

# PLANT DENSITY CONSEQUENCES ON NITROGEN UPTAKE DYNAMICS OF MAIZE PLANTS FROM VEGETATIVE TO REPRODUCTIVE STAGES

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## Abstract

There are few studies to date that have intensively examined the simultaneous effects of differences in plant crowding and fertilizer N availability for different hybrids and locations on N uptake and maize (*Zea mays* L.) grain yield. The main research objective was to utilize a quantitative framework to better understand the physiological mechanisms that govern N dynamics in maize plants at varying plant densities and N rates. Two field experiments involving paired near-isogenic hybrids (i.e. with/without transgenic rootworm resistance) investigated possible individual and interacting consequences of plant density (low- 54,000, medium- 79,000 and high- 104,000 pl ha<sup>-1</sup>) and fertilizer N rate (low- 0, medium- 165 and high- 330 kg of N ha<sup>-1</sup>) on maize N uptake and grain yield. Total above-ground biomass was fractionated and both dry matter and N uptake were measured at four developmental stages (V14, R1, R3 and R6). Both N availability and plant density affected growth and grain yield in maize, but hybrid effects were negligible. As expected, in both locations, total aboveground biomass and N content were highly correlated at V14 stage. However, biomass gain was not the only factor driving vegetative N uptake since treatments receiving N fertilizer achieved higher shoot N concentrations even when maize in the zero N treatment accumulated as much aboveground biomass as those treatments with N. Higher plant densities increased pre-silking N uptake, but had no influence on cumulative post-silking N uptake quantities in both hybrids at either location. Treatment differences in grain yield were more associated with variation in total biomass at R6 than with harvest index (HI), which never exceeded 0.54. Total biomass production per unit area from R1 till R6 stages increased with both increasing N supply and plant population; total biomass itself was positively associated with increases in the crop growth rate (CGR) and N uptake rate (NUR) during the critical period bracketing silking. Plant density and N rate interaction effects on the uptake timing, amounts and partitioning of N in high yield corn systems are complex, but hybrid response differences to N and plant density within a genetic family due to trait incorporation were minimal when insect pressure was low.

**Keywords:** Nitrogen fertilization rates; Plant density; Crop growth rate; Nitrogen uptake rate.

## Introduction

Nitrogen uptake is related to the demand for N within the plant, and is also dependent upon the availability of soluble carbohydrates in the roots (Tolley-Henry et al., 1988; Tolley-Henry and Raper, 1991). If plant N uptake continues during grain filling, less N will be mobilized from vegetative organs, which may result in increased leaf area duration (i.e., delayed leaf senescence) and an extended period of dry matter accumulation.

With adequate N supply, prolonged accumulation of dry matter and N by above-ground plant parts of maize during grain filling has been associated with high yields (Swank et al., 1982; Moll et al., 1994). Large yield responses to increased N supply have been found with high-yielding maize hybrids (Moll et al., 1987; Osaki, 1995), whereas low-yielding hybrids generally respond poorly to added N (Anderson et al., 1985; Osaki, 1995).

A roughly parabolic relationship exists between plant density and per-unit-area grain yield (Tokatlidis and Koutroubas, 2004). At suboptimal plant densities, the addition of more plants compensates for an accompanying decline in grain yield per plant due to intraspecific competition, leading to an overall increase in grain yield per area. Overall reductions in grain yield at supraoptimal plant densities are often associated with (i) reductions in per-plant photosynthesis and shoot and (more pronouncedly) ear growth during the critical period bracketing silking (Andrade et al., 1999; Echarte et al., 2008), (ii) minor and major delays in the time to anthesis and silking, respectively, (iii) declines in biomass partitioning to the ear (i.e., HI), (iv) an increase in barrenness, and (v) a rise in plant-to-plant variability (Tokatlidis and Koutroubas, 2004; Borrás et al., 2007).

There are few studies to date that have intensively examined the simultaneous effects of differences in plant crowding and fertilizer N availability for different hybrids and locations. The main objective of this study was to evaluate the grain yield and N uptake of modern maize genotypes using agronomic suboptimal, optimal, and supraoptimal plant densities in conjunction with three levels of side-dress N fertilization.

