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# Progress Report

## 2022 South Dakota Nutrient Research and Education Council Invited Proposals

<b>Progress Report Title:</b>	Interim Report - Due July 1, 2022
<b>Applicant Name:</b>	David Clay
<b>Application Title:</b>	Building a South Dakota Corn No-tillage N Recommendation Algorithm that Considers Improvements in Soil Health
<b>Application ID:</b>	1826
<b>Review Deadline:</b>	07/1/2022 11:59 PM

## Interim Report - Due July 1, 2022

### Project

	Start Date	End Date
<b>Start and End Dates of Funding:</b>	01/1/2022	12/31/2022
<b>Title of Project:</b>	The influence of nitrogen stabilizers and application dates in no-tillage corn production on nitrogen use efficiency and N losses to the atmosphere and groundwater	
<b>Project Description:</b>	<p>Dakota commercial agriculture systems. Such as determining the effect of hydrolysis inhibitor treated urea, and polymer-coated urea (ESN) on gaseous N emissions (NH<sub>3</sub> and N<sub>2</sub>O), NO<sub>3</sub>-N leaching, and the amount of N needed to attain the economic optimum N rate for corn. Based on the research we will conduct a cost benefit analysis.</p> <p>This project was initiated in the spring of 2021. During 2021, soil and plant tissue samples were collected, the greenhouse gas (GHG) sampling and measurement system was installed and information was collected. Harvest occurred in November 2021 and yield was analyzed shortly after. All Fall sampling and application for the 2022 plots were also completed by mid December 2021. All grain samples and stover samples from the 2021 harvest have been dried, shelled, and ground to be analyzed by the Mass Spectrometer. Both these samples will be analyzed for N and C. Soil samples collected for the 2021 plots completed processing for soil moisture December 17th, 2021 and have been analyzed. Inorganic nitrogen extractions have been completed for the 2021 plot samples and have also been analyzed. PLFA to determine the microbial community structure from Fall 2021 samples has also completed analysis. At this point, pre-plant and sampling up to V6 has been completed for the 2022 plots. The GHG fall measurements using the LiCor chambers and Picarro instrument began in the 2022 plots on November 12th, 2021 and fall sampling was completed the same day spring measurements began. GHG measurement for spring treatments began in the 2022 plots May 16th, 2022. GHG measurement for spring application is ongoing and analysis has not yet started. Spring fertilizer treatment applications were also applied to 2022 plots May 16th, 2022 and the second application for the spring/summer split application treatments was applied June 17th, 2022. Nutrient sampling as well as PLFA and tissue sampling has also occurred for the V6 growth stage. Processing and analysis of samples is ongoing.</p>	

## **Publications**

**Publication Title:** none to date  
**Publication Date:** 06/2/2022  
**Status:** none to date  
**Publication Description:** will work on publications following the completion of the study

**Title:** The influence of nitrogen stabilizers and application dates in no-tillage corn production on nitrogen use efficiency and N losses to the atmosphere and groundwater.

**Prepared by** Skye Brugler and David Clay

**Date:** June 29th, 2022

**Research Goal:** The purpose of this project is to answer questions not yet addressed in South Dakota commercial agriculture systems. Such as determining the effect of hydrolysis inhibitor treated urea, and polymer-coated urea (ESN) on gaseous N emissions ( $\text{NH}_3$  and  $\text{N}_2\text{O}$ ),  $\text{NO}_3\text{-N}$  leaching, and the amount of N needed to attain the economic optimum N rate for corn. Based on the research we will conduct a cost benefit analysis.

