2021 Final Report Summary Sheet

Grantee Information

Project Title: Managing the maize microbiome for sustainable nutrient retention in Illinois agricultural soils

Institution: University of Illinois at Urbana-Champaign

Primary Investigator: Angela Kent

NREC Project # NREC 2021-2-360190-334

Is your project on target from an IMPLEMENTATION standpoint? ☒ **Yes** ☐ **No**
If you answered “no” please explain:

Is your project on target from a BUDGET standpoint? ☒ **Yes** ☐ **No**
If you answered “no” please explain:

Based on what you know today, will you meet the objectives of your project on-time and on-budget? ☒ **Yes** ☐ **No**
If you answered “no” please explain:

Have you encountered any issues related to this project? ☐ **Yes** ☒ **No**
If you answered “yes” please explain:
Field trial with N-fixing bacteria + BNI maize was smaller than desired due to seed availability, but included key treatments. Will be repeated in 2022. We confirm that we have produced sufficient seed for the experiment in 2022.

Have you reached any conclusions related to this project that you would like to highlight? ☒ **Yes** ☐ **No**
If you answered “yes” please explain:

Preliminary results indicate that +NIL phenotype enhances N response to diazotroph inoculant.
Preliminary results indicate that nitrification rates are not increased by +N fixing inoculant, suggesting that the additional N is being taken up by the plant.
N fixation by inoculant was elevated in +BNI genotypes at first 2 time points (get growth stage). Treatment effects for N fixation were not observed after VT.

Have you completed any outreach activities related this project? Or do you have any activities planned? ☒ **Yes** ☐ **No**
If you answered “yes” please explain and provide details for any upcoming outreach:
Will report on our results at Maize Genetics Conference in March in St. Louis.
Discussed research strategies at ASA conference and UIUC Agronomy Day in 2021.
Training undergraduates, graduate students, and postdocs in plant breeding and crop sciences, along with microbial ecology and biogeochemistry.

Please write a detailed summary report that includes: Details of each objective and the progress made towards its competition, planned research activities for 2022, major accomplishments, any preliminary findings or data relevant to the project, relevant budgeting, and any publications or outreach accomplished from the research. Please **include a one page summary with relevant data tables or graphs and pictures related to the project that you would like included in the NREC end of the year report.**

Managing the maize microbiome for sustainable nutrient retention in Illinois agricultural soils

Objective 1: Determine if nitrification inhibition in the maize rhizosphere reduces N losses at the ecosystem level

Objective 2: Determine if introgression of teosinte genes that confer nitrification inhibition impacts maize yields

Objective 3: Building on increasing industry interest in N-fixing inoculants, we will investigate synergy between N-fixing inoculants (generating ammonium), BNI (inhibiting nitrification), and DNRA (transforming nitrate/nitrite back to ammonium), potentially reducing the need for fertilizer N addition to fields

Objective 1 & Objective 3 Updates
A field trial was conducted to address both objectives 1 and 3. This experiment was carried out at the UIUC South Farm in Champaign, IL under conventional tillage in a corn-soybean rotation. The field site was maintained weed- and disease-free, is level and well-drained, and was well-suited to provide evenly distributed soil fertility, pH, and soil organic matter. A common maize inbred (B73) and one near isogenic line (Z031E0021) exhibiting BNI (biological inhibition of nitrification) were utilized in this experiment.

Experimental treatments included: three levels of N fertilization and inoculation with a N-fixing inoculant. Nitrogen fertilizer applications will include zero, limiting (30 lbs N acre^{-1}), and a fully sufficient N supply (60 lbs N acre^{-1}). Nitrogen rates will be broadcast applied as urea with a Gandy drop spreader and incorporated into the soil. Corn inbreds have a low sink potential and grain yields are limited compared to modern corn hybrids, so N rates were reduced to 30 and 60 lbs N acre^{-1}. N-fixing inoculant treatments including an untreated control and *Klebsiella variicola* (PROVEN from Pivot Bio). The inoculant was applied in-furrow at planting with a SureFire planter-mounted application system and blended with water for a total rate of 112 liter hectare^{-1}.

