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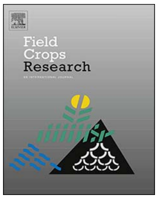

Contribution of the early-established plant hierarchies to maize crop responses to N fertilization

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ABSTRACT

Maize crop production depends on nitrogen (N) availability, N uptake by the crop and the efficiency with which absorbed N is used to produce biomass (NUE_{BIOM}) or grain yield (NUE_{GRAIN}). This framework assumes unique efficiency values for the whole stand, with no distinction among plants in spite of the inherent inter-plant variability of plant growth, especially under crowding stress. In this work we assessed the degree of contribution of different early-established groups of plants to crop responses to N fertilization of two maize hybrids (H) with different tolerance to crowding stress (high for AX820 and low for AX877) cultivated at two stand densities (9 and 12 pl m^{-2}). Groups corresponded to the lower, mid and upper terciles (Ts) of the crop, representing dominated, intermediate and dominant plants, respectively. In most cases, lower and mid Ts had a greater participation in crop biomass and grain yield responses to N fertilization. The response of NUE_{BIOM} and NUE_{GRAIN} to N fertilization was higher for the lower and mid Ts than for the upper T. For each N level, crop NUE_{GRAIN} was negatively related to inter-plant variability in plant NUE_{GRAIN}. When no N was added, the reduction in crop NUE_{GRAIN} of both hybrids was mainly caused by the increased inter-plant variability in plant N uptake (i.e. resource capture). Additionally, the crowding-intolerant AX877 under the most stressful condition (12 pl m^{-2} and no added N) had a reduced crop NUE_{GRAIN} due to the enhanced plant-to-plant variability in grain yield (i.e. resource use). Consequently, the early-established plant-to-plant variability pattern conditioned crop NUE_{GRAIN}; the predominant path was hybrid dependent.

1. Introduction

Maize grain yield responses to N fertilization are mainly associated with the number of kernels per unit land area (Uhart and Andrade, 1995). This grain yield component depends on crop growth rate during a critical 30-day period centered at silking (Andrade et al., 1999), which is affected by N availability (Uhart and Andrade, 1995). From an eco-physiological approach focused on resource supply, crop growth depends on the acquisition of resources (i.e., solar radiation, water and nutrients) and the efficiency with which the acquired resource produces biomass (Galagher and Biscoe, 1978). Focusing on N economy (Moll et al., 1982), crop biomass production depends on N supply, the amount of N uptake by the crop (total N uptake) and N use efficiency for biomass production (NUE_{BIOM}). Similarly, crop grain yield depends on N supply, total N uptake and N use efficiency for grain yield production (NUE_{GRAIN}).

The described framework is generally used to study the performance of maize crops in response to variable N availability. As such, it assumes unique N efficiency values for the whole stand, with no distinction among plants despite the inherent inter-plant variability of plant growth present in most maize crops (Vega and Sadras, 2003; Maddonni and Otegui, 2004). Additionally, plant-to-plant interactions for light

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Abbreviations: CV, coefficient of variation; D, stand density; D9, 9 plants m^{-2}; D12, 12 plants m^{-2}; Exp., experiment; H, hybrid; HI, harvest index; N, nitrogen; N0, no N added; N200, 200 kg N ha^{-1}; Nc, critical N concentration; NHI, N harvest index; NNI, N nutrition index; NUE_{BIOM}, N use efficiency for biomass production; NUE_{GRAIN}, N use efficiency for grain yield production; T, tercile

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acquisition affect total N uptake (Lemaire et al., 2005; Gastal et al., 2015). Several studies have analyzed the competition for N among individuals within communities of different species and plant morphologies (e.g., pastures, mixed crops, forests) and described it as symmetric (Casper and Jackson, 1997; Berntson and Wayne, 2000); i.e., plant N uptake is proportional to the plant size. Hence, inter-plant variability of N uptake would match that of plant biomass. Inter-plant variability of plant biomass exists even in a community of genetically identical individuals (e.g., F1 hybrids) of similar initial plant size and plant architecture, as documented for maize crops (Maddonni and Otegui, 2004). This pattern became evident early in the cycle, and was exacerbated at high stand densities (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007) and under reduced N availability (Rossini et al., 2011). The early-established inter-plant variability of plant biomass (i.e., a proxy of plant hierarchies) held during the critical period around flowering, and generated variability in kernel number per plant (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007; Rossini et al., 2011; Rossini et al., 2012) that penalized crop grain yield (Glenn and Daynard, 1974; Tollenaar and Wu, 1999). There is no information on associated penalties in crop NUE_{GRAIN}.

Few studies have documented the attenuation of early-established plant hierarchies as a result of enhanced resource availability, e.g., by thinning (Pagano and Maddonni, 2007) or N fertilization (Rossini et al., 2011). In the former study, the early suppressed plants of the stand (i.e., dominated individuals) were the most responsive to thinning, suggesting an asymmetric nature of plant competition for light under crowding (Weiner, 1990; Casper and Jackson, 1997). In the latter study, N fertilization smoothed the initial plant-to-plant variability in plant biomass (i.e., a reduced coefficient of variation was recorded for this trait after N fertilization), but the extent of this benefit was genotype dependent; it was larger in a hybrid tolerant to crowding stress than in an intolerant one. Hence, genotypic differences in the capacity of the early-established plant hierarchies to respond to N fertilization could be expected. Additionally, inter-plant variation in kernel protein concentration was increased under reduced N availability (Mayer et al., 2012), limiting our capacity to infer the nature of N competition (i.e., symmetric or asymmetric) based exclusively on the temporal analysis of plant biomass variability.

To our knowledge, little information exists about the effects of inter-plant variability in N uptake and NUE_{GRAIN} on the performance of maize crops (e.g., Ciampitti et al., 2012), and no attention has been given to differences produced on these variables by plant hierarchies and stand densities. Caviglia and Melchiori (2011) identified the dominated plants as those individuals of the stand with the highest grain yield response to N fertilization, but they classified plants based on their biomass at physiological maturity (Maddonni and Otegui, 2004) rather than at the time of hierarchy establishment early in the cycle (Pagano and Maddonni, 2007). The differential sensitivity to N fertilization among early-established plant hierarchies was not explored, which may be crucial for the correct interpretation of genotypic differences in grain yield stability across environments (Pagano and Maddonni, 2007).

In this work, we studied the N economy at the crop and plant levels, of two maize hybrids classified *a priori* as contrasting in their tolerance to crowding (Rossini et al., 2011). For this purpose and early in the cycle, we used plant biomass to classify plants in three terciles (Ts), representative of dominated (lower T), mid-size (mid T) and dominant (upper T) plants of the stand. We analyzed the contribution of each group of plants to crop responses to N fertilization in terms of total biomass, grain yield, total N uptake and N in grains. We also estimated the response of NUE_{BIOM} and NUE_{GRAIN} of each group of plants to N fertilization. The hypothesis of the present investigation was that the group of dominated plants (lower T) of the crowding-tolerant hybrid would contribute to crop responses to N fertilization in a greater proportion than the group of dominant plants (upper T). Conversely, the different group of plants of the crowding-intolerant hybrid would contribute more evenly to crop responses to N fertilization. We also hypothesized that the enhanced variability in NUE_{GRAIN} among plants of a stand would penalize crop NUE_{GRAIN}.

