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Potential physiological frameworks for mid-season field phenotyping of final plant N uptake, N use efficiency and grain yield in maize

Ignacio A. Ciampitti*, Hao Zhang, Pete Friedemann and Tony J. Vyn*

I.A. Ciampitti and T.J. Vyn, Agronomy Department, Purdue University, 915 W State Street, West Lafayette, Indiana 47907-2054, United States; H. Zhang, Department of Statistics, Purdue University, 536 Mathematical Science Building, West Lafayette, Indiana 47907-2054, United States; P. Friedemann, Dow Agrosciences, Mycogen Seeds, 2310 County Road 1050N, Homer, Illinois 61849, United States. *Corresponding authors (iciampit@purdue.edu; tvyn@purdue.edu).

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ABSTRACT

Improved phenotyping tools for simultaneously characterizing maize (*Zea mays* L.) genotypes with superior grain yield (GY) and N use efficiency (NUE) would be beneficial for breeding progress. Possible phenotypic predictors of the crowding intensity and N availability effects on maize plant N uptake, GY and NUE were evaluated for different genotypes in two environments. Our objectives were to develop phenotyping framework tools to predict plant N uptake, GY and NUE via: (i) identification of important mid-season morpho-physiological traits (from a total of 80 parameters), (ii) assessment of correlations between predictive traits (Principal Component Analyses identified 21 traits), and (iii) arrangement of key traits into sequential pathways of mechanistic functions (3 traits). Plant phenotyping measurements taken during vegetative stages were poor predictors of GY and NUE. Plant N status at silk emergence was strongly associated with grain components. At silking, SPAD readings were highly correlated to leaf N concentration, and the latter with the N nutrition index (NNI). As expected, NNI fairly reflected plant N uptake at silking, and correlated well to relative GY. Maize plant biomass and N uptake at maturity were predicted via stem volume estimation at silking. The latter predictive model accurately simulated both GY and NUE in other field experiments. Physiologically-based frameworks for mid-season prediction of maize GY and NUE require further testing but hold promise.

Abbreviations: GY, grain yield; NUE, Nitrogen use efficiency; NNI, Nitrogen Nutrition Index; G, genotypes; E, environments; M, management practices; N_E, ear N uptake; Gl, number of green leaves; SD, stalk diameter; PH, plant height; plant BM, plant biomass; Sink/Source; ratio of Kn/LAI; LAId, LAI% reduction; HI, grain harvest index; NHI, Nitrogen harvest index; Cw, cob weight; Kn, kernel number; Kw, kernel weight; $B_{G,}$ grain biomass; N_E(%), ear N increment; LAI, green leaf area index; NLAI, plant N uptake over green LAI; NUE, nitrogen use efficiency; NIE, nitrogen internal efficiency; NRE, nitrogen recovery efficiency; PGR, plant growth rate; NUR, nitrogen uptake rate; SLN, specific leaf nitrogen; PCA, principal component analysis.

INTRODUCTION

The three-way interaction of genotypes, environment and management practices (G x E x M; Messina et al., 2009) is highlighted in the diverse tolerances and yield responses of modern maize (*Zea mays* L.) genotypes to specific abiotic and biotic stresses under varying management practices and environments. During the last century, productivity improvements in maize have been attributed to farmer adoption of changes in genetics (from double- to single-cross hybrids), overall management practices (irrigation, nutrient management, conservation tillage, planting dates, soil testing, integrated pest control), and transgenic pest resistance and herbicide tolerance technologies (CAST, 2006). The conjunction of all these changes allowed maize productivity in the United States to rise from ~4 Mg ha^{-1} in the 1960s to ~9 Mg ha^{-1} in 2011 (USDA, 2012).

Breeding progress for maize grain yield (GY) was achieved, in part, through an increase in the number of kernels (Kn) per unit area (Tollenaar et al., 1992; Edmeades et al., 2000; Duvick et al., 2004; Duvick, 2005), but the kernel weight (Kw) influence cannot be overlooked (Borrás and Gambín, 2010). In addition, direct breeding progress in GY has also indirectly impacted the N use efficiency (NUE) (Moose and Below, 2008; Ciampitti and Vyn, 2012). Future improvement in maize GY and NUE might also benefit from direct selection for “phenotypic traits” that govern physiological processes (Donald, 1968), but only if sufficiently predictive physiological assessment tools are made available. In that regard, combined source-sink evaluations are important for future maize progress, and incremental gains in resource capture and efficiency (“source”) should be coupled with a larger sink capacity to allocate additional plant-acquired resources (Tollenaar and Lee, 2011).

Total maize N uptake over the entire growing season is dependent on management practices such as plant density and N fertilization rate (Lemcoff and Loomis, 1994; Ciampitti and

Vyn, 2011). At low N supply, reduced plant growth rate (PGR) and partitioning to reproductive structures during the period bracketing silking were accompanied by a low N uptake rate (NUR) (Ciampitti and Vyn, 2011). Low Kn was observed under the combined stresses of crowding intensity and N deficiency (Lemcoff and Loomis, 1994), but for apparently dissimilar reasons. When the crowding intensity was intensified, yields were limited more by the failure of grains to establish; yet when the primary stress was low N supply, yields were limited by delays or failure of silk emergence (Lemcoff and Loomis, 1994). Low N and C levels around the period bracketing silking (e.g. due to abiotic stresses), can exert a substantial impact on GY and its components (Jacobs and Pearson, 1991; Lemcoff and Loomis, 1994; Ciampitti and Vyn, 2011).

Advances in the physiological understanding of the interacting effects of hybrids, plant densities and N rates are possible through improved awareness of the morpho-physiological parameters most relevant to achieving gains in maize GY and NUE. In this context, a morpho-physiological trait selected to construct a physiological framework should be sufficiently predictive as to be used as a “physiological marker” for the selection process in maize breeding programs. Some common morpho-physiological traits used previously when phenotyping for hybrids and inbreds were stem diameter (SD), chlorophyll content (SPAD measurements), plant height (PH), biomass (BM) and N accumulation, partitioning indices, leaf area index (LAI), number of green leaves (Gl), and GY and its components among others (Greef, 1994; Bänzinger and Lafitte, 1997; D’ Andrea et al., 2006; Cirilo et al., 2009). The incorporation of a measurement for the efficient use of N, such as that based on GY per unit of N fertilizer, is needed (Moll et al., 1982). Despite numerous reports of individual or multiple plant phenotype relationships to final maize GY or NUE outcomes, few studies are published showing a functional and comprehensive approach in using those tools at mid-season (before or at silk emergence) for predicting future plant behavior related to GY, plant N uptake and NUE. Earlier and more accurate predictions can speed up the phenotyping process (discarding bad phenotypes, and selecting for superior materials) for testing hybrids as well as inbreds.

The primary objective of this work was to develop conceptual frameworks for the mid-season estimation of the final plant N uptake, GY and NUE. Two different phenotyping frameworks were investigated following the determination of the associations of multiple phenotyping parameters during vegetative and reproductive stages to final GY and NUE responses to a wide range of plant density and N rate treatments in multiple hybrids. One framework related to the estimation of plant BM through the determination of the stem diameter and plant height by silk emergence, and the second one related to the estimation of the leaf N concentration (leaf %N) at canopy-level with the determination of the SPAD at leaf-level. The statistical approach proceeded logically in a step-like fashion from a more complex analysis to a final simple validation. Briefly, those steps were to (i) evaluate the morpho-physiological traits associated with maize GY and plant N uptake differing in environments, plant density and N supply (from a total of 80 parameters), (ii) assess correlations among predictive traits (identified through Principal Component Analysis, PCA) and (iii) arrange key physiological traits into a sequential pathway of mechanistic functions for the estimation of N uptake, GY, and then, by autocorrelation, to quantify the NUE parameter.

MATERIALS AND METHODS

Management Practices, Experimental Design, and Treatments

The approach is novel in that it builds a physiological framework from previously published information such as plant BM, N uptake and GY (Ciampitti and Vyn, 2011), and then validates the framework with additional datasets from studies conducted at the same locations.

A brief description of the experiment is presented in this section; further details of site characterization (soils, slope, and nutrient content), management practices (planting, harvest and phenological measurement dates, etc.), and experimental conditions at the two sites evaluated can be reviewed at Ciampitti and Vyn (2011). One site was located at the Purdue University Agronomy Center for Research and Education (ACRE) (soil type, Typic Endoaquoll) near West Lafayette, IN and, the second site at the Pinney-Purdue Agricultural Center (PPAC) (soil type, Typic Argiaquoll) near Wanatah, IN. In each site, the study was arranged as a split-split plot design with six blocks. The eighteen treatments evaluated in these experiments come from the combination of two hybrids (main plot), three plant densities (54000, 79000 and 104000 plants ha^{-1}, subplot) and three side-dress N rates (0, 165 and 330 kg N ha^{-1}, sub-subplot). All three N rates included a starter N application (25 kg N ha^{-1}) applied at planting.

