

## THE EFFECTS OF N INPUT LEVEL ON N UPTAKE, N REMOBILIZATION AND AGRONOMIC TRAITS UNDER DEFICIT IRRIGATION CONDITION IN WINTER WHEAT

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### ABSTRACT

Improvement of the nitrogen use efficiency (NUE) positively impacts on the growth and development of plants as well as the crop productivity. In this study, we investigated the characterization on N uptake, N remobilization, and agronomic traits of winter wheat under N input treatments combined by deficit irrigation. Under the sufficient-N treatment (N240), the accumulative N amounts and internal N remobilization rates in plants of the tested cultivars (Jimai 585 and Shimai 22) were elevated relative to those under the deficient-N treatment (N120), together with improvement on plant biomass and the grain yields. Compared with Jimai 585, a control cultivar to be acclimation to affluent irrigation condition, the drought tolerant cultivar Shimai 22 enhanced the accumulative N amount, N harvest index (NHI), NUE, and agronomic traits under both N input treatments, especially under N120. In addition, Shimai 22 also displayed higher activities of nitrate reductase (NR), nitrite reductase (NIR), and glutamine synthetase (GS) than Jimai 585 under the both N level conditions. The soil N contents (NC) in 2 m profile were elevated whereas the soil moisture contents were lowered in plots planted by the wheat cultivars during late stages under N240 with respect to those under N120. Moreover, reduced soil moisture and N contents in 2 m soil profile were found in the plots grown by Shimai 22 with respect to those by Jimai 585 under N120, suggesting that Shimai 22 improved the N uptake, N remobilization, and the agronomic traits under low N input treatment to be associated with the enhanced N assimilation-related enzymatic activities and the elevated capacity for usage of the soil N storage. Therefore, it is feasible to cultivate winter wheat under the water- and N saving conditions by using the drought tolerant cultivars due to their high NUE and effective usage for the soil nutrient storage.

**Keywords:** agronomic trait, deficit irrigation, nitrogen level, plant N acquisition, wheat (*Triticum aestivum* L.)

### INTRODUCTION

In North China Plain, winter wheat and summer maize constitute the major cropping system that contributes largely to the sustainable agriculture in this ecological region. However, the double planting model around whole year consumes much more water resource due to the high-yielding purpose together with the low rainfall amount during growth seasons. The long term of irrigation management during winter wheat seasons has drastically reduced the underground water table and limited on the sustainable crop development (Sun et al., 2010; Zhang et al., 2018). Aside from the shortage of water resource to restrict the crop production, unsuitable management of the inorganic fertilizers, especially N fertilizer, also negatively impact on the yield formation and the product quality of

the winter wheat (Ping et al., 2009; Wang et al., 2010). The frequently overdose of the N fertilizer in winter wheat has intensified the environmental pollution due to the reduced NUE (Liu et al., 2013; Gao et al., 2016). Therefore, suitable management on N fertilizer in winter wheat production can help to improve NUE and contribute to the sustainable development of the crops.

A suite of investigations have been performed focusing on understanding of the N uptake and remobilization properties in wheat under various irrigation and N input treatments (Sharma et al., 1990; Zhang et al., 2015). The N acquisition and the N distribution patterns in plants of the wheat cultivars were modified by the irrigation treatments (Wang et al., 2002; Zhang et al., 2008). Under high N level condition, the plants in wheat cultivars

generally accumulated much more amount of N nutrition but displayed lower internal N remobilization rate than those under the low N amount treatment (Gregory et al., 1981; Sharma et al., 1990). In addition, the N acquisition and N remobilization rate were drastically varied across the wheat cultivars (genotypes). The cultivars sharing high NUE possess strong capacity on N uptake and internal N translocation upon the N deficiency (Gao et al., 2016; Nehe et al., 2018), due to the improvement on physiological and biochemical processes associated with N acquisition and N assimilation (Zhang et al., 2015). These findings suggest that it is an effective strategy to cultivate winter wheat by using the high NUE cultivars.

Although the properties on the N acquisition and internal N remobilization have been reported in plants of winter wheat (Gregory et al., 1981; Sharma et al., 1990; Zhang et al., 2015), the physiological mechanisms underlying the N uptake and internal N translocation in wheat plants following the water- and N-saving treatments are still needed to be further understood. In this study, we investigated the behaviors on N uptake, N remobilization, and agronomic traits in plants under the deficit irrigation condition using two wheat cultivars displaying contrasting drought tolerance. The main objectives in this investigation are as follows: (i) To characterize the N accumulation and internal N remobilization of plants at various stages as well as the biochemical process associated with N assimilation in plants challenged by limited water; (ii) To understand the N contents in soil profile at late growth stage and agronomic traits of the wheat cultivars under various N input treatments combined by deficit irrigation; (iii) To elucidate the relation between yield and the N-associated traits, such as N amount, N remobilization rate, and the contents of N and moisture in soil.

