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RESEARCH ARTICLE

Increased plant density and reduced N rate lead to more grain yield and higher resource utilization in summer maize

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Abstract

Planting at an optimum density and supplying adequate nitrogen (N) to achieve higher yields is a common practice in crop production, especially for maize (*Zea mays* L.); however, excessive N fertilizer supply in maize production results in reduced N use efficiency (NUE) and severe negative impacts on the environment. This research was conducted to determine the effects of increased plant density and reduced N rate on grain yield, total N uptake, NUE, leaf area index (LAI), intercepted photosynthetically active radiation (IPAR), and resource use efficiency in maize. Field experiments were conducted using a popular maize hybrid Zhengdan 958 (ZD958) under different combinations of plant densities and N rates to determine an effective approach for maize production with high yield and high resource use efficiency. Increasing plant density was clearly able to promote N absorption and LAI during the entire growth stage, which allowed high total N uptake and interception of radiation to achieve high dry matter accumulation (DMA), grain yield, NUE, and radiation use efficiency (RUE). However, with an increase in plant density, the demand of N increased along with grain yield. Increasing N rate can significantly increase the DMA, grain yield, LAI, IPAR, and RUE. However, this increase was non-linear and due to the input of too much N fertilizers, the efficiency of N use at N_{CK} (320 kg ha^{-1}) was low. An appropriate reduction in N rate can therefore lead to higher NUE despite a slight loss in grain production. Taking into account both the need for high grain yield and resource use efficiency, a 30% reduction in N supply, and an increase in plant density of 3 plants m^{-2}, compared to LD (5.25 plants m^{-2}), would lead to an optimal balance between yield and resource use efficiency.

Keywords: summer maize, increased plant density, reduced N rate, N use efficiency, resource use efficiency

1. Introduction

Increasing plant density is a common technique for achieving higher grain yield, as it increases the potential capacity of the crop canopy to capture resources including solar radiation, water and nutrients (Duan 2005). Although high plant density also increases interplant competition for resources and reduces grain yield per plant (Tetio-Kagho and Gardner 1988; Tollenaar and Wu 1999; Rossini *et al*. 2011; Al-Naggar *et al*. 2015), the use of hybrids tolerant of high densities and responsive to improvements in fertilization management

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practices could overcome the negative impacts of competition and lead to maximized maize (*Zea mays* L.) productivity per unit area (Bangizer *et al*. 1999; Rossini *et al*. 2011).

In recent years, nitrogen (N) rate in maize production has far exceeded the agronomic and economic optimums, leading to a sharp reduction in the recovery of N in soil-plant systems (Raun and Johnson 1999) and severe negative impacts on the environment and human health (Snyder *et al*. 2009; Huang and Tang 2010; Chen *et al*. 2011). Thus, it is imperative to develop agronomic practices that allow for reduced N rates in agricultural production.

Grain yield in maize is determined mainly by the final number of kernels per unit area that reach maturity, a measurement that is determined by kernel numbers per ear (KN) and ear density. Management practices, such as N fertilization (Uhart and Andrade 1995; Rossini *et al*. 2011) and plant density (Maddonni *et al*. 2006), could affect both KN and ear density, and, consequently, final KN. Under limited N conditions, the N fertilizer supply usually leads to increases in kernels per unit of ground area and mean kernel weight (KW) (Lemcoff and Loomis 1986; Thiraporn *et al*. 1987, 1992; Oikeh *et al*. 1998). However, studies have found that, when the N rate reaches a sufficiently high level, these increases diminish, and both KN and mean KW might even decline (Kniep and Mason 1989).

High plant density is also needed to obtain high yield in maize (Tokatlidis *et al*. 2011). However, as plant density increases, the KN per plant is reduced (Edmeades and Daynard 1979; Maddonni and Otegui 2004), while KW is either not affected (Tetio-Kagho and Gardner 1988) or is reduced slightly (5–30%; Borrás *et al*. 2003; Sangoi *et al*. 2002), resulting in a decline in grain yield per plant (Edmeades and Daynard 1979; Tetio-Kagho and Gardner 1988).

Maize grain yield is determined by the product of total dry matter and its partitioning to kernels. When other factors are not limited, crop biomass production can be represented as the product of two major components: the amount of accumulated intercepted radiation (RI_{acc}) (Sinclair and Muchow 1999; Boomsma *et al*. 2009) and the efficiency of conversion into dry matter, also referred to as radiation use efficiency (Monteith 1977; Sinclair and Muchow 1999; Boomsma *et al*. 2009). The RI_{acc} depends on the fraction of radiation intercepted (RI_{frac}) by the canopy each day, the days over which radiation is intercepted and total incident solar radiation. The RI_{frac} by a crop canopy depends on the canopy leaf area index (LAI) and the canopy light extinction coefficient (*k*) according to Beer's law. Consequently, many studies have focused on these two parameters (Nilson 1971; Suits 1971; Campbell 1990; Wang *et al*. 2007). For maize, authors have reported values of *k* ranging from 0.40 (Kiniry *et al*. 1989) up to 0.72 for inbred strains with more horizontal leaves (Pepper *et al*. 1977).

Numerous studies have reported that increases in N rate and plant density lead to significant increases in maize dry matter accumulation (DMA, Ciampitti and Vyn 2011; Cheng *et al*. 2015). This rise in DMA is due to an increase in the LAI and a corresponding increase in the RI_{frac} (Maddonni *et al*. 2006). The radiation use efficiency (RUE) also appears to increase with N rate and plant density increased (Muchow and Davis 1988). Massignam *et al*. (2009) reported that the RUE of maize in the whole crop cycle rose from 1.07 to 2.08 g MJ^{-1} after the addition of 30 g m^{-2} of N and from 1.08 to 1.24 g MJ^{-1} when plant density was increased from 3.33 to 6.67 plants m^{-2}.

In the North China Plain (NCP), smallholder farmers typically double-crop summer maize with winter wheat (*Triticum aestivum* L.), producing average maize yield of 7.81 t ha^{-1} (Jin *et al*. 2012). Average N rate in the region reach 360 kg ha^{-1} (Zhang 2008); however, the density of summer maize in smallholder fields in the NCP is generally less than 60 000 plants ha^{-1}. Therefore, agricultural strategies that are able to achieve higher yield and resource use efficiency, while focusing on an increase in plant density and reduction in N rate, would be welcome. The specific objectives of this study were to assess the effects of higher plant density and less N rate on (i) total N uptake, N use efficiency (NUE), (ii) DMA, LAI, RI_{frac}, and RUE and (iii) grain yield and its components in maize grown under field condition, in order to determine an effective approach for maize production with high yield and high resource efficiency in the NCP.

2. Materials and methods

2.1. Field site and growing conditions

Field experiments were conducted in 2013 and 2014 at the Corn Research Center (36°10´N, 117°09´E) of Shandong Agricultural University, Tai'an, Shandong Province, China. The soil type was a neutral sandy loam, composed of 9.84 g kg^{-1} organic matter, 0.79 g kg^{-1} total N, 47.19 mg kg^{-1} available phosphate, and 84.23 mg kg^{-1} available potassium in the top 0–20 cm arable soil layer. Meteorological data during the maize growing seasons were recorded by an automatic weather station installed in the field (Fig. 1). There were no significant differences between the weather data from the two years, except for precipitation.