**Summary:** This project was initiated in the spring of 2021. During 2021, soil and plant tissue samples were collected, the greenhouse gas (GHG) sampling and measurement system was installed and information was collected. Harvest occurred in November 2021 and yield was analyzed shortly after. All Fall sampling and application for the 2022 plots were also completed by mid December 2021. All grain samples and stover samples from the 2021 harvest have been dried, shelled, and ground to be analyzed by the Mass Spectrometer. Both these samples will be analyzed for N and C. Soil samples collected for the 2021 plots completed processing for soil moisture December 17th, 2021 and have been analyzed. Inorganic nitrogen extractions have been completed for the 2021 plot samples and have also been analyzed. PLFA to determine the microbial community structure from Fall 2021 samples has also completed analysis. At this point, pre-plant and sampling up to V6 has been completed for the 2022 plots. The GHG fall measurements using the LiCor chambers and Picarro instrument began in the 2022 plots on November 12th, 2021 and fall sampling was completed the same day spring measurements began. GHG measurement for spring treatments began in the 2022 plots May 16th, 2022. GHG measurement for spring application is ongoing and analysis has not yet started. Spring fertilizer treatment applications were also applied to 2022 plots May 16th, 2022 and the second application for the spring/summer split application treatments was applied June 17th, 2022. Nutrient sampling as well as PLFA and tissue sampling has also occurred for the V6 growth stage. Processing and analysis of samples is ongoing.

**Justification:** One of the outcomes of this project is to identify management practices that optimize productivity while minimizing the environmental effects of agriculture. This outcome will be achieved by exploring the effects of management on N losses and utilization by corn. Fertilizer derived N can be lost to the environment through many different mechanisms that include leaching, denitrification, or nitrification (Figure 1). The amount lost through each mechanism is affected by interactions among the fertilizer material, the soil processes, the climate, and management. For example, research that we conducted in 2018 and 2019 showed that the urea-N losses to the atmosphere were much lower when applied after the soil had cooled to < 50 F. In a second example, research conducted by David Clay in the 1980's showed that the

treatment of urea with nitrification and hydrolysis inhibitors reduced ammonia volatilization from over 20% of the applied N to less than 1%. In summary, these studies had multiple combined findings. First, a one-size fits all product is not available. Second, the use of nitrogen stabilizers works in some environments but not others and that minimizing losses through one mechanism can increase losses through another. Third, reducing losses leads to improved efficiency and lower N rates and carbon footprints. This project will provide guidance on how to select fertilizer products that fit different problems.

**Work Plan:** This replicated field and laboratory study tests different fertilizer products for their ability to improve nitrogen use efficiency and reduce N losses to the atmosphere and groundwater. This field research was conducted at the SD experimental farm located at Aurora SD in 2021. This site was selected because our previous work showed that it is uniquely suited to conduct detailed experiments with a minimal variation and that we can detect relatively small changes in economic optimum N rates. The treatments were 5 N rates (0, 60, 100, 140, and 180 lbs N/a), 3 application dates (Fall temperature < 50F, preplant, and between V4 and V6), 3 urea products (untreated, poly coated, and hydrolysis inhibitor treated) and 2 yield potentials. Each treatment was replicated 4 times. Hyperspectral remote sensing data was collected from the site to identify N and water sensitive bands and create remote sensing algorithms for characterizing water and N stress in corn on May 30th, 2021 (following emergence), June 15th 2021, (V4), July 15th, 2021 (V8), and August 24th, 2021 (prior to tasseling). No-tillage was used at the site and DKC 97-97 was seeded at 32000 plants/a.

The low yield potential plot was not irrigated and the high yield potential plot was irrigated. Each plot was approximately 30 ft long and 15 ft wide with 6 rows of corn. Each plot has one of four different treatments: 100% untreated urea, 100% treated urea with NBPT (also known as Factor), 75% ESN combined with 25% urea, and 100% ESN. The treatments have four N rates (0, 60, 100 and 140 lbs) and 3 application dates: fall, spring, and split application in spring/summer. Each treatment will be replicated 4 times in experiments conducted in 2021, 2022, and 2023. This project uses no-tillage and the findings will be used to create Nitrogen fertilizer fact sheets.

## **Progress**

### **Field study 2021**

The field was planted on May 12th, 2021 and harvested between October 15th and 19th, 2021. Grain, stover, and grain moisture percentages were determined. Corn ears were shelled December 15th, 2021, and grounded December 21st, 2021. These grounded samples will be analyzed by the Mass Spectrometer. Preseason and postseason soil samples, including Bulk Density, have been collected from the 0- to 6-, 6- to 12- and 12-to 24- inch soil depths on June 16th, July 19th, and October 30th, 2021. Preseason samples were sent to Ward labs in Nebraska to measure and obtain background information. Extractions to analyze inorganic N from the preseason and postseason soil samples began January 4th, 2021 and have since been completed. Soil moisture measurement information was completed December 17th, 2021. PLFA samples were collected October 31st, 2021 at a depth of 0- to 6- inches. Following collection, the samples

