

GENETIC IMPROVEMENT FOR NITROGEN UTILIZATION IN MAIZE

BY

JESSICA MARIE BUBERT

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2014

Urbana, Illinois

Master's Committee:

Professor Stephen Moose, Chair
Professor Fred Below
Professor Patrick Brown

Abstract

Increased nitrogen use efficiency (NUE) is an important target for future maize improvement. Essential to the design of an effective breeding program to select maize hybrids with enhanced NUE is an understanding of past progress, variation among maize germplasm for NUE and its component traits, and identification of phenotyping approaches to optimize genetic gain. We documented genetic variation for NUE and its component agronomic traits among a diverse collection of historical and recent elite maize inbreds and hybrids grown in field trials with different levels of soil N supply. Many of the genotypes evaluated also represent important resources for maize functional genomics. The results confirm previously reported trends for modern elite compared to historical hybrids, where grain yields have increased as a result of superior tolerance to higher plant densities, greater harvest index, and reductions in grain protein concentration. In addition, we demonstrate that past breeding has likely optimized N uptake for high grain yields, but that significant opportunities exist to further improve how maize plants utilize acquired N. We developed a phenotyping approach that estimates N utilization as the ratio of total biomass relative to total plant N, which effectively controls for the significant impacts of N-level, relative maturity, and heterosis on this trait. Using this measure of total N utilization, we identified the allelic genotypes associated with enhanced N utilization in the IBM population at nine previously identified potential NUE Quantitative Trait Loci (QTL). Selection of IBM lines showing maximum enrichment of alleles associated with high grain yield at low nitrogen at the potential QTLs did not shift the population means for grain yield or nitrogen utilization efficiency. The results from the QTL enrichment project substantiate the need to understand the mechanisms of heterosis before using QTL for marker assisted selection across populations and direct future research to elucidate these genetic mechanisms.

Table of Contents

CHAPTER 1: VARIATION FOR NITROGEN UTILIZATION EFFICIENCY AND ITS COMPONENT TRAITS AMONG DIVERSE MAIZE LINES.....	1
Introduction	1
Materials and Methods	5
Results and Discussion	10
Conclusions	19
References	19
Figures and Tables.....	27
 CHAPTER 2: QTL ENRICHMENT FOR IMPROVING NITROGEN UTILIZATION EFFICIENCY IN MAIZE	 42
Introduction	42
Materials and Methods	45
Results and Discussion	50
Conclusions	56
References	56
Figures and Tables.....	62
Appendix	80

Chapter 1 – Variation for Nitrogen Utilization Efficiency and its Component Traits Among Diverse Maize Lines

Introduction

As available land for agricultural production has stabilized, farmers rely on fertilizer application rates to boost yields. Consequently, nitrogen (N) use in the United States has increased nearly six fold in the last fifty years to nearly 13 million tons applied in 2012 (USDA, 2013). This trend is consistent worldwide with nitrogen demand at 105.3 million tons in 2011 and expected to increase 1.7 percent annually through 2015 (UN-FAO, 2011). While nitrogen fertilizer use is associated with yield increases, fertilization can be expensive for the producer. In 2010, Illinois farmers applied an average of 167 pounds per acre of nitrogen on their corn crop. At a price of just over \$1000 per short ton of nitrogen in farm fertilizers, N applications cost a producer \$88 per acre (USDA, 2013). Nitrogen fertilizer is a product of the Haber-Bosch process which is dependent on energy inputs to attain high pressure and temperature necessary to convert dinitrogen from the atmosphere to a form useable by plants. Therefore, as energy prices continue to climb the cost of nitrogen fertilizers will increase accordingly.

Additionally, the direct cost incurred by the producers does not include the indirect cost of nitrogen loss on water and air quality. Due to the volatile nature of N fertilizers, it has been estimated that between 35% and 65% of applied nitrogen is lost from soil systems. (Cassman et al., 2002; Jayasundara et al., 2007; Koocheki et al., 2013). The detrimental environmental effects on water and air quality of these nitrogen losses from denitrification, volatilization, and leaching have been well documented. Nitrates in groundwater supplies have been of particular interest from both a human health standpoint and because of the growth of the hypoxic zones on the United States coastal regions (Cole et al., 2006; Comly, 1945; Paulot & Jacob, 2014; Robertson & Vitousek, 2009). According to a recent analysis by Bodirsky et al. (2014), in order to feed the world and keep nitrogen pollution levels below critical levels, world N losses need to decrease to less than 15% by 2050. Furthermore, as a more immediate concern to producers, the Environmental Protection Agency has recently started enforcing more rigorous nutrient load limits in several areas, including the Chesapeake Bay. These conditions are indicative of the need to improve the efficiency with which plants can use available nitrogen.

Nitrogen use efficiency (NUE) is commonly defined as yield per unit of nitrogen applied (Moll et al., 1982). It can be described as the product of two main component traits, nitrogen uptake and nitrogen utilization efficiency which describe different physiological processes in the nitrogen pathway. Nitrogen uptake efficiency (NUpE) is the ratio of total N in the plant at maturity over the total N supply to the plant and acts as a measure of how well the plant absorbs available N, including applied N fertilizer. Nitrogen utilization efficiency (NUtE) is the ratio of grain weight to total N in the plant at maturity and describes how well the plants used the absorbed N to make grain (Moll et al., 1982).

Nitrogen uptake efficiency has been widely studied and is known to be highly influenced by agronomic practices and environmental factors. Nitrogen uptake has been shown to be greater with higher planting density as well as high plant populations and narrow row spacing (Barbieri et al., 2008; Ciampitti & Vyn, 2011; Cox & Cherney, 2001; Rosolem et al., 1993). These effects are likely seen because increased root length density in the interrow allows the root distribution in these plots to be more advantageous for nitrogen absorption (Sharratt & McWilliams, 2005). Several other studies have supported the importance of root architecture in plant nitrogen acquisition (Lynch, 2013; Mi et al., 2010; Pace & McClure, 1986; Wiesler & Horst, 1994). Additionally, cultural agronomic fertilization practices including N application rate, form of supplied N and application timing, can affect NUpE (Jayasundara et al., 2007; McTaggart & Smith, 1995; Subedi & Ma, 2005; Wang & Below, 1992). Finally, environmental factors including soil quality, water availability, and the residence time of nitrogen in the rhizosphere, impact nitrogen uptake (Kirda et al., 2005; Scholberg et al., 2002; Zotarelli et al., 2008).

In contrast to the sensitivity of NUpE to environmental conditions and cultural practice, NUtE is highly dependent on the physiological processes of the plant itself. Utilization efficiency is indicative of how the N in the plant is used to stimulate earshot and kernel growth. NUtE is effectively a measure of the sink capacity of the plant and how well it can remobilize accumulated N stored in the vegetative tissue to the ear (Pollmer et al., 1979). Considered the optimal combination between N remobilization efficiency and N assimilation efficiency, NUtE is governed by complex network of nitrogen cycling enzymes and processes (Masclaux-Daubresse et al., 2010).

As the two components of nitrogen utilization efficiency, NUpE and NUtE have a dynamic relationship. Previous studies have indicated that maize plants can compensate for a lack of N during their later growth stages by utilizing stored N (Friedrich et al., 1979). Others showed that N response was affected by genetic differences, management practices, soil factors and climate (Asghari & Hanson, 1984; Sabata & Mason, 1992; Tremblay et al., 2012). For these reasons, the consistency of field and sampling methods are extremely important in any study examining nitrogen use efficiency and its component traits – especially when trying to compare genetic differences across diverse germplasm.

NUE and its component traits in maize are usually assessed by growing inbreds or hybrids under a series of high and low applied nitrogen conditions in plots under a long term fertility management regime. Plants are sampled from the experimental plots and measured for both biomass and N accumulation at various growth stages as well as measured for the amount of N partitioned to the vegetative tissues versus the grain (Moll et al., 1982; Pollmer et al., 1979). Total plant N content is a crucial measurement used to estimate the component traits of nitrogen utilization efficiency so it is important to use methods that accurately quantify both grain and stover N. N availability within a field varies at a range of 1 to 20 meters, so an experimental design that minimizes the spatial variation between nitrogen treatments is imperative (Robertson et al., 1988).

In studies stretching over the past thirty years, diversity for NUE and its component traits have been observed in a variety of maize germplasm (Gallais & Hirel, 2004; Moll et al., 1982; Pace & McClure, 1986; Pollmer et al., 1979; Tsai et al., 1992). Several studies have specifically looked at the diversity available for use in maize producing regions in the developing world where low soil nitrogen and tropical climates are a concern. Abe et al. (2013) evaluated the performance of both experimental and commercial tropical hybrids under high and low N conditions and determined that the grain yield and yield component response varied with N supply. They concluded that some hybrid lines exhibited high grain yields across N levels ranging from no-N to high-N and would be ideal for use in developing countries with poor grain yield and inconsistent N fertilizer availability. These results confirmed several other studies that found sufficient diversity in tropical germplasm to allow for improvement in grain yield in low N environments using direct selection for the trait (Bänziger et al., 1997; Lafitte & Edmeades, 1994a, 1994b). Similar results

were found in both North American temperate and European germplasm (Balko & Russell, 1980; Presterl et al., 2002). Comparable variation has been identified and utilized to find alleles to improve NUE traits in other crop plants including rice, wheat, and barley (Hirel et al., 2007; Muurinen et al., 2006).

Previous studies that have included both hybrids and their respective inbred parents have been inconclusive in determining if inbreds can be used to predict hybrid performance. Several studies found positive but non-significant correlations between the inbred parent and hybrid progeny N use efficiencies. Their conclusions ranged from inbred performance being an ineffective predictor of hybrid efficiency (Balko & Russell, 1980) to the suggestion that selection on inbred lines in some environments could work to predict hybrid performance (Lafitte & Edmeades, 1995). Others found a positive and significant correlation between the inbred and hybrid NUE. Presterl et al. (2002) recommends using indirect selection on inbreds as a preliminary screening for NUE, while Zaidi et al. (2003) maintains that *per se* performance of inbred lines can be used to predict hybrid NUE.

While there is sufficient evidence that inbred lines are responsive to N, the mechanism of their contribution to their hybrid progeny has not yet been clearly defined (Balko & Russell, 1980; Beauchamp et al., 1976). In tropical and temperate germplasm, a comparison of general combining ability (GCA) and specific combining ability (SCA) indicated that the inheritance pattern for N traits showed an additive effect rather than dominant (Below et al., 1996; Di Fonzo et al., 1982; Rizzi et al., 1993). Others, however, suggest that non additive effects are significant for nitrogen use efficiency traits (Gama et al., 2002; Meseke et al., 2013).

Balko and Russell (1980) reported that inbred response to N is significantly lower than hybrid response to N and inbred have lower values for NUE. This phenomenon could be due to the small stature and lower grain yield of inbreds as compared to their hybrid progeny. Decreased stover and grain biomass would result in a lower amount of available N needed to reach the peak of the nitrogen response curve. It is possible that soil nitrogen is sufficient for inbreds to reach this peak and their maximum growth potential, so additional nitrogen application does not improve performance. The absence of a response in the inbreds is an indication that inbred lines may not be a good predictor of hybrid performance.

In addition to the importance of using hybrids in breeding programs for NUE, the N level under which selections occur can influence the resulting lines. There is a negative correlation between the two NUE component traits and available N (Worku et al., 2007). NUpE decreases from high N to low N and the opposite is true for NUtE (Bertin & Gallais, 2000). Additionally, high heritability exists for grain yield (GY) and other traits under both high and low N levels, so selection under the N environment of interest would be ideal for improving NUE (Presterl et al., 2002). Most improvement in NUE over the last several decades is a result of improved NUpE, which could be the result of continued selection under high N in modern North American maize breeding programs (Haegele et al., 2013).

Since most of the improvement for maize NUE has come from the improvement of NUpE, there is potentially untapped genetic variation for NUtE that could be exploited to further enhance NUE, especially for low N environments. The purpose of this study was to identify that genetic variation for NUtE using a comprehensive, multi-year experiment grown in managed N environments. In order to capture the diversity available in maize today, six different populations were included in the testing which took place over the ten years between 2003 and 2013.

Materials and Methods

Germplasm

IPS

The Illinois Long Term Selection project was developed in the late 19th century as a tool to look at divergent selection for protein and oil content in maize. Now one of the longest continuous selection experiments in the world, the Illinois Protein Strains (IPS) that come from that experiment provide a unique tool to look at the effect of high and low protein on NUE and its component traits. The populations were developed as described by Dudley and Lambert (2004). Illinois High Protein (IHP) and Illinois Low Protein (ILP) are the derivatives of selection for the ears with the highest and lowest protein in the population with 32% and 4% protein, respectively, after 110 cycles of selection. At cycle 90, thirty S₁ lines were selected from both the ILP and IHP populations and self-pollinated to create S₆ inbreds for high protein (IHP1) and low protein (ILP1) (Uribelarrea et al., 2004). Illinois Reverse High Protein (IRHP1) and Illinois Reverse

Low Protein (IRLP1) are inbreds formed from reverse selection initiated at cycle 48 and self-pollinated at cycle 90.

NAM

The maize Nested Association Mapping (NAM) is a population designed to capture the diversity of maize and preserve historic linkage disequilibrium. The first reduced iteration of this population was for use in QTL mapping and association studies. It consisted of 302 lines, termed the maize association population, selected from thousands of inbreds to capture the majority of alleles in cultivated maize (Flint-Garcia et al., 2005). These 302 lines were then narrowed down based on simple sequence repeats (SSR) to 25 inbreds that captured the maximal amount of diversity. These lines were then crossed by B73 and self-pollinated to form 200 recombinant inbred lines (RILs) per inbred family as described by McMullen et al. (2009). The parents of the NAM population were made into hybrid families with B73, IHP1, ILP1, and LH82 as testers (Table A.1).

Tropicals

A subset of the 302 diverse lines selected by Flint-Garcia et al. (2005) are considered tropical germplasm. These lines are photoperiod sensitive compared to temperate maize, so growing them at northern latitudes results in distinct phenotypic attributes including delayed flowering, increased plant height and a greater total leaf number as well as decreased grain formation. As a result of these traits, and their impact on harvest index (HI), tropical germplasm is a unique source to explore the effect of harvest index on NUE and its component traits. Additionally, it was recently shown that tropical by temperate maize crosses could produce the amount of ethanol per hectare as modern grain hybrids with a lower N requirement, implying that these tropical lines may be a unique source of alleles for NUtE or NUpE (White et al., 2012). The tropical lines were made into hybrid families with B73, IHP1, ILP1, and LH82 as testers (Table A.1).

IBM

The intermated B73 x Mo17 (IBM) population was originally developed to improve genetic resolution for mapping efforts by increasing the number of recombination events and decreasing linkage disequilibrium before fixing the alleles through self-pollination to RILs (Lee et al.,

2002). These IBM RIL lines were first mapped with 1000 restriction fragment length polymorphism (RFLP) loci and over 700SSR loci. Cone et al. (2002) extrapolated loci from non-IBM maps to their nearest neighbor onto the framework formed by the IBM map to create the IBM Neighbors map with more than 4,900 markers. Recent advancements in genotyping using a genotyping-by-sequencing as described by Elshire et al. (2011) have resulted in the addition of more than 2 million single nucleotide polymorphism (SNP) markers to the maize map. IBM RIL lines were made into hybrids with IHP1, ILP1, and LH82 as testers (Table A.1).

ex-PVP

The recent available germplasm are those lines that have recently come off of plant variety patents (ex-PVP). These lines are often from the private maize breeding industry so their pedigree is less defined than those from older public breeding programs, but they would be considered elite lines when compared to the older public material. More than 90 of the ex-PVP lines that came off patent in the mid 2000's were genotyped with more than 750 SNP markers to provide a clearer understanding of the background of and relationship between these lines and the private maize breeding industry as a whole (Nelson et al., 2008). These lines provide opportunities to look at the diversity for NUE currently available in private maize breeding programs, as well as identify particular families that may have a particularly high affinity for NUE or its component traits. The ex-PVP inbreds were crossed to B73, IHP1, ILP1, and LH82 as testers (Table A.1).

A complete list of lines included in each population are included in Table A.2.

Environmental Measurements

Average monthly precipitation as well as maximum and minimum temperatures were recorded daily by the State Climatologist Office via an electronic maximum-minimum temperature sensor (MMTS) and standard 8-inch rain gauge as described by Dr. Jim Angel (Angel, 2009). The data was collected at a weather station with the coordinates 40° 5' 3", -88° 14' 26" which is less than half a mile from the experimental sites used for the NUE trials.

Field Trials

Field trials were conducted during each growing season between 2003 and 2013 at the Department of Crop Sciences Research and Education Center in Urbana, IL on field sites previously shown to be N responsive (Gentry et al., 2001). The soil pH averaged 6.2 with 3.7% organic matter and is classified as Drummer silty clay loam. Based on N recovery from plots with no applied N fertilizer, the soil N capacity was around 90 kg/ha. The trial sites were subjected to a corn-soybean rotation in order to homogenize the soil N as much as possible. Irrigation was applied to the entire experiment upon observation of drought stress symptoms (leaf rolling). Weed pressure was eliminated using both chemical spray applications and hand cultivation.

Experiments were arranged in a replicated split plot design with hybrid as the main plot and nitrogen treatment as the sub plot. This was achieved by pairing the high and low plots for each genotype in adjacent ranges. The paired plot design was selected to minimize spatial variation of nitrogen. Genotypes were arranged by maturity rather than being randomized within the main plot in order to facilitate sampling and avoid inter-row shading stress. Hybrid plots were 5.3 m long with .76 m spacing between rows. They were hand-planted at a target density of 74,000 plants per hectare. Inbred plots were 3.66 m long with .76 m spacing between rows and were hand-planted at a target density of 70,000 plants per hectare. In 2003, nitrogen was applied at rates of 0, 56, 112, 200 kilograms. In 2004 and 2005, supplemental nitrogen was applied at rates of 0, 75, 150, 225 kilograms per hectare. From 2006-2013, low nitrogen plots received no supplemental nitrogen while high nitrogen hybrid plots received 200 kilograms per hectare and high nitrogen inbred plots received 100 kilograms per hectare. All nitrogen was applied at V3-V4 in the inter-row space as a diffuse band of granular ammonium sulfate (21-0-0-24S) and incorporated with either hand cultivation or a rain event.

Sampling

Plant biomass and N content were measured at the V8, R1, and R6 growth stage in 2003, 2004 and 2005. For V8 and R1 sampling, the aboveground biomass of three plants was harvested from each plot. If stands were low, only two plants were used in order to maintain an end of season stand of 65,000 plants per hectare. R6 measurements were taken every year from 2003 to

2013. The R6 growth stage, or physiological maturity, was determined by the appearance of a visible black layer at the base of the kernels in half of the plants in that plot. Four plants per plot were selected for R6 measurements. Evenly spaced plants from the middle of the row were selected; end plants and double plants were excluded from consideration. Once all aboveground biomass was harvested, all ears and cobs were partitioned from the stover portion of the plants.

The combined stover fresh weight of the plants was recorded. The plants were then chopped in a Vermeer wood chipper and an aliquot (100-250 grams) of the chopped stover was weighed and saved for further analysis. Stover aliquots were dried for approximately seven days in forced air dryers at approximately 75°C. Dry samples aliquots were weighed. These measurements were then used to calculate the moisture adjustment and, subsequently, the total sample dry weight. The stover was then ground in a Wiley laboratory mill to pass through a 2 mm mesh screen and analyzed for percent nitrogen using combustion analysis in a Fissions NA 2000 N Analyzer.

The ears removed from the R6 plants were dried at 35°C to 9-10% moisture. Each plot was shelled either mechanically (hybrids) or by hand (inbreds) and each partition, grain and cob, was weighed. From 2003-2011, a representative sample of approximately 30g of the grain was ground and concentrations of starch, oil and protein were determined using a Dickey-John Instalab 600 near infrared reflectance analyzer. In 2012 and 2013, the whole kernel samples were run on a Perten DA 7200 Near Infrared (NIR) analyzer for moisture, oil, protein, and starch measurements. Grain nitrogen was calculated from % protein by using the %N to %protein conversion factor of 6.25 as used in the Kjeldahl method of composition analysis (Jones, 1931).

Table A.1 shows a summary of the populations, crosses, N rates and samples taken for each year of the experiment.

Analysis

Total Nitrogen Utilization is calculated as total biomass divided by total plant nitrogen. Grain Nitrogen Utilization is calculated as grain biomass divided by total plant nitrogen. Unless marked as grain N Utilization, all NUtE shown are total N utilization. Grain yields were mathematically adjusted to 0% moisture. Correlations and P-values were calculated using the cor.Prob function in R, using the Pearson's method to determine correlation coefficients (R, 2008). The lm function in R was used to fit linear regressions with fixed effects.

Normality was tested using the Anderson-Darling test via the `as.test` function as a part of the R package `nortest`. Two sample means separation tests were done using the `t.test` function in R, which uses a two-sample Student's *t* for equal variances and a Welch corrected two sample *t*-test for non-equal variances. Wilcoxon rank sum means separation *t*-tests were used for non-normal two sample tests. Fisher's least significant difference test was used for multiple means separations, with an $\alpha=0.05$. Density functions were constructed using the `plot_density` option in the `ggplot2` package of R which uses a kernel density method to construct the functions. The y-axis for kernel density plots was scaled to one.

Sources of variation and variance components were calculated using PROC MIXED in SAS (SAS, 2009). A linear mixed model was fit with year and N rate as fixed effects and genotype as a random effect. Since every genotype in a population was not included in the test, the use of genotype as a random effect allows for the results to be applied to the entire population. Within the PROC MIXED function, Restricted Estimation of Maximum Likelihood (REML) was used to calculate the variance components of the random effects and their standard errors

Results and Discussion

Environmental Variation

A comparison of environmental conditions among the ten years during which these trials were done show levels of variation in precipitation and temperature that are not unexpected. Precipitation in May and August tended to be below the twenty year average while precipitation in June, July, September, and October tended to fluctuate above and below the twenty year average. Of particular interest are the 2011 and 2012 growing seasons. 2011 was dry through flowering and grain set in July and August and may have had an effect on yield. 2012 was dry through planting, flowering and grain set, May through August. This would be considered an extremely dry growing season and should be considered accordingly when looking at analysis of NUE and its component traits. Figure A.1 contains a summary of average monthly precipitation compared to the 20 year averages (panel A) as well as maximum, minimum and average monthly temperatures compared to the 20 year average (panel B).

NUE Assay: Growth Stage

The early years of these NUE studies, from 2003-2005, included measurements at three different growth stages – V8, R1, and R6. The values from the early sampling, V8 and R1, can be used to determine if early sampling could be a more time efficient early screening for terminal NUtE by comparing them to the NUtE values at R6. Neither of the early sampling times provide measurements of grain biomass or grain N, so the NUtE measurement provided at those points is more a measure of N uptake ability of the plant at each growth stage and provides little information about remobilization. Linear regression indicates that the strong significant relationship between R1 and R6 NUtE could be used in a predictive manner, as the regression equation $y=0.723x-.2432$ for that relationship has an $R^2=0.6067$ (Figure 1.1). Linear regression analysis of V8 NUtE with R6 NUtE (Figure 1.1) as well as R6 grain NUtE with V8 and R1 early measurements resulted in coefficients of determination of 0.1583, 0.187, and .0101 respectively indicating that these sampling times have little utility as predictors of terminal grain NUtE

NUE Assay: Confounding Effect of Maturity

In 2008 and 2009, the NAM diversity parents and a subset of elite (ex-PVP) lines were all grown as hybrids with B73 as a tester. These inbreds show a range for the days to tasseling phenotype, which can be used as a proxy for maturity, when grown in Urbana, Illinois (Buckler et al., 2009; Hauck et al., 2014). One elite line (PHJ40) and one diversity line (P39), which is a sweet corn line, show the earliest maturity with 62 days to tassel. As expected, the photoperiod sensitive tropical lines are the latest maturing, needing up to 93 days to tassel. Since there is such diversity for maturity within maize germplasm, maturity is often considered a confounding effect for many analyses of phenotypic traits including grain yield and biomass. This consideration is confirmed in our data set when grain N Utilization Efficiency is compared to relative maturity. As maturity increases, the time at which plants transition from vegetative to reproductive phase nears the end of the growing season so stover biomass is greater and grain biomass is decreased. This is exemplified by the lower grain NUtE in the tropical lines (Figure 1.2). With maturity considered a fixed effect, a linear regression of grain NUtE against maturity showed a slope of -1.0672. The corresponding Analysis of Variance (ANOVA) showed maturity as a highly significant effect ($p<0.0001$). Comparing total NUtE against maturity does not show a confounding effect (Figure 1.2). Linear regression of total NUtE against maturity showed no

significant effect of maturity. This indicates that total NUtE is an NUE phenotype that can be analyzed without the confounding effect of maturity.

Historical Perspectives

Historical trends in NUE component traits are observed when comparing inbred parent lines from each decade from the beginning of modern hybrid breeding in the late 1940's through current commercial breeding lines. Data shown in Figure 1.3 shows the results of a comparison of total biomass, grain yield (grain biomass) and total plant N content for six representative lines for those decades as hybrids with the tester LH82 grown from 2011-2013. This comparison shows little to no increase in total plant N content over the last 40 years, indicating that N uptake has not been improved by maize breeding over the last seventy years. Additionally, total plant N at high nitrogen rates across all eras is not significantly higher than at low nitrogen rates. This shows that there is little room for improvement of NUpE and breeding could be better focused on NUtE. Remobilization has changed over the decades of breeding as evidenced by the increases in grain biomass as total N content remains constant, which indicates that N is being remobilized for use in growth more efficiently. As it is the main focus of most breeders, grain biomass has steadily increased at both low and high N rates since 1950. Total biomass increases until the 1970's when breeders tried to alter the harvest index (HI; ratio of grain biomass to total above-ground biomass) of maize as a strategy for increasing yield. This resulted in decreasing stover biomass while keeping grain biomass constant or increasing and is confirmed by the decrease in total biomass while grain biomass is constant between 1970 and 1990. Since then, harvest index has been fixed and yield increases are due to a general increase in plant size, as shown by the spike in total biomass and grain biomass. Each representative line shows a response to N, the grain biomass is greater at the high N rate as compared to the low N rate. This supports the theory put forth by Carlone et al. (1987) that maize lines have been bred in high N environment for performance in high N environments, resulting in the selection of N responsive lines and leaving genetic potential for the development of lines that have superior yields with lower N requirements.

