NITROGEN FERTILIZER TIMING, RATE, AND SOURCE EFFECTS ON CORN AND WHEAT PRODUCTION IN THE RED RIVER VALLEY

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MASTER OF SCIENCE

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ABSTRACT

Experiments were conducted in 2015-2016 to determine if yield and protein concentration in corn (*Zea mays* L.) and hard red spring wheat (HRSW) (*Triticum aestivum* L.) could be enhanced through different nitrogen (N) fertilizer application timings, rates, and sources when compared to urea applied pre-plant and to quantify the dissolution rate of a controlled release fertilizer (CRF). The CRF effectively released N slowly over time. Placement depth did not impact release rate. Soil NH_4^+-N and NO_3^--N levels differed in diverse environments when different fertilizer sources were applied. Corn grain yield was not impacted by N application treatments in 2016, but the 100% N rate increased yield over other treatments in 2015. Nitrogen applied in October or November at the 100% rate increased HRSW yield over other treatments in 2016. Total grain protein and percent protein in the grain were not improved with N treatment over the check in 2015.

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DEDICATION

This thesis is dedicated to my best friend Cody and our sassy dog Cinch. Cody, the never-ending support, patience, and adoration I received from you motivated me to strive for my personal best every day.

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INTRODUCTION

More than 1.1 million ha of corn (*Zea mays* L.) and 2.4 million ha of hard red spring wheat (*Triticum aestivum* L. emend. Thell.) (HRSW) were planted in North Dakota in 2016 (North Dakota Agricultural Statistics Service, 2016). The recent decline in corn and wheat prices in the Red River Valley (RRV) led farmers to search for ways to increase input efficiencies to maximize grain yield and wheat protein content without the addition of unnecessary costs. One way producers in the RRV can improve returns is to increase the efficiency of nitrogen (N) fertilizers by minimizing environmental N losses. This can be achieved through the use of best management practices such as choosing the right fertilizer source, improved application timing, and improved decisions regarding application rate. Proper N management has the potential to decrease environmental losses of N to denitrification, volatilization, leaching to groundwater, and run off to surface waters, and thereby improving the efficiency of applied N fertilizers. The need for producers to minimize input costs to increase grain yield and protein in wheat supports the need of further research on N fertilizer sources in the RRV.

LITERATURE REVIEW

Protein Importance

Nitrogen is an essential element for plant development due to it being a key constituent of nucleic acids, amino acids, and chlorophyll. Plants take up N in the forms of ammonium (NH_4^+) or nitrate (NO_3^-) (Meisinger et al., 2008). However, N transformations generated by biological, chemical, and physical processes in the soil can reduce its availability to plants. Knowledge of these processes allows for the design and implementation of N management programs that can increase N use efficiency, and lead to higher yields and grain protein content per unit of applied N.

Grain protein is an important factor in many crops for a variety of reasons. In the United States, HRSW growers experience price discounts at the elevator when grain protein content is below, and protein premiums when grain protein is above the market standard of 14%, particularly in years when US wheat protein levels are low. Premiums can result in up to 50% increase in the HRSW crop's market value while protein discount percentages commonly exceed the amount paid for a premium protein difference (Brown et al., 2005). As an example, Montana wheat producers lost \$10-\$24 ha^{-1} due to low protein that many experienced in the 2010-2011 marketing year (Jones and Olson-Rutz, 2012). The current recommendation in Montana to achieve 14% protein is to apply 1.5 kg of available N for every 25.4 kg^{-1} of expected yield. Based on a 2010 survey, growers only applied 1.2 kg of N for every 25.4 kg^{-1} of yield achieved. This under fertilization cost those growers \$10-24 ha^{-1} (Jones and Olson-Rutz, 2012). Part of the reason for this disparity is that if farmers use a yield-goal based formula to determine N rate, their choice of a lower-than-actually achieved yield will most often result in lower grain protein.

Higher grain protein content can be influenced by variety selection, water management, fertilizer application timing, and fertilizer source. However, the most important factor impacting grain protein is sufficient N availability to the crop during key periods of crop development (Brown et al., 2005). A deficiency in N availability to wheat causes a dilution effect of protein in the grain mass as yield increases. Grain protein formation utilizes N taken up before heading or flowering, then transported to the kernel during grain fill (Jones and Olson-Rutz, 2012). Through N fertilization, yield and protein can increase until maximum levels are limited by varietal genetics and environment. Maximum yield can be achieved with less N than maximum protein. The current N recommendations for most crops in North Dakota, including HRSW and corn, is based on the amount of nitrate (NO_3^-) found in the top 60 cm of the soil in the fall, the yield potential of the area, historical productivity, previous crop N credits, crop price, and fertilizer cost (Franzen, 2014). Hard red spring wheat utilizes a small portion of the total seasonal N requirements to establish a vegetative canopy during the first two to four weeks of growth. The majority of the N used by plants is taken up in the next 30 days (Franzen, 2017). Therefore, ensuring that adequate nutrient resources are available throughout the growing season is imperative.

Nitrogen Cycle and Losses

Ammonium is converted to NO_3^- by bacteria present in the soil using a two-step process called nitrification. The first step occurs when the autotrophic bacteria, *Nitrosomonas,* oxidizes ammonia into plant toxic nitrite (NO_2^-) (Nelson and Huber, 2001). The second step requires further oxidation by the *Nitrobacter* bacteria to result in an unbound, negatively charged nitrate ion. Temperatures below 10 °C and water filled pore space greater than 60% are examples of environmental conditions that can retard the nitrification conversion rate; however, N losses due to leaching, volatilization, and denitrification can still occur (Mulvaney, 2008.

Nitrate leaching occurs when the nitrate molecule moves with the water as it percolates through the soil. High rainfall intensity and distribution, highly irrigated fields, and coarse textured soils are conditions that favor nitrate leaching. A study at Iowa State University found that with 2.5 cm of rainfall, nitrate can move downward 6.4 cm in a coarse textured soil or a field with tile drainage, whereas in a clay loam soil, the movement downward was 2.54 cm (Nelson and Huber, 2001). Leaching distance with different rainfall totals vary greatly due to preferential flow at small horizontal spatial scales. Preferential flow always occurs in soils, but is also influenced greatly by presence of soil cracking, root channels, insect and other animal burrows and general discontinuities in the soil matrix.

Ammonium volatilization can occur when N is applied at or near the soil surface in the form of urea (Rochette et al., 2013). Urea applied to the soil reacts quickly with water (if present) and urease enzymes to produce NH_4^+ through hydrolysis; N released as ammonia gas to the atmosphere is lost permanently for crop uptake. Urease enzymes are most active when the soil pH is between 6 and 6.5. However, NH_4^+ is lost most readily from the soil surface when the soil pH is greater than 7 (Kissel et al., 2008). Alkaline soils surrounding the granule or droplet increases the potential for NH_3 volatilization to occur, since more free NH_4^+ is partitioned in soil water at higher pH and the partial pressure of dissolved ammonia drives the reaction towards release from solution; however, losses can still occur in acidic soils (Rochette et al., 2013). Urea undergoing hydrolysis initially increases the soil pH near the urea pellet to greater than pH 8. Unstable NH_4^+ carbonate is formed, which then dissociates quickly to release NH_3 gas (Kissel et al., 2008). Soils with high clay content and/or high organic matter tend to have less risk of NH_3

loss due to higher pH buffering capacities and greater cation exchange capacities. Greater volatilization losses from surface applications occur when soils are moist and warm, thereby increasing the rate at which fertilizers are dissolved through heightened biological and chemical reactions (Kissel et al., 2008).

