

RESEARCH

Open Access



# Split nitrogen fertilizer application improved grain yield in winter wheat (*Triticum aestivum* L.) via modulating antioxidant capacity and $^{13}\text{C}$ photosynthate mobilization under water-saving irrigation conditions

Zhen Zhang, Zhenwen Yu, Yongli Zhang and Yu Shi\*

## Abstract

A water-saving cultivation technique of supplementary irrigation based on soil moisture levels has been adopted for winter wheat production in the Huang-Huai-Hai Plain of China, due to the enhanced water-use efficiency. However, appropriate split nitrogen management may further improve crop growth and grain yield. Here, we conducted a 2-year field experiment to determine if split nitrogen management might improve wheat productivity by enhancing  $^{13}\text{C}$  photosynthate mobilization and the antioxidant defense system under water-saving conditions. Split nitrogen management involved a constant total nitrogen rate ( $240 \text{ kg ha}^{-1}$ ) split in four different proportions between sowing and jointing stage, i.e., 10:0 (N1), 7:3 (N2), 5:5 (N3), and 3:7 (N4). The N3 treatment significantly enhanced “soil-plant analysis development” values, superoxide dismutase antioxidant activity, soluble protein content, sucrose content, and sucrose phosphate synthetase activity, although it reduced the accumulation of malondialdehyde (MDA). The N3 treatment ultimately increased the amount of dry matter assimilation after anthesis significantly. In addition, the  $^{13}\text{C}$  isotope tracer experiment revealed that the N3 treatment promoted the assimilation of carbohydrates after anthesis and their partitioning to the developing grains. Compared to the unequal ratio treatments (N1, N2, and N4), the equal ratio treatment (N3) increased grain yield by 5.70–16.72% via increasing 1000-grain weight and number of grains per spike in both growing seasons. Therefore, we recommend the use of a 5:5 basal-topdressing split nitrogen fertilizer application under water-saving irrigation conditions to promote antioxidant enzyme activity and the remobilization of photosynthate after anthesis for improving wheat grain yield.

(Continued on next page)

\* Correspondence: [ndxiaomai@163.com](mailto:ndxiaomai@163.com)

Key Laboratory of Crop Ecophysiology and Farming System, Ministry of Agriculture, Shandong Agricultural University, Tai-an 271017, Shandong, China

(Continued from previous page)

**Keywords:** Split nitrogen fertilization, Modulating antioxidant capacity,  $^{13}\text{C}$  photosynthate mobilization, Water-saving irrigation

## Introduction

Winter wheat is a widely cultivated crop in the Huang-Huai-Hai Plain of China (HPC), a very important area of wheat production in China (He et al. 2017). The identification of methods to increase wheat grain yield in HPC is increasingly crucial for grain productivity and food security in China (Liu et al. 2020a). Water is an important factor for wheat development and grain yield. However, recurrent water shortages in the region of the HPC, mainly due to climate change and increased water demand, threatens the sustainability of winter wheat production. Therefore, reasonable irrigation management recommendations are urgently needed.

Traditionally, farmers in the region of the HPC irrigate winter wheat by flooding four to six times during the growth season, for a total irrigation water use of 300–400 mm. However, serious water consumption affects the sustainable development of this irrigation method in the HPC (Dar et al. 2017; Mostafa et al. 2018; Jha et al. 2019). Methods that reduce irrigation water consumption require urgent attention. Our research group previously reported a water-saving cultivation technique of supplementary irrigation based on measuring soil moisture at critical stages. Compared to traditional flood irrigation, this reported technique maintained high grain yield and water use efficiency and decrease water consumption (Man et al. 2014). Our previous research also showed that this irrigation method can promote wheat root growth to a greater soil depth and, consequently, improve the absorption and utilization of soil moisture (Guo et al. 2015). Compared with flood irrigation, the total irrigation water amount was reduced by 200–300 mm under this water-saving irrigation technique (Zhang et al. 2019).

As nitrogen is an essential macronutrient for wheat growth and yield, fertilizer management is generally the most effective way to increase grain yield in winter wheat production (Azam et al. 2020). However, unreasonable nitrogen fertilizer application has led to serious environmental problems, higher fertilizer loss, and lower returns for farmers (Singh et al. 2014; Tan et al. 2017). Therefore, appropriate nitrogen fertilizer management is essential for a sustainable strategy to improve crop yield while reducing environmental risk (Trost et al. 2016). An appropriate basal-topdressing ratio of nitrogen application is the most important component of nitrogen fertilizer management (Zhang et al. 2018). In many areas

of the world, farmers commonly tend to apply nitrogen fertilizer at pre-planting due to the convenience of such an application and its priming effect on wheat seedling growth (Santis et al. 2020). However, the nitrogen requirements of wheat have been shown to vary among growth stages (Santis et al. 2020). In addition, nitrogen fertilizer availability can be affected by wheat processes (Lollato et al. 2019). Therefore, wheat nitrogen requirements may not be satisfied if the total amount of nitrogen fertilizer is applied at once, at pre-planting. Alternatively, split nitrogen management at the jointing may contribute towards satisfying the plant nitrogen demand via soil supply, thereby, enhancing nitrogen use efficiency, and ultimately improving grain yield. In addition, the response of wheat to nitrogen fertilization is dependent on the soil-water condition. Li et al. (2012) reported that an appropriate soil moisture level led to improved nitrogen availability and the simultaneous utilization of water and that supplemental irrigation is the main factor that affects the soil water condition. Previous studies reported the effects of the nitrogen application rate, fertilization ratio, and fertilization period on wheat yield under the sufficient irrigation condition (Zhao et al. 2020). However, the effect of split nitrogen fertilization on wheat yield under water-saving irrigation condition requires further investigation. The appropriate nitrogen application under water-saving irrigation may contribute significantly to the sustainable production of winter wheat in the region of the HPC.

Leaf senescence is a developmental process that involves the degradation of macromolecules, such as nucleic acids and proteins, the accumulation of reactive oxygen species, and the transition from nutrient assimilation to nutrient remobilization (Nehe et al. 2020). The commencement of grain growth from anthesis onwards coincides with leaf senescence (Kitonyo et al. 2018a, 2018b). The plant leaves are the major sites for photosynthesis, which, together with the pre-anthesis reserves, supply the assimilates for grain-filling (Kitonyo et al. 2018a, 2018b). Premature leaf senescence can lead to a reduction in wheat canopy size (Yang et al. 2017), loss of light use efficiency, and decreased crop yield (Thapa et al. 2018). A delayed onset of senescence with longer functional photosynthesis (stay green) may produce more assimilates for developing grains and, thus, has the potential to maximize grain yield (Wu et al. 2019; Li et al. 2019). Nitrogen is a key factor in regulating the leaf senescence of crops (Roche et al. 2017). Previous studies

have demonstrated that nutrient stress due to nitrogen deficiency induces the premature senescence of flag leaves (Jiang et al. 2020). In contrast, high nitrogen levels delay the initiation of flag leaf senescence and prolong the leaf functional period (Luo et al. 2018). However, previous nitrogen management studies have focused predominantly on the agronomic and physiological bases of yield potential under sufficient irrigation conditions. More research is needed to develop an optimal water and nitrogen management system to support the use of water-saving irrigation, especially involving fertigation techniques, for winter wheat production in the HPC. Therefore, in this study, the working hypothesis is that split nitrogen management techniques would significantly affect the grain yield by impacting the antioxidant capacity and  $^{13}\text{C}$  photosynthate mobilization under water-saving irrigation condition. The objectives of the present study were to (1) examine and compare the antioxidant capacity of flag leaves between different nitrogen treatments after anthesis; (2) investigate differences in  $^{13}\text{C}$  photosynthate assimilation, distribution, and redistribution between different nitrogen treatments under water-saving irrigation; and (3) determine the optimal nitrogen management technique under water-saving irrigation.

