



Nitrogen economy of early and late-sown maize crops

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ABSTRACT

Late sowing dates of maize are widely adopted in the Pampas region of Argentina, stabilising grain yields due to a more favourable water balance around flowering. However, late-sown crops are exposed to high soil N availabilities (N_{av}), high temperatures during the pre-flowering period and declining photo-thermal conditions during grain filling, which may affect nitrogen use efficiency (NUE, kg of grain per kg of N_{av}). These effects could be exerted through nitrogen uptake efficiency (N_{upE} , kg of N uptake per kg of N_{av}) and/or nitrogen utilisation efficiency (N_{utE} , kg of grain per kg of N uptake). Environmental conditions could affect i) pre (N_{uppre}) and/or post-flowering N uptake (N_{uppost}) and, consequently, N_{upE} and ii) the determinants of N_{utE} , such as N harvest index (NHI) and N source per grain. Early- and late-sown maize were cropped in order to analyse i) grain yield, N_{av} and NUE and ii) relationships among NUE and related-N efficiencies. The experiments were carried out in Paraná (31°48' S 60°32' W), Argentina, during 2014–2015 and 2015–2016. Treatments were combinations of two sowing dates (early and late), three N rates (0, 90, and 270 kg N ha⁻¹) and two genotypes (DK 70-10 VT3P and DK 73-10 VT3P). NUE decreased in late-sown crops (ca. 32 to 26 kg grain kg N_{av} ⁻¹), mediated by lower grain yields (ca. 8564 kg ha⁻¹ and 7832 kg ha⁻¹ in early- and late-sown crops, respectively) and higher N_{av} (ca. 267–312 kg N_{av} ha⁻¹). DK 73-10 VT3P exhibited the highest NUE (ca. 31 kg grain kg N_{av} ⁻¹) and N_{utE} (ca. 63 kg grain kg N_{upE} ⁻¹). N rate affected more strongly N_{av} than grain yield; and there was a greater association between NUE and N_{upE} ($P < 0.0001$, $R^2 = 0.72$) relative to N_{utE} ($P < 0.01$, $R^2 = 0.65$). In both sowing dates, N_{uppre} had a positive impact on N_{upE} , which strongly declined with N rate especially in late-sown crops. The lower N_{utE} of late-sown crops (66 vs. 52 kg grain kg N_{upE} ⁻¹ in early and late sowing dates, respectively) was related to the highest post-flowering N source per grain (2.5 vs. 3.5 mg N grain⁻¹). Thus, our study highlights the components of N economy of late-sown crops with the highest impact on NUE, i.e., N_{uppre} and N_{utE} . Therefore, nutritional management of late-sown maize crops should be focused on these NUE components. High plant densities could be useful to increase N_{uppre} . Finally, the choice of a genotype with high N_{utE} appears as a valid strategy to mitigate NUE reductions, promoted by the high N_{av} typical of late sowing dates.

Abbreviations: AcETO, accumulated potential evapotranspiration; AcPP, accumulated rainfall; AcRg, accumulated global radiation; ETO, potential evapotranspiration; N_{av} , soil N availability during crop cycle; NHI, N harvest index; N_{min} , soil N mineralised during crop cycle; N_{remAP} , apparent N remobilised during the post-flowering period; N_s , soil N at sowing; NUE, N use efficiency; N_{upE} , N uptake efficiency; N_{upt} , total N uptake; N_{uppost} , N uptake during the post-flowering period; N_{uppre} , N uptake during the pre-flowering period; N_{utE} , N utilisation efficiency for kernel; Tm, mean temperature; WBAp, apparent water balance in vegetative V critical (CP) and reproductive (R) periods

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1. Introduction

Traditional sowing dates of rainfed maize in the Argentinean Pampas usually occur from early September to late October. For these early-sown crops, the critical period for grain setting (i.e., ± 15 d around flowering) is exposed to favourable photo-thermal conditions (Cirilo and Andrade, 1994; Otegui et al., 1995), but moderate and highly variable water restrictions (Maddonna, 2012). High inter-annual variability of rainfalls around flowering of early-sown crops may limit grain setting, increasing yield gaps between actual and potential grain yields (Aramburu et al., 2015). Among biotic factors, pressures of insects (e.g., *Diatraea saccharalis* (Fabricius) and *Spodoptera frugiperda* (Smith)) and diseases (e.g., *Puccinia sorghi* (Schwein), *Puccinia polysora* (Schwein) and *Excerohilum turcicum* (Pass.)) are usually low, compared to those experienced by late-sown crops, i.e., December sowings.

The introduction of hybrids carrying Bt genes (Shi et al., 2013), conferring better crop performance against insects and the need to improve grain yield stability across years (i.e., yield gaps reductions), have driven to an important enlargement of the temporal window of maize sowing dates, from early (September–October) to late sowings (December) (Maddonna, 2012; Caviglia et al., 2014). In fact, the highly adopted late sowings (more than 45% of maize cropped area in Argentina) have stabilised current maize grain yields at the expense of a lower potential yield, due to a sensible decrease in photo-thermal conditions during grain setting and the post-flowering period (Maddonna, 2012). Studies conducted in the Argentinean Pampas report potential yield reductions of 9–35% when the sowing date was delayed from September to mid-January, which were mainly due to the decline in photothermal conditions during the reproductive period (Cirilo and Andrade, 1994; Otegui et al., 1996; Mercau and Otegui, 2014).

The delay of sowing, from early spring to the end of spring or early summer, enlarges the fallow period prior to maize sowing, which allows the replenishment of soil water content (Maddonna, 2012) and an increase in initial soil N availability (Bruun et al., 2006; Caviglia et al., 2014). For the Pampas region, Coyos et al. (2018) report that soil N availability at sowing in late maize crops was higher than 60 kg N ha⁻¹ in fifteen out of seventeen sites, which is in accordance with Díaz Valdez et al. (2014), who report values higher than 60 kg N ha⁻¹ in all sites. These reported values of soil N availability are higher than those that have usually been reported for early sowing dates (e.g., Orcellet et al., 2017). Consequently, a lower N fertiliser requirement has been suggested for late-sown maize crops, compared with early-sown crops (Melchiori and Caviglia, 2008; Caviglia et al., 2014; Mercau and Otegui, 2014). Overall, results describing fields or genotypes with differential yield response, in late-sown maize, to N fertilization are common in the Pampas region (Caviglia et al., 2014; Mercau and Otegui, 2014; Gambin et al., 2016; Coyos et al., 2018), which indicates the need to study the N economy, with the aim of generating knowledge for adequate N rate recommendations.

