Exploring New Microbial Pathway for Nutrient Control to Increase Agroecosystem Sustainability

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Summary: Excess fertilizer applied to agriculturally important crops such as corn can leach into nearby waterbodies and lead to harmful algal blooms. To retain the excess fertilizer from leaching, cover crops are being used to retain nutrients and promote soil health. However, the cover crop decomposition rate is still unknown and the type/mixes of cover crop that releases nutrient at a slower rate for an even load on tile water upon decomposition is unclear. Our goal was to improve understanding of the importance of cover crop decomposition and microbial processes in nutrient leaching and retention in SD agricultural soil. As microbial activities are central to cover nutrient cycling, transfer and decomposition, the functional genes central to nitrogen cycling, including the dissimilatory nitrate reduction to ammonium (DNRA) and anammox pathways are being compared. The role of these activities in nitrate reduction affected by cover crop decomposition on soil health and tile water quality will help us gain knowledge on modulating the nitrogen and phosphate leaching into the waterways. The objectives included 1. Compare nutrient levels of leachate from rye planted in corn-soybean rotation with different timing of suppression; 2. Compare the N cycling gene expression; 3. Enrich DNRA-annamox microbes by cover cropping. Overall, we tested the effectiveness of a more diverse cropping system with cover crops to decrease tile nitrate loss with improved soil health. Our field site was selected to be at the Southeast Experimental Station in Beresford, SD. So far, the results showed that there was a 10% increase in soil protein in plots with cover crop, but no statistical difference in soil health between the plots prior to and after cover crop rye planting. Soil protein is a measure of soil health and a measure of easily accessible N in the organic form that is not subject to leaching from the system. We also compared phosphorus in the leachate at the greenhouse between oats, rye, and no cover crop. No significant differences were found between the three cover crop treatments. Therefore, the growers should not have concern over phosphorus leaching from decomposing rye and oats. We will further determine whether termination dates will play a role in phosphorus leaching from decomposing rye in the second year, and continue to determine the effects of cover crop on soil health.

Objectives:

1. Characterize the microbial communities capable of DNRA-anammox in SD agricultural soils associated with corn roots and determine the abundances of N-cycling genes; 2. Compare nutrient levels of leachate from rye and oats planted in the greenhouse for different timing of suppression; 3. Compare the effects of cover crop on soil health indicators.

Results:

1. Compare the microbial communities capable of DNRA-anammox in SD agricultural soils associated with corn roots and determine the abundances of N-cycling genes: In the field, we have collected corn roots on 6/22/2018 to quantify the abundance and diversity of functional genes related to N cycling transformations of interest by quantitative polymerase chain reaction (qPCR). Therefore, soil DNA extraction is on the way. qPCR based quantification of the *nrf*A and *hzo* genes involved in DNRA and anammox, respectively, will enable the comparisons of gene abundance. Similarly, qPCR based quantification of fungal co-denitrification gene will be measured by *P450nor* genes. In the greenhouse experiment, we performed preliminary evaluation of the nutrient release from the decomposition of different cover crops. Leachates were analyzed for phosphorus by colorimetric method to determine nutrient levels comparing rye and oats.

Progress (up to December 2018): A postdoctoral research associate, Dr. Huma Saleem, was hired to oversee the day-to-day activity of the project from June till December. However, due to a 6-month gap in the available NREC funding for this project, Dr. Saleem had to be redirected to work on a different project since November 2018 when the funding ran out. In the field, on June 21st, corn roots were collected from the tiled fields after the rye/cover crop planting in the SE experimental station. Soil DNA were extracted, and nitrogen cycling genes from the bacterial community have been submitted for NiCE-chip quantification. However, due to a lack of supporting personnel in the U. of Minnesota lab we collaborate with, we will collect the 2019 spring samples and combine for NiCE chip measurements along with the 2018 samples together with our staff here from SDSU. We will go up to Minneapolis to use their Fluidyme access array-qPCR setup, and we will be able to summarize the results in the June 2019 report to NREC.

