2019 Final Report Summary Sheet

Grantee Information

Project Title:	**Towards Management of Dissimilatory Nitrate Reduction to Ammonium for Nitrate Retention in Agricultural Soils**
Institution:	University of Illinois at Urbana-Champaign
Primary Investigator:	Angela Kent and Wendy Yang
NREC Project #	**NREC #2016-01781**

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:

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

We observed meaningful DNRA activity at soil moisture levels low enough to that oxygen prevents denitrification. This suggests that **DNRA may act as an alternative nitrate reduction pathway when reduction via denitrification has been inhibited by the presence of oxygen.** Meanwhile, at higher soil moistures where oxygen diffusion is inhibited and nitrate transport is promoted, we observed the co-occurrence of denitrification and DNRA. **Our findings thus far demonstrate that DNRA can occur both within the conventionally recognized reducing conditions characteristic of saturated soils—such as during the early growing season—and also within the substantially drier soils characteristic of the late growing season.**
Additionally, the presence of gene transcripts (mRNA) in soils exhibiting meaningful DNRA rates supports our previous hypothesis that observed **DNRA activity can be attributed to gene expression, rather than gene abundance at the DNA level**.

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:
A peer-reviewed publication resulting from this work is currently in preparation for submission to Nature Communications in Spring 2020. Results as of August 2019 were presented at the 2019 Ecological Society of America conference, and we have created a website "The Story of DNRA" (http://sada-egenriether.com) as both a companion to the poster presentation and public outreach. We will be presenting our results at the 2020 NREC Investor Insights meeting.

NREC #2016-01781

Towards Management of Dissimilatory Nitrate Reduction to Ammonium for Nitrate Retention in Agricultural Soils

2019 Final Research Report

Kent & Yang

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. Our preliminary data showed that soils sampled from Urbana, Illinois performed DNRA at rates sufficient to serve as an important N retention process. Despite dramatic differences in microbial community structure and N-cycling gene abundance across the management treatments tested, DNRA rates responded only to two specific edaphic conditions: soil moisture and NO_3^-. This indicates that the genetic potential for DNRA to occur in these soils exists independently of soil conditions and is simply "activated" under the certain circumstances. Further investigation was required to comprehensively characterize the effects of these soil conditions on DNRA rates, and ultimately translate this to management practices that encourage DNRA activity in agricultural soils.

The *overall goal* of this project is to improve understanding about the importance of and controls on DNRA in Illinois agricultural soil. This goal is being achieved through the following *specific objectives*:

Objective 1: Quantify drivers of DNRA rates across agricultural management treatments,
Objective 2: Identify controls on DNRA gene expression in soil microbial communities, and
Objective 3: Assess identified drivers to more accurately evaluate the combined effects of soil moisture and NO_3^-.

To address these objectives, we have collected and analyzed soil samples from a diversity of agricultural management treatments in central Illinois to identify the role management may play in shaping DNRA rates as a function of soil moisture and soil NO_3^-.

SUMMARY OF ACTIVIES TO DATE

The work proposed for the 2018-2019 funding period has been completed. Work began in May 2018 with the identification of agricultural plots suitable for an N manipulation experiment, and the development of new protocols and sampling methods to assess movement of dissolved oxygen and NO_3^- through soils. Preliminary results from the 2016-2017 field seasons indicated that meaningful DNRA rates were possible in all agricultural fields tested, especially after rainfall events occurring during the high-NO_3^- post-fertilization period. To assess the roles of soil moisture and soil NO_3^- independently, collected soils were subjected to dual gradients of moisture and NO_3^- in the lab and evaluated for DNRA and other N-cycling rates using ^{15}N stable isotope techniques. To complement these rate measurements, soil was flash-frozen in liquid N_2 for later DNA-RNA coextraction to evaluate expression of relevant N-cycling genes. In addition, the movement of dissolved oxygen and NO_3^- through the soils was assessed as a function of soil moisture and NO_3^- addition.

Sampling to date. For the 2018 growing season, we chose to focus sampling efforts at the Crop Sciences Research and Education Center in Urbana, IL. The goal of this sampling campaign was to thoroughly characterize the response of DNRA activity against soil moisture and NO_3^-, in order to create a robust baseline model against which to compare future measurements (e.g., differing soil types) in later sampling campaigns. By the end of the 2019 growing season, we had sampled each replicate plot 5 times, with each sampling event representing a unique (but overlapping) range of soil moisture. For each sampling event, 4 soil samples from 0-10 cm depth were collected at random locations within each replicate plot and composited together for experimentation. The composited soils were then divided into 8 different NO_3^- addition treatments, ranging from no addition to 75 $\mu g\text{-}N\ g^{-1}$. These were administered the evening prior to experimentation. Overall, this yielded a total of 160 data points which we have used to regress DNRA rate against soil moisture and NO_3^-.