2.2. Experimental design

Zhengdan 958, a compact maize cultivar tolerant of high plant density, was used as the experimental material. Three planting used were 52 500, 67 500 and 82 500 plant ha^{-1} (henceforth referred to as LD, MD and HD, respectively, and five N rates were imposed in this experiment: (i) no N (N_0), (ii) a maximum N rate of 320 kg ha^{-1} (N_{ck}), (iii) a 15% reduction in the N rate ($N_{-15\%}$, 272 kg ha^{-1}), (iv) a 30% reduction in the N rate ($N_{-30\%}$, 224 kg ha^{-1}), and (v) a 45% reduction in the N rate ($N_{-45\%}$, 176 kg ha^{-1}). The experiments were established in a split-plot design consisting of three replicates (45 subplots), with three planting densities as the main plot and five N rates as the sub-plot. The size of each subplot was 15.0 m×3.0 m (rows spaced 60 cm apart).

Basal fertilization of each subplot, which was applied before tillage on 7 June during both years, included phosphorus as calcium superphosphate and potassium as potassium chloride at rates of 120 kg ha^{-1} P_2O_5 and 240 kg ha^{-1} K_2O, respectively. The N fertilizer source for side-dress application was urea half of which was applied at V6, and the remainder at the 12th leaf stage (V12). The seeds were sown on 17 June 2013 and 13 June 2014. Thinning was performed at the 3rd leaf stage (V3) and V6.

Four irrigations were imposed after seeding and at V6, tasseling stage (VT), and blister stages (R2) on 17 June, 8 July, 5 August, and 9 September 2013, respectively, with approximately 60 mm rate at each time. No irrigation was applied at V6 and VT, because 358.30 mm of precipitation occurred from seeding to July (Fig. 1). The irrigation strategy in 2014 was the same as that in the previous season and occurred on 17 June, 8 July, 6 August, and 7 September 2014 (Fig. 1).

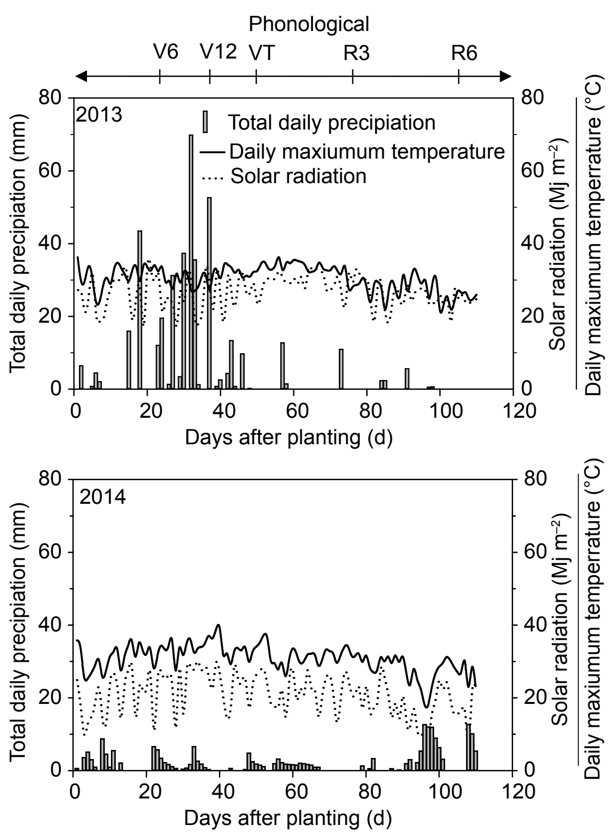

Fig. 1 Total daily precipitation (mm), daily maximum temperature (°C) and solar radiation (Mj m^{-2}) recorded during the growing seasons in 2013 (from 17 June to 5 October) and 2014 (from 13 June to 30 September). Timing of planting and the physiological stages V12 (the 12th leaf stage), VT (tasseling stage), R3 (milk stage), and R6 (physiological maturity) (Ritchie *et al.* 1996) are indicated for each growing season.

A prophylactic program of insecticides, fungicides and herbicides was applied during both years to control pests, diseases, and weeds, respectively. No significant incidences of pest, diseases or weeds were observed in any of the subplots.

All subplots were harvested on 5 October 2013 and 1 October 2014.

2.3. Sample collection

Five representative maize plants in the center three rows were sampled per plot to determine dry matter at V6, V12, VT, R2, milk stage (R3), dent stage (R5), and R6, and N uptake at R6. These five plants were cut at the stem base and dried at 80°C to a constant weight. At R6, prior to drying, maize plants were separated into leaves, stems, tassels, husks, cobs, and kernels.

At R6, a 5 m×3 row quadrant of each subplot was marked during both years, and all ears in the quadrant were collected, and tagged and bagged, separately. Grains were manually separated from cobs for each individual ear. The resulting grain samples were weighted to determine the individual plant grain yield (adjusted to a moisture content of 0.14 H_2O g^{-1} fresh weight). KN per plant was determined for all tagged plants. For each ear, KW was calculated as the quotient of that ear's grain weight and KN. The harvest index (HI) of each subplot was calculated as the ratio of the grain weight to the total aboveground plant DMA.

2.4. N uptake and N indices

Plant and grain samples were ground and then digested by adding 10 mL H_2SO_4-H_2O_2 to each sample. The N content in each fraction was measured using the semi-micro Kjedahl method (Kjeltec^{TM} 8200 Auto Distillation Unit, Foss, Hillerød, Denmark.; Yuen and Pollard 1953; Bremner 1960).

The NUE, NRE and NIE were calculated to compare the performance of plant density and N application treatments (Cassman *et al.* 2003; Ciampitti and Vyn 2011). The indices were calculated as follows:

$$\text{NUE (kg kg}^{-1}\text{)=}\frac{\text{Grian yield with applied N}-\text{Grain yield without applied N}}{\text{N applied amount}}$$

$$\text{NRE (\%)}=\frac{\text{N uptake with applied N}-\text{N uptake without applied N}}{\text{N applied amount}}\times 100$$

$$\text{NIE (kg kg}^{-1}\text{)}=\frac{\text{Grian yield with applied N}-\text{Grain yield without applied N}}{\text{N uptake with applied N}-\text{N uptake without applied N}}$$

2.5. Light interception measurements

At each sampling date, light interception was measured in each plot using a digital plant canopy imager (CI-110, CID, WA, USA). This instrument measured radiation in the photosynthetically active radiation (PAR) range (400–700 nm). Measurements were taken on clear cloudless days within 2 h of either side of the solar noon. For each measurement, one above-canopy reading was recorded to determine the incident photosynthetically active radiation (IPAR; mol m^{-2} s^{-1}) and five below-canopy readings were recorded below the lowermost green leaves of the canopy to determine the transmission photosynthetically active radiation (TPAR; mol m^{-2} s^{-1}). The RI_{frac} at midday was calculated from these measurements as:

$$RI_{frac}=1-\frac{TPAR}{IPAR}$$

Daily solar radiation data (Fig. 1) were obtained from the weather site equipment installed in the field. Total incident solar radiation data were converted to PAR by assuming that PAR made up 0.5 of the total solar radiation (Trapani *et al*. 1992; Sinclair and Muchow 1999). On days where the RI_{frac} was not measured directly, it was estimated by linear interpolation between measured values. Daily IPAR for each plot was calculated as the RI_{frac} of daily incident PAR. IPAR was summed from emergence to the first sampling date, and for each sampling interval assuming a linear change between measurements. RUE was calculated for each treatment by linear regression of sequential crop biomass measurements against accumulation of IPAR.