The most used physiological framework with which to study the N economy at the crop level, involves the partition of N use efficiency (the ratio between crop grain yield and soil N availability; NUE) in two components: N uptake efficiency (the ratio between total N uptake and soil N availability; N_{up}E) and N utilisation efficiency (the ratio between grain yield and total N uptake; N_{ut}E) (Moll et al., 1982). Recent studies comparing hybrids released in different decades have documented a genetic gain in NUE that is related to the high yields of modern hybrids (Ciampitti and Vyn, 2011, 2012, 2013; Ferreyra et al., 2013; Mueller and Vyn, 2016). The proportionally greater increases in grain yield than in total N uptake (N_{up}) which are determined by breeding were reflected in the highest N_{ut}E of modern hybrids, although these reports involve only data that were obtained from maize crops at optimal sowing dates (Ciampitti and Vyn, 2012; Haeghele et al., 2013; Mueller and Vyn, 2016). These findings, however, could not be extrapolated to

late-sown maize crops, due to the contrasting environmental conditions during the pre- and post-flowering periods. Solar radiation and air temperatures during the pre-flowering period of early-sown crops are lower than those of late-sown crops (Cirilo and Andrade, 1994; Caviglia et al., 2014). On the other hand, the post-flowering period of late-sown crops is exposed to both declining incident solar radiation values and air temperatures (Hall et al., 1992; Cirilo and Andrade, 1994; Caviglia et al., 2014). Due to higher temperatures, N availability and soil water content are close to field capacity, typical of late-sown crops, and anticipate a higher biomass production and N uptake during the pre-flowering period (N_{up}pre) than early-sown crops. However, the impoverished post-flowering environment of late-sown crops would decrease crop growth, N uptake during this period (N_{up}post), and N harvest index (the ratio between N in grains and total N_{up}; NHI) (Cirilo and Andrade, 1994). Hence, apparent N remobilisation (N_{rem}AP) could be a relevant N source to provide the N demand for kernel growth (Tsai et al., 1991; Gallais and Coque, 2005; Coque and Gallais, 2007; Abe et al., 2013). Tradeoffs among N_{rem}AP, N_{up}pre and N_{up}post have been documented (Weiland and Ta, 1992; Triboi and Triboi-Blondel, 2002; Ciampitti and Vyn, 2013). Thus, a framework based on the analysis of NUE components (Ciampitti and Vyn, 2012) could be useful to account for variations of NUE across environments (i.e., sowing dates, years) and genotypes. We propose a measure of post-flowering N source per grain, estimated from the ratio between total N source during the post-flowering period (N_{up}post + N_{rem}AP) and grain number per unit area. This estimator of N source limitation for kernel growth is similar to that used in terms of carbon (C) balance (post-flowering biomass production per grain) and both could be used together in order to compare simultaneous N and C limitations for post-flowering crop growth in contrasting environments.

In this work, N fertilisation field experiments were carried out in early- and late-sown maize crops in order to analyse i) grain yield, N_{av} and NUE and ii) relationships among NUE and related-N efficiencies.

2. Materials and methods

2.1. Experiments and crop management

Two field experiments were conducted at Paraná, in the experimental station (31°48' S 60°32' W) of the National Institute for Agricultural Technology (INTA), Argentina, during two consecutive growing seasons (2014–2015 and 2015–2016, Exp1 and Exp2; respectively).

Treatments included a combination of i) two single-cross maize hybrids (DK 73-10 VT3P and DK 70-10 VT3P) that are tolerant to glyphosate [N-(phosphonomethyl)glycine] and are characterised as contrasting in N_{up}E (high and low for DK 73-10 VT3P and DK 70-10 VT3P, respectively) (Robles et al., 2015) and with similar cycle duration (Cultivio, 2018), ii) two sowing dates: mid-September (early sowing) and mid-December (late sowing), and iii) three N rates: 0, 90, and 270 kg N ha⁻¹ (hereinafter, 0N, 90N and 270N; respectively) applied as urea (46%N). Treatments were distributed in a split-plot design with three replicates. Sowing date was randomised in the main plots and the combination of hybrids and N rates in the sub-plots (hereinafter, plots). Each plot (26 m²) included five rows, 0.52 m apart and 10 m long, with a plant population of 7 plants m⁻². Exps were conducted under rainfed conditions.

The experimental field has been cropped in a continuous wheat/soybean-maize sequence for, at least, the last 20 years. Hence, soybean was the previous crop of each Exp, which was harvested in mid-April. The soil is a fine textured Aquic Argiudol, with moderate levels of P availability (ca. 10 mg kg⁻¹ P Bray). Triple superphosphate was applied (100 kg ha⁻¹) before the sowing of each Exp. Weeds were chemically controlled with glyphosate at 3 L ha⁻¹ rate, from 60 days before sowing

to the end of the Exps. Insects and diseases were adequately controlled whenever necessary. Meteorological variables were obtained from a conventional weather station that is located near to the Exps (< 1000 m).

2.2. Crop measurements and soil samples

Crop phenology was recorded weekly on ten tagged plants per plot, from seedling emergence to physiological maturity (R6), using the Ritchie et al. (1986) scale. The plant stand was measured at R6 by counting plants in two central rows of each plot.

Aerial biomass samples were determined at silking (R1) and R6 by cutting five consecutive plants per plot at ground level. Aerial biomass per unit area at R1 and R6 was estimated, based on stand count in each plot and on individual plant biomass. In R1 and R6, stem + sheaths, leaf blades and ear weights were determined. Samples were dried in a forced air circulation oven at 65 °C, until constant weight. An aliquot of samples was ground using a Willey-type mill (< 1 mm mesh) in order to determine N concentration using the micro-Kjeldahl method (Nelson and Sommers, 1973).

Grain yield, corrected to 145 g kg⁻¹ of moisture, was determined by the mechanical harvest of the two central rows of each plot. Harvest index (HI) was calculated as the ratio between grain yield (0% moisture) and total aerial biomass at R6.

To determine N-NO₃⁻ concentrations in the soil, soil samples at sowing, R1 and R6 on plots of each treatment without N fertilization were taken at 0–0.2, 0.2–0.4 and 0.4–0.6 m depth. Samples were composed from 20 sub-samples per plot.

Total available soil N (N_{av}) during crop cycle was calculated as the sum of N_s (0–60 cm) at sowing, N applied as fertiliser and N mineralised from sowing to R6 (N_{min}). N_{min} was estimated in 0 N plots (Alvarez and Steinbach, 2011), neglecting N losses via volatilisation, denitrification, and/or leaching (Eq. 1).

$$N_{min} = (N_{upt} R6 + N_s \text{ at } R6) - N_s \text{ at sowing} \quad (1)$$

2.3. Calculations

N_{upt} at R1 and N_{upt} at R6 were obtained from the product of total aerial biomass and N concentration at each stage. NHI was calculated as the ratio of N content in grains and N_{upt} at R6. N_{uptpost} was calculated as the difference between N_{upt} at R6 and N_{upt} at R1 (i.e., N_{upt} pre). N_{remAP} was calculated as the difference between N_{upt} in leaves plus stem at R6 and N_{upt} in leaves plus stem at R1 (Chen et al., 2015). Senesced leaves during the reproductive period were collected at R6, because most of them remained attached to the plant.