2. Compare nutrient levels of leachate from rye and oats planted in the greenhouse for different timing of suppression;

For the cover crop decomposition assay in the greenhouse, we suppressed the rye cover crop, performed based on the recommended growth stage by the Midwest Cover Crop Council (MCCC) that allowed the cover crop to establish for 1 month (Fig 1). Leachat data did not show significantly higher concentration of phosphorus from the decomposing oats or rye compared to the negative control (Fig 2). We are currently comparing the effect of termination dates (2 weeks vs. 4 weeks) on phosphorus leaching from decomposing rye for which we can report the results by the June report deadline to NREC.

Figure 1. One week old rye planted in the green house for studying the leachate between control, rye, and oats treated soil for nutrient retention.

Statistics associated with cover crop decomposition:

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> t.test(Oats,Control)

        Welch Two Sample t-test

data:  Oats and Control
t = 1.2754, df = 2.0702, p-value = 0.3266
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.9894413  1.8626410
sample estimates:
mean of x mean of y
1.2947443 0.8581445

> t.test(Rye,Control)

        Welch Two Sample t-test

data:  Rye and Control
t = 0.61391, df = 2.0607, p-value = 0.6002
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.313044  1.764699
sample estimates:
mean of x mean of y
1.0839720 0.8581445
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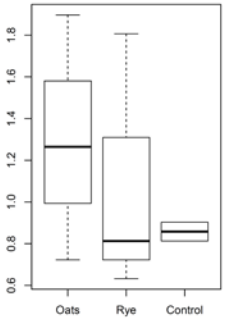


Figure 2. Greenhouse cover crop treatments comparing treatment with rye, oats and without cover crop for the phosphorus leaching

3. Compare the effects of cover crop on soil health indicators

Samples were taken from the Beresford, SD plots to compare the effects of cover crop rye treatment on soil health measures from October 2017 before treatment (Fig 3) and May 2018 after (Fig 4) the first cover crop treatment. The data showed that all measures had the same trends for the treatments and across the paired plots. The rye cover crop plots are higher on average for all measures compared to the no cover cropped. Doubt differences are significant, but would have to be tested with a paired t-test. Samples from Fall 2018 were taken by Dr. Sexton and will be tested by Dr. Lehman soon.

