2017 Final Report Summary Sheet

Grantee Information

Project Title: Dissimilatory nitrate reduction to ammonium: An unexplored microbial pathway for nitrate retention in agricultural soils

Cereal Ry

Institution: University of Illinois

Primary Investigator: Kent

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[redacted]

Is your project on target from an IMPLEMENTATION standpoint? ☒ **Yes** ☐ **No**

If you answered "no" please explain:

We completed the proposed work on schedule.

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:

Have you reached any conclusions related to this project that you would like to highlight? ☒ **Yes** ☐ **No**

If you answered "yes" please explain:

Our results indicate that soils sampled from corn-soy rotations in Urbana, IL performed DNRA at rates sufficient to serve as an important N retention process. Depending on soil NO_3^- concentration, DNRA rates are correlated with one of two edaphic conditions (soil NO_3^- and soil moisture). Our results indicate that DNRA currently plays a role in N retention in agricultural soils, and that this desirable process can be maximized by management efforts.

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:

Additional Notes:

Based on our successful results, we proposed objectives and activities to extend this work with additional funding.

BACKGROUND

Economic and regulatory factors are increasing the pressure on Illinois producers to improve nutrient management, with nitrogen (N) run-off presenting a major challenge. Options like precision agriculture practices and denitrifying buffer zones are being used and improved, but may provide only partial solutions. Microbially mediated dissimilatory nitrate reduction to ammonium (DNRA) can lead to nitrogen retention by returning NO_3^- to the less mobile form of inorganic N, NH_4^+, rather than losing NO_3^- to leaching or gaseous nitrous oxide and dinitrogen via denitrification. DNRA can thus mitigate the water pollution and climate change impacts of fertilizer N inputs to agricultural systems while also potentially increasing crop yields by improving N retention for crop uptake. Despite the important role DNRA could play in creating sustainable agricultural systems, it has been understudied in agricultural soils, due to the prevailing conceptual model which suggests that DNRA occurs only under highly reducing conditions such as found in flooded soils. However, mounting evidence indicates that DNRA rates can be comparable to or even many times greater than NO_3^- leaching and denitrification rates in unsaturated soils, likely due to the activity of facultative anaerobes within anoxic soil microsites. Therefore, DNRA should no longer be ignored in assessments of soil N cycling.

The long-term goal of the research team is to reduce NO_3^- losses from agricultural systems in the Midwest U.S., thereby improving water and air quality while possibly increasing crop yields. DNRA is an unexplored pathway for NO_3^- retention in agricultural soils that may be optimized through management practices. However, the environmental and genetic potential for DNRA to occur in agricultural soil is currently unknown.

The *overall goal* of this project is to improve understanding about the importance of and controls on DNRA in Illinois agricultural soil. During the 2016 – 2017 funding period, we have progressed toward this goal through the following *specific objectives*:

Objective 1: Quantification of DNRA rates to determine if DNRA is an important process in Illinois agricultural soils,
Objective 2: Characterization of abundance and diversity of microbial communities capable of DNRA in Illinois agricultural soils, and
Objective 3: Determination of drivers of DNRA rates and microbial community composition to identify treatments and management practices that affect DNRA rates in these soils.

To address these objectives, we collected and analyzed soil samples from a diversity of agricultural systems at the UIUC South Farms in Urbana, Illinois to identify which treatments and conditions facilitate a meaningful level of DNRA potential, and of these, how management practices might be used to encourage increased DNRA rates within these soils.

SUMMARY OF ACTIVIES TO DATE

The project has concluded its second year. Work began in April 2016 with the identification of relevant and accessible sites with differing treatments (fertilization regimes, tillage, residue application), and the development of protocols and sampling methods to measure DNRA activity using ^{15}N tracer methods. Preliminary results from 2016 indicated that soils sampled from corn-soy rotations in Urbana, Illinois performed DNRA at rates sufficient to serve as an important N retention process. Depending on soil NO_3^-

concentration, DNRA rates correlated with one of two edaphic conditions (soil NO_3^- and soil moisture, **Figure 1**). The tight correlation of DNRA rates to soil moisture in high NO_3^- conditions—with no similar correlation to soil NO_3^-—implies that once a minimum NO_3^- threshold is met, DNRA activity is likely controlled by oxygen dynamics associated with soil moisture. In contrast, the strong correlation of DNRA rates to soil NO_3^- in low NO_3^- conditions with only a weak relationship to soil moisture implies that NO_3^- is the primary limiting factor in these situations.

