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Combined Effect of Plant Density and Nitrogen Input on Grain Yield, Nitrogen Uptake and Utilization of Winter Wheat

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Abstract

The nitrogen input and plant density have significant effects on nitrogen uptake and utilization of winter wheat. The objective of this work was to optimize nitrogen input and plant density for higher nitrogen uptake and utilization while maintaining grain yield. Field experiments were performed during the 2011-2012 and 2012-2013 growing seasons using two cultivars under different nitrogen input rates (0,180 and 240 kg ha^{-1}) and plant densities (120, 180 and 240 plants m^{-2} for Jimai 22 and 135, 270 and 405 plants m^{-2} for Tainong 18, respectively). The results showed that increasing plant density improved yield and increased nitrogen-use efficiency owing to the increased nitrogen uptake efficiency. The highest plant density at nitrogen input of 180 kg ha^{-1} obtained equivalent grain yield, and higher nitrogen uptake efficiency and nitrogen utilization efficiency compared with that at 240 kg nitrogen ha^{-1}. Nitrogen-use efficiency positively correlated to nitrogen uptake efficiency at all nitrogen input rates. Negative relationship between nitrogen-use efficiency and nitrogen utilization efficiency was only found at nitrogen input of 240 kg ha^{-1}, indicating that the low nitrogen utilization efficiency limited nitrogen-use efficiency increasing at high nitrogen input.

Keywords

Winter wheat; Nitrogen input; Plant density; Nitrogen-use efficiency; N-uptake efficiency; N-utilization efficiency

Abbreviations

AGN: Aboveground Nitrogen Uptake; GNC: Grain Nitrogen Concentration; NHI: Nitrogen Harvest Index; NUE: Nitrogen-Use Efficiency; UPE: Nitrogen Uptake Efficiency; UTE: Nitrogen Utilization Efficiency

Introduction

Nitrogen (N) is a primary nutrient limiting the grain yield of winter wheat (*Triticum aestivum* L) [1] and represents a significant cost for the grower. Recently, agricultural practices have focused on maximizing yields by increasing N fertilization [2,3], but global recovery of N fertilizer in wheat systems is low at 30–50% [4,5]. Large amounts of external N inputs are lost through leaching, surface runoff, denitrification, volatilization, and microbial consumption, which can cause severe environmental degradation, including the pollution of rivers and lakes, global warming, acid rain and soil acidification, and other forms of air pollution that can affect human health [6]. Therefore, improving N-use efficiency (NUE) while maintaining acceptable grain yield is important to reduce costs and environmental damage in wheat production [7,8].

Moll [9] defined NUE as the grain dry matter (DM) yield (kg DM ha^{-1}) per unit of available N (from both soil and fertilizer) and can be divided into two components: N-uptake efficiency [aboveground N uptake (AGN)/N available; UPE] and N-utilization efficiency (UTE, grain DM yield/AGN). UPE depends mainly on the AGN and available N, and reflects the N-absorption capacity of crops from both soil and fertilizer. UTE is a reflection of grain yield production per unit of AGN. Thus, agronomic management can be used to improve NUE by recovering more N from both soil and fertilizer (better UPE) and/or utilizing the absorbed N to produce more grain (better UTE).

Under a fixed N level, a higher NUE was associated with improved yield [10]. However, under different N availabilities, variations in NUE were more strongly associated with its components (such as UPE and UTE) than with grain yield [11]. The relationship of yield, AGN, N harvest index (NHI) and grain N concentration (GNC) could be expressed by the followed formula:

$$\text{Yield} = (\text{AGN} \times \text{NHI}) / \text{GNC}$$

Therefore, the NUE could be calculated by:

$$\text{NUE} = (\text{AGN}/\text{N available}) \times (\text{NHI}/\text{GNC}) = \text{UPE} \times \text{UTE}$$

Based on the formula, methods to improve NUE include (i) increasing AGN, (ii) reducing N available, (iii) increasing NHI and (iv) reducing GNC. These four parameters are closely associated, but also restrict each other. Practical methods to improve NUE may involve optimizing one or several indices using agro-technical approaches. Additional, the variation in UTE could be explained by NHI and GNC.

N [12] and plant density [13] are two important factors in winter wheat production. Optimal N input [14,15] or plant density [16,17] maximizes the grain yield. Although the N supply drives wheat productivity and high AGN are commonly associated with a high N input [18,19], the UPE, UTE, NHI, and consequently NUE were relatively low [20,21]. Therefore, obtaining NUE profits with reduced N inputs is important while maintaining or improving grain yield [18,22].

Previous studies have focused mostly on improving yield and NUE by augmenting N management practices, such as optimizing the time of N application and the ratio of base N to topdressing N [23-25], as well as combining sulfur fertilization availability [26,27], water availability [28], and tillage systems [29,30] with N application. However, plant density significantly influences the use of environmental resources such as light, water, and N by plants [31,13]. The highest AGN is observed at an optimal plant density in wheat' [32-34]. Our previous study also indicated that increasing plant density could improve the uptake of available N from both soil and fertilizer to improve AGN and grain yield in wheat [35]. As an alternative, we could improve AGN for the crop growth through the enhanced N absorption capacity resulting from increasing the plant density of

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winter wheat at relative low N input rather than using high quantity of N fertilize and consequently improve UPE and NUE. Additionally, lower N input can increase the UTE [19,36], which would facilitate the NUE. However, the response of NUE, UPE, and UTE in winter wheat when combining N input with plant density management strategies has not been characterized.

In this study, under field conditions, we investigated the effects of different N levels, plant density, and their interactions on (i) grain yield and its components, and (ii) NUE and its components UPE, UTE, AGN, NHI, and GNC. We also explored whether NUE could be improved and N input could be reduced while maintaining acceptable grain yield by optimizing plant density and N rates.