## MATERIALS AND METHODS

### *Experimental design*

The field experiments were conducted at Liujiashuang, Gaocheng city, China, during the 2016-2017 and 2017-2018 growth seasons. The climate in this region is typical temperate continental monsoon with the precipitation concentrated in summer season. Meteorological factors of experimental field at late stage during two growth seasons are shown in Table S1. The soil for experiment was loamy and contained follow nutrients: organic matter 16.5 g kg<sup>-1</sup>, available N 66.5 mg kg<sup>-1</sup>, available P 21.22 mg kg<sup>-1</sup>, and exchangeable K 122.32 mg kg<sup>-1</sup>. The plots in experiments were arranged in a split plot design with three replicates, in which, N application amount (sufficient-N, 240 kg N ha<sup>-1</sup> simplified by N240 and deficient-N, 120 kg N ha<sup>-1</sup> by N120) was set

up as main plot, whereas cultivar (Jimai 585, a control cultivar acclimated to affluent water and Shimai 22, a drought tolerant cultivar) as sub-main plot. In two growth seasons, the N120 treatment was initiated by 48 kg N ha<sup>-1</sup> (source of complex fertilizer, with N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O of 15-15-15) as basal after the straw broken of summer maize and by topdressing of 72 kg N ha<sup>-1</sup> (urea as N source) at jointing stage, together with jointing irrigation; N240 treatment was established by basal 96 kg N ha<sup>-1</sup> using same complex fertilizer mentioned above and by topdressing of 144 kg N ha<sup>-1</sup> at jointing stage together with the irrigation. For all of plots, deficit water irrigation management was applied as previous suggestion, namely, one spring irrigation was performed at jointing stage with water amount of 750 m<sup>3</sup>/ha by which to sustain maximum net profit of winter wheat (*Triticum aestivum* L.) in the North China Plain under limited supplemental water resource. The dates for irrigation were arranged at April 8 and 10 during the 2016-2017 and the 2017-2018 seasons, respectively. Plot area was 24 m<sup>2</sup> (6 m in length and 4 m in width) and the row distance of seedlings was 15 cm. The sowing dates were October 8 and 7 whereas the harvest dates were next June 12 and 10 for the 2016-2017 and 2017-2018 seasons, respectively. Seeding rates for the tested cultivars maintained the seedling amounts of 3,700 to 3,800 thousands per hectare. Other management practices performed were same as the conventional ones that are used in the high-yielding wheat production.

### *Assay of yields and yield components*

At maturity, the spikes in 2 m<sup>2</sup> planting area were counted by which to calculate the spike amounts per planting area. After that, the grains in the spikes were threshed using a mini harvest machine. Yields were calculated based on the harvested grains after air-drying. Thirty representative plants sampled in each plot were subjected to assay of the kernel numbers per spike and the grain weights following the conventional methods.

### *Measurements of plant biomass and N-associated traits*

At growth stages of seedling, jointing, flowering, and maturity, the phenological dates were recorded. Meanwhile, at each stage, thirty representative plants were sampled for assessment of the biomass, N content, N accumulative amount, and the N accumulation rate. Of which, the plant biomass was obtained after oven drying 48 h under 80°C. The N concentrations in the oven dried plant samples were assessed as described previously (Guo et al., 2011). The plant N accumulative amounts were calculated by multiplying the plant biomass and the N concentrations. The N accumulation rates in plants were defined at growth phase covering two adjacent stages by dividing the N accumulative amount to the phase duration.

**Table S1.** Meteorological factors of experimental field at late stage during two growth seasons

Year	10 d	Arverage temperature (°C)		Precipitation (mm)		Total sunshine (hour)		Solar radiation (W/m <sup>2</sup> )	
		May	June	May	June	May	June	May	June
2017	First	21.4	24.2	0.1	3.6	86.8	84.6	233	250
	Second	24.8	27.2	0	3.4	113	84.9	283	252
	Third	24.5	27	17.9	43.7	110.7	80.4	266	224
2018	First	20.3	26.5	5.5	31.8	87.8	86.1	242	232
	Second	22.1	26.6	23.8	21.7	45.7	82.8	250	239
	Third	23.6	30.1	43	0.4	111.4	85.8	274	231