Morpho-Pheno-Physiological and Agronomic Measurements

Individual plants were tagged (30 plants per plot; ~3240 plants in total for both sites) in nondestructive areas for each treatment combination. Maize phenology was tracked from V5 to R6 for tagged plants in each plot. Various morpho-physiological measurements were taken primarily at V10 and V14 stages (vegetative period), and at R1, R3 and R6 stages (reproductive period). The PH parameter was recorded at V10, V14 (measured from the stem base to the upmost developed leaf tip), R1, R3 and R6 (measured from the stem base to the collar of the upmost leaf) stages. The SD variable was measured using a Mitutoyo ABSOLUTE Digimatic caliper (Mitutoyo America Corporation, Aurora, IL) at V14, R1, R3 and R6 stages (i.e. by recording maximum diameter at the middle of the sixth internode). The SPAD measurements were determined using the Konica Minolta SPAD-502 Chorophyll Meter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ) at V10, V14, R1, R3 and R6 phenological stages; three readings were obtained on each uppermost developed leaf (vegetative stages) or earleaf (reproductive stages). The LAI estimates at R1 stage (via leaf area meter; Model LI-3100, Li-Cor, Inc., Lincoln, NE) were derived from Ciampitti and Vyn (2011). Declination of LAI during the post-silking period was estimated from the Gl measurements (>50% of the leaf area was green) from tagged plants five times during the grain filling period (Ciampitti and Vyn, 2011). In addition, the anthesis-silking interval was measured in all plots, but due to inconsistencies in the proportion of developed tassels actually shedding pollen, this parameter is not reported.

Data for plant BM and N uptake at different phenological stages (V14, R1, R3 and R6 stages), and GY and its components at maturity, were incorporated into the statistical analysis and used to enhance the conceptual physiological framework. Details about plant BM, N uptake, GY and its components for these field studies are found in Ciampitti and Vyn (2011).

The NUE was calculated from the multiplication of its main components, the N internal efficiency (NIE) and N recovery efficiency (NRE). As a result, NUE was determined as the ratio of GY to N applied (Note: in 0N plot, starter N fertilizer equaled 25 kg N ha^{-1}). For this reason, the NIE and NRE were calculated as:

$$\mathrm{NUE}\ (\mathrm{g\ g^{-1}}) = \left[\mathrm{NIE} = \left(\frac{\mathrm{GY_A}}{\mathrm{Nupt}}\right)\right] * \left[\mathrm{NRE} = \left(\frac{\mathrm{Nupt}}{\mathrm{Nfert}}\right)\right]$$

where $\mathrm{GY_A}$ is the GY per unit-area (g m^{-2}); Nupt is the plant N uptake (g m^{-2}) at R6 stage; and the Nfert is the sum of the starter N and the N side-dress applications (g m^{-2}). As a pre-condition for derivation of biologically meaningful NUE, a treatment-specific boundary was set such that the maximum NRE never exceeded the N rate applied.

Furthermore, the reduction in green LAI from R1 to R3 (LAId %), during the post-silking period, was calculated with the following ratio:

$$\text{LAId (\%)} = \left[\frac{\text{LAI at R1-LAI at R3}}{\text{LAI at R1}}\right] * 100$$

where LAI is the leaf area index (m^{-2} m^{-2}) measured at silking (R1), and milk stage (R3).

Following a similar reasoning, the proportion of N accumulated in the ear (N_E%) from R1 to R3 with respect to the total ear N uptake achieved at (R6) was determined as:

$$N_E\ (\%) = \left[\frac{N_E \text{ at R3} - N_E \text{ at R1}}{N_E \text{ at R6}}\right] * 100$$

where N_E is the proportion of N accumulated in the ear (%) calculated at silking (R1), milk (R3) and at physiological maturity (R6) stages.

To investigate the relative proportions of the plant BM and N uptake partitioned to the ear organ, the ratio $\Delta B_E/\Delta N_E$ at R1 stage was determined as:

$$\Delta B_E/\Delta N_E \text{ (dimensionless)} = \frac{(B_E/BM)}{(N_E/N_t)}$$

where B_E is the ear biomass, BM is the plant BM (aboveground), N_E is the N accumulated in the ear and N_t is the N taken up by the plant, (g m^{-2}) at the R1 stage.

The N nutrition index (NNI) was calculated to evaluate the N status within the plant at V14, R1, R3 and R6 stages. The NNI was determined as the ratio of the actual N concentration (%Na) to the critical N concentration (%Nc), and the latter calculated as:

$$\%Nc\ (g\ g^{-1}) = 3.4 * BM^{-0.37}$$

where BM is the plant BM ranging from 1 to 22 Mg ha^{-1} (Plénet and Lemaire 2000). The NNI for maize crop was first proposed by Lemaire et al. (1996) and, more recently, by Ziadi et al. (2008a, b; 2009) as a reliable index of the N stress level.

Information regarding the plant N uptake to the green LAI ratio (g N m^{-2} LAI) at silk emergence, understood as the capacity of the plant to store N per unit of green LAI, was utilized from Ciampitti and Vyn (2011), and included in the statistical analyses. Lastly, an indicator of the sink strength relative to source supply during silking was quantified as the ratio of Kn at R6 to the green LAI at R1.

Physiological Frameworks

Two conceptual physiological frameworks were developed to capture the functionality of plant N uptake and GY formation processes. Both physiological frameworks estimated plant N uptake at silk emergence with the intent of correlating N status with final GY. The latter relationship was evident in a recent large-scale review by Ciampitti and Vyn (2012). For the first physiological framework, data from three growing seasons were utilized to develop and calibrate the model. The SPAD values collected at R1 stage for the 2009, 2010 and 2011 seasons (Ciampitti and Vyn, 2011; Ciampitti, 2012) were correlated with the leaf %N (at canopy-level) at R1 stage (for 2010 and 2011) or at V14 stage (in 2009, when no leaf %N at R1 stage was available). Except for 2009, all the experiments (four site-years, 2010-11) involved the same hybrids, densities and N rate levels (further details are presented in this section). The framework relied on NNI estimation from the knowledge of the leaf %N at the canopy-level. Next, plant N uptake was estimated by auto-correlation with the NNI because both parameters shared the same component (plant %N). Lastly, NNI was associated with the relative GY (RGY), calculated as the ratio of GY_A for a given treatment with the maximum GY_A.

The development of the second physiological framework was based on PH and SD measurements at silk emergence. Model development, based on the 2009 data, for predicting both GY and NUE was tested with other experimental data involving maize response to plant density and N rate interactions in the same crop sequence (maize-soybean) from the same location(s) in 2007, 2010 and 2011. Data of PH and SD at the silk emergence stage (required for the calibration of model) from the 2007 growing season was cordially provided by Dr. Boomsma (Boomsma et al., 2009). The latter 2007 data involved identical plant densities and N rate treatments [but different hybrids Pioneer 31G68 (2830 GDD to R6, CRM of 118) and Pioneer 31N28 (2910 GDD to R6, CRM of 119) (Pioneer Hi-Bred Intl., Inc., Johnston, IA)], as those in 2009. Calibration data from 2010 and 2011 experiments conducted at the same two locations by Ciampitti (2012) involved different N rates but equivalent plant densities levels as for the 2009 experiment. Data from the latter four site-years (2010 and 2011 seasons) involved N rates of 0, 112 and 224 kg ha^{-1}, and the same two hybrids at each site-year [Mycogen 2T789 and Mycogen 2M750, both with similar CRM at 114 days (Dow AgroSciences, Inc., Indianapolis, IN)]. Calibration data from a third 2010 field experiment conducted at the West Lafayette location (courtesy of Kovacs and Vyn, unpublished), involved N rates of 0, 90, 146 and 202 kg N ha^{-1} at one plant density (equivalent to the medium density) and another hybrid (Pioneer 1395XR, CRM of 113).

Except for the 0N treatment, calculations were simply based on interpolation of the slopes and intercepts for each specific N rate. For the 90, 112 and 146N rates, interpolations were calculated using the 2009 season equations for 0N and 165N as reference; whereas, for estimation of the 202 and 224N rate formulas, the reference equations were those for the 165N and 330N treatments from the same year (2009). The resulting equations were the following:

$NUE = 0.05*GY_A + 11$, for 90N; $NUE = 0.046*GY_A + 12$, for 112N; $NUE = 0.040*GY_A + 13$, for 146N;

$NUE = 0.036*GY_A + 12$, for 202N and $NUE = 0.035*GY_A + 12$, for 224N.

For the 2007 calibration data (Boomsma et al., 2009) NUE simulation was limited to the N fertilized treatments (165-330N), but the lack of plant N uptake values from physiological maturity prevented calculation of the NIE component (n=94 from 418 data calibration points). For a portion of the 2010 (Kovacs, unpublished) data, the NUE simulation was evaluated for 90-146N/medium N rates and 202/high N rate). For the 2010 and 2011 dataset (n=216; Ciampitti, 2012), the calculation of NUE at all N levels was similar to that utilized for 2009.

Statistical Analyses

A principal component analysis (PCA) was performed to identify patterns or correlations among traits and to select those having the largest impact on plant response. Variables (measured at V14 or R1 stage) with a predictive value for N uptake and grain yield were emphasized due to our goal of finding morpho-physiological traits for predicting phenotyping purposes. A biplot graph was constructed by plotting the symmetrically scaled components (Dim 1 and Dim 2) obtained via the PCA (Fig. 1) and the most responsive trait(s) for that specific combination were identified. Analyses were conducted using the R program (R Development Core Team, 2009).