2. Materials and methods

2.1. Experiments

Two field experiments were conducted in Argentina during the growing seasons of 2006/2007 (Exp. 1) and 2007/2008 (Exp. 2) in the Experimental Station of the National Institute of Agricultural Technology (INTA) located at Pergamino (34°56′ S 60°34′ W) on a silty-clay loam soil (Typic Argiudoll). Methodologies of Exp. 1 and Exp. 2 were partially published in Rossini et al. (2011). Briefly, two hybrids (H) classified *a priori* as contrasting in their tolerance to crowding stress (Rossini et al., 2011) were used: the tolerant AX820 CL–MG (hereafter AX820) and the intolerant AX877 CL–MG (hereafter AX877). Both single-cross hybrids were produced by the same seed company (Nidera Argentina). Each hybrid was grown at two stand densities (Dn) and two N levels (Nn). Tested stand densities were 9 (D9) and 12 (D12) plants m^{-2}. Nitrogen levels were a control with no added N (N0) and a high N availability treatment fertilized with 200 kg of N ha^{-1} (N200), added as urea at V6 (Ritchie and Hanway, 1982). Fertilization was applied close to the stage when the largest differences in plant biomass among plants of the stand are recorded (Maddonni and Otegui, 2004). Treatments were distributed in a split-plot design with three replicates. N level was randomized in the main plots and all hybrid per stand density combinations (HD) in the sub-plots (herein termed plots). Plots had six rows with an E-W orientation, 0.7 m between rows and 18 m length.

Manual sowing took place on 20-Oct (Exp. 1) or 22-Oct (Exp. 2) at a rate of 3–4 seeds per hill. Plots were thinned to one plant per hill at the end of the heterotrophic phase (ca. V3; Pommel, 1990). All experiments were kept free of weeds by means of chemical (4 L of atrazine 0.5 a.i. ha^{-1} plus 2 L of acetochlor 0.9 a.i. ha^{-1} at sowing) and manual controls. Water stress was prevented by means of sprinkler irrigation, with the uppermost soil profile (1 m) near field capacity throughout the crop cycle.

2.2. Measurements

A total of 10 (Exp. 1) or 12 (Exp. 2) consecutive plants in a row of similar size (visual assessment) and ontogeny were tagged at V3 in each plot. Plant biomass was estimated at V6 (i.e., immediately before N fertilization) by means of allometric models based on nondestructive morphometric measurements. Details of the non-destructive technique and the fitted allometric models were presented in the previous paper (Rossini et al., 2011). All tagged plants were harvested at physiological maturity (R6). Plants were oven dried at 70 °C until constant weight to quantify final plant biomass. Ears were hand shelled, and grains were weighed to compute plant grain yield. Harvest index (HI) was estimated from the ratio between plant grain yield and total plant biomass.

N concentration (%N) in vegetative tissues and grains was assessed for each plant harvested at R6. Micro-Kjeldahl analysis was used for the vegetative fraction, and near infrared transmittance (Infratec, 1227, Tecator, Sweden) for the grain fraction. Calibration of the near-infrared transmittance instrument was performed by Monsanto Argentina with maize hybrids that are highly representative of those grown throughout the world. N content (in g $plant^{-1}$) of each fraction (vegetative and grain) was obtained as the product between N concentration and the corresponding dry weight, and the sum of these contents was used to quantify plant N uptake at R6. N use efficiency (NUE) was computed at the crop and plant levels, as the quotient between total biomass (NUE_{BIOM}) or total grain (NUE_{GRAIN}) yields and N uptake. Nitrogen harvest index (NHI) was also computed at the crop and plant levels, as the ratio between N content in grains and total N uptake.

The N nutrition index (NNI) was calculated to evaluate the N status of crops at R6 (Lemaire et al., 1996; Ziadi et al., 2008a; Ziadi et al., 2008b; Ziadi et al., 2009). The NNI was established as the ratio between actual %N and the estimated critical %N (%Nc) in Eq. (1).

$$\%Nc = 3.4 \times B^{-0.37} \tag{1}$$

where B is crop biomass (Plénet and Lemaire, 1999). In the original model, B was computed up to R2 and ranged between 1 and 22 Mg ha^{-1}, but its use has been expanded for crops up to R6 and biomass levels as large as 27.5 Mg ha^{-1} (Ciampitti et al., 2012).

2.3. Classification of early-established plant hierarchies

Estimated plant biomass at V6 (see Section 2.2) was taken as an indicator of the capacity of plants for resource capture within the stand (Pagano and Maddonni, 2007) early in the season. All plant biomass data recorded for each plot were ranked in ascending order, and the cumulative frequency was calculated for each record. Plants were assigned to three Ts (i) upper T (i.e. group of dominant plants), when plant biomass ranked within the uppermost 33% of the data set, (ii) lower T (i.e. group of dominated plants), when plant biomass ranked within the lowermost 33% of the data set, and (iii) mid T (i.e. group of intermediate plants), when plant biomass ranked between (i) and (ii). All evaluated traits (plant biomass at R6, plant grain yield, HI, %N grain, %N vegetative biomass, total N uptake, N content in grains, NHI, NUE$_{\mathrm{BIOM}}$, NUE$_{\mathrm{GRAIN}}$, and NNI) were linked to this classification. Total biomass, grain yield, total N uptake and N content in grains of each T was expressed per unit land area as the mean of all plant values of each T, affected by the 33% of the corresponding stand density (i.e. by 3 or 4 for D9 and D12, respectively). The sum of the three Ts represents the value of each trait at the crop level.

HI, NHI, NUE$_{\mathrm{BIOM}}$, NUE$_{\mathrm{GRAIN}}$ were estimated for each T as explained above (Section 2.2). N concentration in grains and vegetative biomass of each T was estimated using the mean values of plants included in each T.

NNI of each T was estimated as the ratio between actual %N and the estimated critical %N (%Nc) in Eq. (1). For this purpose, biomass of each T (i.e., 33% of total biomass on *a per* ha level) was scaled to total biomass (i.e., 100% on a *per ha* level) dividing each value by 0.33.

2.4. Data analysis

The effect of treatments and their interactions on traits measured at the crop level was evaluated across years by ANOVA (Di Rienzo et al., 2017). The ANOVA was performed combining the experiments, with Exp., N, H, and D as fixed variables (Tables 1 and 4).