While DNRA rates varied drastically in response to edaphic conditions, high-throughput DNA sequencing revealed that the soil microbial community structure in these samples did not vary significantly across sites, management practices, or sampling timepoints. Similarly, genetic potential for DNRA evaluated via qPCR as abundance of gene copies of *nrfA*, the gene encoding nitrite reductase in DNRA, did not differ across treatments, sites, or growing season timepoints. This surprising consistency despite the variable rate response indicates that while the potential for DNRA to occur existed in all corn-soy treatments sampled throughout the growing season, edaphic conditions directly influenced the observed DNRA rates by "activating" this potential. **These data, therefore, indicate that DNRA currently plays a role in N retention in these agricultural soils, and that this desirable process can be maximized by management efforts.**

Sampling locations. Based on the preliminary data from 2016, we chose to focus on a subset of sites with both perennial and annual treatments in 2017. DNA sequencing revealed that microbial communities in perennial cropping systems differed substantially from those in annual cropping systems; however, like the contrasting treatment communities in our annual systems, *nrfA* gene abundance did not vary. To evaluate if DNRA rates in perennial systems exhibited a similar pattern to the annual systems previously tested, we selected two sites with both perennial and annual treatments and developed a sampling plan to evaluate DNRA rates pre- and post-rain events at these sites. Each site included four replicate plots per treatment:

- Energy Farm: plots include corn-soy rotation (fertilized during corn years, unfertilized during soy years; 2017 is a corn year), miscanthus (half fertilized, half unfertilized), and switchgrass (fertilized). The treatments at this site were used to compare the effects of crop type to DNRA rates before and after rainfall events (to evaluate response to soil moisture), and at varying levels of fertilization (to evaluate response to NO_3^-).
- Perennial Buffers: plots at this NREC-funded site include corn-soy rotation (both cover cropped and non-cover cropped, 2017 is a soy year and thus unfertilized) and bioenergy grasses plots (complex vs. simple), to compare DNRA rates between crop types before and after rainfall events.

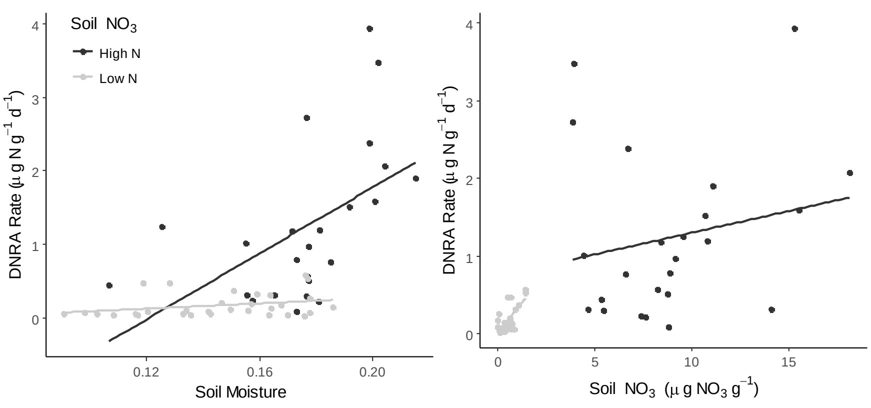

Figure 1. Rates respond differently to environmental variables depending on the existing soil conditions. Rates for higher soil NO_3^- (black circles) correlated strongly to soil moisture ($p < 0.01$). Rates for lower NO_3^-, (gray circles) correlated strongly to NO_3^- concentration ($p < 0.0001$).

In spring 2017, a new treatment was introduced at Energy Farm, in which one half of each switchgrass plot was tilled under and planted in corn. We chose to include these plots in our analysis this year to additionally evaluate the effect on DNRA rates of transitioning from a perennial cropping system to an annual one. In addition to the measurements already outlined in our experimental approach, we collected *in situ* trace gas samples on these two treatments at biweekly intervals beginning in March, including a daily sampling regime immediately after fertilization. This gas flux data will supplement in-lab measurements of N-cycling process rates to help elucidate how this crop type transition may alter the fate of N in agricultural systems.

Sampling to date. During 2017, each site was sampled twice, once prior to any rain events (no rainfall for week prior to sampling; 0.01 inches of rain during two weeks prior) and once after 0.76” at Energy Farm and after 0.13” at Perennial Buffers). For each sampling event, two soil samples from 0-10cm depth were collected at two random locations within each replicate plot and composited together for experimentation. Activities at each site are summarized in the table below.