Models were fitted with GraphPad Prism 4 software (Motulsky and Christopoulos, 2003) using the equation: $Y1= I1 + B1X + B2X^2$ (Fig. 2). Models were selected by comparing independent fits with a global fit. All parameters were selected to test whether one curve fitted the entire data. Similar procedures guided development of the frameworks (Figs. 4 and 5). In addition, quantile regression (R program) was utilized to estimate quantiles and interquantile ranges (Koenker, 2005) for the RGY and the NNI relationship (Fig. 3D).

For the validation procedure (Fig. 6), the observed versus simulated data points for the maize GY_A and NUE relationships were fitted to a 1:1 line and lines within +-20% of the measured values define a “boundary region”. For the outlier determination and detection, the robust standard deviation of the residuals (RSDR) was calculated (Motulsky and Brown, 2006).

RESULTS AND DISCUSSION

Treatment Factors and Morpho-Physiological Traits

The PCA analysis (dimensionality reduction) suggested that there were 21 significant variables (predictive and physiologically meaningful) from the 80 traits measured. A synthesis of the morpho-physiological traits selected (Table 1) are presented as means across hybrids and sites. The PCA method explained ~60% of the total variation using just two components (Fig. 1).

The biplot graph confirmed that GYs in 2009 were more influenced by plant density and N rate than by hybrids and environments (Fig. 1). Utilization of more contrasting hybrids (e.g. divergent grain %N, HI, GY at low N and N responsiveness) or environments (soils, climate) would naturally change the relative factor influences on GY. In our case, GY_A improved as both plant density and N rate increased (Table 1). According to the biplot, hybrids responded to treatments similarly regardless of the environments, and each environment resulted in similar GY range regardless of hybrids evaluated (no changes in ranks in the G x E plot). Thus, the emphasis in the discussion that follows is on the plant density and N rate interactions (the two important M factors).

From the PCA analysis, one of the most striking results was the strong association documented between plant N uptake at silking and the GY at maturity (Fig. 1). Additionally, the evaluation of morpho-physiological plant traits before silk emergence showed either poor GY or NUE prediction power (confirming the challenge and futility of early-stage phenotyping). Plant BM, N uptake, GY and its components (Kn, Kw, Cw) were all reduced as crowding intensity and N deficiency intensified (Table 1). Similar effects of plant density and N rate on GY were previously documented by Lemcoff and Loomis (1986; 1994), Camberato (1987), and Boomsma et al. (2009). The HI response followed a similar trend (Table 1). Nitrogen partitioning to the grain (NHI) did not present any evident trend. The ratio of N uptake pre-silking versus cumulative post-silking was lowest at low plant density and with 0N level (0.56), suggesting lower N uptake during the reproductive period (Table 1). Further details for the abovementioned traits at different stages, sites, and hybrids can be reviewed at Ciampitti and Vyn (2011).

Little association seemed apparent between the GY_A and NUE (Fig. 1), confirming that highest NUE is not automatically related to high GY_A. Additionally, the NUE showed weak correlation with the other parameters (Fig. 1). Under severe N deficiency, NUE was higher but the N content per unit of LAI (NLAI) was reduced (Table 1). The NLAI was similar within an N rate across plant density levels, but the highest N rate showed superior NLAI ratio presumably via (luxury N uptake at similar LAI levels; Table 1). Variation in the NLAI ratio at equivalent LAI was well documented by Lemaire et al. (2008a): these authors showed contrasting scenarios (two different slopes) with lower plant N content in N-limiting versus non-limiting N environments. Nonetheless, in our research the leaf area ratio (LAI to plant BM ratio, LAR) attained at silk emergence clearly changes with plant density. Due to lack of data for leaf and stem fractions during silking, it is not certain if plant LAR changes result from modifications in the specific leaf weight (leaf BM per LAI) or in the leaf:stem BM ratio.

The sink/source ratio and proportional LAI reduction (LAId) were neither associated with GY nor with treatment factors. In accordance with our results, only minor changes in the post-flowering sink/source ratio were documented by Borrás et al. (2003) for three plant density levels (3, 9 and 12 pl m^{-2}). The onset of LAId and the leaf senescence processes are programmed by the genetic component (Noodén et al., 1997; Dangl et al., 2000). Leaf senescence rate is known to be affected by both N deficiency and crowding stresses (Eik and Hanway, 1965; Pearson and Jacobs, 1987; Borrás et al., 2003). Although minor differences were evident in the

LAId, in absolute terms, greater LAId occurred at highest plant density (Table 1) presumably due to lower assimilate supply (Tollenaar and Daynard, 1982). Thus, in agreement with Borrás et al. (2003), differences in the sink/source ratio were unrelated to the absolute LAI changes observed during the early post-silking period.

Lastly, ear N uptake during early-grain filling period (mg N per grain) exhibited only small differences among plant densities, but substantial increases from ~ 0.21 to 0.31 mg N per grain in response to N rates between 0N and 330N (Table 1). Little or no variation in N uptake rate responses to varying N supply levels during early grain filling was also observed in sorghum (van Oosterom et al., 2010) and wheat (Martre et al., 2006). In our research, the ear N uptake represented the cumulative uptake by the grains, husk and cob. Thus, higher N reservoir in husk and cobs at higher N levels might have masked the uniqueness of the grain N uptake rate. Both Crawford et al. (1982) and Cliquet et al. (1990) documented that cob, husk and shank acted as a sink of N, at least until the kernel dough stage, and then became a N source.

Parameters including Gl, LAI, SPAD, PH and SD are only presented at the R1 stage for the purposes of predicting plant N uptake, GY_A, and NUE responses at maturity. However, the seasonal trend can be synthesized as a constant decrease (except for the PH) in all these morpho-physiological traits during the post-silking period. Throughout the reproductive period, the Gl, LAI, and SPAD were proportionally more affected by plant density, but still showed the N effect (data not shown). Faster decline rates in all three parameters were documented as the crowding intensity and N deficiency stresses intensified). Additionally, the peak value for all traits was mostly achieved at R1 stage (greatest treatment differences). Plant-level investigations for these parameters, and also GY, were then pursued to select the individual parameters for the physiological framework.

Pre-Silking N Status Effect on Maize Grain Yield and its Components

The PCA analysis confirmed that plant N uptake at silking stage was not only highly correlated with GY but also with NNI (at R1 stage), and grain HI (Fig. 1). In addition, an association was observed between the N status at silking time and the grain components (Kn, Kw, grain %N) and total N uptake at maturity (Fig. 2). Lower Kn and Kw were associated with N deficient environments, but Kw factor responded proportionately less than Kn factor. Additionally, for both Kn and Kw, as plant N uptake increased the relationship was curvilinear, a trend which was more prominent for the Kn parameter (plateau at 1.5 g N pl^{-1} at R1; Fig. 2A, B). Low levels of per-plant N uptake (<1 g N pl^{-1}) were correlated with low N supply environments (0N), resulting ultimately in low grain %N and final per-plant grain N uptake (Fig. 2C, D). Highest %N was reached with the 330N rate at the low plant density treatment. Such pre-silking N uptake influences on Kn and the grain N were previously reported (Lemcoff and Loomis, 1986; Plénet and Cruz, 1997; Uhart and Andrade, 1995). In addition, Ta and Weiland (1992) suggested a minimum ear N supply required for high maize GY.

In maize, the NNI utility and the consequent critical %N calculation was first proposed by Lemaire et al. (1996) and then re-confirmed by Plénet and Lemaire (2000). These studies employed different site-years, genotypes and N rates at one plant density. Other authors have also documented NNI increments as the N rates increased in maize (Ziadi et al. 2008a, b; 2009). In our research, NNI increased as N rates increased (regardless of plant density; Table 1), and it was also consistently lower under 0N for all densities during the entire season (data not shown). Overall, NNI declined with time towards silk emergence, and then tended to increase slightly towards maturity (data not shown). Similar NNI trends near silking were documented by Lemaire et al. (1996) and Plénet and Cruz (1997).

Calculation of the NNI index is dependent on the critical N dilution curve determination for the %Nc at different stages, in different crop species (Greenwood et al., 1990; Justes et al., 1994; Lemaire et al., 1996). The question of whether the equation used to calculate %Nc and, and subsequently NNI, is independent of the plant density factor must still be answered. A synthesis-analysis was therefore performed to attempt an answer to that question. Data for the plant density and N rate interactions from Ciampitti and Vyn (2011) and Ciampitti (2012) enabled investigation of the correlation between plant %N and plant BM as the crop aged. Treatment mean data were gathered from six site-years (288 data points; during the entire maize growing season). An assumption was made that the highest N rate represented the non-limiting N environments (330N for 2009, and 224N for 2010 and 2011 years). The equation proposed by Plénet and Lemaire (2000) adjusted well to all data points (R^2=0.66; n=288), but as expected (except for the points below 1 Mg ha^{-1}), a better fit was obtained with the highest N rate (R^2=0.75; n=94) regardless of the plant density evaluated (Fig. 3). As maize development progressed, the 0N and medium N treatments fell below the critical N curve, but greatest discrepancy occurred for the most limiting N treatment (0N; Fig. 3). Few treatment means (related to 224N) were above the critical N level. Discrepancies in the fitted critical N dilution curves have also been reported for wheat by Justes et al. (1994) and Greenwood et al. (1991) and to a small extent for forage maize (Herrmann and Taube,2004). Additionally, in our study, the critical N dilution curve corresponded very well to superior plant BM levels (22-27 Mg ha^{-1}) supporting the hypothesis offered by Herrmann and Taube (2004) that the curve proposed by Plénet and Lemaire (2000) can be extended beyond the 22 Mg ha^{-1}. From all these considerations, we can answer the question that the N dilution curves were modified most by the

N supply with only a minor influence of plant density. Proportionality between the plant %N and BM ratio was maintained within each N rate level evaluated.