The model described in Eq. (2) was used for the analysis of data at the crop level.

$$Y_{ijkl} = \mu + Exp._{i} + (Exp.\ N)_{ik} + (Exp.\ HD)_{il} + (Exp.\ NHD)_{ikl} + B_{j(i)} + N_{k} + [BN]_{jk} + HD_{l(k)} + (NHD)_{kl} + [BNHD]_{jkl} + [E]_{ijkl} \tag{2}$$

where μ is the grand mean; $Exp._i$ is the effect of the Experiment i, and i = 1, 2; $B_{j(i)}$ is the effect of the block j nested within the Experiment i, and j = 1, 2, 3; N_k is the effect of the level of N k, and k = 1, 2; $HD_{l(k)}$ is the effect of the combination of hybrid and stand density l nested within the level of N k, and l = 1, 2, 3, 4. Terms in parenthesis correspond to the interaction among factors; $[B\ N]_{jk}$ is the error (a); $[B\ N\ HD]_{jkl}$ is the error (b); $[E]_{ijkl}$ is the error (c).

Similarly, the ANOVA of traits computed at the T level was performed combining the experiments, with Exp., N, H, D, and T as fixed variables (Table 2). The model described in Eq. (3) was used for the analysis of data at the T level.

$$Y_{ijklm} = \mu + Exp._{i} + (Exp.N)_{ik} + (Exp.HD)_{il} + (Exp.NHD)_{ikl} + (Exp.NHDT)_{iklm} + B_{j(i)} + N_{k} + [BN]_{jk} + HD_{l(k)} + (NHD)_{kl} + [BNHD]_{jkl} + T_{m(l)} + (HDT)_{lm} + (NHDT)_{klm} + [BNHDT]_{jklm} + [E]_{ijklm} \tag{3}$$

where μ is the grand mean; $Exp._i$ is the effect of the Experiment i, and i = 1, 2; $B_{j(i)}$ is the effect of the block j nested within the Experiment i, and j = 1, 2, 3; N_k is the effect of the level of N k, and k = 1, 2; $HD_{l(k)}$ is the effect of the combination of hybrid and stand density l nested within the level of N k, and l = 1, 2, 3, 4; $T_{m(l)}$ is the effect of the tercile m nested within the combination of HD l, and m = 1, 2, 3. Terms in parenthesis correspond to the interactions among factors; $[B\ N]_{jk}$ is the error (a); $[B\ N\ HD]_{ikl}$ is the error (b); $[B\ N\ HD\ T]_{iklm}$ is the error (c); $[E]_{ijklm}$ is the error (d).

The response of each T to N fertilization in terms of biomass, grain yield, N uptake, and N in grains was calculated as the difference between the trait value of each T in N200 and the trait value of each T in N0 (Table 3). The sum of the responses of the three Ts represents crop response to N fertilization.

The response of NUE$_{\mathrm{BIOM}}$ and NUE$_{\mathrm{GRAIN}}$ of each T to N fertilization was calculated (Eqs. (4) and (5)).

$$Response\ NUE_{BIOM}\ Tn = \frac{(Biomass\ N200_{Tn} - Biomass\ N0_{Tn})}{Total\ N\ uptake\ N200_{Tn} - Total\ N\ uptake\ N0_{Tn}} \tag{4}$$

$$Response\ NUE_{GRAIN}\ Tn = \frac{(Grain\ Yield\ N200_{Tn} - Grain\ Yield\ N0_{Tn})}{Total\ N\ uptake\ N200_{Tn} - Total\ N\ uptake\ N0_{Tn}} \tag{5}$$

The ANOVA of responses of each T to N fertilization was performed combining the experiments, with the Exp., H, D, and T as fixed variables (Table 3). The model described in Eq. (6) was used for the analysis of T responses to N fertilization.

$$Y_{iklm} = \mu + Exp._{i} + (Exp.\ HD)_{il} + (Exp.\ HDT)_{ilm} + B_{j(i)} + HD_{l} + [BHD]_{jl} + T_{m(l)} + (HDT)_{lm} + [BHDT]_{jlm} + [E]_{ijlm} \tag{6}$$

where μ is the grand mean; $Exp._i$ is the effect of the Experiment i, and i = 1, 2; $B_{j(i)}$ is the effect of the block j nested within the Experiment i, and j = 1, 2, 3; HD_l is the effect of the combination of hybrid and stand density l, and l = 1, 2, 3, 4; $T_{m(l)}$ is the effect of the tercile m nested within the combination of HD l, and m = 1, 2, 3. Terms in parenthesis correspond to the interactions among factors; $[B\ HD]_{jl}$ is the error (a); $[B\ HD\ T]_{jlm}$ is the error (b); $[E]_{ijlm}$ is the error (c).

The coefficient of variation among plants (CV, in %) was computed at the plot level (i.e. inter-plant variability) for plant grain yield, plant N uptake and NUE$_{\mathrm{GRAIN}}$ and their responses to treatments and treatments interactions were also analyzed using ANOVA (Table 4). Crop NUE$_{\mathrm{GRAIN}}$ was related to CV of NUE$_{\mathrm{GRAIN}}$, and CV of NUE$_{\mathrm{GRAIN}}$ was related to CV of plant grain yield and CV of plant N uptake. Linear regressions were fitted between variables. Differences between variables and their interactions were evaluated by means of a *Duncan* test.

3. Results

3.1. Crop responses to N fertilization

Crop biomass and crop grain yield of tested hybrids in both Exps. and stand densities were significantly (P < 0.01) affected by N fertilization (Table 1). These responses were in agreement with NNIs, which were smaller (P < 0.001) in N0 than in N200 (Table 1). N fertilization increased crop biomass by ca. 9.5 Mg ha^{-1} (i.e., 65.3%) and crop grain yield by ca. 6.1 Mg ha^{-1} (i.e., 84.5%). Stand density increased crop biomass only for AX820 (interaction H × D, P < 0.10). At all tested conditions (Exps. and stand densities), harvest index (HI) of both

Table 1
Treatments effect on evaluated traits at the crop level and ANOVA of results. Data correspond to Experiments 1 and 2.