2.6. Statistical analysis

Treatment and their interactions effects on grain yield, yield components, N uptake, NUE, and related parameters in two years were analyzed according to the principles of analysis of variance, using the general linear model (GLM) in SPSS 19.0 (SPSS Inc., Chicago, USA). Significant differences among means were determined by Duncan's multiple range tests at 5% level. Graphs were constructed using SigmaPlot 12.5 software.

3. Results

3.1. Grain yield and yield components

The effects of plant density, N rate, and their interaction on grain yield were significant in both years studied (Fig. 2; Table 1). After averaging the effect of N rate, grain yield rose significantly by 19.0–40.5% as plant density was increased from the lowest plant density (LD, 52 500 plants ha^{-1}) to the medium density (MD, 67 500 plants ha^{-1}) or the high density (HD, 82 500 plants ha^{-1}) levels over the two years. The N rate was responsible for a 21.8–26.6% increase in grain yield at LD, a 25.7–33.3% increase at MD and a 26.5–39.3% increase at HD over the two years. Grain yield varied, however, with different N rates. The maximum yield was obtained with the addition of N at a rate of 320 kg ha^{-1} (N_{CK}) at MD and HD or with a 15% reduction in the N rate ($N_{-15\%}$, 272 kg ha^{-1}) at LD. Similarly, maize yield decreased gradually as the N rate was reduced. Notably, there were

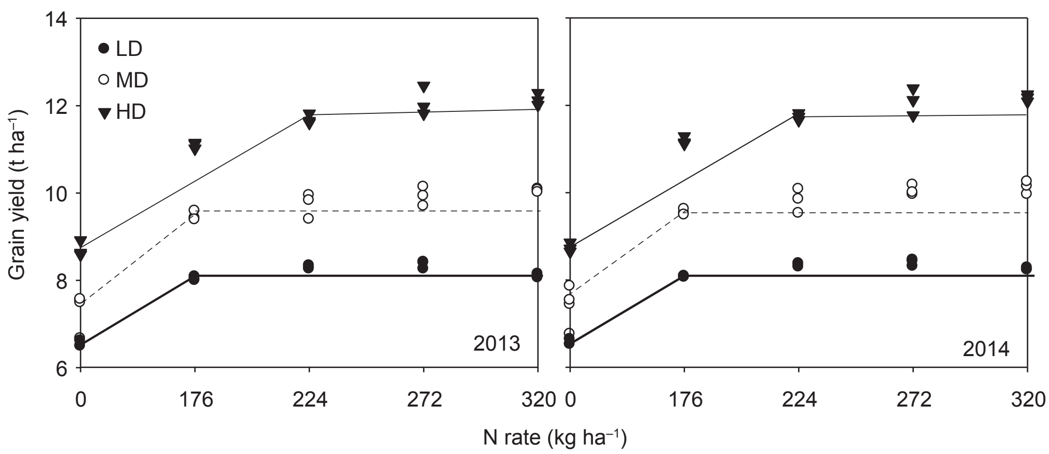

Fig. 2 N response on yield under different plant densities. LD, low density treatment (52 500 plants ha^{-1}); MD, medium density (67 500 plants ha^{-1}); HD, high density (82 500 plants ha^{-1}). The same as below.

Table 1 Effects of plant density and nitrogen rate on grain yield, kernel number (KN), 1 000-kernel weight, and harvest index (HI) in both seasons

Plant density[1)]	Nitrogen rate[2)]	Grain yield (t ha^{-1})		KN (number ear^{-1})		1 000-kernel weight (g)		HI	
		2013	2014	2013	2014	2013	2014	2013	2014
LD	N_0	6.58 c	6.63 c	421.6 b	423.5 c	300.3 c	301.5 c	0.494 a	0.491 a
	N_{CK}	8.10 b	8.24 b	482.3 a	491.0 b	333.4 a	334.3 a	0.493 a	0.494 a
	$N_{-15\%}$	8.35 a	8.39 a	495.5 a	501.9 a	334.2 a	335.3 a	0.511 a	0.509 a
	$N_{-30\%}$	8.29 a	8.33 ab	488.4 a	490.4 b	331.2 a	333.4 a	0.512 a	0.513 a
	$N_{-45\%}$	8.03 b	8.06 b	483.9 a	482.4 b	325.6 b	326.5 b	0.494 a	0.493 a
MD	N_0	7.52 c	7.60 c	381.5 b	383.9 c	293.5 c	294.2 c	0.473 b	0.474 c
	N_{CK}	10.05 a	10.11 a	464.2 a	471.4 a	323.0 a	323.0 a	0.504 a	0.506 b
	$N_{-15\%}$	9.91 ab	10.00 a	458.5 a	463.2 ab	322.6 a	323.4 a	0.507 a	0.508 b
	$N_{-30\%}$	9.88 ab	9.94 ab	456.5 a	461.5 ab	320.5 a	321.6 a	0.517 a	0.515 ab
	$N_{-45\%}$	9.46 b	9.54 b	450.4 a	450.9 b	314.4 b	315.2 b	0.526 a	0.528 a
HD	N_0	8.71 c	8.74 c	372.2 c	375.2 c	286.8 d	286.3 c	0.457 b	0.456 b
	N_{CK}	12.14 a	12.17 a	457.6 a	457.1 a	322.7 a	323.8 a	0.512 a	0.506 a
	$N_{-15\%}$	11.95 a	11.96 a	452.2 a	453.0 a	321.5 ab	321.5 a	0.506 a	0.511 a
	$N_{-30\%}$	11.59 a	11.64 a	446.8 ab	448.9 a	318.6 b	318.2 ab	0.517 a	0.513 a
	$N_{-45\%}$	11.03 b	11.05 b	434.1 b	436.7 b	312.2 c	313.5 b	0.505 a	0.506 a
Year (Y)		2.69 ns		5.76*		1.68 ns		0.01 ns	
Planting density (D)		2 590.7***		414.4***		181.8***		2.60 ns	
Nitrogen rate (N)		651.1***		551.6***		438.9***		27.9***	
Y×D		0.09 ns		0.34 ns		0.21 ns		0.03 ns	
Y×N		0.02 ns		0.38 ns		0.03 ns		0.03 ns	
D×N		25.9***		3.92*		1.74		5.70***	
Y×D×N		0.06 ns		0.34 ns		0.10 ns		0.09 ns	

1) LD, low density treatment (52 500 plants ha^{-1}); MD, medium density (67 500 plants ha^{-1}); HD, high density (82 500 plants ha^{-1}).
2) N_0, no N; N_{CK}, a maximum N rate of 320 kg ha^{-1}; $N_{-15\%}$, a 15% reduction in the N rate of 272 kg ha^{-1}; $N_{-30\%}$, a 30% reduction in the N rate of 224 kg ha^{-1}; $N_{-45\%}$, a 45% reduction in the N rate of 176 kg ha^{-1}.
Different small letters indicate significantly differences at P<0.05 as determined by the LSD test. ns, no significant; *, ** and ***, F-values significant at 0.05, 0.01 and 0.001 levels. The same as below.

no significant differences between N_{CK} and a 30% reduction in the N rate ($N_{-30\%}$, 224 kg ha^{-1}) at MD and HD or a 45% reduction in the N rate ($N_{-45\%}$, 176 kg ha^{-1}) at LD.

