

RESEARCH

Changes in Nitrogen Use Traits Associated with Genetic Improvement for Grain Yield of Maize Hybrids Released in Different Decades

Jason W. Haegele, Kevin A. Cook, Devin M. Nichols, and Frederick E. Below*

ABSTRACT

Further enhancement of maize (*Zea mays* L.) N-use efficiency (NUE) will benefit from a thorough understanding of how genetic improvement has shaped N use parameters. Since selection for grain yield has occurred at high N fertilizer rates, our hypothesis was that modern hybrids would have a greater response to supplemental N than hybrids from earlier eras. In 2009 and 2010, 21 single-cross maize hybrids released between 1967 and 2006 were characterized for grain yield and N use traits. While the ability to acquire mineralized soil N did not change over era, the utilization increased with decade of introduction (0.24 kg kg^{-1} of plant N [$\text{kg}_{\text{plantN}}^{-1}$] yr^{-1} ; $R^2 = 0.37$). Increases of grain yield at high N ($86 \text{ kg ha}^{-1} \text{ yr}^{-1}$; $R^2 = 0.68$) over era were accompanied by increases at low N of $56 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ($R^2 = 0.69$). Grain yield improvements at all levels of N were associated with decreased barrenness and increased kernel number expressed on a per-plant and per-area basis. Fertilizer N response, NUE, increased at a rate of 0.16 kg kg^{-1} of fertilizer N (kg_N^{-1}) yr^{-1} ($R^2 = 0.40$). Increased NUE was positively correlated with improved N-uptake efficiency ($r = 0.76$, $P \leq 0.001$), due to the greater postflowering N uptake of more recent hybrids. The response of grain yield to fertilizer N in current hybrids is more dependent on uptake of fertilizer N than the efficiency of fertilizer N utilization, and approximately two-thirds of genetic gain for grain yield at high N can be explained by improvements in grain yield at low N.

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Abbreviations: BLUP, best linear unbiased predictor; GU, genetic utilization; GY_0 , grain yield of the unfertilized check plot treatment; GY_X , grain yield at an X level of fertilizer application; kg_N , kg of fertilizer N; $\text{kg}_{\text{plantN}}$, kg of plant N; NT_0 , total plant N content at the 0 N rate; NT_X , total plant N content at the X N rate; NUE, nitrogen-use efficiency; NUpE, nitrogen-uptake efficiency; NUtE, nitrogen-utilization efficiency; σ^2_{Hybrid} , variance component associated with the effect of hybrid; $\sigma^2_{\text{N rate} \times \text{Hybrid}}$, variance component associated with the N rate \times hybrid interaction; $\sigma^2_{\text{Year} \times \text{Hybrid}}$, variance component associated with the year \times hybrid interaction; $\sigma^2_{\text{Year} \times \text{N rate} \times \text{Hybrid}}$, variance component associated with the year \times N rate \times hybrid interaction.

AVERAGE MAIZE YIELDS in the United States have experienced a nearly five-fold increase between the beginning of the 20th century and the present (Egli, 2008; USDA-NASS, 2012). This increase in yield has been influenced by a combination of genetic and cultural factors, but it has been estimated that approximately 60% can be attributed to genetic improvement (Russell, 1974; Duvick, 1977, 1992). Increased N fertilizer application accompanied early gains in grain yield of single-cross maize hybrids (Duvick and Cassman, 1999); however, average N fertilizer rates in the United States have remained relatively static during the past 30 yr (Fig. 1; USDA-ERS, 2011). This relationship between increased productivity and a high input of N suggests that maize breeders may have selected for germplasm that has an enhanced response to N fertilizer and, potentially, intolerance to low soil N (Carlone and Russell, 1987).

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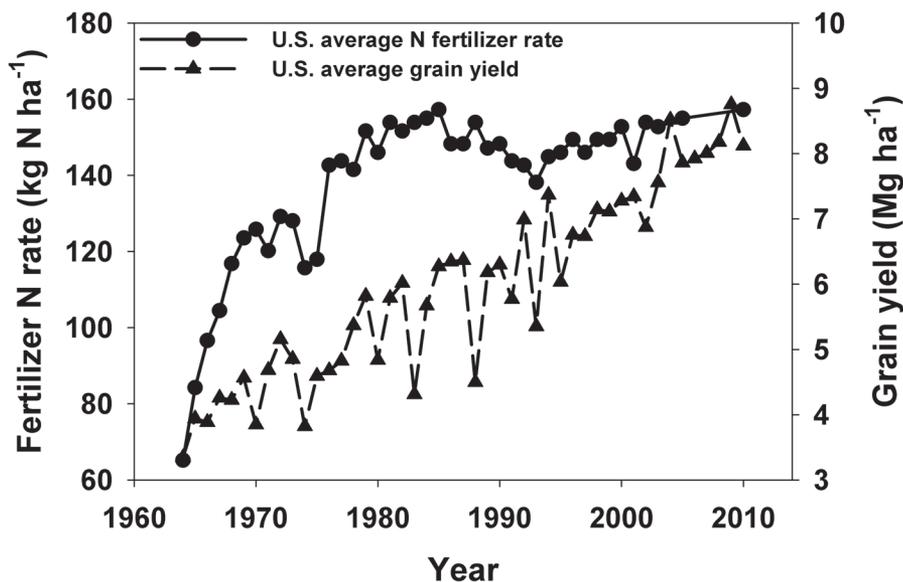


Figure 1. Average fertilizer N application rates for maize production in the United States for years between 1964 and 2010 (USDA-ERS, 2011) and average U.S. maize grain yields (Mg ha^{-1} at 0 g kg^{-1} moisture concentration) during the same period (USDA-NASS, 2012). N application rates were not reported for 2004 and 2006 through 2009.

Era studies (i.e., comparisons of older and newer hybrids) are a common approach to understanding how genetic selection has shaped economically important traits such as grain yield in maize (Castleberry et al., 1984; Duvick, 2005; Campos et al., 2006; Wang et al., 2011); however, the impact of past selection on N use is not as thoroughly documented. Several comparisons of older and newer hybrids under contrasting N levels have been reported (Castleberry et al., 1984; Tollenaar et al., 1997; Sangoi et al., 2001; O'Neill et al., 2004), but these studies are usually limited in the number of genotypes, and as a result it is difficult to completely ascertain the genetic gain that has been made for grain yield in relationship to N fertility in more recent hybrids.

In a comparison of 25 open-pollinated and hybrid maize cultivars used between the 1930s and 1980s, Castleberry et al. (1984) showed that genetic gains for grain yield under low and high soil fertility had been 51 and $87 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively. Similarly, with four hybrids representing Brazilian maize germplasm released between the 1960s and 1990s, the most recent hybrid produced higher grain yields at all levels of N (Sangoi et al., 2001). Likewise, a newer hybrid (Pioneer 3902, released in 1988) produced approximately 25% more grain than an older hybrid (Pride 5, released in 1959) at both low N and high N (Tollenaar et al., 1997). In contrast to these studies, O'Neill et al. (2004) showed that a hybrid widely grown in the United States during the 1970s (B73 \times Mo17) produced approximately 8% more yield under the deficit N treatment than hybrids released in the early and late 1990s while the latter had greater yield responses to applied fertilizer N. Although these studies demonstrate that progress has been made in improving grain yield under both low and high N, characterization of changes in the agronomic and physiological components of

maize N use could identify strategies for continued genetic improvement of yield.