Experiment	Nitrogen	Hybrid	Stand density	Crop biomass	Crop grain yield	HI[a]	% N		N grain	Total N uptake	NHI	NUE$_{BIOM}$	NUE$_{GRAIN}$	NNI
	kg ha^{-1}		pl m^{-2}	Mg ha^{-1}	Mg ha^{-1}		Grain	Biomass	Mg ha^{-1}	Mg ha^{-1}		Mg ha^{-1}	Mg ha^{-1}	
1	0	AX820	9	16.0	9.1	0.55	0.94	0.74	0.095	0.130	0.71	146.9	80.0	0.62
			12	18.2	9.7	0.53	0.87	0.64	0.085	0.118	0.72	157.9	83.9	0.55
		AX877	9	13.5	6.9	0.51	0.74	0.55	0.051	0.075	0.68	183.2	92.7	0.42
			12	14.4	6.9	0.48	0.81	0.61	0.059	0.090	0.64	168.2	80.3	0.48
	200	AX820	9	23.8	14.2	0.60	1.27	1.02	0.181	0.243	0.75	99.8	59.8	0.97
			12	24.9	14.7	0.59	1.26	1.01	0.185	0.251	0.74	99.3	58.7	0.97
		AX877	9	25.7	15.0	0.58	1.36	1.08	0.204	0.278	0.73	93.6	54.3	1.06
			12	26.6	15.6	0.59	1.27	1.00	0.199	0.266	0.75	101.1	59.5	0.99
2	0	AX820	9	10.4	5.1	0.49	1.05	0.68	0.053	0.070	0.76	148.2	72.1	0.47
			12	13.8	7.0	0.51	1.08	0.73	0.077	0.101	0.76	138.3	70.3	0.56
		AX877	9	14.6	6.9	0.47	1.04	0.67	0.072	0.098	0.73	150.0	70.6	0.53
			12	15.5	6.6	0.39	1.03	0.64	0.067	0.102	0.61	158.0	59.4	0.51
	200	AX820	9	20.4	11.7	0.57	1.49	1.06	0.174	0.217	0.80	94.6	54.6	0.95
			12	21.9	11.8	0.54	1.48	1.07	0.176	0.235	0.75	94.4	51.2	0.99
		AX877	9	24.2	12.3	0.50	1.56	1.07	0.189	0.258	0.73	94.0	47.7	1.02
			12	24.9	11.9	0.48	1.62	1.06	0.192	0.265	0.73	94.1	45.3	1.03
Exp				†[b](2.1)	*(1.7)	***(0.03)	***(0.07)					†(7.9)	***(4.2)	
Exp. × N													†(4.7)	
Exp. × HD														
Exp. × N × HD														
N				**(3.5)	**(2.5)	**(0.02)	***(0.02)	***(0.04)	**(0.02)	***(0.013)	†(0.04)	**(15.6)	**(8.5)	***(0.03)
HD				†(1.7)		*(0.04)					*(0.04)			
N × HD							†(0.09)				†(0.05)		†(6.3)	
MSE			**DF**											
(a)			2	8.11	4.08	3.6 e^{-4}	4.3 e^{-4}	1.1 e^{-3}	2.6 e^{-4}	1.1 e^{-4}	2.1 e^{-3}	158	46.6	7.3 e^{-4}
(b)			12	6.15	2.94	2.0 e^{-3}	0.01	0.01	5.2 e^{-4}	9.4 e^{-4}	2.2 e^{-3}	155	39.7	0.01
(c)			16	18.9	7.47	2.1 e^{-3}	0.01	0.01	1.2 e^{-3}	1.9 e^{-3}	3.4 e^{-3}	260	47.4	0.01

[a] DF: Degrees of freedom; Exp.: Experiment; HD: combination of hybrid and stand density; HI: Harvest index; MSE: Mean square error; N: Nitrogen; NHI: Nitrogen harvest index; NNI: Nitrogen nutrition index; NUE$_{BIOM}$: Nitrogen use efficiency for biomass production; NUE$_{GRAIN}$: Nitrogen use efficiency for grain yield production.

[b] †, *, **, ***: significant at 0.10, 0.05, 0.01 and 0.001, respectively. For each factor, the critical values for the comparison of means are detailed in brackets.

hybrids was also increased (ca. 13.7%) by N fertilization ($P < 0.01$). At high stand density, AX820 had a larger HI than AX877 (interaction H × D, $P < 0.05$), whereas at low stand density both hybrids had similar HI.

Nitrogen fertilization increased %N of total biomass and grains of both hybrids ($P < 0.001$). Both hybrids at both stand densities did not differ in %N in grains at N200, but at low stand density under N0 this trait was larger for AX877 than for AX820 (N × H × D interaction, $P < 0.10$).

Total crop N uptake and N content in grains of both hybrids increased ($0.001 < P < 0.01$) by N fertilization (ca. 157%) but were not modified by stand density (Table 1).

NHI of AX877 at N0 decreased in response to increased stand density (N × H × D interaction, $P < 0.10$). This trait was similar across all stand densities and N availabilities combinations for AX820.

The NUE$_{BIOM}$ and NUE$_{GRAIN}$ of crops were smaller ($0.001 < P < 0.01$) in N200 than in N0 (Table 1). When no N was added, AX877 had reduced NUE$_{GRAIN}$ in response to increased stand density (N × H × D interaction, $P < 0.10$).

3.2. Biomass, grain yield and N economy of the early-established group of plants

For both hybrids at high stand density and for AX877 at low stand density, significant differences were recorded among Ts established at V6 in total biomass and grain yield registered at R6 (upper T > mid T > lower T) (H × D × T interaction, $P < 0.01$, Table 2).

In both Exps, stand densities and N availabilities, HI of upper and mid Ts of AX877 were higher than that of the lower T, while all groups of plants of AX820 had similar HI (H × T interaction, $P < 0.05$).

In both Exps. and N conditions, all Ts of both hybrids at high stand density and of AX877 at low stand density differed significantly in total N uptake and N in grains (upper T > mid T > lower T) (H × D × T interaction, $P < 0.01$). In both Exps. and N availabilities, upper and mid Ts of AX877 at high stand density had higher NHI than lower T (H × D × T interaction, $P < 0.05$). All Ts had similar %N in grains and total biomass and NUE$_{BIOM}$ (Table 2). In both Exps. all Ts of AX877 at D12 N0 differed in NUE$_{GRAIN}$ (upper T > mid T > lower T) (N × H × D × T interaction, $P < 0.05$), while NUE$_{GRAIN}$ was similar for the other combinations of N × HD × T.

N fertilization improved the NNI at all T levels ($P < 0.001$). Lowest NNI values corresponded to the lower Ts, and highest values to the upper T ($P < 0.001$, Table 2). Among plants of upper T, however, a few cases (ten) had NNI > 1 (i.e., luxury uptake), and only in five of these cases the luxury uptake was larger than 5%.

3.3. Contribution of the early-established group of plants to crop responses to N fertilization

In both Exps, contribution of the different Ts to crop biomass and grain yield responses to N fertilization was not affected by stand density or genotype (Table 3). In most cases, lower and mid Ts had a greater ($P < 0.10$) participation in crop biomass and grain yield responses to N fertilization. Contrary, contribution of the different Ts to the response of crop N uptake to N fertilization was mainly defined by the mid and upper Ts, although these differences were not statistically significant (Table 3).

In both Exps, hybrids and stand densities, the lower and mid Ts had a higher response ($P < 0.05$) of NUE$_{BIOM}$ to N fertilization (~66 Mg Mg^{-1}) than the upper T (45 Mg Mg^{-1}) (Table 3). Similarly,

Table 2
Treatments effect on evaluated traits at each tercile level and ANOVA of results. Data correspond to Experiments 1 and 2.