*Assay of the N remobilization traits during late growth stage*

At flowering and maturity, thirty representative plants were departed into different tissues, including grain, stem, leaf, and glume. The N concentration in each tissue was analyzed using the approach as reported by Guo et al. (2011). The N accumulative amounts in the tissues were determined based on the biomass and the N concentration. The N harvest index (NHI), N remobilized amount during seed filling (NRA), N remobilized rate during seed filling (NRR), N use efficiency (NUE), and the N fertilizer production efficiency (NFPE), were calculated based on the tissue biomass, yields, and N accumulative amounts as reported by Zhang et al. (2015). Of which, NHI is defined by the ratio of grain N amounts and the plant N accumulative amount (NAA) at maturity; NRA is calculated by the plant NAA at maturity to subtract that at flowering; NRR is shown by the ratio of NRA and plant NAA at maturity; NUE is defined by the ratio of grain yield and plant NAA at maturity; NFPE is shown by the ratio between grain yield and the applied fertilizer N.

*Assay of the N assimilation-associated enzymatic activities*

To characterize the relation between the N assimilation processes and the N-associated traits in plants under various N input treatments, the activities of nitrate reductase (NR), nitrite reductase (NIR), and glutamine synthetase (GS) that reflect the N assimilation efficiencies in plants were analyzed by using the upper leaves during the 2017-2018 seasons. The NR activities were assayed as described by Gaudinová (1990), the NIR activities were measured according to the method reported by Wray et al. (1990), and the GS activities were determined following the descriptions by Lea et al. (1990).

*Assay of the soil moisture and N contents in soil profile*

To understand the connection between plant N uptake and plant usage for the water and N storage in soil profile, the moisture and N contents in various soil depth layers were evaluated during the late growth stage grown by the wheat cultivars under the N input treatments. With this aim, at stages of flowering, mid-filling (19 d after flowering), and maturity (38 d after flowering), the soil samples were collected at various soil depths, including 0.2, 0.4, 0.8, 1.2, 1.6, and 2 m at 2017-2018 season, were subjected to assay of moisture contents. After assay of the soil moisture contents, the soil samples were oven-dried and analyzed for the N contents as described previously (Guo et al., 2011).

*Data calculation and statistical analysis*

The averages and standard errors were calculated based on the Excel tool supplemented in Windows system. The significant tests among the treatments were determined based on SPSS 16.0 statistical software.

## RESULTS

*The yields and yield components*

Compared with those under sufficient-N treatment (N240), the yields and yield components (i.e., spike numbers per area, kernel numbers per spike, and grain weight) in the tested cultivars were all decreased under deficient-N treatment (N120). For the cultivars, Shimai 22 displayed elevated yields and yield components with respect to Jimai 585 under both N240 and N120, especially under N120 (Table 1). These results suggested that Shimai 22 possesses enhanced yield formation capacity under the limited water and N level conditions.

**Table 1.** The grain yields and the yield components of tested cultivars under the N treatments

Growth season	N treatment	Cultivar	Spike number (10 <sup>4</sup> ha <sup>-1</sup> )	Kernel numbers	Grain weight (mg)	Yield (kg ha <sup>-1</sup> )
2016-2017	N120	Jimai 585	641.96 b	31.37 c	41.12 b	7206.52 c
		Shimai 22	655.80 b	33.63 ab	41.86 ab	8056.60 b
	N240	Jimai 585	673.86 a	32.76 bc	42.13 a	8328.06 a
		Shimai 22	678.89 a	34.65 a	42.28 a	8488.61 a
2017-2018	N120	Jimai 585	622.73 b	29.81 c	39.47 b	6768.41 d
		Shimai 22	636.45 ab	32.20 b	41.21 a	7810.20 c
	N240	Jimai 585	654.63 a	32.22 b	41.48 a	8045.24 b
		Shimai 22	659.66 a	34.09 a	41.63 a	8225.53 a

Data are shown by averages from triplicates together with standard errors. Different lowercase letters indicate to be statistical significance between two tested cultivars across the N treatments at each growth season.

#### *Plant biomass and N-associated traits*

Enhancement of N input level increased the plant biomass (PB), N contents (NC), and the N accumulative amounts (NAA), displaying higher on above traits in the wheat cultivars under N240 than those under N120. Compared with Jimai 585, Shimai 22 elevated PB and NAA under both N240 and N120, especially under N120. However, these two cultivars were comparable on behavior of NC under same N input treatment (Table 2). The N accumulation rates (NAR) at middle phase

(jointing to flowering) and late phase (flowering to maturity) were higher than those at early phase (seedling to jointing). In addition, Shimai 22 displayed higher NAR at various phases than Jimai 585 under N120 and N240, except at late phase under 240 (Table 3). The decrease on NAR at late phase for Shimai 22 was ascribed to the strong N uptake capacity at early and middle stages whereas relative low ability on N taken up during late stage (flowering to maturity). These results were inconsistent with the behaviors on agronomic traits in the wheat cultivars under the N input treatments.