Genetic improvements in maize yield have generally been attributed to increased resource acquisition and greater stress tolerance including the ability to maintain harvest index at increased plant density (Tollenaar and Wu, 1999; Duvick, 2005; Campos et al., 2006; Tollenaar and Lee, 2006; Hammer et al., 2009). Many of these changes are associated with increased dry matter partitioning to the ear during the critical period for kernel set (Echarte et al., 2000; Luque et al., 2006) and sustained leaf photosynthesis during grain fill (Ding et al., 2005; Echarte et al., 2008). Genotypic differences in kernel set in response to low soil N have been linked to differences in N utilization (Moll et al., 1982), which likely reflect the ability to partition biomass and N to the developing ear (Ta and Weiland, 1992; Lafitte and Edmeades, 1995; D'Andrea et al., 2009) or variation for N assimilate metabolism in the maize cob tissue (Seebauer et al., 2004; Moose and Below, 2009). Differences in grain yield at high N have been attributed to contributions from both N uptake and utilization with varying degrees of importance being assigned to each of these traits by past studies (Kamprath et al., 1982; Moll et al., 1982; Presterl et al., 2002; Worku et al., 2007). Although the relative importance of N uptake and utilization is not completely understood and likely varies by population, it is clear that N-use efficiency (NUE) can be improved by changes in one or both component traits (Moll et al., 1982; Presterl et al., 2002). As such, understanding how N uptake and utilization has changed over time in commercially representative maize germplasm could identify breeding strategies to further accelerate genetic gain for yield and NUE.

The objective of this study was to characterize changes in grain yield, yield components, and NUE components in response to contrasting levels of N availability for single-cross maize hybrids representing four decades of selection for grain yield. Our hypothesis was that the most recently released hybrids would have larger responses to fertilizer N compared to hybrids from earlier eras as a result of the common practice of selecting and evaluating hybrids under a high input of N fertilizer.

MATERIALS AND METHODS

Germplasm

Twenty-one single-cross maize hybrids were provided by Monsanto (Table 1). These hybrids represent nearly 40 yr (hybrids released between 1967 and 2006) of DEKALB's commercial breeding pipeline. Conventional, nontransgenic versions of all hybrids were used in the study. Although a range of maturities (661–724°C·d) as determined by time to 50% silk exsertion existed in the panel of era hybrids (Table 1), this range of maturities is typical for commercial maize hybrids that might be grown in east-central Illinois.

Cultural Practices, Experimental Design, and Treatments

Field experiments were conducted during the 2009 and 2010 growing seasons at the University of Illinois Department of Crop Sciences Research and Education Center in Champaign, IL. The soil at this site is a Drummer-Flanagan soil association (fine-silty, mixed, superactive, mesic Typic Endoaquolls) typical of east-central Illinois. Soybean [*Glycine max* (L.) Merr.] was the previous crop in both years. Plots were mechanically planted on 23 May 2009 and 29 May 2010. The average plant density at harvest was 82,800 plants ha⁻¹. Tefluthrin [2,3,5,6-tetrafluoro-4-methylphenyl)methyl-(1a,3a)-(Z)-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] was applied in-furrow at a rate of 0.11 kg a.i. ha⁻¹ to control Western corn rootworm (*Diabrotica virgifera virgifera*) larvae. Preemergence weed control consisted of applications of S-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide), atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine), and mesotrione ([2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione).

A strip-plot arrangement in a randomized complete block design with three replications was used in which hybrids and N rates were randomly assigned to the whole-plot experimental units. Each subplot experimental unit (intersection of whole plot experimental units) consisted of two rows 5.3 m in length with 0.76 m spacing. Nitrogen was hand applied and incorporated as granular ammonium sulfate ((NH₄)₂SO₄; 21-0-0-24S) in a diffuse band between the rows after emergence during the V2 to V3 growth stages (Ritchie et al., 1997). These applications occurred on 5 June 2009 and 16 June 2010. Three supplemental N rates (0, 67, and 252 kg N ha⁻¹) were chosen to evaluate a hybrid's tolerance of low N as well as its initial and maximum responses to fertilizer N.

Table 1. Years of introduction, decade grouping, and modified growing degree days (MGDD) to 50% silk exsertion for 21 era hybrids evaluated at Champaign, IL, in 2009 and 2010. Days to silking was measured in 2009 for plants grown with the high N treatment (252 kg N ha⁻¹).

Entry	Year of introduction	Decade	MGDD to 50% silking °C·d
1	1967	1970	678
2	1971	1970	661
3	1975	1970	724
4	1981	1980	720
5	1982	1980	720
6	1989	1980	724
7	1989	1980	699
8	1991	1990	712
9	1991	1990	716
10	1994	1990	699
11	1994	1990	716
12	1996	1990	712
13	1999	1990	716
14	2000	2000	716
15	2001	2000	661
16	2003	2000	703
17	2004	2000	712
18	2005	2000	712
19	2005	2000	682
20	2006	2000	699
21	2006	2000	712

Biomass Sampling, Nitrogen Uptake, and Yield Measurements

The 2009 trial was evaluated for thermal time to 50% silk emergence to document treatment effects on female flowering time associated with genotype. This assessment was made to determine if an altered experimental design (i.e., blocking by maturity) would be required in 2010. Plots were visually rated, and the dates on which 50% of the plants within a plot exhibited at least one exposed silk were recorded. Calendar dates were converted to thermal time (modified growing degree days) using minimum and maximum temperature limits of 10 and 30°C, respectively (Cross and Zuber, 1972). Daily minimum temperatures less than 10°C were set to the lower limit while daily maximum temperatures exceeding 30°C were set to the maximum limit.

Plant N uptake and partitioning of biomass and N were estimated from plant samples collected at silk emergence (R1) and at physiological maturity (R6) when at least 50% of the plants exhibited a visible black layer at the base of the kernels (Ritchie et al., 1997). Five equally spaced, representative plants per plot were sampled (aboveground biomass only) and separated into ear (grain and cob) and stover (leaf, stem, and husk) fractions. An approach was used in which two adjacent plants were sampled at approximately 1.2 m along the length of the sampled row, a third plant at approximately 2.7 m, and two adjacent plants at approximately 4.1 m. The criterion for choosing representative plants was based on a visual assessment of the "average" characteristics of plants in the plot (height, stalk diameter, spacing, etc.). Plants sampled at R1 contained only the stover fraction. The fresh weight of the stover fraction was determined before shredding using a commercial brush chipper

(Vermeer BC600XL; Vermeer Corporation). A representative aliquot of the shredded material was dried to constant weight in a forced-draft oven (75°C). Dried stover aliquots were ground in a Wiley mill (Thomas Scientific) to pass a 20-mesh screen and analyzed for total N concentration (g kg⁻¹) using a combustion technique (EA1112 N-Protein; CE Elantech, Inc.). Total stover N content was calculated by multiplying the per plant dry stover biomass by the stover N concentration. Total dry plant biomass (g per plant) was calculated as the sum of the dry stover, grain, and cob fractions. Grain protein concentration was measured using near-infrared transmittance spectroscopy (Infratec 1241 Grain Analyzer; FOSS). Grain N concentration was estimated from protein concentration using a factor of 6.25. Grain N content was calculated by multiplying the per plant grain weight by the grain N concentration. Total N content (g per plant) was calculated as the sum of the stover and grain N contents. Nitrogen uptake on an area basis was estimated by multiplying per plant N content by the plant density at harvest.