Experiment	Nitrogen	Hybrid	Stand density	Tercile	Total biomass R6	Grain yield	HI[a]	% N		N grain	Total N uptake	NHI	NUE_{BIOM}	NUE_{GRAIN}	NNI
	kg ha^{-1}		pl m^{-2}		Mg ha^{-1}	Mg ha^{-1}		Grain	Biomass	Mg ha^{-1}	Mg ha^{-1}		Mg ha^{-1}	Mg ha^{-1}	
1	0	AX820	9	Lower	4.63	2.64	0.55	0.90	0.70	0.028	0.037	0.70	151.3	81.4	0.56
				Mid	5.50	3.11	0.55	0.96	0.76	0.034	0.047	0.71	146.2	80.1	0.65
				Upper	5.86	3.29	0.55	0.95	0.73	0.033	0.045	0.73	146.0	79.8	0.63
			12	Lower	4.73	2.49	0.52	0.87	0.64	0.021	0.030	0.71	156.1	82.0	0.50
				Mid	5.85	3.09	0.52	0.90	0.65	0.028	0.038	0.73	158.6	82.6	0.55
				Upper	7.65	4.18	0.54	0.83	0.63	0.036	0.050	0.71	162.7	87.9	0.59
		AX877	9	Lower	3.46	1.74	0.49	0.73	0.55	0.013	0.019	0.66	187.3	91.7	0.38
				Mid	4.57	2.32	0.50	0.74	0.55	0.017	0.025	0.68	183.6	92.2	0.42
				Upper	5.49	2.82	0.51	0.73	0.55	0.020	0.030	0.67	183.2	94.5	0.46
			12	Lower	4.08	1.86	0.40	0.82	0.65	0.016	0.026	0.52	155.5	62.8	0.45
				Mid	4.43	2.13	0.48	0.84	0.60	0.019	0.028	0.65	170.5	80.5	0.46
				Upper	5.95	3.00	0.50	0.79	0.59	0.024	0.036	0.66	172.9	86.0	0.51
	200	AX820	9	Lower	8.02	4.96	0.62	1.28	1.03	0.064	0.083	0.77	99.9	61.4	0.99
				Mid	7.88	4.76	0.60	1.28	1.03	0.061	0.081	0.75	98.5	59.4	0.98
				Upper	7.89	4.52	0.57	1.24	0.99	0.056	0.079	0.72	102.0	59.0	0.95
			12	Lower	7.73	4.78	0.62	1.24	0.98	0.059	0.075	0.78	102.3	63.2	0.92
				Mid	8.19	4.61	0.56	1.27	1.01	0.058	0.083	0.70	98.7	55.4	0.98
				Upper	9.01	5.44	0.60	1.27	1.03	0.069	0.092	0.75	97.6	58.9	1.02
		AX877	9	Lower	6.88	3.95	0.57	1.30	1.03	0.051	0.071	0.73	98.6	56.6	0.93
				Mid	9.18	5.37	0.59	1.38	1.10	0.075	0.101	0.74	92.6	53.7	1.10
				Upper	9.48	5.52	0.58	1.37	1.10	0.075	0.104	0.73	91.5	53.4	1.12
			12	Lower	8.44	4.97	0.59	1.24	0.96	0.062	0.082	0.76	104.8	61.6	0.94
				Mid	8.97	5.31	0.59	1.24	0.99	0.066	0.089	0.74	102.3	60.7	0.99
				Upper	9.11	5.30	0.58	1.34	1.04	0.071	0.094	0.76	97.0	56.5	1.04
2	0	AX820	9	Lower	2.88	1.37	0.47	1.10	0.69	0.015	0.020	0.75	145.9	68.8	0.45
				Mid	3.28	1.57	0.48	1.01	0.65	0.016	0.021	0.75	154.8	74.0	0.44
				Upper	4.25	2.14	0.50	1.05	0.69	0.022	0.029	0.77	144.9	72.8	0.52
			12	Lower	3.61	1.73	0.48	1.08	0.71	0.019	0.026	0.73	141.6	68.1	0.50
				Mid	4.62	2.44	0.52	1.10	0.74	0.027	0.035	0.78	135.3	70.6	0.57
				Upper	5.58	2.86	0.51	1.07	0.73	0.031	0.041	0.75	138.7	70.5	0.61
		AX877	9	Lower	3.24	1.23	0.37	1.08	0.64	0.013	0.021	0.62	158.3	58.7	0.44
				Mid	4.86	2.41	0.50	1.04	0.69	0.025	0.033	0.75	146.0	72.5	0.54
				Upper	6.49	3.27	0.50	1.03	0.67	0.034	0.044	0.77	150.7	75.3	0.60
			12	Lower	3.31	1.05	0.26	0.88	0.61	0.011	0.021	0.41	168.2	40.1	0.42
				Mid	5.33	2.26	0.37	1.05	0.65	0.024	0.036	0.59	158.2	55.0	0.53
				Upper	6.86	3.24	0.45	1.01	0.64	0.032	0.044	0.71	155.3	70.3	0.58
	200	AX820	9	Lower	6.81	3.72	0.55	1.45	1.02	0.054	0.070	0.77	99.3	54.3	0.92
				Mid	6.67	3.80	0.57	1.47	1.07	0.056	0.071	0.78	93.8	53.4	0.95
				Upper	6.94	3.86	0.56	1.54	1.09	0.059	0.076	0.78	91.9	51.2	0.99
			12	Lower	5.61	2.84	0.50	1.49	1.07	0.043	0.061	0.71	95.6	48.2	0.90
				Mid	7.61	4.14	0.54	1.51	1.10	0.062	0.083	0.75	92.1	50.3	1.03
				Upper	8.66	4.84	0.56	1.45	1.05	0.070	0.091	0.77	96.3	53.9	1.04
		AX877	9	Lower	6.77	3.48	0.51	1.48	1.04	0.050	0.070	0.72	97.9	50.6	0.93
				Mid	8.67	4.19	0.48	1.60	1.08	0.067	0.093	0.72	93.2	45.1	1.06
				Upper	8.79	4.59	0.52	1.57	1.08	0.072	0.095	0.76	92.5	48.4	1.07
			12	Lower	6.20	2.68	0.42	1.68	1.04	0.044	0.064	0.67	96.4	40.6	0.90
				Mid	9.03	4.55	0.50	1.57	1.04	0.071	0.094	0.76	96.0	48.5	1.04
				Upper	9.67	4.69	0.49	1.64	1.09	0.077	0.106	0.73	91.5	44.9	1.12
Exp.					**[b](0.49)	***(0.31)	***(0.02)	***(0.04)	**(0.03)		†(0.004)		**(5.5)	***(3.3)	
Exp. × N													†(6.3)	*(4.7)	
Exp. × HD					†(0.80)						†(0.008)				
Exp. × N × HD								†(0.09)	†(0.07)				†(12.6)		