NUE and its components (N_{utE} and N_{upE}) were calculated using Eqs (2), (3), and (4); respectively.

$$NUE = \frac{\text{Grain yield}}{N_{av}} \quad (2)$$

$$N_{utE} = \frac{\text{Grain yield}}{N_{upt} \text{ at } R6} \quad (3)$$

$$N_{upE} = \frac{N_{upt} \text{ at } R6}{N_{av}} \quad (4)$$

N source per grain was calculated according to Eq. (5).

$$N_{source} \text{ per grain} = \frac{N_{upt} \text{ post} + N_{remAP}}{\text{Grain number per unit area}} \quad (5)$$

Biomass (B) source per grain was calculated according to Eq. (6).

$$B_{source} \text{ per grain} = \frac{B_{post} + B_{remAP}}{\text{Grain number per unit area}} \quad (6)$$

where B_{post} is the post-flowering biomass production, calculated as the difference between total biomass at R1 and at R6, and B_{remAP} is the apparent biomass that is remobilised during the post-flowering period, and which was calculated as the difference between the biomass of leaves plus the stem at R6 and the biomass of leaves plus the stem at R1. This estimator might underestimate the carbon source for kernel growth demand under optimum post-flowering environmental conditions.

2.4. Statistical analysis

The dataset was subjected to an analysis of variance (ANOVA) to evaluate the effect of factors (Exp, sowing date, hybrid, and N rate) and their interactions on all of the tested variables. We considered the Exp, the main plot (sowing date) and the sub-plot (factorial combination of hybrids x N rates) as fixed factors.

Statistical analysis also considered correlations, and multivariate analysis (principal components analysis; PCA), and simple linear regressions in order to evaluate the relationships among variables. Some correlations are spurious because they share their variables that are used in their calculation (e.g., NUE vs. N_{upE} and N_{utE}, NHI vs. N_{utE}, and B source per grain vs. N source per grain). Consequently, the Pearson correlation coefficient (r) was calculated according to Dunlap et al. (1997). New coefficients of determination (R²) were calculated as the square of r for reporting the fit of the aforementioned relationships.

Statistical analyses were carried out using the InfoStat software (Di Rienzo et al., 2011). For PCA, environmental conditions, including weather and soil variables, grain yield, NUE and its components were considered. The weather variables are summarised as: accumulated rainfalls during crop cycle (AcPP); accumulated potential evapotranspiration during crop cycle (AcET0), accumulated global radiation during crop cycle (AcRg); mean temperature (Tm), and apparent water balance (WBAp) during the vegetative (V), critical (CP) and reproductive (R) periods. Only the first two axes (axis PC1 and axis PC2) are graphically presented, and positive correlations among variables are represented by vectors with angles close to 0°, while those variables that are negatively correlated are represented by vectors with angles close to 180°. Angles of 90° between vectors represent uncorrelated variables.

3. Results

3.1. Weather conditions

The weather conditions during the Exps are summarised in Fig. 1. Mean solar radiation was similar between Exps, but mean air temperature during the post-flowering period of early-sown crops was higher in the period of 2015-16 (Exp2) than in the period of 2014-15 (Exp1). The pre-flowering period of early-sown crops was exposed to lower air temperatures and higher solar radiation values than the pre-flowering period of late-sown crops. Contrarily, the post-flowering period of late-sown crops occurred with lower daily air temperatures and solar radiation values than the post-flowering period of early-sown crops (Figs. 1c, d, e, f).

The length of crop cycle from sowing to R6 (ca. 138 days) was similar among hybrids and Exps (Fig. 1). The delay of the sowing date determined a shortening by ca. 15 days in the length of the pre-flowering period but by ca. 8 days in total crop cycle, because the post-flowering period was ca. 7 days longer.

Total rainfall (ca. 844 mm) and accumulated ET0 (ca. 823 mm) from October to March were similar between Exps (Figs. 1a, b). The critical period of early-sown crops (i.e., December) was exposed to lower rainfall and higher ET0 (ca. 131 mm and 134 mm for rainfall and ET0, respectively) than those recorded during the same period (i.e., February) of late-sown crops (ca. 215 mm and 139 mm for rainfall and ET0,