Denitrification is the conversion of NO_3^- to gaseous N that can be lost into the atmosphere. Denitrifying bacteria, generally located in the topsoil, can utilize organic matter and the oxygen in NO_3^- to complete metabolic processes when oxygen supply is restricted in waterlogged, anaerobic soils (Kissel et al., 2008). Denitrification increases when soil temperatures are above 15.6˚C, the soil pH is above 7, and water filled pore space is above 60% for more than 15 minutes (Coyne, 2008). Although N losses cannot be eliminated, the use of N fertilizer additives or controlled-release N fertilizers and proper application timing have the potential to retard N losses caused by leaching and denitrification; therefore, more N may be available to the crop at critical growth periods.

Nitrification Inhibitor

Products have been developed to increase N efficiency by delaying the nitrification process (Franzen, 2017). A nitrification inhibitor (NI) prevents or hinders activities of the soil bacteria, *Nitrosomonas spp.*, by slowing the transformation of NH_4^+ to nitrate for four to ten weeks depending on soil pH and moisture (Trenkel, 1997).

The use of a NI in the fall can potentially reduce nitrification until the process stops when temperatures drop below 4˚C, and may prevent NO_3^- formation when plant uptake is low and precipitation is high in the early spring (Nelson and Huber, 2001). When a NI is utilized in growing seasons that receive above average rainfall, increased grain protein content has been recorded in wheat when compared to treatments without an inhibitor. This is due to increased soil available inorganic N and plants remaining green longer during a growing season, thereby decreasing lodging and pathogen effects. (Nelson and Huber, 2001).

Nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) (NP) is the most common commercially used NI. The formulation, N-Serve 24, is labeled for use with anhydrous ammonia that is injected into the soil and had to be incorporated immediately (Wilson et al., 2013). Recently, however, a new formulation of nitrapyrin was developed using a microencapsulation process that allows it to be applied to granular urea (Franzen, 2017) and remain on the soil surface for up to ten days. This microencapsulated NP is sold under the trade name Instinct (ENP) (Dow Agrosciences, Indianapolis, IN), or more recently Instinct II. The product patent states the efficacy of the active ingredient in ENP surpasses unencapsulated NP, thereby decreasing the required application rate. Furthermore, it can be incorporated with rainfall instead of mechanically (Wilson et al., 2013). However, a study by Goos refuted these findings. In all four soil types tested, unencapsulated NP inhibited nitrification more effectively than ENP at equivalent rates (Goos, personal communication, 2016).

Further studies conducted at the University of Nebraska summarized by Franzen (2017), showed no yield benefits with the use of nitrapyrin (GF-2017, Instinct) in 2008 or 2009. However, a laboratory study by Goos (2011) indicated nitrapyrin and the NI dicyandiamide (DCD) inhibited the nitrification process. A review on DCD was conducted in Midwest states and was published by Malzer et al. (1989). Inconsistent results in yield were seen when DCD was compared to fertilizer alone, but DCD showed similar nitrification inhibition to that of NP (Malzer et al., 1989).

The DCD produces a bacteriostatic effect on *Nitrosomonas bacteria*. The bacteria are not killed, but their ability to convert NH_4^+ to NO_3^- is inhibited or reduced allowing for stable NH_4^+-N to be present for six to eight weeks (Trenkel, 1997). The NI active ingredient in Super-U (SU) (IMC Phosphate Co., licensed exclusively to Koch Agronomic Services, Wichita, KS, USA) is DCD.

Urease Inhibitors

Fertilizers containing amide-N, such as urea and urea-ammonium nitrate (UAN), can be rapidly transformed into unstable NH_4^+ carbamate when urease enzymes are active in the soil (Trenkel, 1997). Utilization of a urease inhibitor at a proper rate binds with the active site of the urease enzyme, and essentially halts enzyme activity until soil microorganisms decompose the inhibitor to the point where it falls away from the enzyme and activity resumes. The time of inhibition is usually about 10 days plus or minus 3 days depending on soil temperature and moisture (Franzen, 2017).

Super-U not only contains a NI, but the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) as well. This compound has shown consistent reductions in urea volatilization in controlled independent experiments. The NBPT locks onto urease enzyme binding sites, which prevents the urease from interacting with the enzyme (Franzen, 2017).

Four separate greenhouse studies conducted by Goos (2013) found increased NH_4^+ loss 15 days after fertilizer application when NBPT applied with UAN and UAN alone were compared. The studies were conducted on bare soil with small droplet size, bare soil with large droplet size, wheat residue ground and mixed with soil with small fertilizer droplet size, wheat residue ground and mixed with soil with large fertilizer droplet size. However, a study by Dell et al. (2014) found Super-U, as well as Environmentally Smart Nitrogen (PCU) (Agrium Inc., Calgary, Canada), and UAN did not show any yield benefit in poorly drained field sites in 2009, 2010, and 2011 when compared to urea or the unfertilized check. In this experiment (Dell et al., 2014) higher yields were achieved with N fertilization in the 2012 growing season, but no improvement in yield was realized with N sources of PCU or SU.

Controlled-Release Nitrogen Fertilizer

In addition to nitrification and urease inhibitors, controlled-release N fertilizers (CRF) have the ability to reduce N losses and increase N utilization by presenting a physical barrier to urea release. Urea is slowly into the soil solution, according to product literature and some independent studies, at the same approximate rate that N is needed by the crop (Maharjan et al., 2016). Plant toxicity caused by elevated ionic concentrations of rapidly dissolved urea is reduced with CRF. Therefore, larger quantities of fertilizer can be applied in close proximity to seeds/plants in one application. This has the potential to decrease labor costs to growers, while reducing the potential for NO_3^--N losses and NH_4^+ volatilization (Trenkel, 1997).

Environmentally Smart Nitrogen is a polymer-coated, controlled-release urea fertilizer containing 44% N. Water must enter through the polymer coating to dissolve the urea and allow it to diffuse into the soil through cracks or after it degrades (Sullivan et al., 2015). The time it takes for coating degradation and urea dissolution depends on soil temperature and moisture (Trenkel, 1997). Due to imperfections in the polymer coating, 20-25% of the N is available at the time of application. Soil temperatures at 20°C will cause the remaining fertilizer to release in 50 to 70 days (Sullivan et al., 2015).

A study conducted by Sullivan et al. (2015) at Oregon State University found there was no yield benefit in corn when PCU was applied when compared to urea. However, work by Geng et al. (2016) refuted these findings. The 100% PCU rate resulted in a corn yield of 8355 kg ha^{-1} and a wheat yield of 8025 kg ha^{-1}. These yields were significantly higher than the 100% urea rate yields of 7620 kg ha^{-1} and 7170 kg ha^{-1} for corn and wheat, respectively (Geng et al., 2016). Effectiveness of NI, urease inhibitors, and CRF vary depending on the location of use and environmental conditions.

RESEARCH OBJECTIVES

The objectives of this research were to: 1) evaluate the dissolution rate of N fertilizers in a field setting based on incorporation depth, N source, and duration in the field; 2) evaluate the effectiveness of time of application, rate, and N source on supplying the N needs of corn and wheat relative to urea.

MATERIALS AND METHODS

Site Description

Dissolution Rate of N Fertilizers

The field experiment was conducted in 2016 at the North Dakota State University (NDSU) Seed Farm near Casselton, ND (Latitude = 47.00^{o} N, Longitude = -97.64^{o} W). Table 1 provides the soil series, soil taxonomy, and slope for this location.