(*continued on next page*)

Table 2 (*continued*)

Experiment	Nitrogen	Hybrid	Stand density	Tercile	Total biomass R6	Grain yield	HI[a]	% N		N grain	Total N uptake	NHI	NUE_{BIOM}	NUE_{GRAIN}	NNI
								Grain	Biomass						
	kg ha^{-1}		pl m^{-2}		Mg ha^{-1}	Mg ha^{-1}				Mg ha^{-1}	Mg ha^{-1}		Mg ha^{-1}	Mg ha^{-1}	
Exp. × N × HD × T															
N					**(1.17)	**(0.80)	**(0.01)	***(0.03)	**(0.06)	**(0.006)	**(0.004)	*(0.05)	**(13)	**(8.0)	***(0.03)
HD					†(0.58)		**(0.04)					**(0.04)		*(5.8)	
N × HD								†(0.10)				*(0.06)		†(6.5**)	
T					***(0.32)	***(0.19)	***(0.01)		†(0.02)	***(0.003)	***(0.003)	**(0.02)		**(2.6)	***(0.02)
HD × T					**(0.65)	**(0.38)	*(0.03)			**(0.005)	**(0.006)	**(0.04)		*(5.1)	
N × HD × T												*(0.06)		*(7.3)	
MSE	**DF**														
(a)	2				2.67	1.26	4.7 e^{-4}	1.4 e^{-3}	0.01	7.0 e^{-5}	3.3 e^{-5}	0.01	329	125	1.9 e^{-3}
(b)	12				2.01	0.97	0.01	0.03	0.02	1.8 e^{-4}	3.0 e^{-4}	0.01	569	128	0.03
(c)	32				0.61	0.21	1.4 e^{-3}	0.01	3.5 e^{-3}	4.6 e^{-5}	6.0 e^{-5}	2.7 e^{-3}	118	38	3.9 e^{-3}
(d)	60				2.18	0.89	4.4 e^{-3}	0.02	0.01	1.5 e^{-4}	2.2 e^{-4}	0.01	271	100	0.01

[a] DF: Degrees of freedom; Exp.: Experiment; HD: combination of hybrid and stand density; HI: Harvest index; MSE: Mean square error; N: Nitrogen; NHI: Nitrogen harvest index; NNI: Nitrogen nutrition index; NUE_{BIOM}: Nitrogen use efficiency for biomass production; NUE_{GRAIN}: Nitrogen use efficiency for grain yield production; T: Tercile.

[b] †, *, **, ***: significant at 0.10, 0.05, 0.01 and 0.001, respectively. For each factor, the critical values for the comparison of means are detailed in brackets.

the response of NUE_{GRAIN} to N fertilization of lower and mid Ts (~41.7 Mg Mg^{-1}) was higher ($P < 0.01$) than those of the upper T (29.5 Mg Mg^{-1}).

3.4. Variability in NUE_{GRAIN} among plants of a stand and crop NUE_{GRAIN}

N fertilization reduced crop NUE_{GRAIN} (ca. 76.5 and 54 Mg Mg^{-1}, for N0 and N200; respectively; Table 1) of both hybrids, but interplant variability in this trait decreased (from 49% to 21%) only for AX877 at high stand density (N × H × D interaction, $P < 0.05$) (Table 4). N fertilization reduced the CV of grain yield of AX877 at the highest stand density (N × H × D interaction, $P < 0.05$, Table 4) and the CV of plant N uptake of both hybrids at both stand densities ($P < 0.05$). At both stand densities and N levels, CV of N uptake was higher for AX877 than for AX820 (hybrid effect in the HD combinations, $P < 0.05$; Table 4).

For each N level, crop NUE_{GRAIN} was negatively related to interplant variability in NUE_{GRAIN} (Fig. 1a). Linear functions fitted to the data set of each N level differed in the ordinate value (value for N0 was higher than that for N200, $P < 0.05$). The negative trends, however, were almost identical (slopes 0.4 Mg Mg^{-1} %$^{-1}$) for both N conditions and can be attributed to the interplant variability in both determinants of NUE_{GRAIN}; i.e. the CVs of grain yield and of total N uptake. For the former, the impact of the N × H × D interaction on plant-to-plant variability in grain yield (mainly for AX877) explained 80% of interplant variation in NUE_{GRAIN} (Table 4 and Fig. 1b). For the latter, the N and H effects on plant-to-plant variability in plant N uptake explained 50% of the variation in NUE_{GRAIN} (Table 4 and Fig. 1c).

4. Discussion

In several agricultural regions of the world, the use of high N fertilization rates in grain crops is producing negative environmental impacts (e.g., contamination of water resources) and a decrease in crop NUE_{GRAIN} (Cassman et al., 2003). New management practices and breeding programs are needed to improve this efficiency in order to reduce environmental risks and to increase the gross margin of fertilized grain crops. Among new management practices, precision farming is expected to improve fertilization practices, reducing the differences among plants within a stand (Raun et al., 2005; Haboudane et al., 2002). Among breeding programs, high stand densities are currently recommended in maize crops by seed companies for attaining high grain yields (Fischer and Edmeades, 2010), a trend that may promote a strong interaction among plants for light capture (Maddonni and Otegui, 2004). This interaction affects total N uptake and N partitioning to kernels at the crop level (Lemaire et al., 2005; Gastal et al., 2015). Hence, studies of maize crop responses to N fertilization should consider the contribution of individual plants of the stand to crop NUE_{GRAIN}, particularly across optimum and above-optimum stand densities.

In this work we assessed the contribution of the early-established plant hierarchies, grouped in Ts (lower, mid and upper), to crop responses to N fertilization of two maize hybrids with different tolerance to crowding stress (Rossini et al., 2011). We quantified the contribution of each group of plants to crop response to N fertilization in terms of biomass, grain yield, total N uptake and N in grains, and we estimated NUE_{BIOM} and NUE_{GRAIN} of each T. Finally, we established the impact of plant-to-plant variability in NUE_{GRAIN} and N uptake on crop NUE_{GRAIN}.

We hypothesized that "the group of dominated plants (lower T) of the crowding-tolerant hybrid would contribute to crop responses to N fertilization in a greater proportion than the group of the dominant plants (upper T). Conversely, the different groups of plants of the crowding-intolerant hybrid would contribute more evenly to crop responses to N fertilization". This hypothesis was partially rejected. Responses of crop biomass and crop grain yield to N fertilization were predominantly caused by the response of the early-established lower

Table 3
Treatments effect on the response to N fertilization of evaluated traits at the tercile level and ANOVA of results. Data correspond to Experiments 1 and 2.