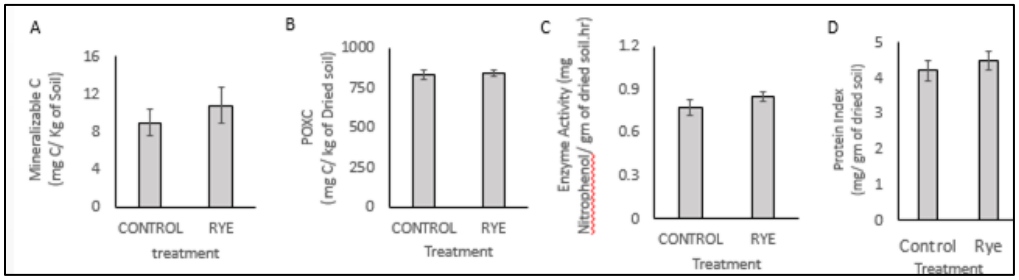
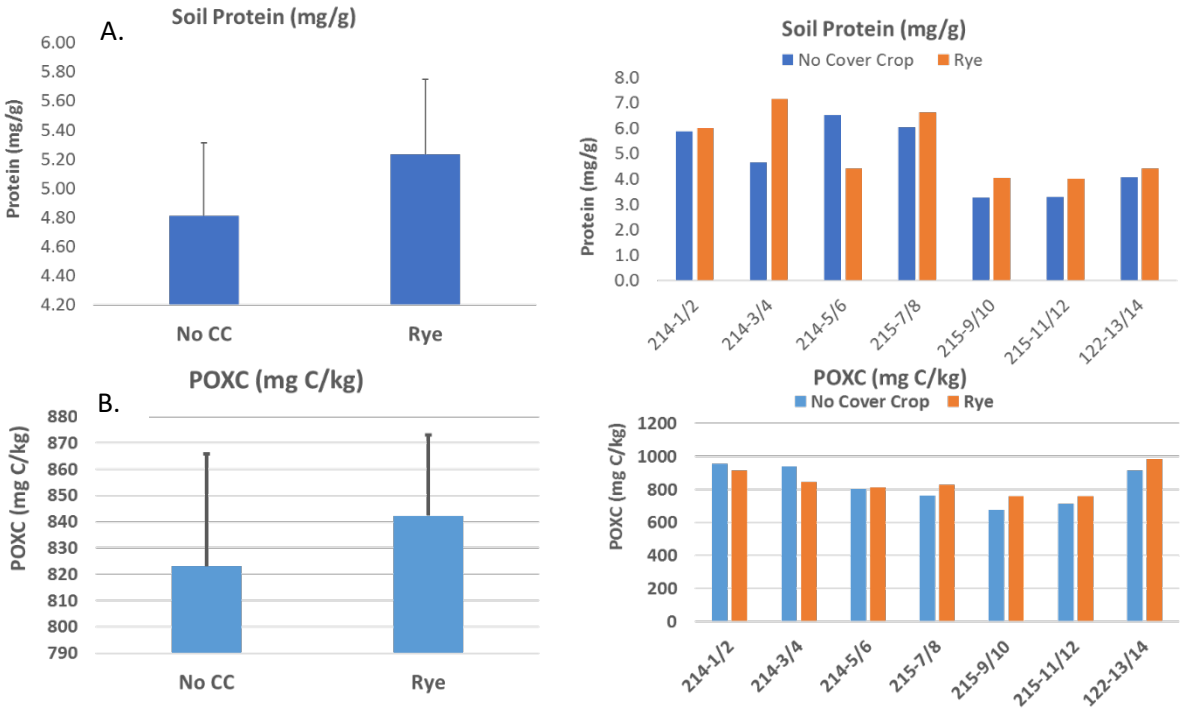


Figure 3. Soil health indicators in plots prior to cover crop treatment (RYE) and no-cover crop treatment (CONTROL). Samples were taken October 2017 A) Short term mineralizable C was measured using soil burst test at 72hours and B) Permanganate oxidizable C measured using POXC assay. C) β-glucosidase extracellular enzyme activity assay. D) Total glomalin measurement using autoclaved citric extractable soil protein.



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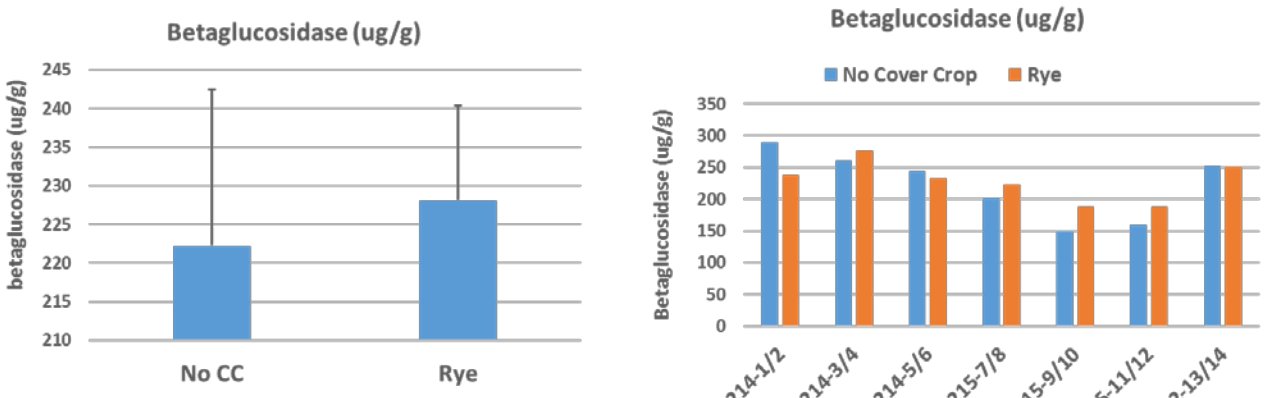

D.