Plots consisted of four 76-centimeter wide, 5.3 meter long rows with a 0.76 meter walk alley between each range of plots with four replications of each treatment. We measured total plant nitrogen (N) accumulation and lysimeters were installed to monitor N losses to determine the fate of applied and fixed N. Microbial community structure and nitrogen-cycling functions (N-fixation, nitrification and BNI, and DNRA) were assessed across the growing season. Lysimeters will be recovered in Spring 2022 to evaluate N losses.

Limited seed stock for the BNI near isogenic line for the 2021 field season required a reduced from the original plan. The resulting experiment was not fully balanced, but included all key treatments and comparisons to achieve Objectives 1 and 3:

- B73 was planted with: 0, 30, 60 lb N acre^{-1}, ± ProveN inoculant at each fertilizer level
    - This allows establishment of the background response to inoculant and N losses against which to compare these phenotypes in the BNI germplasm
- The BNI NIL was planted with: 0 and 60 lb N acre^{-1}, ± ProveN inoculant at 60 lb N acre^{-1} only
    - This will allow us to determine N retention and synergy with N fixation resulting from the ProveN inoculant.

Results
Biomass and plant N
Biomass and plant N were assessed at V8 and VT growth stages. There were minimal effects of N rate on total aboveground biomass at both growth stages. However, increasing N rate from 30 to 60 lbs/acre increased VT N accumulation, partially driven by differences in plant N concentration (Figures 1 and 2). Both inbreds, B73 and the BNI NIL, tended to accumulate similar biomass N when treated with the same N rate. However, the response to the ProveN N-fixing inoculant (N uptake and accumulation) tended to be larger with BNI NIL inbreds, especially at the VT growth stage.

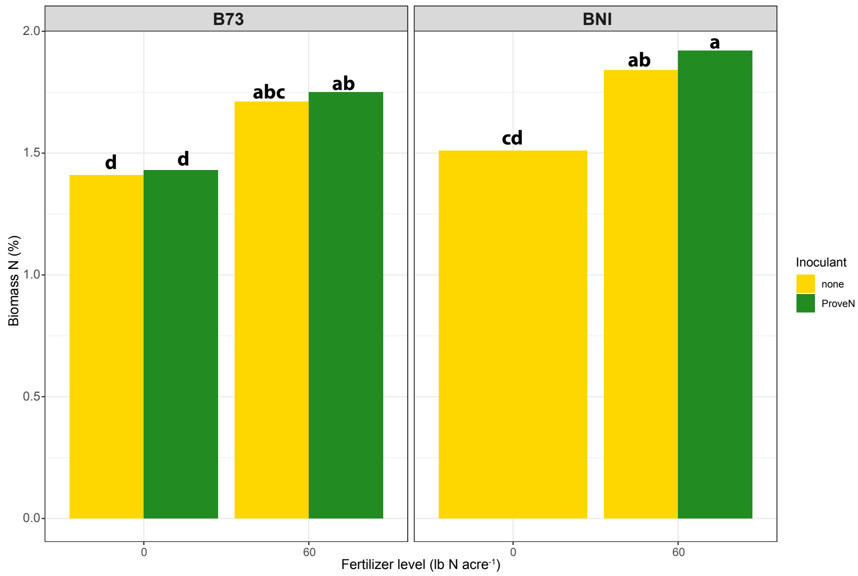


Figure 1. Biomass N concentrations (%) were enhanced by the BNI phenotype compared to the B73 genotype, particularly in combination with the N-fixing inoculant. Results shown here for the VT growth stage.

Key comparisons:

- BNI vs B73 at 0 lb N fertilizer
- BNI vs B73 at 60 lb N fertilizer ± ProveN
- Response to ProveN at 60 lb N fertilizer, compared between genotypes.
- Increase in biomass N between 0 and 60 lb N fertilizer, compared between genotypes.

Nitrification inhibition (BNI) is associated with greater biomass N.