Separate rows of each two row plot were sampled at R1 and R6. The row sampled at R6 was also used to determine grain yield (excluding the first plant on the ends of each row), and shelled grain weights from the ears sampled at R6 and the remaining ears in the row were combined to calculate yield (total harvested area of 3.8 m²). Grain yields are expressed as megagrams per hectare at 0 g kg⁻¹ moisture. Individual kernel weights were estimated by bulk weighing 300 kernels from a representative grain subsample using an electronic seed counter with the resultant data expressed as milligrams per kernel at 0 g kg⁻¹ moisture. Kernel number (m⁻²) was algebraically derived from the total plot grain weight and the estimate of individual kernel weight.

Using the data from grain yield and plant N uptake, NUE (kg kg⁻¹ of fertilizer N [kg_N⁻¹]) and its components, N-uptake efficiency (NUpE) (kg of plant N [kg_{plantN} kg_N⁻¹]) and N-utilization efficiency (NUtE) (kg kg_{plantN}⁻¹), were calculated according to Eq. [1–3]:

$$\text{NUE} = (\text{GY}_X - \text{GY}_0) / \text{NR}_X, \quad [1]$$

$$\text{NUpE} = (\text{NT}_X - \text{NT}_0) / \text{NR}_X, \text{ and} \quad [2]$$

$$\text{NUtE} = (\text{GY}_X - \text{GY}_0) / (\text{NT}_X - \text{NT}_0), \quad [3]$$

in which GY_X corresponds to the grain yield (kg ha⁻¹) at an X level of fertilizer application (≥67 kg N ha⁻¹) and GY₀ corresponds to the grain yield (kg ha⁻¹) of the unfertilized check plot treatment (0 kg N ha⁻¹), NR_X is the fertilizer N rate (kg N ha⁻¹), and NT₀ and NT_X represent the total plant N contents at the 0 and X N rates (kg plant N ha⁻¹). Similarly, genetic utilization (GU) (kg kg_{plantN}⁻¹), which quantifies the physiological efficiency of N utilization for grain yield under unfertilized conditions was calculated according to Eq. [4]:

$$\text{GU} = \text{GM}_0 / \text{NU}_0, \quad [4]$$

in which GM₀ corresponds to the per-plant grain mass (kg per plant) of the unfertilized check plot treatment (0 kg N ha⁻¹) and NU₀ (kg N per plant) is the per-plant N uptake at physiological maturity derived from residual or mineralized soil N.

Statistical Analysis

Statistical analysis using a linear mixed model approach was accomplished with PROC MIXED in SAS (SAS Institute, 2009).

Year and N rate were included in the model as fixed effects while replication, hybrid, and the interactions of replication and hybrid with the fixed effects were included as random effects. Hybrid was treated as a random effect because we assumed that the 21 entries evaluated in this study were a subsample of the entire population of hybrids released during the time span represented. The phenotypic observations (y_{ijkl}) were modeled as

$$y_{ijkl} = \mu + \alpha_i + r_{il} + \beta_j + \alpha\beta_{ij} + g_{ijl} + \gamma_k + \alpha\gamma_{ik} + m_{ikl} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijkl}, \quad [5]$$

in which μ is the overall mean, α_i is the fixed effect of year i , r_{il} is the random effect of replication l within year i , β_j is the fixed effect of N rate j , $\alpha\beta_{ij}$ is the interaction between year and N rate, g_{ijl} is the random interaction between replication and N rate within year, γ_k is the random effect of hybrid k , $\alpha\gamma_{ik}$ is the random interaction between year and hybrid, m_{ikl} is the random interaction between replication and hybrid within year, $\beta\gamma_{jk}$ is the random interaction between N rate and hybrid, $\alpha\beta\gamma_{ijk}$ is the random interaction between year, N rate, and hybrid, and ε_{ijkl} is the residual error.

Variance components and their standard errors for the random effects were calculated using restricted estimation of maximum likelihood. Hybrid performance within each level of N fertilizer was calculated using best linear unbiased predictors (BLUPs). Furthermore, BLUPs were also used to calculate the predicted mean performance of the hybrids released within the same decade. PROC REG in SAS (SAS Institute, 2009) was used to estimate genetic gain for each trait at each level of N by regressing each individual hybrid's BLUP on year of introduction. A linear regression model was deemed significant at $P \leq 0.05$. Pearson's pairwise correlations were calculated between traits using PROC CORR in SAS.

RESULTS

Temperature and Precipitation

The years 2009 and 2010 represented two very different environments for evaluating yield and N use traits. Temperatures in 2009 were generally cooler than average, particularly at flowering and during grain filling (Table 2). During July and August of 2009, maximum and minimum temperatures were approximately 2.6 and 1.7°C less than the 10-yr average at Champaign, IL. Cool temperatures were accompanied by above average precipitation; a total of 54.7 cm of precipitation fell during the May to September period, an increase of approximately 15% over the 10-yr average. In contrast to 2009, 2010 was characterized by above-average temperatures and unevenly distributed precipitation. During May through September, daily minimum and maximum temperatures were on average 1.4 and 1.2°C greater than the 10-yr average, respectively. August of 2010 experienced a +2.5°C deviation from the 10-yr average for maximum temperature, which was accompanied by a -4.7 cm deviation from the 10-yr average for precipitation (Table 2). Together, these conditions resulted in significant stress at flowering and during grain filling.

Table 2. Average monthly weather data at Champaign, IL, for the period between 1 May and 30 September in 2009 and 2010. Tmin. and Tmax. are the minimum and maximum daily temperatures, respectively. Values in parentheses are the deviations from the 10-yr average (2001–2010) at Champaign, IL.

Year	Month	Temperature		Precipitation
		Tmin.	Tmax.	
		°C		cm
2009	May	11.4 (+0.5)	23.1 (–0.3)	13.0 (+4.2)
	June	17.3 (+0.8)	28.4 (+0.0)	10.8 (+1.0)
	July	16.5 (–1.7)	26.6 (–3.1)	15.6 (+3.2)
	Aug.	16.1 (–1.6)	27.2 (–2.0)	13.7 (+5.0)
	Sept.	14.3 (+0.9)	25.0 (–1.4)	1.6 (–6.1)
2010	May	12.7 (+1.7)	24.2 (+0.8)	7.8 (–1.0)
	June	18.7 (+2.2)	29.4 (+1.0)	19.8 (+10.0)
	July	19.7 (+1.6)	31.1 (+1.4)	9.0 (–3.3)
	Aug.	19.1 (+1.4)	31.8 (+2.5)	4.0 (–4.7)
	Sept.	13.2 (–0.1)	26.6 (+0.2)	7.6 (–0.1)

Responses of Measured Traits to Year and Nitrogen Rate

Year and N rate were included in the linear mixed model analysis as fixed sources of variance. Although year was not a significant source of variation for grain yield, the main effect of N rate and the interaction effect of year and N rate were highly significant ($P \leq 0.0001$; Table 3). Averaged across N rates, grain yields in 2009 and 2010 were 7.4 and 8.1 Mg ha⁻¹, respectively. When averaged across hybrids, grain yield at low N (0 kg N ha⁻¹) was 2.1 Mg ha⁻¹ greater in 2010 compared to 2009 ($P = 0.0026$). We attribute the decreased low N grain yield of 2009 to excessive precipitation and cool temperatures, which likely promoted early season N loss and decreased net soil N mineralization. Although 2009 had greatly reduced grain yield at low N, the average N response (increase in yield between 0 and 252 kg N ha⁻¹) in 2009 was nearly 2.1 times greater than that of 2010. As such, at 252 kg N ha⁻¹, grain yields were 11.3 and 9.6 Mg ha⁻¹ in 2009 and 2010, respectively ($P = 0.009$). The decreased N response of 2010 was likely a function of greater residual soil N (i.e., increased grain yield at low N) as well as high temperatures at flowering and grain fill, which contributed to increased kernel abortion and possibly a shortened duration of grain filling.