Materials and Methods

Site and growing conditions

Field experiments were conducted in 2011–2012 and 2012–2013 at the experimental station of Yangzhuang Village (35°33'N, 116°44'E), Xinyan Town, Ji'ning, Shandong, China. The mean annual temperature was 13.6°C and the average annual frost-free period was 225 days. The long-term (1974–2013) average annual precipitation was 658.5 mm, 28% of which occurred during the winter wheat growing stage. The soil type was clay loam [37] (Typic Cambisols; FAO/EC/ISRIC, 2003) with a pH of 7.35, a bulk density of 1.35 g cm-3, 15.45 g kg^{-1} of organic matter (Walkley and Black method), 1.25 g kg^{-1} of total N (Kjeldahl method), 23.64 mg kg^{-1} of available phosphorus (Olsen method), and 84.94 mg kg^{-1} of available potassium (Dirks-Sheffer method) in the topsoil (0-0.20 m).

Weather data were obtained from a meteorological station located less than 500 m from the experimental field. The monthly rainfall and temperature during the two growing seasons are shown in Figure 1. In both years, temperatures during the growing season were similar to the long-term mean, whereas rainfall was above the 40 year average (197.70 mm), with 243.70 mm in 2011–2012, and 291.00 mm in 2012–2013. However, a significantly different distribution was observed during each season. In 2011-2012, pluvial November brought excessive water to wheat plants, while the drought May implied a relatively dry grain filling stage. In 2012–2013, a heavy rain of 133.5 mm occurred on May 25 and 26, providing high soil moisture during the mid-grain filling stage.

Experimental design and treatments

Two widely planted cultivars in the target region, Jimai 22 (a cultivar with high tillering capacity) and Tainong 18 (a cultivar with low tillering capacity) were used as the experimental materials (henceforth referred to as "J22" and "T18," respectively). The current conventionally and widely adopted plant density used by farmers and researchers for winter wheat production is around 160-180 plants m^{-2} for the cultivar J22 [38-40] and 225-270 plants m^{-2} for the cultivar T18 [41-43], respectively, while the widely adopted N input was 240 kg ha^{-1} [38,41,43,44] or much higher [39,45,46]. In consideration of the different tillering capacities, plant densities of 120, 180, and 240 plants m^{-2} were designed for cultivar J22, while plant densities of 135, 270, and 405 plants m^{-2} were utilized for T18. The N fertilization treatment was applied at three levels using urea, i.e., no fertilization N (N0), 180 kg ha^{-1} of N (N180, the optimized N input), and 240 kg ha^{-1} of N (N240). Since the plant density differed between cultivars, the field experiment was designed specifically for each cultivar. The experiment in each cultivar was established as a split-plot design of three replicates (27 subplots) with N input as the main plots and plant density as the subplot. Each subplot consisted of a 20.0 m × 2.0 m plot (8 rows spaced 0.25 m apart).

Crop management

The previous crop was summer maize, and all straws and leaves were returned to the soil before tillage in both years. In each subplot, urea was divided into two equal amounts and applied before sowing and at the beginning of stem elongation. A basal fertilization including phosphorus as calcium superphosphate and potassium as potassium chloride at a rate of 105 kg ha^{-1} of P_2O_5 and 105 kg ha^{-1} of K_2O, respectively, was applied on October 7 during both years. Mixed fertilizer was ploughed to a 0.2 m depth and the seeds were sowed on October 8 during both years.

In 2011–2012, no irrigation was applied before wintering owing to the large amount of precipitation from seeding to November; therefore, three irrigations were performed after seeding, at jointing and anthesis on October 9, 2011, April 5, 2012, and May 6, 2012, respectively. The irrigation strategy in 2012–2013 was carried after seeding, before wintering, at jointing and anthesis on October 9, 2012, December 6, 2012, April 8, 2013, and May 8, 2013, respectively. An approximate rate of 60 mm was applied each time.

During both seasons, a prophylactic program including insecticides, fungicides, and herbicides were applied to control pests, diseases, and weed infestations and avoid biomass and yield losses. All subplots were harvested on June 13, 2012 and June 10, 2013 during both seasons, respectively.

Plant sampling and laboratory procedures

In each year, soil in the 0-1.0 m soil profile at 0.2 m intervals was sampled before any fertilizer was applied, and residues of previous summer maize were returned. These samples were analyzed for the soil mineralized N (including NO_3-N and NH_4-N) using a continuous flow analyzer (Bran+Lubbe, Norderstedt, Germany). In 2011–2012, the N_{min} in the top 1.0 m soil profile was 103.65 kg ha^{-1}. In 2012–2013, a significant difference in the N_{min} of the top 1.0 m soil profile was observed across N input levels, while no significant difference was detected between density regimens at each N input level. The average values were 85.26, 139.92, and 181.38 kg ha^{-1} at N0, N180, and N240, respectively.

At maturity during both seasons, a sample of plant material was obtained by manually cutting all plants in a quadrant of 0.5 m × 6 rows (1.5 m) at ground level and mixing them. Thirty single stems were sampled to calculate N accumulation in different organs. Plant samples were separated into four parts: leaves sheath+stems, grains, and glumes+ ear rachis. All separated samples were oven-dried at 105°C for 30 min and then at 70°C to achieve a constant weight for dry matter accumulation estimation. The oven-dried samples were then milled and analyzed for N concentration (semi-micro Kjeldahl method; Kjeltec TM 8200 Auto Distillation Unit; Foss, Denmark), and N accumulation was calculated by multiplying the N concentration by dry weight.

At harvest during both seasons, all ears in the 2.0 m × 8 rows (2.0 m) quadrant of each plot were cut manually and threshed using a pint-size seed threshing machine. The grain was air-dried, weighed, and adjusted to a standard 12% moisture content (88% DM, kg ha^{-1}).