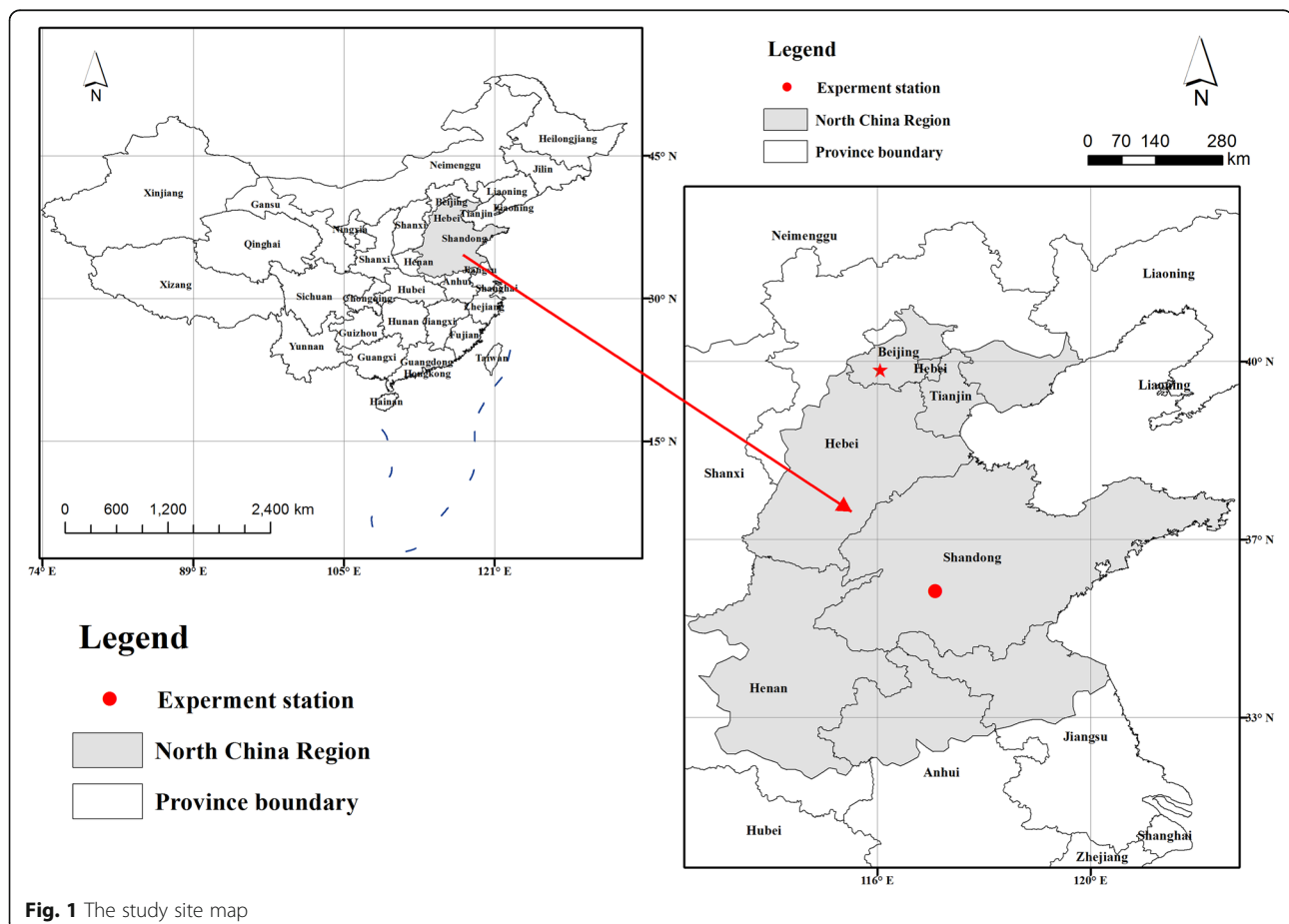
## Materials and methods

### Experimental site

Field experiments were conducted during the winter wheat growing seasons in 2016–2017 and 2017–2018 at the experimental station in Shijiawangzi Village, Xiaomeng Town, Yanzhou, Jining City, Shandong Province, China ( $35^{\circ} 40' \text{ N}$ ,  $116^{\circ} 41' \text{ E}$ ) (Fig. 1). This region is a typical plain region of the HPC, which has a warm temperature continental climate. The soil in the region is classified as clay loam. The top 0–20 cm soil layer contained  $14.20 \text{ g kg}^{-1}$  organic matter concentration,  $1.13 \text{ g kg}^{-1}$  total nitrogen,  $122.60 \text{ mg kg}^{-1}$  available nitrogen,  $129.44 \text{ mg kg}^{-1}$  available potassium, and  $38.11 \text{ mg kg}^{-1}$  available phosphorus. The crop previously grown at the study was corn, and all residual straw was incorporated into the soil after harvest.

### Experimental design and crop management

Winter wheat variety “Jimai 22”, a cultivar widely used in the HPC, was selected for the field experiments. The experiment included four treatments at a nitrogen application rate of  $240 \text{ kg ha}^{-1}$ , with fertilizer ratios of base to topdressing of fertilizer, namely, 10:0 (N1), 7:3 (N2), 5:5 (N3), and 3:7 (N4). A randomized complete block design was used in a split-plot arrangement with three



replications. All plots were 20 m<sup>2</sup> in size. The distance between two adjacent plots receiving different application treatments was 2 m to avoid the interference of nitrogen and irrigation factors. Soil moisture management in the experimental plot was based on the water-saving cultivation technique of supplementary irrigation by measuring soil moisture. Briefly, the relative water content in the 0–40 cm soil layer was supplemented to 70% at the jointing and anthesis stages of wheat.

The irrigation amount was calculated using the formula:  $M = 10 \times r \times H \times (\beta_i - \beta_j)$ , where  $M$  represents the amount of irrigation and  $r$  represents the bulk density of the quasi-moist soil depth,  $H$  represents the pseudo-moist soil depth,  $\beta_i$  represents the design water content, and  $\beta_j$  represents the water content of the soil before irrigation. Irrigation was conducted with a hose and measured using a meter (Man et al. 2014).

Urea (N 46%), calcium superphosphate (P<sub>2</sub>O<sub>5</sub> 12%), and potassium chloride (K<sub>2</sub>O 60%) were used as the nitrogen, phosphate, and potassium fertilizers, respectively. The basal nitrogen fertilizer and the phosphate (150 kg ha<sup>-1</sup>) and potassium (112.5 kg ha<sup>-1</sup>) fertilizers were spread on the soil surface prior to sowing. Immediately after application, the fertilizers were mixed into the top 0–20 cm layer of soil using a rotary cultivator. At the joining stage of the cultivated wheat, nitrogen fertilizer was applied in furrows, which were immediately covered with soil. After fertilizer application, wheat seeds were sown at a depth of 4 cm on October 12, 2016, and October 24, 2017, and the three-leaf basic determining was 1.8 million ha<sup>-1</sup>. Plants were harvested on June 8, 2017, and June 7, 2018. All other agronomic management protocols were maintained consistent in all treatments following the recommendations of local farmers for high yield.

### Sample collection, analyses, and calculations

#### SPAD value determination

The soil-plant analysis development (SPAD) value of the flag leaves was measured in 10 randomly selected plants per treatment, at 7-day intervals from anthesis to maturity (measuring 5 times altogether, every year from 2016 to 2018), using an SPAD-502 chlorophyll meter model (SPAD-502, Soil-plant Analysis Development Section, Minolta Camera Co., Osaka, Japan) (Ren et al. 2020).

#### Superoxide dismutase, malondialdehyde content, soluble protein content, sucrose content, and sucrose phosphate synthetase activity determination

One hundred single stems that flowered on the same day in each plot were tagged and sampled from anthesis to maturity within a 7-day interval, while 20 flag leaves were sampled at each sampling stage (sampling 5 times altogether, every year from 2016 to 2018). Sampled flag leaves were frozen in liquid nitrogen for 1 min and then

stored at –80 °C. One half of the sampled flag leaves was used to measure superoxide dismutase (SOD), malondialdehyde (MDA) content, and soluble protein content, while the other half was used to measure the sucrose content and sucrose phosphate synthetase (SPS) activity.