## Materials and Methods

Research during the 2009 growing season was conducted in two locations, at ACRE (West Lafayette, IN) and at PPAC (Wanatah, IN), Purdue University's experimental farms. At both locations, the study was arranged as a split-split plot design with six blocks. Hybrid (main plot), plant density (subplot), and N application rate (sub-subplot) served as the three treatment factors. Each sub-subplot consisted of six rows (76-cm interrow spacing) and was approximately 4.5 m in width and 27.5 m in length. Two near-isogenic hybrids with and without transgenic corn root worm (CRW) insect resistance were planted in each location: at ACRE Mycogen 2T780 (without CRW resistance) and its near-isoline Mycogen 2T787 (with CRW resistance) were compared, while at the PPAC location the hybrid treatment comparison involved Mycogen 2M749 (non-CRW resistant) versus 2M750 (with CRW resistance). Feeding from the nodal root was minimal, if any root injury was observed, and no significant differences in root worm injury due to hybrids, plant densities or N rates were detected at either location (data not presented). The planting rates were 58,000, 83,000, and 108,000 seeds  $\text{ha}^{-1}$  to achieve the desired final plant densities of 54,000, 79,000, and 104,000 plants  $\text{ha}^{-1}$ . For all sub-subplots in each year, starter fertilizer (10-34-0) was applied at planting 5 cm to the side and 5 cm below the seed at a rate equivalent to 25 kg N  $\text{ha}^{-1}$ . Urea Ammonium Nitrate (UAN) (28-0-0) was applied via side-dressing at a rate equivalent to 165 kg N  $\text{ha}^{-1}$  once (V3), twice (V3, V5), or not at all, depending on each sub-subplots' prescribed N application rate. In general, the 0, 165, and 330 kg  $\text{ha}^{-1}$  side-dress N rates were chosen for investigating (i) an environment with markedly high N stress, (ii) an N application rate similar to that recommended and used by local commercial maize growers, and (iii) an environment with no N limitations, respectively.

Total aboveground biomass (BM) was fractionated and dry matter (DM) and N uptake were measured at four developmental stages from three of the six replications at each location. Aboveground BM was determined from six consecutive plants with a treatment-representative plant density from either row three or four of each sub-subplot's per-plant sampling area. These six plants were cut at the stem base, individually chopped to a fine consistency, and dried to a constant weight at 60°C to determine aboveground per-plant vegetative BM at the stages of fourteen leaves (V14), silking (R1), milk stage (R3) and physiological maturity (PM) (Ritchie et al., 1996). Nitrogen content in each stage was analyzed based on combustion method (AOAC International, 2000).

## **Results and Discussion**

Grain yield per unit area, presented as an average over hybrids (two at each location in 2009) responded positively to increasing plant density and total plant N uptake (Table 1; Fig. 1). The difference in grain yields among N rate and plant population treatments were more associated with total plant BM rather than HI (data not shown). Similar effects were found by Muchow (1994) and Massignam et al. (2009). Differences in N grain concentration (%) were higher at PPAC (~1.4%) compared to ACRE site (~1.1%), across hybrids, densities and N rates (Table 1).

The N stress effect on BM accumulation differed among plant population treatments and locations. At ACRE, the greatest differences between N rates were observed at the highest plant density treatment (one experimental site is presented; Fig. 2). Furthermore, strong N stress (0N) was observed to reduce BM accumulation in all treatments starting well before anthesis.

Table 1. Maize yields at 15.5% of moisture, expressed in g m<sup>-2</sup> (bushel/acre within parenthesis), whole plant biomass (0% moisture) at physiological maturity (g m<sup>-2</sup>), and N% in the grain (%) across two hybrids within a location at three different populations (54,000; 79,000 and 104,000 pl ha<sup>-1</sup>) and three N rates (0, 165 and 330 kg ha<sup>-1</sup>) at PPAC and ACRE locations in 2009 growing season. GY= Grain yield, BM= Biomass, NG%=N Grain concentration.

