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Nitrogen utilization efficiency in maize as affected by hybrid and N rate in late-sown crops

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ABSTRACT

After the introduction of Bt-maize, late sowing is becoming an important strategy to stabilize yields in many areas of the Argentine Pampas. Increased nitrogen (N) availability and sharp reduction in radiation and temperature during grain filling period are dominant features of late-sown maize. Deployment of late sowing therefore requires a better understanding of the nitrogen economy of the crop in a deteriorating photothermal environment. Our aims were to: (i) evaluate the effect of late sowing on the components of maize nitrogen utilization efficiency, i.e. grain yield per unit of N uptake, (ii) assess the interactions among sowing date, hybrid and N rate on N economy, (iii) study the links between biomass and N accumulation and partitioning involved in nitrogen utilization efficiency in late sown maize. Two irrigated experiments were conducted in Paraná, Argentina (−31°50; −60°31; 110 m.a.s.l) during two consecutive seasons. Treatments included the factorial combination of two hybrids with low (DK752MG) and high (DK682MG) harvest index (HI), two rates of N fertilization (0 and 200 kg N ha^{-1}) and two contrasting sowing dates (September and December). Grain yield, shoot biomass, N concentration in grain, stover and biomass were measured. From these measurements we calculated N uptake, N accumulated in stover and grain and, N utilization efficiency for yield ($N_{ut}E_Y$) and biomass ($N_{ut}E_B$) production as the ratio between yield or biomass and N uptake. A nitrogen nutrition index (NNI) was calculated to compare treatments at a similar N status. Late sowing increased soil N availability, hence reducing the response to N fertilization in comparison to traditional sowing, i.e. there were significant interactions between sowing date and N rate for most traits. The NNI accounting for the allometry of nitrogen and biomass proved to be an effective procedure in interpreting these interactions. The increase in N status reduced the $N_{ut}E_B$, although at an equivalent NNI it was higher in traditional than in late sowing, which reflects the lower crop ability to use nitrogen in producing biomass when constrained by late growth. The hybrid DK682MG, showed more ability than DK752MG to allocate both biomass and N to grain in late sowing, as reflected by the higher HI and NHI as well as the lower amount of N accumulated in stover. Overall, our results support adaptive practices for late-sown maize in the Northern Pampas, including the use of hybrids with high partitioning of N and biomass to grain as well as the use of more conservative N fertilizer rates.

1. Introduction

The efficient use of nitrogen (N) is critical to meet the dual need of increasing productivity and profitability of the cropping system and reducing the risk of unproductive and pollutant nitrogen loss (Raun and Johnson, 1999; Cassman et al., 2003; Dobermann and Cassman, 2004). However, nitrogen use efficiency is a complex trait spanning scales from molecular to cropping system (Ortiz-Monasterio et al., 1997; Hirel et al., 2007; Andrews et al., 2009; Sylvester-Bradley and Kindred, 2009; Hirose, 2011). At the crop level, the focus of this paper, nitrogen use efficiency (NUE) indicates the amount of shoot biomass (B) o grain yield produced per unit of available N (Nav) (Moll et al., 1982). In turn, NUE can be partitioned in two main components (Moll et al., 1982), N uptake efficiency ($N_{upt}E$), i.e. N uptake (N_{upt}) per unit of N_{av}, and N utilization efficiency ($N_{ut}E$), i.e., biomass or yield per unit of N_{upt} ($N_{ut}E_B$ or $N_{ut}E_Y$, respectively). It has been suggested that $N_{upt}E$ is a more important component under high N availability, whereas $N_{ut}E$ is

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more important under low N availability (Moll et al., 1982; Ma and Dwyer, 1998).

Increases in biomass per unit nitrogen uptake, harvest index or both can enhance yield per unit nitrogen uptake, in accordance with the following equation:

$$N_{ut}E_Y = N_{ut}E_B \times HI \quad (1)$$

$$N_{ut}E_Y = \frac{B}{N_{upt}} \times HI \quad (2)$$

Accordingly, yield per unit nitrogen uptake is directly related to HI and inversely related to N concentration in shoots ($\%N_B$) (Ciampitti and Vyn, 2012):

$$N_{ut}E_Y = \frac{HI}{\%N_B} \quad (3)$$

The framework outlined above (Eqs. (1)–(3)) is widely used to assess agronomic and breeding avenues to improve the efficiency in the use of nitrogen. However, nitrogen uptake – a key trait in this model – is related to both soil N availability and crop growth rate (Lemaire and Gastal, 2009). For example, selection for yield has improved N uptake of maize in USA (Haegele et al., 2013) and wheat in UK (Foulkes et al., 1998), but these studies did not attempt to separate the putative effect of increased biomass. To account for the allometry between nitrogen uptake and crop growth, the components of nitrogen use efficiency must be linked to the nitrogen nutrition index, NNI (Lemaire and Gastal, 2009; Sadras and Lemaire, 2014), in turn quantified using species-specific nitrogen dilution curves as illustrated for maize (Ciampitti et al., 2012) and wheat (Sadras and Lawson, 2013).

In temperate and subtropical areas of Argentina, early spring sowing (September-mid October) of maize (*Zea mays* L.) allows for high yield potential and avoidance of late season damage of stem borer [*Diatraea saccharalis* (*Frabricius*) *Lepidoptera*: *Crambidae*] and fall armyworms [*Spodoptera frugiperda* (*Smith*) *Lepidoptera*: *Noctuidae*]. After the introduction of Bt-maize the key trade-offs have been simplified. In fact, growers now have two options: (i) the traditional early spring sowing seeking high yield potential at the expense of actual yield reductions caused by water deficit at critical stages in summer, or (ii) late spring sowing (November–December) seeking lower risk of water deficit at critical stages at the expense of yield potential and actual yield in wetter seasons (Maddonni, 2012).

Deploying this late sowing strategy requires, however, a better understanding of the effects of sowing date on the nitrogen economy of the crop and related traits including biomass production and harvest index (Eqs. (1)–(3)) as well as the nitrogen nutrition index as a measure of crop nitrogen status. Delayed sowing, and consequently higher temperature during the vegetative period, usually shortens the crop growing period (Cirilo and Andrade, 1994; Otegui et al., 1995), although not necessarily reduces the capture of solar radiation and biomass production due to higher incident solar radiation. Additionally, lower temperature during grain filling may decrease radiation use efficiency in late sown crops (Andrade et al., 1993). The reduced radiation and radiation use efficiency during grain filling in late sown maize may reduce the source/sink ratio, compel remobilization of carbon and nitrogen from the vegetative organs to grain, and reduce HI. However, these physiological responses to late sowing can vary among hybrids. For example, maize hybrids vary in leaf senescence and N relocation in response to source:sink ratio (Crafts-Brandner and Poneleit, 1987). Hence, hybrids with an improved ability to partition biomass and nitrogen to grain may be more suitable for late sowing.

Our aims were to: (i) evaluate the effect of late sowing on the components (Eqs. (1)–(3)) of maize nitrogen utilization efficiency for yield, (ii) assess the interactions among sowing date, hybrid and N rate on nitrogen traits, (iii) study the links between biomass and N accumulation and partitioning involved in nitrogen utilization efficiency in late sown maize.

2. Materials and methods

2.1. Site, experiments and crop management

The experiments were described in Melchiori and Caviglia (2008). Briefly, crops were grown at INTA experimental station in Paraná, Argentina (−31°50; −60°31; 110 m.a.s.l) during 2002–2003 (Year 1) and 2003–2004 (Year 2). The maize growing season in our region is characterized by moderate to high average temperatures (20.7 °C from September to April, ranging from 15.2 to 24.8 °C) and a frost free period of ca. 240 d. Traditional sowing date is September–October, whereas late sowing ranges from mid December to mid January. Meteorological variables were recorded in a standard weather station located <400 m of experiments.