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

Corn and wheat field experiments were conducted in 2015 and 2016 in North Dakota and Minnesota. The locations consisted of the NDSU NW22 Research Station, Fargo, ND, (Latitude = 46.93^{o} N, Longitude = -96.86^{o} W), a cooperator's field in Steele County, ND, (Latitude = 47.46^{o} N, Longitude = -97.64^{o} W), the NDSU Seed Farm near Casselton, ND, (Latitude = 46.88^{o} N, Longitude = -97.23^{o} W), and at a cooperator's field near Ada, MN, (Latitude = 47.34^{o} N, Longitude = -96.42^{o} W). Corn experiments were established in Steele County, ND and NW22 in 2015, followed by Steele County and Casselton, ND, in 2016. Wheat field experiments in 2015 consisted of Steele County and Casselton, ND. The 2016 experiments were located in Casselton, ND and Ada, MN. Table 1 lists the soil series, soil texture, soil taxonomy, and slope of each location. Soil samples were collected in the fall of 2014 and 2015 to determine the levels of NO_3^- in the soil at each location (Table 2). Five random core samples were collected throughout the experimental area and combined prior to analysis.

Table 1. Soil series, taxonomy, and slope of NW22, Casselton, Steele County, ND, and Ada, MN, in 2015 and 2016.

Location	Year	Soil Series†	Soil Texture†	Soil Taxonomy‡	Slope
					%
NW22	2015	Fargo	Silty Clay	Fine, smectitic, frigid Typic Epiaquerts	0-1
Casselton	2015-16	Kindred	Silty Clay Loam	Fine-silty, mixed, superactive, frigid Typic Endoaquolls	0-2
Steele County	2015-16	Heimdal	Loam	Coarse-loamy, mixed, superactive, frigid Calcic Hapludolls	0-40
Ada	2016	Ulen	Sandy Loam	Sandy, mixed, frigid Aeric Calciaquolls	0-3

† Soil data obtained from Web Soil Survey (USDA-NRCS, 2016).
‡ Soil taxonomy listed on individual lines based on hyphenated soil series name.

Table 2. Initial soil test nitrate-N levels, from the 0-60 cm depth, and preceding crop for corn and wheat sites in 2015 and 2016 in the fall preceding planting.

		2015		2016	
Crop	Location	NO_3^- Levels	Previous Crop	NO_3^- Levels	Previous Crop
		--kg ha^{-1}--		--kg ha^{-1}--	
Corn	NW22	51.6	Corn	-	-
	Steele County	31.4	Soybean	35.9	Dry Bean
	Casselton	-	-	40.4	Wheat
Wheat	Steele County	65.0	Wheat	-	-
	Casselton	38.1	Wheat	37.0	Wheat
	Ada	-	-	9.0	Soybean

Treatments and Experimental Design

Dissolution Rate of N Fertilizers

This experiment was conducted at a single location near Casselton in 2016. The experimental design was a randomized complete block (RCBD) with a 3x2x4 factorial arrangement and three replicates. The factors included were: fertilizer treatment bag application depth, (0, 7.6, and 15.2 cm), N sources (urea and PCU), and fertilizer treatment bag removal dates (26 April, 10 May, 24 May, and 7 June). A complete treatment list can be found in Table 3.

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

The corn experiments were conducted at two site locations in 2015 (NW22 and Steele County) and two site locations in 2016 (Casselton and Steele County). The wheat experiments were performed at two site locations in 2015 (Casselton and Steele County), and three site locations in 2016 (Casselton, Steele County, and Ada). The experimental design for all locations was a RCBD with four replications. The corn and wheat treatments were derived from a factorial combination of application dates (October and November), N rates (75 and 100% rate of location N requirements), N sources (urea, PCU, a 50:50 ratio of urea and PCU (Urea-PCU), SU, and urea impregnated with ENP (Urea+ENP)), plus the addition of an untreated check, and a 75 and 100% rate of urea and a 50:50 ratio of urea applied at the same time as other spring treatments followed by urea ammonium nitrate solutions, 28-0-0 (UAN) applied at the 4 leaf stage (Urea-UAN) for a total of 25 treatments in 2015. A 150% rate of N, applied as urea, to serve as an N rich plot, along with 75 and 100% rates of spring applied urea and PCU were added in 2016 for a total of 30 treatments. The treatment list for 2015 and 2016 can be found in tables 4 and 5, respectively.

In 2015, the corn trials had fertilizer treatments applied on 15 Oct. 2014, 10 Nov. 2014, and 18 Apr. 2015 at the NW22 location and 14 Oct. 2014, 7 Nov. 2014, and 27 Apr. 2015 in Steele County. The date of fertilizer application for 2015 and 2016 corn and wheat trials can be found in tables 6 and 7, respectively.

Table 3. Treatment list for dissolution rate of N fertilizer trial in a RCBD factorial arrangement applied in 2016.

Treatment	Depth†	Source‡	Date§
	--cm--		--2016--
1	0	Urea	26 Apr.
2	7.6	Urea	26 Apr.
3	15.2	Urea	26 Apr.
4	0	PCU¶	26 Apr.
5	7.6	PCU	26 Apr.
6	15.2	PCU	26 Apr.
7	0	Urea	10 May
8	7.6	Urea	10 May
9	15.2	Urea	10 May
10	0	PCU	10 May
11	7.6	PCU	10 May
12	15.2	PCU	10 May
13	0	Urea	24 May
14	7.6	Urea	24 May
15	15.2	Urea	24 May
16	0	PCU	24 May
17	7.6	PCU	24 May
18	15.2	PCU	24 May
19	0	Urea	7 June
20	7.6	Urea	7 June
21	15.2	Urea	7 June
22	0	PCU	7 June
23	7.6	PCU	7 June
24	15.2	PCU	7 June

† Depth of treatment burial
‡ Type of N fertilizer
§ Date of removal
¶ Environmentally Smart Nitrogen

General Procedures

Dissolution Rate of N Fertilizers

Forty fertilizer granules of the respective fertilizer source were counted then weighed using a GX-200 scale (A&D Company Ltd., Tokyo, Japan) for each experimental unit. The granules were then placed in 6 cm by 3 cm, polypropylene 2 mm mesh bags. The tops of the bags were folded over 1 cm then stapled closed. The bags were placed in the field 30.5 cm apart on 13 Apr. 2016. The surface application treatments were placed on top of the tilled soil surface. A 40.5 cm high by 15 cm wide spade shovel was used to create a furrow in the soil to reach the 7.6 and 15.2 cm depths. The bags were placed at the appropriate depth in the soil and were then covered.

The bags were removed on the designated date, then placed in a dryer for approximately 18 h at 34 °C to remove excess moisture. The bags were removed from the dryer and the remaining fertilizer granules were removed from the bag and weighed. Weather data was monitored and recorded from the NDAWN weather station in Prosper, ND, about 15 km from the experimental location (Table 8).

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

Trials were grown using standard growing procedures for the location and region in regards to cultivation and pesticide application (Wiersma and Ransom, 2012). The plot size for an experimental unit in corn was 5.9 m long by 3.8 m wide, and consisted of 4 rows with a 76-cm spacing between rows. Corn trials were planted using a four row Almaco SeedPro 360 planter (Almaco, Nevada, IA) at a rate of 87 900 seeds ha^{-1}. The corn plots were harvested using a Zurn 150 combine (Zürn Harvesting GmbH and Co, Waldenburg, Germany).

The size of the wheat experimental unit in Ada, MN was 5.9 m long by 1.5 m wide, and consisted of 7 rows with an 18 cm spacing between rows. The plot size for Steele County and Casselton was 3.7 m long by 1.5 m wide, and consisted of 7 rows with an 18 cm spacing between rows. All wheat trials were planted using a 3P605NT drill (Great Plains Mfg Inc., Salina, KS) with 18 cm row spacing, at a rate of 2.96 million seeds ha^{-1}. Experimental units were harvested with a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria).