**Table 2.** The plant biomass and N-associated traits of the tested cultivars under the N treatments

Growth stage	N treatment	Cultivar	2016-2017			2017-2018		
			Plant biomass (kg ha <sup>-1</sup> )	N content (%)	N accumulation (kg ha <sup>-1</sup> )	Plant biomass (kg ha <sup>-1</sup> )	N content (%)	N accumulation (kg ha <sup>-1</sup> )
Seedling	N120	Jimai 585	142.28 c	2.23 b	3.17 c	130.90 c	2.18 b	2.85 c
		Shimai 22	159.22 ab	2.20 b	3.50 b	146.48 ab	2.15 b	3.15 b
	N240	Jimai 585	150.26 b	2.38 a	3.58 b	138.24 b	2.33 a	3.22 b
		Shimai 22	164.23 a	2.39 a	3.93 a	155.09 a	2.30 a	3.57 a
Jointing	N120	Jimai 585	4292.31 d	1.57 a	67.39 d	4016.93 d	1.52 a	61.06 c
		Shimai 22	4467.23 c	1.58 a	70.58 c	4285.85 c	1.51 a	64.72 bc
	N240	Jimai 585	4623.23 b	1.62 a	74.90 b	4453.37 b	1.57 a	69.92 ab
		Shimai 22	4784.23 a	1.65 a	78.94 a	4701.49 a	1.56 a	73.34 a
Flowering	N120	Jimai 585	7773.35 d	1.18 a	91.73 d	7571.48 d	1.13 a	85.56 c
		Shimai 22	8237.34 c	1.21 a	99.67 c	8098.35 c	1.12 a	90.70 c
	N240	Jimai 585	8898.34 b	1.28 a	113.90 b	8474.47 b	1.23 a	104.24 b
		Shimai 22	9893.08 a	1.26 a	124.65 a	9301.63 a	1.21 a	112.55 a
Maturity	N120	Jimai 585	15666.36 d	0.83 b	130.03 c	14413.05 c	0.78 b	112.42 c
		Shimai 22	17141.7 c	0.86 b	147.42 b	16270.36 b	0.79 b	128.54 b
	N240	Jimai 585	18506.67 b	1.02 a	188.77 a	17726.14 a	0.97 a	171.94 a
		Shimai 22	18749.13 a	0.96 a	179.99 a	17997.20 a	0.94 a	169.17 a

Data are shown by averages from triplicates together with standard errors. Different lowercase letters indicate to be statistical significance between two tested cultivars across the N treatments at each stage and same growth season.

**Table 3.** The N accumulation rates (NAR) at various growth phases in the tested cultivars under the N treatments

Growth phase	N treatment	Cultivar	2016-2017		2017-2018	
			Duration (d)	N accumulation rate (kg ha <sup>-1</sup> d <sup>-1</sup> )	Duration (d)	N accumulation rate (kg ha <sup>-1</sup> d <sup>-1</sup> )
Seedling-Jointing	N120	Jimai 585	173	0.37 c	172	0.34 b
		Shimai 22	171	0.39 bc	172	0.36 ab
	N240	Jimai 585	174	0.41 ab	173	0.39 a
		Shimai 22	172	0.44 a	173	0.40 a
Jointing-Flowering	N120	Jimai 585	26	0.94 d	25	0.98 c
		Shimai 22	26	1.12 c	25	1.04 c
	N240	Jimai 585	26	1.50 b	25	1.37 b
		Shimai 22	26	1.76 a	25	1.57 a
Flowering-Maturity	N120	Jimai 585	39	0.98 d	36	0.75 d
		Shimai 22	40	1.19 c	36	1.05 c
	N240	Jimai 585	41	1.83 a	37	1.83 a
		Shimai 22	41	1.35 b	37	1.53 b

Data are shown by averages from triplicates together with standard errors. Different lowercase letters indicate to be statistical significance between two tested cultivars across the N treatments at each stage and same growth season.

#### *Tissue N contents and N accumulative amounts at maturity*

At maturity, the grains contained the highest N contents, followed by stem and glume, and the leaves contained the lowest N contents in the wheat cultivars under both N input treatments. Compared with those under N120, the N contents and N accumulative amounts in each tissue of the tested cultivars were increased under N240 (Table 4). Although the N contents in grains were comparable in the two tested cultivars during two growth seasons, the grain N accumulative amounts per planting area for the cultivars displayed a little variation due to the modified grain yields in two growth seasons. For the cultivars, Shimai 22 displayed higher grain N contents and grain N accumulation under both N240 and N120 relative to Jimai 585. In contrast, the former was lower on the N contents in stem, leaf, and glume under both N input treatments with respect to the later (Table 4). These results suggested that Shimai 22 possesses strong capacity on remobilization of the N storage in vegetative tissues to harvest organ during the late growth stages under the limited N condition combined by deficit irrigation.