were immediately placed into a cooler with dry ice and once sampling was complete they were placed into a -80°C freezer. PLFA analysis was initiated December 10th, 2021 and PLFA results for July 19th, 2021 are currently being analyzed with R statistics software. Plant tissue samples were collected July 7, 2021 to assess gene expression. Following collection, the plant tissue samples were immediately placed into a cooler with dry ice and were then placed into a -80°C freezer for storage. The RNA extraction for 2021 corn tissue samples are still in progress. The Licor and Picarro GHG measurement and analysis system was placed into the field on May 16 and measured CH<sub>4</sub>-C, CO<sub>2</sub>-C, NH<sub>3</sub>-N, and N<sub>2</sub>O-N emissions from fertilized and unfertilized plots until harvest. Following harvest, they were moved to the new site for the 2021-2022 treatment year. The fall fertilization application was completed for the 2022 plots on November 15th, 2021 for the irrigated plots and November 16th, 2021 for the dryland plots. Nutrient sampling for residual nitrogen occurred April 26th, 2022. PLFA for each plot was also taken April 11th, 2022 to determine the microbial community change from harvest to spring. These samples are currently being processed and analyzed.

### **Field study 2022**

Pre-plant nutrient sampling for 2022 plots were taken between May 4th and May 10th, 2022 at three depths 0-to 6" 6-to 12" and 12- to 24". These samples are currently being processed for soil moisture, and inorganic nitrogen concentration. Pre-plant PLFA was also taken for the plots with fall treatments on May 7th, 2022 these samples are still being analyzed and processed. Planting occurred May 11th, 2022 with a population of 32000 and 30 inch rows. Treatments for the spring application were applied May 16th, 2022. This includes the first application of the split treatments, 70N was applied. The GHG measurement for spring also began on May 16th, 2022 and is still being continuously collected. Nutrient soil sampling at three depths 0-to 6" 6-to 12" and 12- to 24" we taken at V6 from the split treatments on June 22, 2022. This occurred before the second application of 70N later on the same day June 22, 2022. PLFA and RNA tissue samples were collected from treatments that correspond to the GHG treatments on June 23rd, 2022. All samples are still being processed and analyzed. Currently the corn is at V6.

### **Greenhouse Gas Emissions Results**

N<sub>2</sub>O-N, NH<sub>3</sub>-N, and CO<sub>2</sub>-C, CH<sub>4</sub>-C concentrations using 4.01 LI-COR SoilFluxPro™ software (v. 4.01; LICOR, Lincoln, NE) have been analyzed using 4.01 LI-COR SoilFluxPro™ software (v. 4.01; LICOR, Lincoln, NE). Soil Moisture and Soil temperatures for each chamber have also been analyzed.

ESN has a polymer coating that degrades and releases the urea when moisture is available. The post hoc Tukey test results from R software shows that Spring 140N ESN treatment did produce reduced N<sub>2</sub>O emissions. However, the experiment took place in drought conditions and so, this possibly impeded the ability of the Spring 140N ESN fertilizer treatment to reduce emissions more significantly.

Spring 140N urea treated with NBPT is an urease inhibitor. Urease is a ubiquitous enzyme in the soil that degrades urea and releases NH<sub>3</sub>. The urease inhibitor prolongs the process of the urea breakdown. This therefore will show a decrease in the emission of NH<sub>3</sub>. A post hoc Tukey test from R software indicated that Spring 140N treated urea with NBPT had a decrease of NH<sub>3</sub> emissions. Compared to the untreated Spring 140N urea.

The analysis of the GHG measurements for fall and spring in the 2022 plots, are currently ongoing.