Table 4. Nitrogen fertilizer timing of application, N rate, and N source trial treatment list for corn and wheat trials in 2015.

Treatment	Timing	Rate	Source
		--%--†	
1	Spring	0	-
2	Spring	75	Urea
3	Spring	100	Urea
4	Spring	75	Urea-UAN ‡
5	Spring	100	Urea-UAN ‡
6	October	75	Urea
7	October	100	Urea
8	October	75	PCU §
9	October	100	PCU §
10	October	75	Urea-PCU §¶
11	October	100	Urea-PCU §¶
12	October	75	SU
13	October	100	SU
14	October	75	Urea+ENP
15	October	100	Urea+ENP
16	November	75	Urea
17	November	100	Urea
18	November	75	PCU
19	November	100	PCU
20	November	75	Urea-PCU §¶
21	November	100	Urea-PCU §¶
22	November	75	SU
23	November	100	SU
24	November	75	Urea+ENP
25	November	100	Urea+ENP

† Percent of rate considered optimum
‡ 50% of rate applied as urea in spring: 50% of rate applied as UAN at the four leaf stage of crop
§ PCU is Environmentally Smart Nitrogen
¶ 50% of rate applied as urea: 50% of rate applied as PCU

Table 5. Nitrogen fertilizer timing of application, percent of optimum N rate, and N source trial treatment list for 2016. The optimum N rate in corn for 2016 was calculated by multiplying the yield goal (9,416 kg ha^{-1}) by the constant 0.5, and then subtracting the fall N soil test and previous crop credits. The optimum N rate in wheat for 2016 was calculated by multiplying the yield goal (4,394 kg ha^{-1}) by the constant 1.1, then subtracting the fall N soil test and previous crop credits.

Treatment	Timing	Rate	Source †
		--%--	
1	Spring	0	-
2	Spring	75	Urea
3	Spring	100	Urea
4	Spring	75	PCU
5	Spring	100	PCU
6	Spring	75	Urea-PCU
7	Spring	100	Urea-PCU
8	Spring	75	Urea-UAN
9	Spring	100	Urea-UAN
10	Spring	150	Urea
11	Oct.	75	Urea
12	Oct.	100	Urea
13	Oct.	75	PCU
14	Oct.	100	PCU
15	Oct.	75	Urea-PCU
16	Oct.	100	Urea-PCU
17	Oct.	75	SU
18	Oct.	100	SU
19	Oct.	75	Urea+ENP
20	Oct.	100	Urea+ENP
21	Nov.	75	Urea
22	Nov.	100	Urea
23	Nov.	75	PCU
24	Nov.	100	PCU
25	Nov.	75	Urea-PCU
26	Nov.	100	Urea-PCU
27	Nov.	75	SU
28	Nov.	100	SU
29	Nov.	75	Urea+ENP
30	Nov.	100	urea+ ENP

† PCU is environmentally Smart Nitrogen, UAN is urea ammonium nitrate, SU is Super-U, and ENP is encapsulated nitrapyrin

Table 6. Treatment application timing for North Dakota corn experiment locations in 2015 and 2016.

	2015		2016	
Treatment Timing	Fargo †	Steele County	Casselton	Steele County
	---------------------------Day--------------------------			
Spring	18 Apr.	27 Apr.	29 Apr.	3 May
Early Fall ‡	15 Oct.	14 Oct.	9 Oct.	14 Oct.
Late Fall ‡	10 Nov.	7 Nov.	3 Nov.	2 Nov.

† NW22 is located in Fargo, ND
‡ Treatments occurred the fall before growing season

Table 7. Treatment application date for North Dakota and Ada, MN wheat experiment locations in 2015 and 2016.

	2015		2016		
Treatment Timing	Steele County	Casselton	Casselton	Steele County	Ada
	------------------------------------Day--				
Spring	7 Apr.	7 Apr.	12 Apr.	30 Mar.	13 Apr.
October †	14 Oct.	15 Oct.	9 Oct.	14 Oct.	13 Oct.
November †	12 Nov.	7 Nov.	3 Nov.	2 Nov.	2 Nov.

† Treatments occurred the fall before growing season

Table 8. Total accumulated rainfall from the date of fertilizer burial (1 Apr. 2016) until removal date.

Date	Rain †
	--cm--
26 Apr. 2016	4.32
10 May 2016	4.45
24 May 2016	5.41
7 June 2016	13.74

† Weather data was monitored and recorded from the NDAWN weather station in Prosper, ND (Latitude= 47.00° N, Longitude = -97.12° W)

The corn hybrid Pioneer 8640AM was used in 2015 corn experiments. It is an 86 day relative maturity hybrid that is well adapted to growing conditions in the experimental areas of North Dakota. This hybrid was not available in 2016 and was substituted with P8673AM. This hybrid was selected based on agronomic characteristic similarities to the previously used hybrid.

The spring wheat cultivar used was ‘Faller’. Faller was selected based on high characteristic yield potential and lower characteristic protein content compared to the range of tested HRSW varieties common in North Dakota and the region.

The optimum N rate for both wheat and corn trials in 2015 was set at 100.89 kg ha^{-1}. The optimum N rate in corn for 2016 was calculated by multiplying the yield goal (9 416 kg ha^{-1}) by the constant 0.5, and then subtracting the fall N soil test and previous crop credits. The optimum N rate in wheat for 2016 was calculated by multiplying the yield goal (4 394 kg ha^{-1}) by the constant 1.1, then subtracting the fall N soil test and previous crop credits (Table 9). This method is based on old procedures that do not consider the price of grain or urea and forces a grower to predict yield at least 3 months in advance of achieving it. These N recommendations would be similar to \$5.00 27 kg^{-1} of wheat and \$0.50 0.45 kg^{-1} of urea in regards to the N recommendations by Franzen (2009).

The total N content of urea, SU, UAN, and PCU is 46, 46, 32, and 44%, respectively. Nitrapyrin was added to the urea at a rate of 456 g of active ingredient ha^{-1}; nitrapyrin was added to the plastic bags containing the urea that had been weighed out per plot which was then shaken by hand for 45 s to ensure the urea was evenly coated before application.

The granule fertilizer containing treatments were uniformly hand broadcast over the entire plot area at the specified application time, then incorporated into the soil 10 cm deep immediately after application using a field cultivator operated at 2.7 m s^{-1}. The UAN treatments were applied at Feekes 4 in wheat and V4 in corn (NDSU, 2016; Ransom, 2013) with a CO_2 pressurized backpack sprayer and streamer bar boom in wheat and modified with drip hose to dribble between the rows in corn. The boom was held directly above canopy height and offset from crop rows to prevent crop damage. Speed of UAN application was adjusted to achieve the application rate needed for each treatment.

Table 9. The 100% rates of N and planting and harvest dates for corn and wheat experiments in 2015 and 2016.