All of the measured traits were significantly affected by N rate, and the interaction of year and N rate was significant for all traits with the exception of NUtE (Table 3). The significant year × N rate interactions were generally explained by the previously discussed differences between years in response to low N.

Variance Components

Although we evaluated 21 hybrids released between 1967 and 2006, there were certainly many more hybrids released during this time period. Therefore, we included hybrid in the model as a random effect since our main interest was

Table 3. Tests of fixed effects. A linear mixed model approach was used to analyze the measured traits. Year and N rate were included as fixed sources of variation in the model.

Measured trait	Source of variation		
	Year	N rate	Year × N rate
	$P > F$		
Grain yield	NS [†]	0.0001	0.0001
Area kernel number	0.0004	0.0001	0.0001
Ear kernel number	0.0001	0.0001	0.0001
Kernel weight	0.0194	0.0001	0.0001
Barren plants	0.0470	0.0001	0.0133
Harvest index	0.0015	0.0001	0.0039
R1 N content	NS	0.0001	0.0278
R6 N content	0.0009	0.0001	0.0002
Protein concentration	0.0001	0.0001	0.0020
Genetic utilization [‡]	NS	–	–
N-use efficiency	0.0092	0.0002	0.0465
N uptake	NS	0.0001	0.0001
N utilization	0.0339	0.0005	NS

[†]NS, not significant (at $P \geq 0.05$).

[‡]Genetic utilization is relevant at low N only, and N rate was not included in the statistical model for this trait.

not in individual hybrids per se but rather in the improvement of traits by decade of hybrid introduction.

Hybrid was a significant variance component (σ^2_{Hybrid}) for yield traits such as total grain yield, kernel number, and kernel weight (Table 4). The variance component associated with the year × hybrid interaction ($\sigma^2_{\text{Year} \times \text{Hybrid}}$) as well as the variance component associated with the N rate × hybrid interaction ($\sigma^2_{\text{N rate} \times \text{Hybrid}}$) were also significant but relatively small in comparison to σ^2_{Hybrid} . Significant year × hybrid and N rate × hybrid interactions did not result in a change in the relative rankings of hybrids in each year. Therefore, it is possible to estimate genetic gain for each trait by combining data across years of the experiment.

Relative to other yield components, individual kernel weight had small interactions with year and N rate; σ^2_{Hybrid} for kernel weight was over five times larger in magnitude relative to the variance component associated with the year × N rate × hybrid interaction ($\sigma^2_{\text{Year} \times \text{N rate} \times \text{Hybrid}}$) (Table 4). This result supports the concept of a hierarchy of plasticities for yield components and that individual kernel weight has the least plasticity, particularly in comparison to kernel number (Sadras and Slafer, 2012). The proportion of barren plants increased with the low N treatment, particularly for older hybrids (i.e., those from the 1970s and 1980s). This was especially true of the severely N deficient environment of 2009. As such, the $\sigma^2_{\text{Year} \times \text{Hybrid}}$ and $\sigma^2_{\text{Year} \times \text{N rate} \times \text{Hybrid}}$ variance components were significant for percent barren plants (Table 4).

Aboveground plant N content was measured at flowering and physiological maturity, which allows us to assess potential differences in timing of N uptake between genotypes. There were no significant variance components associated with hybrid for N content at R1,

Table 4. Variance component estimates for random model effects. A linear mixed model approach was used to analyze the measured traits. Hybrid was included as a random effect in the model.

Measured traits	Variance components [†]				
	σ^2_{Hybrid}	$\sigma^2_{\text{Year} \times \text{Hybrid}}$	$\sigma^2_{\text{N rate} \times \text{Hybrid}}$	$\sigma^2_{\text{Year} \times \text{N rate} \times \text{Hybrid}}$	$\sigma^2_{\text{Residual}}$
Grain yield	1.05**	0.11*	0.19**	0	0.58***
Area kernel number	$1.68 \times 10^{5**}$	$2.32 \times 10^{4*}$	$1.17 \times 10^{4*}$	0	$9.56 \times 10^{4***}$
Ear kernel number	$1.24 \times 10^{3**}$	$2.98 \times 10^{2*}$	$2.65 \times 10^{2**}$	$1.48 \times 10^{2*}$	$6.74 \times 10^{2***}$
Kernel weight	$1.46 \times 10^{2**}$	13.72	0	27.18**	88.96***
Barren plants	13.90	11.35*	8.37	20.43***	26.61***
Harvest index	$7.52 \times 10^{-4**}$	$1.47 \times 10^{-4*}$	2.80×10^{-5}	0	$9.14 \times 10^{-4***}$
R1 N content	0	0	0	2.67×10^{-3}	$4.95 \times 10^{-2***}$
R6 N content	$1.09 \times 10^{-2*}$	8.66×10^{-4}	$1.09 \times 10^{-2*}$	4.16×10^{-3}	$4.68 \times 10^{-2***}$
Protein concentration	13.34**	2.00	3.29**	1.24	9.83***
Genetic utilization	27.41**	2.58	–	–	0.98
N-use efficiency	16.14	5.22	0	7.12	78.05***
N-uptake efficiency	8.31×10^{-3}	6.05×10^{-4}	0	0	$4.16 \times 10^{-2***}$
N-utilization efficiency	2.27	6.04	0	16.27	$6.24 \times 10^{2***}$

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

***Significant at $P \leq 0.001$.

[†] σ^2_{Hybrid} variance component associated with the effect of hybrid; $\sigma^2_{\text{Year} \times \text{Hybrid}}$ variance component associated with the year \times hybrid interaction; $\sigma^2_{\text{N rate} \times \text{Hybrid}}$ variance component associated with the N rate \times hybrid interaction; $\sigma^2_{\text{Year} \times \text{N rate} \times \text{Hybrid}}$ variance component associated with the year \times N rate \times hybrid interaction; $\sigma^2_{\text{Residual}}$ variance component associated with the residual error variance of the statistical model (Eq. [5]).

which suggests that N uptake before flowering is mostly influenced by N supply and environment (Tables 3 and 4). The absence of genotypic differences for plant N content at flowering is consistent with other studies that have shown that dry matter accumulation, and hence, potential for N uptake at R1 is similar between older and newer hybrids (Tollenaar, 1991; Rajcan and Tollenaar, 1999). In contrast, plant N content at R6 was associated with significant σ^2_{Hybrid} and $\sigma^2_{\text{N rate} \times \text{Hybrid}}$ variance components indicating that genotypic differences in N uptake are driven by postflowering N uptake ability.

The main fixed effect of year was not significant for GU, which quantifies the physiological efficiency of converting plant accumulated N to grain at low N (Table 3). Genotypic variance for GU was significant while there was not a significant year \times hybrid interaction (Table 4). Furthermore, the variance component associated with hybrid was nearly 28 times larger than the residual error variance for GU, indicating that this trait is highly heritable and consistent across environments with contrasting levels of residual or mineralized soil N availability.