Nitrogen Response

The twenty five diversity parents were crossed to B73 as a tester and grown in 2004 and 2005 under a series of N rates spanning from 0 kg/ha applied N to 225 kg/ha applied N. The N response varies among the diversity lines, but can be separated into four different groups. Table 1.2 shows the resulting grain yields for each hybrid and the groups they belong to across all N rates. The first group is those lines that show little response to N, their yields did not increase notably with increased applied N. This group included a popcorn line (Hp301), a sweetcorn line (P39), and a temperate line (MS71). The popcorn and sweet corn would have low levels of heterosis with a temperate line like B73 and MS71 is one of the most highly related lines to B73 also resulting in low heterosis, which could account for their low N response. Lines that had a large response with only the first additional dose of N were included in the second group. B73 x Ki3 is an example of one of these lines, exhibiting an increase of almost 3 Mg/ha for GY with the first 75 kg/ha of applied N followed by a GY increase of less than .5 Mg/ha over the following 150 kg/ha of applied N. The third group are those lines which showed a maximum GY after 150 kg/ha of applied N. B73 x CML103 falls in this category with increases of 1.1 and 1.5 Mg/ha GY for the first and second N applications respectively but no increase in GY when 225 kg/ha N was applied. The final group includes those lines that increased across all N rates like B73 x Oh7B which showed a GY increase of approximately 1.5 Mg/ha with each subsequent N increase.

As seen in previous studies, most lines exhibited the greatest response with the first increment of applied N, followed by varying levels of response to successive applications. Many of the lines reached maximum yields at either 150 or 225 kg/ha applied N. The overall average for all 25 diversity hybrids followed the same trend, with the greatest response at the first N rate and then smaller increases through the highest rate. To further illustrate the variation for N response in the diversity population, several hybrids are bolded in Table 1.1 that all show similar GY at 0N but vary in their response to the applied N resulting in dissimilar GY at 225N. B73 x M37W recorded the greatest response with an increase of 3.5 Mg/ha GY at 225N, while B73 x MS71 had the smallest response with an increase of only 0.4 Mg/ha from 0N to 225N.

Temperate versus Tropical Germplasm

To determine if tropical adapted germplasm is a viable source of NUtE genes, a comparison of the NUtE of tropical germplasm to the NUtE of temperate germplasm is useful. Under low N conditions, tropical germplasm shows a wider range of values for NUtE and a tail of high NUtE values that exceed those of temperate germplasm (Figure 1.4, panel A). When looking at the temperate and tropical hybrids across the entire data set and all four testers, the mean of the tropical germplasm was 192.7 g/plant while the temperate germplasm averaged only 157.9 g/plant, a highly significant difference ($p=1.03e-8$). These high NUtE lines may exist because many tropical lines were developed in low N environments and are adapted to perform at low N. Because the temperate germplasm was developed under high N environments, these lines are less efficient under low conditions. This highlights the importance of developing NUtE efficient lines under the condition in which they will be grown. Under high N, as mentioned previously, the NUtE values are generally lower as the plants are provided enough N that they don't need to be as efficient with it (Figure 1.4, panel B). The difference between the temperate and tropical populations diminishes under high N environments, because both populations are able to produce their maximum amount of biomass without being limited by N metabolism. At high N, the means for tropical and temperate germplasm were 104.6 and 93.2 respectively, a difference that was not significant at the stringent $\alpha = .001$ level.

Hybrid versus inbred NUtE

As indicated in previous studies, this dataset exhibited a widespread trend of hybrid NUtE being generally higher than that of inbreds. One example of such a relationship is shown in Figure 1.5. A diverse set of inbreds including NAM parents and ex-PVP lines were grown as both inbreds and as hybrids with an LH82 tester in 2012-2013. The distribution of the inbreds at low N (Figure 1.5, panel A) ranged from 25 to 175 for NUtE, while the distribution for hybrids ranged from 75 to over 200. The means for the inbreds and hybrids were 82.1 and 127.7 respectively. Comparison of the inbred and hybrid populations resulted in a highly significant adjusted two-sample t-test statistic of -15.59 ($p<2.2e-16$), indicating that the inbreds and hybrids are significantly different. The same hybrids and inbreds were also grown at high N (Figure 1.5, panel B). The inbred mean for NUtE was 71.8 while the hybrid mean for the same trait was

85.3. The difference between means was less defined than at low N, but the difference was still statistically significant with an adjusted 2 sample t-test statistic of -5.70 ($p < 1.06e-7$).

Predicting hybrid performance from inbred parents

An examination of the suitability of using per se inbred performance to predict that of hybrids resulted in variable data similar to that previously reported. The diversity parents and some IBM recombinant inbred lines (RILs) were grown as inbreds and as hybrids at the same N rate in the same year with B73 (2010), IHP (2010), ILP (2010), and LH82 (2012, 2013) as testers. Since the inbreds and their corresponding hybrids were grown within the same year, environmental variation is minimal and direct comparisons of their NUE component traits is possible.

With LH82 as a tester, inbred performance showed no significant correlation with hybrid NUtE values under low or high. Under high N, inbred biomass was significantly correlated with hybrid biomass, but not with any other traits. Under IHP and ILP, however, inbred traits were more significantly correlated with hybrid performance. With IHP as a tester, inbred stover biomass, stover N, total biomass and Grain NUtE were all highly significantly correlated with hybrid grain NUtE. While most inbred traits were significantly correlated to hybrid stover biomass and stover N, none were significantly correlated to NUtE. Most all of these correlations disappear under high N, with only inbred stover biomass, total biomass, and grain NUtE remaining significantly correlated to hybrid grain NUtE. Similar results are seen with ILP as a tester. At low N, inbred stover biomass, stover N, total biomass, total N and Grain NUtE are highly significantly correlated to hybrid grain NUtE, but no inbred traits are correlated to NUtE. Under high N, the levels of significance decrease between the inbred lines and their hybrid parents. With B73 as a tester, hybrid grain N and stover biomass could be predicted via the inbred phenotype, but other traits showed no significant correlation. The correlation between inbred and hybrid stover biomass is consistently significant across all testers indicating that it is less sensitive to heterosis when compared to the other traits. Significant correlations found in the complete correlation tables for B73 (Table A.4a), IHP (Table A.4b), ILP (Table A.4c) and LH82 (Table A.4d) as testers demonstrate that tester plays an important role in hybrid NUE and its component traits.

Variation for NUE and Component Traits

General descriptive statistics of 0N GY, GY response to N and NUtE at both high and low N levels, indicate that there exists a high amount of variation for those traits within both the Diversity (NAM) population and the ex-PVP elite lines. This variation persists across three different testers (Table 1.2). Within each tester group, the means and ranges are similar for Diversity and Elite for GY response to N and NUtE. The more recent ex-PVP lines have slightly higher means for GY at 0N. There is no clear trend in the amount of variance between the older Diversity germplasm and the newer ex-PVP lines, but in both the IHP1 and ILP1 tester groups, the diversity lines show higher variance for GY at 0N, but lower variance for response to N.

Sources of Variation

The sources of the variation seen in the hybrids can be further partitioned through the use of a linear mixed model. Both year and N rate were considered fixed effects. Within the NAM population with B73 as a tester, year and N rate were significant effects for most traits (Table 1.3). This was consistent across testers and populations (data not shown). The interaction effect was significant for all traits with B73 as a tester, but not with the other three testers.

Within the ex-PVP population, both main effects of year and N rate and the interaction effects are significant for all three testers. The main effects of N rate and year are more highly significant when LH82 is the tester as compared to IHP1 and ILP1 as testers. This may indicate that LH82 itself is highly responsive to N availability, or is contributing genes that increase response to N. Finally, in the IBM population, all three testers had significant variance contributions from year, N rate, and the interaction for all traits. Since the lines in the IBM population are highly related, it is reasonable that variation would come from the environment.

Variance components associated with the random effect of genotype were significant for several traits across the different combinations of population and tester. Grain NUtE was significantly associated with genotype in four of the ten combinations, while genotype explained a significant portion of the variation for stover biomass in five of the combinations. For some traits, however, there is enough genetic variation to support the possibility for improvement of NUtE and its component traits. The high significance of the main effects of N rate and year across populations

and testers are an indication that further analysis should be done on the years and N rates individually

Tester effects

Variation in combining ability is apparent when the means and ranges of grain yield, N utilization efficiency and harvest index are compared across testers (Figure 1.6). The effects on grain yield are especially obvious in the low N environments where lines testcrossed to IHP1 showed the lowest yield, which is to be expected as IHP1 as an inbred has extremely low yield. The IHP1 testcrosses also had the lowest N utilization at high N, although NUtE at low N did not vary across testers under low N in 2010 and 2011. LH82 as a tester resulted in the highest grain yields and harvest index, likely because it is a recent elite line selected for its ability to produce yield. It was not, however, a standout in N utilization efficiency, which may be an artifact of being developed under high N conditions. Additionally, the distribution for all traits at all N rates tend to be more condensed for LH82 testcrosses than for other testers which may be indicative of the ability of LH82 to combine well with poor performing inbreds, but not with well performing inbreds, decreasing the range of resulting phenotypes.

B73 as a tester results in high grain biomass, NUtE and harvest index across N rates. It is possible that the high performance of B73 as a tester is due to its combining ability as a stiff stalk with the rest of the NAM parents which are all non-stiff stalk lines. The effect of environment on the overall performance of the testers can be visualized in the IHP1 and ILP1 testcross populations which were grown in both 2010 and 2011. Under both N conditions for both testers, 2011 had lower grain yield than in 2010. The opposite is true of N utilization efficiency, as 2011 was higher than 2010 for both testers at both N levels. The means for harvest index did not differ between the two years at either N condition, but the ranges for harvest index values across the populations were much wider in 2010 than in 2011 for both N levels.

The behavior of the testers shown in Figure 1.6 are confirmed when the same testers are crossed to other populations and trends in grain yield (Figure 1.7a), NUtE (Figure 1.7b) and harvest index (1.7c) are examined. For B73 and LH82, the means for grain yield did not change significantly when they were testcrossed to the NAM (diversity) lines and the ex-PVP lines, but the means showed a higher trend under high N and the range of values increased with the ex-

PVP population. Since that population comes from a large background pool including several heterotic groups, the additional variation in grain yield is not unexpected. Additionally, the high performance of LH82 testcrossed to the ex-PVP lines indicates that it may have good general combining ability and could be used as an elite tester for future experiments involving multiple heterotic groups. IHP1 and ILP1 had similar grain yield means regardless of which population they were crossed to, however, at high N in 2010 the ex-PVP lines had significantly higher grain yields than the NAM lines. It is possible that the weather that year was such that the ex-PVP alleles were contributing more positive effect alleles than the NAM lines due to previous selection under the same environment.

When B73 and LH82 are used as testers, NUtE tended to be lower in ex-PVP hybrids when compared to NAM hybrids. There was no difference in NUtE between the ex-PVP hybrids and the NAM hybrids when ILP1 and IHP1 were used as testers, but ILP1 testcrosses had higher values than IHP1 testcrosses, confirming trends previously shown. Harvest index shows the same tendencies as grain yield, with the ex-PVP hybrids showing higher harvest index than the NAM hybrids when B73 and LH82 are used as testers, a trend most likely caused by recent improvement in ex-PVP germplasm compared to the NAM parent lines. In 2010, at both high and low N the NAM hybrids showed a greater range of harvest index than the ex-PVP hybrids. Finally hybrids with B73 or LH82 as testers tended to have higher harvest index values than those hybrids with ILP1 and IHP1 as testers, confirming the superior performance and combining ability of B73 and LH82 as testers.

Commercial maize hybrids are typically the result of a cross between inbred lines of two different heterotic groups. The main groups are the stiff stalks (SS), of which B73 is a member, and the non-stiff stalks (NSS). It would be expected that testers from different heterotic groups would perform differently in hybrids with lines from the different heterotic groups. B73 shows a significant increase in both grain yield (Figure 1.8a) and harvest index (Figure 1.8c), but not NUtE (Figure 1.8b) at both N levels when used as a tester with non-stiff stalk lines as opposed to stiff stalk lines. This is to be expected as the NSS crosses would be crosses made across heterotic group and SS crosses would be made within a heterotic group. IHP and ILP as testers show no preferential combining ability with either the stiff stalks or the non-stiff stalks, perhaps because they have not undergone the selection for heterotic patterns at traits related to NUE as

commercial lines have. LH82 is a member of the iodent subgroup of the non-stiff stalk group but does not show preferential performance when crossed with the stiff stalk or non-stiff stalk groups, possibly because most of the non-stiff stalk lines used in this study belong to the Lancaster subgroup.

Conclusions

Historically, maize lines have been under selection in high N environments, resulting in indirect selection for inefficient nitrogen use. With proper sampling that involves testcross hybrids lines and a whole biomass measurement, NUE and its component traits can be evaluated and subject to direct selection. Variation exists for stover traits, grain traits, and the resulting NUpE traits within both historical and more recent elite germplasm, including tropical low N adapted lines. This variation can be exploited to improve the performance of maize hybrids in low N environments, which may become prevalent as government regulations on nitrogen applications are a perceived eventuality.

References

- Abe, A., Adetimirin, V., Menkir, A., Moose, S., & Olaniyan, A. (2013). Performance of Tropical Maize Hybrids Under Conditions of Low and Optimum Levels of Nitrogen Fertilizer Application *Maydica*, 58(1), 141-150.
- Angel, J. (2009). Weather Instruments for Measuring the Climate of Illinois. *State Climatologist Office for Illinios*. 2014, from http://www.sws.uiuc.edu/atmos/statecli/Instruments/weather_instruments.htm
- Asghari, M., & Hanson, R. G. (1984). Climate, Management, and N Effect on Corn Leaf N, Yield, and Grain N1. *Agronomy Journal*, 76(6), 911. doi: 10.2134/agronj1984.00021962007600060012x
- Balko, L., & Russell, W. (1980). Effects of rates of nitrogen fertilizer on maize inbred lines and hybrid progeny. I. Prediction of yield response. *Maydica*, 25(2), 65-79.
- Balko, L. G., & Russell, W. A. (1980). Response of Maize Inbred Lines to N-Fertilizer. *Agronomy Journal*, 72(5), 723-728.

- Bänziger, M., Betrán, F. J., & Lafitte, H. R. (1997). Efficiency of High-Nitrogen Selection Environments for Improving Maize for Low-Nitrogen Target Environments. *Crop Science*, 37(4), 1103.
- Barbieri, P. A., Echeverria, H. E., Rozas, H. R. S., & Andrade, F. H. (2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal*, 100(4), 1094-1100. doi: DOI 10.2134/agronj2006.0057
- Beauchamp, E. G., Kannenberg, L. W., & Hunter, R. B. (1976). Nitrogen Accumulation and Translocation in Corn Genotypes Following Silking¹. *Agronomy Journal*, 68(2), 418. doi: 10.2134/agronj1976.00021962006800020055x
- Below, F. E., Brandeau, P. S., Lambert, R. J., & Teyker, R. H. (1996). *Combining Ability for Nitrogen Use in Maize*. Paper presented at the Developing Drought and Low N-Tolerant Maize, CIMMYT, El Batán, Mexico.
- Bertin, P., & Gallais, A. (2000). Genetic Variation for Nitrogen Use Efficiency in a set of Recombinant Maize Inbred Lines. I. Agrophysiological Results. *Maydica*, 45(1), 53-66.
- Bodirsky, B. L., Popp, A., Lotze-Campen, H., Dietrich, J. P., Rolinski, S., Weindl, I., . . . Stevanovic, M. (2014). Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nat Commun*, 5, 3858. doi: 10.1038/ncomms4858
- Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., . . . McMullen, M. D. (2009). The genetic architecture of maize flowering time. *Science*, 325(5941), 714-718. doi: 10.1126/science.1174276
- Carlone, M. R., & Russell, W. A. (1987). Response to Plant Densities and Nitrogen Levels for Four Maize Cultivars from Different Eras of Breeding¹. *Crop Science*, 27(3), 465.
- Cassman, K. G., Dobermann, A., & Walters, D. T. (2002). Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio*, 31(2), 132-140.
- Ciampitti, I. A., & Vyn, T. J. (2011). A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. *Field Crops Research*, 121(1), 2-18. doi: DOI 10.1016/j.fcr.2010.10.009
- Cole, M. L., Kroeger, K. D., McClelland, J. W., & Valiela, I. (2006). Effects of Watershed Land use on Nitrogen Concentrations and δ^{15} Nitrogen in Groundwater. *Biogeochemistry*, 77(2), 199-215. doi: 10.1007/s10533-005-1036-2

- Comly, H. H. (1945). Cyanosis in Infants Caused by Nitrates in Well Water. *Jama-Journal of the American Medical Association*, 129(2), 112-116. doi: 10.1001/jama.1945.02860360014004
- Cone, K. C., McMullen, M. D., Bi, I. V., Davis, G. L., Yim, Y. S., Gardiner, J. M., . . . Coe, E. H., Jr. (2002). Genetic, physical, and informatics resources for maize. On the road to an integrated map. *Plant Physiol*, 130(4), 1598-1605. doi: 10.1104/pp.012245
- Cox, W. J., & Cherney, D. J. R. (2001). Row spacing, plant density, and nitrogen effects on corn silage. *Agronomy Journal*, 93(3), 597-602.
- Di Fonzo, N., Motto, M., Maggioro, T., Sabatino, R., & Salamini, F. (1982). N-uptake, translocation and relationships among N-related traits in maize as affected by genotype. *Agronomie*, 2(9), 789.
- Dudley, J. W., & Lambert, R. J. (2004). 100 Generations of Selection for Oil and Protein in Corn. In J. Janick (Ed.), *Plant Breeding Reviews: Long-Term Selection: Maize* (Vol. 24, pp. 79). Oxford, UK.: John Wiley & Sons, Inc.
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 6(5), e19379. doi: 10.1371/journal.pone.0019379
- Flint-Garcia, S. A., Thuillet, A. C., Yu, J., Pressoir, G., Romero, S. M., Mitchell, S. E., . . . Buckler, E. S. (2005). Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant J*, 44(6), 1054-1064. doi: 10.1111/j.1365-313X.2005.02591.x
- Friedrich, J. W., Schrader, L. E., & Nordheim, E. V. (1979). N Deprivation in Maize During Grain-Filling. I. Accumulation of Dry Matter, Nitrate-N, and Sulfate-S1. *Agronomy Journal*, 71(3), 461.
- Gallais, A., & Hirel, B. (2004). An approach to the genetics of nitrogen use efficiency in maize. *Journal of Experimental Botany*, 55(396), 295-306. doi: Doi 10.1093/Jxb/Erh006
- Gama, E., Marriel, I. E., Guimaraes, P., Parentoni, S., Dos Santos, M., Pacheco, C., . . . de Oliveira, A. C. (2002). Combining Ability for Nitrogen Use in a Selected Set of Inbred Lines From a Tropical Maize Population. *Brasileira de Milho e Sorgo*, 1(3), 68-77.

- Gentry, L. E., Below, F. E., David, M. B., & Bergerou, J. A. (2001). Source of the soybean N credit in maize production. *Plant and Soil*, 236(2), 175-184. doi: Doi 10.1023/A:1012707617126
- Haegel, J. W., Cook, K. A., Nichols, D. M., & Below, F. E. (2013). Changes in Nitrogen Use Traits Associated with Genetic Improvement for Grain Yield of Maize Hybrids Released in Different Decades. *Crop Science*, 53(4), 1256. doi: 10.2135/cropsci2012.07.0429
- Hauck, A. L., Johnson, G. R., Mikel, M. A., Mahone, G. S., Morales, A. J., Rocheford, T. R., & Bohn, M. O. (2014). Generation Means Analysis of Elite Ex-Plant Variety Protection Commercial Inbreds: A New Public Maize Genetics Resource. *Crop Science*, 54(1), 174.
- Hirel, B., Le Gouis, J., Ney, B., & Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J Exp Bot*, 58(9), 2369-2387. doi: 10.1093/jxb/erm097
- Jayasundara, S., Wagner-Riddle, C., Parkin, G., Bertoldi, P., Warland, J., Kay, B., & Voroney, P. (2007). Minimizing nitrogen losses from a corn–soybean–winter wheat rotation with best management practices. *Nutrient Cycling in Agroecosystems*, 79(2), 141-159. doi: 10.1007/s10705-007-9103-9
- Jones, D. (1931). *Factors for Converting Percentages of Nitrogen in Foods and Feeds Into Percentages of Proteins*. United States Department of Agriculture Circular No 183.
- Kirda, C., Topcu, S., Kaman, H., Ulger, A. C., Yazici, A., Cetin, M., & Derici, M. R. (2005). Grain yield response and N-fertiliser recovery of maize under deficit irrigation. *Field Crops Research*, 93(2-3), 132-141. doi: DOI 10.1016/j.fcr.2004.09.015
- Koocheki, A., Nassiri Mahallati, M., Mansoori, H., & Moradi, R. (2013). Assessing nitrogen flow in production and utilization cycle of wheat and maize as a tool to monitor N loss in Iran. *Acta Scientiarum. Biological Sciences*, 35(3). doi: 10.4025/actascibiols.v35i3.16039
- Lafitte, H. R., & Edmeades, G. O. (1994a). Improvement for tolerance to low soil nitrogen in tropical maize I. Selection criteria. *Field Crops Research*, 39(1), 1-14. doi: 10.1016/0378-4290(94)90066-3

- Lafitte, H. R., & Edmeades, G. O. (1994b). Improvement for tolerance to low soil nitrogen in tropical maize II. Grain Yield, biomass production, and N accumulation. *Field Crops Research*, 39, 15-25.
- Lafitte, H. R., & Edmeades, G. O. (1995). Association between Traits in Tropical Maize Inbred Lines and Their Hybrids under High and Low Soil-Nitrogen. *Maydica*, 40(3), 259-267.
- Lee, M., Sharopova, N., Beavis, W. D., Grant, D., Katt, M., Blair, D., & Hallauer, A. (2002). Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Mol Biol*, 48(5-6), 453-461.
- Lynch, J. P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann Bot*, 112(2), 347-357. doi: 10.1093/aob/mcs293
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Ann Bot*, 105(7), 1141-1157. doi: 10.1093/aob/mcq028
- McMullen, M. D., Kresovich, S., Villeda, H. S., Bradbury, P., Li, H., Sun, Q., . . . Buckler, E. S. (2009). Genetic properties of the maize nested association mapping population. *Science*, 325(5941), 737-740. doi: 10.1126/science.1174320
- McTaggart, I. P., & Smith, K. A. (1995). The effect of rate, form and timing of fertilizer N on nitrogen uptake and grain N content in spring malting barley. *Journal of Agricultural Science*, 125, 341-353.
- Meseka, S., Menkir, A., Ibrahim, & Ajala, S. (2013). Genetic analysis of maize inbred lines for tolerance to drought and low nitrogen. *Jonas*, 1, 29-36.
- Mi, G., Chen, F., Wu, Q., Lai, N., Yuan, L., & Zhang, F. (2010). Ideotype root architecture for efficient nitrogen acquisition by maize in intensive cropping systems. *Sci China Life Sci*, 53(12), 1369-1373. doi: 10.1007/s11427-010-4097-y
- Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and Interpretation of Factors Which Contribute to Efficiency of Nitrogen-Utilization. *Agronomy Journal*, 74(3), 562-564.
- Muurinen, S., Slafer, G. A., & Peltonen-Sainio, P. (2006). Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop Science*, 46(2), 561-568. doi: DOI 10.2135/cropsci2005-05-0046

- Nelson, P. T., Coles, N. D., Holland, J. B., Bubeck, D. M., Smith, S., & Goodman, M. M. (2008). Molecular Characterization of Maize Inbreds with Expired U.S. Plant Variety Protection. *Crop Science*, 48(5), 1673.
- Pace, G. M., & McClure, P. R. (1986). Comparison of Nitrate Uptake Kinetic-Parameters across Maize Inbred Lines. *Journal of Plant Nutrition*, 9(8), 1095-1111. doi: Doi 10.1080/01904168609363512
- Paulot, F., & Jacob, D. J. (2014). Hidden cost of U.S. agricultural exports: particulate matter from ammonia emissions. *Environ Sci Technol*, 48(2), 903-908. doi: 10.1021/es4034793
- Pollmer, W. G., Eberhard, D., Klein, D., & Dhillon, B. S. (1979). Genetic-Control of Nitrogen Uptake and Translocation in Maize. *Crop Science*, 19(1), 82-86.
- Presterl, T., Groh, S., Landbeck, M., Seitz, G., Schmidt, W., & Geiger, H. H. (2002). Nitrogen uptake and utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. *Plant Breeding*, 121(6), 480-486. doi: 10.1046/j.1439-0523.2002.00770.x
- R. (2008). R: A language and environment for statistical computing Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>
- Rizzi, E., Balconi, C., Nembrini, L., Stefanini, F. M., Coppolino, F., & Motto, M. (1993). Genetic-Variation and Relationships among N-Related Traits in Maize. *Maydica*, 38(1), 23-30.
- Robertson, G. P., Huston, M. A., Evans, F. C., & Tiedje, J. M. (1988). Spatial Variability in a Successional Plant Community - Patterns of Nitrogen Availability. *Ecology*, 69(5), 1517-1524. doi: Doi 10.2307/1941649
- Robertson, G. P., & Vitousek, P. M. (2009). Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. *Annual Review of Environment and Resources*, 34(1), 97-125. doi: 10.1146/annurev.enviro.032108.105046
- Rosolem, C. A., Kato, S. M., Machado, J. R., & Bicudo, S. J. (1993). Nitrogen redistribution to sorghum grains as affected by plant competition. *Plant and Soil*, 155-156(1), 199-202. doi: 10.1007/bf00025018
- Sabata, R. J., & Mason, S. C. (1992). Corn Hybrid Interactions with Soil-Nitrogen Level and Water Regime. *Journal of Production Agriculture*, 5(1), 137-142. doi: 10.2134/jpa1992.0137

- SAS. (2009). SAS 9.2 User's Guide. Cary, NC: SAS Inst.
- Scholberg, J. M., Parsons, L. R., Wheaton, T. A., McNeal, B. L., & Morgan, K. T. (2002). Soil temperature, nitrogen concentration, and residence time affect nitrogen uptake efficiency in citrus. *J Environ Qual*, 31(3), 759-768.
- Selig, L., Lambert, R. J., Rocheford, T. R., & da Silva, W. J. (1999). RFLP and Cluster Analysis of Introgression of Exotic Germplasm into U.S. Maize Inbreds. *Maydica*, 44, 85-92.
- Sharratt, B. S., & McWilliams, D. A. (2005). Microclimatic and rooting characteristics of narrow-row versus conventional-row corn. *Agronomy Journal*, 97(4), 1129-1135. doi: DOI 10.2134/agronj2004.0292
- Subedi, K. D., & Ma, B. L. (2005). Nitrogen uptake and partitioning in stay-green and leafy maize hybrids. *Crop Science*, 45(2), 740-747. doi: 10.2135/cropsci2005.0740
- Tremblay, N., Bouroubi, Y. M., Belec, C., Mullen, R. W., Kitchen, N. R., Thomason, W. E., . . . Ortiz-Monasterio, I. (2012). Corn Response to Nitrogen is Influenced by Soil Texture and Weather. *Agronomy Journal*, 104(6), 1658-1671. doi: DOI 10.2134/agronj2012.0184
- Tsai, C. Y., Dweikat, I., Huber, D. M., & Warren, H. L. (1992). Interrelationship of Nitrogen Nutrition with Maize (*Zea-Mays*) Grain-Yield, Nitrogen Use Efficiency and Grain Quality. *Journal of the Science of Food and Agriculture*, 58(1), 1-8. doi: DOI 10.1002/jsfa.2740580102
- UN-FAO. (2011). *Current world fertilizier trends and outlook to 2015*.
- Uribelarrea, M. (2007). *Physiological Characterization of Nitrogen Use in Maize: Opportunities for Improvement*. (Ph.D), Unviersity of Illinois at Urbana - Champaign. (UMI Microform 3290410)
- Uribelarrea, M., Below, F. E., & Moose, S. P. (2004). Grain composition and productivity of maize hybrids derived from the Illinois protein strains in response to variable nitrogen supply. *Crop Science*, 44(5), 1593-1600.
- USDA. (2013). Fertilizer Use and Price. from <http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26720>
- Wang, X. T., & Below, F. E. (1992). Root-Growth, Nitrogen Uptake, and Tillering of Wheat Induced by Mixed-Nitrogen Source. *Crop Science*, 32(4), 997-1002.