	ACRE experimental site								
	Low Density			Medium Density			High Density		
Traits	0N	165N	330N	0N	165N	330N	0N	165N	330N
GY @ 15.5%	720 (115)	1037 (165)	1014 (162)	814 (130)	1250 (199)	1203 (192)	956 (152)	1325 (211)	1353 (216)
BM-R6 @ 0%	1385	1650	1649	1516	1955	1938	1677	2132	2206
NG% @ 0%	1.1	1.2	1.3	1.1	1.1	1.2	1.0	1.1	1.1
	PPAC experimental site								
	Low Density			Medium Density			High Density		
Traits	0N	165N	330N	0N	165N	330N	0N	165N	330N
GY @ 15.5%	718 (114)	894 (143)	866 (138)	989 (158)	1114 (178)	1356 (216)	979 (156)	1275 (203)	1323 (211)
BM-R6 @0%	1385	1576	1624	1807	1887	2196	1836	2257	2225
NG @ 0%	1.3	1.6	1.6	1.0	1.5	1.3	1.1	1.5	1.5

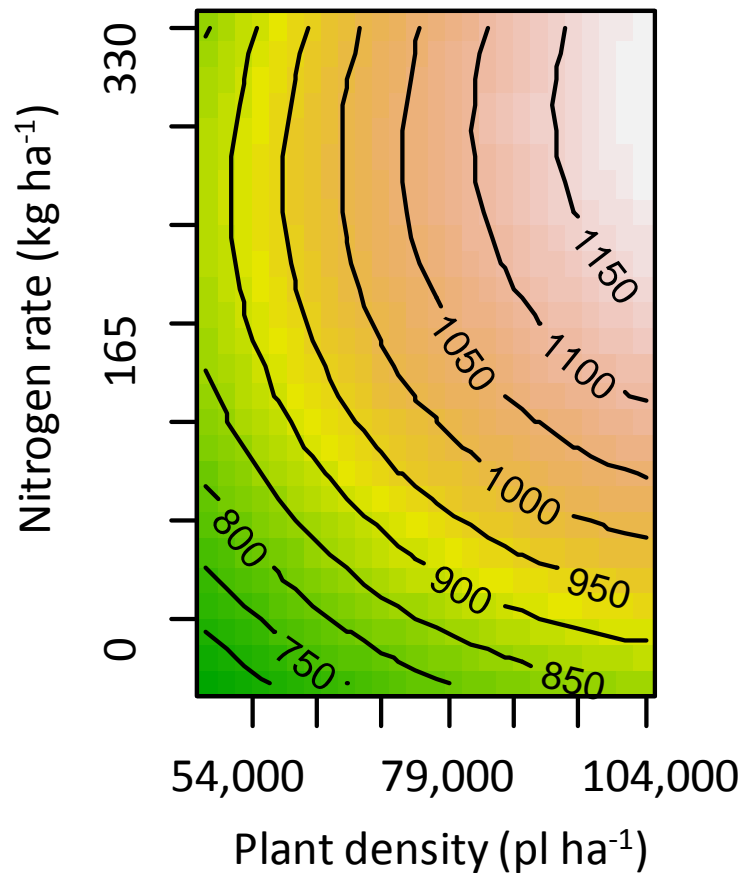


Figure 1. Grain yield (0% moisture), expressed in  $\text{g m}^{-2}$ , at physiological maturity (R6) for maize experiencing different N supply (zero N sidedress treatment,  $0 \text{ kg ha}^{-1}$ ; medium N,  $165 \text{ kg ha}^{-1}$ ; and high N treatment;  $330 \text{ kg ha}^{-1}$ ) and plant density levels (low plant density,  $54,000 \text{ pl ha}^{-1}$ ; medium plant population,  $79,000 \text{ pl ha}^{-1}$ ; and high plant density,  $104,000 \text{ pl ha}^{-1}$ ).

As expected, higher N uptake was observed when N fertilizer rate increased. The total N content was similar between hybrids (non-RW vs. RW) compared at the same plant density and N rate (one location is presented; Fig. 3). Similar results were presented by Subedi and Ma (2005). The 0N treatment resulted in the lowest productivity and N uptake under the high-density stress conditions (Fig. 1). At the medium N rate (165N), maize plants did not demonstrate apparent N deficiency symptoms across all different plant density treatments, suggesting that soil plus fertilizer N may have been enough to meet the crop N demand at this N uptake level.