Year	Crop	Location	Planting	Harvest	100% N Rate
			---------Date---------		--kg ha^{-1}--
2015	Corn	NW22	27 Apr.	29 Sept.	100.89
		Steele County	27 Apr.	14 Oct.	100.89
	Wheat	Steele County	7 Apr.	4 Aug.	100.89
		Casselton	7 Apr.	12 Aug.	100.89
2016	Corn	Casselton	2 May	10 Oct.	161.42
		Steele County	3 May	15 Oct.	121.07
	Wheat	Casselton	14 Apr.	1 Aug.	142.37
		Ada	13 Apr.	30 July	142.37

Data Collection

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

Experimental plots in 2016 were sampled for the amount of NH_4^+-N and NO_3^--N in the soil for fall applied treatments on 27 April at the Casselton corn location, 3 May for Steele County corn location, and 9 May for the Ada wheat location. Spring applied treatments were sampled on 2 June for Casselton and Steele County corn and wheat, and 3 June for Ada wheat

locations. Data was collected only from the plots containing the 100% rate of any fertilizer and the untreated check. Five, 30 cm deep soil core samples were taken from each experimental unit using a 30 cm long by 1.9 cm in diameter sampling tube attached to a JMC Backsaver Handle (JMC Soil Samplers, Newton, IA). The five soil core samples were dried for 18 h at 29.5 °C. General sample preparation and analysis procedures followed the recommended chemical soil test procedures for the North Central Region (Geldermann et al., 2015). The NH_4^+-

N test followed the nitroprusside catalyzed indophenol reaction procedure (O’Dell, 1993). The soil was extracted using 2N KCl solution. An Autoanalyzer 3 High Resolution Digital Colorimeter (Seal Analytical, Mequon, WI) was used to measure the extracted NH_4^+-N colormetrically. Nitrate levels were determined using the transnitration of salicylic acid method (Vendrell and Zupancic, 2008). The soil was extracted using deionized water with added gypsum. A Brinkmann PC 910 Colorimeter (Metrohm, Riverview, FL) at a wavelength of 420 nm was used to measure NO_3^--N. Total N was calculated by adding the NO_3^--N and NH_4^+-N together for each plot.

Corn stand counts were obtained by counting the two interior rows of each experimental unit at V4 growth stage. Wheat stand counts were collected by counting plants that were between two wooden stakes placed 0.91 m apart in two interior rows of a plot before Feekes 3 growth stage.

Normalized Difference Vegetation Index (NDVI) measurements using an active-optical sensor were not taken in 2015 for corn or wheat. In 2016, NDVI measurements for corn were acquired using a GreenSeeker (GS) handheld device (Trimble, Sunnyvale, CA) at the V6 growth stage and Crop Circle (CC) RapidScan handheld device (Holland Scientific, Lincoln, NE) at the V8 growth stage, according to North Dakota State University corn growth stages. These devices utilize the red or visible bands of the electromagnetic spectrum to estimate leaf area index (Bu et al., 2017). Red-NDVI (RNDVI) measurements can be calculated based on red and near-infared light in the following formula:

$$\mathrm{NDVI} = \frac{\textit{Near infrared reading} - \textit{Red reading}}{\textit{Near infrared reading} + \textit{Red reading}}$$

The sensing devices were held approximately 65 cm above the ground and measurements were taken at a walking speed of approximately 1.8 m s^{-1}. Wheat plots in 2016 had RNDVI measurements taken with the GS when wheat growth stages were at Feekes 2, Feekes 7, and Feekes 10, while the CC measured RNDVI at Feekes 10.51; plant growth staging was based on the NDSU Feekes scale.

Harvested weights expressed at 12.5% moisture were obtained at the time of combining using a Harvest Master (Juniper Systems, Logan, UT) combine weighing system. A subsample of grain from each harvested plot was retained for further analysis. This subsample was cleaned using a Clipper Office Tester and Cleaner (Seedburo Equipment Co., Chicago, IL). Moisture and test weight were obtained from processed grain using a GAC 2100 moisture tester (Dickey-John Corp., Minneapolis MN). Corn seed protein content was measured based on 12.5% moisture using near-infa-red spectrometry with an Infratec 1241 Grain Analyzer (Foss, Eden Prairie, MN), while the wheat seed protein was analyzed using a DA 7250 NIR analyzer (Perten Instruments, Hägersten, Sweden). Total protein in wheat was calculated by multiplying the yield by the percent protein concentration.

Statistical Analysis

Dissolution Rate of N Fertilizers

Data were statistically analyzed using PROC GLM in SAS 9.3 (SAS Institute, SAS Circle, Cary, NC). Application depth, fertilizer source and removal date were considered fixed effects, while replicate was considered a random effect. Proc mixed method=type3 was used for LSMEANS. Mean separations were done through a least significant difference calculation. An alpha level of 0.05 was used to test all hypotheses.

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

Data were analyzed with PROC MIXED in SAS 9.3. Application time, fertilizer source, and application rate were considered fixed effects in the model, while replicate and environment were considered random effects. All fixed effect interactions were considered fixed, while any interactions containing a random term were considered random. PROC MIXED method=type3 was used for LSMEANS. Mean separations were done through a least significant difference calculation. Corn yield, GS NDVI, and CC NDVI correlations were analyzed in Xcel. Yield, protein, GS NDVI, and CC NDVI correlations were analyzed for wheat in Xcel. An alpha level of 0.05 was used for all hypothesis tests.

RESULTS AND DISCUSSION

Weather Information

Weather impacts crop yield and N status in the soil. Average monthly air temperatures in the 2015 and 2016 growing seasons in corn and wheat locations were comparable to the long term location averages (Tables 10 and 11). Despite the near average temperatures, differences in rainfall were observed (Tables 12 and 13). In 2015, Fargo recorded high rainfall in May (200 mm), while June (64 mm), September (41 mm), and October (32 mm) experienced below average rainfall. The 2015 growing season in Casselton experienced above normal rainfall in May (149 mm), June (110 mm), and July (88 mm), and minimal rainfall in September (22 mm). In 2016, Casselton received slightly above normal rainfall in May (82 mm compared to 72 mm). On the other hand, June received below normal rainfall (38 mm). Ada also experienced high rainfall in May (119 mm) of 2015, while the 2016 growing was below normal in May (51 mm), but noted above average normal rainfall in July (156 mm), August (173 mm), and September (111 mm). The Finley ND weather station, located in Steele County, could not provide historical weather due to its establishment in 2014. However, rainfall differences between the 2015 and 2016 growing seasons are evident in May when 2015 received 106 mm compared to 70 mm in 2016. These differences in rainfall may have impacted corn and wheat response to treatments.

Dissolution Rate of N Fertilizers

The urea was completely dissolved by the first sampling date, two weeks after the initiation of the experiment. The site received 4.2 cm of rainfall by 25 April, shortly after installing the treatments, which was adequate to dissolve all the urea at all depths. The urea data were excluded from further analysis. When considering just the PCU, the main effect of depth was not significant for the release of N. Treatments with the main effect of date, however, differed significantly (P <0.0001). Moreover, the date by depth interaction was not significant for the amount of PCU fertilizer dissolved (Table 14). Significantly less N remained in the PCU granules at each consecutive two week sampling date (Fig. 1).

Table 10. Average air temperature for the months of planting to harvest in corn experiment locations, in 2015 and 2016, along with normal (1990-2016)†.

	Fargo			Steele County ‡§		Casselton ¶		
Month	2015	2016	Normal	2015	2016	2015	2016	Normal
	°C							
Apr.	8	6	7	7	4	8	6	5
May	13	15	14	12	14	12	15	13
June	20	20	19	19	19	19	19	18
July	22	22	21	21	20	21	21	20
Aug.	20	21	21	20	20	19	21	19
Sept.	18	17	16	17	15	17	16	15
Oct.	10	10	8	9	7	9	8	7
Nov.	2	6	-1	1	4	2	4	-2

† Information collected from NDAWN, 2016.
‡ Weather information collected from the Finley, ND, weather station 47.526° N, -97.847° W.
§ Normal data are not available due to the recent establishment of this site.
¶ Weather information collected from the Prosper, ND, weather station 47.002° N, -97.115° W.