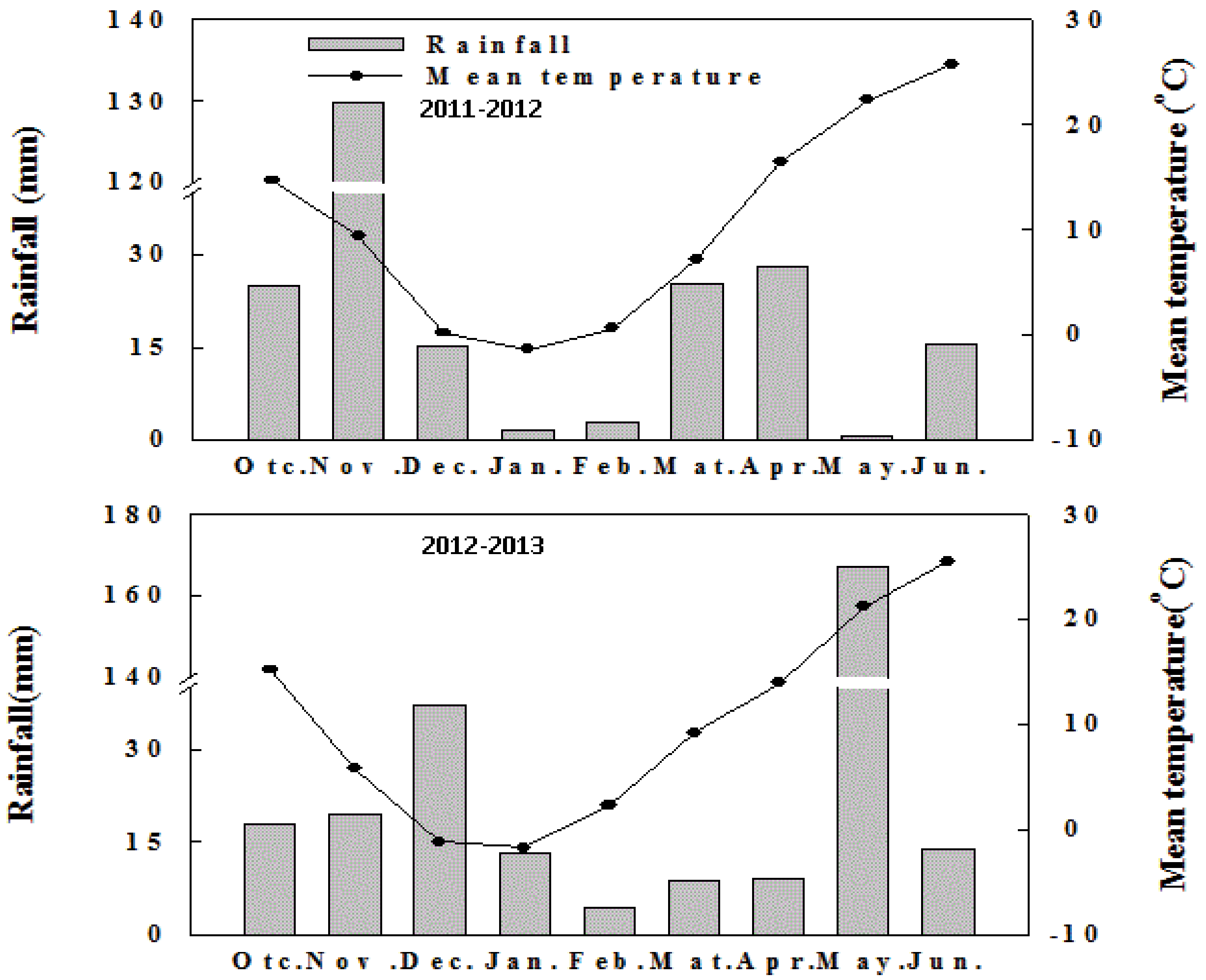

Figure 1: Monthly rainfall and mean temperature (ºC) recorded during the period of winter wheat growth (October to next June).

Calculation of the N evaluation indicator

- NUE was calculated as grain dry matter yield per unit of N available (from both soil and fertilizer, kg kg^{-1}).
- AGN represents total aboveground N uptake per unit area at maturity (kg ha^{-1}).
- UPE refers to the AGN per unit of N available (%).
- UTE is the grain dry matter yield per unit of AGN (kg kg^{-1}).
- NHI is calculated as the proportion of AGN in the grain at maturity × 100% (%).

Statistical analysis

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) procedures were used to examine the effects of N input, plant density, year, and their interactions on grain yield, NUE, and related parameters in individual cultivars due to differences in plant densities. Analysis of differences was determined based on least-significant differences (LSD) at the 5% confidence level (significant at $P<0.05$). When interactions were not significant ($P>0.05$), primary effects are discussed.

Results

Grain yield and its components

The effects of N input, plant density, and all two-way interactions were significant on grain yield for the cultivar J22, while N input, plant density, and N input × plant density, N input × year, and N input × plant density × year interactions significantly affected the grain yield of T18 (Table 1).

The main effects of N input, plant density and year on the grain yield and yield components were shown in Table 2. Applying N fertilizer averagely increased grain yield by 39.48 and 38.90% for the cultivar J22 and T18, respectively, compared with the N0 treatment. The increased grain yield mostly resulted from the 37.68 and 34.81%

Table 1: Analysis of variance of grain yield and yield components as affected by nitrogen (N) input, plant density (D) and year(Y) for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively.