The soil was a fine, mixed, thermic Aquic Argiudoll under no-till since 1998. Organic matter in the top 0.20 m was 2.90–3.05 g kg^{-1} and previous crops were soybean in 2002 and maize in 2003. The soil showed no physical restriction, had an adequate P availability (>20 mg kg^{-1} P Bray) and was further fertilized with 20 kg P ha^{-1} as triple superphosphate at sowing. Crops were sown with a pneumatic planter and plant population was adjusted to 8.5 plants m^{-2} by thinning at V2 stage. Experiments were kept free of weeds, diseases and insects and sprinkler irrigated to maintain soils above 60% of the potential plant available water. All treatments were irrigated with the same amount of water when needed.

Treatments were a combination of three factors; two single cross hybrids: DK682MG (hereafter DK682) with higher HI and relative maturity = 118 and DK752MG (hereafter DK752) with lower HI and relative maturity = 125, two sowing dates: traditional in the region (15 Sep 2002, 3 Sep 2003) and late (26 Dec 2002, 30 Dec 2003) and two N rates (0 and 200 kg N ha^{-1}, hereafter N0, and N200, respectively). Treatments were arranged in a randomized complete block design with four replicates. Each plot had 5 rows, 0.70 m apart and 20 m long. Nitrogen was broadcast as urea (46% N) immediately after sowing.

2.2. Traits related to yield and crop nitrogen economy

The phenological development (Ritchie and Hanway, 1982) of 10 consecutive plants, in the central row of the well fertilized plots (N200), was recorded twice a week. The duration of the sub-periods was expressed both in days and in degree-days (base temperature = 8 °C) (Ritchie and NeSmith, 1991).

At physiological maturity (R6), 10 consecutive plants in the central row of each plot were sampled and oven-dried at 65 °C. After drying, ears were removed, manually shelled and cobs were weighed together with stalks and leaves to determine stover dry weight. Harvest index (HI) was calculated as the ratio between grain yield and total aboveground biomass. Grain yield and its numerical components (grain number per unit area and grain size) were reported in Melchiori and Caviglia (2008).

Grain and stover were milled and nitrogen (N) concentration ($\%N_g$ and $\%N_{st}$, respectively) was determined by a Kjeldhal microdistillation technique (Nelson and Sommers, 1973). Nitrogen accumulated in grains (N_g) and in stover (N_{st}) was calculated as the product of biomass of grain or stover and the N concentration in the plant material. Nitrogen uptake (N_{upt}) was calculated as the sum of the amount of nitrogen in grain and stover. Nitrogen concentration in total aerial biomass ($\%N_B$) was calculated as the quotient between N_g and total aerial biomass, i.e. grain and stover.

Nitrogen utilization efficiency was calculated as the quotient between biomass ($N_{ut}E_B$) or yield ($N_{ut}E_Y$) and nitrogen uptake.

Nitrogen harvest index (NHI) was calculated as the quotient of N_g and N_{upt}.

To evaluate the N status of the crops, we calculated the nitrogen nutrition index (NNI, Plénet and Lemaire, 1999) for each replicate, as $N_{act}\ N_c^{-1}$, where N_{act} is the actual and N_c the critical N concentration in biomass, respectively. N_c was calculated as $3.4\ B^{-0.37}$ (Plénet and Lemaire, 1999), where B is biomass expressed in t ha^{-1}. Although the theoretical validity of NNI is restricted to silking + 25 d, it may be used for N diagnostics in maturing maize crops (Plénet and Lemaire, 1999). In Section 4.1, we consider further the consequences of using Plénet and Lemaire (1999) parameters in mature crops.

2.3. Statistical analysis

We used two complementary approaches to evaluate the effect of sowing date interacting with hybrid and nitrogen rate on the crop traits under study. First, we used analysis of variance accounting for sowing date, hybrid, nitrogen rate and their interactions; analysis was performed for each year separately. Second, we plotted trait values vs the NNI to allow for the interaction between sowing date and crop nutrition status after accounting for the effects on biomass (Lemaire and Gastal, 2009; Sadras and Lemaire, 2014).

The coincidence and the slope of the linear regressions between NNI and crop traits for each sowing dates were evaluated using dummy variables, i.e. including an additional, dichotomous variable (0 or 1) representing the sowing date in the linear model. Briefly, in addition to x values, the dummy variable (d) and the product of the dummy variable and x (d*x) were included as regressors into a multiple linear model to test the following hypotheses:

$$d * x = 0$$

for parallelism, i.e. the slope of the two linear models not differ between sowing dates

$$d * x = d = 0$$

for coincidence, i.e. the two linear models not differ between sowing dates.

3. Results

3.1. Growing conditions, phenology and photothermal environment

Table 1 and Fig. 1 summarize the phenology of the crops and the photothermal environment during key phenostages. Time from sowing to maturity was reduced from 122 to 133 days in traditional sowing to 102–97 days in late sowing (20–37%). Similarly, time to silking was shortened by 22–38% whereas the reduction in time from silking to maturity was 15–36%. The period from sowing to the beginning of critical period for grain number determination, i.e. 15 days before R1, was 62–69 days in traditional sowing and 48–46 days in late sowing, i.e. a shortening of 29–50%. In contrast, the period from sowing to the beginning of critical period for grain number determination expressed as thermal time increased slightly in late sowing, i.e. 10–14%. Thermal time during the critical period for grain number determination of 30 days bracketing silking (R1 ± 15 d) was similar between sowing dates in both years.

Year 1 had higher average temperature during March and April and solar radiation during December than Year 2. Rainfall (not shown) was higher in Year 1 than in Year 2, nonetheless soil water level was kept at an established level (see Section 2.1). Although monthly mean temperature were similar to the historical records (not shown) in both years, monthly rainfall in Year 1 was markedly higher with the exception of January and March, as much as two fold than the historical records. In contrast, rainfall during Year 2 was similar or higher than the historical records with exception of January, February and March. The rainfall pattern in Year 1 seems to be related with the occurrence of the phase El Niño of the El Niño-Southern Oscillation (ENSO) (NWS-NOAA, 2014), which is the most prominent year-to-year global climate fluctuation (McPhaden, 2004). In contrast, the rainfall pattern of Year 2 was unrelated with that phenomenon, which remained in a neutral phase during this year.

The photothermal environment during vegetative and reproductive period differed markedly between sowing dates (Fig. 1 and Table 1). In comparison to traditional sowing, late sowing reduced moderately the total amount of solar radiation between sowing and the beginning of the critical period for grain number determination (14–18%) but the reduction was dramatic between the end of critical period for grain number determination and R6 (38–51%). Small differences in the total amount of incident solar radiation during critical period for grain number determination were recorded between sowing dates (<11%). Mean temperature between sowing and the beginning of the critical period for grain number determination was 20–28% (Year 1 and 2, respectively) higher in late than in traditional dates, whereas there were small differences during the critical period for grain number determination. In the grain filling period, mean temperature was 5% higher in late than traditional sowing in Year 2 but 18% lower in Year 1.