For SOD enzyme extraction, 500 mg of the fresh leaves was ground in 5 mL extraction buffer consisting of 100 mmol L<sup>-1</sup> potassium phosphate buffer (pH 7.0), 1 mmol L<sup>-1</sup> ethylenediaminetetraacetic acid (EDTA), and 1% polyvinylpolypyrrolidone (PVPP). The extract was centrifuged at 20,000 rpm for 20 min at 4 °C. The supernatant was collected and used to determine SOD activity, MDA content, and soluble protein content, following the methods described by Lv et al. (2017). SOD activity was evaluated based on its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT). In turn, MDA content was calculated using the thiobarbituric acid reaction. Soluble protein content was calculated by Coomassie Brilliant Blue staining.

For SPS enzyme extraction, 500 mg of fresh leaves was ground in 7 mL extraction buffer consisting of 50 mmol L<sup>-1</sup> HEPES buffer (pH 7.5), 5 mmol L<sup>-1</sup> ethylenediaminetetraacetic acid (EDTA), 1 mmol L<sup>-1</sup> DL-Dithiothreitol (DTT), 2 mmol L<sup>-1</sup> potassium chloride (KCl), and 1% polyvinylpolypyrrolidone (PVPP). The extract was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was collected and used to determine SPS activity and sucrose content, following Feng et al.'s method (Feng et al. 2019).

#### Dry matter accumulation and translocation

Overall, 20 single stems were randomly sampled from each plot by cutting at ground level at anthesis and maturity stages, and separated into vegetative organs and grains (only at maturity). All plant samples were oven-dried to a constant weight at 70 °C to determine dry matter biomass. Dry matter accumulation and translocation indices were calculated using the following equations (Latifmanesh et al. 2018):

$$\text{DMT} = \text{DMA} - \text{DMM} \quad (1)$$

$$\text{CDMT} = \text{DMT} / \text{GY} \times 100 \quad (2)$$

$$\text{DMAA} = \text{GY} - \text{DMT} \quad (3)$$

$$\text{CDMAA} = \text{DMAA} / \text{GY} \times 100 \quad (4)$$

where DMT is the dry matter translocation amount (kg ha<sup>-1</sup>), DMA is the dry matter accumulation amount at anthesis, DMM is the dry matter of vegetative plant parts at maturity, CDMT is the contribution of pre-anthesis assimilates to grain (%), GY is the grain yield, DMAA is the dry matter accumulation amount after anthesis, and CDMAA is the contribution of dry matter accumulation amount after anthesis to grains.

### Carbon isotope analysis

We conducted a leaf isotope-tagging experiment using  $^{13}\text{CO}_2$  in both growing seasons. Ten representative single stems in each experimental plot were selected at anthesis. We encased the flag leaf of each selected single stem in a 0.1-mm-thick Mylar plastic bag, which permitted sunlight to pass at levels up to 95% of the natural intensity. The bags were sealed at the base with adhesive tape and subsequently injected with 3.5 mL of  $^{13}\text{CO}_2$ . After allowing photosynthesis to proceed for 30 min,  $^{13}\text{CO}_2$  in each bag was extracted through a KOH washer to absorb the remaining  $^{13}\text{CO}_2$ , and the bag was removed. This experiment was conducted from 09:00 to 11:00 a.m. on sunny days. At 72 h after processing from anthesis and maturity stages, the wheat plants were randomly sampled from each plot by cutting at the ground level. All plants were separated into stems and sheaths, leaves, glumes (spike axis and kernel husks), and grains (only at maturity), and oven-dried to a constant weight at 70 °C to determine the aboveground biomass. All samples were milled to a fine powder using a ball mill, for use in the carbon isotope analysis. The carbon isotope content of milled samples (5 mg) was determined using an online system composed of an elemental analyzer, a TripleTrap, and a mass spectrometer (Carlo Erba 2100, Milan, Italy). The distribution of  $^{13}\text{C}$  photosynthates among different organs was determined (Gao et al. 2017).

### Grain yield and yield component determination

Grain yield and yield components were evaluated at the harvest of winter wheat stage (Gaju et al. 2014).

### Statistical analysis

Correlations between the photosynthesis performance parameters and grain filling after anthesis were analyzed

using the SPSS 13.0 software ( $\alpha = 0.05$ ). An analysis of variance was used to assess the effects of irrigation and nitrogen fertilizer treatments on wheat photosynthetic performance and grain-filling parameters using GLM in SPSS 13.0. A logistic equation of grain filling was modeled using SPSS 13.0. All graphics were produced using Excel and SigmaPlot 12.5.

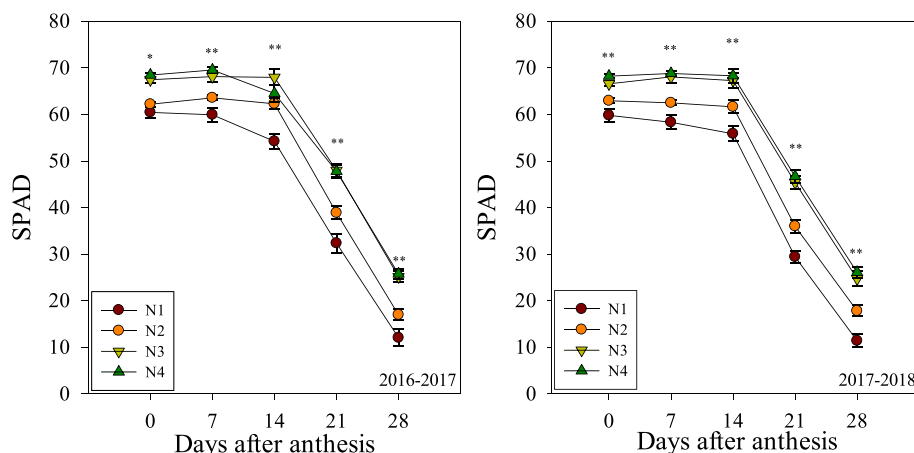
## Results

### The chlorophyll content (SPAD value)

Changes in SPAD value of flag leaves after anthesis are shown in Fig. 2. Split nitrogen treatment had significant effects on the SPAD value in both growth seasons (Additional file 1: Table 1). In 2016–2017, the mean SPAD value of the N3 treatment was significantly higher than that of the N1 and N2 treatments, and no significant differences were detected between the N3 and N4 treatment. Similar results were obtained in both growth seasons. These results indicated that plants under the N3 treatment maintained a longer effective duration of photosynthesis, compared with the other treatments.