Cultivar	Factors	Grain yield	Spikes per square meter	Kernels per spike	Grain weight
J22	Nitrogen input (N)	***	***	***	***
	Plant density (D)	***	***	***	***
	Year (Y)	ns	ns	*	*
	N × D	***	***	***	***
	N × Y	***	***	***	ns
	D × Y	***	***	ns	ns
	N × D × Y	ns	***	ns	ns
T18	Nitrogen input (N)	***	***	***	***
	Plant density (D)	***	***	***	***
	Year (Y)	ns	**	*	*
	N × D	***	ns	ns	ns
	N × Y	***	***	***	*
	D × Y	ns	ns	ns	ns
	N × D × Y	***	ns	ns	ns

* Significance at the *P*<0.05 level
** Significance at the *P*<0.01 level
*** Significance at the P<0.001 level

Table 2: The grain yield and yield components as affected by nitrogen (N) input, plant density and year for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively.

Cultivar	Treatment	Yield (kg ha^{-1})	Spikes per square meter (No. m^{-2})	Kernels per spike (No. spike^{-1})	Grain weight (mg)
J22	N input(kg ha^{-1})				
	0	5802.09 c	412.47 c	30.83 c	45.68 a
	180	7905.65 b	537.68 b	34.75 a	42.54 b
	240	8272.07 a	598.12 a	33.83 b	41.32 c
	Plant density (plants m^{-2})				
	120	6802.94 c	443.80 c	34.23 a	44.78 a
	180	7424.56 b	518.37 b	33.23 b	43.05 b
	240	7752.32 a	586.07 a	31.94 c	41.72 c
	Year				
	2012	7320.86 a	516.07 a	33.80 a	42.27 b
	2013	7332.34 a	516.12 a	32.47 b	44.09 a
T18	N input(kg ha^{-1})				
	0	5720.35 c	404.93 c	35.26 c	40.32 a
	180	7833.48 b	513.62 b	39.62 a	38.63 b
	240	8064.15 a	578.17 a	38.08 b	36.67 c
	Plant density (plants m^{-2})				
	135	6906.52 c	456.35 c	38.47 a	39.48 a
	270	7219.49 b	493.26 b	37.78 b	38.75 b
	405	7491.96 a	547.11 a	36.72 c	37.40 c
	Year				
	2012	7145.96 a	530.37 a	36.52 b	37.06 b
	2013	7266.03 a	467.45 b	38.79 a	40.03 a

[a] For each cultivar, within year and treatment (nitrogen input or plant density) values followed by the same letter are not significantly different at P<0.05 as determined by the least significant difference test

higher spikes per unit area and 11.22 and 10.18% higher kernels per spike for J22 and T18, respectively, although the kernel weight was reduced by 8.21 and 6.22%, respectively. Reducing the N input from 240 to 180 kg ha^{-1} significantly decreased grain yield by 4.35 and 2.85% for J22 and T18, respectively, mainly because of 10.10 and 11.65% lower spikes per unit area, respectively, although the kernels per spike was 2.72 and 4.04% higher and kernel weight was 2.95 and 5.34% higher, respectively.

Grain yield increased significantly as the plant density increased, with significant differences among all plant densities for each cultivar. Increasing plant density significantly increased the grain yield by 13.97 and 8.39% as the plant density increased from 120 to 240 plants m^{-2} for cultivar J22 and from 135 to 405 plants m^{-2} for T18, respectively, averaged over the two years. The improvement in grain yield with increasing plant density is mainly due to the 32.06 and 19.89% higher spikes per unit area, despite the 6.69 and 4.55% lower kernels per spike and 6.83 and 5.27% lower kernel weight for J22 and T18, respectively.

The significant N input × plant density interaction on grain yield showed that the yield value was the highest with N240 at all plant densities except plant density of 240 plants m^{-2} for J22 and 405 plants m^{-2} for T18, respectively, for which no differences were observed between N240 and N180 (Table 3). The N input × plant density interaction was also significant on the yield components of cultivar J22 (Table 3). This interaction showed that the increased spikes per unit area with increasing plant density was lower under N0 than that

Table 3: Significant effect of nitrogen (N) input and plant density (D) on the grain yield and yield components for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively.

Cultivar	Yield indices	Plant density (plants m^{-2})	N input kg ha^{-1}			LSD$_{0.05}$
			0	180	240	
J22	Yield	120	5266.68	7263.14	7878.99	168.71 (420.09) ^{a}
	(t ha^{-1})	180	5800.71	7996.71	8476.28	
		240	6338.87	8457.13	8460.96	
	Spikes per square meter	120	364.01	458.23	509.27	14.19 (26.75)
	(No. m^{-2})	180	411.28	542.85	600.99	
		240	462.14	611.98	684.10	
	Kernels per spike	120	31.25	35.95	35.50	0.72 (1.41)
	(No. spike^{-1})	180	30.84	34.77	34.08	
		240	30.39	33.53	31.91	
	Kernel weight	120	46.20	44.24	43.90	0.89 (1.81)
	(mg)	180	45.64	42.21	41.32	
		240	45.21	41.19	38.76	
T18	Yield	135	5367.31	7460.97	7891.26	90.04 (236.90)
	(t ha^{-1})	270	5659.77	7848.80	8149.90	
		405	6133.96	8190.66	8151.27	

^{a} The first value is for comparisons within a nitrogen input and the second value within bracket is for comparisons between nitrogen inputs

under N180 and N240, and showed that only plant density of 240 plants m^{-2} prompted significant differences between N180 and N240 with regard to the kernels per spike and kernel weight.