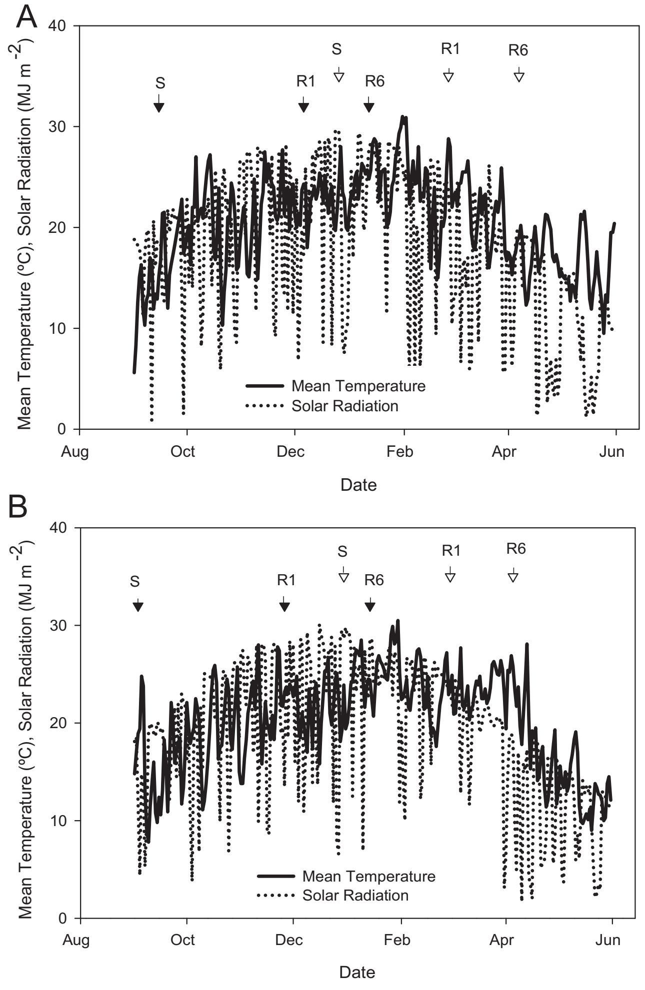

Fig. 1. Daily mean temperature and solar radiation during the maize growing season at traditional (closed arrows) and late (open arrows) sowing dates in Paraná, Argentina. (A) Year 1 (2002–2003), (B) Year 2 (2003–2004). S: sowing R1: silking, R6: physiological maturity.

Table 1
Duration of different phenological phases, average daily mean temperature, average daily solar radiation and cumulative solar radiation during the maize growing season at traditional and late sowing dates in two years at Paraná, Argentina. Phenological phases are S-BCP: sowing to beginning of critical period for grain number determination, BCP-ECP: beginning to the end of critical period for grain number determination, ECP-R6: end to critical period for grain number determination to physiological maturity (R6). BCP: 15 days before silking. ECP: 15 days after silking. Values are averages for the two hybrids, which had negligible differences in phenology.

		Year 1		Year 2	
		Sowing date			
		Traditional	Late	Traditional	Late
Phase duration (d)	S-R1	77	63	84	61
	R1-R6	45	39	49	36
	S-BCP	62	48	69	46
	BCP-ECP	30	30	30	30
	ECP-R6	30	24	34	21
	S-R6	122	102	133	97
Phase duration (°C d)	S-R1	975	1046	893	995
	R1-R6	690	502	679	559
	S-BCP	753	829	688	779
	BCP-ECP	444	435	410	433
	ECP-R6	468	285	474	343
	S-R6	1665	1548	1572	1554
Temperature (°C)	S-R1	20.5	24.4	18.7	24.2
	R1-R6	23.5	21.0	22.6	23.7
	S-BCP	20.1	25.3	18.0	24.9
	BCP-ECP	22.8	22.5	21.7	22.4
	ECP-R6	24.1	19.9	23.0	24.3
	S-R6	22.4	21.6	20.1	24.0
Solar radiation ($MJ\,m^{-2}$)	S-R1	19.7	22.1	19.6	23.9
	R1-R6	22.3	16.3	23.3	19.3
	S-BCP	19.6	21.9	19.2	23.7
	BCP-ECP	21.7	19.3	21.9	22.4
	ECP-R6	22.4	16.8	23.4	18.4
	S-R6	20.7	19.9	20.9	22.2
Cumulative solar radiation ($MJ\,m^{-2}$)	S-R1	1517	1390	1642	1455
	R1-R6	1004	634	1142	694
	S-BCP	1216	1050	1328	1091
	BCP-ECP	651	579	658	673
	ECP-R6	671	403	796	387
	S-R6	2520	2026	2783	2151

3.2. *Crop nitrogen status, grain yield and biomass production*

Table 2 summarizes NNI, grain yield, total and stover biomass, and HI in response to sowing date, nitrogen rate, hybrid and their interactions. A significant interaction between sowing date and N rate was detected in both years for the NNI. As a result, high nitrogen rate improved the nitrogen nutrition index in all cases except for the late sown crop in Year 1. In Year 1, the hybrid DK752 had a higher ($P < 0.01$) NNI in traditional sowing date than DK682, whereas there were not differences between hybrids otherwise.

Grain yield was affected by N rate and hybrid in both years. The effect of N on grain yield was, however, greater for traditional than for late sowing date, i.e. a significant sowing date × N interaction was detected. The yield response to N was 100% (Year 1) and 204% (Year 2) in traditional sowing date, compared to 10% (Year 1) and 78% (Year 2) in late sown-crops. The yield response to N was closely related to NNI in the unfertilized controls (Fig. 2). In late sowing grain yield in well fertilized treatments was 6–19% (DK682 and DK752, respectively) higher in Year 2 than in Year 1, whereas the opposite occurred in traditional sowing. In fact, grain yield of late-sown crops was 15–11% higher in Year 1 (DK682 and DK752, respectively) than in Year 2.

Total biomass increased with high N rate and was reduced in late sowing although N effect was higher in the traditional than in the late sowing date (significant interaction N × sowing date, Table 2). On average, fertilized crops had 34–48% less biomass in late than in traditional sowing date, which resulted from a higher reduction of yield (38–54%) than in stover biomass (29–43%). The relative reduction in total biomass with late sowing was larger than the reduction in the duration of growing season, i.e. 16–27% (Table 1); this suggests that late sowing also reduced crop growth rate. In both years, the stover biomass was consistently higher in traditional than in late sowing and in DK752 than in DK 682 (Table 2). Only in Year 2, the stover biomass was higher in fertilized treatments than in unfertilized controls.

The hybrid with high partitioning to grain, i.e. DK682, outyielded DK752 on average 12 and 10% in Year 1 and 2, respectively, independently of N rate and sowing date. Harvest index was 16 and 14% (Year 1 and 2, respectively) higher in DK682 than in DK752 and 16 and 24% (Year 1 and 2, respectively) higher in fertilized treatments than in unfertilized controls. The effect of N on HI was significant in both years; it was larger in traditional (35–54%) than in late sowing (6–14%), i.e. significant interaction N × sowing date. In late sowing HI was 3% higher in Year 1 for the well fertilized hybrid DK682 than in Year 2, in contrast with DK752, which had a lower HI in Year 1 than in Year 2. In traditional sowing HI was 6–8% higher in Year 1 (DK682 and DK752, respectively) than in Year 2.

3.3. *Nitrogen uptake and its partitioning*

Table 3 summarizes nitrogen uptake and its partitioning to grain and stover in response to sowing date, nitrogen rate, hybrid and their interactions. Nitrogen rate affected N uptake more strongly in traditional than in late sowing date (significant interaction N × sowing date). In fact, N rate increased N uptake by 135% in Year 1 and 234% in Year 2 for the traditional sowing date compared with 0 and 80% for late sowing. As a result, the range of nitrogen uptake was broader in traditional (80–219 $kg\,N\,ha^{-1}$) than in late

Table 2
Nitrogen nutrition index (NNI), grain yield, shoot biomass, stover biomass, and harvest index (HI) of two maize hybrids grown under contrasting N rates at traditional and late sowing dates during two seasons in Paraná, Argentina. NNI was estimated using actual N concentration and optimal N concentration as proposed by Plénet and Lemaire (1999).