- White, W. G., Vincent, M. L., Moose, S. P., & Below, F. E. (2012). The sugar, biomass and biofuel potential of temperate by tropical maize hybrids. *Global Change Biology Bioenergy*, 4(5), 496-508. doi: DOI 10.1111/j.1757-1707.2012.01158.x
- Wiesler, F., & Horst, W. J. (1994). Root-Growth and Nitrate Utilization of Maize Cultivars under Field Conditions. *Plant and Soil*, 163(2), 267-277. doi: Doi 10.1007/Bf00007976
- Worku, M., Banziger, M., Erley, G. S. A., Friesen, D., Diallo, A. O., & Horst, W. J. (2007). Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. *Crop Science*, 47(2), 519-528. doi: DOI 10.2135/cropsci2005.05.0070
- Zaidi, P. H., Srinivasan, G., & Sanchez, C. (2003). Relationship between line per se and cross performance under low nitrogen fertility in tropical maize (*Zea mays* L.). *Maydica*, 48(3), 221-231.
- Zotarelli, L., Scholberg, J. M., Dukes, M. D., & Munoz-Carpena, R. (2008). Fertilizer residence time affects nitrogen uptake efficiency and growth of sweet corn. *J Environ Qual*, 37(3), 1271-1278. doi: 10.2134/jeq2007.0460

Figures and Tables

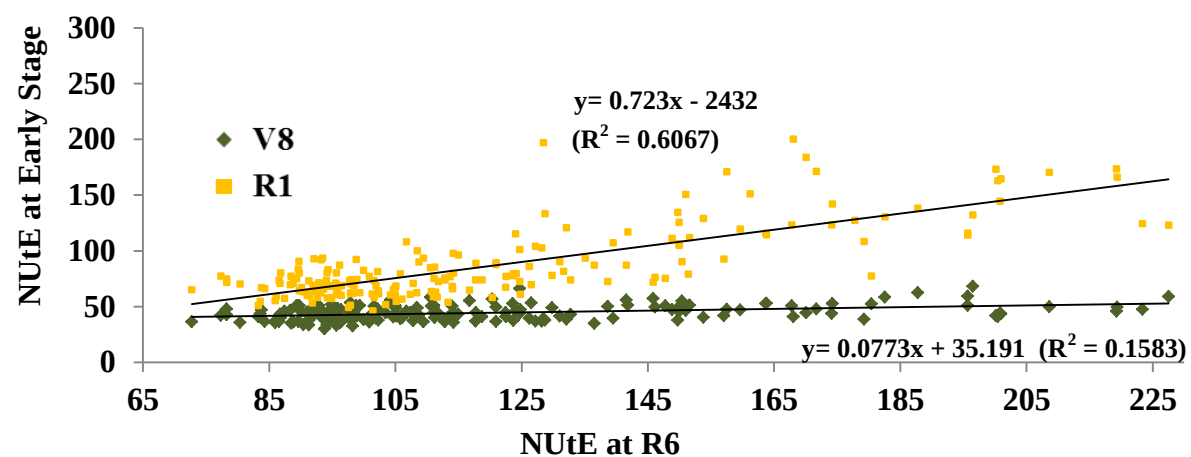


Figure 1.1 Linear regressions of the early sampling prediction of terminal NUtE. Each point represents a plot sampled at V8 or R1 and again at R6 between 2003-2005 at any N rate.

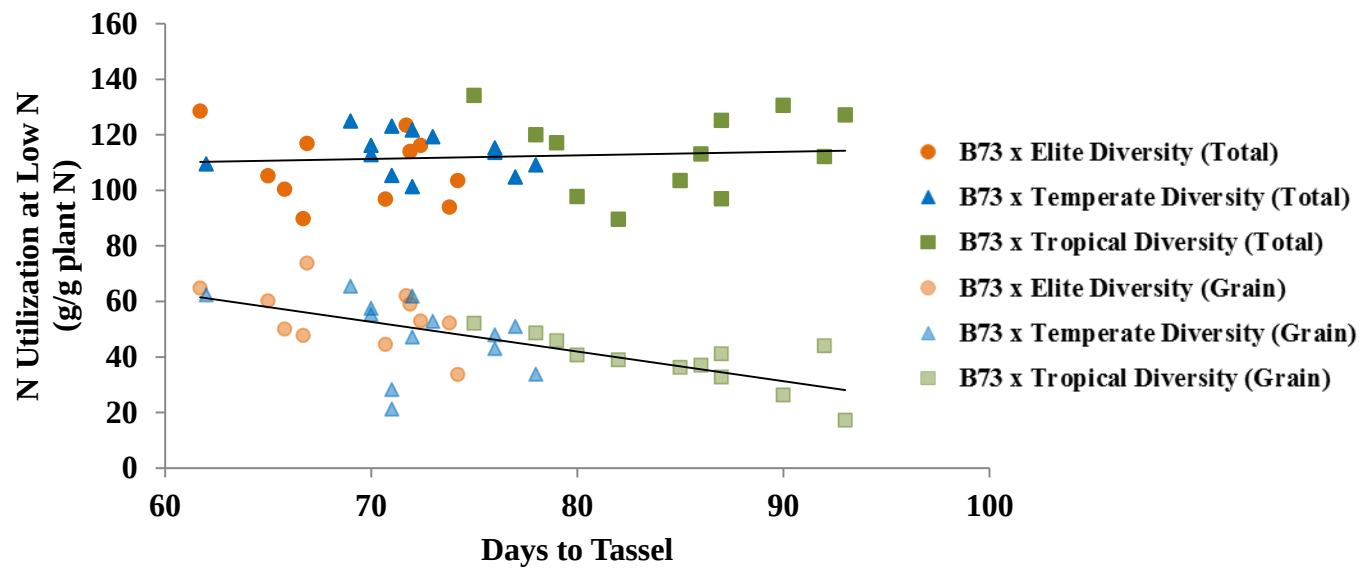


Figure 1.2 Effect of Maturity on Grain and Total NUtE. Members of three groups with diverse days to tassel grown in 2008-2009 plotted as both grain NUtE and total NUtE. Regression of the lines show grain NUtE is confounded by maturity while total NUtE is not.

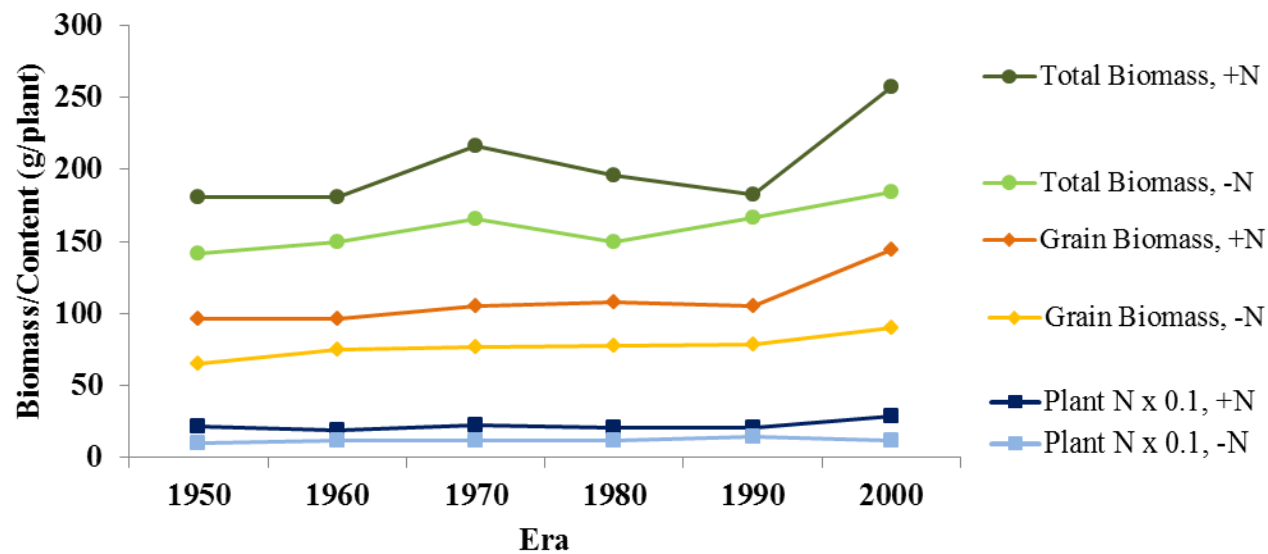


Figure 1.3 Historical trends in NUE component traits. Inbreds representing each decade were crossed to LH82 and evaluated from 2011-2013. Data points represent the content of each fraction in the plots grown at high and low N. Plant N shows little increase while grain and total biomass reflect the changing goals of plant breeders. Data and lines provided in Table A.3

Table 1.1 Grain yield (Mg/plant) of NAM x B73 lines grown at four N rates in 2004 and 2005. Groups are according to N response A) very little response, B) GY maximized at 75 kg/ha N, C) GY maximized at 150 kg/ha N, D) GY response at every level through

		N Rate (kg/ha)			
		0	75	150	225
Group A					
	B73 x Hp301	4.5	5.6	6.6	6.9
	B73 x MS71	5.7	5.6	5.7	6.1
	B73 x P39	4.1	4.2	4.7	4.1
	<i>Average</i>	4.8	5.1	5.7	5.7
Group B					
	B73 x H99	4.9	5.4	6.0	6.3
	B73 x Ki3	4.6	7.0	7.1	7.2
	B73 x Tzi8	2.2	4.1	4.6	4.7
	B73 x CML322	4.0	6.8	7.3	7.9
	B73 x NC350	4.1	7.7	8.5	8.3
	B73 x CML333	2.0	5.3	5.7	7.3
	<i>Average</i>	3.6	6.1	6.5	7.0
Group C					
	B73 x CML103	5.8	7.0	8.4	8.4
	B73 x CML228	1.2	3.5	5.1	4.4
	B73 x Ki11	1.0	1.7	4.1	4.3
	B73 x Ky21	2.5	4.5	6.5	5.8
	B73 x NC358	4.5	6.6	8.0	8.2
	B73 x Tx303	3.0	4.6	7.7	7.5
	B73 x CML69	3.3	5.2	6.7	6.7
	<i>Average</i>	3.0	4.7	6.6	6.5
Group D					
	B73 x B97	5.5	6.3	6.5	8.2
	B73 x CML247	4.4	5.3	5.6	6.4
	B73 x CML277	1.0	3.5	5.3	5.9
	B73 x Il14H	4.4	5.5	6.3	7.2
	B73 x M37W	5.5	7.3	8.5	9.1
	B73 x Mo17	5.9	7.3	8.1	8.7
	B73 x Mo18W	0.3	3.2	4.7	5.6
	B73 x Oh43	5.5	6.3	7.1	7.6
	B73 x Oh7B	4.5	7.0	8.6	10.2
	<i>Average</i>	4.1	5.8	6.8	7.7
	Overall Average	3.8	5.5	6.5	7.0

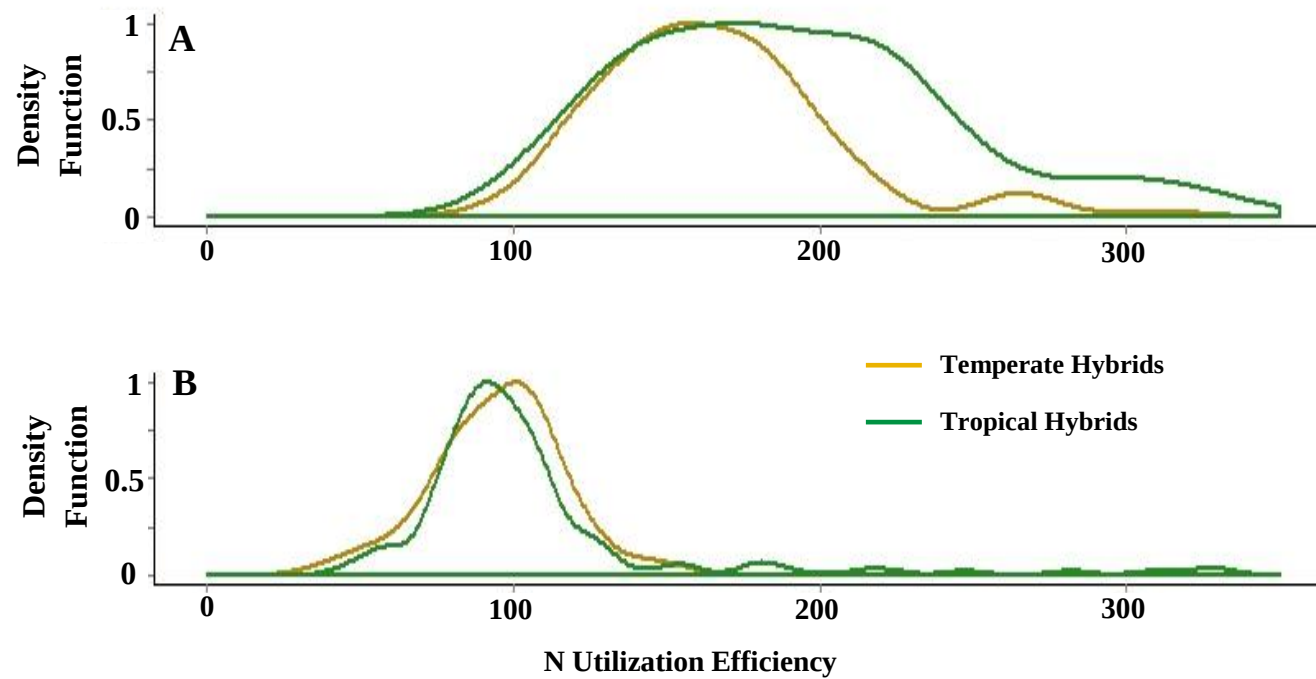


Figure 1.4 Temperate versus tropical NUtE values. Kernel density plots of tropical and temperate germplasm across all years. Panel A) Low N plots. Tropical hybrids show greater diversity for NUtE and more lines with high NUtE. Panel B) High N plots. The variation seen at low N is not apparent at high N and the NUtE values are lower with sufficient N.

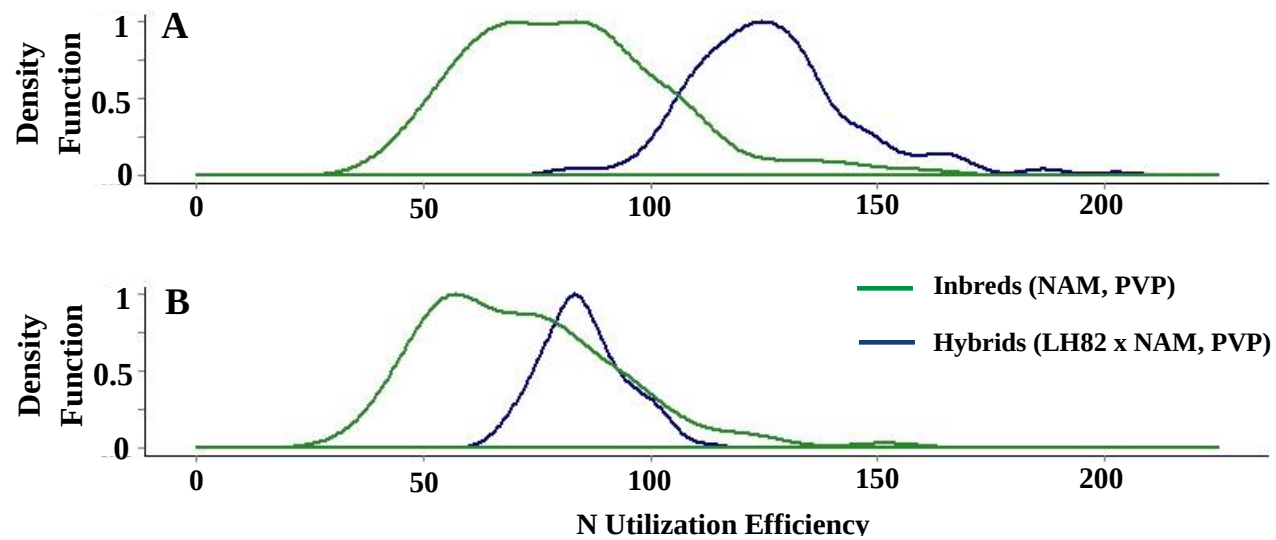


Figure 1.5 Comparison of inbred and hybrid NUtE performance under low (panel A) and high (panel B) N conditions. Inbreds and hybrids, made with LH82, were grown in 2012 and 2013.

Table 1.2 Summary statistics for NAM and ex –PVP lines with three different testers for two GY traits and NUtE at high (225 kg/ha) and low (0 kg/ha) N grown in 2010.

	B73					
	Diversity (9 lines)			ex-PVP (11 lines)		
Trait	<i>Mean</i>	<i>Range</i>	σ^2	<i>Mean</i>	<i>Range</i>	σ^2
<i>GY (g/plant) at 0N</i>	63.3	19.8-103.0	406.0	69.9	29.6-147.1	611.8
<i>ΔGY</i>	39.4	4.0-75.0	351.5	30.1	1.7-76.7	283.0
<i>NUtE 0N</i>	136.7	109.5-177.0	266.5	126.6	105.6-171.1	213.3
<i>NUtE 225N</i>	96.6	85.6-112.0	53.6	98.3	83.2-125.3	107.3

	IHP1					
	Diversity (21 lines)			ex-PVP (18 lines)		
Trait	<i>Mean</i>	<i>Range</i>	σ^2	<i>Mean</i>	<i>Range</i>	σ^2
<i>GY (g/plant) at 0N</i>	25.2	0.17-60.7	387.7	44.8	8.2-66.7	235.4
<i>ΔGY</i>	63.2	44.4-83.45	148.0	61.3	36.8-110.2	304.8
<i>NUtE 0N</i>	171.6	121.0-246.8	1061.1	156.0	99.8-254.4	1407.5
<i>NUtE 225N</i>	76.8	52.9-124.8	251.4	74.4	61.3-101.7	90.9

	ILP1					
	Diversity (19 lines)			ex-PVP (12 lines)		
Trait	<i>Mean</i>	<i>Range</i>	σ^2	<i>Mean</i>	<i>Range</i>	σ^2
<i>GY (g/plant) at 0N</i>	47.0	2.5-101.4	560.8	68.4	39.8-89.5	240.1
<i>ΔGY</i>	64.5	29.5-105	618.3	58.4	7.2-137.4	1446.3
<i>NUtE 0N</i>	142.2	99.0-253.8	1209.1	147.2	129.0-169.3	190.7
<i>NUtE 225N</i>	110.8	96.9-124.4	74.4	99.6	80.4-119.8	150.0

Table 1.3 Sources of variation and variance estimates for NAM population with B73 as a tester

Population	Tester	Trait	Source of Variation			Variance Estimates						
			N Rate	Year	N Rate * Year	Genotype	Genotype*Year	Genotype*Nrate	Genotype*Nrate*Year	Residual		
				P>F								
NAM	B73	Grain Biomass	<.0001	0.0001	0.0005	0	3.91×10^3	***	80.43	1.52×10^3	***	909.26 ***
		Grain N	<.0001	<.0001	<.0001	9.59×10^{-3}	3.93×10^{-2}	*	1.78×10^{-2}	1.16×10^{-3}		0.14 ***
		Grain NUtE	NS	<.0001	<.0001	0	693.66	***	27.36	316.47	***	122.41 ***
		Stover Biomass	NS	<.0001	0.0224	681.24 *	534.13	*	0	597.72	***	752.46 ***
		Stover N	<.0001	<.0001	<.0001	1.43×10^{-2} *	3.19×10^{-2}	*	0	2.63×10^{-2}	*	9.41×10^{-2} ***
		Total NUtE	<.0001	<.0001	<.0001	318.28	457.41	*	96.21	350.55	*	917.76 ***
		Harvest Index	<.0001	<.0001	NS	1.97×10^{-3}	7.66×10^{-3}	***	0	3.53×10^{-3}	***	4.74×10^{-3} ***

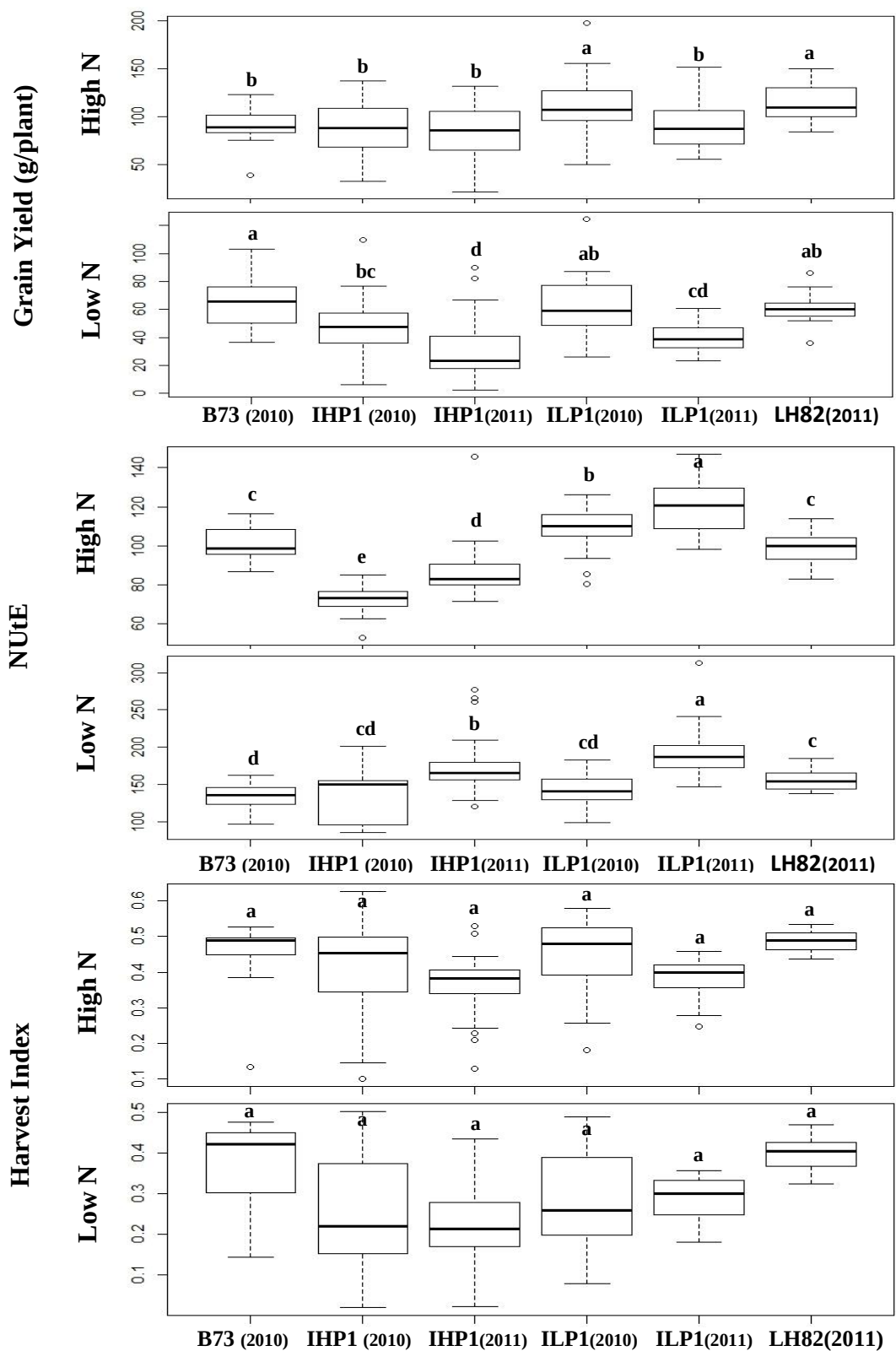


Figure 1.6 Comparison of the effects of different testers with the NAM population on grain yield, N utilization efficiency, and harvest index in plots grown in 2010 and 2011 in Urbana, IL at low and high N. Means with the same letters are not significantly different ($\alpha=0.05$).