#### *Plant NUE-associated parameters*

Among the NUE-associated parameters, the N remobilized amount (NRA) and N remobilized rate (NRR) were higher during late growth stage whereas the NHI, N use efficiency (NUE), and N fertilizer production efficiency (NFPE) were lower in the wheat cultivars under N240 than those under N120. For the cultivars, Shimai 22 displayed much higher NRA, NHI, NUE, and NFPE than Jimai 585 under N120. The cultivar Shimai 22 was also higher on NHI, NUE, and NFPE than cultivar Jimai 585 under the sufficient-N treatment (N240). In contrast, the NRA and NRR in Shimai 22 were lower than Jimai 585 under N240 (Table 5). These results suggested that the N input level drastically affects the NUE-associated parameters in winter wheat plants. The improved plant N accumulation and agronomic traits in cultivar Shimai 22 are associated with the enhanced N uptake and efficient N remobilization efficiency during the middle and late growth stages.

**Table 4.** The tissue N contents and N accumulative amounts at maturity in tested cultivars under the N treatments

Tissue	N treatment	Cultivar	2016-2017		2017-2018	
			N content (%)	N accumulation (kg ha <sup>-1</sup> )	N content (%)	N accumulation (kg ha <sup>-1</sup> )
Grain	N120	Jimai 585	2.13 b	153.49 c	2.22 b	152.94 c
		Shimai 22	2.22 ab	178.84 b	2.25 ab	173.28 b
	N240	Jimai 585	2.18 ab	181.55 b	2.25 ab	179.13 b
		Shimai 22	2.32 a	193.68 a	2.33 a	185.96 a
Stem	N120	Jimai 585	0.52 a	26.25 b	0.50 ab	24.13 c
		Shimai 22	0.48 a	27.07 b	0.45 c	24.26 c
	N240	Jimai 585	0.56 a	32.61 a	0.54 a	30.07 a
		Shimai 22	0.50 a	29.22 ab	0.47 bc	26.26 b
Leaf	N120	Jimai 585	0.35 a	10.56 b	0.33 ab	9.52 b
		Shimai 22	0.32 a	9.82 b	0.30 b	8.80 c
	N240	Jimai 585	0.38 a	9.82 b	0.36 a	8.89 c
		Shimai 22	0.37 a	12.51 a	0.35 a	11.31 a
Glume	N120	Jimai 585	0.42 a	8.32 b	0.41 ab	7.76 b
		Shimai 22	0.38 a	8.13 b	0.37 c	7.57 b
	N240	Jimai 585	0.44 a	8.13 b	0.43 a	7.60 b
		Shimai 22	0.41 a	9.35 a	0.39 bc	8.50 a

Data are shown by averages from triplicates together with standard errors. Different lowercase letters indicate to be statistical significance between two tested cultivars across the N treatments at each stage and same growth season.