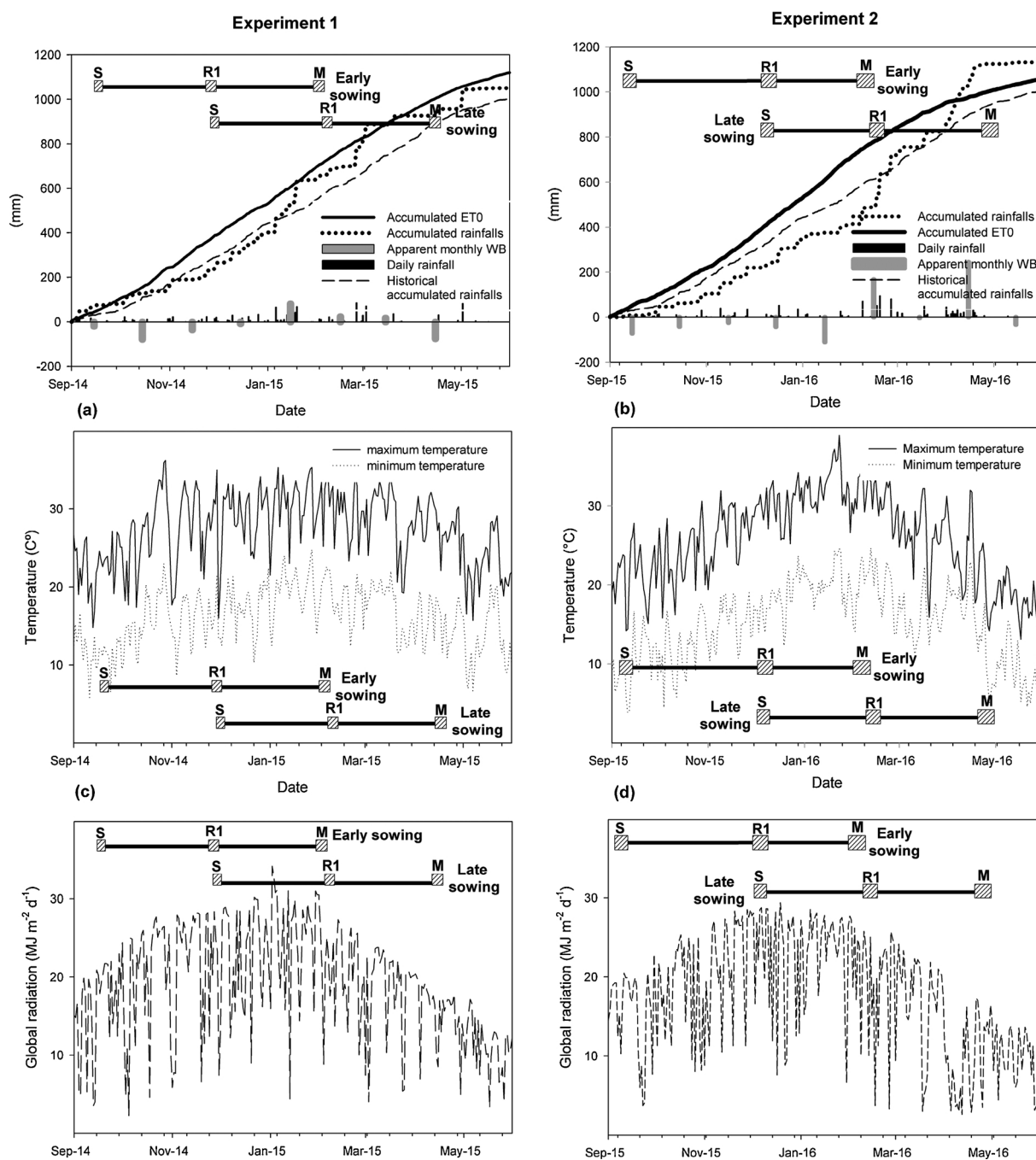


Fig. 1. Accumulated rainfall and ET0 (potential evapotranspiration), daily rainfall, historical rainfall data, apparent monthly water balance (WB Ap) (a, b), maximum and minimum air temperature (c, d) and global solar radiation (e, f) in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina. The black segments represent crop cycles of early- and late-sown crops. Squares identify sowing date (S), flowering (R1), and physiological maturity (R6).

respectively).

Although rainfall levels along the crop cycle were higher than the historical accumulated rainfall levels (Figs. 1a, b), the apparent water balance (difference between rainfall and ET0, WBAp) during the critical period was slightly deficient for early-sown crops, and positive for late-sown crops. In Exp2, total rainfall recorded during the post-flowering period of early-sown crops (January) were 44% lower than the historical record.

3.2. N availability and grain yield

N_s at sowing did not exhibit a common pattern across sowing dates

Table 1

Soil N-NO₃⁻ (0–60 cm) at sowing (N_s) and N mineralised (N_{min}) from sowing to R6 of early and late-sown crops in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina.

Sowing date	Exp 1		Exp 2	
	N _s (kg N ha ⁻¹)	N _{min}	N _s	N _{min}
Early	51	94	19	131
Late	46	111	55	173

Table 2

Analysis of variance and mean values for total N available (N_{av}) during the crop cycle, grain yield, harvest index (HI), total N uptake (N_{upt}) during the crop cycle, N uptake during the pre- and post-flowering periods (N_{uptpre} and $N_{uptpost}$, respectively), apparent N remobilisation during the post-flowering period (N_{remAP}), N harvest index (NHI) and grain N concentration in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina. At each Exp x sowing date combination, two hybrids were cultivated with three N rates.

	N_{av} (kg N ha ⁻¹)	Grain yield (kg ha ⁻¹)	HI	N_{upt} (kg N ha ⁻¹)	N_{uptpre} (kg N ha ⁻¹)	$N_{uptpost}$ (kg N ha ⁻¹)	N_{remAP} (kg N ha ⁻¹)	NHI	grain N concentration (%)
Experiment (Exp)									
Exp1	271 a	10144 b	0.54 b	155 b	99 b	56 b	52 b	0.67 b	1.18 a
Exp2	308 b	6252 a	0.47 a	116 a	77 a	39 a	34 a	0.60 a	1.22 b
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0031	0.0361
N rate (kg N ha ⁻¹) (N)									
0 N	170 a	6483 a	0.46 a	96 a	59 a	37 a	30 a	0.66 b	1.12 a
90 N	260 b	8996 b	0.54 b	135 b	92 b	43 a	50 b	0.68 b	1.19 b
270 N	440 c	9115 b	0.51 b	176 c	114 c	62 b	49 b	0.56 a	1.28 c
P-value	< 0.0001	< 0.0001	0.003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Hybrid (H)									
DK70-10VT3P	290	7801 a	0.47 a	137	93 b	44	46	0.60 a	1.21
DK73-10VT3P	290	8595 b	0.53 b	134	84 a	50	40	0.67 b	1.19
P-value	ns	0.0016	0.0011	ns	0.0094	ns	ns	0.002	ns
Sowing date (SD)									
Early	267 a	8564 b	0.53 b	131	89	42 a	45	0.68 b	1.15 a
Late	312 b	7832 a	0.47 a	140	88	52 b	41	0.59 a	1.24 b
P-value	< 0.0001	0.0033	0.0009	ns	ns	0.0114	ns	0.0002	0.0002
Interactions					<u>P-value</u>				
Exp x SD	< 0.0001	0.0231	0.0022	< 0.0001	0.0001	0.0013	0.0188	0.0271	< 0.0001
Exp x H	ns	ns	ns	ns	ns	ns	ns	ns	ns
Exp x N	ns	0.0045	ns	ns	ns	ns	ns	0.0006	ns
SD x H	ns	ns	ns	ns	ns	ns	ns	ns	ns
SD x N	ns	0.0013	ns	< 0.0001	< 0.0001	ns	0.0139	ns	0.0001
N x H	ns	ns	ns	ns	0.0355	ns	ns	ns	ns
Exp x SD x H	ns	ns	ns	ns	ns	ns	ns	ns	ns
Exp x SD x N	ns	ns	ns	ns	ns	ns	ns	ns	0.0026
Exp x H x N	ns	ns	ns	ns	0.002	ns	0.0499	ns	ns
SD x H x N	ns	ns	ns	0.0098	0.0491	ns	ns	0.0148	0.0208
Exp x SD x H x N	ns	ns	ns	ns	ns	ns	ns	ns	0.0012

^aValues followed by different letters indicate significant differences (LSD, $P < 0.05$) within each sources of variation.

(Table 1). In Exp1, N_s was similar between sowing dates, while in Exp2 late-crops were sown with higher N_s than early crops.

In both Exps, however, N_{min} during the growing season of late crops was higher (ca. 21%) than during the same period of early crops (Table 1). On the other hand, a significant Exp x sowing date interaction was detected on N_{av} ($P < 0.0001$), where Exp1 had a lower range of N_{av} between sowing dates than Exp2 (Table 2). Hence, N_{av} was significantly ($P < 0.0001$) increased (ca. 14%) by the delay in sowing, which was mainly due to the higher N_{min} . The range of N_{av} , mediated by variations in N_s , N_{min} and N rates, extended from ca. 170 up to more than 440 kg N ha⁻¹ (Table 2).

Grain yield was higher in early- rather than in late-sown crops, only in Exp1. In Exp2, no differences were detected between sowing dates (Exp x SD interaction, $P < 0.05$, Table 2). Additionally, a significant Exp x N rate interaction ($P < 0.01$) was detected for grain yield, where grain yield response to N rate was higher in Exp1 than in Exp2, regardless of the sowing date. On the other hand, grain yield of DK 73-10 VT3P out-yielded ($P < 0.01$) DK 70-10 VT3P. Across Exps, N fertilisation significantly ($P < 0.0001$) increased the grain yield of both hybrids, but the magnitude of this response differed between sowing dates (N x sowing date interaction, $P < 0.01$, Table 2). Early-sown crops showed a greater grain yield response to N rate (on average, 1838 kg ha⁻¹) than late-sown crops (on average, 794 kg ha⁻¹). Kernel number per unit area varied in a similar way to grain yield and ranged from 1939 to 4776 kernels m⁻² (results not shown).