Hyatt et al. (2010) also found that the release of urea from PCU was impacted by date. Their study was conducted on a Hubbard loamy sand with a May through September 30 year average temperature and precipitation of 6.8 °C and 752 mm, respectively. They found 73% of the N remaining after four weeks, while 35% of N remained six weeks after burial. This compares to the 78 and 64% of the N fertilizer remaining at four and six weeks after burial, respectively, in our study. The differences in the remainder of fertilizer between the two studies may be attributed to difference in the mesh size of the sample bags. A study by Wilson et al. (2009) found larger mesh spacing resulted in significantly higher N release rates caused by increased soil to fertilizer contact. Greater rainfall events occurred in the study by Hyatt et al. (2010) compared to our study and could also explain the large difference in fertilizer remaining six weeks after burial. The urea inside the polymer granule is dissolved when water passes through the polymer coating through diffusion (Hyatt et al., 2010), which degrades and cracks overtime.

Table 11. Average air temperature for the months of planting to harvest in wheat experiment locations, in 2015 and 2016, along with normal (1990-2016 for Casselton and 2007-2016 for Ada)†.

	Casselton ‡			Steele County §¶		Ada		
Month	2015	2016	Normal	2015	2016	2015	2016	Normal
	--------------------	--------------------	--------------------	°C	--------------------	--------------------	--------------------	--------------------
Mar.	0	3	-3	0	2	-1	2	-3
Apr.	8	6	5	7	4	7	5	5
May	12	15	13	12	14	12	15	12
June	19	19	18	19	19	18	19	16
July	21	21	20	21	20	21	21	19
Aug.	19	21	19	20	20	19	20	20
Sept.	17	16	15	17	15	17	16	15

† Information collected from NDAWN, 2016.
‡Weather information collected from the Prosper, ND, weather station 47.002° N, -97.115° W.
§ Weather information collected from the Finley, ND, weather station 47.526° N, -97.847° W.
¶ Normal data are not available due to the recent establishment of this site.

Optimum Nitrogen Fertilizer Timing, Rate, and Source for Corn and HRSW Production

NH_4^+-N and NO_3^--N Soil Levels

The error variances in the combined location analysis of either spring or fall application timing treatments were found to be homogenous using Bartlett's test. The fall applied N ANOVA showed NH_4^+-N did not differ significantly between treatments at Ada, Casselton, Steele County, or combined across all locations (Table 15). However, lower than expected NH_4^+-N values were found across all locations. Ammonium levels in Ada were consistently less than those found in Casselton and Steele County. The percent of organic matter in the top 30.5 cm of soil in Ada (2.2%), Casselton (4.7%), and Steele County (4.8%) is one possible explanation for this. The biological decomposition of organic matter releases NH_4^+-N into the soil at an increased rate when a larger amount of organic matter is present, provided that environmental conditions are similar.

Table 12. Monthly rainfall averages for corn experiment locations in 2015, 2016 and historical (1990-2016)†.

	Fargo			Steele County ‡§		Casselton ¶		
Month	2015	2016	Normal	2015	2016	2015	2016	Normal
	----------	----------	----------	mm	----------	----------	----------	----------
Apr.	16	59	35	12	39	20	43	26
May	200	33	73	106	70	149	82	72
June	64	69	110	108	93	110	38	101
July	71	132	69	107	112	88	88	75
Aug.	54	48	60	33	54	36	26	54
Sept.	41	80	65	28	73	22	61	60
Oct.	32	64	54	21	17	31	49	49

† Information collected from NDAWN, 2016.
‡ Weather information collected from the Finley, ND weather station 47.526 °N, -97.847 °W.
§ Normal data are not available due to the recent establishment of this site.
¶ Weather information collected from the Prosper, ND weather station 47.002 °N, -97.115 °W.

Table 13. Monthly rainfall averages for wheat experiment locations in 2015, 2016 and historical (1990-2016 for Casselton and 2007-2016 for Ada)†.

	Casselton ‡			Steele County §¶		Ada		
Month	2015	2016	Normal	2015	2016	2015	2016	Normal
	----------	----------	----------	mm	----------	----------	----------	----------
Apr.	20	43	26	12	39	20	41	34
May	149	82	72	106	70	119	51	66
June	110	38	101	108	93	100	65	106
July	88	88	75	107	112	65	156	70
Aug.	36	26	54	33	54	26	173	58
Sept.	22	61	60	28	73	12	111	74

† Information collected from NDAWN, 2016.
‡Weather information collected from the Prosper, ND weather station 47.002 °N, -97.115 °W.
§ Weather information collected from the Finley, ND weather station 47.526 °N, -97.847 °W.
¶ Normal data are not available due to the recent establishment of this site.

Table 14. F-values for percentage of fertilizer remaining in granules in Casselton, ND, in 2016.

SOV	F
Rep	0.43
Depth	0.92
Date	40.01***
Depth*Date	20.31

*** Significant at the 0.001 probability level.

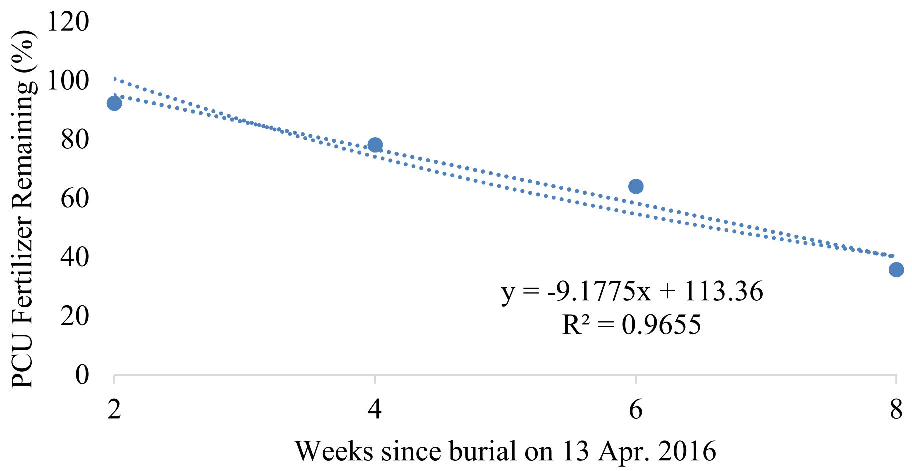

Fig. 1. Influence of date of removal on the dissolution rate of Environmentally Smart Nitrogen (PCU), averaged over depths in a field setting at Casselton, ND in 2016 (LSD=11). Points in the graph represent observed data and the line is the trend line.

Fall applied treatments had statistically different NO_3^--N levels at each location, as well as in the combined analysis. The Ada untreated check had significantly less NO_3^--N than all other fall treatments (Table 15). The November applied SU had a NO_3^--N level of 32.9 ppm, which was significantly higher than all other fall applied treatments. The untreated check had less NO_3^--N than any of the fall treatments sampled in Casselton (Table 15). At the Steele County site, October applications of PCU and Urea+ENP had similar NO_3^--N levels to the untreated check, whereas PCU and Urea+ENP treatments were higher in $NO3^-$-N than the check at Ada and Casselton (Table 15). Overall, the fall combined locations showed the untreated check to have statistically less NO_3^--N levels than all other treatments (Table 15). A six-week field study by Sullivan et al. (2014) found PCU, SU, and Urea+ENP had statistically lower NO_3^--N levels than urea up to four weeks after fertilizer application occurred, supporting the results in this study.

Table 15. The fall applied 100% N rates in 2016 studies were soil sampled to evaluate the location and combined location average for ammonium (NH_4^+-N), nitrate (NO_3^--N), and total N through 0-60 cm deep soil sampling in Ada, Casselton, and Steele County on 9 May, 27 April, and 3 May, respectively.

