Genotypic differences in maize grain yield at low N are consistent across years and are predicted by genetic N utilization

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Introduction:

- N use in maize hybrids can be improved by increasing grain yield under low N (i.e., unfertilized conditions) and by increasing the response of grain yield to fertilizer N.
- There is a scarcity of data on how these two traits are influenced by genetic and environmental factors.
- Furthermore, selection for improved N use might be enhanced by advanced phenotyping methods like metabolite profiling that complement traditional measures of grain yield, N uptake, and other physiological parameters. Metabolite profiling as a tool to predict N use in maize has been largely unexplored.

Research questions:

1. Which components of maize nitrogen use are most influenced by genotype and are stable across environments?
2. Are there specific metabolites or metabolic pathways which predict nitrogen response?

Rationale for hybrid selection:

- An initial 2003 experiment identified four genotypes with different combinations of grain yield at low N and response to fertilizer N (Figure 1).
- CML103 and NC350 had similar grain yields at low N (average of 4.4 Mg ha⁻¹); however, CML103 had a 52% greater response to fertilizer N (200 kg N ha⁻¹).
- A second pair of genotypes (Mo18W and Tx303) had reduced grain yields at low N (average of 2.7 Mg ha⁻¹), yet had markedly different responses to fertilizer N.

Research approach:

- This experiment was conducted over three years (2003, 2009, and 2010) at the University of Illinois Department of Crop Sciences Research and Education Center at Champaign-Urbana, IL. The soil type was a Drummer-Flanagan soil association (Typic Endoaquols) with adequate P and K fertility.
- Four genotypes (CML103, NC350, Mo18W, and Tx303) were evaluated. These genotypes represent available diversity lines. In 2003, these lines were evaluated in hybrid combinations with B73, and in 2009-2010, a proprietary line from Syngenta was used as the tester.
- The plots were planted to achieve a final population of approximately 74,000 plants ha⁻¹.
- N fertilizer treatments consisted of ammonium sulfate [(NH₄)₂SO₄; 21-0-0-245] incorporated in a diffuse band between the rows at V3-V4. Five N fertilizer rates were used (0 – 225 kg N ha⁻¹) in 56 kg N ha⁻¹ increments.
- In 2009, unpolinated earsheaths were collected 3-5 days after silk emergence and flash frozen in liquid N. Global metabolite profiling was provided by Syngenta using GC/MS and LC/MS instrumentation.
- Measurements of biomass and N concentration were taken at both flowering (R1) and physiological maturity (R6). Total N content at R1 and R6 was calculated using the measures of N concentration (combustion for slower and NIT for grain) and total biomass. Grain yields are expressed at 0% moisture concentration.

Phenotypic traits for hybrids:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Grain yield at low N</th>
<th>Maximum response to fertilizer N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML103</td>
<td>2 1 1 2 4 2</td>
<td>1 2 3 1 1 1</td>
</tr>
<tr>
<td>NC350</td>
<td>1 2 2 3 3 4</td>
<td>3 3 3 4 1 1</td>
</tr>
<tr>
<td>Tx303</td>
<td>3 3 3 4 1 1</td>
<td>1 2 3 3 3 4</td>
</tr>
<tr>
<td>Mo18W</td>
<td>4 4 4 1 2 3</td>
<td>1 2 3 1 1 1</td>
</tr>
</tbody>
</table>

Table 1. Rankings of the four hybrids grown in 2003, 2009, and 2010 for grain yield at low N and maximum response to fertilizer N. A ranking of one indicates the highest mean value for that trait.

Table 2. Phenotypic traits for hybrids grown at low N (0 kg N ha⁻¹). Values are averages of measurements taken in 2009 and 2010. Genetic utilization describes the efficiency with which plant accumulated nitrogen (measured at R6) is used to produce grain under low N conditions.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>R1 biomass</th>
<th>R1 N content</th>
<th>Grain yield</th>
<th>Genetic utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML103 and NC350</td>
<td>77</td>
<td>0.63</td>
<td>3.6</td>
<td>65</td>
</tr>
<tr>
<td>Mo18W and Tx303</td>
<td>80</td>
<td>0.61</td>
<td>1.6</td>
<td>48</td>
</tr>
</tbody>
</table>

Nitrogen responses in 2009 and 2010:

- 2009 and 2010 represented two very distinct N response environments (Figure 2).
- N loss was prevalent in 2009, resulting in a small average check plot yield. Favorable weather conditions during the reproductive period led to a large response to fertilizer N and high average grain yields.
- In contrast, warmer than average summer temperatures in 2010 limited yield potential and responsiveness to N fertilizer.

Conclusions:

1. Maize genotypes can be selected for increased grain yield under low N, and genetic rankings for this trait are consistent across environments with low and high average low N grain yields.
2. Genetic differences in grain yield at low N are mostly reflective of differences in N utilization rather than N uptake.
3. Certain earsheet metabolites (e.g. glutamine) are highly predictive of final grain yield. These metabolites provide opportunities to develop biomarkers for diagnosing N deficiency, as well as for selecting superior genotypes.