Conceptual Framework for Estimating Plant N Uptake and Relative Grain Yield

The proposed framework construction and parameter validation were based on previously proven concepts (Chapman and Barreto, 1997; Lemaire et al., 2008b; Ziadi et al., 2008a, b; 2009; among others). Three steps were followed in the estimation of plant N uptake. The first association (Fig. 4A) between the SPAD values (determined at ear-leaf level at silk emergence) and the leaf %N (measured at the canopy-level), was based on previous research that conclusively confirmed high correlations for these parameters (Blackmer et al., 1994; Dwyer et al., 1995; Chapman and Barreto, 1997; Wang et al., 2011). Nonetheless, past associations were restricted to individual leaves since SPAD and leaf %N measurements typically involved the same leaf. A sufficiently positive correlation between SPAD (at leaf-level) and leaf %N (at canopy-level) will permit a more reliable estimation of the overall canopy-leaf N status. Utilizing six site-years (2009, 2010 and 2011 seasons; Ciampitti and Vyn, 2011; Ciampitti, 2012) a strong correlation was found between canopy-level leaf %N and ear-leaf SPAD values at silk emergence (R^2=0.67; n=108). In addition, the previous correlation was independent of the site-year, hybrid, plant density, and N rate levels. The high SPAD and leaf %N correlations were plausible due to the stoichiometry relationship (close to 1:1 ratio) between ear leaf %N and canopy leaf %N, calculated from different environments and plant densities (Sadras et al., 2000; Drouet and Bonhomme, 1999; 2004). This novel concept allowed the calculation of this step of the framework. However, we acknowledge that SPAD and leaf %N correlations at specific maize stages can also be affected by contrasting water supply environments and perhaps by a wider range of genotypes than those evaluated in our work to date (Schepers et al., 1992; 1996).

The second step in this estimation was to relate canopy-leaf %N with the plant %N (i.e. aboveground plant) at silk emergence. Strong associations have recently been reported between maize NNI and the leaf %N in the uppermost collared leaves (~V12) (Ziadi et al., 2009). Following the previous principle, a strong correlation was found between the NNI and canopy-leaf %N (R^2=0.76; n=108; Fig. 4B). A similar association has been previously reported for maize, but using the leaf %N per unit of leaf area (specific leaf N-SLN) rather than leaf %N (Lemaire et al., 1997), and for perennial grasses, but for leaf %N in the upper layer leaves (Gastal et al., 2001; Farrugia et al., 2004; Gonzalez-Dugo et al., 2005). During the vegetative period, SPAD values (for the uppermost fully developed leaf) correlated as well with the SLN as the leaf %N (Ciampitti, 2012). Nonetheless, as documented by Ziadi et al. (2009), the SLN and NNI association was weakened (data not shown) due to the small range of variation (~1.1 to 2.1 g N m^{-2}) as compared with the canopy-leaf %N (~14 to 34 mg g^{-1}). Direct correlations of SPAD with NNI were weaker (data not shown) than those between SPAD and leaf %N (as observed by Ziadi et al., 2008b) and inconsistent (as reported by Houlès et al., 2007). Following a similar rationale postulated by Lemaire et al. (2008b), a pathway was built to indirectly estimate NNI (as a plant N status indicator) after canopy- leaf %N was estimated based on ear-leaf SPAD.

The highest and strongest goodness of fit for the association between NNI and plant N uptake on a per-unit-area basis occurred at silk emergence (Fig. 4C). Utilizing all individual-plot R1-stage data for the six site-years (n=324), the correlation between the plant N uptake (per-unit-area) and the NNI was strong (R^2=0.92) as well as reliable (*$P<0.0001$*; RSDR=1.1). The relationship demonstrated independency from the site-year, hybrid, plant density and N rate factors. A key novel aspect of these investigations is that the proportionality between the NNI and plant N uptake did not change for either plant densities or N rates. However, the latter parameters are not independent because both share the plant %N (and plant BM) as a component. In our case, the NNI versus plant N uptake correlation is only needed to acquire the function required to use NNI as an input to estimate plant N uptake at silking. A similar interrelation between NNI and plant N uptake at silking was reported by Bertin and Gallais (2000).

The estimation of the final GY using the NNI presented a higher goodness of fit when GY was expressed as relative GY (RGY; Fig. 4D). Accordingly, Ziadi et al. (2008a) also reported a very strong association between RGY and NNI, but in their report the relationship represented the average over all sampling dates (5 timings across the maize growing season). In our case, the NNI levels plateaued at 0.95 (similar to Ziadi et al.; 2008a), but at a RGY close to 0.7 (50% quantile line, 50%Q). The maximum GY_A value achieved at the individual plot-level was close to 1600 g m^{-2} (on dry weight basis; medium plant density and highest N rate), and this corresponded with a NNI of ~1.3 units. When NNI dropped below 1.0 unit, RGY also declined. It was evident that NNI clearly identified 0N versus N side-dress treatments (Fig. 4D). The lower boundary (1%Q) dashed line portrays conditions in which the NNI is maximum at equivalent RGY level and stresses (e.g. heat, drought, nutrient deficiencies, pest pressure, etc.) are restricting grain productivity. In contrast, the upper boundary dashed line (99%Q) indicates an environment in which NNI is at the lowest level (restricted by N) and, thus, the conversion of N into GY is maximized. The range of NNI values reported in this paper (~0.37 to 1.51; Fig. 4D) is similar to that recorded by Lemaire et al. (1996), Plénet and Lemaire (2000) and Ziadi et al. (2008a, b). Highest NNI suggests occasional luxury N uptake at the medium to high N rates.

Conceptual Physiological Framework for Phenotyping for Actual Maize Grain Yield and NUE

This framework construction began with estimation of plant BM at silk emergence. To pursue this goal, the allometric relationship between the per-plant stem volume (estimated via the cylindrical formula based on PH and SD, both measured at silk emergence) and the plant BM was determined (R^2=0.83; Fig. 5A). The latter is in accordance with previous findings (Miles, 1993; Vega et al., 2000; Borrás and Otegui, 2001; Maddonni and Otegui, 2004; Pagano and Maddonni, 2007; D'Andrea et al., 2008). A similar relationship was observed in our research; high correlations were observed regardless of site, hybrid, plant density and N rate (Fig. 5A). Overall, the stem volume calculation can express potential plant BM at a given phenological stage; moreover, this parameter can facilitate genotypic selection for higher productivity.

Plant N uptake is a controlling factor in determining both LAI and plant BM, but the latter parameters feed-back regulate plant N uptake at different N supplies (Lemaire et al., 2008a). Per-plant N uptake for limiting and non-limiting N environments was estimated through the plant BM, at R1, resulting in two slopes, with (165/330N) and without (0N) N applied (Fig. 5B). Each slope represents a plant %N and show relative proportional consistency. For the same dataset, a strong PGR and NUR relationship occurred during the period bracketing silking (Ciampitti and Vyn, 2011). Lemaire et al. (2008a) observed a similar trend, with diverse slopes for non-limiting and limiting N environments in dissimilar maize production environments (France and Australia). Additionally, due to the well documented relationship between maize plant BM and LAI (Plénet and Lemaire, 2000), the association presented in Figure 5B is related to the plant N uptake and LAI relationship (Lemaire et al., 2008a). Therefore, the N uptake capacity per unit of plant BM was restricted in the most limiting N environment, but was only negligibly affected by plant density. Per-unit-area N uptake was calculated from the adjustment of the per-plant N uptake within each plant density level.

Our conceptual framework for simulating GY is fairly robust since its foundation is based on prior documented scientific concepts, and it follows justified steps to simulate plant N content at silk emergence. An evident connection between the present framework and the earlier one proposed for the plant N uptake estimation is that the former bases the estimation on the plant BM simulation, while the latter reaches the same goal through the quantification of the NNI.

For the next step, an association was built between the plant N uptake at silk emergence with GY from concepts previously developed (Jacobs and Pearson, 1991; Lemcoff and Loomis, 1994; D'Andrea et al., 2006; 2008; 2009; Ciampitti and Vyn, 2011), but more comprehensively outlined in a recent review paper by Ciampitti and Vyn (2012). The previous research clearly identified the effect of N deficiency around the silking time over the grain components (also documented in Fig. 2). It is noteworthy that these effects were documented for inbreds as well as hybrids under contrasting N rates (D'Andrea et al., 2006; 2008; 2009). A strong correlation was also observed between the maize GY and the plant N uptake (both at 0% moisture; Fig. 5C). From this result, the effect of N status at silking time over grain components (Fig. 2), and consequently, on final GY (Fig. 5C), was clearly demonstrated.