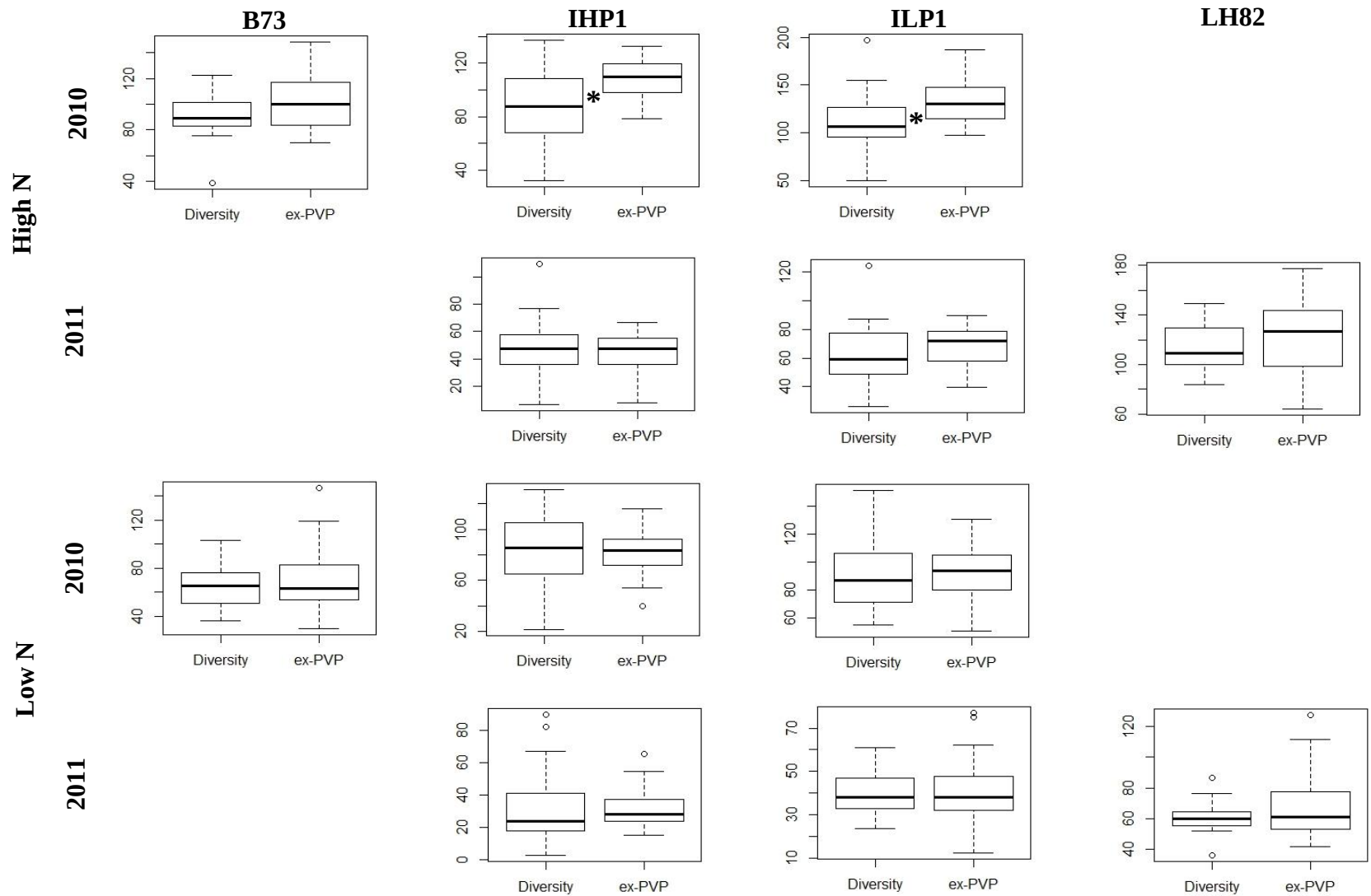


Figure 1.7a Comparison of the grain yield (g/plant) in the NAM (Diversity) and ex-PVP populations under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 and 2011 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

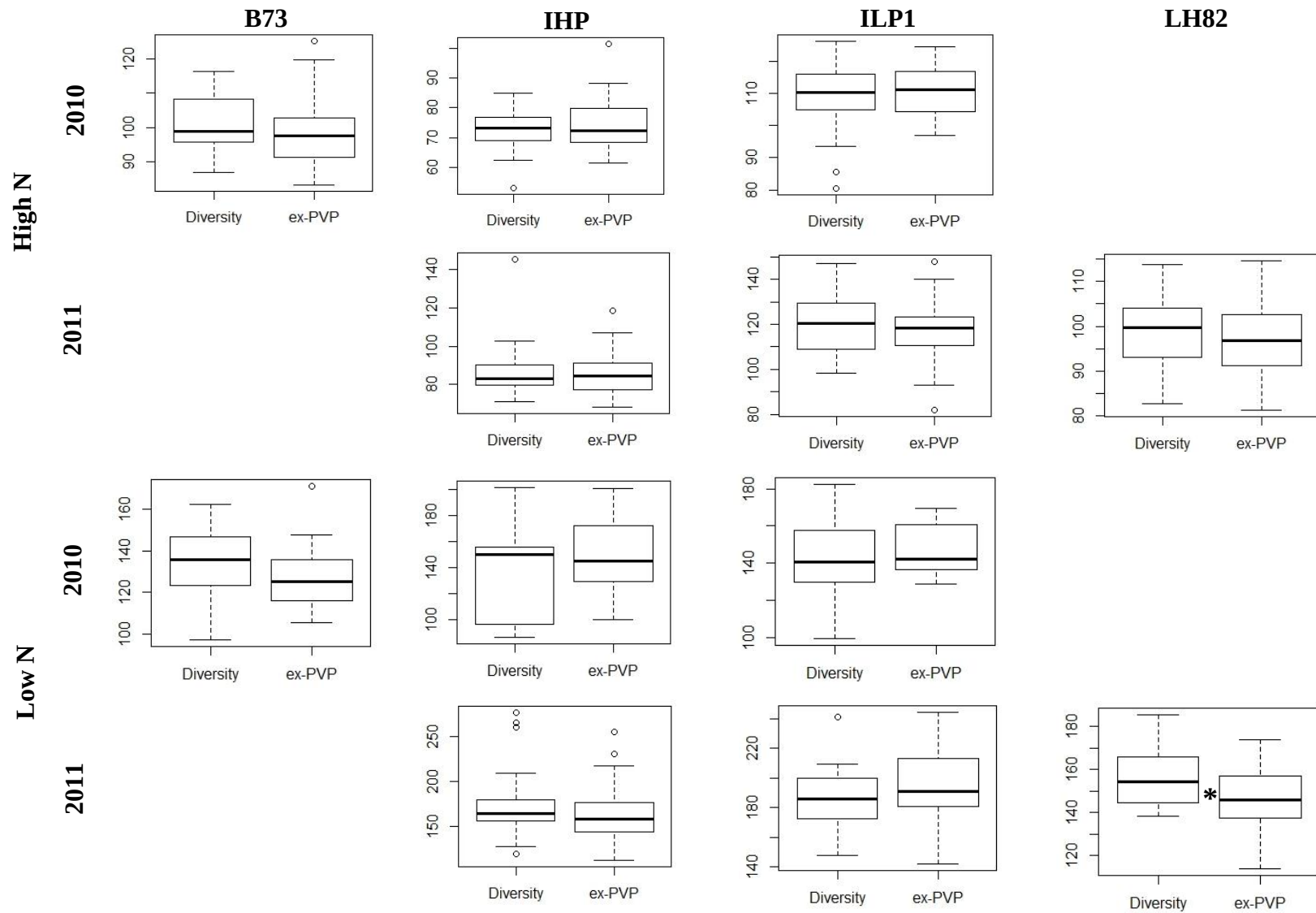


Figure 1.7b Comparison of the N utilization efficiency in the NAM (Diversity) and ex-PVP populations under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 and 2011 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

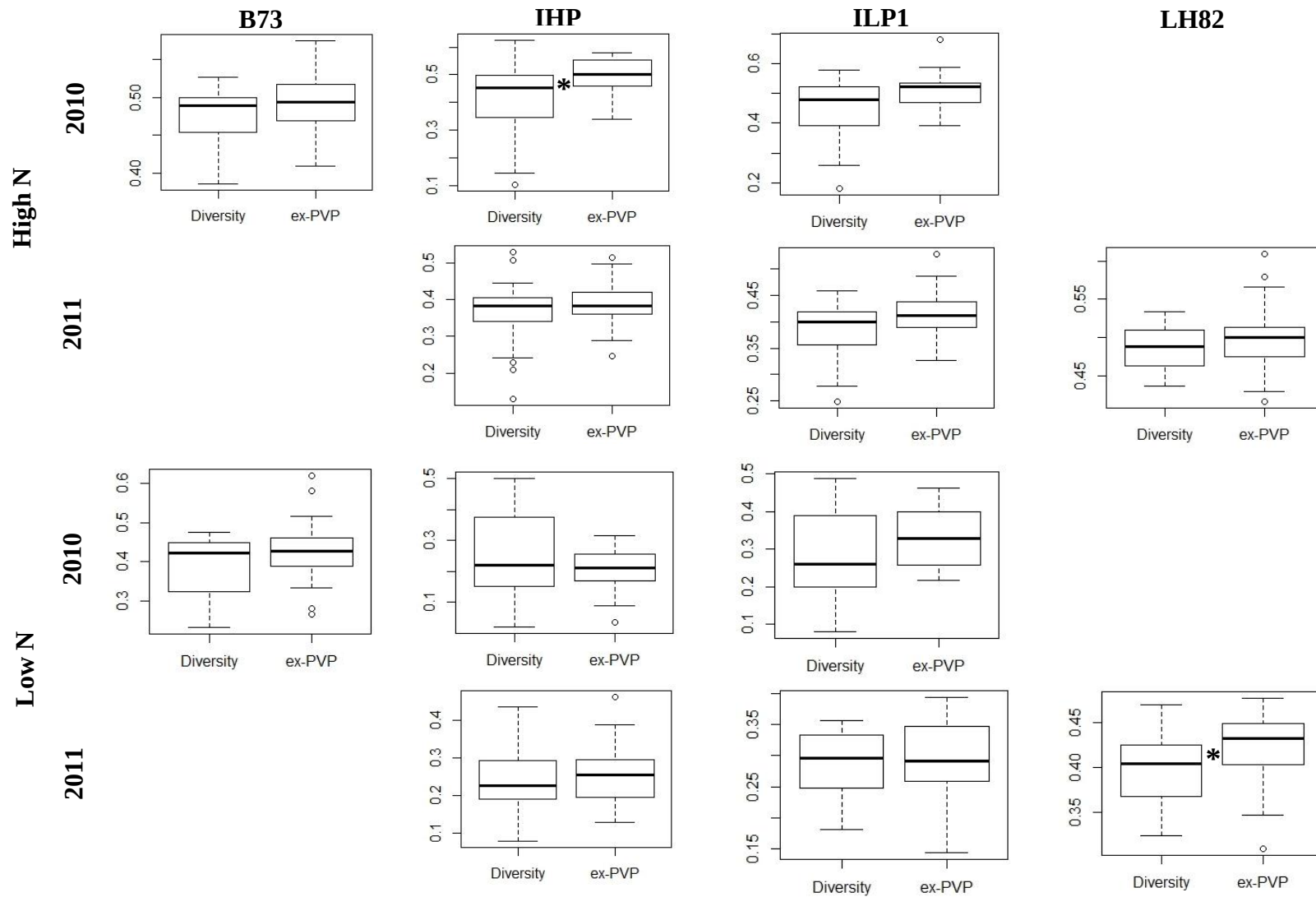


Figure 1.7c Comparison of the harvest index in the NAM (Diversity) and ex-PVP populations under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 and 2011 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

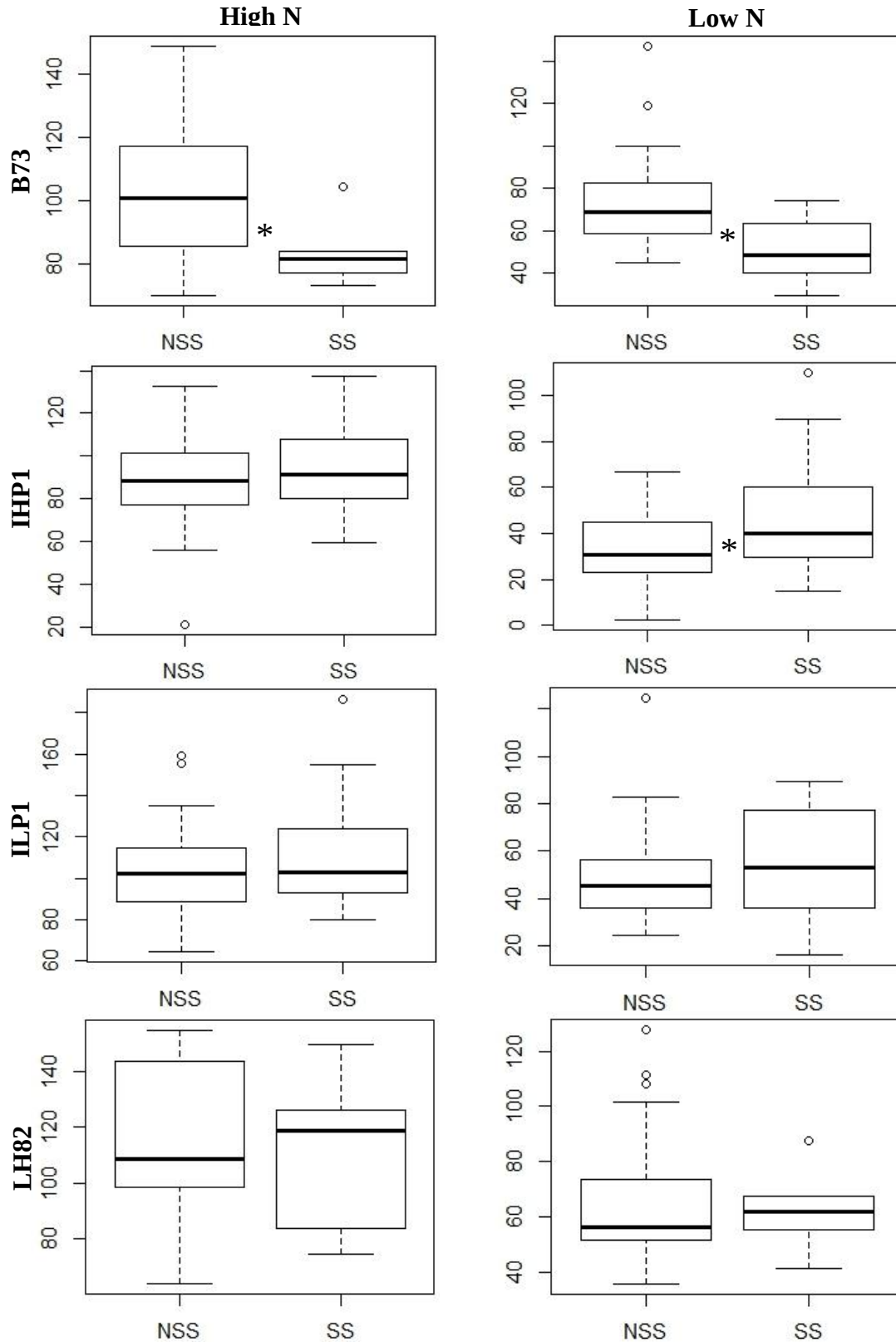


Figure 1.8a Comparison of the grain yield (g/plant) for hybrids of non- stiff stalk (NSS) inbreds and stiff stalk (SS) inbreds under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

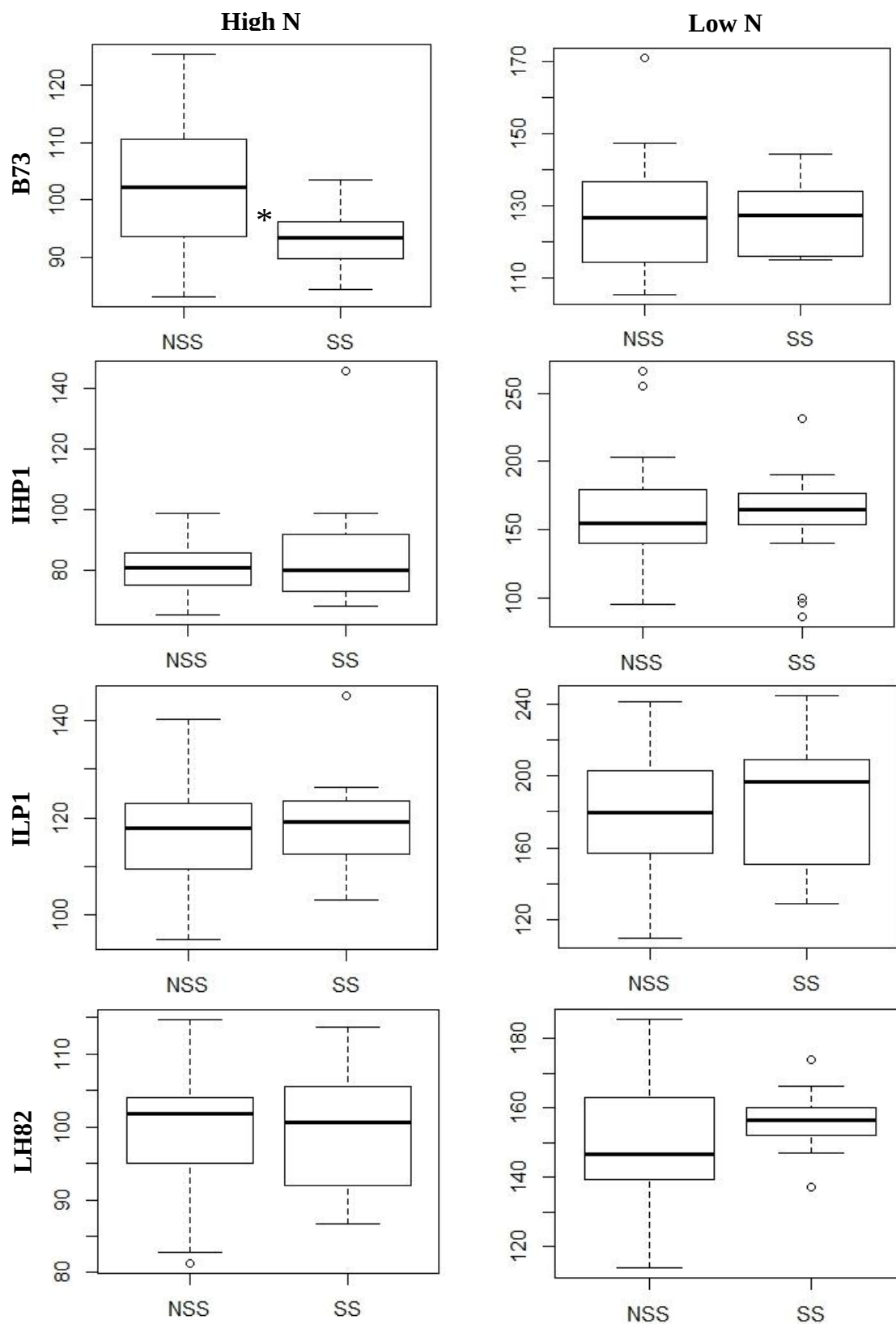


Figure 1.8b Comparison of the N utilization efficiency for hybrids of non-stiff stalk (NSS) inbreds and stiff stalk (SS) inbreds under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

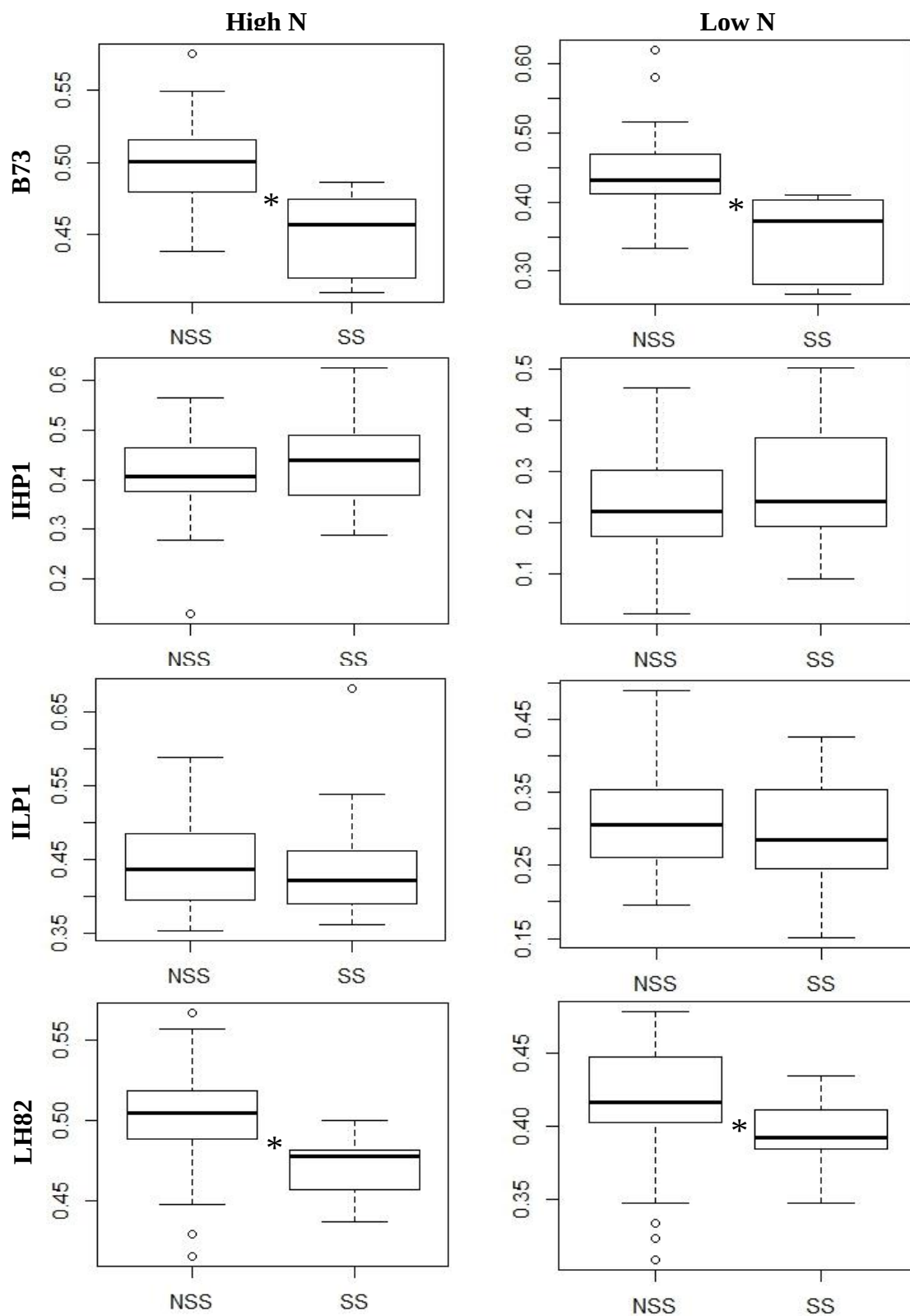


Figure 1.8c Comparison of the harvest index for hybrids of non- stiff stalk (NSS) inbreds and stiff stalk (SS) inbreds under low and high N when crossed to different testers (B73, IHP1, ILP1, LH82) grown in 2010 in Urbana, IL. Starred means are significantly different at $p < 0.1$.

Chapter 2 – QTL Enrichment for Improving Nitrogen Utilization Efficiency in Maize

Introduction

It is widely reported that maize (*Zea mays* L) yield shows a response to applied nitrogen (N). Historically, the N response of grain yield (GY) from low N to high N increased from a 73% increase in the 1970's to 102% in the early 1990's and continued increasing to 111% percent in the late 1990's (O'Neill et al., 2004). Further research suggests the high N conditions under which most maize breeding occurs is contributing to the adaptation of maize to perform well under high N but not low N, thereby inflating the grain response values (Haegerle et al., 2013; Presterl et al., 2003). Regardless, the presence of such a drastic N response has encouraged application of some form of supplemental N to 98% of maize grown in Illinois and 97% of maize grown nationwide (USDA, 2013).

In addition to widespread acreage receiving N fertilizer applications, the rate of fertilizer applied per acre has also increased more than two fold over the past fifty years from 80.7 kg/ha to 187 kg/ha in Illinois and from 65.0 kg/ha to 156.6 kg/ha nationwide (USDA, 2013). While the direct cost of nearly \$90/acre for N fertilizer are incentive to improve nitrogen use efficiency, the indirect costs are those that may cause regulatory reform in the area of nutrient management and implementation of nutrient application ceilings for producers. These indirect costs are the well documented detrimental environmental effects of N loss through leaching, volatilization, and denitrification which are estimated to result in the loss of between 35% and 65% of applied N from the soil system (Cassman et al., 2002; Jayasundara et al., 2007; Koocheki et al., 2013).

These issues are not confined to the United States. In fact, it is estimated that in the next thirty-five years, world N losses need to decrease to less than 15% in order to keep N pollution levels below critical levels and feed the worlds growing population (Bodirsky et al., 2014). Nitrates in groundwater supplies have been a salient point in the debate about nutrient use and pollution. The recent enactment of total maximum daily loads to control those nitrate levels in the Chesapeake Bay could set a standard for watersheds nationwide to limit nutrient losses from point sources and nonpoint sources. The likelihood of mandatory pollution reduction programs highlights the importance of research surrounding nitrogen use efficiency (NUE) and its component traits and the urgency of utilizing that knowledge to develop maize lines with increased N use efficiency.

Moll et al. (1982) coined the most widely used definition of nitrogen use efficiency (NUE), which is yield per unit of nitrogen applied. It is comprised of the two component traits, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE). NUpE is a measure of how well the plant absorbs available N and is measured as total plant N over total N supply. NUtE describes how the plant uses the N in its system to make grain and is measured as total grain or total biomass uptake over total plant N.