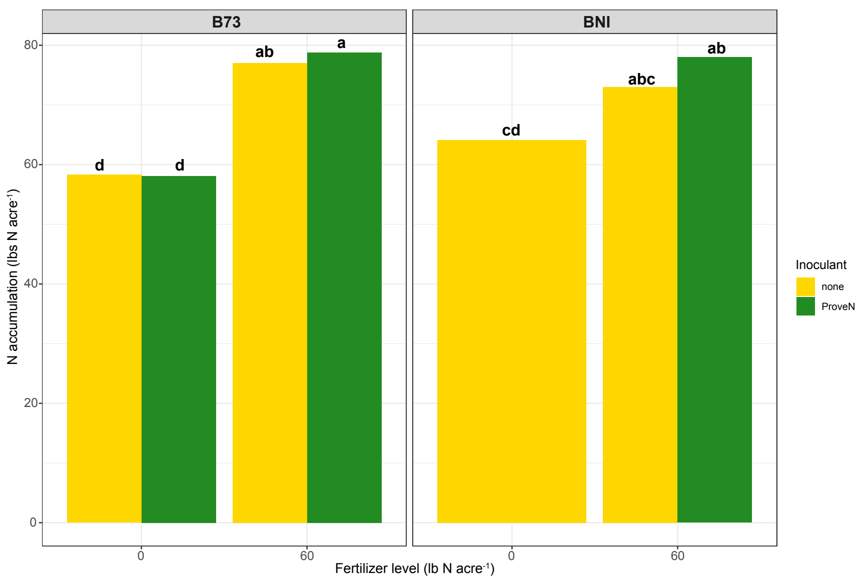
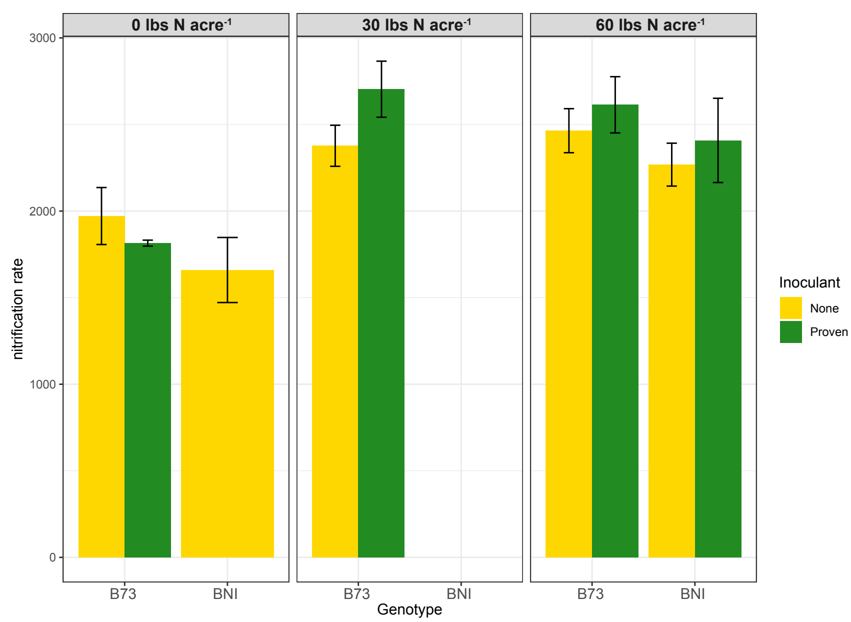


Figure 2. Biomass N accumulation was enhanced by the BNI phenotype compared to the B73 genotype, and BNI enhances the response in N accumulation due to the N-fixing inoculant. Results shown here for the VT growth stage.

Key comparisons:

- BNI vs B73 at 0 lb N fertilizer
- Response to ProveN at 60 lb N fertilizer, compared between genotypes

Nitrification inhibition (BNI) is associated with greater N accumulation at 0 lb N and greater response to the N fixing inoculant.



Nitrification: The only treatment that significantly impacts nitrification is fertilizer level. There is a trend that ProveN raises nitrification rates at V5 (Figure 3), but this effect is absent by V12. At 30 lb N acre^{-1}.

Figure 3. Potential nitrification rates at the V5 growth stage, compared among genotypes, fertilizers, and inoculant treatments. Nitrification responds significantly ($p < 0.05$) to fertilizer level.

While not significant here, nitrification trends lower in the rhizosphere of the BNI NIL, and nitrification tends to increase (p=0.0561) in the presence of the diazotroph inoculant, particularly in the fertilized treatments.