Source of variation			Year 1					Year 2				
Sowing date	Hybrid	N rate (kg N ha^{-1})	NNI	Grain yield (kg ha^{-1})	Shoot biomass (kg ha^{-1})	Stover biomass (kg ha^{-1})	HI	NNI	Grain yield (kg ha^{-1})	Shoot biomass (kg ha^{-1})	Stover biomass (kg ha^{-1})	HI
Traditional	DK682	0	0.56	6565	14,218	8605	0.399	0.42	3986	10,213	6805	0.338
Traditional	DK682	200	1.00	14,037	20,890	8888	0.577	0.94	11,892	18,772	8605	0.542
Traditional	DK752	0	0.60	6951	15,410	9467	0.385	0.44	3732	10,924	7733	0.300
Traditional	DK752	200	1.16	13,058	23,208	12,044	0.481	0.99	11,602	22,525	12,605	0.441
Late	DK682	0	1.00	8358	14,002	6856	0.515	0.61	5874	10,768	5747	0.464
Late	DK682	200	0.98	9680	15,319	7043	0.540	0.88	10,303	16,810	8001	0.526
Late	DK752	0	0.84	6894	14,811	8917	0.405	0.62	4831	10,565	6435	0.402
Late	DK752	200	0.84	7101	13,902	7831	0.437	0.80	8790	16,393	8877	0.463
Analysis of variance							P > F					
Hybrid (H)			ns	+	ns	**	**	ns	+	ns	*	*
Sowing date (SD)			*	***	***	**	ns	ns	ns	+	*	*
N rate (N)			***	***	***	ns	*	***	***	***	***	***
H × SD			***	Ns	*	ns	ns	ns	ns	ns	ns	ns
H × N			ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns
N × SD			***	***	***	ns	+	***	***	*	ns	+
H × N × SD			ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns
SE			0.035	606	699	655	0.031	0.040	477	886	744	0.029

ns: not significant; SE: standard error.

+ $P < 0.05$.

* $P < 0.01$.

** $P < 0.001$.

*** $P < 0.0001$.

sown crops (119–155 kg N ha^{-1}). In traditional sowing, DK752 had 17–14% (Year 1 and 2, respectively) higher nitrogen uptake than DK682, whereas the opposite occurred in late sowing (significant interaction hybrid × sowing date), i.e. DK682 had 15–7% (Year 1 and 2, respectively) higher nitrogen uptake than DK752.

Nitrogen rate effect on nitrogen accumulated in grain at harvest (N_g) was grater in traditional than in late sowing (significant interaction N × sowing date). In Year 1, N rate increased N_g by 173% in traditional sowing and 8% in late sowing, whereas in Year 2 N_g was increased by 313 and 127%. In late sowing DK682 had 21% more N_g than DK752 in both years, although hybrids did not differ in traditional sowing (significant interaction hybrid × sowing date).

Nitrogen in stover (N_{st}) increased with N fertilization, more in traditional than in late sowing (significant interaction N × sowing date), except in late sowing of Year 1 where N_{st} was reduced by 16% in the fertilized treatment as compared with the unfertilized control. In turn, in traditional sowing date, nitrogen fertilization increased the N_{st} by 133–163% (Year 1 and 2, respectively) for DK752 compared with 15–118% (Year 1 and 2, respectively) for DK682. In late sowing, fertilization increased little (Year 2) or even reduced (Year 1) the N_{st}, without evident differences between hybrids.

Nitrogen harvest index increased with high N rate and was 7–14% (Year 1 and 2, respectively) higher in DK682 than in DK752. In Year 1, fertilizer increased NHI by 17% and 7% in traditional and

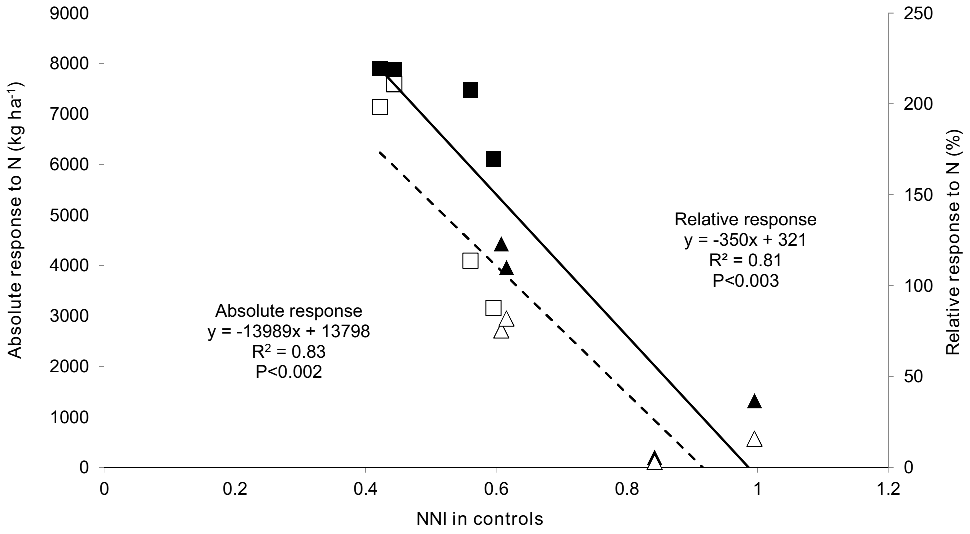

Fig. 2. Yield responses to fertilizer as a function of the nitrogen nutrition index in unfertilized controls. Absolute (closed symbols) and relative (open symbols) yield response to N in late (triangles) and traditional (squares) sown maize grown under contrasting N rates during two seasons in Paraná, Argentina. Responses were calculated as the yield difference between the fertilized and non-fertilized crops. Relative response was calculated using the non-fertilized as reference. Data for two hybrids aligned in the same linear relationships.

Table 3

Nitrogen uptake (N_{upt}), N accumulated in grain (N_g), in stover (N_{st}) and N harvest index (NHI) in two maize hybrids grown under contrasting N rates at traditional and late sowing dates during two seasons at Paraná, Argentina.

Source of variation			Year 1				Year 2			
Sowing date	Hybrid	N rate (kg N ha^{-1})	N_{upt} (kg ha^{-1})	N_g (kg ha^{-1})	N_{st} (kg ha^{-1})	NHI (kg N kg N^{-1})	N_{upt} (kg ha^{-1})	N_g (kg ha^{-1})	N_{st} (kg ha^{-1})	NHI (kg N kg N^{-1})
Traditional	DK682	0	96.1	55.1	40.9	0.574	59.3	33.4	25.9	0.564
Traditional	DK682	200	218.3	164.3	54.0	0.754	190.9	134.4	56.5	0.709
Traditional	DK752	0	107.6	64.2	43.4	0.583	65.0	33.3	31.7	0.516
Traditional	DK752	200	269.0	161.2	107.8	0.599	225.0	141.5	83.5	0.629
Late	DK682	0	169.2	110.0	59.2	0.649	88.5	45.0	43.4	0.510
Late	DK682	200	176.9	126.0	50.9	0.712	167.8	109.7	58.1	0.658
Late	DK752	0	149.6	92.1	57.5	0.617	88.1	39.6	48.5	0.455
Late	DK752	200	143.1	92.9	50.1	0.648	149.2	82.2	67.0	0.552
Analysis of variance						P > F				
Hybrid (H)			ns	ns	*	+	ns	ns	+	*
Sowing date (SD)			ns	ns	ns	ns	ns	**	ns	+
N rate (N)			***	***	*	+	***	***	***	***
H × SD			**	+	*	ns	+	+	ns	ns
H × N			ns	ns	*	ns	ns	ns	ns	ns
N × SD			***	***	***	ns	***	***	+	ns
H × N × SD			ns	ns	*	ns	ns	ns	ns	ns
SE			9.8	9.1	6	0.04	10	5.7	6.3	0.031

ns: not significant; SE: standard error.