The year, plant density, N rate, and the interaction between plant density and N rate all had significant effects on KN, while only plant density and N rate affected 1 000-kernel weight significantly. A reduced N rate led to reductions in both KN (~13.7%) and KW (~9%) per plant (with *vs.* without applied N). The plant density factor also affected KN and KW significantly (P<0.05), leading to a 7% decrease in KN and a 3% decrease in KW at MD (compared to LD), and a 9% decrease in KN and a 4% decrease in KW at HD. The HI was affected significantly by the N rate and the interaction of N rate and plant density, but there were no significant differences among treatments with applied N.

3.2. Plant dry matter accumulation

N supply led to a significant increase in DMA for all sampling dates, except V6 (the first application of N fertilizer), at all plant densities (Fig. 3). However, similar to the grain yield results, the maximum biomass was obtained with the N_{CK} treatment at all plant densities, and there were no significant differences in DMA among the treatments with applied N at LD or among $N_{-30\%}$, $N_{-45\%}$ and N_{CK} at MD or HD. Plant density also affected DMA significantly. As plant density increased, the dry matter per unit area increased significantly.

3.3. Plant N uptake and N efficiency indices

We observed significant effects from plant density, N rate and their interaction on total N uptake (Table 2). As N rate increased, the total N uptake increased at all plant densities. N supply led to an increase in total N uptake of 44.0–63.5% at LD, 46.7–69.4% at MD, and 56.4–82.5% at HD over the two years (Table 2). Increasing plant density led to an average increase in total N uptake per unit area of 22.0% at MD (compared to LD) and 39.2% at HD.

In terms of N indices, we observed the highest NUE values (4.74–13.18 kg kg^{-1}) and nitrogen recovery efficiency (NRE) values (24.35–50.82%) in the treatment that combined HD and $N_{-45\%}$. This combination was responsible for the large difference in grain yield (from 14.4 to 24.2 g m^{-2}) and total N uptake (from 5.41 to 8.85 g m^{-2}) per unit area observed between the no N (N_0) and $N_{-45\%}$ treatments at HD. As the N rate increased, the NUE and NRE decreased by 7.0–40.3% and 6.5–22.6%, respectively, over the two years. The interaction between plant density and N rate had a significant effect on NUE and NRE. For the MD treatments, NUE and NRE were 40.6 and 25.0% higher on average, respectively, than at LD, while for HD, increases in NUE and NRE were even greater (79.2 and 64.3%, respectively) compared to LD.

The N internal efficiency (NIE) value reflects the efficiency in the use of total N uptake by maize plants for grain yield formation. As mentioned, we observed larger total N uptake values with higher N rates and plant densities, and this higher total N uptake led to an increase in NIE from 19.46 to 29.87 kg kg^{-1} (Table 2). As N rates increased,

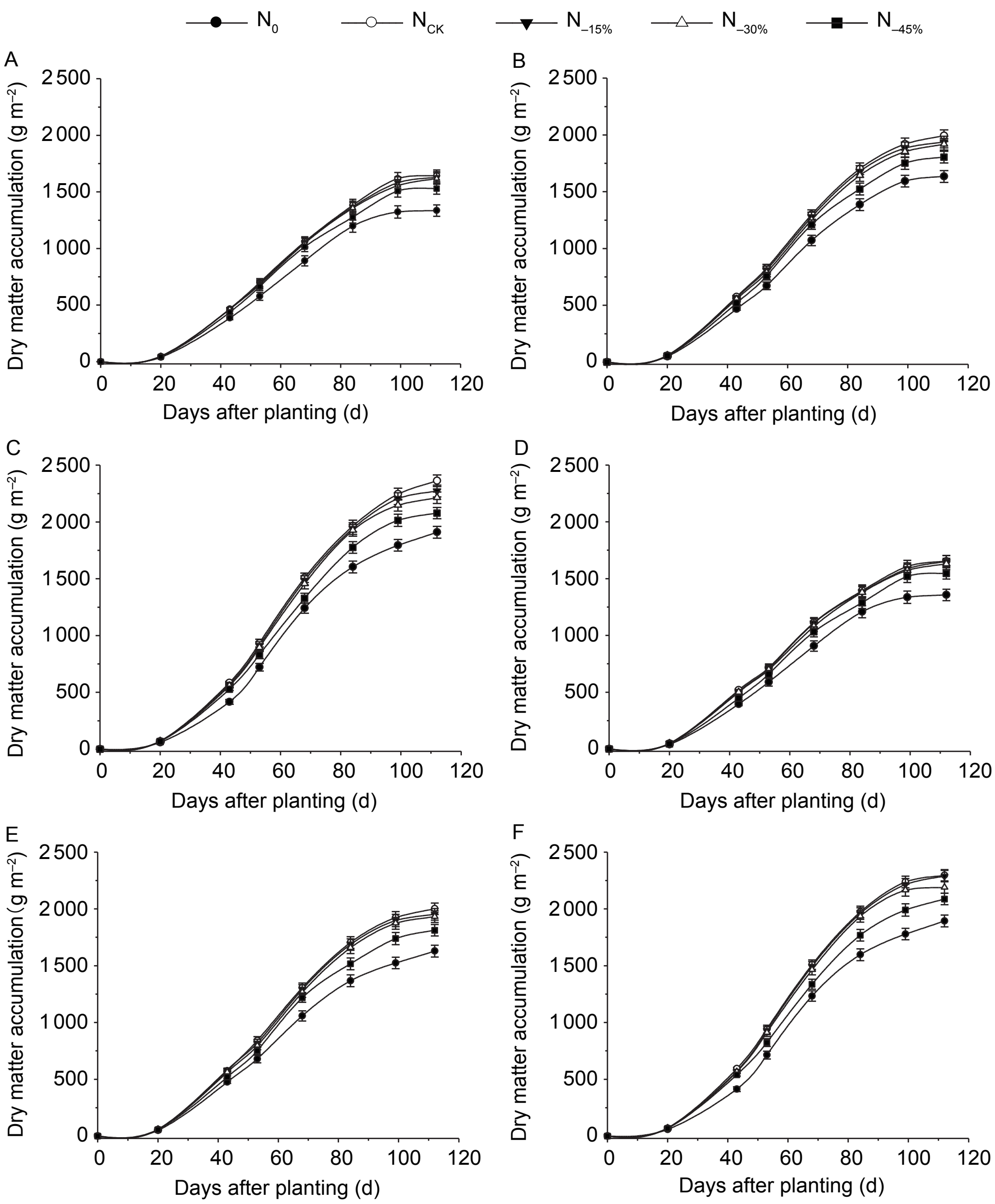

Fig. 3 Dry matter accumulation±SE (standard error) *vs*. days after planting for the different plant densities and N rates in 2013 and 2014 growing seasons. For the year factor: A, B and C, 2013; D, E and F, 2014. For the plant density factor: A and D, LD; B and E, MD; C and F, HD. N_0, no N; N_{CK}, a maximum N rate of 320 kg ha^{-1}; $N_{-15\%}$, a 15% reduction in the N rate of 272 kg ha^{-1}; $N_{-30\%}$, a 30% reduction in the N rate of 224 kg ha^{-1}; $N_{-45\%}$, a 45% reduction in the N rate of 176 kg ha^{-1}.