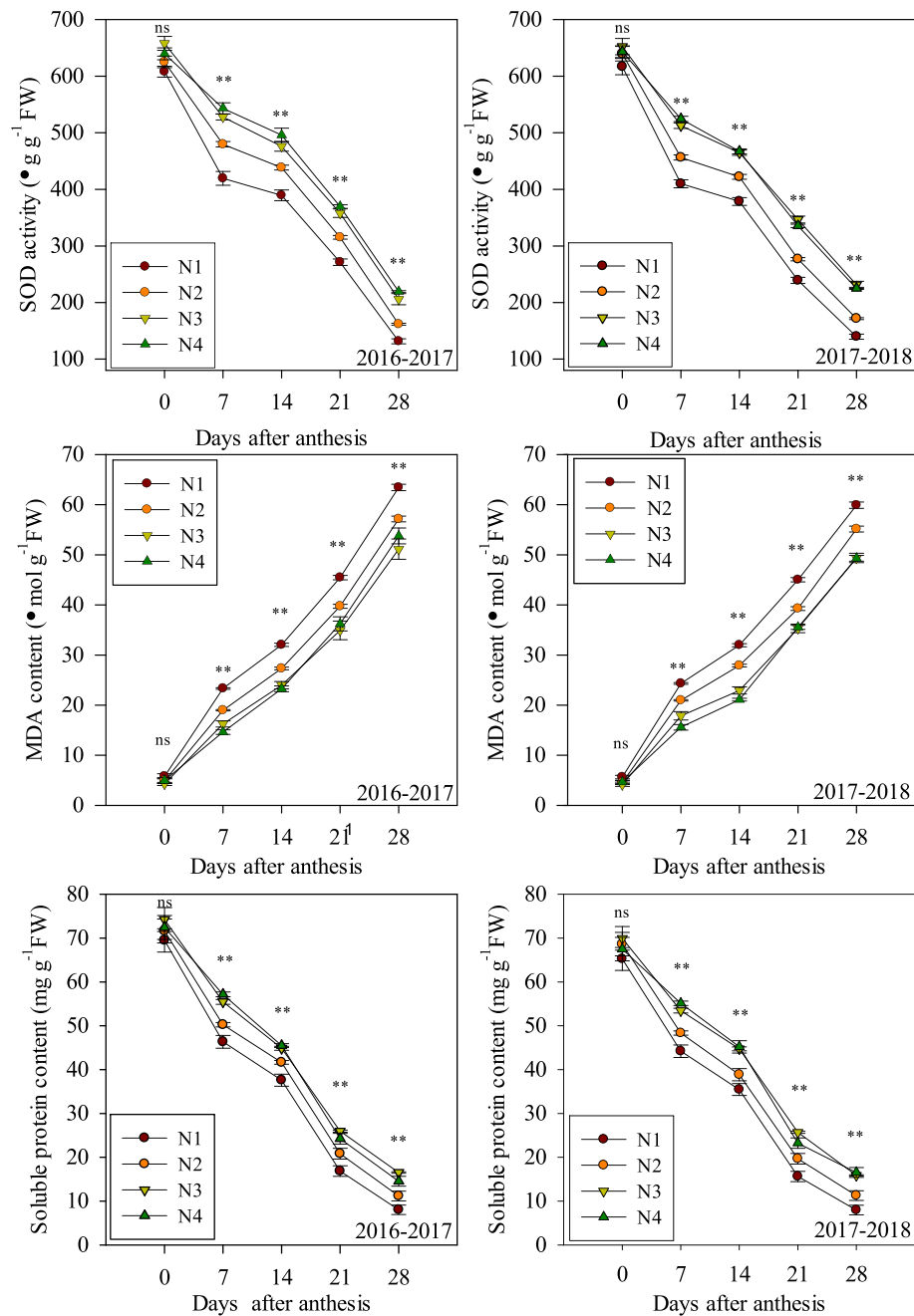
### Antioxidation indexes of flag leaves

Changes in MDA content, SOD activity, and soluble protein content in the flag leaves after anthesis are shown in Fig. 3. Split nitrogen treatments had no significant effects on MDA content, SOD activity, and soluble protein content at 0 DAA after anthesis (Additional file 1: Table 2). However, significant differences were detected among the different treatments at 7, 14, 21, and 28 DAA. Compared with the N1 and N2 treatments, the N3 treatment showed significant increases in SOD activity and soluble protein content at 7, 14, 21, and 28 DAA after anthesis and a significant decrease in MDA content. No significant differences were detected between N3 and N4 treatments. These results indicated that the



**Fig. 2** Effects of four split nitrogen fertilizer treatments on flag leaf SPAD value at 0 days, 7 days, 14 days, 21 days, and 28 days after anthesis. \*\* $P < 0.01$ ; \* $P < 0.05$





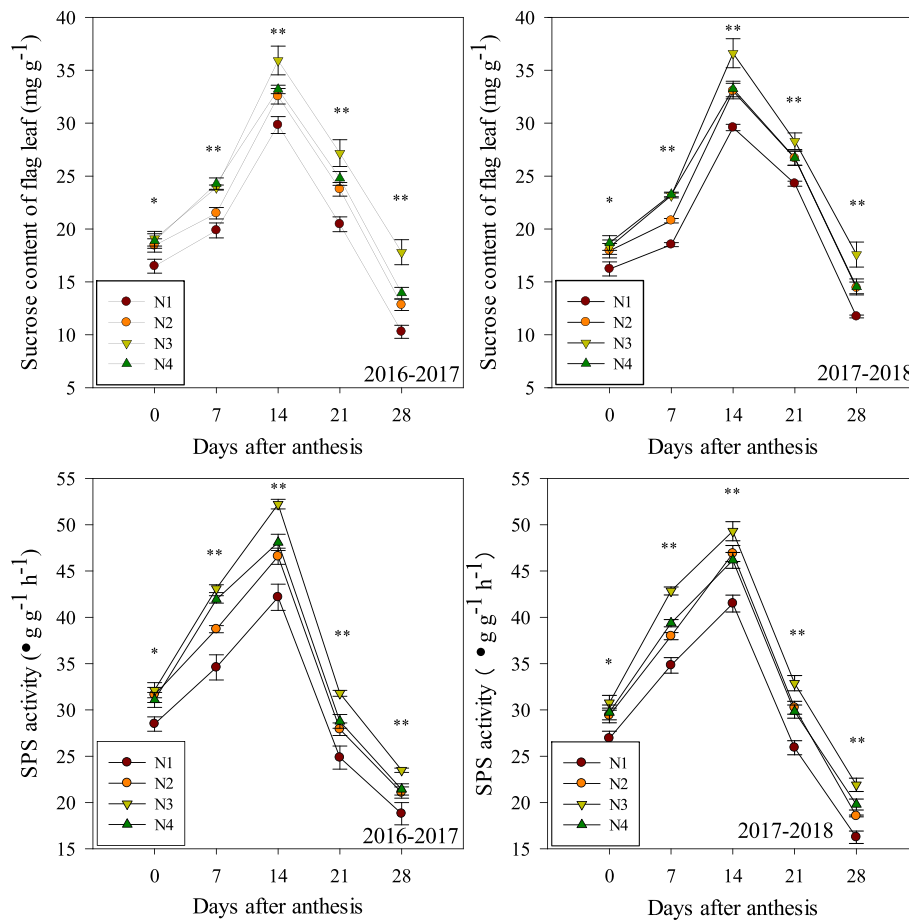
**Fig. 3** Effects of four split nitrogen fertilizer treatments on flag leaf MDA content, SOD activity, and soluble protein content at 0 days, 7 days, 14 days, 21 days, and 28 days after anthesis. ns, not significant; \*\* $P < 0.01$ ; \* $P < 0.05$

N3 treatment extended the duration of high SOD activity and soluble protein content, which was conducive to delaying the senescence and apoptosis of leaf cells.

#### Sucrose content and SPS activity of flag leaves

Changes in the sucrose content and SPS activity levels in the flag leaves after anthesis are shown in Fig. 4. The sucrose content and SPS activity first increased and then decreased as senescence progressed from 0 to 28 DAA.

Sucrose content and SPS activity were highest at 14 DAA. Split nitrogen treatments had significant effects on sucrose content and SPS activity (Additional file 1: Table 3). Significantly higher levels were detected in the N3 treatment compared to the N1 treatment, whereas no significant differences were detected between N3 and N2 treatments or the N3 and N4 treatments at 0 DAA. At 7 DAA, the sucrose content in the N3 treatment was significantly higher than that in the N1 and N2 treatments,



**Fig. 4** Effects of four split nitrogen fertilizer treatments on flag leaf source content and SPS activity at 0 days, 7 days, 14 days, 21 days, and 28 days after anthesis. ns, not significant; \*\* $P < 0.01$ ; \* $P < 0.05$

while no significant differences were detected between N3 and N4 treatments. From 14 to 28 DAA, sucrose content was highest in the N3 treatment, followed by the N2 and N4 treatments, while the lowest content was detected in the N1 treatment. SPS activity was similar across treatments. Overall, sucrose content and SPS activity in the flag leaves were improved by the N3 treatment.