Experiment	Hybrid	Stand density pl m^{-2}	Tercile	Total Biomass R6 Mg ha^{-1}	Grain yield Mg ha^{-1}	N grain Mg ha^{-1}	Total N Mg ha^{-1}	NUE$_{\text{BIOM}}$[a] Mg ha^{-1}	NUE$_{\text{GRAIN}}$ Mg ha^{-1}
1	AX820	9	Lower	3.39	2.32	0.036	0.046	62.6	44.7
			Mid	2.38	1.66	0.027	0.034	101.2	56.4
			Upper	2.03	1.23	0.023	0.034	57.7	30.4
		12	Lower	3.00	2.29	0.037	0.045	59.1	47.4
			Mid	2.34	1.52	0.031	0.045	47.8	29.7
			Upper	1.36	1.25	0.033	0.043	1.7	12.4
	AX877	9	Lower	3.42	2.21	0.038	0.052	67.6	43.6
			Mid	4.61	3.05	0.058	0.076	62.6	40.8
			Upper	3.98	2.70	0.055	0.074	54.3	36.8
		12	Lower	4.36	3.11	0.047	0.056	76.7	54.9
			Mid	4.54	3.18	0.047	0.061	74.2	51.8
			Upper	3.16	2.31	0.047	0.058	52.8	38.7
2	AX820	9	Lower	3.93	2.35	0.039	0.051	80.7	48.9
			Mid	3.38	2.23	0.040	0.050	67.4	44.5
			Upper	2.69	1.72	0.037	0.047	58.3	37.4
		12	Lower	2.00	1.11	0.024	0.035	56.3	29.7
			Mid	2.99	1.70	0.035	0.049	57.9	32.4
			Upper	3.08	1.98	0.039	0.050	60.9	39.5
	AX877	9	Lower	3.53	2.25	0.037	0.049	69.4	44.7
			Mid	3.82	1.77	0.042	0.060	63.4	29.6
			Upper	2.29	1.33	0.038	0.051	42.7	23.8
		12	Lower	2.88	1.64	0.033	0.044	66.2	37.7
			Mid	3.70	2.29	0.047	0.058	50.1	31.9
			Upper	2.82	1.44	0.045	0.062	31.4	17.5
Exp.									
Exp. × HD									
Exp. × HD × T									
HD							†[b](0.57)		
T				†(0.56)	†(0.34)			*(15.9)	**(8.8)
T × HD									
MSE	**DF**								
(a)	6			3.76	2.28	2.5 e^{-4}	4.0 e^{-4}	1580	827
(b)	16			1.35	0.49	9.9 e^{-5}	1.5 e^{-4}	677	206
(c)	26			3.77	1.51	2.3 e^{-4}	3.2 e^{-4}	1068	352

[a] DF: Degrees of freedom; Exp.: Experiment; HD: combination of hybrid and stand density; HI: Harvest index; MSE: Mean square error; NUE$_{\text{BIOM}}$: Nitrogen use efficiency for biomass production; NUE$_{\text{GRAIN}}$: Nitrogen use efficiency for grain yield production; T: Tercile.

[b] †, *, **, ***: significant at 0.10, 0.05, 0.01 and 0.001, respectively. For each factor, the critical values for the comparison of means are detailed in brackets.

and mid Ts, but this response was similar for the two hybrids evaluated (Table 3). These results are consistent with previous studies, which documented that N fertilization reduced inter-plant variability of grain yield (Boomsma et al., 2009) due to the increased responsiveness of dominated plants, especially for crops severely limited by N supply (Caviglia and Melchiori, 2011). However, in previous studies (Caviglia and Melchiori, 2011) plants were classified based on their biomass at physiological maturity (R6), without considering plant-to-plant variability established early in the cycle (V7-V9; Maddonni and Otegui, 2004) or the different capacity of maize hybrids to dampen this early established plant-to-plant variability (Pagano and Maddonni, 2007; Rossini et al., 2011).

The present study is the first one that shows how different early-established hierarchical groups of plants affect crop N economy. Differences among Ts in the responses of NUE$_{\text{BIOM}}$ and NUE$_{\text{GRAIN}}$ to N fertilization (Table 3) have never been documented for genetically identical individuals (plants of F1 hybrids). Mentioned trends could be caused by photo-morphogenic responses triggered by light signals in dense canopies (Kasperbauer and Karlen, 1994; Maddonni et al., 2002). The reduced response in NUE$_{\text{BIOM}}$ of the upper T may have been caused by the high leaf:stem ratio and leaf N concentration of these plants in dense canopies (Lemaire et al., 2005). Additionally, under crowding stress, plants of the lower T could be more limited by N supply than plants of the upper T due to the enhanced shoot:root ratio of the former (Kasperbauer and Karlen, 1994). These hypotheses would be confirmed by the differences in NNI between groups of plants, where plants of the upper T reached the sufficiency level (NNI $\cong$ 1) or even an exacerbated (*luxury*) N uptake (NNI $>$ 1) respect to plants of the lower T.

In the second hypothesis, we stated that “inter-plant variability in NUE$_{\text{GRAIN}}$ would penalize NUE$_{\text{GRAIN}}$ at the crop level” and this hypothesis could not be rejected (Fig. 1a). As expected, N fertilization reduced crop NUE$_{\text{GRAIN}}$ because the increase in crop N uptake (ca. 157%) was proportionally larger than that registered in crop grain yield (ca. 84.5%) (Section 3.1). Interestingly, at each N level, crop NUE$_{\text{GRAIN}}$ was negatively related to inter-plant variability in this trait promoted by (i) the N × H × D effect on plant-to-plant variability in grain yield, (ii) the N and the H effects on plant-to-plant variability in plant N uptake. At the most stressful conditions (high stand density in N0), the crowding-intolerant hybrid AX877 had a reduced crop NUE$_{\text{GRAIN}}$ due to increased population variability in this trait, promoted by the great plant-to-plant variability in grain yield. The mentioned response was due to the presence of plants with low biomass partitioning to the ear around silking and enhanced barrenness when this hybrid was cultivated at high density in N0 (Rossini et al., 2011). These results are in line with the theory proposed by Tollenaar et al. (2006), based on the carbon balance of maize plants in crops exposed to crowding stress, where variations in crop grain yield were mainly promoted by the increase in inter-plant variability of grain yield. Hence, for the crowding-intolerant hybrid AX877, cultural practices that tend to reduce both N stress (N fertilization) or crowding stress (optimum plant density) would increase NUE$_{\text{GRAIN}}$. Moreover, genetic efforts to improve tolerance to high plant density should enhance NUE$_{\text{GRAIN}}$ under low N environments (Boomsma et al., 2009). For both hybrids, however, the reduction of crop NUE$_{\text{GRAIN}}$ was mainly caused by the increased inter-plant variability in plant N uptake when no N was added. Additionally, we detected an inherent higher inter-plant variability in plant N uptake

Table 4
Coefficient of variation, ANOVA, significance levels and least significant differences for Experiments 1 and 2.