Protocol development. To assess the roles of NO_3^- and soil moisture separately, as well as the interaction between them, soils were subjected to dual gradients in soil moisture and soil NO_3^-. For the NO_3^- gradient treatment, we evaluated initial soil NO_3^- colorimetrically, and added a calculated volume of KNO_3 solution to achieve a total concentration ranging from the initial, unaltered concentration up to 75 μg-N g^{-1}, a range representative of what soils under conventionally managed corn-soy rotations experience throughout the growing season. The moisture gradient was primarily achieved by scheduling sampling dates during times of varying *in situ* soil moisture. We chose to include a range of soil moisture from ~8% to 33%, which is representative of moisture ranges typically observed during the growing growing season, from the saturated conditions that occur in early spring, to the much drier conditions in late summer.

Within a soil profile, soil moisture acts as both a conduit for movement of dissolved NO_3^- and an impediment to movement of gaseous oxygen into soil pore space. Because of its opposite effects on these two important aspects of DNRA activity, an assessment of the movement of these two constituents through the soils will provide key insights into how and why these edaphic conditions are driving DNRA rates. To evaluate this, used PreSens oxygen sensor spots arranged vertically inside of transparent soil-filled sleeves. These sensor spots are read non-destructively with a PreSens Microx 4 oxygen sensor through the sleeve wall. The soil cores were subjected to a gradient of soil moisture, similar to the experimental conditions described above, and placed into an anaerobic glovebox flushed with N_2 until all oxygen was been depleted from the soil. Then, the cores were removed and incubated under ambient room conditions to evaluate the speed with which dissolved oxygen traveled into the soil column as a function of soil moisture. Similarly, to assess movement of NO_3^- through the soil profile, a solution of ^{15}N-labeled KNO_3 was added to the top of 10 cm soil cores subjected to differing moisture levels, and the these were dissected at 1 cm intervals and sampled destructively for isotopic analysis to evaluate the speed with which NO_3^- moves through the soil as a function of soil moisture.

Although no difference in functional gene abundance or microbial community composition was detected at the DNA level, DNRA rates clearly differed as a function of soil moisture in all previous years. **This indicates that while the genetic potential for DNRA exists regardless of rainfall conditions, relevant functional genes are being "activated" under favorable conditions.** To evaluate the effect of soil moisture and NO_3^- on which functional genes are being expressed (i.e. to determine the soil conditions that favor DNRA), we have developed a DNA-RNA co-extraction method. This has enabled us to compare the abundance of functional genes in DNA (the genetic "potential" in the soil) vs. the abundance of these genes in RNA (what is actively being expressed at the time of experimentation), and is being used to help us better understand how edaphic factors are controlling DNRA.

RESULTS TO DATE

Oxygen and nitrate transport. The results from the O_2 and NO_3^- transport experiments have shed valuable insight into the mechanism by which soil moisture has been observed to control DNRA rates under adequate NO_3^- conditions. We found that, even at high soil moistures, dissolved O_2 was able to rapidly diffuse into the upper layers of the 10 cm soil cores (Figure 1). In the upper 1.6 cm, there was no difference in the rate at which each soil regained oxygen saturation, regardless of soil moisture. By contrast, NO_3^- transport was severely hampered at low soil moisture and required 24 hours to move a comparable distance in the soil profile compared to the ~30 minutes for O_2.