#### Translocation after anthesis in vegetative organ

In 2016–2017, total dry matter biomass at anthesis and maturity and the DMT did not differ significantly among treatments (Table 1). The CDMT did not differ significantly among the N2, N3, and N4 treatments, but the CDMT from the N1 treatment was always higher than that of the other nitrogen treatments. The DMAA was the highest in the N3 treatment, followed by N2 and N4 treatments, and lowest in the N1 treatment. The CDMAA was significantly higher in the N3 treatment compared to the N1 treatment but did not differ significantly with respect to the N2, or the N4 treatments. The

results obtained in 2017–2018 showed almost the same trends as those obtained in 2016–2017. Altogether, our data indicated that the dry matter assimilation after anthesis was higher under the N3 treatment and that the dry matter redistribution from the vegetative organs to the developing grain remained at a higher level after anthesis.

#### $^{13}\text{C}$ photosynthate distribution in different organs of wheat

The changes in distribution of  $^{13}\text{C}$  photosynthate among different organs at 72 h after labeling and at maturity are shown in Table 2. In 2016–2017, stems, sheaths, spike axes, and husks under the N3 treatment had higher  $^{13}\text{C}$  photosynthate contents by averages of 15.80% and 10.52% and 11.99% and 8.17% compared to those under the N1 and N2 treatments, respectively. However, no significant differences were detected between the N3 and N4 treatments. Additionally, the leaf  $^{13}\text{C}$  photosynthate content did not differ significantly among treatments at 72 h after labeling. At maturity, the  $^{13}\text{C}$

**Table 1** Effects of four split nitrogen fertilizer treatments on dry matter accumulation amount at anthesis and maturity and dry matter translocation after anthesis. Different letters in each column represent significant differences in each tissue among four treatments at each sampling date according to LSD at 5% level

Year	Treatments	Dry matter accumulation amount at anthesis (kg ha <sup>-1</sup> )	Dry matter accumulation amount at maturity (kg ha <sup>-1</sup> )	DMT (kg ha <sup>-1</sup> )	CDMT (%)	DMAA (kg ha <sup>-1</sup> )	CDMAA (%)
2016–2017	N1	12,197.10a	16,999.66a	2490.61a	34.15a	4802.56c	65.85b
	N2	11,973.31a	17,495.29a	2395.95a	30.26b	5521.98b	69.74a
	N3	11,977.48a	17,983.05a	2474.11a	29.17b	6005.57a	70.82a
	N4	12,345.23a	17,907.85a	2460.01a	30.66b	5562.62b	69.34a
2017–2018	N1	11,594.27a	16,032.37a	2468.70a	35.74a	4438.10c	64.26b
	N2	11,227.28a	16,256.94a	2374.28a	32.07b	5029.66b	67.93a
	N3	11,391.45a	16,897.80a	2555.17a	31.70b	5506.35a	68.30a
	N4	11,583.62a	16,731.20a	2465.35a	32.38b	5147.58b	67.62a

DMT Dry matter translocation amount, CDMT Contribution of pre-anthesis assimilates to grain, DMAA The dry matter accumulation amount after anthesis, CDMAA The contribution of dry matter accumulation amount after anthesis to grains

photosynthate contents in the leaves, stems, and sheaths were significantly lower in the N3 treatment than in the other split nitrogen treatments. The spike axis and husk <sup>13</sup>C photosynthate content were significantly higher in the N3 treatment than in the N1 and N2 treatments; however, no significant differences were detected between the N3 and N4 treatments. The grain <sup>13</sup>C photosynthate content was higher under the N3 treatment by averages of 24.43%, 11.83%, and 6.44% compared to the N1, N2, and N4 treatments, respectively. Similar responses to treatments were observed in both growth

seasons. Overall, the N3 treatment significantly increased carbohydrate assimilation after anthesis and their distribution from vegetative organs to the grains, which likely accounts for the higher grain yield observed for plants in the N3 treatment.

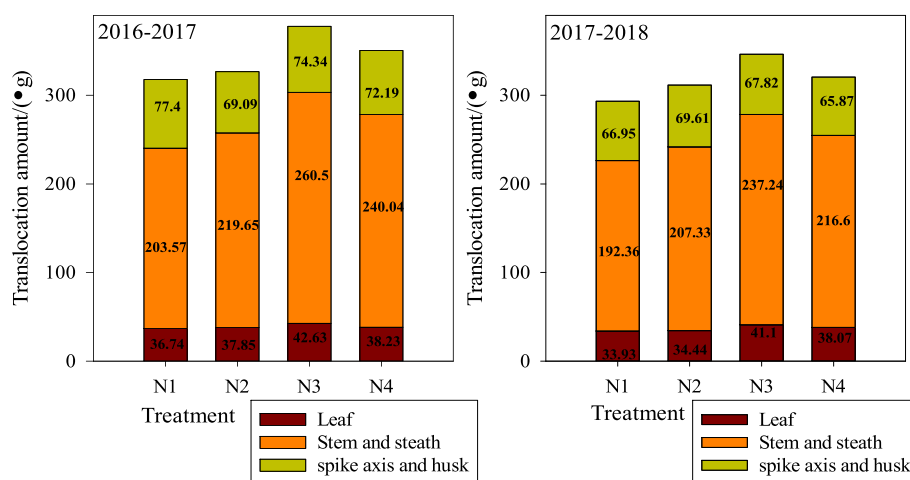
#### <sup>13</sup>C photosynthate translocation after anthesis

In both growth seasons, split nitrogen treatments had no effect on the translocation amount of the spike axis and husk <sup>13</sup>C photosynthates but did significantly affect the translocation amounts of leaf and stem <sup>13</sup>C

**Table 2** Effects of four split nitrogen fertilizer treatments on <sup>13</sup>C distribution amount in different organs at 72 h after labeling and maturity in wheat plants. Different letters in each column represent significant differences in each tissue among four treatments at each sampling date according to LSD at 5% level

Stage	Year	Treatments	<sup>13</sup> C distribution amount (μg stem <sup>-1</sup> )			
			Leaf	Stem and sheath	Spike axis and husk	Grain
At 72 h after labeling	2016–2017	N1	70.88a	295.89c	112.06b	-
		N2	67.04a	310.02b	116.02b	-
		N3	68.95a	342.64a	125.50a	-
		N4	66.89a	326.75a	120.81a	-
	2017–2018	N1	69.52a	276.01b	110.11a	-
		N2	65.72a	285.30b	112.48a	-
		N3	68.92a	309.35a	115.92a	-
		N4	68.98a	294.79b	113.70a	-
At maturity	2016–2017	N1	34.14a	92.32a	34.66b	274.80d
		N2	29.19b	87.37b	49.93a	305.75c
		N3	26.32c	81.99c	51.16a	341.92a
		N4	28.66b	86.71b	48.62a	321.22b
	2017–2018	N1	35.59a	83.65a	43.16b	263.24c
		N2	31.28b	77.97b	42.87b	288.20b
		N3	27.82c	72.11c	48.10a	324.96a
		N4	30.91b	78.19b	47.83a	300.96b





**Fig. 5** Effects of four split nitrogen fertilizer treatments on  $^{13}\text{C}$  photosynthate translocation amount in vegetative organ after anthesis

photosynthates (Fig. 5). In 2016–2017, the translocation amounts of leaf and stem  $^{13}\text{C}$  photosynthates under the N3 treatment were higher by averages of 16.03%, 12.63%, and 11.51%, and 27.97%, 17.00%, and 8.52% compared to the N1, N2, and N4 treatments, respectively. The second growth season showed similar results. Collectively, these results indicated that the N3 treatment substantially promoted the translocation of photosynthates to the grain, especially from the leaves and stems.