The influence of agronomic practices and environmental factors on NUpE has been widely reported. High plant populations and narrow row spacing have been shown to positively influence NUpE (Barbieri et al., 2008; Ciampitti & Vyn, 2011). Additionally, the importance of root architecture, agronomic fertilization practices, and environmental conditions have been widely cited as influential on the rate of NUpE (Kirda et al., 2005; Lynch, 2013; McTaggart & Smith, 1995; Mi et al., 2010; Zotarelli et al., 2008).

Since NUpE is a measure how well a plant uses N to stimulate growth, it is dependent on the physiological processes of the plant and has a more significant genetic component than NUpE. The decreased dependency on environmental conditions as compared to NUpE makes NUtE a more realistic target of genetic association studies and targeted breeding. Many era studies include the effect of nitrogen on yield and some include NUE as a phenotype for their analysis, but few report the historical selection for the separate component traits NUpE and NUtE (Castleberry et al., 1984; Sangoi et al., 2001). Those that have included a breakdown of the components report that past improvement in NUE has been associated with increased NUpE which may now be at levels that support the plants remobilization capacity, consequently implying that future improvement in NUE should be focused on NUtE (Ciampitti & Vyn, 2012; Haegele et al., 2013; O'Neill et al., 2004). These observations are supported by experiments reported in Chapter 1.

NUtE can be partitioned further into N remobilization efficiency and N assimilation efficiency, which are controlled by a network of N cycling enzymes and processes (Masclaux-Daubresse et al., 2010). As such, the amino acids that act as carriers for the N molecules during assimilation and remobilization can be measured as a genotype to supplement direct measures of NUE and its components. Plants take up N in the form of ammonium (NH_4^+) or nitrate (NO_3^-), but once in the plant, nitrate is reduced to ammonia via nitrate and nitrite reductases. Ammonia is then assimilated into organic N and attached to transfer amino acids as part of an amino group for

transportation to sink tissues where they are used in the biosynthesis of other amino acids, nucleotides or metabolites. They can also be integrated into structural, transport, or storage proteins. Glutamine, glutamate (glutamic acid), asparagine, and aspartate (aspartic acid) are the important transport amino acids in the N cycling pathway and their measurement provides an assay for N remobilization in the plant (Masclaux-Daubresse et al., 2006). By sampling earshoots at R1 as silks first emerge, the transport amino acids are captured as measureable free amino acids after they are transported into the reproductive sink but before incorporation into grain storage proteins (J Boddu, personal communication, 2011). Previous studies in other crops have indicated a relationship between free amino acid levels and plant growth phenotypes that are related to NUtE (Giannino et al., 2007; Lam et al., 2003; Lightfoot et al., 2007).

Quantitative Trait Loci (QTL) mapping offers information on the processes of complex traits by associating quantitative traits with their underlying genetic factors. The scope of the body of work for QTL mapping is extremely broad, as QTL have been identified for many traits in most species with a developed genetic map. QTL for NUE, specifically, have been identified in maize and other species. Wei et al. (2012) reported finding fifteen QTL for NUE and its related phenotypes including grain biomass/N content, stover biomass/N content, and N response. Several studies in maize have identified QTL for grain yield and its components (kernels number, kernel weight) as well as NUE under low and high N environments (Agrama et al., 1999; Bertin & Gallais, 2001; Liu, 2007; Ribaut et al., 2007). Others have identified traits including grain yield and amino acid synthesis enzyme activity at a single nitrogen level (Coque et al., 2008; Hirel et al., 2001). A meta-analysis of 190 QTL for yield and yield components from the previously cited studies resulted in 18 consensus QTL for low-N environments and 19 consensus QTL for high N environments, many of which were co-localized across N levels in the same chromosome regions (Liu et al., 2012). The maps used in these studies have low marker density, so the QTL they identify have limited use in breeding because of the large size of their intervals (Agrama, 2005). A study in *Arabidopsis*, a plant with a smaller genome and higher density markers, resulted in gene-resolution QTL (Loudet et al., 2003). With the advent of inexpensive and high-throughput genotyping like genotyping-by-sequencing, this high resolution QTL mapping will become commonplace in maize genetics in the near future (Elshire et al., 2011).

The most common strategy for marker assisted selection is the use of markers to predict the breeding value of an individual for use in an indirect selection approach to breeding (Johnson, 2004). Direct use of QTL mapping in marker assisted selection as used in this experiment is less common, but it has been used in successful single gene integration (Tanksley & Hewitt, 1988). Additionally, selections based on a summation index based on the number of positive alleles were successful on NUE QTL in perennial ryegrass. The transfer of marker assisted selection technology to NUE in maize, however, has been unsuccessful thus far (Hirel et al., 2007). The goal of this study is to use NUE QTL mapping results in a summation index to improve NUE in maize.

Materials and Methods

Initial QTL Determination

The QTL used in this study were mapped by Devin Nichols based on a population developed in 2004 and phenotyped in 2006 and 2007 (Nichols, 2008). The mapping population was established by crossing 243 IBMRILs as females to Illinois High Protein (IHP1). IHP1 was selected as the tester for this experiment because it represents the high extreme for N uptake, so resulting phenotypic differences would be due to the effects of the alleles contributed by the IBM population. The IBMRILx IHP mapping population was grown in 2006 and 2007 in field trials as previously described in Chapter 1. B73xMo17, as well as parental hybrids B73xIHP1 and Mo17xIHP1, were included in each range as reference hybrids. R6 sampling and subsequent phenotypic measurements were as described in Chapter 1.

Windows QTL Cartographer Version 2.5 was used to perform the QTL analysis using the genetic map and markers available from the Maize Genetics and Genomics Database. After performing composite interval mapping (CIM), experiment-wise LOD significance thresholds were determined for each trait using permutation tests. Analysis was done for 2006 and 2007 separately on the trait means across the three replications for high N and the trait means for the three replications at low N. For the combined analysis, years were combined for one analysis on the six high N replications and one on the six low N replications. Across the seven included traits at high and low N, one hundred and sixty QTL were identified. The QTL that are consistent across two

years were assimilated into nine genomic regions distributed over six chromosomes. These QTL, their locations, flanking markers and effects are summarized in table 2.1.

Germplasm

IBM

The intermated B73 x Mo17 (IBM) population was originally developed to improve genetic resolution for mapping efforts by increasing the number of recombination events and decreasing linkage disequilibrium before fixing the alleles through self-pollination to recombinant inbred lines (RIL) (Lee et al., 2002). These IBMRIL lines were first mapped with 1000 restriction fragment length polymorphism (RFLP) loci and over 700SSR loci. Cone et al. (2002) extrapolated loci from non-IBM maps to their nearest neighbor onto the framework formed by the IBM map to create the IBM Neighbors map with more than 4,900 markers. Recent advancements in genotyping using a genotyping-by-sequencing approach as described by Elshire et al. (2011) have resulted in the addition of more than 2 million single nucleotide polymorphism (SNP) markers to the maize map.

During the 2008-2011 growth seasons, testcross hybrids were created with LH82 as a male onto a subset of 262 of the IBMRIL lines. Genotypes for the IBMRILs were assigned as B73 or Mo17 alleles at each region of interest based on the marker data found on the Maize Genetics and Genomics Database (maizegdb.org). Flanking markers used to determine the region of interest are listed in table 2.1. If the allele within that region was inconclusive it was considered missing data.

Ideotype and Population Selection

Further summarization of the QTL results found in table 2.1 was based on the additive effect for each trait at that QTL. Based on the methods of analysis, a positive value for additive effect was considered the effect of the B73 allele on the trait while a negative value was considered the effect of a Mo17 allele on the trait. Regardless of which allele was favored, additive effects from 0 to .5 were considered low effect, .5 to 4.0 were considered to be medium effects and any over 4.0 was considered a high effect region for the trait of interest.

Alleles that comprised the desired ideotype were selected such that the resulting combination of alleles would theoretically result in high yield in a low N environment. Low N was the target environment, but high N effects were considered if the QTL had no effect on traits at low N. Since

increased grain yield was the target objective, grain biomass and kernel number were the primary traits for which positive effect alleles were selected. Conversely, previous studies have reported that grain N is inversely related to GY, so grain N was a primary trait for which negative effect alleles were selected (Duvick & Cassman, 1999). Only one region had an effect on N Utilization at low N, so this trait would be considered a secondary trait for which positive alleles were selected. Finally, surveys of historical trends in maize yields have shown that harvest index (HI) has increased as yield has increased so stover biomass was considered a secondary trait for which allele combinations were selected that would result in negative or null effects on stover biomass but positive effects on grain biomass (Lorenz et al., 2010).

Using the desired ideotype for high yield at low N, an enriched and depleted population were developed from the IBMRIL lines based on their genotypes at the same regions. The enriched population included the ten IBMRIL lines with the high number of regions that matched the desired ideotype. In order to maximize stringency, missing data for this selection were considered to be the undesired allele. The ten IBMRIL lines with the most undesirable alleles, or alleles that mismatched the desired ideotype, were included in the depleted population. Again, in order to maximize stringency, any missing data was considered the desired allele. Any lines that were not testcrossed to LH82 were excluded from consideration in the depleted and enriched populations.

Environmental Measurements

The State Climatologist Office collects daily temperature and precipitation data at a weather station less than one half mile from the experimental site used for this experiment (coordinates 40° 5' 3", -88° 14' 26"). Daily temperature was recorded on an electronic maximum-minimum temperature sensor (MMTS) and daily precipitation was measured via a standard 8-inch raingauge (Angel, 2009). Growing degree days (GDD) are calculated as $\left(\frac{T_{max}+T_{min}}{2}\right) - base$ where T_{max} is the maximum daily temperature, T_{min} is the minimum daily temperature and the values follow the standard maize T_{max} ceiling of 30°C, T_{min} floor of 10°C, and base of 10°C.

Field Trials

Field assessments of this experiment were grown at the University of Illinois Department of Crop Sciences Research and Education Center in Urbana, IL. The field sites are on a Drummer silty

clay loam with an average organic matter of 3.7% and an average soil pH of 6.2. Based on N recovery for plots with no applied N fertilizer, soil mineralization provided around 90 kg N/ha. The sites were previously shown to be N responsive by Gentry et al. (2001). In order to homogenize the soil N, trial sites were subject to a two year corn-soybean rotation. Upon observation of drought stress symptoms, including leaf rolling, irrigation was applied evenly over the entire experiment. Weed pressure was controlled using both hand cultivation and chemical applications.

Experiments were arranged with paired high and low N plots for each genotypes in adjacent ranges in order to minimize the effect of spatial variation for available nitrogen. Genotypes were arranged by maturity as measured in Urbana, IL in 2006. Plot planting and nitrogen application were done as described in Chapter 1. For 2012 and 2013, low N plots received no added nitrogen while high N plots received 200 kg/ha and 100 kg/ha for hybrids and inbreds, respectively.

Sampling

At the R1 stage, three earshoots per plot were covered with bags at emergence to prevent pollination. Just after silk emergence, two earshoots were sampled from each row for amino acid analysis. The sampled shoots were husked and placed in tubes, after which they were lyophilized (Millrock LD85) to remove any water. Steel balls were added to each sample and ground to powder at 1500 rpm (GenoGrinder 2000). Approximately 60 to 70 mg of tissue were treated with trichoroacetic acid to extract free amino acids. The free amino acids were quantified via high-performance liquid chromatography (Agilent 1200 HPLC). The HPLC program was adapted from an Agilent protocol for amino acid analysis (Woodward et al., 2007). Hydroxyproline and proline were derivatized with 9-fluorenylmethly chloroformate (FMOC). The remaining 19 amino acids were derivatized with o-phthalaldehyde (OPA). A 4.6 x50 mm, 1.8 micron particle size Zorbax Eclipse Plus C18 column (Agilent 959941-902) was used to separate the metabolites, which were then detected and quantified with a diode array detector. Chemstation (Agilent) software provided a visualization of chromatograms and calculation of the amino acid concentrations within each sample.

Four plants per plot were sampled at physiological maturity for R6 measurements as described in chapter 1. Due to poor seed set when open pollinated during the 2012 growing season, 2013

inbreds received supplemental hand pollinating, but were ultimately open pollinated. Hybrids were open pollinated in both 2012 and 2013. All grain samples from these two years were analyzed for grain protein, starch and oil as whole kernels on the Perten DA 7200 Near Infrared (NIR) Analyzer.

Analysis

Shapiro-wilk tests for normality were done using the `shapiro.test()` function in R. Analysis of Variance tests to determine the effect of year and N rate were done in SAS using the PROC GLM function (SAS, 2009). Group, year, and N rate were all considered fixed effects for the ANOVA. Significant effects in the model were determined using $\alpha=0.1$. Means separation tests were done in R using the `t.test()` function, which compares two populations using a two-tailed Students t-test (R, 2008). Prior to means separation, equal variances were verified using the `var.test()` function in R which uses an F-test to compare the variances. For this test, variances were assumed equal at $p>0.1$. The Welch corrected two sample t-test, which estimates the variances and adjusts the degrees of freedom used in the test to compensate for the difference, was used when variances were unequal. The nonparametric Mann-Whitney U test was used to test the null hypothesis that two populations were identical in instances where the populations were not normally distributed. The `wilcox.test()` function in R produced the significance values for the Mann-Whitney U test.

Mid-parent values were calculated as the average of the parental values. The resulting value was subtracted from the F1 value to determine mid-parent heterosis. High parent value was denoted as the higher of the two parental lines and high parent heterosis was the difference between the F1 value and the high parent value. The distributions of the populations were not normal, so significance values were determined using the Mann-Whitney-Wilcox test (`wilcox.test ()` in R).

Physical locations for the flanking markers for the nine regions of interest were previously determined during a QTL fine mapping project (Liu, 2014). Preliminary LH82 alleles were determined by using TASSEL to filter the un-imputed genotyping by sequencing data set to include only the genotypes to LH82, B73, and Mo17 (Bradbury et al., 2007). Taxa were filtered to only include physical locations between the flanking markers. The LH82 bases were compared to those of Mo17 and B73 to determine if they were the bases found in B73 (B73-like) or those found in Mo17 (Mo17-like).

Results and Discussion

Environmental Variation

The years during which these populations were evaluated represent two very different growing seasons. The maximum temperatures during the 2012 growing season were higher than usual, especially during July, which was the second warmest on record averaging 5.7°C above the 20-year average (Table 2.2). It was also the third driest July on record, at a 10.4 cm deficient compared to the 20-year average. The high temperatures and dry weather persisted throughout vegetative growth and flowering, leading to potential seed set. This is especially concerning on the inbred plots, which are less hardy than the hybrids. Cooler weather and above average rainfall during grain fill may have mitigated some potential yield loss from poor seed set, but the 2012 growing season represents an adverse Illinois growing season.

Temperatures during the 2013 growing season were more in line with the 20-year average than those in 2012 as most were less than one degree from the average. Precipitation was almost 5 cm more than the 20-year average in June, but the remainder of the growing season was drier than average. Since these plots were irrigated, the low precipitation is not expected to have impacted seed set or grain fill. Based on its minimum and maximum temperatures, coupled with the artificial precipitation, the 2013 season is more like the average Illinois growing season.

Ideotype Selection

The ideal ideotype to achieve maximum grain yield (GY) was determined for the nine alleles based on the additive effect of each QTL region on any of six traits (Table 2.3). At location 1-375, the B73 (B) allele had a moderate positive effect on N utilization at both N levels and a slight negative effect on grain N, so it was selected as the desired allele. At regions 3-310 and 4-385, the Mo17 (M) allele had moderate to high positive effect on grain biomass so it was considered the desired allele for both regions. At region 4-539, the B allele had a high positive effect on kernel number at both high and low N while the M allele had an undesired positive effect on stover biomass so this region was assigned B as a desired allele. The B allele had a high positive effect on kernel number at low N, which is desired, but also a low effect on grain N, which is undesired. Because the low strength of the effect on grain N and considering that effect was only at high N, that effect was disregarded and the region was assigned a B allele as desirable. Because region 9-24 only had

effects on traits at high N, these secondary effects were used to assign a B allele as desired. The M allele at regions 2-323 and 6-480 had a positive effect on grain N, so the opposite allele would be desired for high GY. Region 6-79 had a single low positive effect on stover N at low N. The other regions where there was an effect on stover N resulted in the desirable allele being that with a negative effect on stover N, so the B allele was selected as desirable for this region as well.

Population Selection

Six of the regions were considered primary regions because they had a medium or high effect on one or more primary selection traits. Three regions were considered secondary regions because they only had low effects on one trait. When populations were selected, lines that had more of the primary regions desired alleles were included over those with secondary region desired alleles. Within the IBMRIL population, the distributions of the number of desired alleles and the number of undesired alleles showed that no line available in our population of 302 IBMRILs had all desired alleles and no line had all undesirable alleles (Figure 2.1). In the future, stacking all nine desired alleles could be a target goal for breeders working to improve grain yield at low N.

The enriched population was constructed from the ten lines at the right tail of the desired allele distribution. None of the lines had all nine favorable alleles, but one line had 8 favorable alleles, five lines had 7 favorable alleles and four lines were selected with 6 favorable alleles (Table 2.4). The depleted population was constructed from the ten lines at the right tail of the undesired allele distribution. None of the lines had nine or eight undesired alleles, but two lines had seven undesired alleles, five lines had six undesired alleles, and four lines were selected with five undesired alleles to complete the population

QTL Enrichment Effect

As previously shown, inbreds and hybrids have different magnitudes of grain yield, NUtE and harvest index, so they are analyzed separately throughout. An analysis of variance for the inbreds indicated that year was a highly significant main effect and N rate was also a significant main effect for all four traits included (Table 2.5). The group effect was not significant for any trait, and the interaction of N rate and group was only significant for NUtE. An ANOVA for hybrids resulted in N rate being a highly significant main effect for all traits (Table 2.5). This is expected after previous analysis in chapter 1 confirmed that hybrids perform differently under low and high

N. Year was also a significant main effect for all traits. Group was not a significant effect for any trait, but the interaction of N rate and group was significant for all traits. After noting the difference in environmental conditions between 2012 and 2013, it is not unexpected that the year effect be significant in both inbreds and hybrids. Because of this significant, years were not combined and analysis throughout is separated into 2012 and 2013 results. Additionally, high and low N plots are also analyzed separately. This demarcation is essential when looking at the difference between the populations because the desired ideotype was specifically selected for performance in low N environments.

The lack of significance for the main effect of group in all analysis of variance indicates that group did not contribute to variance in the model, which is surprising as we expected stratification between the enriched and depleted populations to contribute to the variation within the experiment. Further analysis of the enriched and depleted populations included grain biomass (grain yield), grain and total N utilization efficiency, and harvest index. These phenotypes represent iterations of the six traits that were initially used to select the desired alleles for the experiment.

Summary statistics for the four traits of interest confirm trends shown to be significant in Chapter 1 (Table 2.6). Across both 2012 (Table 2.6a, Table 2.6b) and 2013 (Table 2.6c, Table 2.6d), hybrids tended to have higher grain yields, N utilization efficiencies and harvest indices than their inbred counterparts. Both inbred and hybrid grain biomass showed a response to applied N with yields higher in the high N environments (Table 2.6a, Table 2.6c) than in the low N (Table 2.6b, Table 2.6d). This response was less pronounced during the dry 2012 growing season, most likely because N was less available.

Mean comparison tests were done on all four traits across years, N rates and heterotic states in order to determine if our selections changed the phenotypic population means (Table 2.7). None of the comparisons showed a significant difference between the enriched and depleted populations. This is an unexpected result that suggests our method of using QTL enrichment to improve grain yield under low N is not effective. Since the QTL mapping was done using a hybrid population with IHP1 as the testcross parent, it is possible that the QTL regions identified do not have the same effect on a population that uses a different tester due to epistatic interactions or even epigenetic difference. This phenomena would be consistent with theories put forth by Bos and Caligari (2008). As previously mentioned, the low density of markers used for the CIM in the

original mapping resulted in wide QTL intervals, possibly encompassing many genes. Fine mapping of these QTL using recently available low-cost, high-throughput genotyping methods could resolve in the QTL intervals closer to a single-gene QTL and reduce possible interaction effect with nearby genes that are inadvertently selected upon. Additionally, recent research has suggested that heterozygosity at some loci is favored for hybrid performance (Y. Liu, 2014). Under that assumption, it is possible that selecting QTL based on the genotypes of one inbred parent is only looking at half of the puzzle and rather parental genotypes need to be compared to select those hybrids that complement each other at the regions of interest.

Rank correlations of the IBMRI x IHP1 tester set with the rankings of the selection inbreds per se and the selection hybrids testcrossed to LH82 as grown in 2012 and 2013 show very few significant correlations and none that have a high correlation coefficient (Table 2.8). Under low N conditions only the correlation of the prediction hybrids and the 2012 inbreds for NUtE was slightly significant, but had a low correlation coefficient of .398. At high N, the rank correlations between 2012 and 2013 were significant with correlation coefficients greater than 0.4 for most traits in inbreds and hybrids indicating that the year effect changed the absolute values of the phenotypes, but those that performed well in 2012 also performed well in 2013. Only the prediction hybrids with the 2013 inbreds under high N for yield showed a highly significant effect but had a highly negative correlation coefficient. The lack of significant correlations between the prediction hybrids testcrossed to IHP1 and the selection inbreds grown in 2012 and 2013 indicate that the level of heterosis plays a role in the ability to predict hybrid line performance. Consequently, the low levels of significant correlation between the hybrids testcrossed to IHP1 and the hybrids testcrossed to LH82 are indicative of variation in combining ability of the IBM lines with those two testers.

Effect on Amino Acids

While the enriched and depleted populations were selected for the specific phenotype of high GY at low N, it is likely that any changes would be the result of alterations in the N cycling pathway. As previously shown, the two populations had no significant differences in whole plant phenotype, but it is still possible to examine a sampling of biological differences on the cellular level. To do so, asparagine, glutamine, glutamic acid, and aspartic acid – four amino acids important to the nitrogen cycling pathway – were included in the comparison of the enriched and depleted

populations (Table 2.9). There were no significant differences in asparagine levels in any of the comparisons, but aspartic acid showed significant differences in both hybrid years under high N. In 2012 the enriched population had a higher level of aspartic acid and in 2013 the depleted population had a higher level of aspartic acid. This indicates that stark difference in environmental conditions could impact the interaction between the populations and their aspartic acid levels. In 2012, glutamine was significantly higher in the enriched population under high N in both the inbreds and hybrids. The inbred enriched population also had higher glutamine in both N levels in 2013. Finally, 2012 glutamic acid levels were higher in the enriched population under both N levels in the inbreds and under high N in the hybrids. Previous research indicated that glutamine is higher in IBM lines that have the B73 allele when compared to those with the Mo17 allele at the asparaginase loci (Postin, 2014). The increase in glutamine in the enriched population could mean that one of the loci included in the desired ideotype may be genetically linked to the desired B allele for asparaginase.

Allele Effects

Since there were no lines with all of the desired alleles of interest in the IBMRIL population, allelic variation existed at each of the nine regions within the selected populations. The effects of this variation at each region was explored by comparing the means of two populations created by sorting the twenty selected IMBRIL lines based on their allele at the region of interest in both the hybrids (Table 2.10a) and the inbreds (Table 2.10b). Region 1-375 showed highly significant positive effects of the M allele on Stover biomass at both high and low N in inbreds and hybrids, which is consistent with the results from the original QTL mapping. That region also showed a significant positive effect of the M allele on NUtE at low N in the inbreds and high N in the hybrids. This is opposite of the positive effect of B73 on NUtE from the original QTL mapping. At region 3-310, a positive effect of the B allele on stover N at high N was consistent with the original mapping. The positive effect of the M allele at this region on NUtE was reported in the original QTL mapping and also in this experiment.

In the original mapping experiment, region 4-385 had a strong effect on grain biomass with M as the positive effect allele. That effect is not visible in this experiment. This region does, however, seem to have an effect on stover biomass. Region 4-539 had an effect on many traits in the original mapping and in this LH82 experiment. The positive effect of B on NUtE and the positive

effect of M on stover N are similar across experiments, but the effect of the M allele on stover biomass used in the selection was not obvious in this population. Unlike many other allele effects in this experiment, the effects at region 6-126 are not stable across hybrids and inbreds as the B allele is the positive effect on NUtE at high N in inbreds but the M allele has the positive effect at high N in hybrids.

The LH82 experiment shown here includes a positive effect of the M allele on biomass that is omitted from the original selection criteria based on the IHP1 mapping experiment. Additionally, regions 2-323 and 6-79 show positive effects of the M allele on Grain Biomass and Grain N which are not in the original mapping results. Finally, region 6-480 does not show the grain N effect that the original mapping indicated would be significant. These discrepancies illustrate differences in allele effects that could be due to interactions with a tester other than that used for the QTL mapping. The changes in significance of alleles on individual traits and the switch in positive effect alleles suggest that the desired alleles selected from the original mapping population would be ideal to obtain improved low N GY in a population of IBMRIL x IHP1 lines but cannot be used when the IBMRIL population is crossed to a different tester, thereby contributing to the lack of selection efficiency observed in this experiment.

Heterosis and the contribution of LH82

LH82, the IBMRIL inbreds, and their F₁ progeny were all grown in 2013 under the same conditions so the level of heterosis can be calculated for each trait. Under high N, all hybrids out-yielded both the mid-parent and high parent value of their inbreds (Table 2.11a). The F₁ hybrid populations also had a higher harvest index than both their mid-parent and high parent values. Under low N, the hybrid populations both out-yielded the mid-parent values, but the enriched population was not significantly different than the high parent (Table 2.11b). The populations both had a higher harvest index than the mid-parent, but were lower than the high parent.

The testcross parent LH82 was one of the inbred lines included in the recent genotyping by sequencing of more than 2800 maize line in the National Plant Germplasm System (GRIN) (Romay et al., 2013). Based on the SNP marker dataset published with the study, it is possible to identify the alleles that LH82 is contributing to the cross. Preliminary analysis shows that alleles can be determined for six of the nine regions (Table 2.12). The other three regions showed indications of

a recombination event within the interval, so these regions cannot be declared as B73-like or Mo17-like until they are narrowed. Three of the regions, 1-375, 2-323, and 9-4 contained Mo17-like alleles. The other three regions, 4-385, 4-539, and 6-480 all contained B73-like alleles.