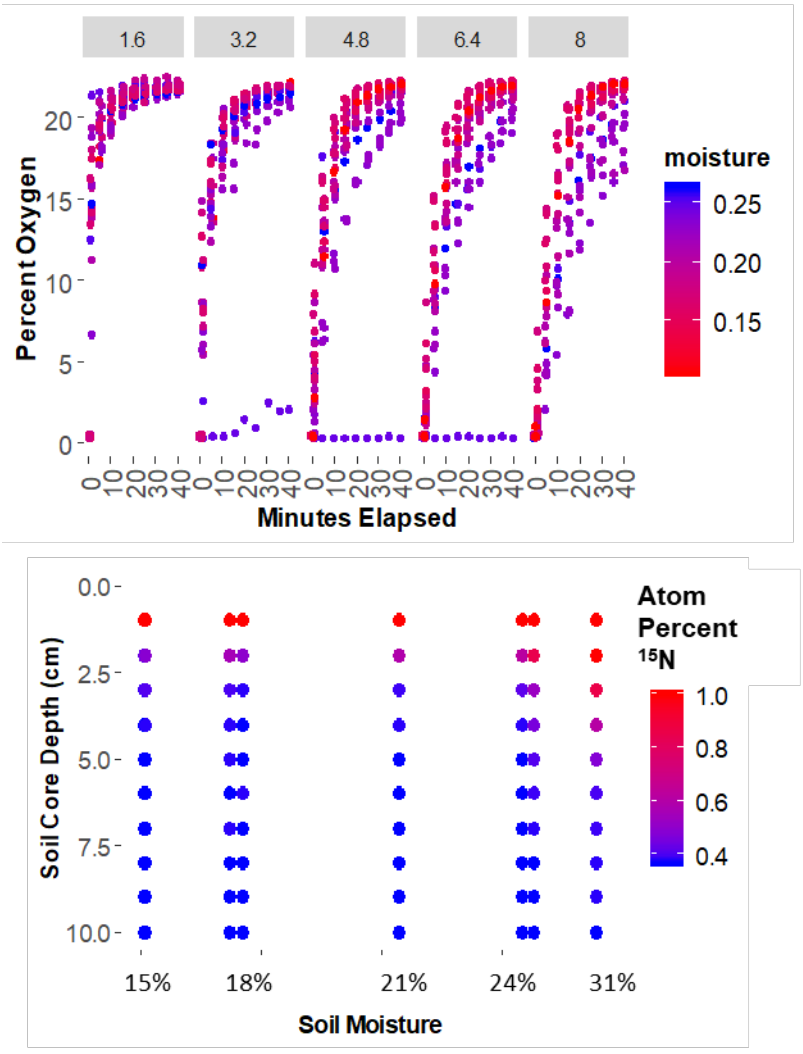

Figure 1. *Top: Oxygen in the upper layer of the soil core (1.6 cm, left column) rapidly returned to saturation levels in approximately 30 minutes after being removed from anoxic conditions, regardless of soil moisture level. Deeper soil levels returned to saturation more slowly at higher soil moistures.*

Bottom: ^{15}N-labeled NO_3^- traveled much more slowly than dissolved O_2 under the same moisture conditions. Only under the highest moisture conditions did NO_3^- travel a comparable distance compared to O_2, and even these required 24 hours, or approximately 50-fold the amount of time it took O_2 to travel the same distance.

Rate assessments. DNRA rates exhibited a pronounced bimodal response across the moisture gradient, peaking at 8 µg-N g^{-1} d^{-1} at 18% gravimetric soil moisture, dropping back to near zero between 22 and 28%, and peaking again at 30% soil moisture **(Figure 2a)**. Across the NO_3^- gradient, rates reached their maximum at 20 µg-N g^{-1}, and began to decline after 40 µg-N g^{-1} **(Figure 2b)**. There was a strong significant interaction between these two soil characteristics, wherein increased nitrate at a given moisture level enhanced DNRA rates.

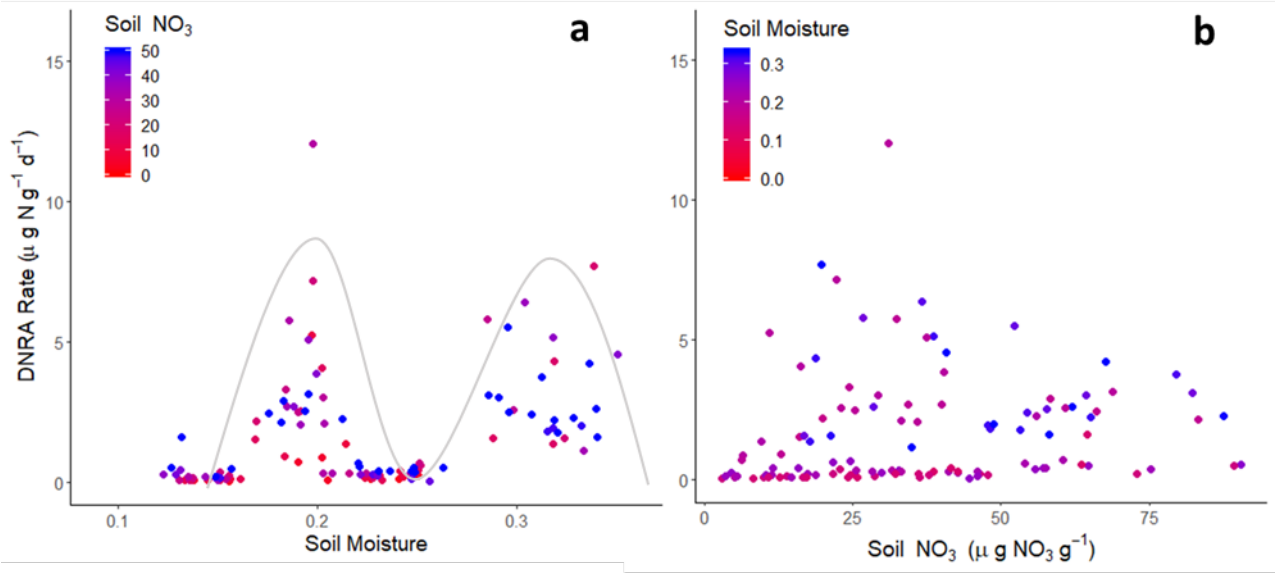

***Figure 2**. DNRA rates exhibited bimodal response across moisture gradient (a), and roughly unimodal response across nitrate gradient (b), with a significant interaction between the two effects.*