Table 2 Effects of plant density and nitrogen rate on total N uptake, nitrogen use efficiency (NUE), nitrogen recovery efficiency (NRE), and nitrogen internal efficiency (NIE) in both seasons

Plant density	Nitrogen rate	Total N uptake (g m⁻²)		NUE (kg kg⁻¹)		NRE (%)		NIE (kg kg⁻¹)	
		2013	2014	2013	2014	2013	2014	2013	2014
LD	N_0	12.26 e	12.31 e						
	N_{CK}	20.05 a	20.13 a	4.74 d	5.02 d	24.35 c	24.44 d	19.46 c	20.54 c
	$N_{-15\%}$	19.73 b	19.82 b	6.51 c	6.47 c	27.46 b	27.62 c	23.69 b	23.42 b
	$N_{-30\%}$	18.85 c	18.88 c	7.63 b	7.59 b	29.40 a	29.34 b	25.96 a	25.86 a
	$N_{-45\%}$	17.63 d	17.77 d	8.22 a	8.14 a	30.52 a	31.01 a	26.96 a	26.28 a
MD	N_0	14.80 e	14.83 e						
	N_{CK}	25.07 a	25.15 a	7.91 b	7.84 c	32.08 c	32.25 c	24.65 a	24.31 a
	$N_{-15\%}$	23.80 b	23.86 b	8.80 ab	8.84 bc	33.08 c	33.20 c	26.61 a	26.61 a
	$N_{-30\%}$	22.73 c	22.83 c	10.55 ab	10.46 ab	35.38 b	35.70 b	29.87 a	29.35 a
	$N_{-45\%}$	21.78 d	21.70 d	11.02 a	11.00 a	39.66 a	39.02 a	27.78 a	28.26 a
HD	N_0	15.72 d	15.75 d						
	N_{CK}	28.69 a	28.77 a	10.73 b	10.73 c	40.54 c	40.68 b	26.49 a	26.40 a
	$N_{-15\%}$	27.65 ab	27.75 ab	11.92 a	11.84 a	43.87 bc	44.10 ab	27.23 a	26.81 a
	$N_{-30\%}$	26.64 b	26.75 b	12.87 a	12.95 a	48.75 ab	49.10 a	26.42 a	26.38 a
	$N_{-45\%}$	24.55 c	24.69 c	13.18 a	13.14 a	50.15 a	50.82 a	26.28 a	25.88 a
Year (Y)		43.4***		0.59 ns		0.12 ns		0.01 ns	
Planting density (D)		56.5***		426.1***		672.6***		24.5***	
Nitrogen rate (N)		19.6***		71.9***		70.3***		16.6***	
Y×D		50.7***		0.02 ns		0.03 ns		0.01 ns	
Y×N		97.9***		0.03 ns		0.03 ns		0.06 ns	
D×N		58.4***		0.76 ns		3.26*		3.70**	
Y×D×N		78.8***		0.09 ns		0.08 ns		0.18 ns	

NIE decreased gradually in the LD treatment. At MD and HD, there was an initial increase in NIE with increasing N rate, before an eventual reduction for these treatments as well.

We examined the effects of total N uptake on grain yield and DAM for all plant densities over the two years (Fig. 4). For all plant densities, both grain yield and dry matter increased with increase in total N uptake, but these increases were relatively small as total N uptake rose above 18.86 g m⁻² at LD, 22.78 g m⁻² at MD and 27.70 g m⁻² at HD.

3.4. LAI and PAR interception

N supply had a large effect on the rate of increase and the maximum value of the LAI for all plant densities in both years (Fig. 5). The LAI rose to a maximum near VT at all plant densities. This was followed by a sharp decline in the LAI as leaves senesced during grain filling. As the N rate decreased, the LAI experienced a gradual decline. However, there were no significant differences in the LAI between the N_{CK} and $N_{-30\%}$ treatments at all plant densities. Plant density, however, did affect the LAI significantly. Averaged for the effect of N rates, peak LAIs of 4.55, 5.56 and 6.98 were observed at LD, MD and HD, respectively, in 2013, while peak LAIs of 4.57, 5.66 and 7.02, respectively, were observed in 2014.

The RI_{frac} increased until VT for all treatments in both years (Fig. 6). Subsequently, the RI_{frac} remained relatively unchanged for some time followed by a slight decline during the latter half of the grain filling stage. There were marked changes in RI_{frac} in response to N fertilizer supply at all plant densities during the 2013 and 2014 growing seasons (Fig. 6). The N_0 treatments produced the maximum RI_{frac} values of 0.84 (LD), 0.86 (MD) and 0.89 (HD), which rose by 3.0–7.2, 2.8–7.5 and 3.2–8.9%, respectively, as the N rate increased. RI_{frac} increased with increasing plant density, starting at V6, during both years. Higher plant density led to average increases in the maximum RI_{frac} of 2.6% (MD *vs*. LD) and 6.8% (HD *vs*. LD) over the two years.

3.5. RUE and the relationship with N uptake and grain yield

There was a strong linear relationship (R^2>0.98) between RI_{acc} and DMA at all plant densities over the two years (Fig. 7). The N rate increased the RUE values by 7.8–10.1, 7.5–12.7 and 4.7–9.7% for LD, MD and HD, respectively

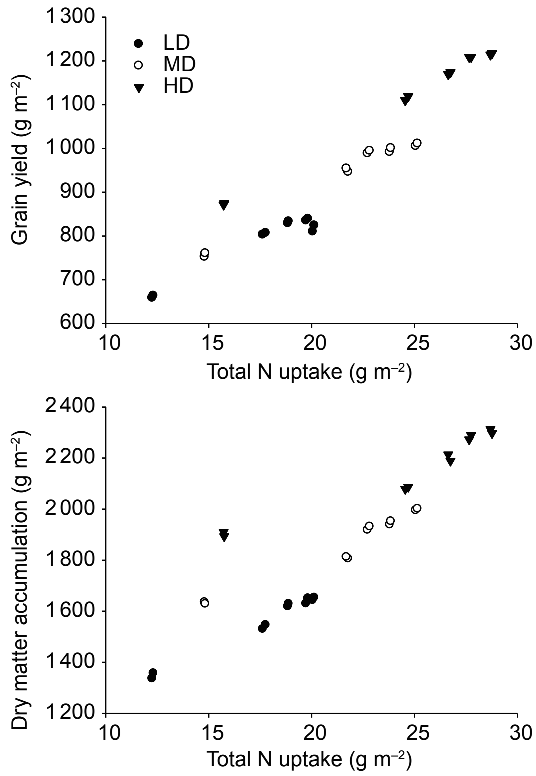

Fig. 4 Relationship between grain yield (A) and dry matter accumulation (B) and total N uptake at R6 under LD, MD and HD for all N supply levels.