$^{+}$ $P < 0.05$.

* $P < 0.01$.

** $P < 0.001$.

*** $P < 0.0001$.

late sowing date, respectively, in contrast with Year 2 where NHI was increased by 24–25% irrespective of sowing date. Sowing date did not affect NHI in Year 1, whereas NHI of fertilized crops was 10% lower in late than in traditional sowing date in Year 2.

3.4. Nitrogen concentration in grain, biomass and stover and N utilization efficiency

Table 4 shows N concentration in grain, biomass and stover and N utilization efficiency for biomass and yield in response to sowing date, nitrogen rate, hybrid and their interactions. Nitrogen concentration in grain (%N_g) was strongly affected by N rate, although the effect was different between sowing dates, i.e. a significant interaction N × sowing date was detected. High N rate increased N concentration in grain by 36–38% in traditional sowing date in both years, did not affect it in late sown crops in Year 1, and increased it by 26% in late sown crops in Year 2.

Similarly, N concentration in biomass (%N_B) increased more in response to N in traditional than in late sowing (significant interaction N × sowing date). Noticeably %N_B was, on average, higher in late than in traditional sowing.

Nitrogen concentration in stover increased 25–98% with N rate only in traditional sowing date, whereas no differences between hybrids and N rates were found in late sowing. The %N_{st} was on average, higher (18–44%, Year 1 and 2, respectively) in late than in traditional sowing.

Fertilizer reduced nitrogen utilization efficiency for biomass in traditional sowing in both years and in late sowing in Year 2. At high N rate, $N_{ut}E_B$ did not differ between hybrids and sowing dates. Nitrogen utilization efficiency for yield was unaffected by N or sowing date in Year 2 but was consistently higher (13–16%) in the hybrid with higher harvest index DK682 in comparison with DK752.

3.5. Relationships between nitrogen nutrition index, crop traits and N utilization efficiency

Nitrogen nutrition index was negatively related with $N_{ut}E_B$ (Fig. 3A), positively related with nitrogen concentration in biomass and HI (Fig. 3B and C, respectively), and poorly related with yield per unit N_{upt} ($N_{ut}E_Y$) (Fig. 3D). In the explored range of NNI, the reduction in $N_{ut}E_B$ as affected by NNI was as high as 100% (Fig. 3A), in contrast with the little reduction in $N_{ut}E_Y$ (Fig. 3D).

At equivalent nutritional status, as indicated by NNI, $N_{ut}E_B$ was higher and %N_B was lower in traditional than in late sowing (Fig. 3A and B), i.e. lineal models were not coincident ($P < 0.0002$). No differences between sowing dates were found for HI (Fig. 3C) and $N_{ut}E_Y$ (Fig. 3D) at equal NNI, i.e. the linear relationships were coincident ($P > 0.1$).

For the pooled data, both $N_{ut}E_B$ and $N_{ut}E_Y$ were negatively correlated with %N_B but the association was stronger for the former ($r = -0.98$, $P < 0.0001$ vs -0.63, $P < 0.009$). Remarkably, $N_{ut}E_B$ was negatively related to HI ($r = -0.82$, $P < 0.0001$) whereas $N_{ut}E_Y$ and HI were unrelated. The reduction in $N_{ut}E_B$ was related to N_{upt} only up to a threshold of ca. 159 kg N ha^{-1} (Fig. 4). Further increases over that threshold of N_{upt} did not reduce the $N_{ut}E_B$, which leveled off at 93.5 kg kg N^{-1} for both hybrids. The linear relationship between NNI and both N_{upt} and N_g (Fig. 3E and F), suggests that the threshold of N_{upt} was probably linked to the lack of relationship between NNI and N_{st} in late sowing ($P > 0.1$) (Fig. 3G).

Nitrogen concentration in grain (%N_g) was negatively associated with both $N_{ut}E_B$ and $N_{ut}E_Y$ ($r = -0.79$ and -0.74, respectively). At an equivalent N concentration, the hybrid with higher HI, i.e. DK682, had a consistently higher $N_{ut}E_Y$ (Fig. 5).

4. Discussion

4.1. Limitations and insights from the application of the nitrogen nutrition index

Nitrogen dilution curves used to derive the NNI assume vegetative plants with two compartments, metabolic and structural (Greenwood et al., 1990). Hence, using the parameters of a dilution curve for maize previous to grain filling (Plénet and Lemaire, 1999) may have biased our estimates of NNI in mature crops. If the change in slope of the nitrogen dilution curve after grain filling for sorghum (Fig. 7 of Greenwood et al., 1990) applies to

Table 4
Nitrogen concentration (%) in grain (%N_g), biomass (%N_B) and stover (%N_{st}), and N utilization efficiency for biomass ($N_{ut}E_B$) and yield ($N_{ut}E_Y$) in two maize hybrids grown under contrasting N rate at traditional and late sowings during two seasons in Paraná, Argentina.

Source of variation			Year 1					Year 2				
Sowing date	Hybrid	N rate (kg N ha^{-1})	%N_g	%N_B	%N_{st}	$N_{ut}E_B$ (kg kg N^{-1})	$N_{ut}E_Y$ (kg kg N^{-1})	%N_g	%N_B	%N_{st}	$N_{ut}E_B$ (kg kg N^{-1})	$N_{ut}E_Y$ (kg kg N^{-1})
Traditional	DK682	0	0.98	0.68	0.48	149.3	58.4	0.98	0.58	0.38	172.7	57.6
Traditional	DK682	200	1.37	1.05	0.60	95.6	55.1	1.32	1.02	0.64	99.3	54.0
Traditional	DK752	0	1.06	0.70	0.45	144.2	54.4	1.04	0.60	0.41	169.4	49.5
Traditional	DK752	200	1.44	1.16	0.90	86.5	41.5	1.43	1.00	0.66	100.4	44.2
Late	DK682	0	1.53	1.21	0.85	82.8	42.2	0.90	0.82	0.76	123.8	56.9
Late	DK682	200	1.52	1.15	0.72	86.9	46.8	1.24	1.00	0.72	101.7	53.4
Late	DK752	0	1.54	1.01	0.65	99.7	40.4	0.96	0.83	0.77	119.1	47.4
Late	DK752	200	1.53	1.03	0.65	97.6	42.4	1.09	0.91	0.77	110.4	50.5
Analysis of variance							P > F					
Hybrid (H)			ns	+	ns	ns	*	ns	ns	ns	ns	*
Sowing date (SD)			***	***	*	***	***	***	*	***	**	ns
N rate (N)			***	***	*	***	Ns	***	***	+	***	ns
H × SD			ns	***	**	*	Ns	*	ns	ns	ns	ns
H × N			ns	ns	**	ns	Ns	ns	ns	ns	ns	ns
N × SD			***	***	***	***	*	+	**	*	***	ns
H × N × SD			ns	ns	ns	ns	Ns	+	ns	ns	ns	ns
SE			0.044	0.032	0.041	4.6	2.5	0.031	0.045	0.061	6.7	3.0

ns: not significant; SE: standard error.
+ $P < 0.05$.
* $P < 0.01$.
** $P < 0.001$.
*** $P < 0.0001$.