The auto-correlation between NUE and GY_A is acknowledged, but for practical purposes the relationship is just used to predict NUE with the GY parameter. In addition, the proposed NUE term is not a straightforward derivation from the equation GY divided by N applied due to the limitations imposed on the NRE (<1 unit). The framework approach permits estimation of N effectiveness for maize production at different N rates and plant densities from the context of a biologically meaningful efficiency term. It is well known that NUE declines with increasing N supply (Cassman et al., 2003; Ladha et al., 2005), even regardless of plant density (Fig. 5D).

Simulation and Validation

For the simulation and validation steps, all equations presented in Figure 5 were combined in a sequential fashion, based on information inputs of SD and PH parameters. A procedure similar to the latter followed using the SPAD values to estimate NNI, plant N uptake, and then, RGY. Both framework approaches estimate the same parameter. However, because leaf %N at the silk emergence stage was not quantified during the 2009 season, the correlation between leaf %N at V14 and SPAD at R1 was weaker (R^2=0.51; n=36; data not shown) as compared to that presented in Figure 4A. Another advantage of the stem volume estimation approach is that fewer steps are required for estimating plant N uptake (Fig. 5), as compared to when the SPAD is used (3 steps; Fig. 4). Although both models can be useful, greater accessibility to SD and PH data from other research efforts prompted selection of the conceptual framework in Figure 5 for validation and calibration.

All equations in Figure 5 were estimated using the 2009 dataset. The effectiveness of these equations and overall framework to estimate GY_A and NUE were tested with other datasets (2007, 2009, 2010 and 2011) from the same locations. The latter resulted in reasonable R^2 (0.41 for GY_A and 0.51 for NUE), and model simulation (62% for GY_A and 69% for NUE of the predicted data points were within ±20% boundaries) for all evaluated years, based on SD and PH inputs (measured at silk emergence; Fig. 6A, B). However, the GY_A was considerably overestimated with low SD and PH values arising from the non-N fertilized treatments for 2010 and 2011 growing seasons (green and blue colors). The greatest discrepancy in estimation occurred in both seasons at ACRE location, where low GY values for 0N treatments (~3-4 Mg ha^{-1}) were documented. At the same site, the peak value (~10-11 Mg ha^{-1}) corresponded to the medium density and highest N rate combination. Data points outside the ±20% lines resulted in greater proportion from model overestimation (58% for GY_A and 64% for NUE; Fig. 6A, B).

The physiological framework developed (simply utilizing the SD and PH inputs) was useful to accurately estimate, for different site-years, hybrids and management practices, the final GY_A and NUE. However, it should be recognized that one of the limitations of using this simulation model -deliberately circled in Figure 6A, B- corresponds to the estimation of the non-N fertilized treatments at different plant density levels. Another limitation of this simulation model is that the equations are based on maize responses from only one growing season. Nevertheless, this novel model approach was comprehensively validated with several growing seasons, hybrids, densities and N rates.

CONCLUSION

The initial PCA analyses provided convincing justification that the maize phenotyping features that mattered to GY and NUE estimation were not those measured during vegetative growth stages but at the silk emergence stage. Early-vegetative-stage phenotyping of the specific parameters reported here, therefore, were less useful as morpho-physiological traits than those determined later. The most striking results that supported the physiological frameworks for the data set of hybrids and conditions used in this study were: i) the use of chlorophyll estimator (SPAD) to predict the NNI; ii) the stoichiometry ratio encountered in the ear leaf %N versus canopy-leaf N status prior to silk emergence; iii) the indirect association (through the plant BM) between the stem volume and the plant N status prior to silk emergence; and iv) the critical and fundamental relationship found between the plant N status prior silk emergence with the GY at physiological maturity (physiological foundation based on the association of the plant N content at silk emergence with grain components-Kn and Kw- and gran N uptake-grain %N). Both conceptual models were successfully related to GY, but one with RGY and the other with GY_A (absolute values). Furthermore, models enabled NUE prediction from their respective GY estimates even when boundaries were imposed on maximum internal N efficiencies that could be reached in response to N fertilizer treatments.

A sensitivity analysis of the stem volume based physiological framework utilized other datasets from 2007, 2009, 2010 and 2011 years to simulate both GY_A and NUE. The calibration analysis showed acceptable goodness of fit, especially considering that plot-level data points (rather than treatment means) were used to validate the framework. This conceptual physiological framework appears to be a promising tool for phenotyping simultaneously for maize plant N uptake, GY and NUE through two relatively simple variables to measure (stem diameter and plant height) at the silking stage.

Future research should focus on testing and calibration of these - or other appropriately modified - pathways under different environments (e.g. soil N supply and weather) and genotypes (diverse NHI, grain %N, and total plant N uptake). More effort should be invested in determining plant N status more directly with NNI, perhaps via an indirect association with a morpho-physiological trait, even though a trade-off between phenotyping simplicity and quality of the NNI tool is acknowledged (Lemaire et al., 2008b).

The suggested physiological frameworks are perhaps more applicable as potential phenotyping tools to maize plant breeders than to advancing the maize physiological science itself. Physiology framework endeavors like these should be pursued to improve efficient phenotyping for hybrids and inbred lines at diverse testing stages of the plant breeding programs for complex stress tolerance traits.

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Figure Captions

Fig. 1. Response variable by morpho-physiological trait biplot derived from principal component analysis of grain yield per unit-area basis (GY_A), nitrogen use efficiency (NUE; GY_A/N applied), and plant N uptake [at R1 (NR1) and at R6 (NR6) stages] at two environments - Env - (ACRE and PPAC sites), four hybrids – Hybrid - (Mycogen 2M749, 2M750, 2T780 and 2T787), three densities – PD - (low, medium and high plant density – 54,000; 79,000 and 104,000 pl ha^{-1}, respectively) and three N rates – Nrate - (0, 165 and 330 kg N ha^{-1}), for maize during the 2009 season. N_ER1= ear N uptake at R1 stage; GlR1= number of green leaves per plant at R1 stage; SPADR1= SPAD measurement at R1; SDR1= stalk diameter at R1 stage; PHR3= plant height at R3 stage; BM= plant biomass (at V14, R1 and R6 stages), expressed per unit area; Sink/Source= ratio of Kn/LAI, expressed per unit area; LAId= LAI% reduction (from R1 to R3 stages); HI= grain harvest index; Cw= per unit area cob weight; Kn= per unit area kernel number; Kw= per unit area kernel weight; B_G= grain biomass, expressed per unit area; N_E(%)= ear N increment (from R1 to R3 stages), $\Delta B_E/\Delta N_E$= [(ear BM/ plant BM)/(ear N uptake/ plant N uptake)]; LAIR1= Leaf Area Index at R1 stage; NNI= Nitrogen Nutrition Index.

Fig. 2. Relationship between per-plant kernel number (Kn), 1000 kernel weight, Kw, (g) at the physiological maturity stage (A), the grain N concentration (%N), and the per-plant grain N uptake (g N pl^{-1}) at R6 stage (B), all relative to per-plant N uptake at the silking time (g N pl^{-1}). Circles refer to 54,000 pl ha^{-1} (low plant density), squares to 79,000 pl ha^{-1} (medium plant density) and diamonds to 104,000 pl ha^{-1} (high plant density).

Fig. 3. Relationship between plant N concentration (mg N g^{-1} DM) and plant biomass (Mg m^{-2}) adjusted to the critical N dilution curve %Nc= $34*BM^{-0.37}$ (by Plénet and Lemaire, 2000), across six site-years, hybrid, plant density and N rate. Dashed line represents the N dilution curve proposed by Plénet and Lemaire (2000), adjusting for all data points presented. When only the black symbols (regardless of plant density) were adjusted to the same equation, the goodness of fit improved to R^2=0.75 (n=94). Circles refer to 54,000 pl ha^{-1} (low plant density), squares to 79,000 pl ha^{-1} (medium plant density) and diamonds to 104,000 pl ha^{-1} (high plant density). Empty symbols represent the 0N rate level, light grey color symbols refer to the 112N or 165N and black to the 224N or 330N rate treatment level. Red borders correspond to the 2009 season, green for the 2010 and blue for the 2011 maize growing seasons. The information is summarized from Ciampitti and Vyn (2011) and Ciampitti et al. (unpublished).

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divided by the maximum grain yield among all treatments, and site-years) (Fig. D). Circles refer to 54,000 pl ha^{-1} (low plant density), squares to 79,000 pl ha^{-1} (medium plant density) and diamonds to 104,000 pl ha^{-1} (high plant density). For all the symbols, red borders correspond to the 2009 season, green for the 2010 and blue for the 2011 maize growing seasons.