There were no significant variance components associated with hybrid for NUE or its component traits, N uptake and N utilization (Table 4). Genetic variation for these traits in maize has previously been documented (Moll et al., 1982; Presterl et al., 2002); however, our derivation of these traits relies on calculating the difference between separate measurements, which may contribute to the large residual error variances associated with these traits. For example, NUtE (Eq. [3]) is calculated from four separate measurements (GY_0 , GY_X , NT_0 , and NT_X). Its residual error variance was approximately eight times

larger compared to NUE (Eq. [1]), which is derived from only two component measurements (GY_0 and GY_X).

Genetic Gain for Yield and Nitrogen Use Traits

Grain yield increased by decade of hybrid introduction at all three levels of N fertility (Table 5). When regressed on year of introduction, the calculated rate of gain for grain yield at 252 kg N ha⁻¹ was 86 kg ha⁻¹ yr⁻¹ (Table 5; Fig. 2B). Grain yield at 0 kg N ha⁻¹ also increased with year of hybrid introduction at a rate of 56 kg ha⁻¹ yr⁻¹ (Table 5; Fig. 2A). Genetic gain for grain yield at 67 kg N ha⁻¹ was intermediate (79 kg ha⁻¹ yr⁻¹) to the other levels of N availability.

Grain yield is a function of the number of plants per area, the proportion of these plants that produce a harvestable ear, kernel number per ear, and the weight of each individual kernel. Grain yield in this study was highly correlated with kernel number and a decrease in percent barren plants (Table 6). At low N, the estimated rates of gain for kernel number on a per-area basis were 21, 24, and 25 kernels m⁻² yr⁻¹ at the 0, 67, and 252 kg N ha⁻¹ levels, respectively (Table 5). Although the regression of kernel number per ear on year of hybrid release was significant at all levels of N, the rate of gain of 1 to 2 kernels per ear yr⁻¹ represents a much lower increase on a percentage basis (average of 0.42% yr⁻¹ relative to 1970s hybrids) compared to the percentage increase in kernel number on a per-area basis (average of 0.87% yr⁻¹ relative to 1970s hybrids). Kernel number on a per-area basis is impacted by kernels per ear but is also strongly affected by the proportion of barren plants. The negative correlation between proportion of barren plants and grain yield was greatest at low N ($r = -0.92$) and became less negative

Table 5. Best unbiased linear predictor (BLUP) estimates (\pm standard error) for grain yield and yield components of era hybrids evaluated under three levels of N fertilizer at Champaign, IL, in 2009 and 2010.

Measured trait	Era or slope	Fertilizer N rate		
		0 kg N ha ⁻¹	67 kg N ha ⁻¹	252 kg N ha ⁻¹
Grain yield, Mg ha ⁻¹				
	1970s	3.7 \pm 0.3	5.9 \pm 0.3	8.3 \pm 0.3
	1980s	4.8 \pm 0.3	7.4 \pm 0.3	9.9 \pm 0.3
	1990s	5.1 \pm 0.3	7.8 \pm 0.3	10.8 \pm 0.3
	2000s	5.7 \pm 0.2	8.6 \pm 0.2	11.3 \pm 0.2
	Slope [†]	0.056 \pm 0.009	0.079 \pm 0.009	0.086 \pm 0.014
Area kernel number, m ⁻²				
	1970s	1827 \pm 103	2882 \pm 103	3609 \pm 103
	1980s	2264 \pm 93	3407 \pm 93	4084 \pm 93
	1990s	2396 \pm 81	3563 \pm 81	4369 \pm 81
	2000s	2588 \pm 75	3742 \pm 75	4481 \pm 75
	Slope	21 \pm 5	24 \pm 6	25 \pm 6
Ear kernel number, per ear				
	1970s	299 \pm 11	400 \pm 11	476 \pm 11
	1980s	308 \pm 10	432 \pm 10	508 \pm 10
	1990s	320 \pm 9	446 \pm 9	548 \pm 9
	2000s	332 \pm 8	463 \pm 8	547 \pm 8
	Slope	1 \pm 0.5	2 \pm 0.6	2 \pm 0.6
Kernel weight, mg per kernel				
	1970s	201 \pm 3.8	208 \pm 3.8	232 \pm 3.8
	1980s	211 \pm 3.6	218 \pm 3.6	241 \pm 3.6
	1990s	215 \pm 3.4	222 \pm 3.4	245 \pm 3.4
	2000s	220 \pm 3.3	227 \pm 3.3	251 \pm 3.3
	Slope	0.58 \pm 0.17	0.58 \pm 0.17	0.58 \pm 0.17
Barren plants, %				
	1970s	17.0 \pm 2.2	8.5 \pm 2.2	6.3 \pm 2.2
	1980s	9.8 \pm 1.9	3.6 \pm 1.9	1.8 \pm 1.9
	1990s	8.6 \pm 1.6	3.4 \pm 1.6	1.6 \pm 1.6
	2000s	5.8 \pm 1.4	2.1 \pm 1.4	0.8 \pm 1.4
	Slope	-0.3 \pm 0.07	-0.2 \pm 0.03	-0.1 \pm 0.03
Harvest index, kg kg ⁻¹				
	1970s	0.33 \pm 0.01	0.43 \pm 0.01	0.47 \pm 0.01
	1980s	0.34 \pm 0.01	0.44 \pm 0.01	0.48 \pm 0.01
	1990s	0.36 \pm 0.01	0.45 \pm 0.01	0.50 \pm 0.01
	2000s	0.38 \pm 0.01	0.48 \pm 0.01	0.52 \pm 0.01
	Slope	0.002 \pm 0.0004	0.002 \pm 0.0004	0.001 \pm 0.0004

[†]Slope (\pm standard error) calculated by regressing BLUP estimates for each individual hybrid on year of introduction. Slopes are reported on a per year basis.

with increasing N rate ($r = -0.84$ and $r = -0.76$ at 67 and 252 kg N ha⁻¹, respectively) (Table 6).

Kernel weight increased at a rate of 0.58 mg per kernel yr⁻¹ and this rate of gain was not influenced by N supply (Table 5). The response of kernel weight to high N was approximately 30 mg per kernel for groups of hybrids representing each decade.

Grain yield was positively correlated with harvest index (i.e., the proportion of dry matter partitioned to the grain) (Table 6). At low N, harvest indices for 1970s and 2000s hybrids were 0.33 and 0.38 kg kg⁻¹, respectively

