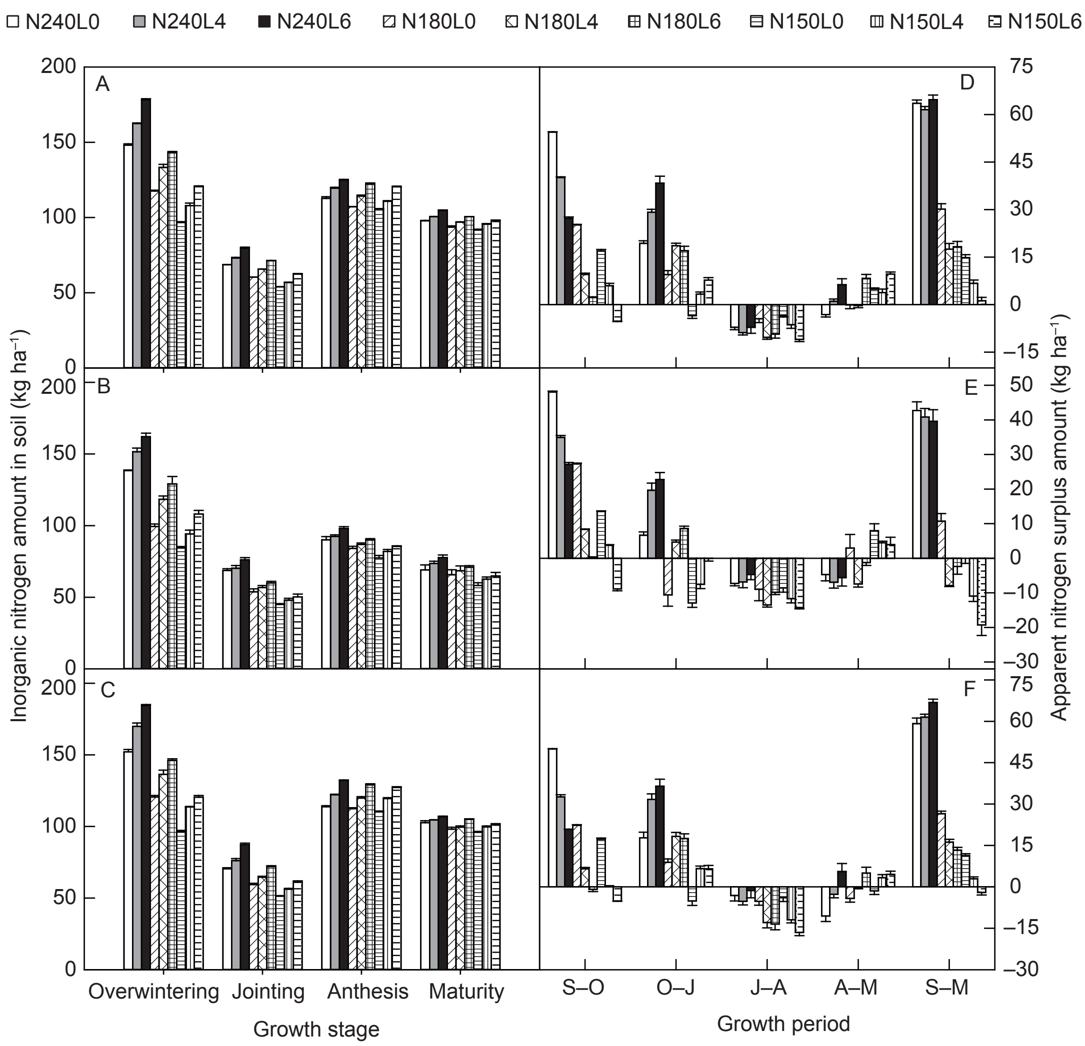

Fig. 7 Effects of nitrogen application rate and basal nitrogen application stage on soil inorganic N contents (A–C) at different growth stages and apparent N surplus amount (D–F) during the seeding to overwintering (S–O), overwintering to jointing (O–J), jointing to anthesis (J–A), anthesis to maturity (A–M), and seeding to maturity (S–M) growth periods of wheat during 2013–2014 (A and E), 2014–2015 (B and F), and 2015–2016 (C and G). N240, N180, and N150 indicate 240, 180, and 150 kg N ha^{-1}, respectively. L0, seeding stage; L4, four-leaf stage; L6, six-leaf stage. The error bars represent the standard error of the mean.