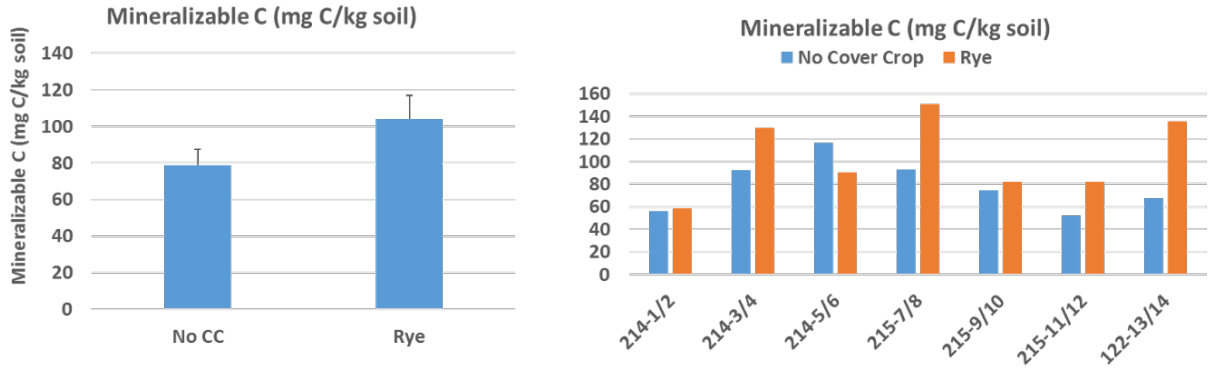

Figure 4. Soil health indicators in plots prior to cover crop treatment (RYE) and no-cover crop treatment (CONTROL). Samples were taken May 2018 for A) Total glomalin measurement using autoclaved citric extractable soil protein. B) Permanganate oxidizable C measured using POXC assay. C) β-glucosidase extracellular enzyme activity assay. D) Short term mineralizable C was measured using soil burst test at 72hours.

Objective 2 – Mycorrhizal fungal activity and P leaching: Arbuscular mycorrhizal fungi (AMF) colonization of plant roots improves plant nutrient uptake and promotes soil health that prevents nutrient leaching. In the soil samples subjected to the cover crop decomposition in the greenhouse, we will plant corn, and determine the arbuscular mycorrizhal levels using the fungal ITS gene as a marker. To decipher the effect of possible virus infection on P uptake of mycorrhizae and the effect on P leaching, we have identified sequences of viral genome in the existing RNA sequences of arbuscular mycorrhizal fungi of *Rhizophagus* spp. downloaded from NCBI database. This identification has spiked an important question of what could be the role of such viral infection on beneficial fungi. Therefore, we will identify the viruses infecting the AMF from the plant roots which will lead to further characterize the biological function in the mutual relationship between plant and fungus to reduce nutrient leaching.

In our previous bridge funding proposal which was not funded (7/1-12/31/2018), we mentioned this additional objective that we would study the mycorrhizal fungal activity and P leaching. In this objective, we sequenced a *Rhizophagus* infected *Medicago* root provided by Dr. Bucking at SDSU. We identified several viral contigs that associate with a colonized root exhibiting the enhanced P uptake of the model plant *Medicago*.

Products:

Neupane, N., Feng, C., Feng, J., Kafel, A., Bucking, H., Marzano, S.-Y. L. 2018. Mycoviruses identified in arbuscular mycorrhizal fungi, Rhizophagus spp. Viruses, 10, 707.