N fixation. We also observed a significant increase (p = 0.00611) in N fixation by indigenous soil diazotrophs in the rhizosphere of the BNI NIL (Figure 4). N fixation in the maize rhizosphere appears to be carbon limited (it is elevated when we add carbon to the acetylene reduction assay).

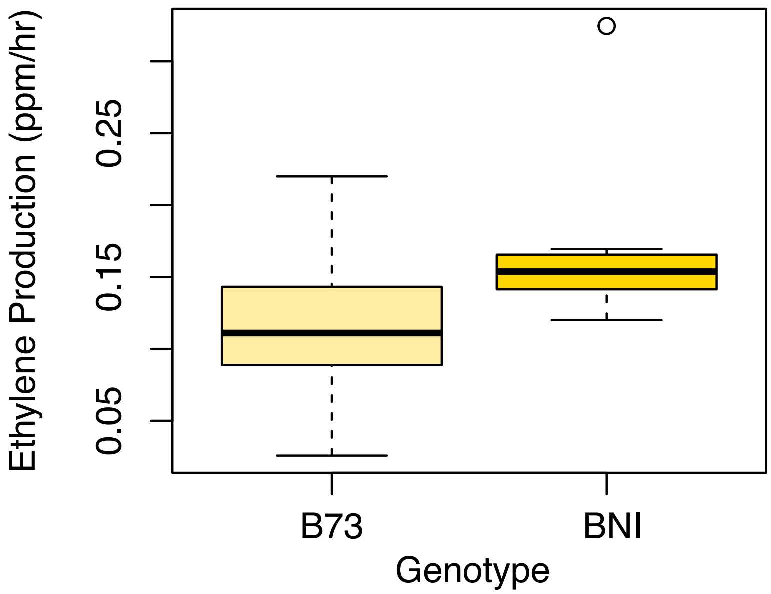


Figure 4. Potential N fixation, as assessed by reduction of acetylene to ethylene by the nitrogenase enzyme. Nitrogenase activity is shown here at the V5 growth stage, for uninoculated plants. This plot represents *potential* N fixation, as we relieved C limitation in this assay. N fixation at the V5 growth stage was significantly (p = 0.00611) enhanced by the BNI maize phenotype.

Conclusions for Objectives 1 and 3: Our initial results show some favorable indicators for the efficiency of N cycling due to the BNI phenotype:

- Greater biomass N and N accumulation: the BNI phenotype appears to enhance N uptake, particularly the added N from N fixing inoculants.
- Lower nitrification: Inhibition of nitrification may be particularly import where N fixing inoculants enhance nitrification.
- Enhanced N fixation: an unexpected result was the observation that elevated N fixation by indigenous soil diazotrophs was paired with inhibition of nitrification. This will be explored further in future field seasons.

Based on these results, we will add additional sampling time points early in the season. We have also generated sufficient seeds for the BNI NIL and B73 inbred to allow for an expanded field trial in 2022.
We are working to complete our analysis of N cycling rates through N isotope pool dilution assays. These results will provide actual (rather than potential) rates of N cycling processes for comparison among our treatments.

We are also evaluating the microbiome associated with B73 and BNI maize to determine if composition and abundance of the microbiome, particularly N cycling functional groups, is altered by BNI maize. Analysis is in progress. To date, DNA has been extracted from all rhizosphere samples, and will be submitted for DNA sequencing and quantitative PCR in January 2022.

Objective 2 Update:
To determine if introgression of teosinte genes that confer nitrification inhibition impacts maize yields, we are generating experimental hybrids, crossing B73 NILs with Non-Stiff Stalk (NSS) inbred lines – two different BNIs and WT B73 were crossed with 4 different NSS genotypes. On average, we generated about 5858 grams of seed for each of the hybrid combinations (estimated around 19,500 seeds).

These experimental hybrids will be evaluated in the 2022 field season.

One page summary

Managing the maize microbiome for sustainable nutrient retention in Illinois agricultural soils

Economic and regulatory factors are increasing the pressure on Illinois producers to improve nutrient management in maize operations in order to reduce environmental issues associated with nitrogen runoff. Agronomic management approaches, such as precision agriculture practices, edge of field treatment of exported nutrients, and denitrifying buffer zones are widely used and are increasing in efficacy. However, these practices provide only partial solutions, and do not address the root cause of nitrogen losses: soil microbial communities and their N transformation functions.