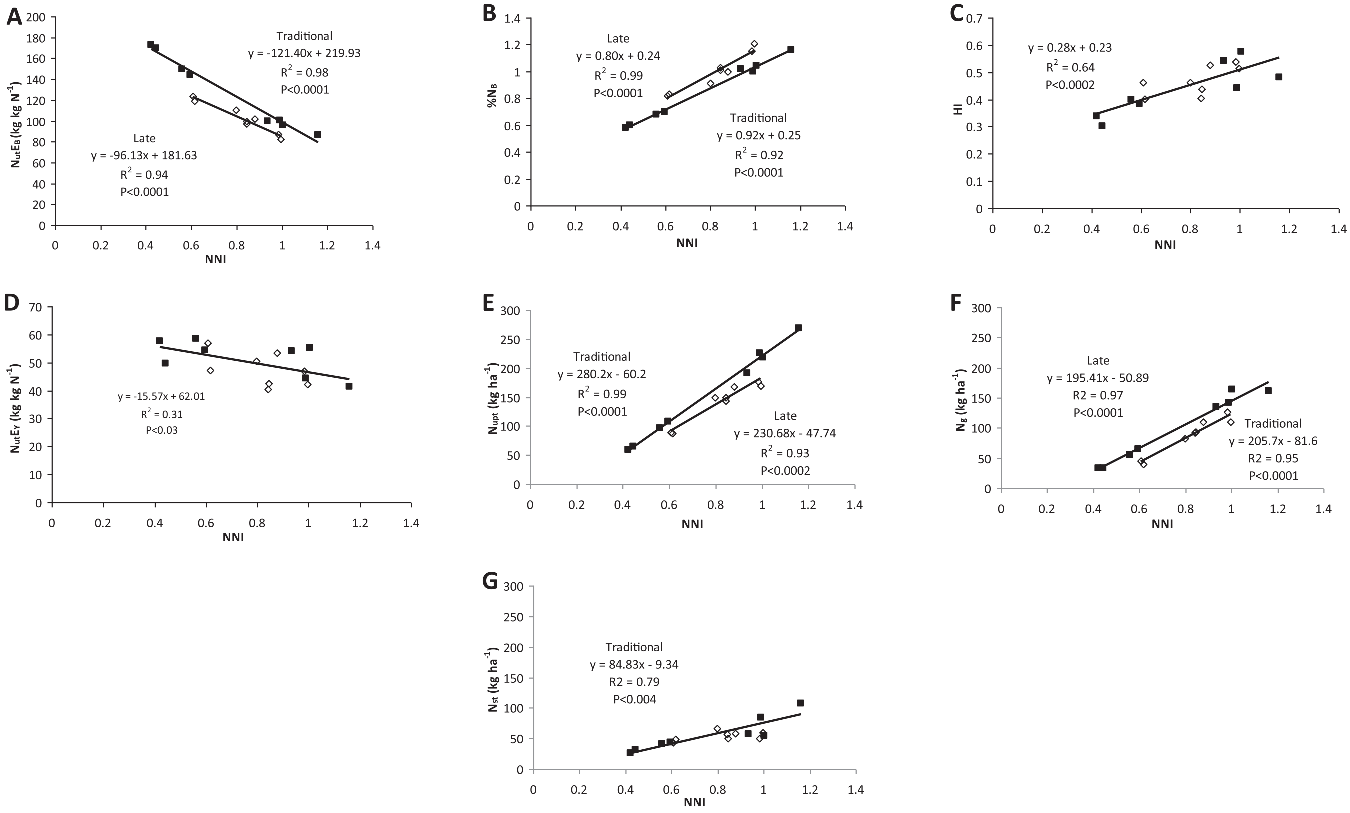

Fig. 3. Values for (A) Biomass per unit of N uptake ($N_{ut}E_B$), (B) N concentration in biomass (%N_B), (C) harvest index (HI) and (D) grain yield per unit of N uptake ($N_{ut}E_Y$) (E) Nitrogen uptake (N_{upt}), (F) N accumulated in grains (N_g) and (G) N accumulated in stover (N_{st}) as a function of a N nutrition index (NNI) during two seasons in Paraná, Argentina. Since functions fitted to traditional and late sowing data were statistically indistinguishable, a single linear fit for both sowing dates in C and D. In G, a linear model was only significant for data from the traditional sowing. NNI was estimated using actual N concentration and optimal N concentration as proposed by Plénet and Lemaire (1999). Open squares are data for late sowing, and closed diamonds are values for the traditional sowing date.

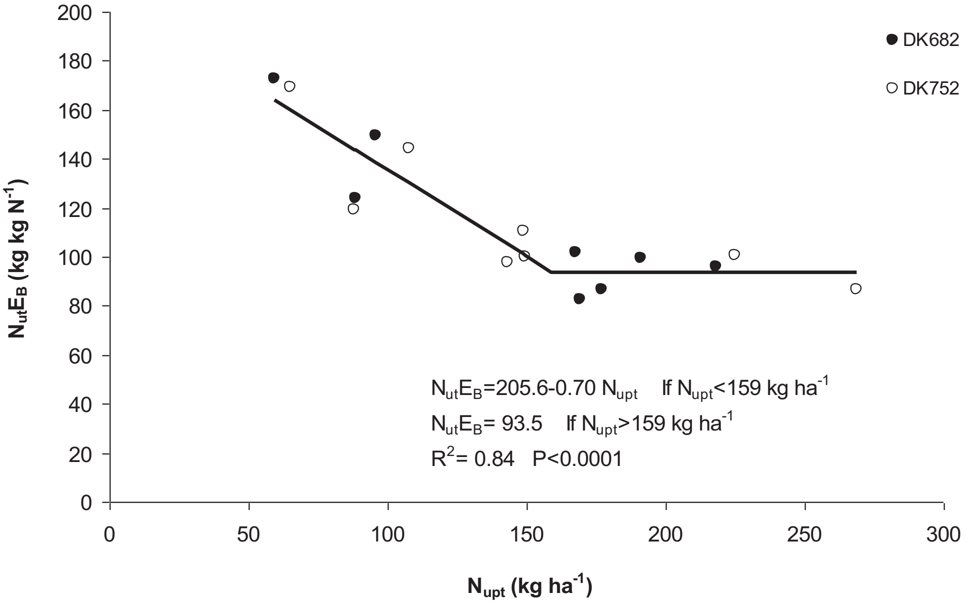

Fig. 4. Biomass per unit of N uptake ($N_{ut}E_B$) as a function of N uptake in two maize hybrids grown at contrasting sowing dates and N rates during two seasons in Paraná, Argentina.

maize, then the NNI may have over-estimated nitrogen deficiency in our crops, but with no changes in the ranking of treatments. Hence, plotting yield response to nitrogen fertilizer as a function of NNI in unfertilized controls returned a robust relationship where sowing dates, hybrids and seasons all aligned in a single, agronomically meaningful function (Fig. 2). Furthermore, yield response to fertilizer was close to zero when NNI approached 1 (Fig. 2), with NNI = 1 indicating, by definition, the crop nitrogen status that maximizes growth (Lemaire and Gastal, 1997, 2009). Herrmann and Taube (2005) have successfully extended the use of NNI until maize silage maturity, i.e. R1 + 25 d. Belanger et al. (2001) also found agronomically meaningful correlations between yield response to fertilizer and NNI in potato despite using nitrogen dilution curves that included tubers, thus violating the assumption of plants with only metabolic and structural compartments.