Exploring a new microbial pathway for nitrate control using cover crops and bioreactors at the Southeast Research Station

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Summary: High N fertilizer application to support corn production can result in nitrate leaching into waters which causes eutrophication. Some commercial corn/soybean producers use cover crops to increase nutrient retention, although there is some concern that nutrient leaching, especially phosphorus, may occur during cover crop decomposition. An unexplored biological pathway for nitrate retention in agricultural soils is dissimilatory nitrate reduction to ammonium (DNRA), a process recently found to be present in agricultural soils with varying degrees. DNRA can also be coupled with anaerobic ammonium oxidation (anammox) to remove nitrogen from tile drainage water. Managing agricultural soils to optimize cover crop benefit and the DNRA-anammox process that competes with nitrification are promising approaches for the control of nutrient leaching. By retaining soil N, available nitrogen to crops will be increased while mitigating the environmental impacts from leached N. The field plots are in a corn and soybean rotation with or without rye cover crops. Soil and plant samples were collected for analysis before and after decomposition of rye in the field, and by varying the timing of suppression in the greenhouse. We hypothesize that microbes capable of biological nitrification inhibition (BNI) are enriched in corn rhizospheric soil. Soil samples associated with corn roots are being characterized for the abundance for N cycling genes. Soil health indicators including soil protein, permanganate oxidizable carbon, enzyme activities, and carbon mineralization activity are being measured. In the two years since the rye cover crop treatments were applied, soil health measures have progressively improved in response to cover crop treatments. Multivariate statistical analyses will determine drivers of nutrient leaching and BNI effect. The ***goal*** of the project is to improve understanding about the importance of cover crop decomposition and microbial processes in nutrient leaching and retention in SD agricultural soil. Data collected in this reporting period are summarized below:

Objective 1. Determine the BNI effect of rye on soil health.

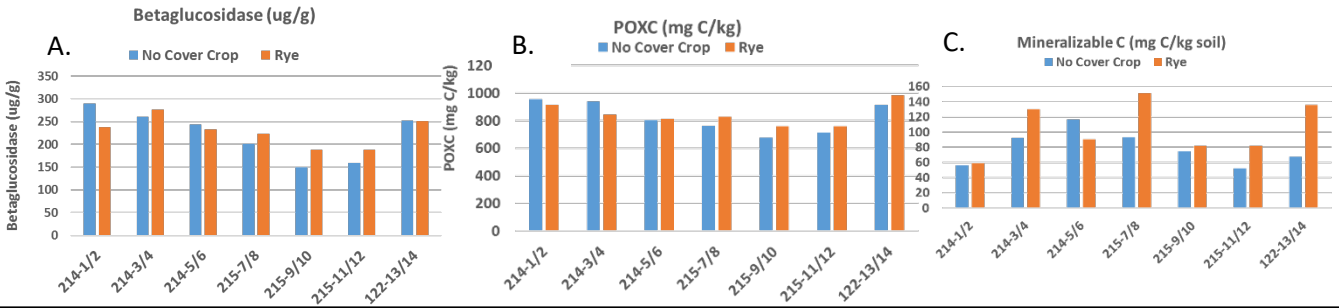

Figure 1. Soil heath indicator measurement. (A) Soil β-glucosidase enzyme activity (P=0.8) (B) Soil oxidizable carbon (P=0.72) (C) Soil mineralizable carbon (P=0.13) were measured from May 2018 samples.

In 2018, even though there were increases in rye-treated plots for the levels of soil protein, soil oxidizable carbon, and mineralizable carbon compared to the control without the cover crop treatment, the differences were not significant statistically (P>0.10) (Fig. 1). However, after two years of rye cover crop, the protein level accumulatively increased significantly, which measures the bioavailable N, general microbial activity, and mineralizable carbon (P<0.05) (Fig. 2). We took a third year of soil samples on May 19, 2020. New soil samples are being dried for the measurements of soil health indicator.