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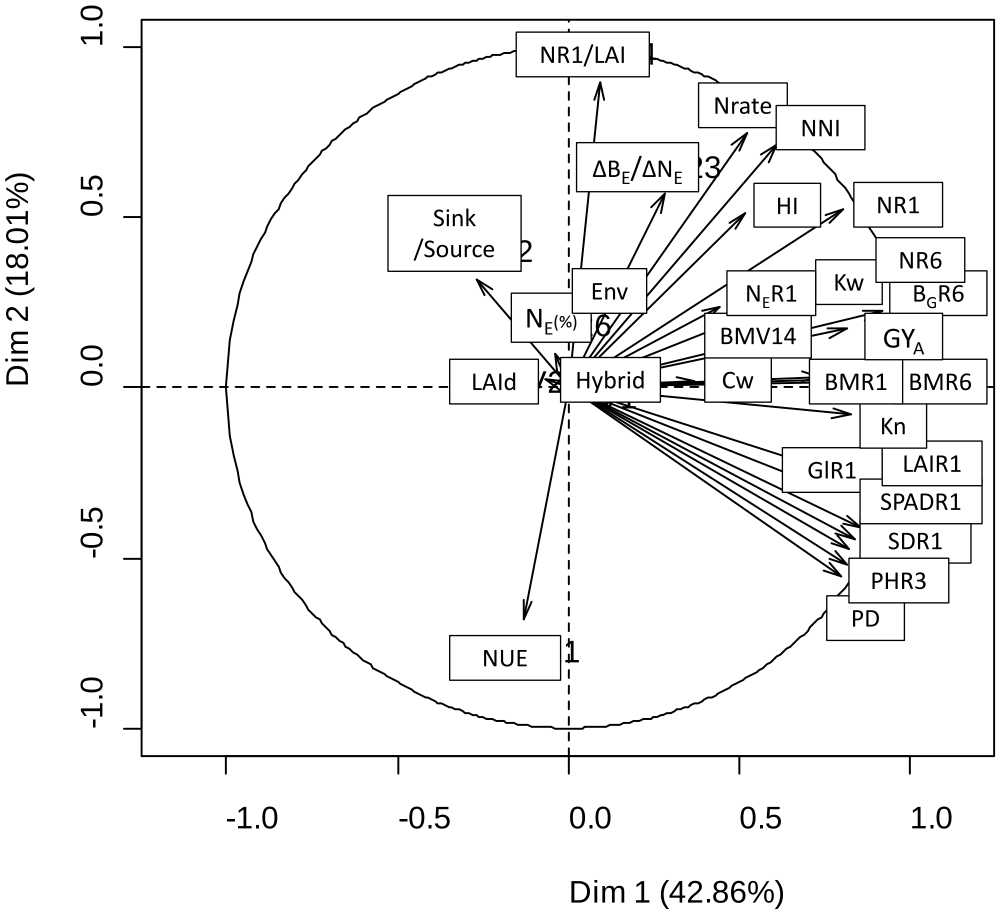
Fig. 6. Observed versus simulated maize grain yields (0% moisture, GY_A; A) and N use efficiency (NUE; B) for a test set of different hybrids, plant densities, N rates and years. Diagonal solid line: 1:1 ratio; dotted lines: ±20% deviation from 1:1 line. Separate robust standard deviation of the residuals (RSDR) for all plant densities and N rates combinations for each simulation are shown. For maize GY_A (A) and NUE (B), data points within the circle mainly correspond to non-N fertilized treatments, which were strongly affected by abiotic stresses during the post-silking period. Circles refer to 54,000 pl ha^{-1} (low plant density), squares to 79,000 pl ha^{-1} (medium plant density) and diamonds to 104,000 pl ha^{-1} (high plant density). Empty symbols represent the 0N rate level (low N), light grey color symbols refer to the 112N or 165N rates (medium N), and black to the 224N or 330N rates (high N). Yellow borders refer to 2007 season, red for the 2009 season, green for the 2010 and blue for the 2011 maize growing seasons.

Table 1. Summary of descriptive statistics -mean and standard error (within parenthesis) - for plant biomass (BM) accumulation (V14, R1, R3 and R6), expressed in g m^{-2}, harvest index, grain yield (GY$_A$; per unit area basis) and its components, expressed on a per plant basis, (kernel number –Kn, kernel weight –Kw and cob weight – Cw), total N uptake (Nupt) accumulation (V14, R1, R3 and R6), expressed in g m^{-2}, dry matter and N harvest indexes (HI and NHI), and morpho-physiological traits [number of green leaves – Gl – leaf area index –LAI (m^{-2} m^{-2})– chlorophyll content –SPAD– plant height –PH (cm) – and stalk diameter –SD (mm) – all these parameters measured at R1 stage] for the combinations of three densities (54,000; 79,000 and 104,000 pl ha^{-1}) and three N rates (0, 165 and 330 kg N ha^{-1}), calculated as an average of 4 hybrids at two different locations (2 hybrids within each site) for the 2009 growing season. LAId (%) = LAI declination from R1 to R3. N$_E$ (%) = proportional increase in ear N content from R1 to R3, expressed as a percentage of the maximum achieved at R6. $\Delta B_E/\Delta N_E$ = proportional ratio of ear biomass relative to plant biomass (ΔB_E) vs. ear N uptake relative to plant N uptake (ΔN_E) at R1. Nupt.R1/LAI = ratio of the plant N uptake to green LAI at R1 (g m^{-2}). NNI= Nitrogen Nutrition Index (%Na/%Nc) at R1 stage. Sink/Source = relationship between the final Kn (sink) achieved at R6 stage to the LAI (source) at R1 stage.

Traits	Low Density (54,000 pl ha^{-1})			Medium Density (79,000 pl ha^{-1})			High Density (104,000 pl ha^{-1})		
	0N	165N	330N	0N	165N	330N	0N	165N	330N
BM-V14	562.3 (10.3)	606.5 (12.9)	649.0 (17.6)	558.7 (12.3)	752.2 (24.3)	712.8 (25.1)	688.5 (34.7)	811.0 (24.2)	782.5 (25.6)
BM-R1	815.5 (26.5)	910.5 (34.4)	908.2 (52.4)	910.0 (44.5)	1075.0 (22.9)	1080.8 (23.5)	1040.7 (35.9)	1212.5 (21.0)	1252.5 (43.0)
BM-R3	1137.0 (32.5)	1333.3 (21.7)	1301.2 (24.3)	1313.2 (39.7)	1505.5 (27.1)	1474.0 (21.3)	1406.2 (38.5)	1707.7 (49.6)	1596.5 (45.2)
BM-R6	1417.8 (18.7)	1644.8 (15.5)	1672.7 (15.3)	1659.7 (31.4)	1920.7 (25.3)	2003.8 (38.5)	1822.8 (34.8)	2188.7 (36.9)	2226.2 (13.5)
HI	0.44 (0.01)	0.48 (0.01)	0.48 (0.01)	0.45 (0.01)	0.50 (0.01)	0.50 (0.01)	0.44 (0.01)	0.49 (0.01)	0.51 (0.01)
GY$_A$	650.5 (38.0)	866.9 (36.4)	839.3 (40.5)	797.0 (39.3)	1005.0 (38.9)	1066.4 (41.8)	851.5 (47.3)	1133.3 (36.1)	1187.3 (47.6)
Kn	605.8 (6.6)	683.0 (10.8)	694.0 (18.6)	504.8 (7.6)	557.3 (9.5)	567.0 (8.7)	402.0 (6.2)	500.8 (8.3)	485.0 (7.3)
1000 Kw	193.0 (4.3)	216.5 (4.7)	217.3 (4.3)	189.0 (3.5)	216.5 (3.7)	225.5 (4.1)	188.3 (4.9)	205.5 (4.6)	223.5 (4.1)
Cw	21.8 (0.9)	26.3 (0.9)	26.3 (0.8)	14.3 (0.7)	20.8 (0.6)	19.7 (0.8)	15.4 (0.8)	18.3 (0.7)	19.9 (0.9)
Nupt-V14	5.8 (0.2)	8.8 (0.2)	9.6 (0.3)	5.9 (0.2)	10.2 (0.4)	10.3 (0.3)	6. 3 (0.3)	10.4 (0.3)	11.1 (0.4)
Nupt-R1	6.9 (0.2)	10.4 (0.3)	11.5 (0.4)	8.2 (0.3)	12.4 (0.4)	13.1 (0.4)	8.3 (0.3)	13.7 (0.4)	14.6 (0.5)
Nupt-R3	8.7 (0.3)	13.1 (0.2)	14.4 (0.2)	10.1 (0.5)	15.2 (0.2)	17.4 (0.4)	9.9 (0.4)	17.4 (0.5)	19.1 (0.6)
Nupt-R6	12.4 (0.2)	17.5 (0.1)	19.5 (0.2)	13.1 (0.2)	19.5 (0.2)	21.1 (0.2)	13.6 (0.2)	21.7 (0.3)	23.5 (0.2)
Gl-R1	12.7 (0.3)	14.3 (0.1)	13.7 (0.4)	12.7 (0.3)	13.4 (0.4)	13.3 (0.3)	12.3 (0.3)	12.8 (0.3)	13.6 (0.3)
NHI	0.56 (0.02)	0.57 (0.03)	0.56 (0.03)	0.57 (0.03)	0.58 (0.02)	0.57 (0.02)	0.55 (0.03)	0.56 (0.02)	0.58 (0.03)
LAI-R1	3.2 (0.1)	3.4 (0.1)	3.5 (0.1)	4.1 (0.1)	4.6 (0.1)	4.7 (0.1)	4.8 (0.2)	5.5 (0.1)	5.5 (0.1)
SPAD-R1	52.3 (0.8)	56.1 (0.7)	56.0 (0.7)	49.5 (1.0)	52.4 (0.9)	53.8 (1.0)	45.6 (0.9)	50.3 (1.1)	50.4 (1.3)
PH-R1	198.9 (3.9)	209.2 (8.2)	207.7 (5.1)	202.8 (6.8)	203.7 (5.3)	205.9 (5.1)	198.5 (7.2)	203.5 (4.0)	203.3 (4.5)
SD-R1	28.1 (0.6)	29.1 (0.7)	30.1 (0.5)	24.6 (0.5)	25.8 (0.4)	26.0 (0.4)	22.3 (0.3)	23.1 (0.3)	23.3 (0.3)
LAId (%)	14.1 (0.9)	11.9 (0.8)	11.6 (0.8)	14.1 (0.7)	9.8 (0.7)	10.0 (0.7)	13.3 (0.6)	11.0 (0.7)	11.6 (0.8)
N$_E$(%)	0.42 (0.01)	0.43 (0.01)	0.40 (0.02)	0.44 (0.01)	0.39 (0.01)	0.41 (0.01)	0.52 (0.01)	0.44 (0.01)	0.39 (0.01)
$\Delta B_E/\Delta N_E$	0.57 (0.01)	0.70 (0.01)	0.73 (0.01)	0.52 (0.02)	0.69 (0.02)	0.69 (0.01)	0.49 (0.02)	0.70 (0.02)	0.68 (0.02)
Nupt.R1/LAI	2.2 (0.03)	3.1 (0.04)	3.3 (0.05)	2.0 (0.03)	2.7 (0.03)	2.8 (0.05)	1.8 (0.02)	2.5 (0.02)	2.7 (0.03)
Sink/Source	1072.2 (11.1)	1176.8 (11.3)	1104.8 (18.1)	1029.1 (12.6)	1014.6 (14.2)	1013.3 (14.4)	954.4 (12.8)	1020.2 (19.7)	967.6 (11.4)
NNI	0.55 (0.01)	0.77 (0.01)	0.85 (0.02)	0.60 (0.01)	0.81 (0.02)	0.86 (0.01)	0.56 (0.01)	0.83 (0.02)	0.87 (0.02)
NUE	52.4 (2.9)	49.5 (2.2)	25.4 (1.2)	60.6 (2.7)	51.6 (2.1)	32.3 (1.3)	62.6 (3.5)	52.5 (2.3)	36.0 (1.4)