Treatment †	Ada			Casselton			Steele County			Combined		
	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N
	ppm											
Check	3.0	5.3	8.3	7.3	16.9	24.2	4.3	12.4	16.7	4.9	11.5	16.4
Oct. Applied												
Urea	3.5	19.4	22.8	6.7	49.3	56.0	6.0	33.8	39.7	5.4	34.1	39.5
PCU	3.6	16.5	20.1	7.2	45.4	52.6	4.5	21.4	25.9	5.1	27.8	32.8
Urea-PCU	3.8	13.5	17.3	6.9	45.0	51.9	4.8	22.1	26.9	5.2	26.9	32.0
SU	3.4	16.3	19.6	6.9	50.1	57.0	4.7	31.6	36.3	5.0	32.7	37.7
Urea+ENP	3.3	16.1	19.4	15.4	54.4	69.8	5.9	20.6	26.5	8.2	30.4	38.6
Nov. Applied												
Urea	3.3	20.5	23.8	8.9	49.3	58.2	4.9	33.6	38.6	5.7	34.5	40.2
PCU	4.1	15.8	19.9	8.6	31.9	40.4	6.6	23.4	30.0	6.4	23.7	30.1
Urea-PCU	4.4	16.9	21.3	10.0	44.9	54.9	5.6	22.4	28.0	6.7	28.0	34.7
SU	4.3	32.9	37.2	8.0	52.6	60.6	10.5	37.5	48.0	7.6	41.0	48.6
Urea+ENP	3.5	21.8	25.3	14.8	39.3	54.0	6.6	29.1	35.7	8.3	30.0	38.3
LSD (0.05) ‡	NS	7.7	8.0	NS	13.1	17.0	NS	9.2	11.6	NS	8.6	9.8

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the nitrogen applied as urea and 50% applied as PCU, SU is Super-U, Urea+ENP is the nitrogen rate applied as urea coated with encapsulated nitrapyrin.
‡ LSD values are to be used to compare all numbers within the same column.

The greatest total N with fall applications at Ada was recorded with the SU November application treatment. The untreated check had significantly less N than all other treatments at

8.3 ppm (Table 15). The untreated check in the fall applied soil samples had a comparable total N content to PCU applied in November (Table 15). The untreated check in Steele County had a total N level of 16.7 ppm, though it was not significantly lower than October applied PCU, Urea+ENP, and Urea-PCU, or November applied PCU. The combined ANOVA showed the untreated check had the lowest total N level statistically, while the November applied SU had the greatest total N level at 48.6 ppm. However, urea applied in October or November were not statistically different from the November applied SU. The combined results compared to a field study by Sullivan et al. (2014) when a 0 to 25.4 cm deep sample was analyzed 6 weeks after the fertilizer was applied; SU, and Urea+ENP total N levels did not differ significantly from urea.

Ammonium levels did not differ statistically in the spring applied N at Ada and Casselton; however, differences were found at the Steele County site and the spring applied combined locations. The Steele County spring applied PCU had greater NH_4^+-N levels than any other treatments (Table 16). The spring applied PCU in combined locations had 8.0 ppm of NH_4^+-N, significantly higher than the untreated check (Table 16). A laboratory study by Dell et al. (2014) also found increased levels of NH_4^+-N when PCU was applied compared to the untreated check when the soil was at 18% volumetric soil water content and sampling occurred 21 days or more after treatment. The lack of differences between the untreated check and other treatments for NH_4^+-N content could be attributed to environmental conditions favoring mineralization, where organic N is converted to NH_4^+-N and the rapid conversion of NH_4^+-N to NO_3^--N. Nitrogen mineralization in southeastern ND was unusually high during the 2016 growing season. One corn N rate study in southeast ND achieved 11 298.6 kg ha^{-1} with low residual nitrate-N in April and no added N fertilizers (Franzen, personal communication, 2017).

Only the spring application timing in Steele County did not have discernable NO_3^--N differences between treatments. In the spring soil samples at Ada, PCU had similar NO_3^--N to the untreated check (Table 16). Similar NO_3^--N levels were found between spring applied urea and the Urea-PCU blend, while Urea-UAN had the highest levels at 20.5 ppm. The highest levels of NO_3^--N are associated with Urea-UAN due to UAN application 1 week prior to spring soil sampling in the wheat locations. Similar to the findings in Ada, soil analysis of the spring applied treatment experimental units found the untreated check and PCU had significantly lower levels of NO_3^--N than Urea-PCU and urea (Table 16), supporting work by Geng et al. (2016). Their study showed urea had significantly higher NO_3^--N levels than PCU when soil samples were taken from a 0-20 cm depth, even under higher than average annual temperature and rainfall at 20.3 °C and 530 mm, respectively. The spring combined locations showed a similar pattern to the individual locations where the PCU NO_3^--N level was not significantly higher than the untreated check (Table 16). The low PCU NO_3^--N levels is caused by the polymer coating slowing the release of N in any form (Trenkel, 1997).

The untreated check had significantly less total N than all other spring applied treatments (Table 16). Urea and Urea-PCU had similar total N concentrations of 14.8 and 17.7 ppm, respectively, while Urea-UAN had the highest amount at 22.6 ppm. In Casselton, the untreated check and PCU had lower total N levels than urea (Table 16). Work by Sullivan et al. (2014) found similar PCU and urea comparisons for total N levels two weeks after fertilizer application occurred. The lower PCU total N concentrations may be attributed to N being retained in the fertilizer granules. Steele County did not show differences between treatments for total N when N was applied, but all N applications, regardless of fertilizer type, were significantly higher than the untreated check (Table 16). The ANOVA combined across locations showed similar statistical differences to those seen in Steele County.

Table 16. The 2016 spring applied 100% N rate treatment experimental units, soil sampled 2 June, Casselton and Steele County and 3 June, Ada and to evaluate ammonium (NH_4^+-N), nitrate (NO_3^--N), and total N by location and combined locations.

	Ada			Casselton			Steele County			Combined		
Treatment †	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N	NH_4-N	NO_3-N	Total N
	ppm											
Check	2.0	3.0	5.0	4.4	11.6	16.0	4.1	12.6	16.7	3.5	9.0	12.5
Urea	2.5	12.3	14.8	4.9	35.0	39.9	4.7	30.1	34.7	4.0	25.8	29.8
PCU	2.6	6.8	9.4	8.3	20.5	28.8	13.2	24.3	37.5	8.0	17.2	25.2
Urea-PCU	3.2	14.5	17.7	6.7	31.2	37.9	5.4	26.6	32.0	5.1	24.1	29.2
Urea-UAN	2.1	20.5	22.6	5.8	26.7	32.5	6.7	28.3	35.0	4.9	25.1	30.0
LSD (0.05)‡	NS	4.7	4.7	NS	10.3	9.9	5.2	NS	12.9	4.4	8.2	10.7

† PCU is Environmentally Smart Nitrogen, Urea- PCU is 50% rate applied as urea: 50% of rate applied as PCU, Urea-UAN is 50% of nitrogen rate applied as urea and 50% applied as UAN at the 4-leaf stage.

‡ LSD values are to be used to compare all numbers within the same column.

Corn

<u>All Treatment Comparisons</u>

Active-optical sensors are currently being used to evaluate in-season plant N content. However, there is some debate among scientists regarding the ideal crop growth stage necessary to predict yield (Shaver et al., 2011; Lopresti et al., 2015). In 2015 and 2016, the variances of error for all locations within a year were evaluated for homogeneity through Barlett's test. Since these variances were found not to differ statistically, locations within the same growing season and crop were combined in an analysis of variance.

GreenSeeker and Crop Circle sensor data were not taken in 2015. GreenSeeker data did not show any discernable differences in 2016 at either location or in the combined location analysis. The 2016 Crop Circle RNDVI readings did not differ statistically at Casselton, but did differ in Steele County. When the 2016 locations were combined, differences were not detected (Table 17).