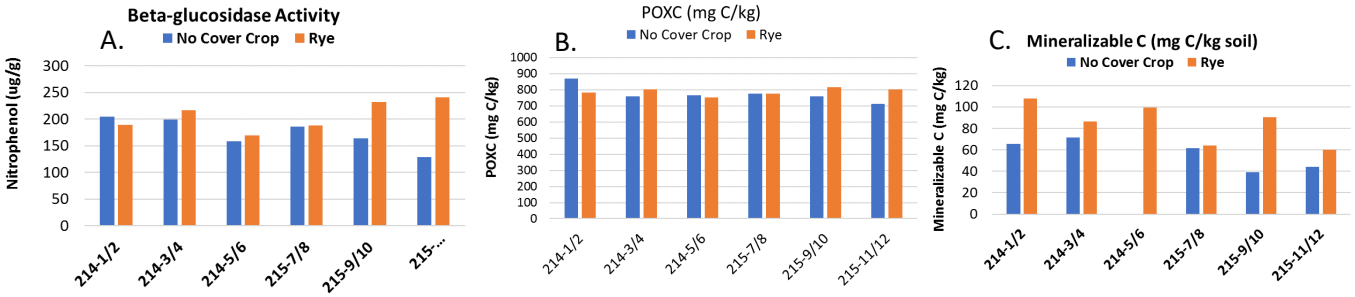

Figure 2. Soil heath indicator measurement. (A) Soil β-glucosidase enzyme activity (P=0.8) (B) Soil oxidizable carbon (P=0.72) (C) Soil mineralizable carbon (P=0.13) were measured from May 2019 samples.

Objective 2. Determine the BNI effect of rye on N-cycling genes

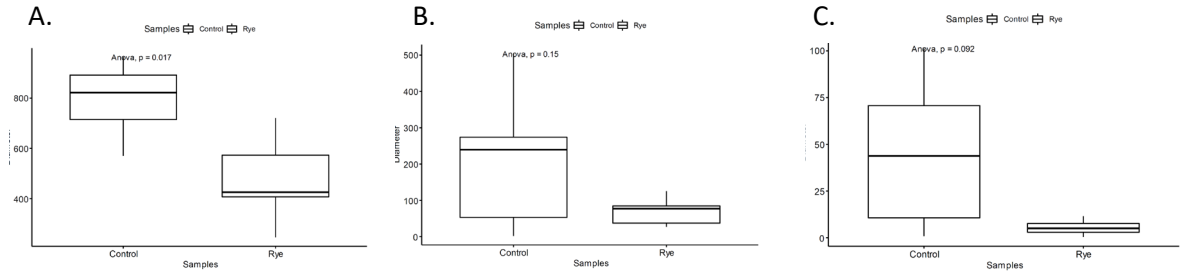

Figure 3. Rye reduces (A) hao enzyme produced by proteobacterial AOB-ammonia oxidizing bacteria responsible for nitrification; (B) *Nitrospira* population (NxrBF) responsible for nitrite oxidation in the second step of nitrification; (C) archaeal amoA (ammonia monooxygenase). Samples were taken in May 2019.

We quantified the amount of N-cycling genes from May 2019 samples, among which 3 genes showed a significantly increase in the rye treated plots (Figure 3). A fraction of the new soil samples has been frozen, and are being extracted for soil DNA to measure the amount of N-cycling genes using the NiCE chip.

Accomplished work:

In 2019, rye treated plots showed a **reduction in nitrification** corresponding to archaeal population. We will measure the amount of N-cycling genes with the 2020 samples. The work has demonstrated that rye cover crop plays a role of biological nitrification inhibition (BNI). Including cover crop with BNI effects will help shift the existing production system towards a low-nitrifying production system. We have taken new samples in May 2020 to compare the accumulative effect of rye cover crop on soil health and N-cycling genes. Soil DNA has been extracted and sent to UM for NiCE chip analysis. A research manuscript will be prepared after we receive the data.