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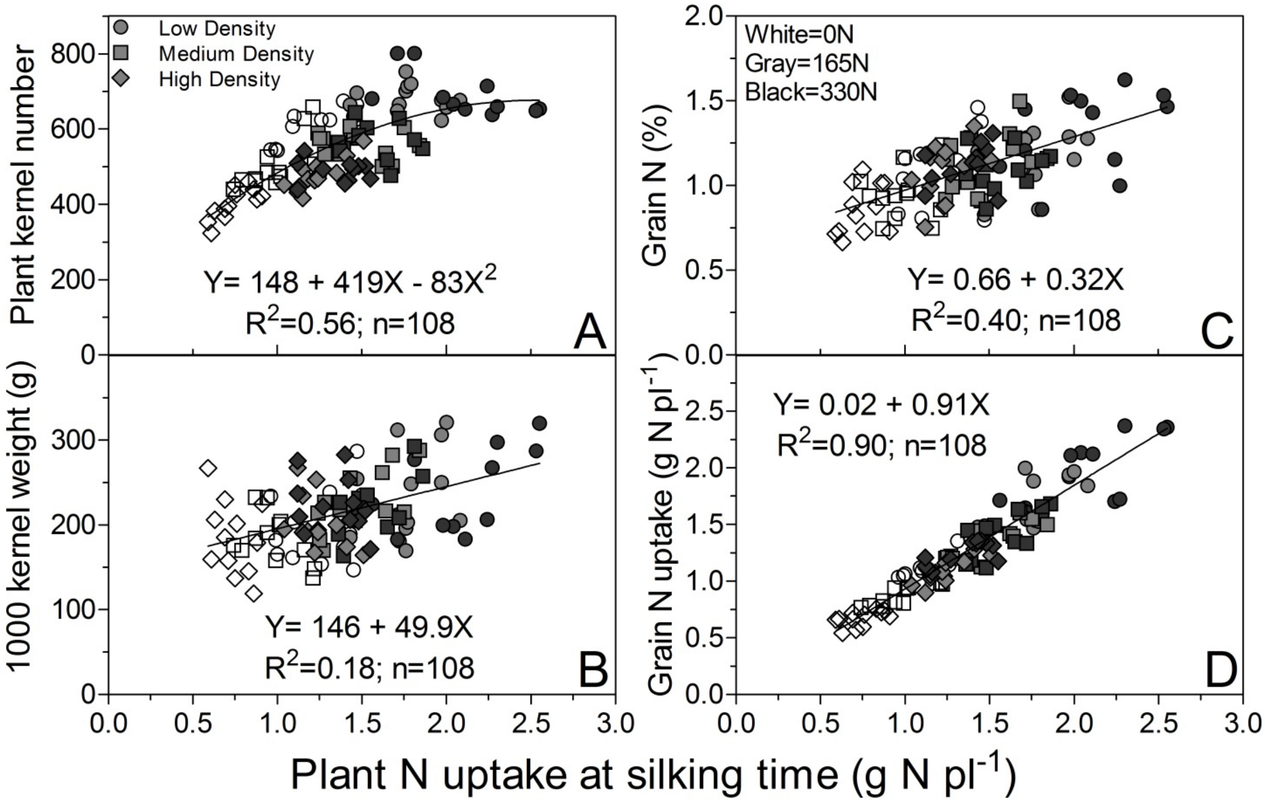

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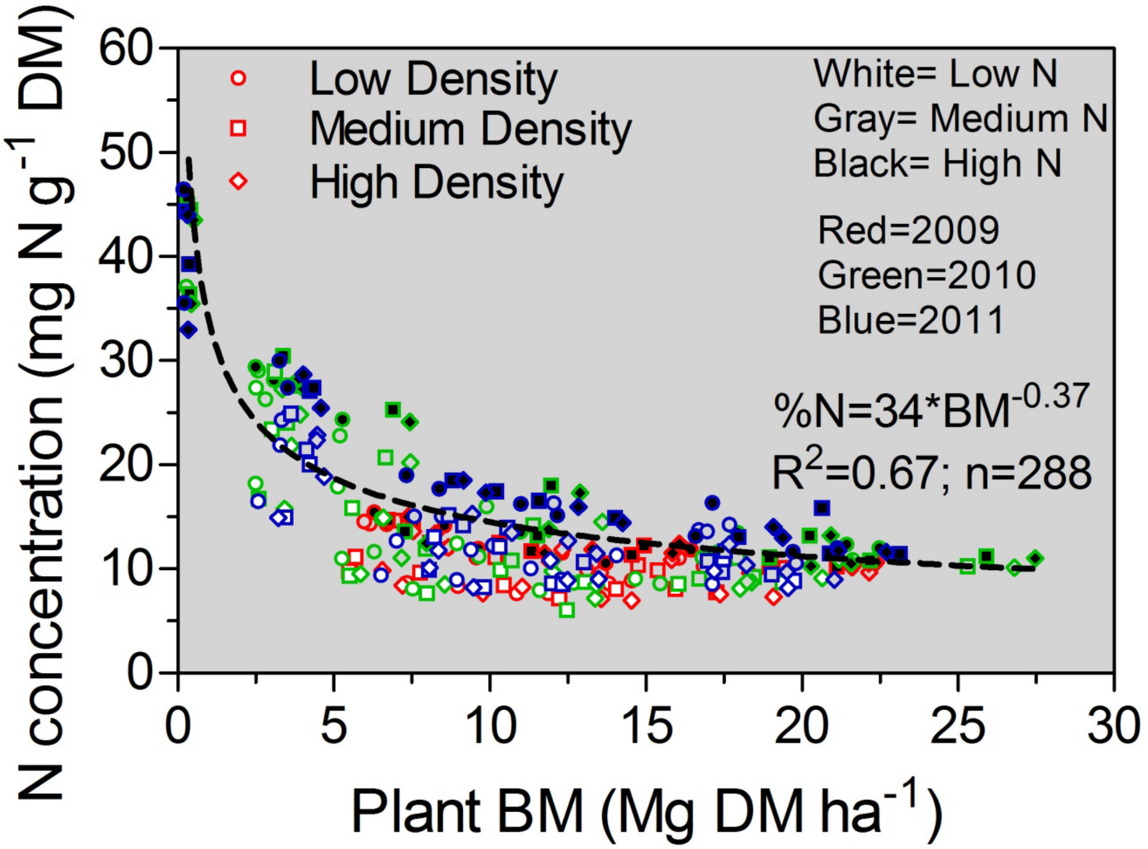