#### Grain yield and yield components

The effects of split nitrogen fertilizer treatments on grain yield and its components in both growth seasons are shown in Table 3. Split nitrogen fertilizer treatments significantly affected grain yield in both the 2016 and 2017 seasons. Compared with N1, N2, and N4 treatments, the N3 treatment increased grain yield by 16.27%, 7.09%, and 5.70% in 2016–2017 and 16.72%, 8.88%, and 5.89% in 2017–2018, respectively. The 1000-grain weight and the number of grains per spike were significantly higher in the N3 treatment, compared with any other split

nitrogen treatments. Spike number did not differ significantly among the treatments in both growth seasons. These results indicated that the N3 treatment led to a significant increase in grain yield that was mainly attributed to the increase in 1000-grain weight and the number of grains per spike.

## Discussion

### Effects of split nitrogen fertilization on flag leaf antioxidant capacity

Chlorophyll is essential for leaf photosynthesis, and its content in wheat leaves reflects their photosynthetic capacity (Hlavacova et al. 2018). Nitrogen affects and participates in chlorophyll synthesis (Christopher et al. 2014; Gaju et al. 2014). Moderate split nitrogen fertilizer application has been shown to increase leaf chlorophyll content, which promotes the growth of aboveground parts, such as leaves, and thus leads to an increase in biomass (Xie et al. 2016; Yang et al. 2017; Tian et al. 2020). In our study, moderate split nitrogen ratios readjusted the flag leaf antioxidant capacity, such that plants under the N3 treatment showed the highest SOD activity and

**Table 3** Effects of four split nitrogen fertilizer treatments on grain yield and components at maturity. Different letters in each column represent significant differences in each tissue among four treatments at each sampling date according to LSD at 5% level

Year	Treatment	Grain yield (kg ha <sup>-1</sup> )	1000-grain weight (g)	Grains per spike	Spike number (×10 <sup>4</sup> ha <sup>-1</sup> )
2016–2017	N1	7293.17c	39.05c	37.96b	566.31a
	N2	7917.93b	41.47b	38.47b	575.67a
	N3	8479.68a	44.31a	40.92a	576.43a
	N4	8022.63b	41.95b	40.56a	593.19a
2017–2018	N1	6906.80c	37.80c	36.73b	559.83a
	N2	7403.94b	40.32b	37.06b	565.92a
	N3	8061.52a	43.21a	39.38a	562.74a
	N4	7612.93b	40.56b	38.97a	576.29a

soluble protein content after anthesis, compared with the other split nitrogen treatments. In contrast, in the unequal ratio treatments (N1, N2, N4), the increase in antioxidant enzyme activity was not sufficient for detoxification of reactive oxygen species, which caused damage to photosystems and contributed to chlorophyll degradation (Zhang et al. 2018). The results of this study showed that flag leaf SPAD values decreased significantly under excessive basal or topdressing nitrogen fertilizer treatments. However, SPAD values significantly improved, and MDA content was significantly reduced under the N3 treatment, which indicated that the N3 treatment was the most appropriate split nitrogen treatment for the protection of chlorophyll integrity and functionality. These responses and the low level of chlorophyll degradation under the N3 treatment may be associated with plants maintaining a higher water status as a result of an improved root system under this treatment (Kitonyo et al. 2018a, 2018b). Overall, these findings indicated that the equal ratio treatment resulted in the flag leaves having a longer duration of photosynthetic capacity than those in any of the unequal ratio treatments (Kitonyo et al. 2017), which favored higher levels of photosynthetic assimilation.

#### **Effects of split nitrogen fertilization on dry matter accumulation amount, $^{13}\text{C}$ -photosynthate translocation, and distribution**

One objective of the experiment was to determine how split nitrogen fertilizer treatment affects photosynthate assimilation, distribution, and redistribution. Our experiment allowed to observe positive effects of the split nitrogen treatments on assimilation in vegetative organs, photosynthesis capacity, and grain yield. SPS enzyme transforms photosynthesis assimilates into sucrose and contributes to grain yield (Liu et al. 2020b). The results of our study showed that sucrose content of flag leaves was higher in the N3 treatment than in the other split nitrogen treatments from 14 DAA to 28 DAA, which coincided with the N3 treatment having the highest SPS activity. The final grain yield of winter wheat is determined by the ability of dry matter assimilation in the grain-filling stage (Kamiji et al. 2014). Our results showed that the dry matter assimilation after anthesis was significantly reduced under excessive basal or topdressing nitrogen fertilizer regimens, which was in accordance with previous observations (Liang et al. 2017).

Our assessment of the amount of  $^{13}\text{CO}_2$  fixed by wheat plants and translocated to each organ showed that the leaves provided an efficient model to appraise the contribution of each vegetative organ to the grain under different split nitrogen treatments. Our  $^{13}\text{C}$ -isotope tracer experiment revealed that the equal ratio treatment (N3) significantly increased the  $^{13}\text{C}$  photosynthate

accumulation post-anthesis and its contribution to grain compared to the unequal ratio treatments (N1, N2, and N4). This is likely the result of an improved source-sink relationship because dry matter partitioning was biased towards the reproductive organs. The equal ratio treatment resulted in a higher accumulation of dry matter compared to the unequal ratio treatments, especially during anthesis. This higher accumulation of dry matter seemingly occurred because a higher abundance of assimilates at anthesis enabled plants grown under the equal ratio treatment to produce more leaves with enhanced longevity, as needed to increase the source capacity and thus, optimize leaf cover for light interception and use (Lv et al. 2017; Si et al. 2020). Photosynthates are produced by photosynthesis, and nutrient deficiencies at grain-filling stage can accelerate leaf senescence by increasing ROS production (Okamura et al. 2018), while the supply of nitrogen fertilizer during grain-filling can delay leaf senescence and increase wheat yield (Zhang et al. 2019).

In addition, our findings demonstrated that split nitrogen application altered the dry matter allocation among plant organs in wheat plants. Total  $^{13}\text{C}$  assimilates translocated to the grain, the stems, and sheaths made the highest contribution, followed by the spike axes and husks, while leaves made the lowest contributions. In this study, as basal or topdressing nitrogen fertilizer increased, wheat plants showed an increase in vegetative dry matter accumulation and a decrease in dry matter allocation to spikes. These effects negatively affected dry matter accumulation in the grain at grain-filling stage, which led to a reduced dry matter accumulation in the grain at maturity (Finnan et al. 2019). However, it is noteworthy that the N3 treatment not only increased the amount of photosynthesis assimilates produced in the post-anthesis period, but also promoted the post-anthesis translocation of carbohydrates from the vegetative organs to the grains as well, thus, further supporting the notion that equal ratio treatments (such as that of N3) can increase grain yield under conditions of water-saving irrigation.

#### **Effects of split nitrogen fertilization on grain yield and yield components**

Previous studies have shown that wheat yield was highly correlated with the number of grains per spike and spike number under the sufficient irrigation condition (Rivera-Amado et al. 2019). Current approaches to increase grain yield are mainly focused on increasing the number of grains per spike or 1000-grain weight, but it has proven difficult to further increase the capacity of the grain sink (Agami et al. 2018). Therefore, 1000-grain weight is the most important restrictive factor affecting grain yield. The results of our experiment under conditions of water-saving irrigation further demonstrated

that the N3 treatment significantly improved grain yield, which was attributed to a higher number of grains per spike concomitantly with a higher 1000-grain weight.