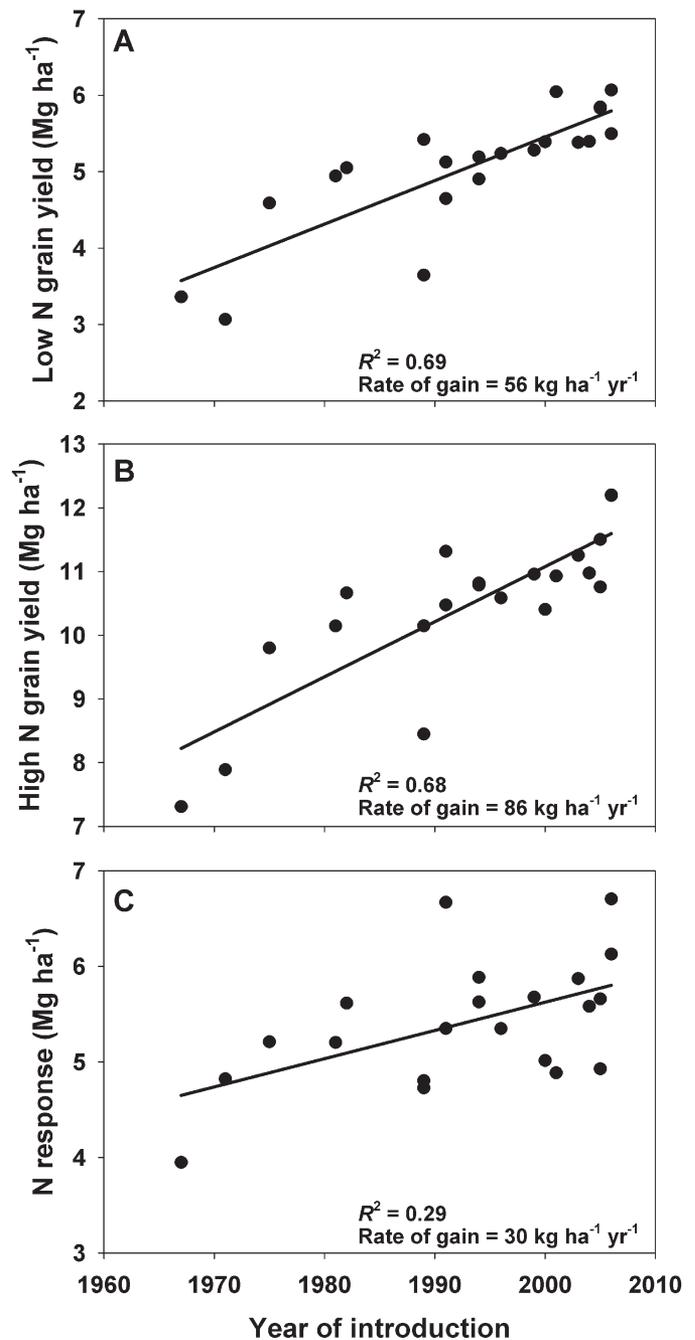


Figure 2. Linear regressions on year of introduction for A) grain yield at low N (0 kg N ha⁻¹), B) grain yield at high N (252 kg N ha⁻¹), and C) N response (difference in yield between 0 and 252 kg N ha⁻¹). Each point represents individual hybrids introduced between 1967 and 2006.

(Table 5). Similarly, at high N, a 0.05 kg kg⁻¹ difference occurred between the oldest and newest hybrids, with an average harvest index of 0.52 kg kg⁻¹ achieved in hybrids representing the 2000s (Table 5).

Total aboveground plant N contents were measured at flowering (R1) and physiological maturity (R6) to ascertain potential differences in timing of N uptake between older and newer hybrids. At R1, no genotypic differences in N uptake were detected (Table 4) while

Table 6. Pearson's pairwise correlation coefficients ($n = 21$) calculated between grain yield at each level of N fertilizer, yield components, N uptake and partitioning, and N-use efficiency traits.

	Correlations with grain yield		
	0 kg N ha ⁻¹	67 kg N ha ⁻¹	252 kg N ha ⁻¹
Area kernel number	0.93***	0.89***	0.89***
Ear kernel number	0.75***	0.81***	0.84***
Kernel weight	0.37	0.51**	0.55**
Barren plants	-0.92***	-0.84***	-0.76***
Harvest index	0.59**	0.56**	0.38
R6 N content	0.39	0.76***	0.72***
Grain protein concentration	-0.61**	-0.54**	-0.75***
N-use efficiency	-	0.68***	0.77***
N-uptake efficiency	-	0.60**	0.68***

**Significant at $P \leq 0.01$.

***Significant at $P \leq 0.001$.

application of N at the 67 and 252 kg N ha⁻¹ rates resulted in 100 and 183% increases in N uptake (Table 7). At R6, there were no differences in plant N content at 0 kg N ha⁻¹ (Table 7), suggesting that the N mineralization capacity

of the soil regulates N uptake potential when fertilizer is not applied. At low N, the correlation between R6 plant N content and grain yield was positive but not significant ($r = 0.39$, $P = 0.08$) (Table 6). In contrast, correlations between plant N content and grain yield at 67 and 252 kg N ha⁻¹ were $r = 0.76$ and $r = 0.72$, respectively ($P \leq 0.001$). With application of N fertilizer, the estimated rate of genetic gain for R6 plant N content was 0.008 g per plant yr⁻¹ ($R^2 = 0.62$ at 67 kg N ha⁻¹ and $R^2 = 0.35$ at 252 kg N ha⁻¹) (Table 7). Furthermore, because there were no apparent genotypic differences in R1 plant N content, this improvement in N uptake at physiological maturity represents postflowering N uptake.

Genetic utilization increased at a rate of 0.24 kg kg_{plantN}⁻¹ yr⁻¹ ($R^2 = 0.37$) (Table 7; Fig. 3A). Genetic utilization was positively and significantly correlated with kernel number per ear ($r = 0.75$, $P \leq 0.001$) and harvest index ($r = 0.80$, $P \leq 0.001$) at low N while it was negatively correlated with barren plants ($r = -0.76$, $P \leq 0.001$) and uncorrelated with kernel weight ($r = -0.07$; not significant) (data not shown). A trend for decreased grain protein concentration accompanied the increase in GU with year of introduction although this trend was not

Table 7. Best unbiased linear predictor (BLUP) estimates (\pm standard error) for N uptake and use traits of era hybrids evaluated under three levels of N fertilizer at Champaign, IL, in 2009 and 2010.

Measured trait	Era or slope	Fertilizer N rate		
		0 kg N ha ⁻¹	67 kg N ha ⁻¹	252 kg N ha ⁻¹
R1 N content, g per plant	Avg.	0.6 \pm 0.1	1.2 \pm 0.1	1.7 \pm 0.1
R6 N content, g per plant	1970s	0.9 \pm 0.06	1.2 \pm 0.06	2.2 \pm 0.06
	1980s	0.9 \pm 0.06	1.4 \pm 0.06	2.4 \pm 0.06
	1990s	0.9 \pm 0.05	1.4 \pm 0.05	2.4 \pm 0.05
	2000s	0.9 \pm 0.05	1.5 \pm 0.05	2.4 \pm 0.05
	Slope†	NS‡	0.008 \pm 0.002	0.008 \pm 0.002
	Protein concentration, g kg ⁻¹			
Genetic utilization, kg kg _{plantN} ⁻¹	1970s	62 \pm 1.1	66 \pm 1.1	85 \pm 1.1
	1980s	59 \pm 1.0	63 \pm 1.0	81 \pm 1.0
	1990s	58 \pm 0.8	62 \pm 0.8	79 \pm 0.8
	2000s	58 \pm 0.7	63 \pm 0.7	79 \pm 0.7
	Slope	NS	NS	-0.18 \pm 0.08
	N-use efficiency, kg kg _N ⁻¹	1970s	57 \pm 2	-
1980s		61 \pm 1	-	-
1990s		63 \pm 1	-	-
2000s		66 \pm 1	-	-
Slope		0.24 \pm 0.07	-	-
N-uptake efficiency, kg kg _{plantN} kg _N ⁻¹		1970s	62 \pm 1.1	66 \pm 1.1
	1980s	59 \pm 1.0	63 \pm 1.0	81 \pm 1.0
	1990s	58 \pm 0.8	62 \pm 0.8	79 \pm 0.8
	2000s	58 \pm 0.7	63 \pm 0.7	79 \pm 0.7
	Slope	-	0.16 \pm 0.04	0.16 \pm 0.04
	N-utilization efficiency, kg kg _{plantN} ⁻¹	1970s	-	38 \pm 2
1980s		-	40 \pm 2	20 \pm 2
1990s		-	42 \pm 2	22 \pm 2
2000s		-	43 \pm 2	23 \pm 2
Slope		-	0.16 \pm 0.04	0.16 \pm 0.04
N-uptake efficiency, kg kg _{plantN} kg _N ⁻¹		1970s	-	38 \pm 2
	1980s	-	40 \pm 2	20 \pm 2
	1990s	-	42 \pm 2	22 \pm 2
	2000s	-	43 \pm 2	23 \pm 2
	Slope	-	0.16 \pm 0.04	0.16 \pm 0.04
	N-utilization efficiency, kg kg _{plantN} ⁻¹	1970s	-	38 \pm 2
1980s		-	40 \pm 2	20 \pm 2
1990s		-	42 \pm 2	22 \pm 2
2000s		-	43 \pm 2	23 \pm 2
Slope		-	0.007 \pm 0.002	0.003 \pm 0.001
N-utilization efficiency, kg kg _{plantN} ⁻¹		Avg.	-	70 \pm 4

†Slope (\pm standard error) calculated by regressing BLUP estimates for each individual hybrid on year of introduction. Slopes are reported on a per year basis.