### **Yield Results**

#### **Least Significant Difference in Yield (kg/ha) Across Treatments**

N rates (lbs/ac)	Dryland Plots Yield (kg/ha)	Irrigated Plots Yield (kg/ha)
140N Untreated Urea Spring app	10422.85 a	13557.97 a
NBPT Treated Urea 140N spring/summer split app	10128.66 ab	13803.09 a
Untreated Urea 180N Spring app	10079.44 ab	13626.66 a
ESN 140N Spring app	9607.09 abc	13646.82 a
Untreated Urea 140N spring/summer split app	9601.69 abc	11683.09 b
Urea Treated with NBPT 140N Spring app	9354.63 bc	12743.47 ab
Untreated Urea 60N Spring app	9307.97 bc	12161.63 b
Untreated Urea 100N Spring app	9221.68 bc	12659.37 ab
75% ESN combined with 25% Urea 140N spring/summer split app	9182.08 c	11866.95 b
ESN spring/summer 140N split app	9169.090 c	11854.33 b
75% ESN combined with 25% Urea 140N Spring app	8920.92 c	11654.02 b

Untreated Urea 0N Spring app (Control)	7852.19 d	12006.58 b
p-value	0.0001221	0.006609

The non-irrigated plots produced less yield overall, due to drought conditions, than the irrigated plots. In the non-irrigated plots, untreated urea 140N Spring application and NBPT 140N treated urea as a spring/summer split application produced the highest yields. In the irrigated plots, these two treatments as well as ESN 140N Spring application, produced the highest yields. Likely, the high temperature low moisture environment of the non-irrigated plots prevented ESN from fully releasing the nitrogen solution through its polymer coating and releasing the nitrogen solution to the crops. Overall, hydrolysis inhibitor treated urea (NBPT) and polymer coated ESN maintained yield when used in corn production compared to the commonly used urea, making their use economical as well as environmentally sustainable.

### **Inorganic N Extraction Results May**

#### **Nitrate ( µg/g) Across Depths in May**

Depth	Dryland NO <sub>3</sub> May µg/g	Irrigated NO <sub>3</sub> May µg/g
0-6"	17.8 a	19.8 a
6-12"	12.3 ab	8.0 b
12-24"	6.4 b	6.3 b
p-value	0.018	0.004

There is a significant difference between nitrate concentration in depths between all blocks in May. This demonstrates the nitrate is being held in place on the surface of the soil pre-plant.

### **Inorganic N Extraction Results June**

#### **Nitrate ( µg/g) Across Split Application Treatments in June**

N rate (lbs/ac)	Dryland NO <sub>3</sub> June µg/g	Irrigated NO <sub>3</sub> June µg/g
NBPT Treated Urea 140N spring/summer split app	34.9 a	41.3 a
Untreated Urea 140N spring/summer split app	33.3 a	35.3 ab

75% ESN combined with 25% Urea 140N spring/summer split app	25.0 ab	29.5 bc
ESN spring/summer 140N split app	20.3 b	24.9 c
p-value	0.05	0.005

In June there is a significant difference in nitrate concentration between treatments. Split 140N NBPT has the greatest amount of nitrate in the soil for both dryland and irrigated. This may be because urea is not being broken down by urease since it is inhibited. However, split 140N ESN has the least amount of nitrate in the soil. This may be because it wasn't collecting enough moisture to release the urea and so there was no new nitrogen.

#### **Ammonium ( $\mu\text{g/g}$ ) Across Split Application Treatments in June**

N rate (lbs/ac)	Dryland $\text{NH}_4$ June $\mu\text{g/g}$	Irrigated $\text{NH}_4$ June $\mu\text{g/g}$
Untreated Urea 140N spring/summer split app	38.0 a	19.8 b
NBPT Treated Urea 140N spring/summer split app	37.5 a	19.0 b
75% ESN combined with 25% Urea 140N spring/summer split app	23.2 b	24.0 a
ESN spring/summer 140N split app	22.1 b	17.7 b
p-value	0.015	0.034

In June there is a significant difference in ammonium concentration between treatments. In the dryland the untreated 140N urea appears to have the most ammonium. However, in the irrigated field the split 75% ESN combined with 25% Urea 140N has the greatest amount of ammonium in the soil. This may be due to the differences in moisture in the dryland and irrigated fields in June.