**Table 5.** Plant NUE-associated parameters of tested cultivars under the N treatments

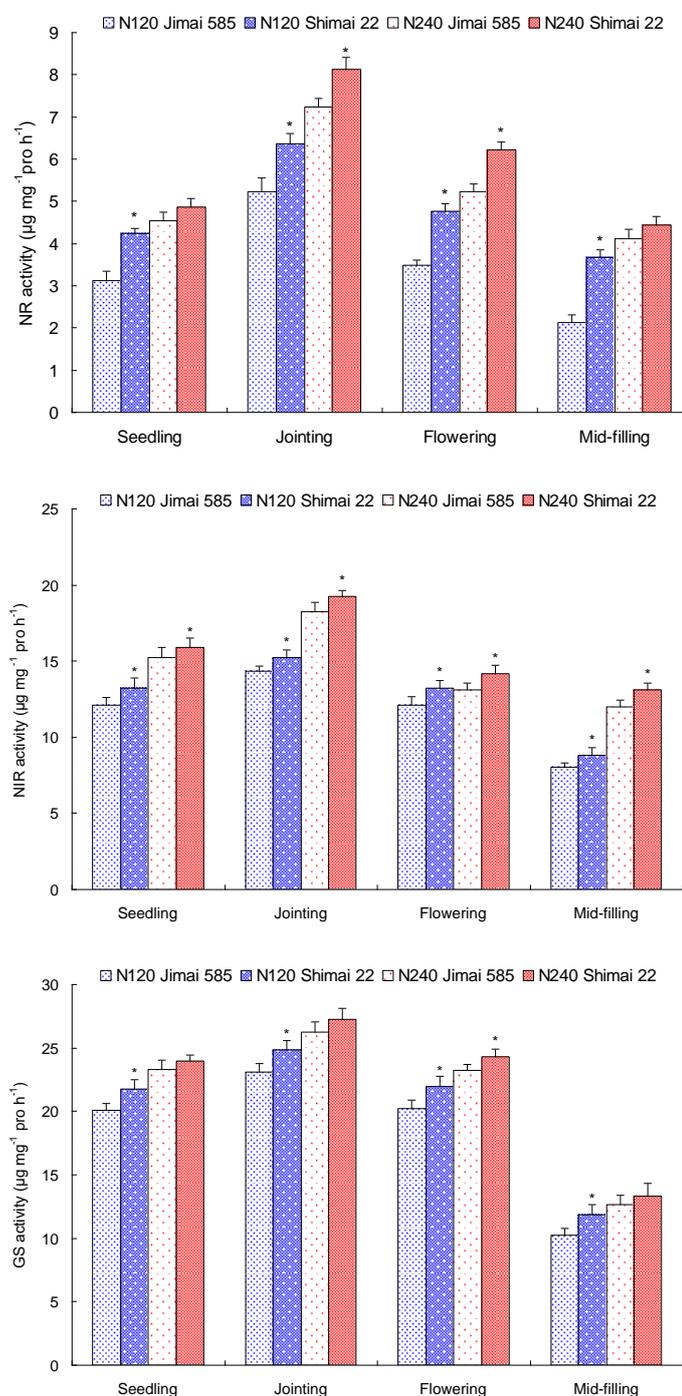
Season	Trait	N120		N240	
		Jimai 585	Shimai 22	Jimai 585	Shimai 22
2016-2017	NHI	0.77 b	0.80 a	0.76 b	0.79 ab
	NRA (kg ha <sup>-1</sup> )	38.30 d	47.75 c	74.87 a	55.34 b
	NRR (%)	29.45 c	32.39 b	39.66 a	32.75 b
	NUE (kg kg <sup>-1</sup> )	55.42 a	54.65 a	44.12 d	47.16 c
	NFPE (kg kg <sup>-1</sup> )	60.05 b	67.14 a	34.70 c	35.37 c
	NHI	0.79 ab	0.81a	0.75b	0.78 ab
2017-2018	NRA (kg ha <sup>-1</sup> )	26.86 d	37.84 c	67.7 a	56.62 b
	NRR (%)	23.89 d	29.44 c	39.37 a	33.47 b
	NUE (kg kg <sup>-1</sup> )	60.21 a	60.76 a	46.79 b	48.62 b
	NFPE (kg kg <sup>-1</sup> )	56.40 b	65.09 a	33.52 c	34.27 c

Data are shown by averages from triplicates together with standard errors. Different lowercase letters indicate to be statistical significance between two tested cultivars across the N treatments at each stage and same growth season.

*Plant N assimilation-associated biochemical parameters*

The activities of NR, NIR, and GS in the tested wheat cultivars were higher at each growth stage under N240 than those under N120, suggesting the promotion effects of the sufficient-N treatment on the activities of the N assimilation-associated enzymes. For the cultivars, Shimai

22 displayed higher enzymatic activities mentioned above than Jimai 585 under both N240 and N120, especially under N120 (Fig. 1). These results indicated that the improved N assimilation processes contribute to the plant N assimilation, biomass production, and the yield formation capacity in winter wheat plants under the N-limited conditions.