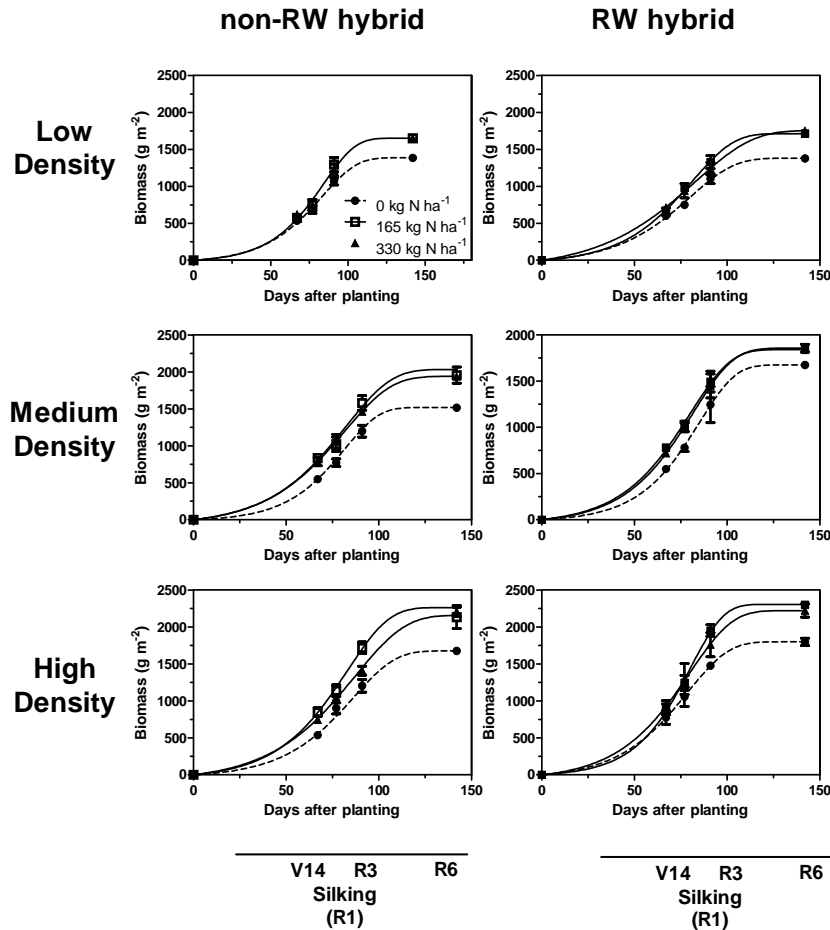


Figure 2. Biomass accumulation versus days after planting for the different hybrids, plant densities and N rates at two different locations. Each point represents the mean of 18 individual plants per treatment. Curves are fitted using Gompertz equations.

The time course of N uptake (expressed in terms of the proportion of total N uptake reached at anthesis relative to that at maturity) varied in response to the treatment combinations (and location) even when total N uptake by the crop at maturity was similar for given N treatments in both locations. On average, the post-anthesis N uptake under 0N ( $\sim 5.2 \text{ g m}^{-2}$ ) was lower than for the N fertilized treatments ( $\sim 7.4$  and  $8.3 \text{ g m}^{-2}$  for 165N and 330N, respectively). Consequently, the 0N treatment required an increase in N translocation from vegetative plant parts to meet grain N demand even though it had fewer grains and lower kernel weight (data not shown). Initial translocation of N from the stem conserves photosynthetic capacity in leaves and hence minimizes the effect of abiotic stress on resource use efficiency (Muchow and Sinclair, 1994). Differences in total post-silking N uptake among plant densities were not significant across hybrids and locations (i.e. these ranged from  $5.0$  to  $5.4 \text{ g m}^{-2}$ ); suggesting that plant population influences the pre-anthesis interval (period in which maize plants start to set the potential kernel number) more than the post-silking N uptake.