Nitrogen use efficiency

The effects of N input, plant density, year, and all two- and three-way interactions were significant on NUE for both cultivars excluding N input × year (Table 4). Applying N fertilizer averagely decreased the NUE by 60.86 and 60.99% for the cultivar J22 and T18, respectively, compared with N0 treatment (Table 5). Reducing the N input from 240 to 180 kg ha^{-1} significantly increased the NUE by 20.47 and 22.59% for J22 and T18, respectively (Table 5). Increasing the plant from 120 to 240 plants m^{-2} for J22 and from 135 to 405 plants m^{-2} for T18 significantly increased the NUE by 16.95 and 10.77%, respectively, averaged over the two years (Table 5). The significant N input × plant density interaction on the NUE showed that the NUE value increased as the plant density increased, with significant differences among all plant densities at all N input rates except N240, for which lack of significant difference in NUE was observed between plant densities of 180 and 240 plants m^{-2} for the cultivar J22 and plant densities of 270 and 405 plants m^{-2} for the cultivar T18, respectively (Table 6).

Nitrogen uptake efficiency and above-ground nitrogen uptake

The UPE was significantly affected by N input, plant density, year, and their two-way interactions for J22, while it was significantly affected by the main effects and N input × plant density and N input × plant density × year interactions for T18 (Table 4).

Applying N fertilizer averagely decreased the UPE by 45.79 and 46.69% for the cultivar J22 and T18, respectively, compared with the N0 treatment (Table 5). Reducing the N input from 240 to 180 kg ha^{-1} significantly increased the UPE by 7.14 and 9.04% for J22 and T18, respectively (Table 5). Increasing the plant from 120 to 240 plants m^{-2} for J22 and form 135 to 405 plants m^{-2} for T18 significantly increased the UPE by 17.76 and 12.07%, respectively, averaged over the two years (Table 5). The significant N input × plant density interaction showed that at all plant densities, the UPE value diminished as N input rates increased, although at plant densities of 120 and 180 plants m^{-2} for the cultivar J22 and of 135 plants m^{-2} for T18, respectively, no statistical differences were observed between N240 and N180 (Table 6). The correlation analysis showed that the NUE positively related to the UPE at all N input rates, indicating the importance of UPE to NUE (Table 7).

The UPE is the product of AGN divided by available N; therefore, the variation in UPE could be explained by fluctuations in both AGN and available N. The positive relationship between UPE and AGN at all N input rates confirmed the importance of AGN to UPE (Table 7). The N input, plant density, year, and their two-way interactions significantly affected the AGN for J22, and all the main effects and their two-way and three-way interactions except plant density × year significantly affected AGN for T18 (Table 4).

Applying N fertilizer averagely increased the AGN by 90.96 and 89.61% for the cultivar J22 and T18, respectively, compared with the N0 treatment (Table 5). Reducing the N input from 240 to 180 kg ha^{-1} significantly decreased the AGN by 14.89 and 13.46% for J22 and T18, respectively (Table 5). Increasing plant density from 120 to 240 plants m^{-2} for J22 and from 135 to 405 plants m^{-2} for T18 significantly increased the AGN by 18.13 and 12.43%, respectively, averaged over the two years (Table 5). The significant interaction between N input and plant density was manifested that the AGN increased much less with increasing plant density at N0 than that at N180 and N240 (Table 6). Significantly positive correlation was observed between grain yield and AGN at all N input rates (Table 7).

Differences in available N resulted from differences in N input during the first season, while these differences may be derived from differences in N input and available N in the soil during the later seasons. Applying N observed 262% higher total available N compared with N0 and N180 observed 18.77% lower total available N than that at N240, averaged over the two years.

Nitrogen utilization efficiency, nitrogen harvest index and grain nitrogen concentration

The N input, plant density, year, N input × plant density and N input × year interactions significantly affected UTE for the cultivar J22, and the N input, plant density, year, and N input × plant density significantly affected UTE for T18 (Table 4).

Table 4: Analysis of variance of nitrogen use efficiency (NUE), nitrogen uptake efficiency (UPE), nitrogen utilization efficiency (UTE), above-ground nitrogen uptake (AGN), nitrogen harvest index (NHI) and grain nitrogen concentration (GNC) as affected by nitrogen (N) input, plant density (D) and year (Y) for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively.

Cultivar	Factors	NUE	UPE	UTE	AGN	NHI	GNC
J22	N input (N)	***	***	***	***	***	***
	Plant density (D)	***	***	*	***	ns	**
	Year (Y)	*	**	**	*	*	*
	N × D	***	***	***	***	ns	***
	N × Y	ns	***	***	***	ns	***
	D × Y	***	***	ns	***	ns	ns
	N × D × Y	**	ns	ns	ns	ns	ns
T18	N input (N)	***	***	***	***	**	***
	Plant density (D)	***	***	**	***	ns	**
	Year (Y)	*	**	*	*	*	ns
	N × D	***	***	***	***	ns	**
	N × Y	ns	ns	ns	***	ns	ns
	D × Y	**	ns	ns	ns	ns	ns
	N × D × Y	***	*	ns	*	ns	ns

* Significance at the $P<0.05$ level

** Significance at the $P<0.01$ level

*** Significance at the $P<0.001$ level

Table 5: The nitrogen use efficiency (NUE), nitrogen uptake efficiency (UPE), nitrogen utilization efficiency (UTE), above-ground nitrogen uptake (AGN), nitrogen harvest index (NHI) and grain nitrogen concentration (GNC) as affected by nitrogen (N) input, plant density and year for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively[a]