(Table 3). As N rate decreased, the RUE decreased gradually. The RUE values for N_{CK} at LD, MD and HD were 2.84, 3.29 and 3.74 g MJ^{-1} PAR, respectively. These values were reduced by 2.1, 4.6 and 4.5% to 2.78, 3.14 and 3.57 g MJ^{-1} PAR, respectively, by the $N_{-45\%}$ treatment. Plant density also had a significant effect on RUE for all N supply treatments over the two years. Averaged over N rates, the RUE grew by 13.3 and 30.2% when plant density was increased from LD to MD and to HD, respectively, over the two years.

The total RI_{acc} values for the N_{CK} treatment at LD, MD, and HD over the two years were 625, 662 and 690 MJ PAR m^{-2}, respectively. With the N rate reduced by 30%, RI_{acc} decreased by 2.7, 3.2 and 2.5% at LD, MD and HD, respectively. On average, plant density increased the total RI_{acc} by 5.5 and 10.0% in the MD and HD treatments, respectively, compared to LD.

To further examine these changes in RUE with changes in N rate and plant density, we compared RUE with total N uptake and grain yield over the two years (Fig. 8). There was a strong linear relationship between total N uptake and RUE for all plant densities (Fig. 8-A). There were, however, clear differences in this relationship among different plant densities. While the RUE increased with increases in total N uptake at all plant densities, the magnitude of the RUE increase was small when total N uptake rose above 18.9 g m^{-2} at LD, 22.8 g m^{-2} at MD and 27.7 g m^{-2} at HD. At the same time, grain yield rose with increases in RUE, but again, the magnitude of this increase was small when RUE values were above 2.78 g MJ^{-1} PAR at LD, 3.17 g MJ^{-1} PAR at MD and 3.66 g MJ^{-1} PAR at HD over the two years (Fig. 8-B).

4. Discussion

4.1. Grain yield and yield components

In the NPC, summer maize is followed by winter wheat in the winter wheat-summer maize double cropping system. There have been many problems in the traditional planting patterns of smallholders. First, the density of summer maize in the fields of small land-holders is usually less than 60 000 plants ha^{-1}, which might produce high grain yield per plant, but achieving high grain yield per hectare is difficult (Nan 2010). However, plant densities of 80 000–120 000 plants ha^{-1} have resulted in high yields in this area (Yang *et al*. 2010; Lü *et al*. 2011). Second, farmers have applied excessive fertilizer lavishly to try to maximize crop yields. Generally, under high N supply, only 5–15% of fertilizers are transformed into food (Erisman *et al*. 2007). The remaining N is lost as gaseous emissions or leached from the soil (Bowman *et al*. 2008).

In this study, maize grain yield responded positively to increases in plant density and N rate. Differences in grain yield over various plant densities and N rates were associated with changes in both DMA and HI. The increase in HI from 0.46 to 0.51, (10.87%) was proportionally lower than the increase in DMA from 1 335 to 2 362 g m^{-2}, (76.9%), indicating the dominant effect of plant density and N treatment was on DAM in maize. Similar results were reported by Muchow (1994), Massignam *et al*. (2009) and Ciampitti and Vyn (2011). Although, we obtained the highest grain yield with the combination of HD and N_{CK}, our results from LD and MD showed that increased N rate did not increase maize grain yield indefinitely. Furthermore, there were no significant differences between N_{CK} and $N_{-30\%}$ at HD, and, due to the input of excessive N fertilizer, N_{CK} failed to obtain high NUE compared to $N_{-30\%}$. Thus, an appropriate reduction in the N rate and an increase in plant density would have little or no effect on grain yield, but could significantly increase NUE. This is consistent with results obtained by Jin *et al*. (2012).

In terms of grain yield components, the most dramatic responses to changes in plant density and N rate were observed in KN and, to a lesser extent, KW. Below *et al*. (2000) reported that the N rate exerts an effect on carbon and N metabolism in developing kernels, which might increase final grain set. However, previous results showed that the

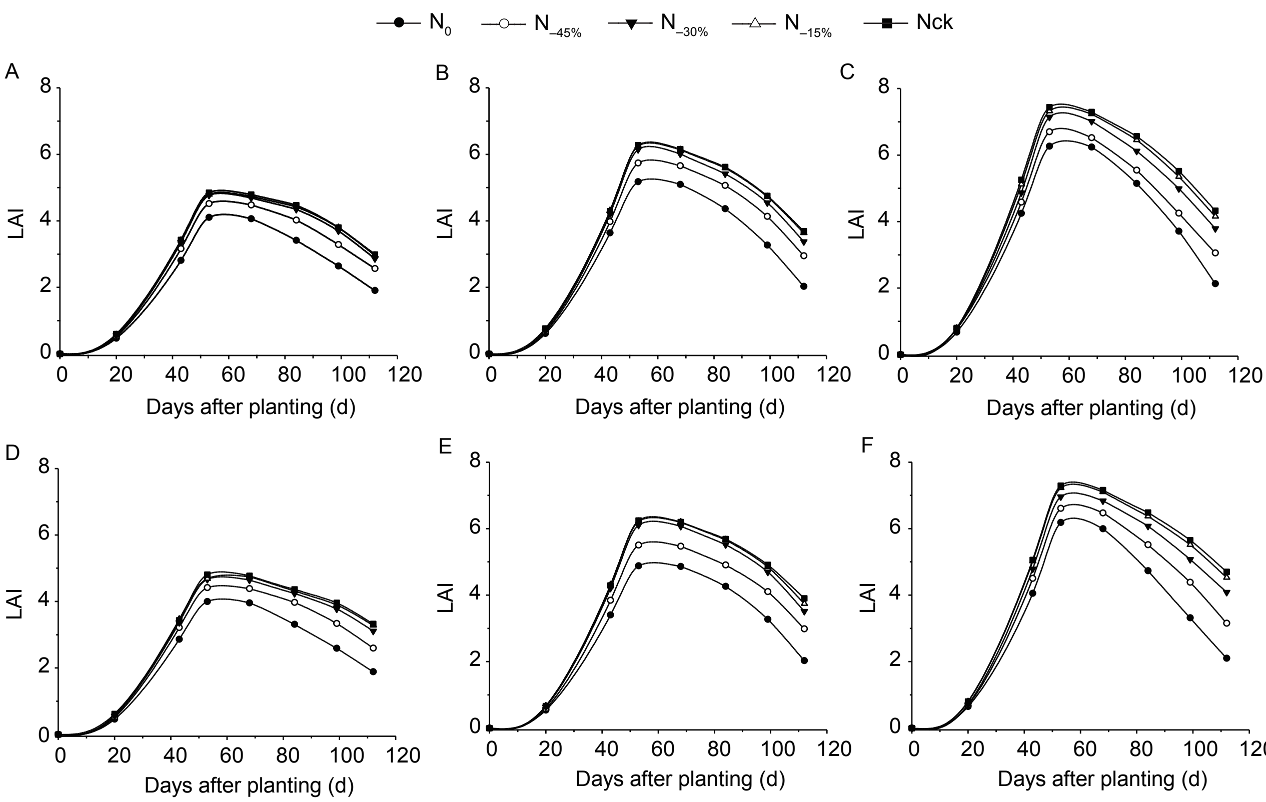

Fig. 5 The leaf area index (LAI) *vs*. days after planting for the different plant densities and N rates in 2013 and 2014 growing seasons. For the year factor: A, B and C, 2013; D, E and F, 2014. For the plant density factor: A and D, LD; B and E, MD; C and F, HD.