stages, and N surpluses and deficits were found during different periods (Fig. 7-D–F). With decreasing N fertilization rate, we observed a decrease in N surplus from the seeding to overwintering stages. Under the same N level, the N surplus was the greatest under L0 treatment. N deficits were first observed under the N180L6 and N150L6 treatments due to delayed topdressing application. From the overwintering to jointing stages, the N rates showed the same trends from the seeding to overwintering growth period. Among the topdressing stages, the N surplus was the greatest under the L6 treatment. From the J–A growth period, all treatments showed N deficits due to the high N demand. Over the entire lifecycle of wheat, the N surplus decreased with decreasing N application rate and postponement of basal N application.

4. Discussion

Many researchers have studied the effects of N management on NUE and grain yield. Hawkesford (2014) found that under N fertilizer applications of 0–200 kg ha^{-1}, both yield and N uptake increased substantially; however, no further yield increases were observed at N rates higher than 200 kg ha^{-1}, despite additional N uptake, and there was no yield response to N applied with high initial SIN levels (average: 212 kg ha^{-1}). Hence, optimizing N use for winter wheat considerably reduces N loss into the environment without compromising yield (Cui *et al*. 2006). In our research, under N rate of 240 kg ha^{-1}, grain yield, NAE, and NRE decreased with increasing delay in basal N application. Combined with the N balance results, excessive N application before jointing stage may have detrimental effects on wheat. Conversely, under N rates of 180 and 150 kg ha^{-1}, grain yield, NAE, and NRE increased with increasing delay in basal N application. This indicated that reducing the N rate and delaying basal N application increased the N uptake and reduced N surpluses before jointing stage. Several other studies have found that no application of basal N fertilizer before overwintering is beneficial. For example, Shukla *et al*. (2004) reported that once soil N reached a threshold concentration, the yield did not further decrease in the absence of basal N fertilizer. Interestingly, in present study, there was no significant difference in grain yield between the N240L0 and N180L4 treatments, and the N180L4 treatment showed the maximum NAE and NRE among the treatments. Thus, the N180L4 treatment is optimal for N management and can improve NUE while sustaining a stable grain yield.

Leaf is not only the main organ that uses N to produce grain yield, but also an important organ that affects NUE (Vogan and Sage 2011). Leaf P_n has a very important influence on grain yield and NUE, and N affects the photosynthetic efficiency of leaves by affecting the stomatal conductance and leaf conductivity (Yoshihira and Karasawa 2003). Previous study showed that the ability of wheat leaves to immobilize CO_2 was related to the leaf N content, high leaf N content increased the rate of CO_2 assimilation, but the assimilation rate would be reduced when the N content exceeded a certain level (Trouwborst *et al*. 2011; Li *et al*. 2013). Therefore, photosynthetic characteristics are closely related to N nutrition and NUE in plants. In present study, the P_n of flag leaves under the N180L4 treatment did not differ significantly from that under N240L0. This indicated that postponing basal N fertilizer application while reducing the N fertilizer application rate could improve the flag leaf P_n and increase photosynthates accumulation to provide sufficient material sources for grain production. Postponing basal N fertilizer application from L0 to L6 resulted in a decrease in the NRE and NAE under N240, and led to an initial increase and subsequent decrease under N180. These results suggest that the basal fertilizer application period should be postponed in conjunction with reduced N application rates for higher photosynthetic efficiency, grain yield, and NUE.

NR and GS are the key enzymes involved in N metabolism, and also key regulators of protein accumulation. Nitrate absorbed by wheat is continuously catalyzed by NR and nitrite reductase (NiR) to form NH_4^+. NR is a NO_3^--inducible enzyme lies in cell protoplasm, and its activity directly affects capability of the N assimilation, N absorption in the plant and N supplement for protein accumulation (Singh *et al*. 2014; Thomsen *et al*. 2014). GS lies in the chloroplasts of leaves and catalyzes the NR reduction product NO_2^- to synthesize NH_4^+ and drive glutamic acid to synthesize glutamine, and its activity can react to the strength of ammonia assimilation ability in the flag leaf. The improvement of GS activity can promote the enhancement of N metabolism, promote the synthesis and transformation of amino acids, and have many functions in the process of plant metabolism and development (Singh *et al*. 2014; Thomsen *et al*. 2014). It is considered that the activities of NR and GS are significantly correlated with NUE, and their activities can be used as an indicator of N nutrition efficiency (Tian *et al*. 2016). In the present study, NR and GS activities in flag leaves decreased along with decreasing N application rate, and NR and GS activities under N180L4 did not differ significantly from those under N240L0 (Fig. 6). This indicated that postponing the basal N fertilizer application time under N deficiency could promote the N assimilation efficiency and improve N uptake and accumulation.