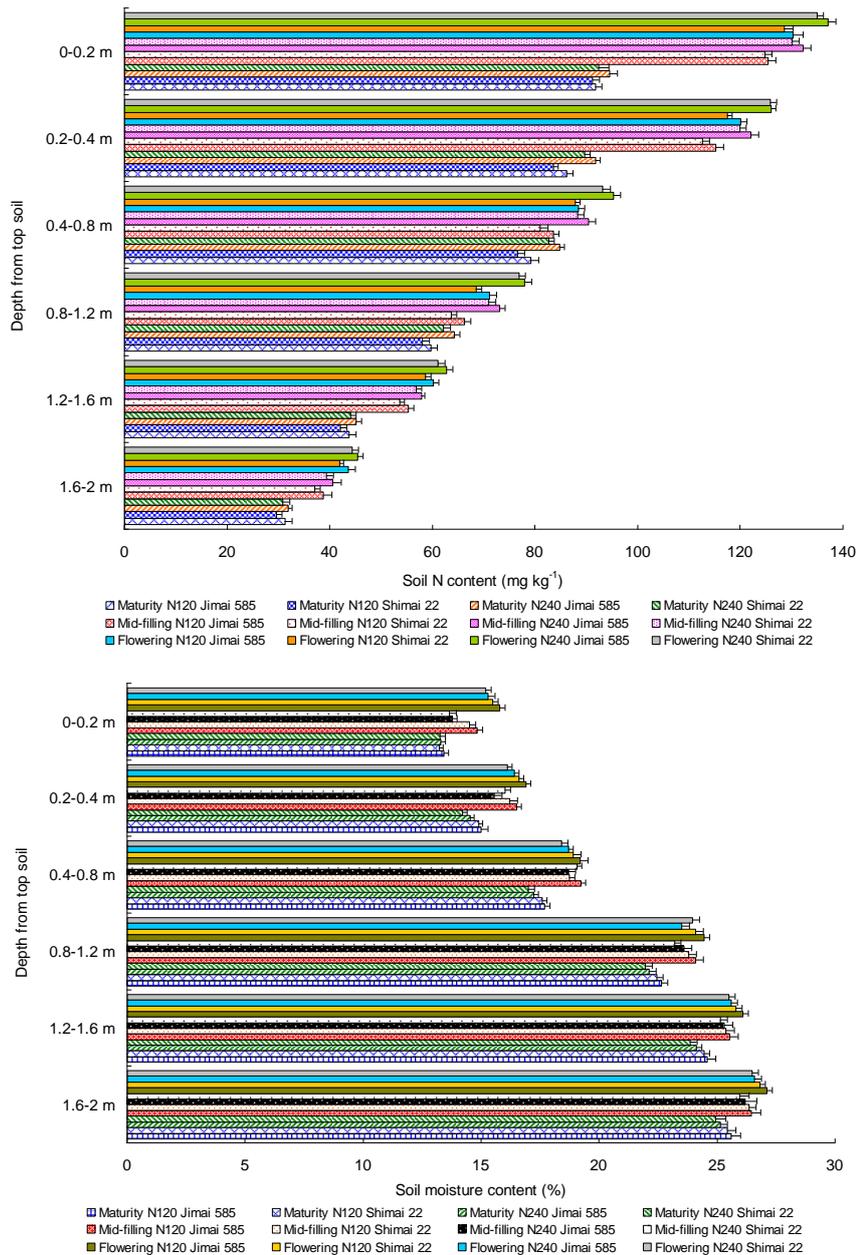
**Figure 1.** The activities of NR, NIR, and GS in the tested cultivars under the N treatments

Data are shown by averages from triplicates together with standard errors. Symbol \* indicates to be statistical significance between two tested cultivars at each assay time.

### Moisture and N contents in soil profile

The soil moisture contents, especially at 0.2 to 0.8 m depth layer, were modified by the N input treatments. The plots cultivated by the tested wheat cultivars displayed reduced moisture contents in soil depth layers under N240 with respect to those under N120 (Fig. 2), suggesting the relation between the much more consumption of soil water storage under sufficient-N treatment and the improved plant biomass and grain yields under N240. In contrast, the soil N contents in 2 m profile under N240

were increased relative to those under N120 at late growth stage (Fig. 2). Under N240, no significant differences on the soil moisture and N contents were found in the plots planted by Shimai 22 compared with those in the plots planted by Jimai 585. However, under N120, the plots cultivated by Shimai 22 were much lower on soil moisture and N contents at various depth layers relative to those cultivated by Jimai 585 (Fig. 2). These results indicated the variation of wheat cultivars in use of the water and N resources in soil storage upon the N deficiency condition combined by deficit irrigation.



**Figure 2.** The contents of N and moisture in soil planted by the tested cultivars at late stage under the N treatments. Data are shown by averages from triplicates together with standard errors. Symbol \* indicates to be statistical significance between two tested cultivars at each assay time.