Cultivar	Treatment	NUE	UPE	UTE	AGN	NHI	GNC
		(kg kg^{-1})	(%)	(kg kg^{-1})	kg ha^{-1}	(%)	(%)
J22	N input (kg ha^{-1})						
	0	53.93 a	135.95 a	40.52 a	130.44 c	79.24 a	1.99 c
	180	23.07 b	76.24 b	30.49 b	229.06 b	72.81 b	2.39 b
	240	19.15 c	71.16 c	27.12 c	269.12 a	69.66 c	2.57 a
	Plant density (plants m^{-2})						
	120	29.44 c	86.55 c	33.06 a	191.42 c	74.22 a	2.29 b
	180	32.28 b	94.87 b	32.80 a	211.09 b	74.21 a	2.32 ab
	240	34.43 a	101.92 a	32.27 b	226.12 a	73.27 a	2.35 a
	Year						
	2012	32.87 a	106.08 a	29.74 b	222.65 a	70.88 b	2.40 a
	2013	31.23 b	82.80 b	35.68 a	196.43 b	76.91 a	2.23 b
T18	N input (kg ha^{-1})						
	0	53.18 a	127.78 a	41.72 a	121.49 c	80.27 a	1.92 c
	180	22.85 b	71.06 b	32.27 b	213.74 b	76.44 b	2.37 b
	240	18.64 c	65.17 c	28.76 c	246.98 a	73.75 c	2.57 a
	Plant density (plants m^{-2})						
	135	30.00 c	83.27 c	34.59 a	183.04 c	77.20 a	2.27 b
	270	31.45 b	87.42 b	34.41 a	193.37 b	76.94 a	2.28 b
	405	33.23 a	93.32 a	33.76 b	205.80 a	76.32 a	2.32 a
	Year						
	2012	32.20 a	94.04 a	32.63 b	197.78 a	73.42 b	2.29 a
	2013	30.92 a	81.97b	35.87 a	188.36 b	80.23 a	2.28 a

[a] For each cultivar, within year and treatment (nitrogen input or plant density) values followed by the same letter are not significantly different at $P<0.05$ as determined by the least significant difference test

Applying N fertilizer averagely decreased the UTE by 28.91 and 26.86% for the cultivar J22 and T18, respectively, compared with the N0 treatment (Table 5). Reducing the N input from 240 to 180 kg ha^{-1} significantly increased the UTE by 12.43 and 12.20% for J22 and T18, respectively (Table 5). Equivalent UTE was observed between plant densities of 120 and 180 plants m^{-2} for J22 and between plant densities of 135 and 270 plants m^{-2} for T18, respectively, while further increase in plant density significantly decreased the UTE for each cultivar (Table 5). The significant N input × plant density interaction on UTE showed that only N240 prompted significant differences between plant densities of 180 and 240 plants m^{-2} for the cultivar J22 and between plant densities of 270 and 405 plants m^{-2} for T18, respectively (Table 6). The correlation analysis indicated that neutrally relationship was found between NUE and UTE at N0 and N180, while significantly negative correlation was observed between NUE and UTE at N240 (Table 7).

UTE could be calculated based on the ratio of the NHI to the GNC; therefore, variations in UTE could be explained by both the NHI and GNC. In the present study, NHI was only significantly affected by N input and year for each cultivar (Table 4). Applying N fertilizer averagely decreased the NHI by 10.10 and 6.45% for the cultivar J22 and T18, respectively, compared with the N0 treatment; while reducing the N input from 240 to 180 kg ha^{-1} significantly increased the NHI by 4.52 and 3.65% for J22 and T18, respectively (Table 5). Lack of significant difference in NHI was observed among plant densities at the same N input for each cultivar.

The primary effects of N input, plant density, year, and N input × plant density and N input × year interactions significantly affected the GNC for the cultivar J22, while only N input, plant density, and N input × plant density interaction affected the GNC for T18 (Table 4). Applying N fertilizer averagely increased the GNC by 24.62 and 28.68% for the cultivar J22 and T18, respectively, compared with the N0 treatment; while reducing the N input from 240 to 180 kg ha^{-1} significantly decreased the GNC by 7.00 and 7.78% for J22 and T18, respectively (Table 5). Significant difference in GNC was only observed between plant densities of 120 and 240 plants m^{-2} for the cultivar J22, while equivalent GNC was observed between plant densities of 135 and 270 plants m^{-2} for T18 and further increase in plant density to 405 plants m^{-2} observed significantly higher GNC. The significant N input × plant density interaction with regard to the GNC showed that the GNC was statistically greater with the highest plant density for each cultivar at all N input rates, except at N0 for J22 and at N0 and N180 for T18, respectively (Table 6). The NHI increased but the GNC diminished as N input decreased from 240 to 180 kg ha^{-1} and therefore the UTE was increased. The UTE positively correlated to NHI and negatively correlated to GNC at N0 and N180, while at N240 only statistically positive relationship was found between UTE and NHI (Table 7).

Table 6: Significant effect of nitrogen (N) input and plant density (D) on the nitrogen use efficiency (NUE), nitrogen uptake efficiency (UPE), nitrogen utilization efficiency (UTE), above-ground nitrogen uptake (AGN) and grain nitrogen concentration (GNC) for the cultivar Jimai 22 (J22) and Tainong 18 (T18), respectively.

Cultivar	NUE index	Plant density (plants m^{-2})	N input (kg ha^{-1})			LSD$_{0.05}$
			0	180	240	
J22	NUE (kg kg^{-1})	120	48.80	21.23	18.29	0.82 (2.07)^a
		180	53.93	23.31	19.60	
		240	59.08	24.67	19.55	
	UPE (%)	120	125.36	68.72	65.57	2.10 (5.91)
		180	135.90	76.99	71.74	
		240	146.58	83.01	76.17	
	UTE (kg kg^{-1})	120	40.02	31.10	28.06	0.89 (1.92)
		180	40.49	30.41	27.49	
		240	41.04	29.96	25.81	
	AGN (kg ha^{-1})	120	120.71	206.11	247.44	4.68 (12.16)
		180	130.32	231.47	271.47	
		240	140.28	249.62	288.45	
	GNC (%)	120	2.01	2.36	2.50	0.05 (0.14)
		180	1.99	2.40	2.56	
		240	1.97	2.42	2.65	
T18	NUE (kg kg^{-1})	135	49.99	21.77	18.24	0.55 (1.15)
		270	52.62	22.89	18.84	
		405	56.95	23.89	18.84	
	UPE (%)	135	121.54	66.59	61.68	2.10 (5.44)
		270	126.25	70.99	65.04	
		405	135.57	75.61	68.80	
	UTE (kg kg^{-1})	135	41.22	32.83	29.72	0.85 (2.00)
		270	41.80	32.34	29.08	
		405	42.14	31.67	27.49	
	AGN (kg ha^{-1})	135	115.38	200.12	233.61	4.38 (11.19)
		270	120.04	213.55	246.50	
		405	129.04	227.52	260.80	
	GNC (%)	135	1.94	2.35	2.51	0.05 (0.13)
		270	1.92	2.37	2.55	
		405	1.91	2.39	2.65	