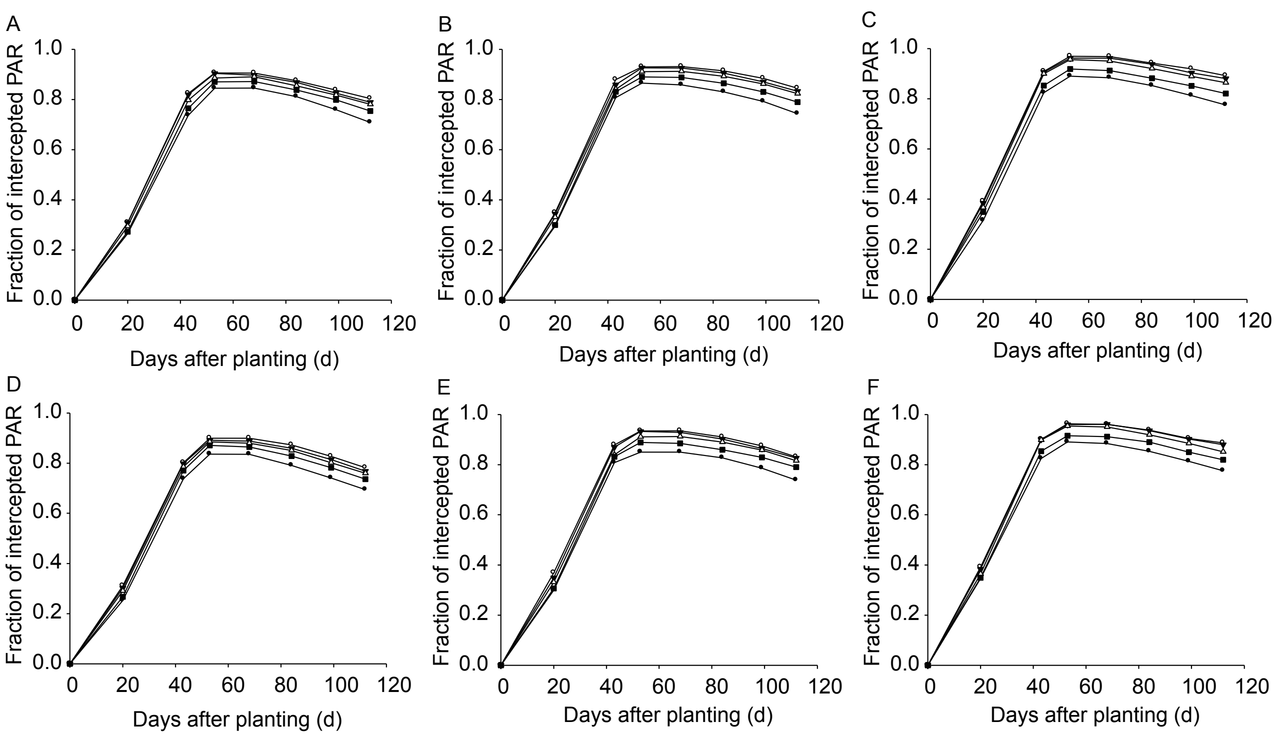

Fig. 6 Fraction of intercepted photosynthetically active radiation (PAR) for maize under LD (A and D), MD (B and E) and HD (C and F) grown with N_0, N_{CK}, $N_{-15\%}$, $N_{-35\%}$, and $N_{-45\%}$ applied in 2013 (A–C) and 2014 (D–F).

increase in KN due to the N rate is not sustained, and there is no significant effect when the N rate reaches very high levels (Uhart and Andrade 1995). We detected similar results in our study. There was no significant difference in KN per plant between the N_{CK} and $N_{-30\%}$ treatments for all plant densities in the two years, although we observed a continual increase in KN per unit area at higher plant densities. With respect to KW, Jones *et al*. (1996) reported that N supply might increase the duration of the effective grain-filling period and the grain-filling rate, while higher plant density has a tendency to reduce the duration and rate of grain-filling (Lemcoff and Loomis 1994).

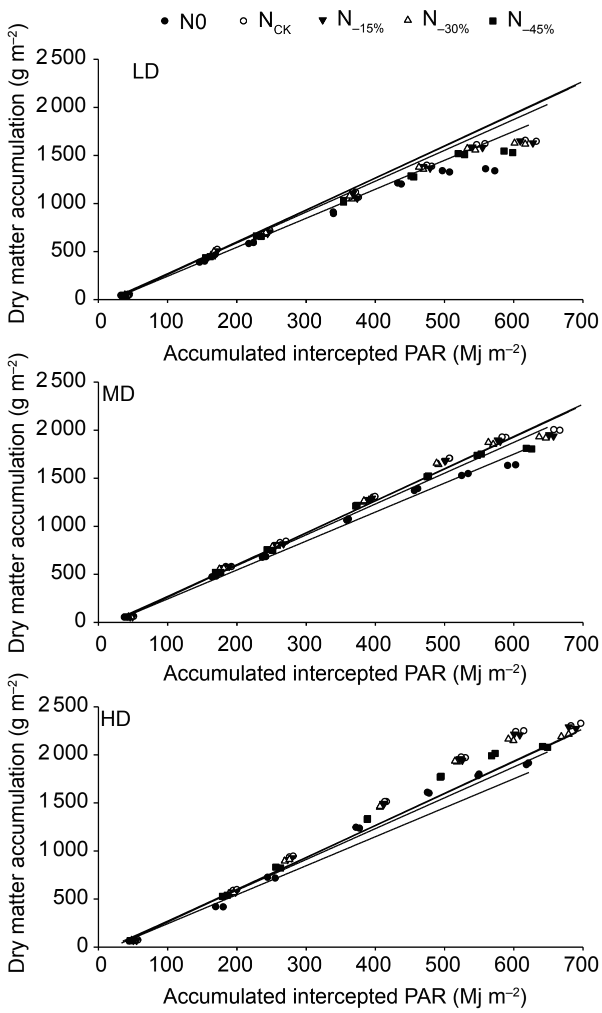

Fig. 7 Dry matter accumulation against accumulated intercepted PAR for summer maize in different N fertilization rates under LD, MD and HD for data combined over two seasons.

4.2. N uptake and N efficiency indices

We observed greater N uptake per unit area at R6 when plant density and N rate increased. However, when the N rate exceeded 224 kg ha^{-1} ($N_{-30\%}$), maize plants did not demonstrate N deficiency symptoms across all plant density treatments, and further increases in N uptake did not produce more DMA or grain yield, suggesting that soil plus fertilizer N might have been enough to meet the crop N demand at this N uptake level. Similar results were reported by Ciampitti and Vyn (2012), who pointed out that the association between maize grain yield and plant N uptake at the end of the growing season is the best represented by a linear-plateau model.