Conclusions

While variation exists in the IMBRIL population for NUE and its component traits, the use of a summation index based on the number of positive or negative QTL was unsuccessful in improving grain yield or other traits used in NUE determination. The lack of selection response could be due to a difference in testers from the mapping population to the selection population which could alter the positive effect allele, resulting in poor selections for the enriched and depleted populations. More likely is that our summation selection index chose the populations based on one parents' positive alleles, but disregarded the possibility that the positive effect is a result of heterozygosity at that allele in the hybrid. The idea that heterozygosity is favored for hybrid performance merits further exploration as it could help elucidate the mechanisms through which inbred parents should be selected to make the ideal hybrid progeny.

References

- Agrama, H. A. (2005). Application of Molecular Markers in Breeding for Nitrogen Use Efficiency. *Journal of Crop Improvement*, 15(2), 175.
- Agrama, H. A. S., Zakaria, A. G., Said, F. B., & Tuinstra, M. (1999). Identification of quantitative trait loci for nitrogen use efficiency in maize. *Molecular Breeding*, 5(2), 187-195. doi: Doi 10.1023/A:10096669507144
- Angel, J. (2009). Weather Instruments for Measuring the Climate of Illinois. *State Climatologist Office for Illinios*. 2014, from http://www.sws.uiuc.edu/atmos/statecli/Instruments/weather_instruments.htm
- Barbieri, P. A., Echeverria, H. E., Rozas, H. R. S., & Andrade, F. H. (2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal*, 100(4), 1094-1100. doi: DOI 10.2134/agronj2006.0057
- Bertin, P., & Gallais, A. (2001). Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines II - QTL detection and coincidences. *Maydica*, 46(1), 53-68.

- Bodirsky, B. L., Popp, A., Lotze-Campen, H., Dietrich, J. P., Rolinski, S., Weindl, I., . . . Stevanovic, M. (2014). Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nat Commun*, 5, 3858. doi: 10.1038/ncomms4858
- Bos, I., & Caligari, P. (2008). *Selection Methods in Plant Breeding* (2nd ed.). The Netherlands: Springer.
- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, 23(19), 2633-2635. doi: 10.1093/bioinformatics/btm308
- Cassman, K. G., Dobermann, A., & Walters, D. T. (2002). Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio*, 31(2), 132-140.
- Castleberry, R. M., Crum, C. W., & Krull, C. F. (1984). Genetic Yield Improvement of U.S. Maize Cultivars under Varying Fertility and Climatic Environments¹. *Crop Science*, 24(1), 33. doi: 10.2135/cropsci1984.0011183X002400010008x
- Ciampitti, I. A., & Vyn, T. J. (2011). A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. *Field Crops Research*, 121(1), 2-18. doi: DOI 10.1016/j.fcr.2010.10.009
- Ciampitti, I. A., & Vyn, T. J. (2012). Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crops Research*, 133, 48-67. doi: 10.1016/j.fcr.2012.03.008
- Cone, K. C., McMullen, M. D., Bi, I. V., Davis, G. L., Yim, Y. S., Gardiner, J. M., . . . Coe, E. H., Jr. (2002). Genetic, physical, and informatics resources for maize. On the road to an integrated map. *Plant Physiol*, 130(4), 1598-1605. doi: 10.1104/pp.012245
- Coque, M., Martin, A., Veyrieras, J. B., Hirel, B., & Gallais, A. (2008). Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences. *Theor Appl Genet*, 117(5), 729-747. doi: 10.1007/s00122-008-0815-2
- Duvick, D. N., & Cassman, K. G. (1999). Post-green revolution trends in yield potential of temperate maize in the north-central United States. *Crop Science*, 39(6), 1622-1630.
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 6(5), e19379. doi: 10.1371/journal.pone.0019379

- Gentry, L. E., Below, F. E., David, M. B., & Bergerou, J. A. (2001). Source of the soybean N credit in maize production. *Plant and Soil*, 236(2), 175-184. doi: 10.1023/A:1012707617126
- Giannino, D., Nicolodi, C., Testone, G., Frugis, G., Pace, E., Santamaria, P., . . . Mariotti, D. (2007). The overexpression of asparagine synthetase A from *E. coli* affects the nitrogen status in leaves of lettuce (*Lactuca sativa* L.) and enhances vegetative growth. *Euphytica*, 162(1), 11-22. doi: 10.1007/s10681-007-9506-3
- Haegerle, J. W., Cook, K. A., Nichols, D. M., & Below, F. E. (2013). Changes in Nitrogen Use Traits Associated with Genetic Improvement for Grain Yield of Maize Hybrids Released in Different Decades. *Crop Science*, 53(4), 1256. doi: 10.2135/cropsci2012.07.0429
- Hirel, B., Bertin, P., Quillere, I., Bourdoncle, W., Attagnant, C., Dellay, C., . . . Gallais, A. (2001). Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol*, 125(3), 1258-1270. doi: DOI 10.1104/pp.125.3.1258
- Hirel, B., Le Gouis, J., Ney, B., & Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J Exp Bot*, 58(9), 2369-2387. doi: 10.1093/jxb/erm097
- Jayasundara, S., Wagner-Riddle, C., Parkin, G., Bertoldi, P., Warland, J., Kay, B., & Voroney, P. (2007). Minimizing nitrogen losses from a corn–soybean–winter wheat rotation with best management practices. *Nutrient Cycling in Agroecosystems*, 79(2), 141-159. doi: 10.1007/s10705-007-9103-9
- Johnson, G. R. (2004). Marker-assisted Selection. In J. Janick (Ed.), *Plant Breeding Reviews* (Vol. 24, pp. 293-309): John Wiley & Sons, Inc.
- Kirda, C., Topcu, S., Kaman, H., Ulger, A. C., Yazici, A., Cetin, M., & Derici, M. R. (2005). Grain yield response and N-fertiliser recovery of maize under deficit irrigation. *Field Crops Research*, 93(2-3), 132-141. doi: DOI 10.1016/j.fcr.2004.09.015
- Koocheki, A., Nassiri Mahallati, M., Mansoori, H., & Moradi, R. (2013). Assessing nitrogen flow in production and utilization cycle of wheat and maize as a tool to monitor N loss in Iran. *Acta Scientiarum. Biological Sciences*, 35(3). doi: 10.4025/actascibiols.v35i3.16039

- Lam, H. M., Wong, P., Chan, H. K., Yam, K. M., Chen, L., Chow, C. M., & Coruzzi, G. M. (2003). Overexpression of the ASN1 gene enhances nitrogen status in seeds of *Arabidopsis*. *Plant Physiol*, 132(2), 926-935. doi: 10.1104/pp.103.020123
- Lee, M., Sharopova, N., Beavis, W. D., Grant, D., Katt, M., Blair, D., & Hallauer, A. (2002). Expanding the genetic map of maize with the intermated B73 x Mo17 (IBM) population. *Plant Mol Biol*, 48(5-6), 453-461.
- Lightfoot, D. A., Mungur, R., Ameziane, R., Nolte, S., Long, L., Bernhard, K., . . . Young, B. (2007). Improved drought tolerance of transgenic *Zea mays* plants that express the glutamate dehydrogenase gene (*gdhA*) of *E. coli*. *Euphytica*, 156(1-2), 103-116. doi: DOI 10.1007/s10681-007-9357-y
- Liu, R. X., Zhang, H., Zhao, P., Zhang, Z. X., Liang, W. K., Tian, Z. G., & Zheng, Y. L. (2012). Mining of Candidate Maize Genes for Nitrogen Use Efficiency by Integrating Gene Expression and QTL Data. *Plant Molecular Biology Reporter*, 30(2), 297-308. doi: DOI 10.1007/s11105-011-0346-x
- Liu, Y. (2014). *Discovery and Characterization of Genes Controlling Nitrogen Utilization in Maize*. (PhD), University of Illinois, Urbana, IL.
- Liu, Z. H. (2007). QTL mapping of ear traits under low and high nitrogen conditions in maize. *Scientia Agricultura Sinica*, 40(11), 2409.
- Lorenz, A. J., Gustafson, T. J., Coors, J. G., & de Leon, N. (2010). Breeding Maize for a Bioeconomy: A Literature Survey Examining Harvest Index and Stover Yield and Their Relationship to Grain Yield. *Crop Science*, 50(1), 1-12. doi: DOI 10.2135/cropsci2009.02.0086
- Loudet, O., Chaillou, S., Merigout, P., Talbotec, J., & Daniel-Vedele, F. (2003). Quantitative trait loci analysis of nitrogen use efficiency in *Arabidopsis*. *Plant Physiol*, 131(1), 345-358. doi: 10.1104/pp.102.010785
- Lynch, J. P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann Bot*, 112(2), 347-357. doi: 10.1093/aob/mcs293
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Ann Bot*, 105(7), 1141-1157. doi: 10.1093/aob/mcq028

- Masclaux-Daubresse, C., Reisdorf-Cren, M., Pageau, K., Lelandais, M., Grandjean, O., Kronenberger, J., . . . Suzuki, A. (2006). Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. *Plant Physiol*, 140(2), 444-456. doi: 10.1104/pp.105.071910
- McTaggart, I. P., & Smith, K. A. (1995). The effect of rate, form and timing of fertilizer N on nitrogen uptake and grain N content in spring malting barley. *Journal of Agricultural Science*, 125, 341-353.
- Mi, G., Chen, F., Wu, Q., Lai, N., Yuan, L., & Zhang, F. (2010). Ideotype root architecture for efficient nitrogen acquisition by maize in intensive cropping systems. *Sci China Life Sci*, 53(12), 1369-1373. doi: 10.1007/s11427-010-4097-y
- Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and Interpretation of Factors Which Contribute to Efficiency of Nitrogen-Utilization. *Agronomy Journal*, 74(3), 562-564.
- Nichols, D. (2008). *Genetic Analysis of Nitrogen Use Efficiency and Related Traits in the IBMRIL x IHP1 Population of Maize*. (PhD), University of Illinois, Urbana, IL.
- O'Neill, P., Shanahan, J., Schepers, J., & Caldwell, B. (2004). Agronomic Responses of Corn Hybrids from Different Eras to Deficit and Adequate Levels of Water and Nitrogen. *Agronomy Journal*, 96, 1660.
- Postin, C. (2014). *Effect of Nitrogen Form and Genetic Variations of the Asparagine-Cycling Pathway on Maize Ear Growth*. (MS), University of Illinois, Urbana, IL.
- Presterl, T., Seitz, G., Landbeck, M., Thiemt, E. M., Schmidt, W., & Geiger, H. H. (2003). Improving nitrogen-use efficiency in European maize: Estimation of quantitative genetic parameters. *Crop Science*, 43(4), 1259-1265.
- R. (2008). R: A language and environment for statistical computing Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>
- Ribaut, J. M., Fracheboud, Y., Monneveux, P., Banziger, M., Vargas, M., & Jiang, C. J. (2007). Quantitative trait loci for yield and correlated traits under high and low soil nitrogen conditions in tropical maize. *Molecular Breeding*, 20(1), 15-29. doi: DOI 10.1007/s11032-006-9041-2

- Romay, M. C., Millard, M. J., Glaubitz, J. C., Peiffer, J. A., Swarts, K. L., Casstevens, T. M., . . . Gardner, C. A. (2013). Comprehensive genotyping of the USA national maize inbred seed bank. *Genome Biol*, 14(6), R55. doi: 10.1186/gb-2013-14-6-r55
- Sangoi, L., Ender, M., Guidolin, A. F., Almeida, M. L. d., & Konflanz, V. A. (2001). Nitrogen fertilization impact on agronomic traits of maize hybrids released at different decades. *Pesquisa Agropecuária Brasileira*, 36(5), 757-764. doi: 10.1590/s0100-204x2001000500005
- SAS. (2009). SAS 9.2 User's Guide. Cary, NC: SAS Inst.
- Tanksley, S. D., & Hewitt, J. (1988). Use of Molecular Markers in Breeding for Soluble Solids Content in Tomato - a Re-Examination. *Theoretical and Applied Genetics*, 75(5), 811-823.
- USDA. (2013). Fertilizer Use and Price. from <http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26720>
- Wei, D., Cui, K., Ye, G., Pan, J., Xiang, J., Huang, J., & Nie, L. (2012). QTL mapping for nitrogen-use efficiency and nitrogen-deficiency tolerance traits in rice. *Plant and Soil*, 359(1-2), 281-295. doi: 10.1007/s11104-012-1142-6
- Woodward, C., Henderson Jr., J., & Wielgos, T. (2007). High-Speed Amino Acid Analysis (AAA) on 1.8um Reversed Phase (RP) Columns. Wilmington, DE: Agilent Technologies.
- Zotarelli, L., Scholberg, J. M., Dukes, M. D., & Munoz-Carpena, R. (2008). Fertilizer residence time affects nitrogen uptake efficiency and growth of sweet corn. *J Environ Qual*, 37(3), 1271-1278. doi: 10.2134/jeq2007.0460

Figures and Tables

Table 2.1 Summary of QTL analysis for seven traits at high and low N. Modified from Nichols (2008)

Trait	Position (cM) ^a	Left	Right	Estimated Mbp ^b	Peak LOD	Additive effect	R ² (%)
Grain biomass -N	3-310	cdo105	csu636	15.10	3.54	-2.76	5.68
Grain biomass -N	4-385	umc104a	mmp115	2.16	6.90	-6.08	13.70
Kernel number -N	4-385	umc104a	mmp115	2.16	4.41	-15.55	6.78
Kernel number -N	6-125	umc1257	umc1595	4.71	3.80	14.31	5.73
Kernel number -N	4-539	umc2139	umc1999	6.69	2.98	10.26	5.00
Kernel number +N	4-538	umc2139	umc1999	6.69	4.19	16.88	11.65
Stover N content -N	1-375	SSR-18 ^c	umc2025	3.80	6.08	-0.03	10.48
Stover N content -N	6-79	uck1	mmp160	7.68	5.05	-0.04	7.71
Stover N content +N	3-310	cdo105	csu636	15.10	3.52	0.06	8.35
Stover N content +N	4-539	umc2139	umc1999	6.68	5.03	-0.05	7.57
Grain N concen -N	1-375	SSR-18	umc2025	3.80	2.59	-0.19	4.87
Grain N concen -N	2-325	mmc0401	umc1079	6.51	3.68	-0.24	6.71
Grain N concen -N	6-480	mmp105	umc2059	1.74	5.82	-0.33	9.10
Grain N concen +N	1-375	SSR-18	umc2025	3.80	3.45	-0.31	5.16
Grain N concen +N	2-325	mmc0401	umc1079	6.51	3.75	-0.27	5.40
Grain N concen +N	6-125	umc1257	umc1595	4.71	4.14	0.28	6.17
Grain N concen +N	9-25	bnlg2122	umc1867	0.32	6.91	-0.36	10.11
Stover biomass -N	1-375	SSR-18	umc2025	3.80	3.60	-3.30	5.16
Stover biomass -N	3-310	cdo105	csu636	15.10	4.34	3.88	7.11
Stover biomass -N	4-538	umc2139	umc1999	6.69	3.56	-3.58	5.32
Stover biomass +N	1-375	SSR-18	umc2025	3.80	2.71	-2.94	4.80
Stover biomass +N	4-538	umc2139	umc1999	6.69	3.78	-4.22	6.25
N utilization -N	1-375	SSR-18	umc2025	3.80	6.17	3.16	10.15
N utilization +N	1-375	SSR-18	umc2025	3.80	2.93	0.92	4.13
N utilization +N	3-310	cdo105	csu636	15.10	3.98	-1.15	10.32
N utilization +N	4-539	umc2139	umc1999	6.69	4.92	1.29	7.16
N utilization +N	9-25	bnlg2122	umc1867	0.32	3.34	0.89	6.00

a. Chromosome positions are provided as Chromosome-centimorgan and are defined as the QTL peak position on the genetic map

b. Physical distance is based on the flanking marker position in the maize genome from www.maizesequence.org. Flanking markers are listed as “left” and “right”.

c. In-house marker, primer sequences being forward “GCAGGCAAGCCGCAAGTG”, reverse “GCGGTGAGATAGCGGAGTAAC”

Table 2.2 Average monthly weather data for May 1 to September 30 in 2012 and 2013 at Champaign IL. $T_{\max}$ and $T_{\min}$ are the maximum and minimum temperatures respectively. GDD are the number of growing degree days accumulated that month. Values in parenthesis for temperature and precipitation are deviations from the 20-year average (1991-2011) at Champaign, IL.

Year	Month	T _{max}		T _{min}		Precipitation		GDD
		°C				cm		
2012	May	27.1	(4.1)	13.3	(2.4)	7.9	(-4.5)	557
	June	29.5	(1.4)	15.3	(-1.3)	5.8	(-5.2)	632.5
	July	35.2	(5.7)	20.6	(2.3)	1.5	(-10.4)	853
	August	30.6	(1.9)	16.2	(-1.1)	14.1	(4.1)	689
	September	24.3	(-1.3)	12.1	(-0.2)	14.5	(6.6)	469
2013	May	23.9	(0.9)	11.9	(1.0)	9.5	(-2.9)	475.5
	June	27.7	(-0.4)	15.9	(-0.7)	15.9	(4.9)	626
	July	27.7	(-1.7)	17.3	(-1.0)	9.0	(-3.0)	678
	August	29.0	(0.3)	16.7	(-0.6)	0.9	(-9.1)	694
	September	28.1	(2.4)	13.7	(1.4)	1.7	(-6.2)	574

Table 2.3 Summary of effects of nine regions on six traits at low and high N. Regions are listed as chromosome-centimorgan. Low additive effect is 0 to .5, medium effect is between .5 and 4.0, high effects are over 4.0. Bottom row is ideotype across the nine regions for increase GY at low N.

Trait	N Rate	Primary QTL							Secondary QTL		
		1-375	3-310	4-385	4-539	6-126	9-24		2-323	6-79	6-480
Stover N	Low	M								M	
	High		B		M						
Grain N	Low	M							M		M
	High	M				B	M		M		
Stover Biomass	Low	M	B		M						
	High	M			M						
Grain Biomass	Low		M	M							
	High									Additive Effect	
Kernel Number	Low			M	B	B					Low
	High				B						Medium
N Utilization	Low	B									High
	High	B	M		B		B				
Desired Alleles for Improved 0N GY		B	M	M	B	B	B		B	B	B

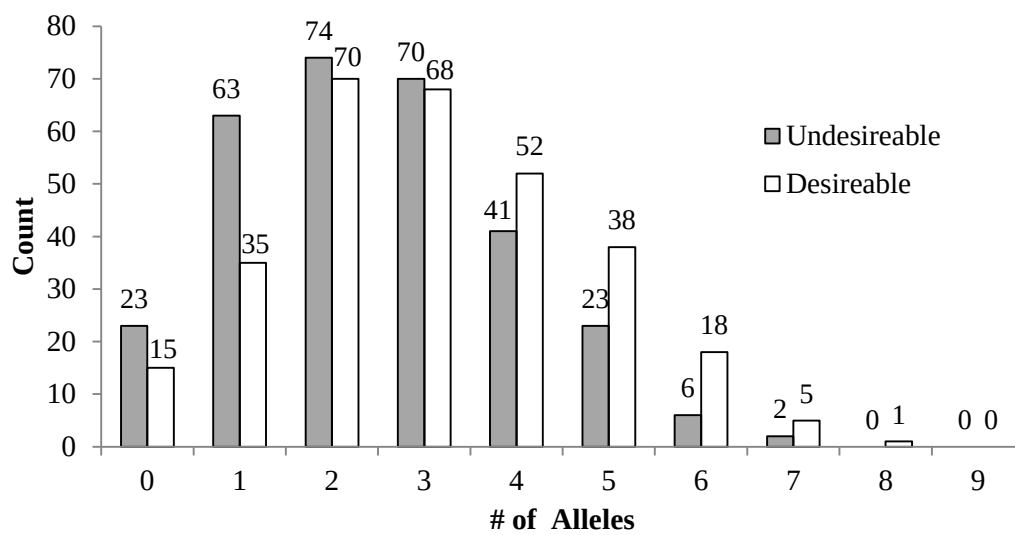


Figure 2.1 Distribution of 302 IBMRIL genotypes when compared to the desired ideotype for high GY at low N.

Table 2.4 Composition of enriched and depleted populations. Letter indicated B73 (B) or Mo17 (M) allele in the indicated region. Slashed cells are missing or inconclusive data. White boxes are desired alleles, grey boxes are undesired alleles. Number of loci is the number of regions with desirable alleles or undesirable alleles for the enriched and depleted populations respectively.

IBMRIL Lines	Primary QTL							Secondary QTL					
	1-375	3-310	4-385	4-539	6-126	9-24		2-323	6-79	6-480	# of Loci	Rank	
MO351	B	M	M	B	B	B		M	B	B	8	1	Enriched
MO138	B	M	M	B	B	B		M	B	M	7	2	
MO003	M	M	M	B	B	B			B	B	7	3	
MO090	B	M	M	B	M	B			B	B	7	4	
MO029	B	B	M	B	B	B		B	B	M	7	5	
MO354	B		M	B	B	B		B	B	M	7	6	
MO304	M	M	M	B	B	B			B		6	7	
MO027	B	M	M	B	M	B			B	M	6	8	
MO289	B	M	M		B	B		B			6	9	
MO142	B	M	M	M	M	B		B	M	B	6	10	
MO190	M	M	B	B	M	M			M	B	5	11	Depleted
MO168	B	M	B	B	M	M		M	M	B	5	12	
MO197	B	B	B	M	B	M		B	B	M	5	13	
MO250		B	B	B	M	M		B	M	M	6	14	
MO219		B	B	M	M	B		B	M	M	6	15	
MO101	B	B	B	B	M	M			M	M	6	16	
MO194	M	B	B	B	M	B			M	B	6	17	
MO277	M	B	B		M	M		B	M		6	18	
MO238		B	B	M	M	M			M	M	7	19	
MO337	M	M	B	M	M	M		B	M	M	7	20	
	B	M	M	B	B	B		B	B	B	Desired Ideotype		

Table 2.5 Analysis of variance for grain biomass, grain N utilization efficiency, stover utilization efficiency, and harvest index for the enriched and depleted populations grown at high and low N in 2012 and 2013 in Urbana, IL. Hybrids are test crosses with LH82.

	Trait	Sources of Variation			
		Year	N Rate	Group(year)	N rate*Group(year)
		P>F			
Inbred	<i>Grain Biomass</i>	<.0001	0.0846	NS	NS
	<i>Grain NUtE</i>	<.0001	NS	NS	NS
	<i>Total NUtE</i>	<.0001	0.0002	NS	0.0711
	<i>HI</i>	<.0001	NS	NS	NS
	Trait	Sources of Variation			
		Year	N Rate	Group(year)	N rate*Group(year)
		P>F			
Hybrid	<i>Grain Biomass</i>	0.0109	<.0001	NS	0.0470
	<i>Grain NUtE</i>	<.0001	<.0001	NS	0.0423
	<i>Total NUtE</i>	<.0001	<.0001	NS	<.0001
	<i>HI</i>	<.0001	0.0001	NS	0.0321

Table 2.6a Summary statistics for grain biomass, grain N Utilization efficiency, total N Utilization efficiency and harvest index for the enriched and depleted populations grown in 2012 at Urbana, Illinois under high N conditions as inbreds and hybrids testcrossed to LH82.

	IBMRIL Line	Inbreds								Hybrids x LH82							
		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index	
		mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
Enriched	IBMRI MO003	25.8	NA	10.9	NA	57.9	NA	0.19	NA	86.9	53.8	35.4	17.0	80.5	11.1	0.43	0.15
	IBMRI MO027	33.1 *	46.8	15.6	22.0	62.4	3.2	0.26	0.37	98.4	12.6	48.5	5.8	86.7	4.4	0.56	0.04
	IBMRI MO029	25.2	11.4	9.8	4.5	49.5	2.9	0.20	0.08	86.4	21.9	43.4	4.1	89.6	1.9	0.48	0.05
	IBMRI MO090	2.5 *	3.6	1.2	1.7	51.9	9.2	0.02	0.03	84.0	27.5	38.8	4.9	90.2	2.8	0.43	0.04
	IBMRI MO138	13.0	NA	3.3	NA	41.9	NA	0.08	NA	85.6	34.8	41.1	16.9	85.4	11.9	0.47	0.14
	IBMRI MO142	2.8 *	3.9	1.4	2.0	41.9	5.0	0.03	0.04	110.9	7.5	42.4	2.5	83.4	4.6	0.51	0.00
	IBMRI MO289	43.4	2.3	27.4	7.6	65.2	13.4	0.42	0.03	97.0	6.8	43.8	9.7	79.8	8.7	0.54	0.06
	IBMRI MO304	28.5	12.9	12.1	4.4	60.9	2.7	0.20	0.06	88.9	32.3	45.1	12.3	98.6	5.6	0.45	0.10
	IBMRI MO351	45.4	10.7	22.7	2.2	60.9	4.4	0.37	0.01	105.7	19.4	48.3	17.8	88.2	18.7	0.53	0.10
	IBMRI MO354	32.1	8.6	13.0	0.1	53.1	4.9	0.25	0.03	98.8	29.1	40.5	12.2	78.0	7.3	0.51	0.12
	Population	25.8	19.7	12.3	10.5	55.1	9.2	0.21	0.16	94.3	23.1	43.1	10.3	86.2	9.7	0.50	0.09
Depleted	IBMRI MO101	28.2	15.9	12.8	5.4	58.2	3.3	0.22	0.08	99.9	43.2	35.1	8.5	80.4	2.7	0.43	0.09
	IBMRI MO168	32.2	22.2	14.8	12.0	63.0	18.0	0.22	0.13	131.6	25.5	52.1	2.3	91.1	3.4	0.57	0.02
	IBMRI MO190	14.5 *	20.5	6.9	9.8	54.7	5.9	0.12	0.17	128.6	28.3	56.6	5.8	99.6	10.2	0.57	0.03
	IBMRI MO194	36.7	31.1	12.4	9.6	59.3	2.1	0.21	0.15	84.5	66.1	33.6	26.8	76.0	14.6	0.42	0.31
	IBMRI MO197	0.1 *	0.1	0.0	0.0	48.8	0.7	0.00	0.00	71.5	20.6	28.7	9.4	76.0	6.4	0.37	0.09
	IBMRI MO219	17.6	9.8	6.2	2.2	49.8	0.1	0.13	0.04	126.7	9.9	50.8	11.1	90.2	10.8	0.56	0.06
	IBMRI MO238	0.0 *	0.0	0.0	0.0	38.5	0.2	0.00	0.00	117.8	6.0	57.2	6.8	100.5	12.0	0.57	0.02
	IBMRI MO250	37.3	NA	12.6	NA	52.1	NA	0.24	NA	82.5	51.4	35.6	20.4	82.1	10.3	0.42	0.20
	IBMRI MO277	Lost Both Replications								103.9	25.0	44.9	8.3	92.5	6.5	0.48	0.06
	IBMRI MO337	31.8	21.5	10.9	7.1	54.7	4.8	0.19	0.11	102.9	4.8	50.2	9.3	93.5	10.6	0.54	0.04
	Population	21.1	19.2	8.3	7.5	53.3	8.7	0.14	0.12	105.9	34.3	44.6	14.4	88.2	11.7	0.49	0.13

IBMRIL MO277 stover and grain were completely lost due to hot weather conditions and were excluded from analysis.