^{a} The first value is for comparisons within a nitrogen input and the second value within bracket is for comparisons between nitrogen inputs

Table 7: Correlation coefficients between grain yield, nitrogen use efficiency (NUE) and related parameters^{a}

Nitrogen input (kg ha^{-1})	Indices	NUE	UPE	UTE	AGN	NHI	GNC
0	Yield	0.63*	0.69*	-0.49	0.61*	-0.16	0.52
	NUE	1.00	0.61*	-0.13	0.47	-0.17	0.12
	UPE		1.00	-0.86**	0.97**	-0.69*	0.84**
	UTE			1.00	-0.92**	0.77**	-0.97**
180	Yield	0.70*	0.37	0.00	0.80**	0.07	0.46
	NUE	1.00	0.82**	-0.46	0.92**	-0.38	0.79**
	UPE		1.00	-0.88**	0.81**	-0.84**	0.95**
	UTE			1.00	-0.49	0.99**	-0.83**
240	Yield	-0.15	-0.10	0.03	0.65*	0.14	0.26
	NUE	1.00	0.96**	-0.85**	0.46	-0.88**	0.19
	UPE		1.00	-0.97**	0.59*	-0.93**	0.36
	UTE			1.00	-0.66*	0.93**	-0.46

[a] UPE: Nitrogen Uptake Efficiency; UTE: Nitrogen Utilization Efficiency; AGN, Above-Ground Nitrogen Uptake; NHI, nitrogen harvest index; GNC: grain nitrogen concentration

[*] Significance at the $P<0.05$ level

[**] Significance at the $P<0.01$ level

Discussion

Increasing the plant density is commonly used to increase the grain yield of winter wheat [47-49]. In the present study, grain yield was significantly increased as plant density increased from 120 to 240 plants m^{-2} and from 135 to 405 plants m^{-2} for the cultivar J22 and T18, respectively. This benefit in grain yield from increasing plant density agreed with plenty of previous studies on wheat [16,17,32,50] and resulted mostly from the increased spikes per unit area. The supply of exogenous N fertilizer highly prompted wheat production in the majority of agricultural growing regions [51]. In the present study, applying N fertilizer significantly increased grain yield, which greed with most previous studies [18-20]. This improvement in grain yield was attributable to the increase of spikes per unit area and kernels per spike. Although reducing N input from 240 to 180 kg ha^{-1} significantly decreased grain yield owing to lower spikes per unit area, most importantly, the significant interaction between N input and plant density showed equivalent yield value between N240 and N180at the plant densities of 240 plants m^{-2} for the cultivar J22 and of 405 plants m^{-2} for T18. This indicated that the utilization of high plant density at relatively lower N input could achieve equal grain yield compared with that at higher N input. Hence, an N input of 240 kg ha^{-1} used in common farming practices may be excessive for the target region with a potential winter wheat yield of 8250 kg ha^{-1}. Therefore, sowing at higher plant densities with lower N input can be used to reduce N input while maintaining acceptable grain yield.

In the present study, the NUE value differed significantly between plant densities for each cultivar, efficiency increasing with grain yield, which indicated that increasing plant density was an efficient pathway to improve the NUE and confirmed our previous results [52] on this mid-yield field. The NUE diminished as N input increased and similar results were published by many previous researchers [4,53-55]. Compared to N240, the higher NUE at N180 is due to the fact that grain yield decreased less (by 4.35 and 2.85% for the cultivar J22 and T18, respectively) than the N available from both soil and fertilizer (by 18.77%). The NUE differed significantly between N input rates at each plant density and therefore the interaction between N input and plant density was expressed in the lack of significant difference at N240 between plant densities of 180 and 240 plants m^{-2} for the cultivar J22 and of 270 and 405 plants m^{-2} for T18, respectively, and this NUE value followed grain yield variation (Table 3). This demonstrated that at high N input, as plant density increase, there comes a point at which grain yield and NUE ceases to follow plant density. There also may be a threshold value of plant density with regard to grain yield at low N input, but it would be higher than that at high N according to present results.

However, the use of NUE alone is not sufficient to explore the impact of management practices on crop N dynamics because it comprises both soil and plant processes [27]. Analysis of UPE and UTE, which is associated with processes of NUE that occur in soil and plant, respectively, could improve the interpretation of interaction results.

The positive relationships between NUE and UPE and between UPE and AGN at each N input rate (Table 7) demonstrated that the improvement in NUE with increasing plant density was due mostly to the increase in UPE resulting from increased AGN and its compensation for the UTE, which confirmed our previous work in Dai [52], although the decreased UTE was mainly attributable to the increased GNC and unchanged NHI was observed among plant densities for each cultivar. The same importance of UPE to NUE has also been demonstrated in Muurinen [54] and Van Sanford and MacKown [56] at all N input rates.