Despite the extrapolation beyond the theoretical limits of nitrogen dilution curves, our derived NNI captured the improved nitrogen status of late-sown unfertilized crops, and helped to untangle the effects of hybrid, sowing date, nitrogen fertilizer and their interactions on yield- and nitrogen-related traits. The NNI allowed the comparison between sowing dates at an equivalent N status (Fig. 3), which reflects a new insight gained by the application of this index.

4.2. Physiological responses to sowing date, nitrogen rate and hybrids, and their agronomic implications

4.2.1. Photothermal conditions and phenology

Photothermal conditions during the critical period for grain number determination for grain set were similar between sowing dates but there was a sharp reduction in incident solar radiation and

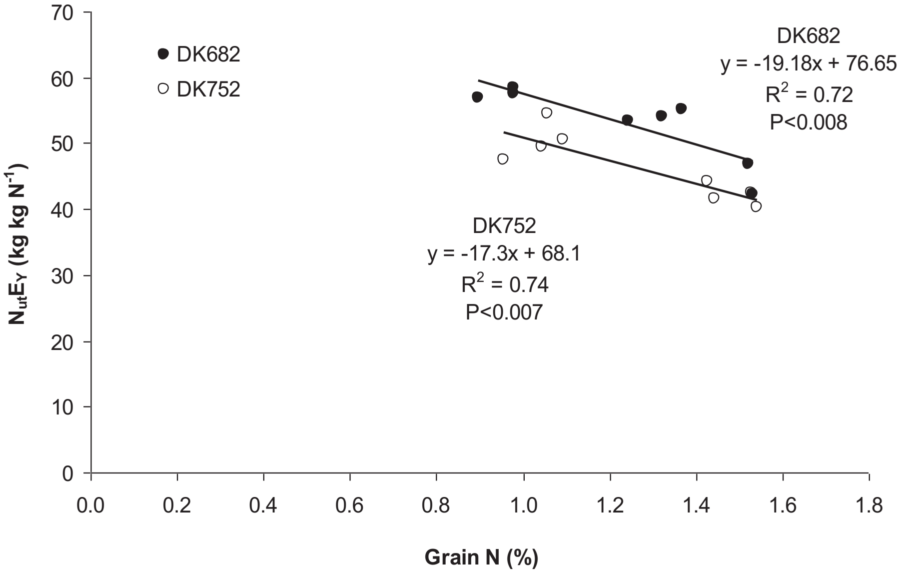

Fig. 5. Yield per unit of N uptake ($N_{ut}E_Y$) as a function of N concentration in grains in two maize hybrids grown at contrasting sowing dates and N rates during two seasons in Paraná, Argentina.

mean temperature during grain filling for late sown crops (Table 1). Maddonni (2012) presented an in-depth analysis of the climatic constraints for late sown maize in the Argentinean Pampas, and highlighted the sharper deterioration of photothermal environment with delayed sowing in a north (latitude −27°05′)-to-south (latitude −35°48′) transect.

The increase in duration of the vegetative period, i.e. from sowing to R1, when it was expressed in degree-days (76–91 °C d) (Table 1) contrasted with the reported reduction in the thermal time duration of the vegetative period for late-sown maize in Waikato (latitude −37°86′) and Manawatu (latitude −40°38′), New Zealand (Tsimba et al., 2013) and in Indiana (four locations ranging from latitude 39°3′–41°1′), USA (Nielsen et al., 2002). The longer photoperiod during the photoperiod sensitive phase in our late-sown crops (ca. 1 h) was probably involved in the increase of the thermal time of the vegetative period (Kiniry et al., 1983).

The shortening of the vegetative period (in days) with late sowing (Table 1), was the main cause for lower cumulative incident solar radiation (Cirilo and Andrade, 1994; Otegui et al., 1995). On the other hand, both the duration in days as well as the lower daily incident solar radiation were involved in the dramatic reduction of cumulative incident solar radiation in late sowing date as compared with the traditional, in coincidence with the reported by Otegui et al. (1995).

4.2.2. Nitrogen status and response to N

Most of measured variables were strongly affected by the interaction between sowing date and N rate. Although mineral nitrogen in the soil was not measured, the combination of soils with about 3% organic matter, and the extension of a warm and wet (not shown) fallow period by 3 months contributed to the putative increase in available nitrogen for late-sown crops in relation to crops in traditional spring sowings, which is reflected in the improved NNI in unfertilized controls (Table 2). Indeed, the effect of long fallow on N availability has been documented in several environments (e.g. Bruun et al., 2006) and is one of the reasons to implement catch crops to uptake the soil mineral nitrogen excess during long fallows (Thorup-Kristensen et al., 2003). Therefore, smaller yield response to fertilizer in late-sown crops than in traditional sowing (sowing date × N interaction in Table 2; Fig. 2) could be attributed, at least in part, to a higher soil N availability.

The better N status recorded in late sowing in N0 plots (Table 1) suggests the use of more conservative N rate, to avoid excess of soil mineral N prone to unproductive and contaminant losses. In fact, the adjustment of N rate to achieve an adequate synchrony between N supply and crop demand is a key to improve NUE (Cassman et al., 2002). However, it should be noted that yield response to N is a complex variable, which depends on multiple factors such as available soil N, growing conditions for growth during critical stages, i.e. attainable yield, and agronomical management, such as genotype, plant density, and fertilizer source and rate, among others (Ciampitti and Vyn, 2011; Cassman et al., 2002; Lemaire and Gastal, 2009). Weather forecasts could be valuable (Bert et al., 2006; Monzon et al., 2012) to capture season-dependent responses to fertilization which are largely mediated by amount and timing of rainfall in rainfed systems (Maddonni, 2012).

4.2.3. Biomass and N accumulation and its partitioning

Reduced total growth with late sowing (Table 2) can be associated with a reduction in the duration of growth period (Table 1), a reduced growth rate or both, hence the need to consider the shifts in photothermal environment and phenology with sowing date (Cirilo and Andrade, 1994; Otegui et al., 1995).

Biomass accumulation (Table 2) and N uptake (Table 3) in well fertilized crops were reduced proportionally more than the shortening of the growing cycle with late sowing (Table 1). This indicates that late sowing also reduced the rates of growth and N uptake. The putative reduction in these rates was probably associated with both lower incident radiation and lower radiation use efficiency with low temperature (Cirilo and Andrade, 1994; Uhart and Andrade, 1995; Andrade et al., 1993).

Although HI was similar between sowing dates at an equivalent N status, HI of fertilized crops was, on average, 8–12% lower in late than in traditional sowing date (see HI with higher NNI in Fig. 3C and Table 2); this may be attributed to the environmental constraints during grain filling as compared with the favorable environment during early and critical period for grain number determination. On the other hand, DK682 had a higher HI and a lower stover biomass than DK752 (Table 2). Moreover, stover biomass of fertilized crops was reduced 21–7% (Year 1 and 2, respectively) in DK682 and 35–30% (Year 1 and 2, respectively) in DK752 with delayed sowing. Echarte and Andrade (2003) showed that the HI of some maize hybrids including DK752 is stable for the range of plant biomass explored in this study, from ca. 120 to 280 g plant^{-1}. However, our results showed larger than expected variation in HI. Three possible causes may explain this apparent inconsistency. First, trait plasticity is dependent on the source of variation (Sadras and Richards, 2014); where Echarte and Andrade (2003) used plant density as source of variation, we used a combination of sowing date (and associated changes in photothermal environment) and nitrogen. Second, late-sown crops in our experiments could have had lower source/sink ratio than the crops of Echarte and Andrade (2003). Third, the relationship between biomass and HI at the plant level in Fig. 2 of Echarte and Andrade (2003) was highly scattered in the range between 100 and 200 g plant^{-1}, which implies high and low HI for plants with similar biomass. Comparisons of maize hybrids in a narrower range of sowing dates also showed greater stability of HI than in our wider photothermal range (Otegui et al., 1995).