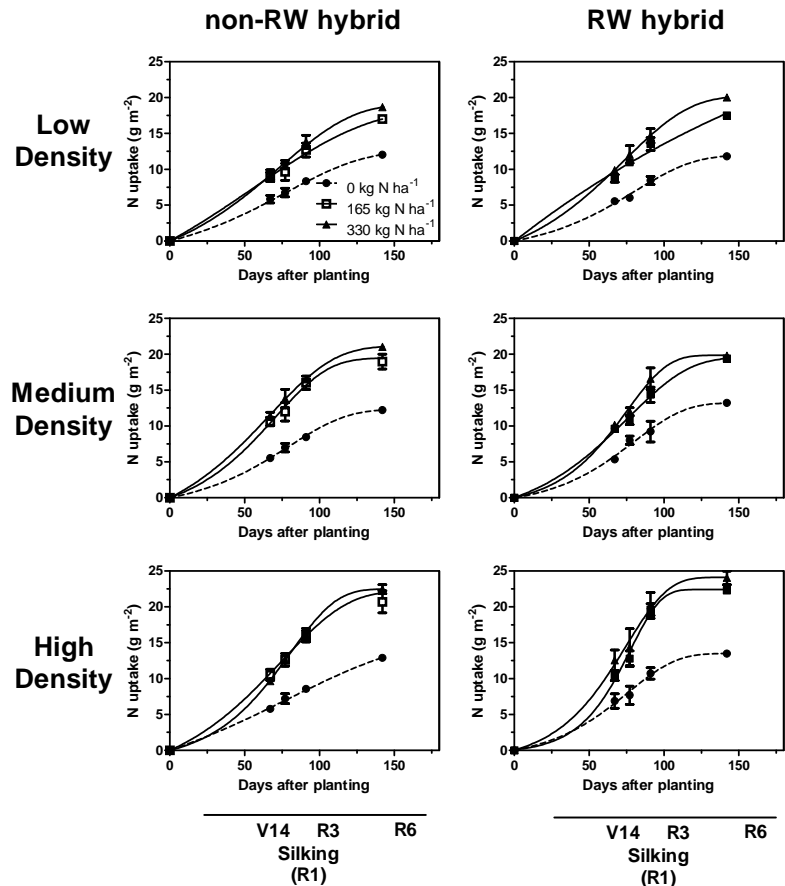


Figure 3. Nitrogen uptake versus days after planting for the different hybrids, plant densities and N rates at two different locations. Each point represents the mean of 18 individual plants per treatment. Curves are fitted using Gompertz equations.

The ratio of N uptake pre-silking versus cumulative post-silking was not clearly related with the hybrid and N rate factors; however, in general terms, the ratio was lower at the lowest plant density (data not shown). The ratio was highly variable, and ranged from 0.51 to 0.71. The total aboveground BM resulted in similar ratio values for the pre- silking period relative to BM at maturity, ranging from 0.45 to 0.65 (data not shown). There is, therefore, tight linkage between the total above ground BM accumulation and N taken up by maize plants.

Environmental conditions during the period bracketing silking (i.e. from V14 to R3 stage) affected CGR and, consequentially, the pattern of N uptake (Fig. 4). Likewise, NUR per plant was dependent on N supply and plant density at both locations (Fig. 4) presumably via the effects of these treatments on CGR; because NUR increased as CGR increased there appeared to be little sink limitation to NUR during this period. Slopes between NUR and CGR ranged from 0.009 to 0.016 for 165-330N and from 0.005-0.008 for 0N (Fig. 4); slopes changed with the N supply regardless of the hybrid and plant population factors. Therefore, it is the CGR during the period bracketing silking that determines the NUR and, as a consequence, the amount of N that needs to be remobilized to meet the N requirement in the ears whenever post-silking N uptake is not sufficient, by itself, to meet N demand in ears.