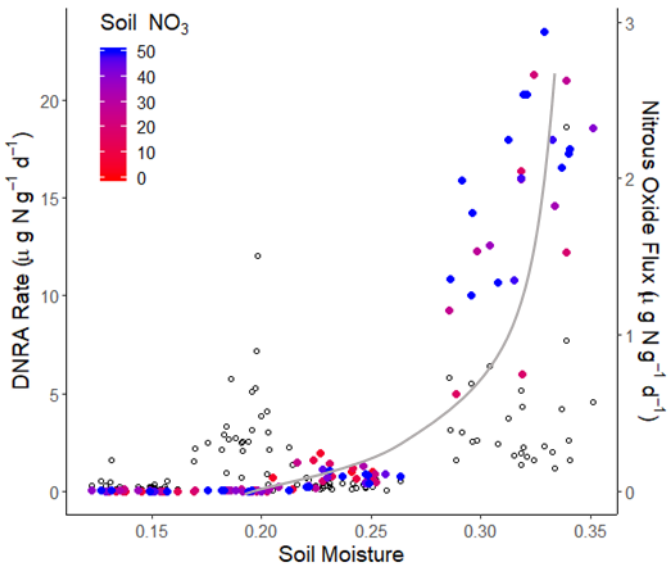

Figure 3. *Nitrous oxide flux (colored circles) increased from 21% soil moisture, which coincided with the decrease in DNRA activity (open circles).*

Nitrous oxide (N_2O) flux from denitrification increased exponentially as soil moisture rose from 21%. The soil moisture range between 22 and 28% – where DNRA was repressed – coincided with the first appearance of denitrification N_2O flux **(Figure 3)**.

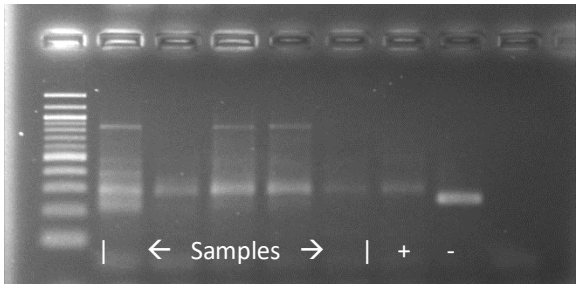

Figure 4. *Gel image of PCR for* nrfA *gene in cDNA generated from extracted mRNA* exhibiting *elevated (~5μg $g^{-1}d^{-1}$) DNRA rates. From left to right:*
Lane 1: size standard
Lanes 2-7: samples with elevated DNRA
Lane 8: positive control
Lane 9: negative control

Gene expression assessments. Results from PCR carried out on gene transcripts (mRNA) from our newly-developed DNA-RNA coextraction protocol confirmed the presence of actively expressed *nrfA* in samples exhibiting elevated DNRA activity (Figure 4), verifying that we can detect and quantify the microbes that are actively carrying out DNRA in agricultural soils.

CONCLUSIONS TO DATE

The results of our oxygen and NO_3^- transport experiments indicate that oxygen diffuses through the top several centimeters of soil rapidly, even at high soil moistures. There is evidence from pure culture studies that increased oxygen concentrations significantly inhibit expression of denitrification genes, whereas the same is not true of DNRA genes. **Our discovery of meaningful DNRA activity at soil moistures low enough to completely prevent denitrification suggests that DNRA may act as an alternative nitrate reduction pathway when reduction via denitrification has been inhibited by the presence of oxygen.**

Meanwhile, at higher soil moistures where oxygen diffusion is inhibited and nitrate transport is promoted, we observed the co-occurrence of denitrification and DNRA. Our findings thus far demonstrate that DNRA can occur both within the conventionally recognized reducing conditions characteristic of saturated soils—such as during the early growing season—and also within the substantially drier soils characteristic of the late growing season.
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UPCOMING WORK

To supplement the results generated from the 2018-2019 funding period, we submitted an extension proposal in Fall 2019 to fund Illumina sequencing of gene transcripts obtained from the same soils used in the laboratory incubation. We have confirmed the presence of *nrfA* gene transcripts in these soils, and preliminary data on similar soils indicate that different microbial taxa are responsible for the two distinct peaks in DNRA activity observed across the moisture gradient. The sequencing data generated during this extension period will strengthen the manuscript currently being drafted, and will help form the basis for the work that will be proposed in the next stage of this exciting research.

BUDGET UPDATE

The 2019 objectives within the projected budget. The extension funding will be used to carry out additional work to identify the active microbial populations responsible for DNRA activity under different soil conditions.