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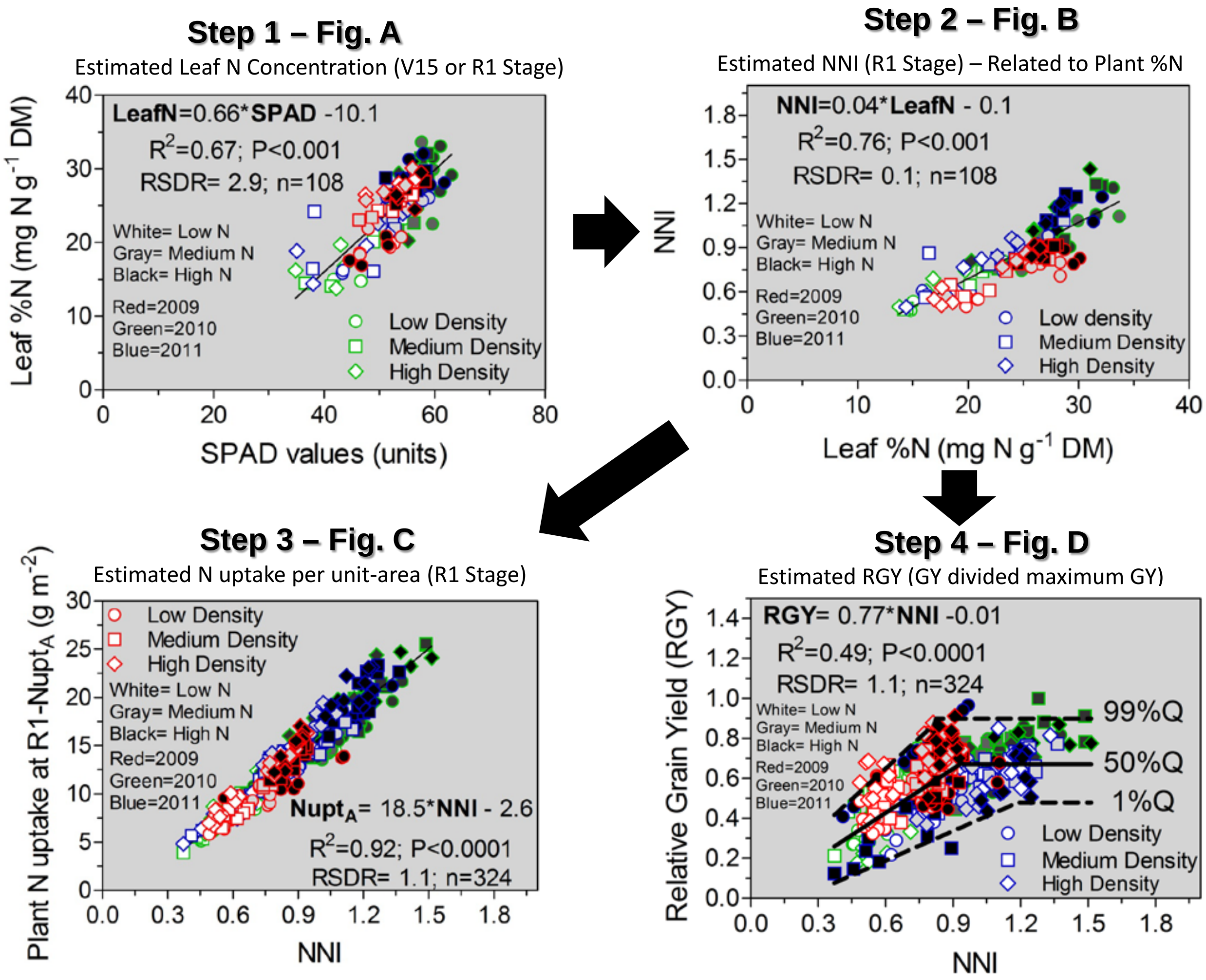

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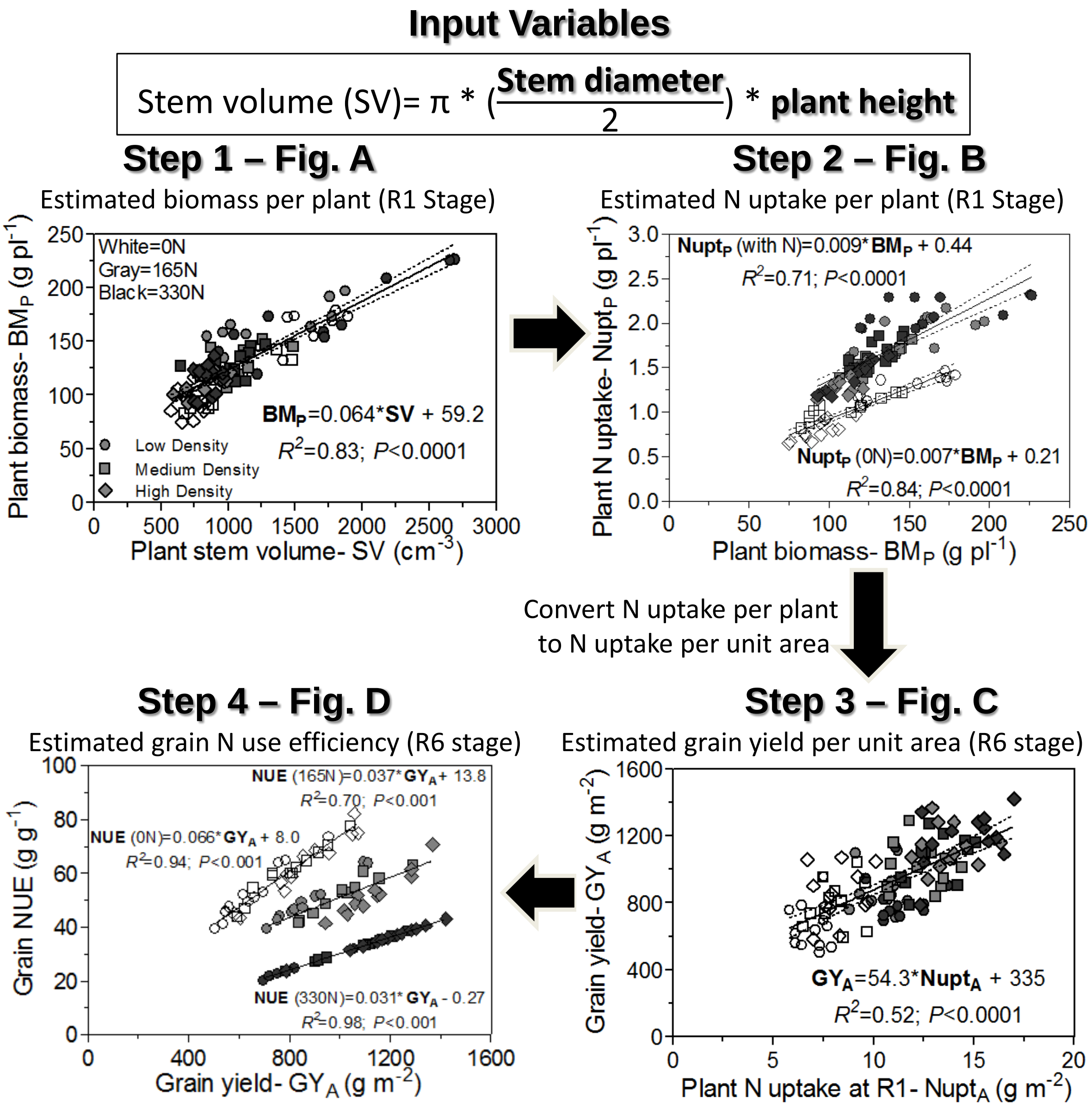

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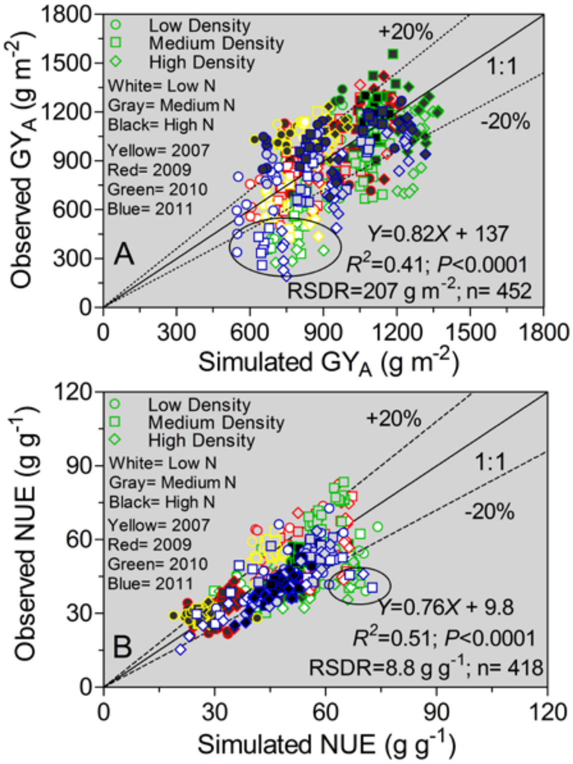

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