Soil inorganic N, which is derived from fertilizer N and soil organic N mineralization, is the main form of plant-available N, and NO_3^--N, and NH_4^+-N are the main components of SIN. Previous research showed that NH_4^+-N in the topsoil changed more rapidly by N application. In contrast, NO_3^--N in the soil profile was greatly altered by the N application rate (Liu *et al*. 2003). Reducing N application rates led to significantly lower concentrations of inorganic N in the soil profile compared to fertilizing according to standard farmers' practices, which resulted in high inorganic N concentrations in the soil after harvest (Hartmann *et al*. 2014). In the present study, reduced N application significantly decreased the NO_3^--N and NH_4^+-N contents in the 0–60-cm soil layer at overwintering, whereas reduced N application significantly decreased the NO_3^--N content in the 0–60-cm soil layer and the NH_4^+-N content in the 0–40-cm soil layer at anthesis stage. The differences among the treatments decreased with increasing soil depth. Under the same N level, the NO_3^--N and NH_4^+-N contents of the L6 treatment were higher than those of the L0 and L4 treatments. This indicated that when N fertilizer was applied as basal fertilizer, N losses were higher. This was consistent with a study that found that most of the N leached from arable soils occurred in the earlier growth stage when plant demand was low. At maturity stage, SIN decreased with decreasing N application rate and increased with increasing delay in topdressing application. The results showed that when the N rate was reduced, delaying the topdressing timing to a later stage increased the SIN residue after harvest and reduced inorganic N loss.

The N balance of a crop, calculated as the difference between the N inputs and N outputs (Hartmann *et al*. 2014), is a useful method for evaluating N management (Guarda *et al*. 2004). N uptake is an important component of N outputs, and improving wheat N uptake can reduce N surpluses and maintain the N balance. Crops generally absorb 70–80% of their N during the vegetative growth stage, and only a small proportion during the seedling stage (Chen *et al*. 2014). Our results showed that, from seeding to jointing stages, reducing the N application rate did not significantly affect N uptake, except under N application rate of 150 kg ha^{-1}. Moreover, N uptake did not differ significantly according to the time of topdressing application. From jointing to anthesis stages, decreasing the N application increased wheat N uptake, especially from jointing to anthesis stages. However, N uptake did not differ significantly between the N240L0 and N180L4 treatments from jointing to anthesis stages. These findings suggested that reducing the N rate while delaying the topdressing application time to a later stage reduced topdressing fertilizer loss before jointing and increased N demand after jointing stage. We also calculated the N balance during different periods. The results showed that soil N was adequate for wheat before jointing, as there was a surplus of soil N from seeding to jointing stages, and the soil N surplus under the reduced-N treatment decreased the N surplus before jointing, in turn reducing N loss before jointing. Moreover, for all observed vegetation periods, Hartmann *et al*. (2014) observed the highest positive N surpluses and balances in the farmers' practice (urea, 550 kg N ha^{-1} yr^{-1}) treatment. Over the whole life of the plants, the soil N surplus also decreased with decreasing N application rate and delayed topdressing application. Farmers prefer to apply fertilizer at seeding stage, which contributes to N loss (Abril *et al*. 2007). However, 84–86.5% or more of basal fertilizer is not absorbed by wheat (Liu *et al*. 2002). Our results are in agreement with previous study in which we recommend reducing unnecessary basal fertilizer in autumn and excessive N topdressing in spring was recommended (Chen *et al*. 2006).

5. Conclusion

Compared with conventional N application (N240L0), postponing basal N application to the four-leaf growth stage under low N supply (N180L4) did not decrease wheat grain yield, but increased NUE significantly. Therefore, the N180L4 treatment represented the optimal N-reduction treatment, based on fertilization period postponement and decreased N application rate. Overall, N180L4 increased the wheat post-jointing photosynthetic production capacity, N assimilation ability, basal N fertilizer recovery rate, and soil residual rate while reducing the basal N fertilizer loss rate to realize the synchronous improvements of NUE while sustaining a high grain yield.

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