## DISCUSSION

The water resource has been drastically reduced in North China plain in the past two decades due to the over-exploited underground water with the aim for high-yielding of the crops (Wei et al., 2008; Fang et al., 2010). Therefore, it is important to adopt the water-saving management for winter wheat cultivation by which to promote the regional sustainable development in agriculture. Previous investigations have defined the N uptake and internal N remobilization patterns in winter wheat under various irrigation treatments (Wang et al., 2002; 2004; Zhang et al., 2008). Under the water-saving conditions, the coupling effects between water and inorganic nutrients suggested that the suitable application of N fertilizer together with other nutrients (i.e., phosphorus and potassium) benefits for the nutrient uptake, plant growth, and the yield formation (Preez et al., 1991; Zhao et al., 2000; Singh et al., 2006). In this study, using Jimai 585 and Shimai 22, two winter wheat cultivars being contrast in drought responses, we investigated the plant N accumulation and N remobilization properties as well as the agronomic traits under two N input treatment combined by deficit irrigation. Both cultivars examined were increase on the plant biomass, N accumulation, and the enzymatic activities associated with N assimilation (i.e., activities of NR, NIR, and GS) together with the improved agronomic traits under N-sufficient treatment (N240), which are in agreement with the previous reports that indicated the enhancement of high N level on plant N uptake and N assimilation metabolism (Wang, 2000; Yuan et al., 2015).

Wheat cultivars are varied on behaviors of N accumulation and N remobilization upon the N-limited condition (Zhang et al., 2015; Gao et al., 2016; Nehe et al., 2018). They can be categorised into the high-, middle-, and low-NUE groups based on the accumulative N amount and biomass (Gao et al., 2016; Nehe et al., 2018). The cultivars in high NUE groups can accumulate more N in plants and produce higher grain biomass under the N-saving management (Gao et al., 2016), due to the enhanced function of root system (Dunbabin et al., 2010; Yu et al., 2015) and the improved photosynthesis of plants (Hirose, 1998; Pang et al., 2015). In this study, Jimai 585 and Shimai 22 were different on behaviors of plant N accumulation, biomass production, N assimilation-associated biochemical parameters, and yields. Compared with Jimai 585, Shimai 22 displayed improved N-associated traits and yields under the N limited treatment, suggesting that it is valuable in the cultivation under the water- and N-saving conditions.

The internal N remobilization from vegetative tissues to kernel during the late growth stage contributes largely to the grain biomass (Fan et al., 2005; Ma et al., 2009). In this study, both cultivars examined were increased on N accumulation amounts in vegetative tissues (i.e., grain, stem, leaf, and glume) at maturity under N240 with respect to those under N120. However, the internal N

remobilized rate, NHI, NUE, and NFPE were lowered under N240 relative to N120 in the tested cultivars. These results are in agreement with the reports in previous studies (Cox et al., 1986; Wang et al., 2003). Therefore, the drastic interaction effect between irrigation and N input level impacts on the plant N-associated traits in winter wheat cultivars under the water- and N-saving conditions.

The biochemical parameters associated with internal N assimilation affect the N physiological efficiency in plants and N storage in grains (Chandna et al., 2012; Zhang et al., 2015). The activities of NR, NIR, and GS, the critical enzymes involving internal N assimilation efficiency, regulate the NUE behaviors of plants (Oaks, 1994; Truax et al., 1994; Esen et al., 2015). In this study, the activities of NR, NIR, and GS in the winter wheat cultivars were enhanced by the N-sufficient treatment. In addition, the activities on obvious enzymes displayed variation across the tested wheat cultivars; higher activities of NR, NIR, and GS were observed in Shimai 22 with respect to those in Jimai 585 under the deficient-N treatment. These results suggested that these enzyme functions impact on the N assimilation metabolism by which to regulate the NUE and agronomic traits of plants treated by N-saving condition combined by deficit irrigation.

Plant acquisition for the nutrients, such as N, is closely associated with the nutrient content in growth media and the root system architecture (RSA) property (Frith et al., 2010; Xi et al., 2013). In this study, we assayed the moisture and N contents in 2 m soil profile in plots under two N input treatments to characterize the effects of them on the plant N accumulation and the agronomic traits. Both soil moisture and N contents were drastically modified by the N input levels. Additionally, compared with those planted by Jimai 585, the plots planted by Shimai 22 were lowered on moisture and N contents at various soil depth layers, especially at 0.2 to 0.8 m under deficient-N treatment (N120). These findings indicated that Shimai 22 improved the N accumulation and biomass production to be partly ascribed to the much more taken up of the soil water and N storage. The mechanisms underlying genetic control on the soil water and N acquisition are needed to be further characterized.

The yield formation capacity in wheat under water-saving condition is closely associated with the N acquisition amount in plants (Gao et al., 2016; Nehe et al., 2018). In this study, our investigation on relations between yield and the N-associated traits, such as N amount, N remobilization rate, and the contents of N and moisture in soil indicated that the wheat yields under water deficit cultivation are largely dependent on the plant N uptake ability and internal N translocation efficiency, which are associated with plant RSA and N assimilation enzyme activities. Thus, plant RSA behavior and enzymatic activities of NR, NIR, and GS can be acted as useful indices in prediction of plant productivity in winter wheat production under deficit irrigation conditions.

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