Experiment	Hybrid	Stand Density (pl m^{-2})	Nitrogen (kg ha^{-1})	CV NUE_{GRAIN} [a] (%)	CV Grain Yield (%)	CV Total N (%)
1	AX820	9	0	13.7	29.9	32.1
			200	7.7	10.9	13.9
		12	0	10.3	32.4	33.1
			200	15.5	21.1	15.7
	AX877	9	0	15.4	30.2	30.9
			200	15.2	36.0	29.6
		12	0	26.3	44.3	38.0
			200	12.3	15.3	14.4
2	AX820	9	0	13.6	30.6	28.3
			200	20.4	16.9	23.8
		12	0	17.4	34.3	30.4
			200	17.3	34.3	26.9
	AX877	9	0	33.8	49.0	41.2
			200	22.2	26.6	25.4
		12	0	72.3	84.1	47.2
			200	28.9	38.4	31.2
Exp.				*[b](10)	†(10)	†(5.5)
Exp. × N						
Exp. × HD						
Exp. × N × HD						
N				†(7.6)	**(5.8)	*(10)
HD				**(9.4)	**(9.7)	*(5.3)
N × HD				*(13.3)	*(13.7)	
	MSE	**DF**				
	(a)	2		0.01	2.2 e^{-3}	0.01
	(b)	12		0.01	0.01	5.3 e^{-3}
	(c)	16		0.03	0.04	0.01

[a] CV: coefficient of variation; DF: Degrees of freedom; Exp.: Experiment; HD: combination of hybrid and stand density; MSE: Mean square error; N: Nitrogen; NUE_{GRAIN}: Nitrogen use efficiency for grain yield production.

[b] †, *, **, ***: significant at 0.10, 0.05, 0.01 and 0.001, respectively. For each factor, the critical values for the comparison of means are detailed in parenthesis.

for AX877, independently of stand density or N availability. This interesting and novel results deserves future studies focused on (i) elucidating the underlying mechanisms involved in mentioned inter-plant variability of N uptake, and (ii) the possible impact of early localized application of N fertilizer based on plant size and plant nutritional status to attenuate variations in N capture among plants of a canopy.

5. Conclusions

Intra-specific competition for resources takes place within a stand, which entails a reduction in individual performance but an improvement at the crop level. In this context, the rule for stand density management indicates to increase it until each new added individual does not represent an improved grain yield per unit land. In practice, for maize crops it means ‘as far as no plant barrenness is detected’. Maize breeding resulted in an improved tolerance to increased stand density; i.e., a less pronounced decline in individual performance in response to crowding or a more ‘communal plant'. Contrary to the idea of “communal” as something uniform, the described responses to crowding presented in this work imply non-uniform changes among plants (i.e., *hierarchization*) despite their almost identical genetic constitution (F1 hybrid). This *hierarchization* increases with increased stand density. We computed the contribution of groups of plants of different hierarchies to the general crop response to N fertilization in terms of biomass, grain yield, N uptake, and N content in grains. The reduced contribution of the upper T to crop biomass and grain yield in relation to that for N uptake denotes an increase in *luxury* N consumption, accompanied by a sharp decline in NUE_{BIOM} and NUE_{GRAIN} when fertilizer was applied. This result was similar for both hybrids. Crop NUE_{GRAIN} was negatively related to inter-plant variability in this trait promoted by (i) the N × H × D effect on plant-to-plant variability in grain yield, and (ii) the N and H effect on plant-to-plant variability in plant N uptake. These

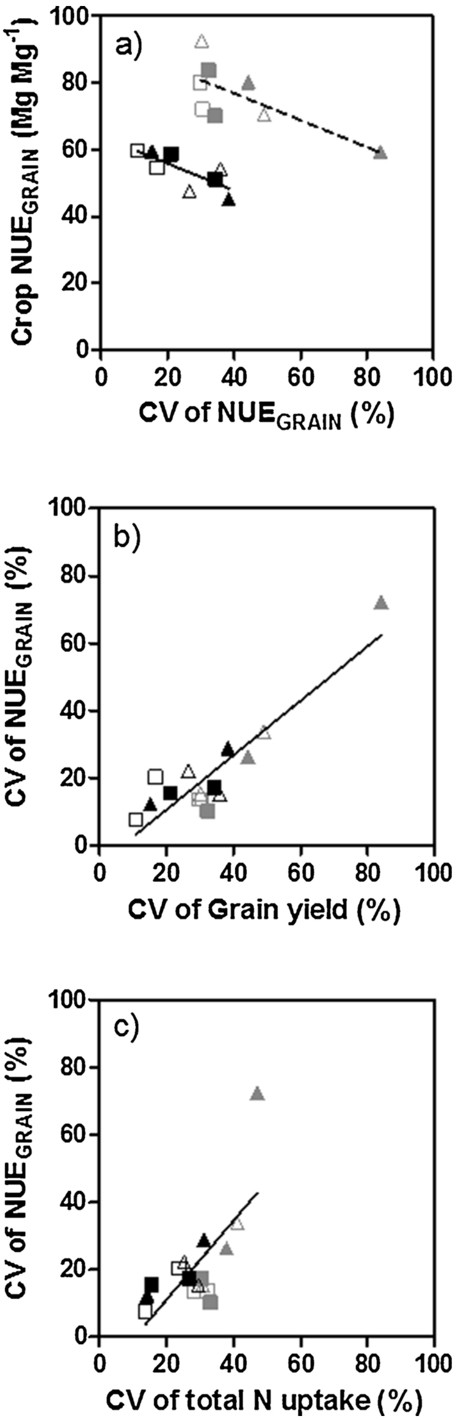

Fig. 1. Crop N use efficiency to produce grain yield (NUE_{GRAIN}) as a function of plant-to-plant variability expressed as coefficient of variation (CV) of NUE_{GRAIN} within the stand (a); CV of NUE_{GRAIN} as a function of CV of grain yield (b) and CV of plant N uptake (c). Squares (AX820) and triangles (AX877) identify hybrids; empty (9 pl m^{-2}) and full (12 pl m^{-2}) symbols correspond to stand densities, grey (N0) and black (N200) symbols represent N levels. Lines indicate linear models fitted to the data set. In (a), the solid line corresponds to N200 and the dashed one to N0. Fitted models are (a) $y = 93 - 0.4x$ ($r^2 = 0.53$, $P < 0.05$) for N0, and $y = 64 - 0.4x$ ($r^2 = 0.59$, $P < 0.05$) for N200; (b) $y = -5.6 + 0.81x$ ($r^2 = 0.80$, $P < 0.001$); (c) $y = -12.6 + 1.18x$ ($r^2 = 0.50$, $P < 0.01$).

results highlight the importance of early-established plant-to-plant variability on N economy at the crop level, and the attenuation capacity of different groups of plants as a trait to be considered both in breeding programs and crop management (e.g., site-specific fertilization) aimed to improve crop NUE_{GRAIN}.

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