* lines in which at least one rep had 0 GY due to dry weather. Included in analysis as barrenness could be a manifestation of poor NUtE

Table 2.6b Summary statistics for grain biomass, grain N Utilization efficiency, total N Utilization efficiency and harvest index for the enriched and depleted populations grown in 2012 at Urbana, Illinois under low N conditions as inbreds and hybrids testcrossed to LH82.

	IBMRIL Line	Inbreds								Hybrids x LH82							
		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index	
		mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
Enriched	IBMRI MO003	23.5	20.8	8.2	6.7	58.0	5.9	0.15	0.13	95.8	31.8	59.4	13.0	123.0	20.6	0.48	0.04
	IBMRI MO027	25.0	24.2	18.1	18.9	56.4	21.4	0.28	0.23	84.9	31.5	56.7	9.7	116.0	19.8	0.49	0.04
	IBMRI MO029	10.0	6.0	4.2	3.0	44.1	7.9	0.09	0.05	86.4	19.7	58.6	10.4	117.8	2.4	0.50	0.08
	IBMRI MO090	0.6 *	0.8	0.3	0.5	50.2	0.6	0.01	0.01	81.9	14.2	53.1	6.9	120.7	12.9	0.44	0.08
	IBMRI MO138	0.0 *	NA	0.0	NA	36.6	NA	0.00	NA	88.3	22.4	64.7	1.2	126.4	10.2	0.51	0.05
	IBMRI MO142	1.3 *	1.9	0.8	1.2	50.8	0.8	0.02	0.02	70.3	22.4	60.1	6.1	133.8	0.5	0.45	0.04
	IBMRI MO289	51.7	26.1	28.6	6.2	66.8	14.1	0.43	0.00	80.8	14.0	59.2	13.1	116.8	13.9	0.50	0.06
	IBMRI MO304	8.3	3.3	3.8	1.0	70.5	8.8	0.05	0.01	102.5	9.5	68.7	1.4	123.6	8.8	0.56	0.05
	IBMRI MO351	31.2	26.9	18.0	14.4	61.9	15.5	0.27	0.16	100.5	6.8	68.9	3.4	126.3	4.1	0.54	0.01
	IBMRI MO354	29.1	14.7	13.3	5.3	61.7	12.1	0.21	0.04	70.7	17.1	56.2	10.3	123.2	14.7	0.45	0.05
	Population	19.0	20.4	10.0	11.2	56.7	12.4	0.16	0.16	85.7	20.2	60.3	8.8	122.7	11.8	0.49	0.06
Depleted	IBMRI MO101	27.1	16.2	17.2	11.2	65.3	7.5	0.25	0.14	83.2	32.5	57.5	16.6	121.7	11.5	0.47	0.10
	IBMRI MO168	29.5	6.8	11.6	1.1	57.6	8.5	0.21	0.05	113.3	19.1	63.2	2.5	120.1	6.4	0.53	0.03
	IBMRI MO190	39.8	5.2	15.1	0.1	61.2	4.9	0.25	0.02	81.2	16.6	61.6	5.2	131.9	2.7	0.47	0.04
	IBMRI MO194	33.0	7.3	12.8	1.5	61.4	2.2	0.21	0.03	72.3	30.9	46.7	26.0	111.0	18.3	0.41	0.17
	IBMRI MO197	0.0 *	0.0	0.0	0.0	55.5	11.9	0.00	0.00	74.6	5.5	52.9	2.4	121.5	9.3	0.44	0.02
	IBMRI MO219	17.1	7.1	7.4	2.4	52.1	5.5	0.14	0.03	75.9	0.9	59.4	8.0	125.2	6.1	0.47	0.04
	IBMRI MO238	0.0 *	0.0	0.0 *	0.0	47.4	2.8	0.00	0.00	99.8	42.2	61.0	9.1	127.2	17.7	0.48	0.01
	IBMRI MO250	6.0 *	8.5	2.0	2.8	42.0	1.1	0.05	0.07	108.8	3.9	62.3	0.1	111.9	0.3	0.56	0.00
	IBMRI MO277	60.6	0.4	24.3	3.7	65.0	6.0	0.37	0.02	75.2	7.3	58.0	4.8	126.6	2.9	0.46	0.03
	IBMRI MO337	12.3	1.9	5.2	1.4	62.2	25.4	0.09	0.01	86.1	24.6	51.0	8.6	120.9	13.2	0.42	0.03
	Population	22.5	19.5	9.6	8.4	57.0	10.5	0.16	0.13	87.2	23.6	57.5	9.6	122.4	10.4	0.47	0.06

* lines in which at least one rep had 0 GY due to dry weather. Included in analysis in case barrenness was a manifestation of poor NUtE

Table 2.6c Summary statistics for grain biomass, grain N Utilization efficiency, total N Utilization efficiency and harvest index for the enriched and depleted populations grown in 2013 at Urbana, Illinois under high N conditions as inbreds and hybrids testcrossed to LH82.

	IBMRIL Line	Inbreds								Hybrids x LH82							
		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index	
		mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
Enriched	IBMRI MO003	71.6	3.1	38.0	5.3	98.6	4.7	0.38	0.04	130.1	14.0	54.2	5.3	92.8	1.3	0.58	0.06
	IBMRI MO027	52.3	14.4	29.0	6.3	69.8	0.7	0.42	0.09	109.0	21.8	55.8	4.7	89.4	7.2	0.62	0.00
	IBMRI MO029	46.5	10.0	33.8	3.1	80.3	11.5	0.42	0.02	117.5	33.7	51.9	3.9	84.9	3.5	0.61	0.03
	IBMRI MO090	52.7	NA	36.9	NA	84.8	NA	0.44	NA	101.6	11.0	52.6	4.1	86.7	3.0	0.61	0.03
	IBMRI MO138	70.6	12.4	36.2	0.7	82.2	2.8	0.44	0.01	98.6	7.1	48.1	1.4	80.0	0.7	0.60	0.02
	IBMRI MO142	35.9	1.9	22.3	1.9	61.6	0.0	0.36	0.03	107.5	12.3	48.6	10.6	83.3	11.7	0.58	0.04
	IBMRI MO289	60.3	32.0	34.4	10.8	92.6	1.2	0.37	0.12	109.8	6.6	51.9	9.4	84.9	10.4	0.61	0.05
	IBMRI MO304	62.9	17.8	34.7	6.5	87.2	5.8	0.40	0.05	129.8	22.8	46.4	4.9	80.4	8.6	0.58	0.00
	IBMRI MO351	68.2	0.8	39.0	8.3	89.0	3.2	0.44	0.11	123.1	9.4	55.0	3.3	87.6	2.5	0.63	0.02
	IBMRI MO354	60.0	1.5	34.6	4.9	79.5	4.6	0.43	0.04	106.4	5.1	48.5	1.8	78.7	1.7	0.62	0.01
	Population	58.4	15.1	33.7	6.5	82.4	11.3	0.41	0.06	113.4	17.6	51.3	5.7	84.9	6.8	0.60	0.03
Depleted	IBMRI MO101	63.9	9.1	33.9	2.2	73.7	1.5	0.46	0.04	105.7	4.6	54.7	6.9	85.3	8.1	0.64	0.02
	IBMRI MO168	75.1	0.4	36.8	4.3	88.0	3.0	0.42	0.06	122.6	2.7	55.4	7.3	89.2	6.6	0.62	0.04
	IBMRI MO190	60.1	16.0	34.2	1.4	80.5	0.1	0.42	0.02	112.7	12.7	53.8	6.5	86.9	7.3	0.62	0.02
	IBMRI MO194	63.1	2.6	34.8	3.5	75.1	2.3	0.46	0.03	99.1	14.1	50.0	1.2	84.4	4.1	0.59	0.04
	IBMRI MO197	52.7	5.4	23.3	3.0	79.4	15.9	0.30	0.02	97.5	23.9	48.9	2.1	81.0	8.0	0.61	0.04
	IBMRI MO219	72.0	6.7	36.0	2.7	78.0	7.4	0.46	0.01	151.6	11.4	57.6	9.1	89.8	10.0	0.64	0.03
	IBMRI MO238	45.6	22.5	15.5	5.1	62.8	9.5	0.24	0.04	118.3	8.3	57.9	5.3	92.6	10.0	0.63	0.01
	IBMRI MO250	78.3	1.5	32.7	0.2	79.3	6.6	0.41	0.03	138.6	17.3	53.4	3.1	86.2	3.0	0.62	0.02
	IBMRI MO277	41.0	11.6	26.4	6.7	75.3	8.8	0.35	0.05	120.3	10.9	52.6	4.0	88.4	5.0	0.60	0.01
	IBMRI MO337	53.1	15.3	31.9	0.4	81.0	3.4	0.39	0.01	117.6	14.7	52.9	6.9	91.2	8.7	0.58	0.02
	Population	60.5	14.7	30.5	7.1	77.3	8.3	0.4	0.1	118.8	19.8	53.7	5.5	87.6	7.0	0.6	0.0

Table 2.6d Summary statistics for grain biomass, grain N Utilization efficiency, total N Utilization efficiency and harvest index for the enriched and depleted populations grown in 2013 at Urbana, Illinois under low N conditions as inbreds and hybrids testcrossed to LH82.

	IBMRIL Line	Inbreds								Hybrids x LH82							
		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index		Grain Biomass (g/plt)		Grain NUtE		Total NUtE		Harvest Index	
		mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
		mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
Enriched	IBMRI MO003	56.2	11.3	38.6	2.3	118.8	4.0	0.32	0.01	89.0	16.8	72.1	5.9	146.1	10.2	0.49	0.01
	IBMRI MO027	53.0	8.0	35.0	0.2	77.5	8.6	0.45	0.05	94.9	25.3	70.3	1.6	116.0	1.3	0.61	0.01
	IBMRI MO029	46.5	10.9	39.3	1.6	101.4	4.4	0.39	0.00	82.4	16.6	77.4	4.3	144.0	7.3	0.54	0.04
	IBMRI MO090	20.9	16.1	16.2	0.8	90.2	28.2	0.19	0.05	81.0	14.1	72.4	3.6	128.5	5.6	0.56	0.03
	IBMRI MO138	79.2	19.0	48.1	4.4	102.3	14.8	0.47	0.02	76.0	21.3	73.6	3.7	130.2	0.5	0.56	0.03
	IBMRI MO142	32.0	5.6	30.0	6.1	77.1	9.7	0.39	0.03	65.4	24.1	67.5	17.3	137.7	5.2	0.49	0.12
	IBMRI MO289	51.9	4.3	41.3	10.7	102.6	9.6	0.40	0.07	86.9	4.6	81.4	2.6	145.8	8.4	0.56	0.03
	IBMRI MO304	34.8	3.7	38.6	4.7	103.8	8.1	0.37	0.02	93.3	28.3	72.2	3.8	146.6	23.9	0.50	0.05
	IBMRI MO351	53.1	23.4	40.0	8.1	89.1	4.1	0.45	0.07	83.5	21.7	73.1	2.9	128.7	0.8	0.57	0.02
	IBMRI MO354	49.9	24.6	39.4	10.8	99.4	3.9	0.40	0.12	79.0	17.6	73.3	7.6	129.2	15.7	0.57	0.01
	Population	47.8	18.8	36.7	9.4	96.2	15.2	0.38	0.09	83.1	18.7	73.3	6.8	135.3	13.3	0.55	0.05
Depleted	IBMRI MO101	55.7	5.8	44.4	1.8	85.5	0.1	0.52	0.02	79.9	17.9	68.8	15.3	146.2	9.7	0.48	0.13
	IBMRI MO168	77.9	4.1	40.9	0.0	95.7	1.8	0.43	0.01	92.3	17.5	77.9	3.9	139.8	6.5	0.56	0.01
	IBMRI MO190	31.7	13.3	30.4	1.9	103.8	13.6	0.30	0.06	95.1	29.5	74.8	5.9	134.3	10.2	0.56	0.03
	IBMRI MO194	59.4	32.2	40.7	15.2	88.0	7.2	0.46	0.14	94.4	21.0	74.9	2.1	133.7	7.3	0.56	0.04
	IBMRI MO197	37.7	1.2	20.3	0.9	85.4	0.1	0.24	0.01	78.6	12.6	76.5	4.5	138.4	3.7	0.55	0.04
	IBMRI MO219	70.2	13.8	36.5	2.3	80.9	7.3	0.46	0.07	90.0	10.7	77.3	1.8	131.5	0.7	0.59	0.01
	IBMRI MO238	18.0	10.1	12.5	6.9	81.6	11.8	0.15	0.06	81.4	14.1	80.2	7.0	151.6	13.5	0.53	0.01
	IBMRI MO250	61.0	4.5	39.6	0.3	95.8	9.3	0.42	0.04	121.2	11.8	72.7	2.2	129.8	5.9	0.56	0.02
	IBMRI MO277	49.6	4.1	40.2	7.2	92.6	0.0	0.43	0.08	77.7	10.7	73.2	5.6	140.2	11.9	0.52	0.05
	IBMRI MO337	68.1	15.8	40.3	1.1	95.6	3.8	0.42	0.01	88.3	26.7	68.7	5.0	142.8	6.1	0.48	0.03
	Population	52.9	20.8	34.6	11.0	90.5	9.0	0.38	0.12	89.9	19.7	74.5	6.5	138.8	9.6	0.54	0.05

Table 2.7 Means comparisons between the enriched and depleted populations for grain biomass (g/plt), grain N utilization efficiency, total N utilization efficiency, and harvest index grown in 2012 and 2013 at Urbana, Illinois under low N and high N conditions as inbreds and as hybrids testcrossed to LH82.

			Grain Biomass		Grain NUtE		Total NUtE		HI		
Hybrid	2012	High N	Enriched	94.3	NS	43.1	NS	86.2	NS	0.50	NS
			Depleted	105.9		44.6		88.2		0.50	
		Low N	Enriched	85.7	NS	60.3	NS	122.7	NS	0.50	NS
			Depleted	87.2		57.5		122.4		0.50	
	2013	High N	Enriched	113.4	NS	51.4	NS	84.9	NS	0.60	NS
			Depleted	118.8		53.7		87.6		0.60	
		Low N	Enriched	83.1	NS	73.3	NS	135.3	NS	0.55	NS
Depleted			89.9		74.5		138.8		0.54		

			Grain Biomass		Grain NUtE		Total NUtE		HI		
Inbred	2012	High N	Enriched	25.8	NS	12.3	NS	55.1	NS	0.21	NS
			Depleted	21.1		8.3		53.3		0.14	
		Low N	Enriched	19.0	NS	10.0	NS	56.7	NS	0.16	NS
			Depleted	22.5		9.6		57.0		0.16	
	2013	High N	Enriched	58.4	NS	33.8	NS	82.4	NS	0.41	NS
			Depleted	60.5		30.5		77.3		0.39	
		Low N	Enriched	52.9	NS	34.6	NS	90.5	NS	0.38	NS
Depleted			47.8		36.7		96.2		0.38		

Table 2.8 Rank correlations between the prediction test crosses of the IBM selections testcrossed to IHP1 (prediction) and each year the IBM selections were grown as inbreds and as hybrids testcrossed to LH82 (2012 and 2013).

			Low N		High N	
			ρ		ρ	
Prediction to 2012	inbred	Yield	-0.05	NS	-0.12	NS
		NUtE	0.40	.091	0.13	NS
		HI	-0.32	NS	-0.43	.078
	hybrid	Yield	-0.08	NS	0.28	NS
		NUtE	0.01	NS	-0.19	NS
		HI	0.14	NS	-0.11	NS
Prediction to 2013	inbred	Yield	0.08	NS	-0.67	.002
		NUtE	0.26	NS	0.25	NS
		HI	0.07	NS	-0.41	.088
	hybrid	Yield	0.05	NS	-0.24	NS
		NUtE	0.09	NS	-0.28	NS
		HI	-0.13	NS	-0.26	NS
2012 to 2013	inbred	Yield	0.14	NS	0.44	.064
		NUtE	0.31	NS	0.47	.047
		HI	0.38	NS	0.25	NS
	hybrid	Yield	0.43	NS	0.39	NS
		NUtE	0.21	NS	0.50	.035
		HI	0.23	NS	0.43	.072

Table 2.9 Means comparisons between the enriched and depleted populations for asparagine, glutamine, glutamic acid, and aspartic acid (all in parts per million). Plots were grown in 2012 and 2013 at Urbana, Illinois under low N and high N conditions as inbreds and as hybrids testcrossed to LH82.

				Asparagine		Glutamine		Glutamic Acid		Aspartic Acid	
Hybrid	2012	High N	Enriched	2708.2	NS	24645.3	0.03	6670.8	0.03	6130.6	0.09
			Depleted	2041.7		17696.5		4857.0		2181.5	
		Low N	Enriched	228.0	NS	4313.5	NS	1489.8	NS	817.1	NS
			Depleted	209.5		4146.6		1420.0		799.1	
	2013	High N	Enriched	473.5	NS	4300.1	NS	1245.7	NS	322.6	0.08
			Depleted	614.7		4963.6		1226.0		567.7	
		Low N	Enriched	208.1	NS	3161.4	NS	1265.5	0.08	216.6	NS
			Depleted	179.5		2551.3		1395.3		347.7	

				Asparagine		Glutamine		Glutamic Acid		Aspartic Acid	
Inbred	2012	High N	Enriched	1242.9	NS	6989.4	0.02	3451.7	0.04	2311.9	NS
			Depleted	2396.2		4410.0		2860.5		2359.2	
		Low N	Enriched	1150.0	NS	9281.6	NS	3823.4	0.10	2286.3	NS
			Depleted	1720.1		7506.3		3239.5		2392.7	
		2013	High N	Enriched	539.7	NS	6641.4	0.08	2318.5	NS	1625.4
	Depleted			330.9	5034.0		2691.6		1732.7		
	Low N		Enriched	287.4	NS	5334.0	0.05	2271.8	NS	1465.7	NS
			Depleted	310.3		4183.1		2126.6		1604.0	

Table 2.10a Means comparisons between hybrid populations grouped by their allele at a particular region. Lines with no data for that region were excluded from analysis. Plots were grown in 2012 and 2013 at Urbana, Illinois under low and high N conditions as hybrids testcrossed to LH82. Italicized comparisons were not significant in the original mapping population with the IHP1 tester. Boxed comparisons have a different positive effect allele with IHP1 versus LH82 as a tester.

Trait	N Rate	Year	1-375	3-310	4-385	4-539	6-126	9-24	2-323	6-79	6-480
StoverN	High	2012	-	B>M*	-	-	-	-	-	-	-
		2013	-	-	-	-	-	-	B>M*	-	-
	Low	2012	-	-	-	-	-	-	-	-	-
		2013	M>B*	-	-	-	-	-	-	-	-
GrainN	High	2012	-	-	-	-	M>B*	-	-	M>B**	-
		2013	-	-	-	-	-	-	-	-	-
	Low	2012	-	-	-	-	-	-	-	M>B*	-
		2013	-	-	-	-	-	-	-	-	-
Stover Biomass	High	2012	-	B>M*	B>M*	-	-	M>B*	-	-	-
		2013	M>B*	-	-	-	-	-	-	-	-
	Low	2012	-	-	B>M*	-	-	-	-	-	-
		2013	M>B***	-	B>M*	-	-	M>B*	-	M>B*	-
Grain Biomass	High	2012	-	-	B>M*	-	M>B*	-	-	M>B**	-
		2013	M>B*	-	-	-	-	-	-	-	-
	Low	2012	-	-	-	-	-	-	-	M>B*	-
		2013	-	-	-	-	-	-	-	-	-
NUtE	High	2012	M>B*	-	-	-	-	-	-	-	-
		2013	-	-	-	-	M>B*	-	-	M>B*	-
	Low	2012	-	-	-	-	-	-	-	-	-
		2013	-	-	-	M>B*	-	M>B*	B>M*	-	-
HI	High	2012	-	-	-	-	-	-	-	-	-
		2013	B>M*	-	-	-	-	-	-	-	-
	Low	2012	-	-	-	B>M**	-	-	-	M>B**	-
		2013	M>B*	-	-	-	-	B>M*	-	-	-

* significant at p<.1

**significant at p<.01

***significant at p<.001

- not significant

Table 2.10b Means comparisons between inbred populations grouped by their allele at a particular region. Lines with no data for that region were excluded from analysis. Plots were grown in 2012 and 2013 at Urbana, Illinois under low and high N conditions. Italicized comparisons were not significant in the original mapping population. Boxed comparisons had different positive effect alleles when in the inbred population and the original mapping results.

Trait	N Rate	Year	1-375	3-310	4-385	4-539	6-126	9-24	2-323	6-79	6-480
StoverN	High	2012	-	B>M*	B>M*	M>B*	-	-	-	-	-
		2013	-	-	-	M>B*	-	<i>M>B*</i>	-	-	-
	Low	2012	-	-	-	-	-	-	-	-	-
		2013	-	-	-	M>B**	-	-	-	-	-
GrainN	High	2012	-	-	-	B>M*	-	-	-	-	-
		2013	-	-	B>M*	-	-	-	M>B*	-	-
	Low	2012	M>B*	-	-	B>M*	-	-	-	-	-
		2013	-	-	-	-	-	-	-	-	-
Stover Biomass	High	2012	M>B**	-	B>M**	-	-	<i>M>B*</i>	-	-	-
		2013	-	-	-	-	-	<i>M>B*</i>	-	-	-
	Low	2012	M>B**	-	-	-	-	-	-	-	-
		2013	-	-	-	-	-	<i>M>B*</i>	-	-	-
Grain Biomass	High	2012	-	-	-	B>M*	-	-	-	-	-
		2013	-	-	-	B>M*	-	-	<i>M>B**</i>	-	-
	Low	2012	-	-	-	-	-	-	-	-	-
		2013	-	-	-	-	-	-	<i>M>B*</i>	-	-
NUtE	High	2012	-	M>B*	-	B>M**	-	-	-	-	-
		2013	-	M>B*	-	B>M*	B>M***	-	<i>M>B*</i>	B>M*	-
	Low	2012	M>B*	-	-	-	-	-	-	-	B>M*
		2013	M>B*	M>B*	-	B>M**	B>M**	-	-	-	-
HI	High	2012	-	-	-	B>M*	-	-	-	-	-
		2013	-	-	-	B>M*	-	B>M*	-	-	-
	Low	2012	-	-	-	B>M**	-	-	-	-	-
		2013	-	-	-	-	-	-	<i>M>B*</i>	-	-

* significant at p<.1

**significant at p<.01

***significant at p<.001

- not significant

Table 2.11a Evaluation of heterosis in the enriched and depleted populations grown in Urbana, IL in 2013 under high N. MPH is mid-parent heterosis, the deviation of the F1 from the mid-parent value. HPH is high parent heterosis, the deviation of the F1 from the high parent value.

			Grain Biomass				NuTE				HI			
			MPH		HPH		MPH		HPH		MPH		HPH	
High N	Enriched	IBMRI MO003	64.3		58.5		1.0		-5.8		0.16		0.13	
		IBMRI MO027	52.8		48.9		12.0		4.4		0.19		0.16	
		IBMRI MO029	64.3		57.5		2.3		-0.1		0.17		0.15	
		IBMRI MO090	45.2		41.5		1.8		1.7		0.16		0.15	
		IBMRI MO138	33.3		28.0		-3.6		-4.9		0.15		0.14	
		IBMRI MO142	59.6		47.5		10.0		-1.6		0.17		0.12	
		IBMRI MO289	49.7		49.5		-3.9		-7.7		0.19		0.15	
		IBMRI MO304	68.3		66.9		-5.7		-6.8		0.15		0.12	
		IBMRI MO351	59.0		54.9		0.6		-1.4		0.18		0.17	
		IBMRI MO354	46.4		46.4		-3.5		-6.2		0.17		0.16	
		Population	54.1	***	53.3	***	1.2	NS	-0.1	NS	0.17	***	0.14	***
	Depleted	IBMRI MO101	43.8		41.8		6.0		0.4		0.18		0.18	
		IBMRI MO168	55.1		47.6		2.7		1.2		0.18		0.16	
		IBMRI MO190	52.6		52.5		4.2		2.0		0.18		0.16	
		IBMRI MO194	37.5		36.0		4.4		-0.5		0.13		0.13	
		IBMRI MO197	41.2		37.5		-1.2		-4.0		0.23		0.15	
		IBMRI MO219	85.6		79.6		8.3		4.8		0.18		0.18	
		IBMRI MO238	65.4		58.2		18.7		7.6		0.28		0.17	
		IBMRI MO250	69.4		60.3		4.1		1.3		0.18		0.16	
		IBMRI MO277	69.8		60.3		8.2		3.4		0.19		0.14	
		IBMRI MO337	61.0		57.6		8.2		6.2		0.15		0.12	
		Population	58.6	***	58.3	***	6.4	**	2.6	NS	0.19	***	0.15	***

* significant at p<.01

**significant at p<.001

***significant at p<.0001

Table 2.11b Evaluation of heterosis in the enriched and depleted populations grown in Urbana, IL in 2013 under low N. MPH is mid-parent heterosis, the deviation of the F1 from the mid-parent value. HPH is high parent heterosis, the deviation of the F1 from the high parent value.