‡NS, not significant (slope not significantly different from zero).

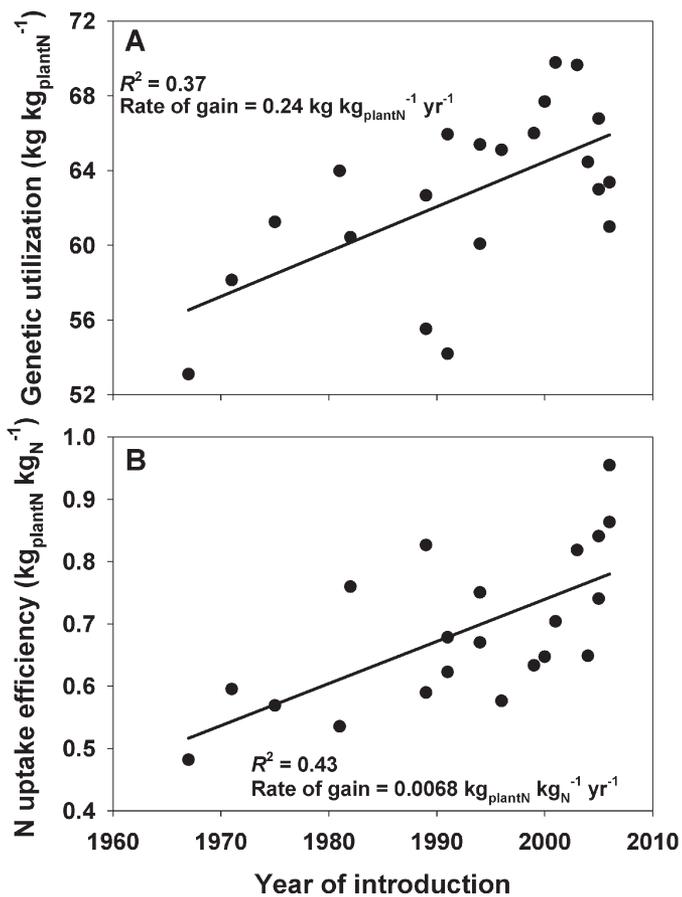


Figure 3. Linear regressions on year of introduction for A) genetic utilization (0 kg N ha⁻¹) and B) N-uptake efficiency at the intermediate N treatment (67 kg N ha⁻¹). Each point represents individual hybrids introduced between 1967 and 2006.

significant (Table 7). However, at high N, grain protein concentration decreased by $0.18 \text{ g kg}^{-1} \text{ yr}^{-1}$ ($R^2 = 0.23$).

Nitrogen-use efficiency, which quantifies the increase in grain yield per unit of applied fertilizer N over the unfertilized check plot, is a measure of N fertilizer response. When grown with 67 or 252 kg N ha⁻¹, NUE increased at a rate of $0.16 \text{ kg kg}_N^{-1} \text{ yr}^{-1}$ ($R^2 = 0.40$) (Table 7). Nitrogen-use efficiency is the product function of NUpE and NUtE. Fittingly, NUpE was highly correlated with NUE, especially at the intermediate level of N supply (Fig. 4). Estimates of genetic gain for NUpE were $0.007 \text{ kg}_{\text{plantN}} \text{ kg}_N^{-1} \text{ yr}^{-1}$ at 67 kg N ha⁻¹ ($R^2 = 0.43$) and $0.003 \text{ kg}_{\text{plantN}} \text{ kg}_N^{-1} \text{ yr}^{-1}$ at 252 kg N ha⁻¹ ($R^2 = 0.38$) (Table 7; Fig. 3B). There were no apparent changes in NUtE from genetic selection, and predicted average values for NUtE were 70 and 45 kg kg_{plantN}⁻¹ at the 67 and 252 kg N ha⁻¹ fertilizer rates, respectively (Table 7).

DISCUSSION

The estimated rate of genetic gain for grain yield at high N in this study ($86 \text{ kg ha}^{-1} \text{ yr}^{-1}$) is consistent with other estimates of $82 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Castleberry et al., 1984) and 57 to

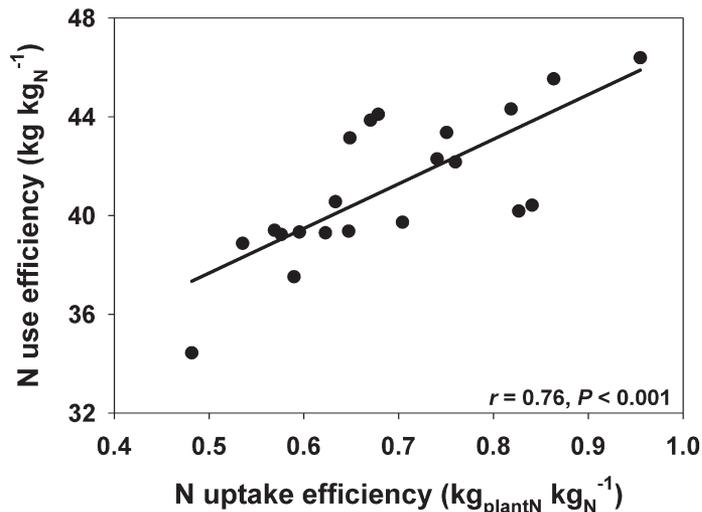


Figure 4. Relationship between N-use efficiency and N-uptake efficiency at the intermediate N treatment (67 kg N ha⁻¹). Each point represents individual hybrids introduced between 1967 and 2006. kg_N, kg of fertilizer N; kg_{plantN}, kg of plant N.

$89 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Duvick and Cassman, 1999) reported in the literature. Grain yield at high N is the sum of grain yield at 0 kg N ha⁻¹ and the response of grain yield to applied N; consequently, the rate of gain for low N grain yield accounts for approximately 65% of improvement for grain yield at high N. By this same logic, response of grain yield to fertilizer N has increased at a rate of approximately $30 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Fig. 2C). The greater increase in grain yield at high N is consistent with common breeding and germplasm testing practices, which aim to evaluate the yield potential of new hybrid combinations at a high level of N. It is not immediately clear how evaluation and selection for grain yield at high N might impact grain yield at low N; however, multilocation testing across different environments with varying potential for N loss might result in selection of genotypes with high average performance. Previous comparisons of direct selection for grain yield at low N versus indirect selection at high N have shown that selection at high N results in greater performance over both levels of N (Brun and Dudley, 1989; Gallais et al., 2008). This response has generally been attributed to greater environmental variance and genotype \times environment interactions at low N and, consequently, decreased heritability of grain yield at low N (Gallais et al., 2008). An alternative yet complementary hypothesis is that physiological mechanisms for efficient assimilation and partitioning of N under deficit N conditions also influence utilization of N for grain production under high N fertility.