The decrease in NUE with N applying resulted from a decrease in UPE and UTE. Generally, decreasing the N input would weaken wheat N uptake, which has been widely demonstrated in previous [18-20] and present studies. However, the AGN often decreased to a lesser extent than N available from soil and fertilizer. Therefore, reducing N input generally improves UPE [36,57]. However, this difference between N 180 and N240 only occurred at relatively higher plant density in present study due to the significant interaction between N input and plant density (Table 6). This lack of statistical difference between N180 and N240 at lower plant density is probably due to its lower N absorption capacity because of lower root length density [35,52] at lower N available, while a higher plant density expressed much higher N absorption capacity mainly because of higher root length density at each soil depth, especially at deeper soil [35], and maintained an equivalently absolute amount of increased AGN compared with lower plant density between N180 and N240 (Table 6). Namely, compared with the unchanged UPE between N180 and N240 at lower plant density, the increased UPE at higher plant density resulted mainly from the enhanced N absorption capacity resulting from increasing plant density, rather than lower N available.

The decrease in the NHI and increase in the GNC with increased N input resulted in a decreased UTE, which agreed with previous researchers [20,27,58] and indicated a reduced grain production per unit AGN. Previous studies have shown that as N fertilizer rates increase, transfer of N to grain no longer follows N uptake by the crop [36], and the NHI trend to be decreased at N inputs above 100 [18], 140 [19], or 150 kg ha^{-1} [36]. Similarly, in the present study, a higher NHI was observed as the N rate reduced from 240 to 180 kg ha^{-1}, indicating that properly reducing N input was an important way to increase the efficiency of N transferred from the un-harvest organs to grains. However, with regard to the interaction of N input and plant density on UTE, the significant difference between 180 and 240 plants m^{-2} for the cultivar J22 and between 270 and 405 plants m^{-2} for T18 at N240 was mainly attributable to the increased GNC with higher plant density.

Although low N input results in high NUE, grain yield is the primary consideration in winter wheat production of China and many countries with high populations. And also, the efficient use of N is another important factor. Possible agro-technical methods for increasing NUE while maintaining acceptable grain yield are of importance for countries similar to China. In the present study, the highest plant density of each cultivar at N180 observed the same high grain yield as that at N240 and observed significant higher NUE due to the higher UPE and UTE. Therefore, optimizing plant density of winter wheat at relatively lower N input could be used as an efficient pathway to obtain high grain yield through improving NUE.

Positive relationships between grain yield and AGN, which was observed at each N input rates in present study (Table 7) and have also been widely reported in previous studies [59-61], indicated that a high accumulation of N was critical to improving or maintaining grain yield, and a high grain yield was often accompanied by a high AGN. In addition, previous studies have confirmed that a high grain yield could be obtained with high AGN, which was achieved through managing S fertilization [26,27], irrigation (Haefele, or tillage) [29,30]. However, the negative relationship between NUE and UTE was only found at N240, (Table 7), indicating that the low grain production per unit AGN limited NUE at high N input. The UTE correlated positively to NHI and negatively to GNC at N0 and N180, while the significant relationship was only found between UTE and NHI at N240, indicating that only the low efficiency of N partitioning to grain drove the UTE at high N input. The UTE and NHI, which played importantly negative role on increasing NUE at N240, were mainly attributable to the higher AGN at present yield level. Then it may be thus summarized that high N input improved N available and consequently raised N accumulation at harvest, which limited the efficiency of N partitioning to grain and therefore reduced UTE. Furthermore, the highest plant density in present study at N180 obtained significantly lower AGN, but still showed equivalent grain yields compared to N 240. Based on these results, hypothesis existed that the AGN at high N input may have been excessive for crop production.

The increase in UTE with the highest plant density at N180 was essentially due to a reduced AGN based on equal grain yield, suggesting that a high grain yield may not require as much AGN as that at N240, and a relatively lower AGN may be sufficient for crop production and grain yield. The absorbed N in winter wheat is used to build structural proteins in supporting tissues and vascular connections of the shoot system [3] and photosynthetic tissues containing large quantities of photosynthetic proteins (principally Rubisco; [63,64]. Any N not allocated to these pools may be considered in a third “reserve N” pool (RN) [21]. The RN in plants can be divided into two components: the “storage RN,” which has a functional role in maintaining canopy photosynthesis, delaying green area senescence during grain filling, and consequently increasing the grain yield, protein concentration, and NUE, and the “accumulation RN,” which occurs through “luxury uptake” of N and has no functional role during the grain-filling phase [64]. Therefore, reducing “accumulation RN” in non-photosynthetic organs may be applicable to improve the UTE [21]. Due to a higher AGN and lower NHI, higher N accumulation in un-harvest organs was observed in wheat plants at N240compared with N180 at the highest plant density. These findings suggested that a greater quantity of “accumulated RN” maybe remain in the straw with high N input than under a low N input, and that the relatively lower level of “accumulated RN” may have accounted for the increased UTE at reduced N inputs. Whatever, quantifying how winter wheat crops accumulate and use nitrogen reserves between different N inputs at high yield level is a topic of academic and practical importance in the following works.

Conclusion

Increasing plant density resulted in increased yield and NUE, owing to the increased UPE resulting from the increased AGN. The grain yield raised but NUE declined as N input increased due to the reduction in UPE and UTE. Significant interaction between N input and plant density showed that the highest plant density at N180 obtained equivalent grain yield, and higher UPE and UTE, compared with that at N240. The increased UPE and UTE accounted for the increased NUE. The increased N capture originating from increasing plant density resulted in the higher UPE at N180, rather than the low N available. The reduction in N input observed increased NHI and reduced the GNC, both of which consequently increased UTE. Therefore, combining high plant density with relatively low N fertilizer input can help to improve NUE and avoid excessive N fertilizer use while maintaining acceptable yields.

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