The higher HI stability in DK682 than in DK752 suggest that the choice of hybrids with an improved ability for biomass partitioning to grain may be useful in late sowing in our environments, characterized by the deterioration of the photothermal conditions during grain filling. It can be speculated, however, that maintenance of high harvest index in late sown crops might increase the risk of lodging associated with lower dry matter allocation to stems.

Nitrogen uptake at an equivalent NNI was higher in traditional than in late sowing (Fig. 5) indicating that soil N availability was not the only factor involved in this result. In maize, a considerable proportion of the nitrogen in grain (35–55%) originates from post-anthesis nitrogen uptake and assimilation (Lemaire and Gastal, 2009). Thus, the low radiation and temperature might have compromised nitrogen uptake during the post-flowering phase of late sown crops. To test this hypothesis, assessment of nitrogen uptake and NNI at R1 would be required. More grains in traditional than in late sown crops could have increased the demand for N and, hence, nitrogen uptake as has been suggested by Ciampitti and Vyn (2013). However an increase in grain number was only recorded in Year 1 (Melchiori and Caviglia, 2008), which suggests that an increase in both sink and photosynthetic source is required to increase N uptake in late sowing, since soil N availability did not limit N uptake. Therefore, the strategy of increasing the post-flowering N uptake to increase total N uptake, would not be valid in late sowing characterized by a strong deterioration of photothermal environment.

Although $\%N_B$ at an equivalent NNI was higher in late than in traditional sowing, it was not enough to reduce the $N_{ut}E_Y$ as suggested by Eq. (3). The higher $\%N_B$ in late sowing may be attributed to the higher restrictions for late growth than for N uptake (Greenwood et al., 1990; Lemaire and Gastal, 2009). Thus, the restrictions for growth late in the season, as well as potential reductions in sink size, may be responsible for the increase in $\%N_B$ in late sowing.

Nitrogen partitioning, as indicated by NHI, was scarcely affected by sowing date, and was higher in DK682, the hybrid with high HI. These results are in agreement with the conservative nature of N in reproductive sinks at expense of vegetative organs (D'Andrea et al., 2008). However, in spite of the reported conservative nature of NHI (Ciampitti and Vyn, 2012), our results indicated that the hybrid with higher HI, with a concomitant higher NHI, reduced the N remaining in the stover (N_{st}). Since N_{upt} did not differ among hybrids, the use of genotypes with an improved ability for N partitioning to grain may be an emerging strategy in environments with strong restrictions for late growth in order to reduce residual N in stover and undesirable environmental consequences. As has been showed recently, the N amount in involved in crop residue is a key factor in it mineralization, which influences the amount of C input required to maintain soil C balance close to neutral (Melchiori et al., 2014).

4.2.4. Nitrogen utilization efficiency and related traits

The $N_{ut}E_B$ was inversely related with N status (Fig. 3). Likewise, at an equivalent N status $N_{ut}E_B$ was higher in traditional than in late sowing. This higher $N_{ut}E_B$ may be attributed to the better conditions during grain filling (Table 1) conducive to higher yield (for details in yield and its components see Melchiori and Caviglia, 2008). Higher N uptake reduced $N_{ut}E_B$, linearly up to a threshold of N_{upt}, which was similar for both hybrids (Fig. 4). This could be attributed to the different response to N of biomass and yield as has been previously suggested (Caviglia and Melchiori, 2011). This differential response to N is involved in the progressive fall in the response of HI to N up to a threshold, as has been recently suggested (Ciampitti and Vyn, 2013). The threshold in Fig. 4 was similar to the N uptake ($15\,g\,m^{-2}$) required to maximize and stabilize harvest index, according to Ciampitti and Vyn (2013).

In contrast with $N_{ut}E_B$, $N_{ut}E_Y$ was scarcely affected by N; this was because the higher $N_{ut}E_B$ at low NNI was counterbalanced by a lower HI. Overall, $N_{ut}E_Y$ was unaffected by the experimental fixed factors, although DK682 had a consistently higher $N_{ut}E_Y$ than DK752 (Table 4) associated with higher HI. These results evidenced the conservative nature of $N_{ut}E_Y$ while, in turn, and highlights the role of biomass partitioning to improve this efficiency. Also, the higher $N_{ut}E_Y$ in the hybrid DK682 at an equivalent N concentration in grain (Fig. 5) suggest that the choice of a hybrid to increase NUE not only should be based on the N in grain, since HI seems to be the main responsible for the differences in $N_{ut}E_Y$ between hybrids.

Although NUE has been shown to be closely related to $\%N_g$ (Lemaire and Gastal, 2009; Ciampitti and Vyn, 2012) our results suggest that biomass partitioning may be an important trait to improve $N_{ut}E_Y$, particularly under sharp deterioration of photothermal environment during grain filling. Moreover, Kemanian et al. (2007) have shown that $\%N_g$ in maize may be predicted accurately using a simple model that only uses HI and $\%N_B$ as inputs. Together these results highlight the connection between HI and $\%N_g$ suggesting that both should be targets for breeding.

Further expansion of maize in the Northern Pampas relies on the development of practices to reduce inter-annual climatic variability. In this context, late sowing appears as a reliable option. However, important constraints remain to be solved for the implementation of complementary practices accounting for both the sharp deterioration of photothermal environment and high soil N availability. Accordingly, our study has implications for both agronomy and breeding of maize adapted to late sowing. Agronomically, the better N status recorded in late sowing suggest the use of conservative rates of N to increase the efficiency and reduce non-productive and potentially contaminant losses. Breeding for adaptation to late sowing, in order to improve NUE, could target hybrids with higher partitioning of biomass and N to grain, which has to be balanced with the potential increase in the risk of lodging with reduced allocation to stems.

There are three ways to increase crop production: better genotypes, improved agronomic practices to grow individual crops, and enhanced spatial and temporal arrangement of crops in cropping systems. These drivers of yield are often non-additive with large synergies contributing to progress in grain production (Fischer, 2009). Globally and on historical time scales, improved availability of N has been critical to sustained increase in food production (Sinclair and Rufty, 2012). Here we showed how new maize hybrids carrying the Bt transgene allow for agronomic practices that were previously not feasible. This study thus starts with locally relevant issues and, with a focus on crop nitrogen economy via a novel use of the NNI, contributes to the globally important questions on the synergy between breeding and agronomy.

5. Conclusions

Higher N availability in late sowing as compared with traditional sowing reduced the yield response to N fertilization. As a result, there were significant interactions between sowing date, hybrid and N rate in most N-related traits. The use of NNI allowed comparisons of traits at an equivalent N status, providing further insight into these interactions. The increase in N status reduced the $N_{ut}E_B$, although at an equivalent NNI it was higher in traditional than in late sowing, whereas comparisons of hybrids showed the benefits from improved partition of biomass and N to grain in late sowing. The $N_{ut}E_Y$ was unrelated with $\%N_B$ and HI, in contrast with the proposition in Eq. (3). The hybrid DK682, with a priori higher HI than DK752, also exhibited a greater capacity for partitioning biomass and N to grain in late sowing, as reflected by the higher HI and NHI and the lower N accumulated in stover (N_{st}). On the other hand, $\%N_g$ was not a clear indicator for an improved $N_{ut}E_Y$.

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