In the 0N treatment, NUR was about 50% lower than in other N treatments from the R1 to the R3 stage (at both locations), and about 50% lower between R3 and R6 stages. Moreover, it is of interest that substantial differences in NUR were already observed from seedling emergence to V14 stage ; mean NUR in the non-fertilized plants averaged  $0.09 \text{ g m}^{-2} \text{ day}^{-1}$ , while mean NUR in fertilized treatments (165N-330N) averaged  $0.15 \text{ g m}^{-2} \text{ day}^{-1}$  (~67% greater compared to the non-N treatment). At both sites, the highest CGRs achieved (i.e.  $40 \text{ g m}^{-2} \text{ d}^{-1}$ ) corresponded with the highest NURs (i.e.  $0.4 \text{ g m}^{-2} \text{ d}^{-1}$ ). At PPAC, the incremental slope was higher than at ACRE, mainly because the low CGRs corresponded with lower NUR (at the low stand density treatment), regardless of the N rates applied (Fig. 4). Moreover, at the low density, more than  $30 \text{ g m}^{-2} \text{ d}^{-1}$  of CGR was required to achieve a NUR above  $0.2 \text{ g m}^{-2} \text{ d}^{-1}$  at PPAC; while at the ACRE location, the same NUR was attained with a lower CGR, around  $20 \text{ g m}^{-2} \text{ d}^{-1}$  (Fig. 4).

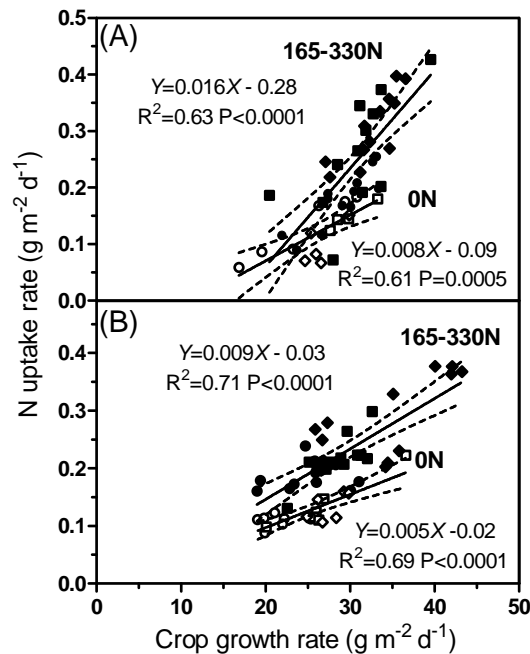


Figure 4. Total N uptake rate and crop growth rate during the bracketing period silking (+15 days from silking stage) for the different plant densities and N rates across hybrids at two different locations (A= PPAC, and B= ACRE sites). Full symbols refer to the N rates, N2 = medium N ( $165 \text{ kg ha}^{-1}$ ), and N3 = high N treatment ( $330 \text{ kg ha}^{-1}$ ): Empty symbols refer to the zero N sidedress applied, N1 = low N ( $0 \text{ kg ha}^{-1}$ ). Circles symbols correspond with low plant density ( $54,000 \text{ pl ha}^{-1}$ ), squares with medium plant population ( $79,000 \text{ pl ha}^{-1}$ ), and diamonds with high plant density ( $104,000 \text{ pl ha}^{-1}$ ).

From R1 to R6, ear growth was highly dependent on total N uptake in the aboveground canopy, and this relationship was constant over time (Fig. 5). Moreover, the ratio of the ear BM to ear N uptake was ranging from 82 to  $98 \text{ g g}^{-2}$  (Fig. 5). Stem plus leaf BM was strongly correlated with N content in these fractions from R1 to R6; however, this relationship was not as strong as observed for the ear BM fraction (Fig. 5). Moreover, the evolution of shoot (stem + leaf) BM and N content were opposite to the ear BM and N content relationship; shoot BM achieved proportionately smaller gains in response to total plant N content within this fraction from R1 to



R6 stages (Fig. 5). The high slopes resulting from this relationship confirm the high N status required for optimal grain development. Because grain N accumulation coincided so directly with ear BM increase, we support the concept of sink (as compared to source-dominant) determination of the potential N accumulated (Ciampitti and Vyn, 2010).