Harvest index did not differ between sowing dates in Exp1, while in Exp2 the delay of sowing date decreased HI (E x SD interaction, $P < 0.01$, Table 2). HI ranged from 0.46 to 0.54 and it was increased

by the N rate ($P < 0.01$). DK 73-10 VT3P had the highest HI ($P < 0.01$).

3.3. Total N uptake, N_{uptpre} , $N_{uptpost}$, N_{remAP} , NHI and grain N concentration

At the early-sowing date, N_{upt} response to N rate was similar between hybrids (Table 2); however, DK 70-10 VT3P had the highest N_{upt} response to N rate when the sowing date was delayed (N x H x SD interaction, $P < 0.01$). A significant Exp x SD was detected for N_{upt} ($P < 0.0001$), where values of N_{upt} of early-sown crops were higher than those of late-sown crops in Exp1, while in Exp2 the opposite trend occurred. Overall, early-sown crops had a greater positive response of N_{upt} to N rate (ca. 55 kg N_{upt} per unit of N applied) than late-sown crops (ca. 25 kg N_{upt} per unit of N applied).

A significant SD x H x N interaction ($P < 0.05$) on N_{uptpre} was recorded. In early sowing, both hybrids did not show differences in N_{uptpre} among N rates. In late sowing, only N_{uptpre} of DK 70-10 VT3P increased with N rate (Table 2). On the other hand, there were no defined response patterns for N_{uptpre} response to N rate of both hybrids between Exps although, in general, the response tended to be greater in Exp1 than in Exp2 (Exp x H x N interaction, $P < 0.01$, Table 2).

$N_{uptpost}$ increased in N rate ($P < 0.0001$), but this did not differ between hybrids ($P < 0.05$, Table 2). A significant Exp x SD interaction ($P < 0.01$) for $N_{uptpost}$ was recorded. In Exp1 there were no differences in $N_{uptpost}$ between sowing dates, but in Exp2 late-sown crops had the lowest $N_{uptpost}$ (Table 2).

N_{remAP} of early crops was positively affected by N rate, however,

Table 4

Values of auto-vectors e1 and e2 resulting from principal components analysis (PCA) for the weather, N soil and crop variables in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina. At each Exp x sowing date combination, two hybrids were cultivated with three N rates.

Variables	e1	e2
Tm _R (°C)	-0.33	-4E-03
Ac ET0 _{CP} (mm)	-0.32	0.07
Ac Rg _{CP} (MJ m ⁻² d ⁻¹)	-0.31	-0.03
Ac Rg _V (MJ m ⁻² d ⁻¹)	-0.26	0.24
Ac ET0 _R (mm)	-0.23	-0.27
N _{ut} E (kg grain kg N _{upt} ⁻¹)	-0.20	0.01
Ac ET0 _{CP} (mm)	-0.19	0.30
Ac Rg _R (MJ m ⁻² d ⁻¹)	-0.18	-0.30
NUE (kg grain kg N _{av} ⁻¹)	-0.10	-0.24
Tm _{CP} (°C)	-0.07	0.21
GY (kg ha ⁻¹)	-0.02	-0.29
N _{up} E (kg N kg N _{av} ⁻¹)	7E-04	-0.28
Ac PP _R (mm)	0.03	0.27
N _{upt} (kg N ha ⁻¹)	0.08	-0.15
N _{min} (kg N ha ⁻¹)	0.10	0.34
Ac PP _V (mm)	0.13	-0.18
WB _R Ap (mm)	0.13	0.31
WB _V Ap (mm)	0.16	-0.25
N _s (kg N ha ⁻¹)	0.24	-0.11
Tm _V (°C)	0.33	0.01
WB _{CP} Ap (mm)	0.33	0.02
Ac PP _{CP} (mm)	0.33	0.04

balance and rainfalls during the post-flowering period of late-sown crops. Thus, NUE reductions of late crops in Exp1 were related to the lower grain yields (associated to lower accumulated radiation during the reproductive period) and the high N_{av} (Table 2). On the other hand, although grain yield in the Exp2 did not vary between sowing dates (Exp x SD interaction, Table 2) late crops were growing with high levels of N_{av} which reduced NUE.

3.5. Correlations among environmental conditions and NUE components

Environmental conditions (including weather and soil variables), grain yield, NUE and its components were subjected to PCA. The first two PC axes explained 69% of total variance (Fig. 2 and Table 4). The auto-vectors e1 and e2 show the coefficients with which each original variable was weighted in order to conform PC1 and PC2. For PC1, Tm_R, Ac ET0_{CP}, and Ac Rg_{CP} received the higher negative weights, which were associated with early-sown maize crops. Tm_V, Ac PP_{CP}, and WB_{CP} Ap had the higher positive weights which were associated with late-sown maize crops (Table 4). Hence, PC1 separated sowing dates, late sowings to positive PC1 values and early sowings to negative PC1 values, and differences between sowing dates were more contrasting in Exp2 than in Exp1. On the other hand, Exps were discriminated by PC2. Higher values of N_{min}, Ac ET0_V, WB_R Ap and PP_R were associated with

Exp2, and higher values of N_{up}E, Ac ET0_R and Ac Rg_R were associated with Exp1.

Additionally, Fig. 2 shows a higher association between N_{up}E and NUE than between N_{ut}E and NUE. NUE is strongly associated with Ac ET0_R and Ac Rg_R. On the other hand, N_{min} is negatively correlated with NUE, and N_{upt} is positively associated to WB_V Ap and Ac PP_V.

3.6. Relationships between NUE components

For the whole dataset (across Exps, SD, N rate and H), variations of NUE were mainly accounting for variations of N_{up}E ($P < 0.0001$; $R^2 = 0.72$) (Fig. 3a). On the other hand, variations of N_{ut}E accounted for 65% of variations of NUE ($P < 0.01$) (Fig. 3b). However, the functions did not have a homogeneous distribution of residuals, due to the negative water balance around flowering in Exp2 (January; Fig. 1b) that strongly affected grain setting and N_{upt}post in early-sown crops (Table 2). These trends are similar between sowing dates. Also, these relationships were consistent with the ordering of variables by the PCA analysis (Fig. 2), where NUE and N_{up}E vectors formed a more acute angle than those of NUE and N_{ut}E.

On both sowing dates, N_{up}E of 0 N crops was positively associated with N_{upt}pre and N_{upt}post (Figs. 4a, b). The value for the coefficient of determination, however, was higher with respect to the N_{up}E vs N_{upt}post relationship. Slope values of linear functions that are fitted to the same variables for the 270 N dataset, are lower than those for the 0 N dataset, and the function fitted to N_{up}E vs. N_{upt}post of late-sown crops was non-significant ($P > 0.05$) (Fig. 4b).