Based on our previous investigations in this area, we are now poised to address this question:

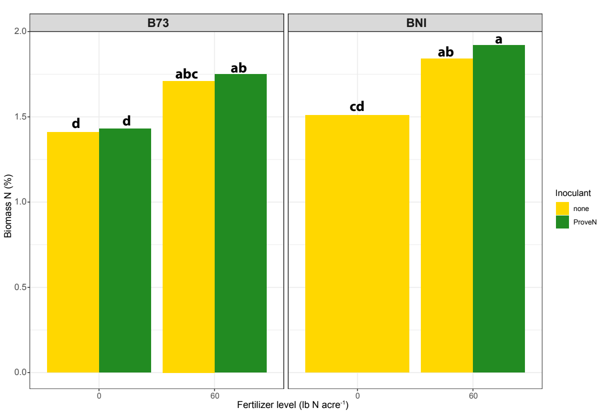
Can we achieve synergy between the plant and microbial functions listed below and potentially reduce the requirement for N fertilizer input?

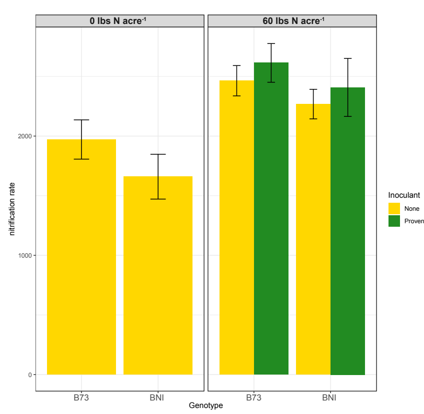
- **N fixation (generating ammonium from atmospheric nitrogen)**
- **BNI – biological inhibition of nitrification (the transformation of ammonium to nitrate)**
- **DNRA – dissimilatory nitrate reduction to ammonium (transforming nitrate/nitrite back to ammonium)**
  Taken together, these have the potential to improve nutrient management in agricultural soils.

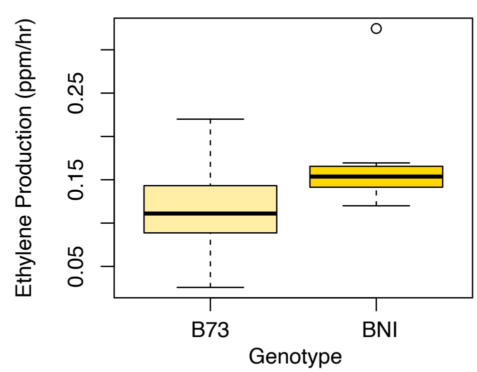
Our prior results demonstrate that nitrification is suppressed in the rhizosphere of older maize lineages and teosinte, and we have ongoing work to *investigate the mechanism* for suppression of undesirable N transformations and *identify the genomic regions* that influence the recruitment of the maize microbiome and nitrification rates.

The 2021 growing season represented our first opportunity to evaluate the potential for BNI to reduce N losses at the ecosystem level, as well as to evaluate synergy between N-fixing inoculants (that generate ammonium), BNI (inhibiting nitrification), and DNRA (transforming nitrate/nitrite back to ammonium), *potentially reducing the need for fertilizer N addition to fields*.

Initial results indicate that the BNI phenotype is associated with enhanced N accumulation when paired with an N fixing inoculant, offering support for a chain of events where diazotrophs add ammonium to the system and the BNI phenotype aids in retention and plant accumulation. This is a valuable synergy, as our initial results also suggest that N fixing inoculants can elevate nitrogen losses through nitrification.







Understanding mechanisms that influence plant-microbe interactions at the plant, rhizosphere, and microbial scale will enable optimization of nutrient retention, nutrient use efficiency, and sustainable crop production. Our perspective represents a shift away from *reactive* approaches to nutrient retention toward *proactive* approaches for retaining nutrients and reducing water quality issues associated with agriculture. We expect this work to lead to novel solutions for nutrient management to complement existing approaches and produce economic and environmental benefits for Illinois corn producers.