Numerous studies have shown that NUE decrease with the increased N rate (McDonald 1992; Timsina *et al*. 2001). In contrary, reducing the amount of N fertilizer applied could achieve a more optimal balance between crop demand and N supply (Cassman *et al*. 2002). In this study, although the highest total N uptake was obtained with the combination of HD and N_{CK}, the NUE was low, which did not reflect effective use of N. Increasing plant density could improve NUE significantly, and the NUE was increased significantly at a lower N rate at any plant density. This study showed that, although the N effects at $N_{-30\%}$ were lower than those at N_{CK}, the density effect and interaction effect of plant density and N rate were significantly better than those at N_{CK}, which effectively improved NUE.

The use of grain NUE by itself does not provide a sufficient basis for understanding the impact of management practices on crop N dynamics, which comprises both soil and plant processes (Salvagiotti *et al*. 2009). To improve our understanding of the impacts of plant density and N application practices on crop N dynamics, we dissected NUE into two main components, NIE and NRE. NIE is

Table 3 Calculated values of radiation use efficiency (RUE, g MJ^{-1} PAR, from Fig. 7) and final amount of accumulated intercepted radiation (RI_{acc}, MJ PAR m^{-2}) for the planting density of LD, MD and HD over the two years[1)]

Nitrogen rate	LD		MD		HD	
	RUE	RI_{acc}	RUE	RI_{acc}	RUE	RI_{acc}
N_0	2.58 (0.9837)	566	2.92 (0.9929)	597	3.41 (0.9902)	620
N_{CK}	2.84 (0.9880)	625	3.29 (0.9902)	662	3.74 (0.9889)	690
$N_{-15\%}$	2.84 (0.9890)	618	3.24 (0.9879)	654	3.72 (0.9886)	684
$N_{-30\%}$	2.84 (0.9898)	608	3.27 (0.9888)	641	3.66 (0.9857)	673
$N_{-45\%}$	2.78 (0.9887)	592	3.14 (0.9900)	621	3.57 (0.9910)	645

1) Data in brackets are R^2 values.

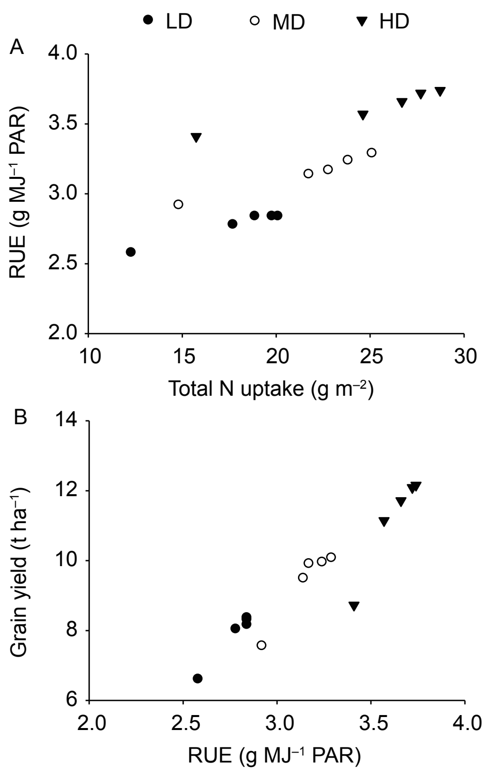

Fig. 8 Relationship between total N uptake and radiation use efficiency (RUE) (A) and the relationship between RUE and grain yield (B) under LD, MD and HD for all N supply levels.

more reflective of the plant's ability to convert or utilize N in producing final grain yield, while NRE is more representative of the plant's N uptake efficiency.

In our work, as was the case for NUE, the highest values observed for both NRE and NIE occurred at low N rates. However, the variations in NRE and NIE in response to N rates at different plant densities differed. As for NUE, the NRE at any plant density and the NIE at LD increased with the N rate declined, while no significant differences were found in NIE among various N rates at MD and HD, and the maximum values were observed at $N_{-30\%}$ (MD) or $N_{-45\%}$ (HD). These results suggest that the increases in NUE obtained by reducing N rate at high plant density were associated more with changes in NRE than in NIE. Hence, the greatest NUE does not necessarily correspond to the highest grain yield. We should therefore focus on both components, NRE and NIE, to achieve a balance of grain yield and NUE.

4.3. Resource use efficiency

We found that the RI_{frac} increased significantly with increase of plant density and N rate in association with an increase in the LAI, which was similar to results of Massignam *et al*. (2009). However, the increase in the LAI observed at higher rate of N supply did not result in a proportional increase in the RI_{frac} at any plant density. This difference might be partially explained by light interception by the tassels and ears (Duncan *et al*. 1967; Rosenthal *et al*. 1985; Tetio-Kagho and Gardner 1988).

The DMA is related to the RI_{acc} and RUE (Gallagher and Biscoe 1978). Increasing plant density could increase the amount of radiation intercepted by the plant canopy (Papadopoulos and Pararajasingham 1997) and, thus, enable plants to use solar radiation more efficiently (Tokatlidis and Koutroubas 2004). In this study, the DMA at R6 increased significantly as plant density increased. In addition, RUE increased from 2.8 to 3.6 g MJ^{-1} PAR, and RI_{acc} increased from 602 to 663 MJ PAR m^{-2}, which was consistent with observations from prior studies by Westgate *et al*. (1997), Konno (2001) and Olsen and Weiner (2007). In addition, because the RI_{frac} reached 0.95 at HD, it is clear that there would be little to no further improvement in light interception at still higher plant densities (Williams *et al*. 1968; Westgate *et al*. 1997; Tollenaar and Wu 1999).

The reduction in DMA under limited N was associated with decreases in both RI_{acc} and RUE (Massignam *et al*. 2009). Andrade *et al*. (1993) also demonstrated that the RUE of maize is reduced under suboptimal conditions. N limitations reduce carbon assimilation in the canopy by directly reducing leaf photosynthesis and accelerating leaf senescence (Massignam *et al*. 2011). The lowest RI_{acc} and RUE values were observed for the N_0 treatment at all plant densities, and these values increased with increases in the N rate; however, no significant increases in RUE were found when the N rate exceeded 224 kg ha^{-1}. Thus, excessive application of N would not continue to lead to increases in NUE. On the contrary, excessive application might result in an imbalance in canopy structure and reduction in RUE.

5. Conclusion

In summary, our results showed clearly that increasing plant density can promote N absorption and LAI, which allowed high total N uptake and interception of radiation to achieve high DMA, grain yield, NUE, and RUE at the same N rate. However, with the increase of planting density, the demands for N fertilizer are also increasing with grain yield. Due to the input of too much N fertilizer, the efficiency of N use for N_{CK} was lower. At the same time, when N rate reach to a high level, no significant increase even decrease were observed in grain yield. In order to achieve synchronization between high yield and high N efficiency, we should decrease the total amount of N supplied. In the current study, considering the demands for high grain yield and high resource use efficiency, a 30% reduction in the N supply and an increase in plant density of 3 plants m^{-2} (compared to LD), would achieve both goals; consequently, we consider this to be an appropriate agronomic management practice. Our research might be valuable for developing radiation-based growth methods for maize while simultaneously complying with the principles of sustainable crop production in the NCP.

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