For the whole dataset, variations in N_{ut}E are positively related to changes of NHI ($R^2 = 0.88$, $P < 0.0001$) (Fig. 5a) that are mediated by changes in N rates, hybrids and sowing dates (Table 2). N_{ut}E is also negatively related to grain N concentration ($R^2 = 0.52$, $P < 0.0001$) (Fig. 5b). These trends are similar between sowing dates.

3.7. Post-flowering N and B sources per grain

In both Exps, late-sown crops had a higher post-flowering B source per grain than early crops; especially in Exp2, with no clear pattern between hybrids (Exp x SD x H interaction, $P < 0.05$, Table 5).

On the other hand, N source per grain has been increased by N rate ($P < 0.001$), while no differences in this variable were found between hybrids ($P > 0.05$, Table 5). A significant Exp x sowing date interaction ($P < 0.0001$) on N source per grain was detected. In Exp1, no significant differences between sowing dates were observed. Conversely, in Exp2 N source per grain was higher in late- rather than in early-sown crops.

N and B sources per grain are positively associated, and a single linear function significantly describes this relationship for all cropping conditions (slope value 72 mg B grain⁻¹ per unit of N grain⁻¹) (Fig. 6a). A similar association is found between grain N concentration and N source per grain (Fig. 6b) with a slope value of ca. 0.11%, which indicates the magnitude of grain N concentration variation per each mg

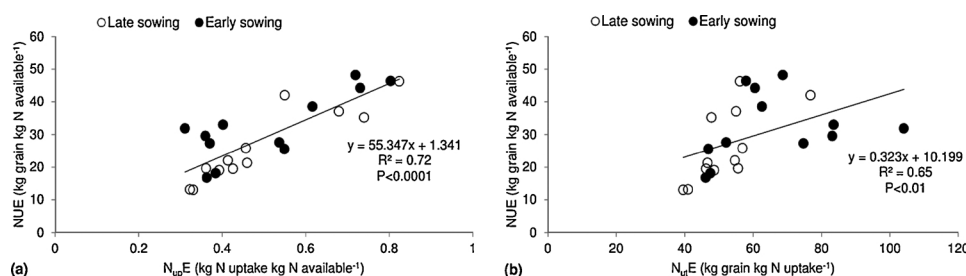


Fig. 3. Nitrogen use efficiency (NUE) as a function of nitrogen uptake efficiency (N_{up}E), and (a) nitrogen utilisation efficiency (N_{ut}E) (b) of early- (close symbols) and late-sown maize crops (open symbols), in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina.

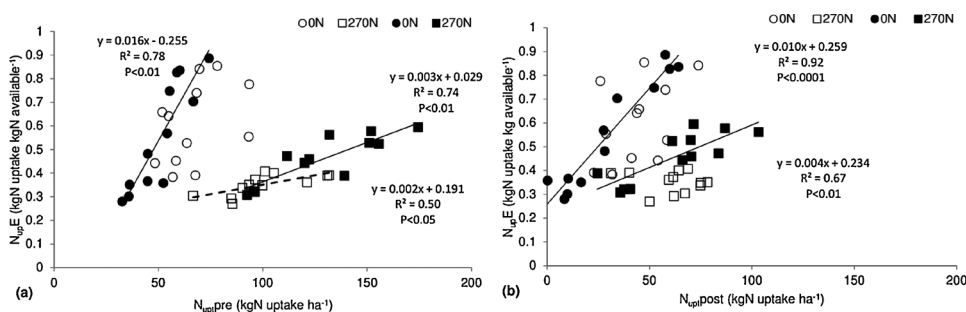


Fig. 4. Nitrogen uptake efficiency (N_{upE}) as a function of nitrogen uptake during pre-flowering (N_{upPre}) (a) and post-flowering period (N_{upPost}) (b) for two N rates, 0 N (circle) and 270 N (squares) of early- (closed symbols) and late-sown maize crops (open symbols), in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) that were carried out in Paraná (Lat. 31.8 °S), Argentina. Continuous and dotted lines represent the lineal functions fit to the dataset of early ($n = 12$) and late-sown maize crop ($n = 12$), respectively. The dataset of 90 N was not included, in order to better describe the differences between contrasting N rates.

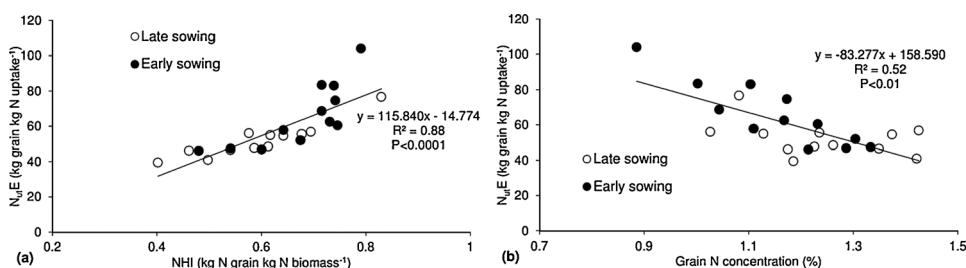


Fig. 5. Nitrogen utilisation efficiency (N_{UE}) as a function of nitrogen harvest index (NHI) (a) and grain N concentration (b) of early- (close symbols) and late-sown maize crops (open symbols) in two experiments (2014-15 and 2015-16, Exp1 and Exp2) carried-out in Paraná (Lat. 31.8 °S), Argentina. Lines represent the lineal function fitted to the whole dataset.

Table 5

Analysis of variance and means values for biomass (B) and N source per grain in two experiments (2014-15 and 2015-16, Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8 °S), Argentina. At each Exp x sowing date combination, two hybrids were cultivated with three N rates.

	B source per grain (mg B grain ⁻¹)	N source per grain (mg N grain ⁻¹)
Experiment (Exp)		
Exp1	280 b	3.1
Exp2	243 a	3.0
P-value	0.0221	ns
N rate (N) (kg N ha ⁻¹)		
0 N	255	2.7 a
90 N	253	3.0 a
270 N	276	3.4 b
P-value	ns	0.0004
Hybrid (H)		
DK70-10VT3P	268	3.1
DK73-10VT3P	255	2.9
P-value	ns	ns
Sowing date (SD)		
Early	216 a	2.5 a
Late	306 b	3.5 b
P-value	< 0.0001	< 0.0001
Interactions	P-value	
Exp x SD	0.0014	< 0.0001
Exp x H	ns	ns
Exp x N	ns	ns
SD x H	ns	ns
SD x N	ns	ns
N x H	ns	ns
Exp x SD x H	0.0296	ns
Exp x SD x N	ns	ns
Exp x H x N	ns	ns
SD x H x N	ns	ns
Exp x SD x H x N	ns	ns

^aValues followed by different letters indicate significant differences (LSD, $P < 0.05$) within each sources of variation.

of N per grain.