#### **Inorganic N Extraction Results October**

#### **Nitrate ( $\mu\text{g/g}$ ) Across Spring and Summer Treatments in October**

N rate (lbs/ac)	Dryland $\text{NO}_3$ October $\mu\text{g/g}$	Irrigated $\text{NO}_3$ October $\mu\text{g/g}$
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140N Untreated Urea Spring app	12.7 a	5.5 abc
75% ESN combined with 25% Urea 140N Spring app	11.3 ab	5.8 ab
ESN spring/summer 140N split app	7.8 abc	5.2 abc
NBPT Treated Urea 140N spring/summer split app	7.4 bc	4.2 abcd
ESN 140N Spring app	6.3 bcd	6.7 a
Untreated Urea 100N Spring app	5.4 cd	4.2 abcd
Untreated Urea 140N spring/summer split app	5.1 cd	5.0 abc
10 Urea Treated with NBPT 140N Spring app	5.1 cd	4.6 abcd
15 75% ESN combined with 25% Urea 140N spring/summer split app	4.9 cd	6.3 ab
6 Untreated Urea 60N Spring app	4.6 cd	2.6 cd
9 Untreated Urea 180N Spring app	4.5 cd	3.3 bcd
5 Untreated Urea 0N Spring app (Control)	1.0 d	1.7 d
p-value	0.003	0.05

In October there is a significant difference in nitrate between treatments in both the dryland and irrigated fields. In the dryland field spring 140N untreated urea has the highest amount of nitrate at the end of the year after harvest while the control has the least. This may be because urea was broken down quickly in the soil but due to a lack of precipitation in the dryland field, the urea was unable to be leached out. However, in the irrigated field ESN spring 140N had the most nitrate while the control also had the least. This may indicate that ESN was released much later than the other fertilizers but remains in the soil because the urea turned nitrate did not have a long enough time period in the soil to be lost by leaching, denitrification or volatilization.

### Ammonium ( $\mu\text{g/g}$ ) Across Spring and Summer Treatments in October

N rate (lbs/ac)	Dryland $\text{NH}_4$ October $\mu\text{g/g}$	Irrigated $\text{NH}_4$ October $\mu\text{g/g}$
Untreated Urea 100N Spring app	9.2 a	6.8 bcd
Untreated Urea 0N Spring app (Control)	9.0 ab	7.0 abcd
Untreated Urea 180N Spring app	8.7 ab	5.1 cd
75% ESN combined with 25% Urea 140N spring/summer split app	8.4 abc	8.2 ab
NBPT Treated Urea 140N spring/summer split app	8.3 abc	5.0 d
140N Untreated Urea Spring app	8.3 abc	6.9 bcd
Urea Treated with NBPT 140N Spring app	8.2 abc	7.7 abc
Untreated Urea 60N Spring app	7.9 bc	7.4 abcd
ESN spring/summer 140N split app	7.9 bc	6.7 bcd
ESN 140N Spring app	7.4 c	9.6 a
75% ESN combined with 25% Urea 140N Spring app	7.4 c	7.4 abcd
Untreated Urea 140N spring/summer split app	7.3 c	6.8 bcd
p-value	0.047	0.07

In October there is a significant difference in ammonium between treatments in both the dryland and irrigated fields. In the dryland field spring 100N urea appears to have the most amount of ammonium, this is also statistically significant to the control. This therefore may indicate that there is very little change in ammonium at the end of the season due to treatment overall. The least amount of ammonium in the dryland field includes spring 140N ESN, 75% ESN combined with 25% Urea 140N Spring app, and split 140N urea. This may be because the ammonium has

been taken up by the crops or that mineralization has occurred but not nitrification. In the irrigated field, spring 140N ESN had the highest amount of ammonium. Possibly because the urea solution was released much later and it is still there because ammonium is more stable in the soil profile. Split 140N NBPT appears to have the least amount of ammonium. Possibly because the release of urea was quickly taken up by the crops.

### **Activities for 2022**

1. Complete the soil and plant sample analysis for 2022
2. Analysis of the hyperspectral data
3. Prepare a spreadsheet containing all data
4. RNA Tissue extraction at V8 growth stage
5. July 14, 2022 nutrient soil sampling, PLFA sampling of all 2022 plots

### **Publications, presentations, and awards**

None to date

### **Training**

Graduate Student Skye Brugler Attended ASA Workshop: Crop Genomics, Bioinformatics, and Variant Calling

*Sponsored by ASA Section: Biometry and Statistical Computing and Statistical Education/Training for Researchers Community*