The unequal ratio treatments (N1, N2, and N4) showed significantly lower yield potential than the equal ratio treatment (N3). This decrease in grain yield can be explained by two factors, mainly: (1) excessive basal nitrogen fertilizer application might have led to an insufficient supply of dry matter for grain filling at the grain-filling stage because the initiation of grain filling requires a large supply of photoassimilate, and (2) excessive topdressing nitrogen fertilizer application delayed plant maturity, as it favored continued vegetative growth. Zhang et al. (2018) reported that nitrogen fertilizer application significantly improved yield by increasing spike number. Interestingly, split nitrogen fertilizer treatments in the present study had no significant effect on spike number (Yang et al. 2017). This discrepancy may be attributed to different climatic conditions and the effects of soil fertility on spike number between the two studies (Li et al. 2020). Nonetheless, our study provides substantial evidence that split nitrogen management can help towards a significant improvement of wheat production. However, the evaluation of the effects of fertilizer management practice on wheat soil environment and wheat yield warrants further research.

## Conclusion

Here, we investigated the effects of split nitrogen fertilizer management on  $^{13}\text{C}$  photosynthate mobilization and antioxidant enzyme activity in winter wheat under water-saving irrigation conditions. Altogether, our findings confirmed that the 240 kg ha<sup>-1</sup> of total nitrogen fertilizer treatment applied in a 5:5 basal-topdressing ratio significantly increased the SPAD values, antioxidant enzymes activities, sucrose content, and SPS activity but reduced the accumulations of MDA in wheat plants. Furthermore, the treatment significantly increased the assimilation of carbohydrates after anthesis and promoted their distribution from vegetative organs to the developing grains. The 5:5 top-dressing N fertilizer treatment effectively increased grain yield by increasing 1000-grain weight and the number of grains per spike. Therefore, combined water-saving irrigation and split nitrogen management are recommended for grain yield improvement of winter wheat in the region of the HPC.

## Abbreviations

HPC: Huang-Huai-Hai Plain of China; SPAD: Soil-plant analysis development; SOD: Superoxide dismutase; MDA: Malondialdehyde; SPS: Sucrose phosphate synthetase; EDTA: Ethylenediaminetetraacetic acid; PVPP: Polyvinylpyrrolidone; NBT: Nitroblue tetrazolium; DTT: DL-Dithiothreitol; KCL: Potassium chloride; DMT: Dry matter translocation amount; DMA: Dry matter accumulation amount at anthesis; DMM: Dry matter of vegetative plant parts at maturity; CDMT: Contribution of pre-anthesis assimilates to grain; GY: Grain yield; DMAA: Dry matter accumulation amount after anthesis; CDMAA: Contribution of dry matter accumulation amount after anthesis to grain

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-021-00290-9>.

**Additional file 1: Table 1.** Results of ANOVA about Fig. 1. **Table 2.** Results of ANOVA about Fig. 2. **Table 3.** Results of ANOVA about Fig. 3.

## Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (31771715, 31771717); Project of Technology System in Modern Wheat Industry, Ministry of Agriculture, China (CARS-3-1-19); and Field Station Union Project of Chinese Academy of Sciences (KFJ-SW-YW035).

## Authors' contributions

Data curation, Zhen Zhang; formal analyses, Zhen Zhang; founding acquisition, Yu Shi and Yongli Zhang; investigation, Zhen Zhang and Yu Shi; project administration, Yu Shi; writing original draft, Zhen Zhang; writing review and editing, Zhen Zhang, Zhenwen Yu, Yongli Zhang, Yu Shi. All authors have read and agreed to the published version of the manuscript.

## Funding

The National Natural Science Foundation of China (31771715, 31771717); Project of Technology System in Modern Wheat Industry, Ministry of Agriculture, China (CARS-3-1-19); and Field Station Union Project of Chinese Academy of Sciences (KFJ-SW-YW035).

## Availability of data and materials

Not applicable

## Declarations

## Ethics approval and consent to participate

Not applicable

## Consent for publication

All authors agreed and approved the manuscript for publication in *Ecological Processes*.

## Competing interests

The authors declare that they have no competing interests.

Received: 25 December 2020 Accepted: 23 February 2021

Published online: 12 March 2021

## References

- Agami RA, Alamri SAM, Abd El-Mageed TA, Abousekken MSM (2018) Role of exogenous nitrogen supply in alleviating the deficit irrigation stress in wheat plants. *Agric Water Manag* 210:261–270
- Azam B, Mir AMS, Ali E (2020) Effects of salt and nitrogen on physiological indices and carbon isotope discrimination of wheat cultivars in the northeast of Iran. *J Integr Agric* 19(3):656–667
- Christopher JT, Veyradier M, Borrell AK, Harvey G, Fletcher S, Chenu K (2014) Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics. *Funct Plant Biol* 41:1035–1048
- Dar EA, Brar AS, Mishra SK, Singh KB (2017) Simulating response of wheat to timing and depth of irrigation water in drip irrigation system using CERES-wheat model. *Field Crop Res* 214:149–163
- Feng LY, Raza MA, Li ZC, Chen YK, Khalid MH, Du JB, Liu WG, Wu XL, Song C, Yu L, Zhang ZW, Yuan S, Yang WY, Yang F (2019) The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front Plant Sci* 9:1952
- Finnan J, Burke B, Spink J (2019) The effect of nitrogen timing and rate on radiation interception, grain yield and grain quality in autumn sown oats. *Field Crop Res* 231:130–140
- Gaju O, Allard V, Martre P, Le Gouls J, Moreau D, Bogard M, Hubbert S, Foulkes MJ (2014) Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crop Res* 155:213–223
- Gao J, Zhao B, Dong ST, Liu P, Ren BZ, Zhang JW (2017) Response of summer maize photosynthate accumulation and distribution to shading stress