Remarkably, variations of N_{UE} (across sowing dates, N rates and hybrids) are strongly and negatively associated with N source per grain through a single exponential function (Fig. 6c).

4. Discussion

Late sowing dates of rainfed maize crops, that have been widely adopted in the Pampas region of Argentina (more than 45% of maize cropped area), contribute to stabilize grain yields due to a more favourable water balance around flowering (Maddoni, 2012). However, late crops are exposed to high soil N_{av} , high temperatures during the pre-flowering period and declining photo-thermal conditions during grain filling, which may affect NUE (kg of grain per kg of N_{av}).

Late-sown crops had a greater decrease in N_{upE} by N rates than early-sown crops (SD x N interaction; $P < 0.0002$; Table 3). This reduction could be associated with the greatest N_{av} (Table 2) promoted by the enhancement in N_{min} (Table 1 and Fig. 2), as was suggested by Cavaglia et al. (2014). The high correlation between NUE and N_{upE} could be related to the fact that N rates more affected N_{av} than grain yields, regardless of sowing date and hybrid (Figs. 2 and 3). An early report of Moll et al. (1982) suggested that at low N_{av} levels the main source of variation of NUE was N_{UE} , while N_{upE} become an important source of variation of NUE at high N_{av} levels as commonly occurred when sowing date is delayed. Thus, in current maize production systems of late sowings with high N_{av} (promoted by high N rates and N_{min}) NUE should be improved through N_{upE} increments (Cassman et al., 2002). Although our results did not indicate a reduction of N_{upE} by the delay of sowing date (Table 3), the Exp x H x SD x N rate interaction indicated that late-sown crops had a higher decreases in N_{upE} by high N rate than early-sown crops (with higher variations in Exp1 than in Exp2), which could imply a constraint for N management in late-sown crops. These results suggest that NUE of late-sown fertilized crops would be improved with agronomical practices that promote higher N_{upE} (Ciampitti and Vyn, 2013) such as high plant density (De-Yang et al., 2016). Moreover, our results indicate that regardless of sowing

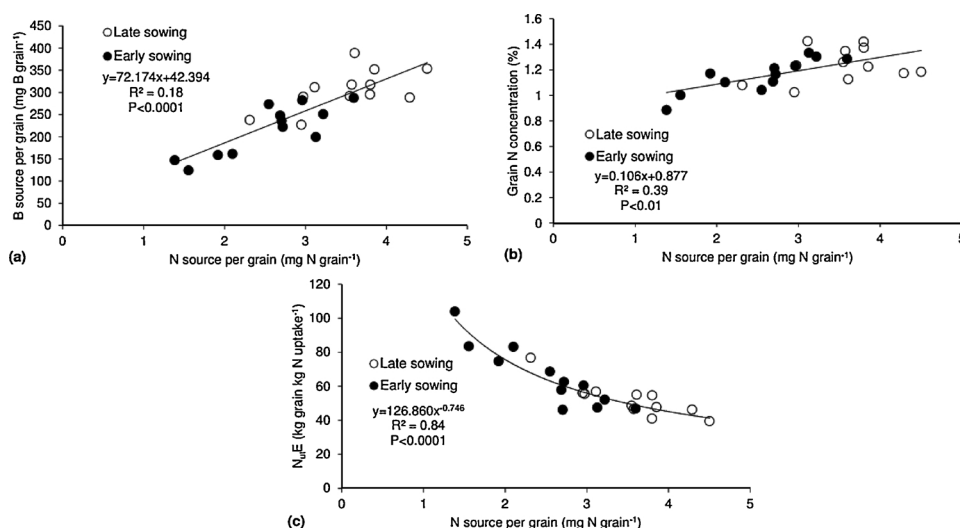


Fig. 6. Post-flowering biomass (B) source per grain: (a) grain N concentration and (b) nitrogen utilisation efficiency (N_{utE}), (c) as a function of post-flowering N source per grain of early- (close symbols) and late-sown maize crops (open symbols), in two experiments (2014-15 and 2015-16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8°S), Argentina. The black lines represent the function fit to the complete dataset ($n = 24$).

date and N rate, N_{upE} had a strong and positive impact on N_{upE} (Fig. 4a). These results highlight the importance of N_{upE} on NUE, suggesting that agronomic practices oriented to improve early N_{upE} could be a useful strategy to improve NUE, mainly in environments with high N_{av} such as late-sown crops.

Interestingly, N rate effect on NUE was greater in early- than in late-crops, especially in hybrid DK 73-10 VT3P, previously characterized by its high N_{upE} (Robles et al., 2015). Although this genotypic pattern was not verified in our results, DK 73-10 VT3P had the highest N_{utE} which was reflected in its higher NUE regardless sowing date (Table 3). Recent studies have documented that variation of NUE among old and new maize hybrids is more related to genotypic differences in N_{utE} than in N_{upE} (see review of Ciampitti and Vyn, 2012; Ferreyra et al., 2013). Thus, the choice of a genotype with high N_{utE} appears to be another valid strategy to mitigate NUE reductions promoted by the high N_{av} typical of N fertilized late-sown crops.

Differences in N_{utE} among genotypes have been often associated with changes in N partitioning, i.e., HI and NHI. For instance, Chen et al. (2015) reported that improvements in N_{utE} of modern hybrids were associated with increases in NHI, which in turn were associated with a higher N_{rem} in late stages of the grain filling period. Accordingly, in our Exps, the higher N_{utE} of DK 73-10 VT3P was associated to a higher NHI (Table 2). However, both hybrids exhibited similar N_{rem} during the entire post-flowering period (Table 2). Studies with a wide genetic bases should be conducted i) to validate the apparent lack of association between NHI and N_{rem} in late-sown crops, ii) the physiological basis of genotype x environment interactions of N_{utE} and iii) the dynamic of N_{rem} and N uptake along the post-flowering period and their relative contribution to NUE in late-sown crops.

Finally, both the sink (grain number) and post-flowering N source ($N_{upE} + N_{remAP}$) were differentially affected by fixed factors, i.e., Exps, hybrids, sowing dates and N rates (Table 2 and 5). N source per grain was positively related to B source per grain (Fig. 6a), suggesting a stable N content of post-flowering biomass production among tested conditions (i.e., combinations of Exps, sowing dates, N rates and hybrids). The B source per grain, ranged from 125 to 389 mg grain⁻¹, and was similar to that obtained from N fertilization Exps of early-sown maize crops (125–400 mg grain⁻¹) by Uhart and Andrade (1995), although results are not strictly comparable since these authors used a shorter period (R3-R5) and a different calculation expression for estimate B source per grain. On the other hand, post-flowering N source per

grain was positively associated with grain N concentration (Fig. 6b), with a slope value of ca. 0.11%, which indicates the magnitude of grain N concentration variation per each mg of N per grain. These results partially agree with those obtained by Abdala et al. (2018) from a dataset covering a wide area of the Pampas region, who reported similar values in grain N concentration between sowing dates. Our studies showed slight differences between sowing dates on grain N concentration, where late-sown crops had a higher grain N concentration as compared with early-sown crops (ca. 1.15 and 1.24 grain N concentration for early- and late-crops respectively; Table 2) by the highest post-flowering N source per grain of the former. Several studies (Ciampitti and Vyn, 2012; Chen et al., 2015) have reported decreases in grain N concentration associated with the higher N_{utE} of modern genotypes, and our results also demonstrated this negative relationship between grain N concentration and N_{utE} (Fig. 5b). For example, in late-sown crops, the higher post-flowering N source per grain (Table 5) increased grain N concentration (Fig. 6b), which explained N_{utE} decreases (Fig. 5b).