Grain yield increases stemming from genetic improvement were associated with a decrease in barren plants and an increase in kernel number on a per-area basis. Barren plants are usually associated with an increased anthesis-silking interval or ear abortion caused by environmental stresses including low N, drought, or high plant density (Buren et al., 1974; Bänziger and Lafitte,

1996; Lejeune et al., 1998; Campos et al., 2006). As such, the large negative correlation between barren plants and grain yield at low N is not unexpected, and barren plants were most prevalent in the 1970s hybrids grown at low N. Hybrids released in the 1970s had an average of 17% barren plants while 2000s era hybrids had an average of 5.8% barren plants at low N (Table 5). At the intermediate and high levels of N, hybrids from earlier decades also had increased barrenness relative to the most recently introduced hybrids, which may be a result of the high plant density under which we evaluated these hybrids. The mean plant density at harvest was 82,800 plants ha⁻¹, which is much greater than the typical plant densities that hybrids from earlier decades would have been grown at (Duvick, 2005). At low plant densities, however, older and newer hybrids have similar yields (Duvick et al., 2004), indicating that the density that we used in our study was a better treatment to discriminate genetic differences in grain yield and N response. As such, estimates of genetic gain in this study probably include not only a genetic component but also the increase in performance associated with an improved genetic × agronomic management interaction (Castleberry et al., 1984).

While individual kernel weight increased modestly as a result of genetic improvement, this increase was unaffected by N supply. Therefore, improvement of kernel weight can be explained by increased genetic potential at low N. Although not measured in this study, increased stay-green (i.e., delayed leaf senescence rate) and prolonged photosynthetic activity are traits that could result in higher individual kernel weight across N rates. Genetic improvement in maize has enhanced stay-green (Duvick and Cassman, 1999) and photosynthetic parameters (Ding et al., 2005; Echarte et al., 2008) although the impacts of changes in these traits on kernel weight potential have not been investigated. A positive correlation between kernel weight and grain yield was present at all levels of N; however, this correlation was only significant at the 67 and 252 kg N ha⁻¹ rates ($P \leq 0.01$) (Table 6). Although kernel weight has increased as a result of selection for grain yield, the apparent lack of an increase in the responsiveness of kernel weight to N supply suggests a potential opportunity for exploiting kernel weight as a contributor to future advances in maize grain yield and NUE.

There were no clear trends in stover biomass yield between hybrids representing different decades (data not shown), indicating that improvements in grain yield have been associated with increased total biomass resulting from greater postflowering accumulation of grain biomass as well as positive changes in harvest index. This result is in partial contradiction to the data summarized by Lorenz et al. (2010), who concluded that historical gains in maize yield in the United States have been mostly accompanied by increasing stover biomass and relatively constant

harvest index. An increase in harvest index supports the conclusion that genetic improvements for grain yield have acted to increase assimilate partitioning to the developing ear, mostly resulting in decreased barrenness and increased kernel number.

At low N, total plant N uptake did not increase with newer genotypes, yet grain yield improved. To examine this phenomenon, we measured GU, the physiological efficiency by which the plant uses accumulated N to produce grain under unfertilized conditions. Genetic utilization increased with genetic improvement at a rate of 0.24 kg kg_{plantN}⁻¹ yr⁻¹ ($R^2 = 0.37$) (Table 7; Fig. 3A) and paralleled the increases in grain yield at low N. We speculate that GU reflects physiological processes that occur at flowering since this composite trait was positively correlated with kernel number per ear and negatively correlated with barren plants. Together these results indicate that GU may represent a trait for efficient partitioning of C and/or N assimilates to the ear at the critical period for kernel set. One hypothesis is that genetic selection has acted on amino acid metabolism pathways within the developing earshoot to favor an increase in the Asn:Gln ratio, which has been previously shown to be reflective of plant N status under different levels of N fertilizer (Seebauer et al., 2004; Moose and Below, 2009). This earlier research suggests that the Asn:Gln ratio may be part of a signal transduction pathway that influences subsequent kernel development. This conclusion is supported by the findings of Martin et al. (2006) who showed that mutations for cytosolic glutamine synthetase isoenzymes (*gln1-3* and *gln1-4*) had distinct effects on maize yield components. Additionally, Cañas et al. (2009) showed that two inbred lines of maize differed considerably in the types and amounts of amino acids that accumulated in the cob and developing kernels.

The trend for decreased grain protein accumulation in response to selection for grain yield has previously been documented, suggesting altered patterns of C and N metabolism in more advanced maize germplasm (Duvick and Cassman, 1999; Scott et al., 2006; Gallais et al., 2008). Additionally, divergent selection for grain protein concentration in the Illinois Protein-Strains has resulted in markedly different patterns of N uptake and utilization (Uribe-larrea et al., 2007). Modeling studies have suggested that genetically decreasing minimum grain N concentration is a strategy for increasing grain yield at low N (Sinclair and Muchow, 1995; Muchow, 1998).

Nitrogen-use efficiency or unit of grain yield increase per unit of fertilizer N applied increased at a rate of 0.16 kg kg_N⁻¹ yr⁻¹ (Table 7). Since NUE is a product of its components, NUpE and NUtE, it can be altered by changes in one or both of its components (Moll et al., 1982). We detected an increase in NUpE but no change in NUtE in the panel of historical germplasm evaluated. Because NUpE quantifies the increase in plant N content

over the unfertilized check plot treatment per unit of applied N and since there were no apparent differences between older and newer hybrids for plant N content at R1 (Table 7), we conclude that improved NUpE must be a consequence of increased postflowering N uptake. Postflowering N uptake relies on sustained photosynthetic activity for NO_3^- assimilation as well as the continued supply of photoassimilates to the root system (Ma and Dwyer, 1998), potentially improving functional stay-green leading to increased individual kernel weight in more modern hybrids. Furthermore, the dependence of advanced maize germplasm on postflowering N uptake suggests that current transgenic technologies for controlling biotic pests such as Western corn rootworm larvae could have an impact on N uptake as a result of season-long protection of the root system (Kahler et al., 1985; Spike and Tollefson, 1991; Vaughn et al., 2005; Haegele and Below, 2013). The lack of improvement in NUtE over hybrid era was due to comparable increases in both grain yield and plant N uptake. Therefore, identifying and exploiting genetic variation for NUtE while maintaining high NUpE could be a promising strategy for improving NUE.

CONCLUSIONS

Maize germplasm has been selected under high input production practices. This approach might have resulted in hybrids that exhibit a large response to fertilizer N and therefore require increased levels of fertilizer N to achieve their genetic potential. Instead, our results demonstrate that genetic gain for grain yield at low N ($56 \text{ kg ha}^{-1} \text{ yr}^{-1}$) was nearly double the rate of genetic gain for response to fertilizer N ($30 \text{ kg ha}^{-1} \text{ yr}^{-1}$). Yield improvements in modern hybrids grown with sufficient N supply were associated with greater postflowering N uptake and NUE, increased kernel number, and decreased percent barren plants. The primary enhancement of grain yield at low N in modern hybrids has important implications for achieving further genetic improvement, yet the ability of the soil to supply N will represent an upper limit to grain yield at low N. As such, identifying and selectively improving traits associated with fertilizer N response will be necessary to maintain and accelerate the rate of genetic gain needed to increase maize yields.

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