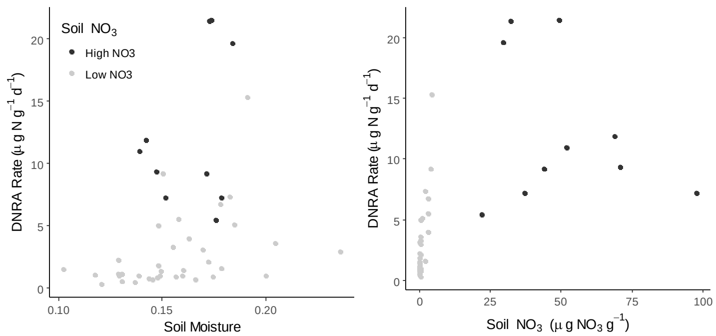

Figure 2. Similar rate response by both annual and perennial cropping systems to soil NO_3^- and soil moisture as previously observed in annuals only. Rates in low NO_3^- samples (switchgrass, Miscanthus, and soy) correlated tightly to soil NO_3^-; rates in high NO_3^- samples (corn and switchgrass-to-corn conversion) did not correlate to soil NO_3^-.

Site	**Activity**
Energy Farm	• 50lbs N applied to switchgrass and half miscanthus; corn planted and 180 lbs N applied during week of May 10-16 • Pre-rain samples collected 6/06/17 • Post-rain samples collected 6/16/17
Perennial Buffers	• Soy planted week of June 29 • Pre-rain samples collected 6/08/17 • Post-rain samples collected 6/19/17

Table 1. Summary of activities at each sampling location to date.

Sample analysis. The samples generated during the pre- and post-rain experiments were processed in the lab to achieve a snapshot of:

- *in situ* NO_3^-, NH_4^+, pH and soil moisture in each replicate plot,
- transformations of N between these pools and into N_2O over the course of 24 hours by use of ^{15}N tracers,
- fluxes of N_2O, CO_2, and CH_4 from each soil sample, and
- the abundance of relevant N-cycling genes within the soil microbial community.

Results. Rate measurements for perennial and annual treatments collected in 2017 revealed a similar pattern of response to soil NO_3^- and soil moisture as previously observed in annual soils. (**Figure 2**). In this study, the low NO_3^- annual soils (from unfertilized soy plots) correlated strongly to soil NO_3^-, as did the perennial soils, which were all low in NO_3^-. The high NO_3^-, recently fertilized corn plots (both regular corn-soy rotation treatments as well as the newly established switchgrass-to-corn conversion plots) exhibited no such correlation. These results confirm that, regardless of microbial community composition, soil NO_3^- and soil moisture are the primary factors controlling DNRA rates in agricultural soils.

Challenges. While a stark differentiation between microbial communities in till vs. no-till treatments was observed in 2016, no similar difference was observed in DNRA rates in these plots. We initially planned to include this site in our sampling efforts in 2017, but unfortunately the no-till site was converted to a tilled site in spring 2017. Similarly, the Biochar site used in 2016 is no longer being maintained as an experimental field site.

Relying on natural rainfall events proved to be an inefficient means of creating a soil moisture gradient against which to regress DNRA rate measurements. To avoid this pitfall in the future, we will instead collect intact soil cores and transport these back to the laboratory for soil moisture and soil NO_3^- manipulations, as described in our renewal proposal for years 2018-2019.

Upcoming work. As of the end of this funding period, we have identified the primary drivers of DNRA activity in agricultural soils (NO_3^- and soil moisture), and have confirmed that rates respond similarly across all treatments regardless of microbial community structure. Genetic potential, indicated by gene copy number of *nrfA*, remains constant across treatments and growing season timepoints despite variable DNRA rates (i.e., variable activity of the enzyme encoded by *nrfA*). This evidence indicates that while the potential for DNRA activity exists in all these soils throughout the growing season, it is being "activated" by edaphic conditions. To further elucidate the independent effects of soil NO_3^- and moisture on the activation of DNRA activity, we will use laboratory manipulations of these two factors on differing soil types (silty-clayey vs. sandy) beginning in the spring of 2018. In addition to rate measurements, we will evaluate gene transcript abundance via mRNA (messenger RNA, which indicates which genes are being actively expressed rather than simply present in a sample). This will allow us to identify the role these factors play in "activating" the genetic potential present in these soils.