- assessed by using  $^{13}\text{CO}_2$  stable isotope tracer in the field. *Front Plant Sci* 8: 1821
- Guo ZJ, Shi Y, Yu ZW, Zhang YL (2015) Supplemental irrigation affected flag leaves senescence post-anthesis and grain yield of winter wheat in the Huang-Huai-Hai Plain of China. *Field Crop Res* 180:100–109
- He G, Cui ZL, Ying H, Zheng HF, Wang ZH, Zhang FS (2017) Managing the trade-offs among yield increase, water resources inputs and greenhouse gas emissions in irrigated wheat production systems. *J Clean Prod* 164:567–574
- Hlavacova M, Klem K, Rapantova B, Novotna K, Urban O, Hlavinka P, Smutna P, Horakova V, Skarpa P, Pohankova E, Wimmerova M, Orsag M, Jurecka F, Trnka M (2018) Interactive effects of high temperature and drought stress during stem elongation, anthesis and early grain filling on the yield formation and photosynthesis of winter wheat. *Field Crop Res* 221:182–195
- Jha SK, Ramatshaba TS, Wang G, Liang Y, Liu H, Gao Y, Duan A (2019) Response of growth, yield and water use efficiency of winter wheat to different irrigation methods and scheduling in North China Plain. *Agric Water Manag* 217:292–302
- Jiang HB, Li HX, Zhao MX, Mei XL, Kang YL, Dong CX, Xu YC (2020) Strategies for timing nitrogen fertilization of pear trees based on the distribution, storage, and remobilization of  $^{15}\text{N}$  from seasonal application of  $(^{15}\text{NH}_4)_2\text{SO}_4$ . *J Integr Agric* 19(5):1340–1353
- Kamiji Y, Pang JY, Milroy SP, Palta JA (2014) Shoot biomass in wheat is the driver for nitrogen uptake under low nitrogen supply, but not under high nitrogen supply. *Field Crop Res* 165:92–98
- Kitonyo OM, Sadras OV, Zhou Y, Denton MD (2018b) Nitrogen supply and sink demand modulate the patterns of leaf senescence in maize. *Field Crop Res* 225:92–103
- Kitonyo OM, Sadras VO, Zhou Y, Denton MD (2017) Evaluation of historic Australian wheat varieties reveals increased grain yield and change in senescence patterns but limited adaptation to tillage system. *Field Crop Res* 206:65–73
- Kitonyo OM, Sadras VO, Zhou Y, Denton MD (2018a) Nitrogen supply and sink demand modulate the patterns of leaf senescence in maize. *Field Crop Res* 225:92–103
- Latifmanesh H, Deng AX, Nawaz MM, Li L, Chen ZJ, Zheng YT, Wang P, Song ZW, Zhang CY, Zhang WJ (2018) Integrative impacts of rotational tillage on wheat yield and dry matter accumulation under corn-wheat cropping system. *Soil Tillage Res* 184:100–108
- Li QQ, Zhao XB, Chen YH, Yu SL (2012) Water consumption characteristics of winter wheat grown using different planting patterns and deficit irrigation regime. *Agric Water Manag* 105:8–12
- Li XN, Ulfat A, Shokat S, Liu SQ, Zhu XC, Liu FL (2019) Responses of carbohydrate metabolism enzymes in leaf and spike to  $\text{CO}_2$  elevation and nitrogen fertilization and their relations to grain yield in wheat. *Environ Exp Bot* 164: 149–156
- Li YJ, Ma LS, Wu P, Zhao XN, Chen XL, Gao XD (2020) Yield, yield attributes and photosynthetic physiological characteristics of dryland wheat (*Triticum aestivum* L.) /maize (*Zea mays* L.) strip intercropping. *Field Crop Res* 248: 107656
- Liang WX, Zhang ZC, Wen XX, Liao YC, Liu Y (2017) Effect of non-structural carbohydrate accumulation in the stem pre-anthesis on grain filling of wheat inferior grain. *Field Crop Res* 211:66–76
- Liu M, Wu XL, Li CS, Li M, Xiong T (2020b) Dry matter and nitrogen accumulation, partitioning, and translocation in synthetic-derived wheat cultivars under nitrogen deficiency at the post-jointing stage. *Field Crop Res* 248:107720
- Liu Y, Zhang XL, Xi LY, Liao YC, Han J (2020a) Ridge-furrow planting promotes wheat grain yield and water productivity in the irrigated sub-humid region of China. *Agric Water Manag* 231:105935
- Lollato RP, Figueiredo BM, Dhillon JS, Arnall DB, Raun WR (2019) Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: a synthesis of long-term experiments. *Field Crop Res* 236:42–57
- Luo YL, Tang YH, Zhang X, Li WQ, Chang YL, Pang DW, Xu X, Li Y, Wang ZL (2018) Interactions between cytokinin and nitrogen contribute to grain mass in wheat cultivars by regulating the flag leaf senescence process. *Crop J* 6: 538–551
- Lv XK, Han J, Liao YC, Liu Y (2017) Effect of phosphorus and potassium foliage application post-anthesis on grain filling and hormonal changes of wheat. *Field Crop Res* 214:83–93
- Man JG, Wang D, White PJ, Yu ZW (2014) The length of micro-sprinkling hoses delivering supplemental irrigation affects photosynthesis and dry matter production of winter wheat. *Field Crop Res* 168:65–74
- Mostafa H, El-Nady R, Awad M, El-Ansary M (2018) Drip irrigation management for wheat under clay soil in arid conditions. *Ecol Eng* 121:35–43
- Nehe AS, Misra S, Murchie EH, Chinnathambi K, Tyagi BS, Foulkes MJ (2020) Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and protein concentration in Indian wheat cultivars. *Field Crop Res* 251: 107778
- Okamura M, Arai-Sanoh Y, Yoshida H, Mukouyama T, Adachi S, Yabe S, Nakagawa H, Tsutsumi K, Taniguchi Y, Kobayashi N, Kondo M (2018) Characterization of high-yielding rice cultivars with different grain-filling properties to clarify limiting factors for improving grain yield. *Field Crop Res* 219:139–147
- Ren BZ, Hu J, Zhang JW, Dong ST, Liu P, Zhao B (2020) Effects of urea mixed with nitrpyrin on leaf photosynthetic and senescence characteristics of summer maize (*Zea mays* L.) waterlogged in the field. *J Integr Agric* 19(6): 1586–1595
- Rivera-Amado C, Trujillo-Negrellos E, Molera G, Reynolds MP, Sylvester-Bradley R, Foulkes MJ (2019) Optimizing dry-matter partitioning for increased spike growth, grain number and harvest index in spring wheat. *Field Crop Res* 240: 154–167
- Roche J, Turnbull MH, Guo QQ, Novak O, Spath J, Gieseg SP, Jameson PE, Love J (2017) Coordinated nitrogen and carbon remobilization for nitrate assimilation in leaf, sheath and root and associated cytokinin signals during early regrowth of *Lolium perenne*. *Ann Bot* 119:1353–1364
- Santis MAD, Giuliani MM, Flagella Z, Reyneri A, Blandino M (2020) Impact of nitrogen fertilisation strategies on the protein content, gluten composition and rheological properties of wheat for biscuit production. *Field Crop Res* 254:107829
- Si ZY, Zain M, Mehmood F, Wang GS, Gao Y (2020) Effects of nitrogen application rate and irrigation regime on growth, yield and water-nitrogen use efficiency of drip-irrigated winter wheat in the North China Plain. *Agric Water Manag* 231:106002
- Singh H, Verma A, Ansari MW, Shukla A (2014) Physiological response of rice (*Oryza sativa* L.) genotypes to elevated nitrogen applied under field conditions. *Plant Signal Behav* 9:7
- Tan Y, Xu C, Liu D, Wu W, Lai R, Meng F (2017) Effects of optimized N fertilization on greenhouse gas emission and crop production in the North China Plain. *Field Crop Res* 205:135–146
- Thapa S, Jessup KE, Pradhan GP, Rudd JC, Liu SY, Mahan JR, Devkota RN, Baker JA, Xue QW (2018) Canopy temperature depression at grain filling correlates to winter wheat yield in the U.S. Southern High Plains. *Field Crop Res* 217: 11–19
- Tian D, Zhang YY, Mu YJ, Liu JF, He KB (2020) Effect of N fertilizer types on  $\text{N}_2\text{O}$  and NO emissions under drip fertigation from an agricultural field in the North China Plain. *Sci Total Environ* 715:136903
- Trost B, Prochnow A, Meyer-Aurich A, Drastig K, Baumecker M, Ellmer F (2016) Effects of irrigation and nitrogen fertilization on the greenhouse gas emissions of a cropping system on a sandy soil in Northeast Germany. *Eur J Agron* 81:117–128
- Wu YW, Li Q, Jin R, Chen W, Liu XL, Kong FL, Ke YP, Shi HC, Yuan JC (2019) Effect of low-nitrogen stress on photosynthesis and chlorophyll fluorescence characteristics of maize cultivars with different low-nitrogen tolerances. *J Integr Agric* 18(6):1246–1256
- Xie Q, Mayes S, Sparkes DL (2016) Early anthesis and delayed but fast leaf senescence contribution to individual grain dry matter and water accumulation in wheat. *Field Crop Res* 187:24–34
- Yang J, Udvardi M (2017) Senescence and nitrogen use efficiency in perennial grasses for forage and biofuel production. *J Exp Bot* 69:855–865
- Yang XL, Lu YL, Ding Y, Yin XF, Raza S, Tong YA (2017) Optimising nitrogen fertilisation: a key to improving nitrogen-use efficiency and minimising nitrate leaching losses in an intensive wheat/maize rotation (2008–2014). *Field Crop Res* 206:1–10
- Zhang HB, Han K, Gu SB, Wang D (2019) Effects of supplemental irrigation on the accumulation, distribution and transportation of  $^{13}\text{C}$ -photosynthate, yield and water use efficiency of winter wheat. *Agric Water Manag* 214:1–8
- Zhang L, Liu HH, Sun JQ, Li JC, Song YH (2018) Seeding characteristics and grain yield of maize grown under straw retention affected by sowing irrigation and splitting nitrogen use. *Field Crop Res* 225:22–31

Zhao HX, Zhang P, Wang YY, Ning TY, Xu CL, Wang P (2020) Canopy morphological changes and water use efficiency in winter wheat under different irrigation treatments. *J Integr Agric* 19:1105–1116

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:**

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)

---