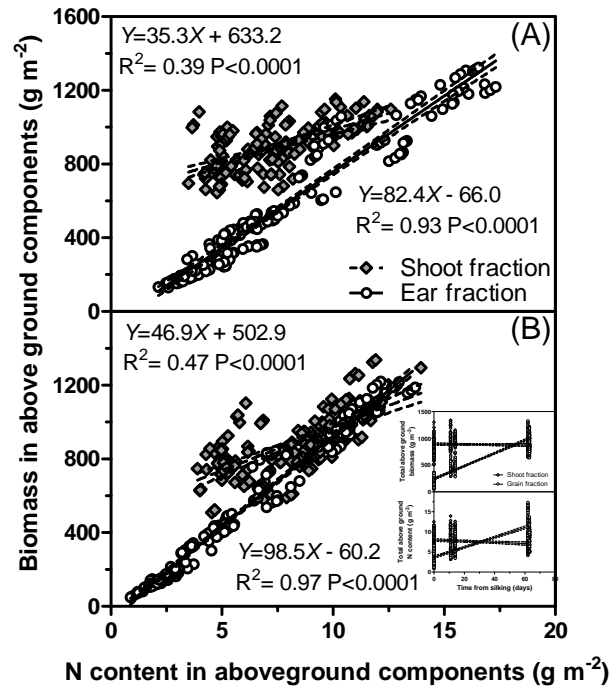


Figure 5. Biomass and N content accumulated in aboveground components for two different fractions (stem + leaves and ear BM) across hybrids, plant densities and N rates factors at two different locations (A= PPAC, and B= ACRE sites) derived from leaf plus stem versus ear shoot samples taken at R1, R3 and R6 stages. Ear BM fraction includes the cob, husk and grains until R6 (at which time the husks were excluded from this fraction). Sub-figures represent the total above ground BM and N content evolution from silking time until physiological maturity developmental stage for two different fractions shoot and ear BM, calculated as an average across hybrids, populations, and N rates.

The enhanced grain N accumulation due to N fertilizer addition was explained by both increases in grain BM (~23% to 26%), and in grain N concentration (~15% to 18%) across plant densities, hybrids and locations (Table 1). Changes in N concentration in grains were observed well beyond the N fertilization rates needed to achieve the maximum grain yield. Other studies have also shown a rise in grain N concentration when a crop is grown with non-limiting N supply (Uhart and Andrade, 1995, Gooding et al., 2007). This suggests that N remobilization is tightly linked to grain BM accumulation and the grain N concentration, which is potentially genetically determined (Uribelarrea et al., 2004). Moreover, grain N concentration was changing with different N rates, plant densities and locations, which suggests a relatively modest genetic effect, but a much larger interaction effect of genotype x environment x management (G x E x M). The size and complexity of the G x E x M interaction, with multiple combinations occurring simultaneously, are difficult to decipher and interpret (Messina et al., 2009).

## Summary

As aforementioned, both N availability and plant density affected grain yield and N uptake in maize, but hybrid effects (non-RW vs. RW) were negligible. The N side-dress applications (165-330N) were much more effective in increasing grain yield, relative to zero sidedress N, as plant density increased in all 4 hybrids evaluated. Total plant N uptake ranged widely, but responded positively to both N rate (as expected) and plant density. Clearly, the dominant influence of plant density and N rate was on total BM gain and N uptake achieved by maize plants in the time period from silking until physiological maturity, rather than in the partitioning components of grain HI and NHI.

A highly significant relationship between NUR and CGR was most dependent on the contrasting N rates (0N vs. 165-330N) regardless of the plant density, hybrid/ location factors. From this result, we suggest that NUR was driven by the potential sink capacity, which was itself modified by the CGR during the critical period bracketing silking (from V14 until R3 phenological stages).

Higher plant densities required more N to be taken by maize plants at physiological maturity, and this can be overemphasized when N is applied (higher yield potential). This requirement is supplied from an increase in the CGR per unit area, which drives an increase in the NUR during the critical period bracketing silking. The ratio of N uptake pre-silking versus cumulative post-silking was lower at the lowest plant density, which gives some clue about the greater N requirement needed by the medium and high plant densities treatments (data not shown).

From the high slope of the grain N and ear BM relationship, we confirm the high N status required for optimal grain development. Furthermore, we support the concept of sink (as compared to source-dominant) determination of the potential N accumulated. Comparing our results with several reports, all suggested that this relationship might not change with maize hybrid era, being independent of hybrids, plant populations and N rates. However, the genotype also plays a large role in determining the kernel's final N concentration.

More research is therefore needed to better understand the relationship between N balance (supply and demand), and N dynamics, specifically during the critical period bracketing silking under the framework of the G x E x M interaction.

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