5. Conclusions

Nitrogen economy of early- and late-sown crops in a humid-temperate region of central Argentina was studied using a comprehensive framework to determine the effect of sowing date on NUE and its components (N_{upE} and N_{utE} and their determinants N_{av} , N_{upE} , and grain yield). The differences in NUE among Exps, sowing dates, hybrids and N rates were more related with N_{upE} , which varied in a wider range than for N_{utE} . Both N rate and sowing date similarly affected N_{upE} , which was strong and positively associated with N_{upE} . The delay in sowing negatively affected NUE, mainly due to decreases in N_{utE} , by the lower grain yields and the higher N_{av} , and post-flowering N sources per grain.

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Appendix A. Average values of total N available (N_{av}) during the crop cycle, grain yield, harvest index (HI), total N uptake (N_{upt}) during the crop cycle, N uptake during vegetative and reproductive periods (N_{uptpre} and $N_{uptpost}$, respectively), apparent N remobilisation (N_{remAP}), N harvest index (NHI), grain N concentration, nitrogen use efficiency (NUE), nitrogen utilisation efficiency (N_{utE}), nitrogen uptake efficiency (N_{upE}), B and N source per grain in two experiments (2014–15 and 2015–16; Exp1 and Exp2, respectively) carried-out in Paraná (Lat. 31.8° S), Argentina. At each Exp x sowing date combination, two hybrids were cultivated with three N rates

Exp	Sowing date	Hybrid	N rate	N_{av}	Grain yield	HI	N_{upt}	N_{uptpre}	$N_{uptpost}$	N_{remAP}	NHI	grain N concentration (%)	N_{upE}	N_{utE}	NUE	B source per grain	N source per grain
				(kg N ha ⁻¹)	(kg ha ⁻¹)		(kg N ha ⁻¹)	(kg N ha ⁻¹)	(kg N ha ⁻¹)	(kg N ha ⁻¹)	(kg N ha ⁻¹)	(%)	(kg N _{up} kg N _{av} ⁻¹)	(kg grain kg N _{upt} ⁻¹)	(kg grain kg N _{av} ⁻¹)	(mg B grain ⁻¹)	(mg N grain ⁻¹)
2014-20-15	Late	DK70-10VT3P	0 N	157	6798	0.43	107	58	49	29	0.62	1.13	0.68	55.0	37.1	389	3.6
2014-20-15	Late	DK70-10VT3P	90 N	247	10187	0.47	182	114	68	58	0.59	1.23	0.74	47.8	35.2	352	3.9
2014-20-15	Late	DK70-10VT3P	270 N	427	9532	0.52	168	123	45	63	0.61	1.26	0.39	48.6	19.1	292	3.5
2014-20-15	Late	DK73-10VT3P	0 N	157	8510	0.55	129	80	49	45	0.57	1.03	0.82	56.1	46.3	227	3.0
2014-20-15	Late	DK73-10VT3P	90 N	247	12157	0.70	136	86	50	46	0.83	1.08	0.55	76.7	42.1	238	2.3
2014-20-15	Late	DK73-10VT3P	270 N	427	9820	0.55	154	88	66	36	0.68	1.23	0.36	55.6	19.6	291	3.0
2014-20-15	Early	DK70-10VT3P	0 N	145	7895	0.48	117	58	59	28	0.64	1.11	0.81	57.9	46.4	248	2.7
2014-20-15	Early	DK70-10VT3P	90 N	235	10623	0.59	145	115	30	70	0.73	1.17	0.62	62.6	38.6	223	2.7
2014-20-15	Early	DK70-10VT3P	270 N	415	12416	0.52	228	160	68	86	0.60	1.29	0.55	46.9	25.6	288	3.6
2014-20-15	Early	DK73-10VT3P	0 N	145	8194	0.51	105	65	40	35	0.71	1.04	0.72	68.7	48.2	274	2.5
2014-20-15	Early	DK73-10VT3P	90 N	235	12168	0.55	172	115	57	65	0.75	1.23	0.73	60.5	44.2	283	3.0
2014-20-15	Early	DK73-10VT3P	270 N	415	13426	0.60	223	132	91	62	0.68	1.30	0.54	52.1	27.6	251	3.2
2015-20-16	Late	DK70-10VT3P	0 N	228	4847	0.35	104	66	38	31	0.54	1.35	0.46	46.6	21.3	318	3.6
2015-20-16	Late	DK70-10VT3P	90 N	318	6205	0.42	136	106	30	60	0.46	1.17	0.42	46.2	19.5	289	4.3
2015-20-16	Late	DK70-10VT3P	270 N	498	6551	0.42	160	99	61	35	0.50	1.42	0.32	41.0	13.2	296	3.8
2015-20-16	Late	DK73-10VT3P	0 N	228	5891	0.39	104	63	41	32	0.70	1.43	0.46	56.9	25.9	312	3.1
2015-20-16	Late	DK73-10VT3P	90 N	318	6992	0.48	131	75	56	35	0.64	1.37	0.42	54.7	22.0	318	3.8
2015-20-16	Late	DK73-10VT3P	270 N	498	6497	0.40	165	93	72	27	0.40	1.18	0.33	39.5	13.1	354	4.5
2015-20-16	Early	DK70-10VT3P	0 N	150	4947	0.48	60	47	13	23	0.71	1.00	0.40	83.5	33.0	124	1.5
2015-20-16	Early	DK70-10VT3P	90 N	240	6552	0.51	88	65	23	34	0.74	1.17	0.37	74.7	27.3	159	1.9
2015-20-16	Early	DK70-10VT3P	270 N	420	7053	0.49	152	103	49	36	0.48	1.21	0.36	46.1	16.8	236	2.7
2015-20-16	Early	DK73-10VT3P	0 N	150	4778	0.53	47	35	12	19	0.79	0.88	0.31	104.0	31.9	148	1.4
2015-20-16	Early	DK73-10VT3P	90 N	240	7088	0.57	86	60	26	31	0.74	1.11	0.36	83.2	29.6	162	2.1
2015-20-16	Early	DK73-10VT3P	270 N	420	7624	0.55	161	118	43	47	0.54	1.33	0.38	47.5	18.2	200	3.1

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