			Grain Biomass		NUtE		HI	
			MPH	HPH	MPH	HPH	MPH	HPH
Low N	Enriched	IBMRI MO003	23.2	13.5	45.0	27.3	0.04	-0.09
		IBMRI MO027	30.7	19.5	35.6	32.7	0.09	0.02
		IBMRI MO029	21.5	7.0	51.7	42.7	0.05	-0.04
		IBMRI MO090	32.8	5.5	41.7	38.3	0.18	-0.02
		IBMRI MO138	-1.4	-3.3	37.4	27.9	0.04	-0.02
		IBMRI MO142	11.6	-10.1	57.5	54.4	0.00	-0.09
		IBMRI MO289	23.2	11.4	52.9	43.3	0.07	-0.02
		IBMRI MO304	38.2	17.9	53.1	42.8	0.02	-0.08
		IBMRI MO351	19.2	8.0	42.5	39.6	0.05	-0.01
		IBMRI MO354	16.4	3.6	37.9	29.9	0.08	-0.01
		<i>Population</i>	21.5 ***	7.7 NS	45.5 ***	39.1 ***	0.06 ***	-0.04 *
	Depleted	IBMRI MO101	14.4	4.5	61.8	60.8	-0.08	-0.11
		IBMRI MO168	15.6	14.4	50.3	44.1	0.05	-0.02
		IBMRI MO190	41.5	19.6	40.7	30.5	0.12	-0.02
		IBMRI MO194	27.0	19.0	48.1	45.7	0.04	-0.02
		IBMRI MO197	22.0	3.2	54.1	53.0	0.14	-0.03
		IBMRI MO219	17.1	14.5	49.5	48.3	0.07	0.01
		IBMRI MO238	34.7	5.9	69.1	68.3	0.16	-0.05
		IBMRI MO250	53.0	45.8	40.2	33.9	0.06	-0.02
		IBMRI MO277	15.3	2.3	52.2	47.5	0.02	-0.06
		IBMRI MO337	16.5	12.8	53.3	47.2	-0.02	-0.10
		<i>Population</i>	25.7 ***	14.5 *	51.9 ***	48.3 ***	0.06 ***	-0.04 ***

* significant at p<.01

**significant at p<.001

***significant at p<.0001

Table 2.12 Preliminary allele calls for the contribution of LH82 based on GBS analysis of the inbred line

Position (cM)	Left Flanking Marker	Right Flanking marker	Left position (bp)	Right position (bp)	Preliminary Allele Call
1-375	csu3	umc2025	81361551	93673942	Mo17
2-323	mmc0401	umc1079	142198250	148709649	Mo17
3-310	cdo105	csu184	144466418	156255753	
4-385	umc19	mmp115	172465264	198339774	B73
4-539	umc2139	umc1999	219582837	226268100	B73
6-79	uck1	uaz232b	3058734	31687703	
6-126	umc1257	umc1595	90544455	95251373	
6-480	mmp105	agp2	166345803	166687872	B73
9-24	bnlg2122	umc1867	4790431	5023949	Mo17

Appendix

		Year	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
		Stages Sampled	V8, R1, R6	V8, R1, R6	V8, R1, R6	R6	R6	R6	R6	R6	R6	R6	R6
IPS	Inbred	N Rates	0, 56, 112				0, 225	0, 225		50	0	0,100	0, 100
	Hybrid	N Rates	0, 200				0, 225	0, 225	0, 200	50, 250	0, 250	0, 200	0, 200
		Testers	FR1064				B73, FR1064	B73, FR1064	B73 *	B73, Mo17*	B73, Mo17*	LH82	LH82
NAM	Inbred	N Rates						0, 225	0	0, 200	0	0, 100	0, 100
	Hybrid	N Rates	0, 200	0, 75, 150, 225	0, 75, 150, 225	90		0, 225	0, 200	50, 250	0, 250	0, 200	0, 200
		Testers	B73	B73	B73	B73, IHP1		B73	B73	B73, ILP1, IHP1	ILP1, IHP1, LH82	LH82	LH82
IBM	Inbred	N Rates						0, 225	0	0	0	0, 100	0, 100
	Hybrid	N Rates						0, 225	0, 200	50, 250	0, 250	0, 200	0, 200
		Testers						ILP1, IHP1	ILP1, IHP1	ILP1, IHP1, LH82	ILP1, IHP1, LH82	LH82	LH82
USB	Inbred	N Rates	0, 56, 112						100	50			
	Hybrid	N Rates		0, 84, 168, 252	0, 84, 168, 253				100	50, 250	0, 225		
		Testers		Mo17	B73, Mo17				B73	B73, Mo17	B73		
ex-PVP	Inbred	N Rates						0, 225	0		0	0, 100	0, 100
	Hybrid	N Rates						0, 225	0, 200	50, 250	0, 250	0, 200	0, 200
		Testers						B73	B73	B73, ILP1, IHP1	ILP1, IHP1, LH82	LH82	LH82
Tropicals	Inbred	N Rates						0, 225	0	0, 200	0	0, 100	0, 100
	Hybrid	N Rates		0, 75, 150, 225	0, 75, 150, 225			0, 225	0, 200	50, 250	0, 225	0, 200	0, 200
		Testers		B73	B73			B73	B73, ILP1	B73, ILP1, IHP1	B73	LH82	LH82

Table A.1 Summary of the germplasm, testers, N Rates and stages sampled from each year of the experiment from 2003-2013. N Rates are in kg/ha and ranged from 0 to 250. Tester options included B73, IHP1, ILP1, FR1064, Mo17, and LH82. V8 and R1 stages were only sampled from 2003-2005. Grey squares indicate populations that were not included in trials that year. * indicates years when those lines were used as testers

Genotype	Population
3IBZ2	Ex-PVP
B73	NAM (Temperate)
B97	NAM (Temperate)
CML103	NAM (Tropical)
CML139	NAM (Tropical)
CML228	NAM (Tropical)
CML246	NAM (Tropical)
CML247	NAM (Tropical)
CML277	NAM (Tropical)
CML322	NAM (Tropical)
CML333	NAM (Tropical)
CML349	NAM (Tropical)
CML52	NAM (Tropical)
CML69	NAM (Tropical)
FR1064	Ex-PVP
GP273	NAM (Tropical)
H99	Ex-PVP
HBA1	Ex-PVP
Hp301	NAM (Temperate)

Genotype	Population
IBMRIMO003	IBM
IBMRIMO005	IBM
IBMRIMO007	IBM
IBMRIMO008	IBM
IBMRIMO010	IBM
IBMRIMO013	IBM
IBMRIMO014	IBM
IBMRIMO016	IBM
IBMRIMO017	IBM
IBMRIMO018	IBM
IBMRIMO021	IBM
IBMRIMO022	IBM
IBMRIMO023	IBM
IBMRIMO025	IBM
IBMRIMO026	IBM
IBMRIMO027	IBM
IBMRIMO028	IBM
IBMRIMO029	IBM
IBMRIMO030	IBM
IBMRIMO031	IBM
IBMRIMO034	IBM
IBMRIMO038	IBM
IBMRIMO039	IBM

Genotype	Population
IBMRIMO041	IBM
IBMRIMO043	IBM
IBMRIMO045	IBM
IBMRIMO051	IBM
IBMRIMO052	IBM
IBMRIMO054	IBM
IBMRIMO056	IBM
IBMRIMO057	IBM
IBMRIMO066	IBM
IBMRIMO067	IBM
IBMRIMO074	IBM
IBMRIMO079	IBM
IBMRIMO080	IBM
IBMRIMO081	IBM
IBMRIMO083	IBM
IBMRIMO086	IBM
IBMRIMO088	IBM
IBMRIMO090	IBM
IBMRIMO092	IBM
IBMRIMO096	IBM
IBMRIMO101	IBM
IBMRIMO106	IBM
IBMRIMO115	IBM

Table A.2 List of genotypes included in the study as well as the population they were classified into.

Genotype	Population
IBMRIMO116	IBM
IBMRIMO118	IBM
IBMRIMO119	IBM
IBMRIMO121	IBM
IBMRIMO123	IBM
IBMRIMO124	IBM
IBMRIMO127	IBM
IBMRIMO132	IBM
IBMRIMO134	IBM
IBMRIMO138	IBM
IBMRIMO142	IBM
IBMRIMO143	IBM
IBMRIMO147	IBM
IBMRIMO151	IBM
IBMRIMO153	IBM
IBMRIMO154	IBM
IBMRIMO156	IBM
IBMRIMO160	IBM
IBMRIMO161	IBM
IBMRIMO162	IBM
IBMRIMO163	IBM
IBMRIMO167	IBM
IBMRIMO168	IBM

Genotype	Population
IBMRIMO171	IBM
IBMRIMO174	IBM
IBMRIMO176	IBM
IBMRIMO177	IBM
IBMRIMO178	IBM
IBMRIMO181	IBM
IBMRIMO182	IBM
IBMRIMO183	IBM
IBMRIMO184	IBM
IBMRIMO186	IBM
IBMRIMO187	IBM
IBMRIMO188	IBM
IBMRIMO189	IBM
IBMRIMO190	IBM
IBMRIMO194	IBM
IBMRIMO197	IBM
IBMRIMO198	IBM
IBMRIMO200	IBM
IBMRIMO202	IBM
IBMRIMO205	IBM
IBMRIMO206	IBM
IBMRIMO209	IBM
IBMRIMO210	IBM

Genotype	Population
IBMRIMO213	IBM
IBMRIMO216	IBM
IBMRIMO219	IBM
IBMRIMO222	IBM
IBMRIMO223	IBM
IBMRIMO228	IBM
IBMRIMO229	IBM
IBMRIMO230	IBM
IBMRIMO233	IBM
IBMRIMO237	IBM
IBMRIMO238	IBM
IBMRIMO240	IBM
IBMRIMO241	IBM
IBMRIMO248	IBM
IBMRIMO250	IBM
IBMRIMO256	IBM
IBMRIMO258	IBM
IBMRIMO262	IBM
IBMRIMO264	IBM
IBMRIMO266	IBM
IBMRIMO269	IBM
IBMRIMO270	IBM
IBMRIMO274	IBM

Table A.2 (cont.)

Genotype	Population
IBMRIMO275	IBM
IBMRIMO276	IBM
IBMRIMO277	IBM
IBMRIMO280	IBM
IBMRIMO281	IBM
IBMRIMO282	IBM
IBMRIMO283	IBM
IBMRIMO284	IBM
IBMRIMO286	IBM
IBMRIMO288	IBM
IBMRIMO289	IBM
IBMRIMO295	IBM
IBMRIMO301	IBM
IBMRIMO304	IBM
IBMRIMO305	IBM
IBMRIMO308	IBM
IBMRIMO309	IBM
IBMRIMO311	IBM
IBMRIMO313	IBM
IBMRIMO315	IBM
IBMRIMO317	IBM
IBMRIMO318	IBM

Genotype	Population
IBMRIMO325	IBM
IBMRIMO327	IBM
IBMRIMO328	IBM
IBMRIMO329	IBM
IBMRIMO331	IBM
IBMRIMO335	IBM
IBMRIMO337	IBM
IBMRIMO340	IBM
IBMRIMO341	IBM
IBMRIMO344	IBM
IBMRIMO347	IBM
IBMRIMO351	IBM
IBMRIMO354	IBM
IBMRIMO358	IBM
IBMRIMO360	IBM
IBMRIMO364	IBM
IBMRIMO368	IBM
IBMRIMO369	IBM
IBMRIMO379	IBM
IBMRIMO380	IBM
IBMRIMO382	IBM
IBMRIMO383	IBM

Genotype	Population
IHP1	IPS
Il14H	NAM (Temperate)
ILP1	IPS
IRHP1	IPS
IRLP1	IPS
Ki11	NAM (Tropical)
Ki3	NAM (Tropical)
Ky21	NAM (Temperate)
LH1	Ex-PVP
LH123	Ex-PVP
LH156	Ex-PVP
LH60	Ex-PVP
LH66	Ex-PVP
LH82	Ex-PVP
M162W	NAM (Temperate)
M37W	NAM (Mixed)
Mo17	NAM (Temperate)
Mo18W	NAM (Mixed)
MS71	NAM (Temperate)
NC350	NAM (Tropical)
NC358	NAM (Tropical)
Oh43	NAM (Temperate)

Table A.2 (cont.)

Genotype	Population
Oh7B	NAM (Temperate)
P39	NAM (Temperate)
PH207	Ex-PVP
PHG35	Ex-PVP
PHG39	Ex-PVP
PHG47	Ex-PVP
PHG84	Ex-PVP
PHJ40	Ex-PVP
PHT55	Ex-PVP
PHW65	Ex-PVP
PHZ51	Ex-PVP
Tx303	NAM (Mixed)
Tzi3	NAM (Tropical)
Tzi8	NAM (Tropical)
Va85	NAM (Temperate)

Table A.2 (cont.)

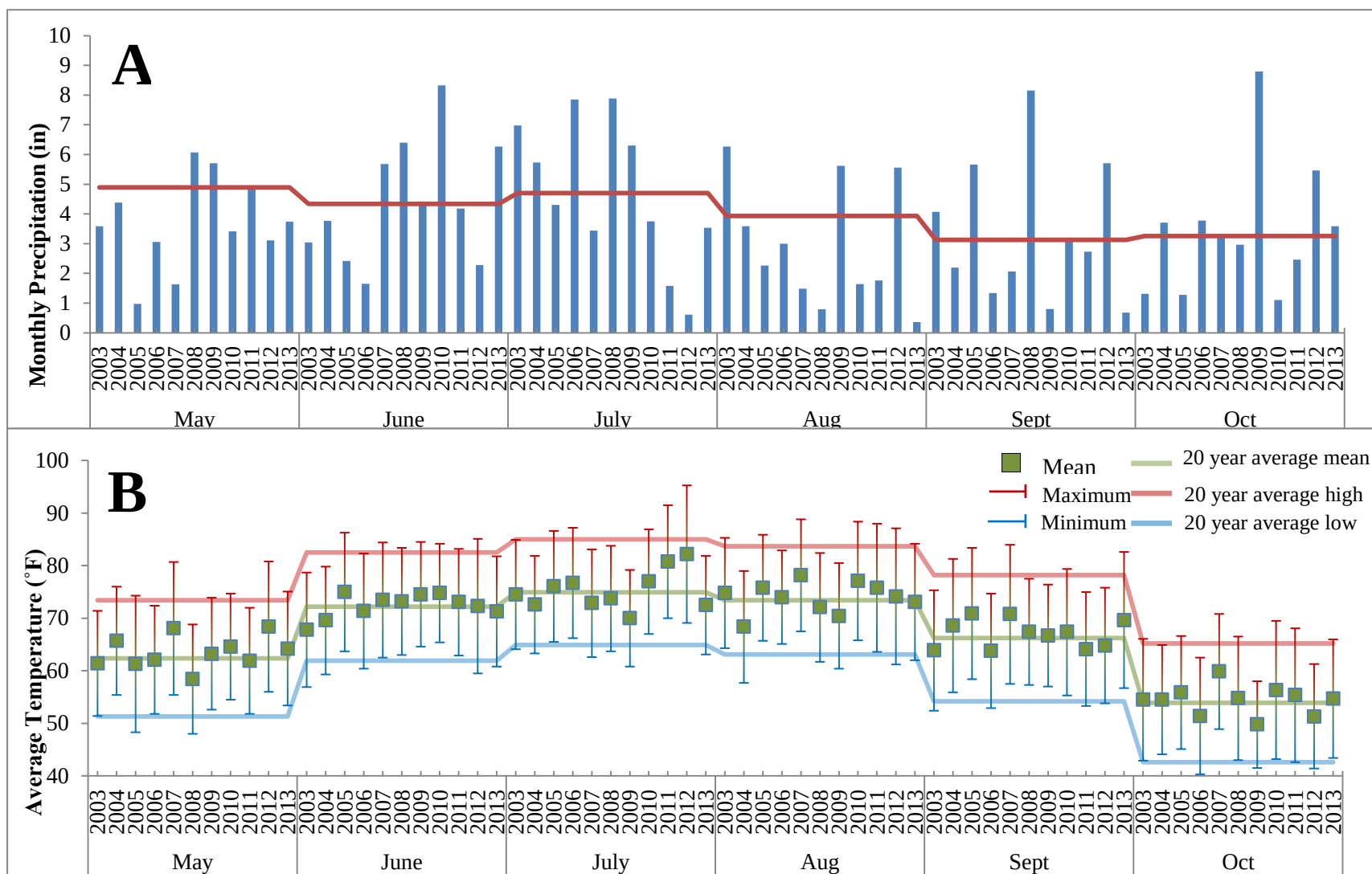


Figure A.1 Summary of environmental conditions for the duration of the studies. Panel A shows average monthly precipitation in inches as (blue bars) compared to the 20 year average for that month (red line). Panel B shows the maximum, minimum and average monthly temperatures (box and whiskers) compared to the 20 year average for that month (red, green, blue lines). The measured values for the term of the experiment are similar to the 20 year averages with the exception of 2011 being dry and a few years with a warm July.

Era	Line	Release Date	Total Biomass (g/plt)		Grain Biomass (g/plt)		Plant N (g/plt)	
			+N	-N	+N	-N	+N	-N
1950	Oh43	1940	181.0	141.2	96.1	65.7	2.1	1.0
1960	Mo17	1964	180.7	149.8	96.6	75.4	1.9	1.2
1970	B73	1972	216.1	166.0	104.9	76.5	2.3	1.2
1980	PHJ40	1985	195.7	149.4	107.6	78.0	2.1	1.2
1990	Elite	1995	182.3	166.6	105.4	78.8	2.1	1.5
2000	Commercial	2005	256.8	184.1	144.3	89.8	2.9	1.2

Table A.3 Inbred lines selected to represent each decade from 1950-2000. NUE component traits are from hybrid (xLH82) plots grown from 2011-2013 averaged for low (0 kg/ha) N and high (225 kg/ha) N.

LOW N		Inbred															
		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass	0.62	**	0.42		-0.6	**	-0.62	**	-0.58	**	0.57	**	0.27		0.83	***
	Stover N	0.36		0.13		-0.4		-0.45		-0.32		0.32		-0.01		0.75	***
	Grain Biomass	-0.24		-0.32		0.0		-0.01		0.07		-0.28		-0.34		0.06	
	Grain N	-0.65	***	-0.56	**	0.5	*	0.53	*	0.56	**	-0.62	**	-0.47	*	-0.44	*
	Grain NUtE	-0.29		-0.36		0.0		0.02		0.09		-0.34		-0.36		-0.03	
	Total Biomass	0.22		0.03		-0.4		-0.39		-0.32		0.16		-0.06		0.57	**
	Total N	0.05		-0.10		-0.1		-0.17		-0.08		0.03		-0.16		0.46	*
	Total NUtE	0.43		0.33		-0.5	*	-0.48	*	-0.46	*	0.37		0.23		0.39	
HIGH N		Inbred															
		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass	0.47		0.45		-0.17		-0.10		-0.53		0.48		0.52		0.02	
	Stover N	0.09		0.23		-0.28		-0.29		-0.32		0.03		0.18		-0.39	
	Grain Biomass	0.74	*	0.79	*	-0.71	*	-0.72	*	-0.70	*	0.64		0.70	*	-0.17	
	Grain N	0.02		-0.05		0.15		0.16		-0.08		0.06		0.00		0.22	
	Grain NUtE	0.72	*	0.76	*	-0.68		-0.68		-0.63		0.63		0.68		-0.14	
	Total Biomass	0.79	*	0.83	**	-0.70	*	-0.69	*	-0.76	*	0.70	*	0.76	*	-0.14	
	Total N	0.19		0.18		0.00		0.02		-0.33		0.21		0.23		0.06	
	Total NUtE	0.74	*	0.77	*	-0.67		-0.66		-0.63		0.66		0.70	*	-0.12	
*** significant at p ≤.0001 ** significant at p≤.001 * significant at p≤.01																	

Table A.4a Correlation table of inbred traits with their corresponding hybrid traits. NAM and ex-PVP inbreds and hybrids grown in 2010 are included with B73 used as a tester.

LOW N				Inbred															
				Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass			0.71	***	0.56	***	-0.39	**	-0.39	**	-0.57	***	0.49	***	0.48		0.1271	*
	Stover N			0.63	***	0.53	**	-0.38	*	-0.39	*	-0.55	**	0.42	***	0.41		0.1304	
	Grain Biomass			-0.51	*	-0.43	*	0.28		0.24		0.42		-0.35	*	-0.36		-0.0090	
	Grain N			-0.49	*	-0.39	*	0.25		0.23		0.39		-0.35	*	-0.33		-0.0473	
	Grain NUtE			-0.61	***	-0.50	***	0.37	*	0.34	*	0.53	**	-0.41	***	-0.41		-0.0694	
	Total Biomass			0.72	**	0.56	*	-0.38		-0.39		-0.57	*	0.51	**	0.49		0.1584	
	Total N			0.31	**	0.25	*	-0.20		-0.23		-0.28	*	0.20	**	0.19		0.1350	
	Total NUtE			0.46		0.34		-0.25		-0.24		-0.36		0.33		0.30		0.0749	
HIGH N				Inbred															
				Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass			0.49		0.57		-0.47		-0.42		-0.63		0.42		0.51		-0.22	
	Stover N			0.33	*	0.45		-0.52		-0.49		-0.55		0.24		0.36		-0.33	
	Grain Biomass			-0.19		-0.24		0.42		0.39		0.41	*	-0.11		-0.15		0.30	
	Grain N			-0.10		-0.12		0.27		0.26		0.29		-0.05		-0.07		0.21	
	Grain NUtE			-0.35	*	-0.44		0.60		0.56		0.58	*	-0.23	*	-0.33		0.40	
	Total Biomass			0.49		0.56		-0.37		-0.32		-0.56		0.44		0.53		-0.14	
	Total N			0.37		0.50		-0.51		-0.49		-0.54		0.28		0.41		-0.31	
	Total NUtE			0.26		0.13		0.33		0.38		-0.03		0.36		0.26		0.39	
				*** significant at p≤.0001				** significant at p≤.001				* significant at p≤.01							

*** significant at $p \leq .0001$ ** significant at $p \leq .001$ * significant at $p \leq .01$

Table A.4b Correlation table of inbred traits with their corresponding hybrid traits. NAM and IBM inbreds and hybrids grown in 2010 are included with IHP used as a tester.

LOW N		Inbred															
		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass	0.85	***	0.69	***	-0.38		-0.30		-0.64	***	0.78	***	0.64	***	0.31	
	Stover N	0.80	***	0.71	***	-0.31		-0.24		-0.56	**	0.75	***	0.65	***	0.30	
	Grain Biomass	-0.34	*	-0.19		0.38	*	0.38		0.42	**	-0.23		-0.13		-0.28	
	Grain N	-0.16		-0.02		0.21	*	0.24		0.21	*	-0.09		0.02		-0.29	
	Grain NUtE	-0.62	***	-0.46	***	0.45	*	0.41		0.60	***	-0.51	***	-0.39	**	-0.29	
	Total Biomass	0.80	***	0.68	***	-0.28		-0.19		-0.55	***	0.77	***	0.65	***	0.26	
	Total N	0.75	***	0.68	**	-0.26		-0.19		-0.50	**	0.72	***	0.64	**	0.23	
	Total NUtE	0.06		-0.04		-0.01		0.06		-0.07		0.07		0.00		0.02	
HIGH N		Inbred															
		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass		Total N		Total NUtE	
Hybrid	Stover Biomass	0.60	*	0.69		-0.44		-0.45		-0.66		0.54		0.64	*	-0.04	
	Stover N	0.59	*	0.73	**	-0.53		-0.54		-0.69	*	0.51		0.65	**	-0.17	
	Grain Biomass	-0.09		-0.20		0.57		0.57		0.35		0.03		-0.05		0.40	*
	Grain N	0.07		-0.03		0.43		0.43		0.19	*	0.17		0.10		0.37	*
	Grain NUtE	-0.54	*	-0.66	*	0.69		0.69		0.72	**	-0.43		-0.53	*	0.26	
	Total Biomass	0.54		0.59		-0.19		-0.20		-0.48		0.53		0.59		0.12	
	Total N	0.61	*	0.72	*	-0.39		-0.40		-0.63		0.56	*	0.68	*	-0.05	
	Total NUtE	-0.33		-0.47		0.67		0.67		0.53		-0.20		-0.33		0.47	

*** significant at $p \leq 0.0001$ ** significant at $p \leq 0.01$ * significant at $p \leq 0.1$

Table A.4c Correlation table of inbred traits with their corresponding hybrid traits. NAM and IBM inbreds and hybrids grown in 2010 are included with ILP used as a tester.

		Inbred											
		LOW N											
Hybrid		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass	
	Stover Biomass	0.69	*	0.31		-0.33		-0.34		-0.06		0.47	0.01
	Stover N	0.45		0.31		-0.35		-0.19		-0.21		0.25	0.09
	Grain Biomass	0.02		-0.16		0.02		-0.09		0.08		0.03	-0.15
	Grain N	-0.09		-0.11		-0.03		-0.01		-0.03		-0.09	-0.08
	Grain NUtE	-0.19		-0.30		0.21		-0.06		0.26		-0.08	-0.22
	Total Biomass	0.52		0.13		-0.23		-0.32		0.01		0.37	-0.10
	Total N	0.27		0.13		-0.31		-0.16		-0.19		0.10	-0.01
	Total NUtE	0.49		0.08		0.01		-0.31		0.25		0.45	-0.13
		Inbred											
		HIGH N											
Hybrid		Stover Biomass		Stover N		Grain Biomass		Grain N		Grain NUtE		Total Biomass	
	Stover Biomass	0.82	***	0.55	**	-0.24	*	-0.21	*	-0.14		0.68	**
	Stover N	0.77		0.55		-0.14		-0.03		-0.16		0.68	0.42
	Grain Biomass	0.60		0.41		-0.12		-0.11		-0.18		0.53	0.26
	Grain N	0.63		0.44		-0.22		-0.07		-0.25		0.50	0.31
	Grain NUtE	-0.35		-0.38		0.19		-0.23		0.20		-0.24	-0.42
	Total Biomass	0.76	*	0.51	*	-0.20		-0.17		-0.17		0.64	*
	Total N	0.77		0.55		-0.21		-0.06		-0.23		0.64	0.40
	Total NUtE	0.22		0.00		-0.02		-0.54		0.25		0.21	-0.30

*** significant at $p \leq 0.0001$ ** significant at $p \leq 0.001$ * significant at $p \leq 0.01$

Table A.4d Correlation table of inbred traits with their corresponding hybrid traits. NAM and IBM inbreds and hybrids grown in 2012 and 2013 are included with LH82 used as a tester.