No statistical differences in RNDVI readings taken with the Crop Circle handheld device at the V8 growth stage in corn were noted in Casselton, which is not surprising given that there were no yield differences due to treatment at that location (Table 18). The RNDVI readings, therefore, predicted the non-response of corn to N at that location. The RNDVI readings at Steele County showed the 75% rate of urea applied in the spring was statistically greater than the 75% rate of PCU applied the previous November, the untreated check, and the 75% rate of Urea+ENP applied in October. The 75% rate of Urea+ENP applied in October had lower RNDVI readings than the 75% rate of urea applied in October and the 100% rate of urea applied in the spring, as well. A study conducted in North Dakota by Sharma et al. (2015) also found increasing the rate of N from 134 kg N ha^{-1} to 179 kg N ha^{-1} did not always lead to elevated reflectance of NIR wavelengths when measurements were taken at the V6 growth stage in corn with the Crop Circle. The Crop Circle measures the top of the corn canopy, while N stress on the plant first appears in lower leaf tissue (Barker and Sawyer, 2013). The Crop Circle may not have been able to pick up early signs of N deficiencies in some treatments. Nitrogen concentrations may also have been adequate at the sensing date so that rate differences may not have been apparent.

No yield differences between treatments were recorded in 2015 at Steele County (Table 19). The moderately well drained soil in Steele County may have resulted in low N loss during the 2015 growing season. In addition, the previous crop credit may have also contributed to lack of response at this site. The 2015 combined location analysis showed the highest yielding treatment was the 100% rate of urea applied in October. Only the 75% rates of October and

November applied PCU, 75% rate of November applied Urea+ENP, and the untreated check produced less yield than the 100% rate of October applied urea. The untreated check yielded less than the 75% rate of November applied PCU, 75% rate of October applied PCU, and the 75% rate of November applied Urea+ENP (Table 19). Lack of yield differences among treatments may be caused by 2015 seasonal weather conditions that limited N losses and favored mineralization. No yield differences due to treatment were found in 2016 (Table 20). There was a positive correlation between yield and CC RNDVI readings taken at Steele County (Table 21), indicating that these readings were able to detect plant color differences at this early stage that ultimately had an impact on yield, later in the season.

Table 17. Statistical F values and level of significance from the 2015 and 2016 corn study location ANOVA tables for RNDVI (GreenSeeker) taken at V6, RNDVI (Crop Circle) taken at V8, yield, and protein variables.

	2015			2016		
Variable	Fargo	Steele County	Combined	Casselton	Steele County	Combined
RNDVI (GreenSeeker)	-	-	-	1.47	1.15	1.62
RNDVI (Crop Circle)	-	-	-	0.71	1.73*	1.15
Yield	2.04*	1.14	2.12*	1.05	0.8	1.51
Protein	-	-	-	1.65*	1.77*	2.33**

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Seed protein content was not determined in 2015; however, protein content was impacted by treatments in 2016 at Casselton, Steele County, and in the combined site analysis (Table 17). Although no premium or discount applies to corn protein, subtle variations caused by the treatments may be seen in protein concentrations even if yield is not impacted by N treatment. Nitrogen treatments applied in excess of what the plant needs for yield accumulate as protein in the grain. At Casselton, the 75% rate of spring applied PCU had statistically greater protein concentrations than the 75% rate of spring and November applied urea (Table 22). At Steele

County, the untreated check, the 100% rate of October applied Urea-PCU, and the 75% rate of Urea-PCU produced less grain protein than the 100% rate of spring applied urea and the 75% rate of urea applied in October. The October application of Urea-PCU applied at a 75% rate produced less protein than the 75% rate of urea applied in either the spring or November, as well (Table 22). The spring applied 150% rate of urea and 100% rate of November applied Urea-PCU resulted in more protein than the 75% rate of urea applied in November when the locations were combined (Table 22). The increased rate of N application and/or the fertilizer applied in the fall may have caused lower protein to be seen with the 75% rate of urea applied in November (Franzen 2009; Brown et al., 2005). These differences in protein may be seen as differences in yield if a growing season favors N losses or minimizes mineralization.

Since the experiment was designed so the effect of specific factors could be analyzed, the data were further analyzed to see if date of application, rate of fertilizer applied, type of fertilizer used, and any interactions between these factors affected RNDVI (GS), RNDVI (CC) yield, or protein.

Fall Factorial Comparisons

In 2015 and 2016, select treatments that were balanced for the factors that were used to develop the full treatment list were analyzed as a factorial to look closer at the effect of the main factors of rate, timing, and fertilizer type, as well as their interactions. There were two rates of N (75% and 100% of 'optimum' N rate), two timings (October and November), and five fertilizer types (urea, PCU, Urea-PCU, SU, and Urea+ENP) in this factorial design. Folded F test for homogeneity error of variance was not significant per year per location; therefore, locations across growing seasons were combined in the ANOVA.

In the combined analysis, there were no yield differences between any the factors and their interactions (Table 23). Similarly, there were no yield differences within individual locations in both 2015 and 2016 (Table 23). Weather conditions that favored N mineralization probably masked any treatment effect. As was noted previously, yield was high even in the unfertilized check, which suggested that only minimal N fertilization was required in to attain optimum yield.

The 2016, RNDVI (GS), RNDVI (CC), and protein data were analyzed using this factorial design. There were no statistical differences for RNDVI (GS) and for protein. However, the rate by fertilizer type interaction was significant for RNDVI (CC) taken at V8 growth stage (Fig. 2). Urea, PCU, and Urea+ENP had a crossover interaction where the 75% rate of urea and the 100% rate of PCU and Urea+ENP had greater RNDVI readings than the urea, PCU, and Urea+ENP at 100%, 75%, and 75%, respectively. It is unclear why the 75% rate of urea had a higher RNDVI reading than the 100% rate. The increased N applied to PCU and Urea+ENP resulted in more chlorophyll production, thus increasing the RNDVI reading. The Urea-PCU and SU RNDVI readings did not vary statistically when the 75 and 100% rates of the same fertilizer were compared. A diverging response occurred between the Urea+ENP and the SU fertilizer. The 100% rate of Urea+ENP had an RNDVI reading of 0.84 compared to the 100% rate of SU at 0.82. The decreased greenness seen with SU may be attributed to N loss through elevated NO_3^--N levels in the SU compared to the Urea+ENP. However, the elevated RNDVI readings did not result in a statistical difference in grain yield.

Table 18. Red-normalized difference vegetative index readings obtained with a handheld Crop Circle device at the V8 growth stage in corn at Casselton, Steele County, and combined across locations in 2016.

Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined
	--%--				
-	-	Check	0.82	0.80	0.81
Spring	75	Urea	0.82	0.84	0.83
Spring	100	Urea	0.83	0.83	0.83
Spring	75	PCU	0.81	0.83	0.82
Spring	100	PCU	0.81	0.83	0.82
Spring	75	Urea-PCU	0.82	0.84	0.83
Spring	100	Urea-PCU	0.83	0.84	0.83
Spring	75	Urea-UAN	0.83	0.84	0.83
Spring	100	Urea-UAN	0.83	0.83	0.83
Spring	150	Urea	0.81	0.83	0.82
Oct.	75	Urea	0.83	0.83	0.83
Oct.	100	Urea	0.82	0.82	0.82
Oct.	75	PCU	0.84	0.81	0.82
Oct.	100	PCU	0.84	0.82	0.83
Oct.	75	Urea-PCU	0.84	0.82	0.83
Oct.	100	Urea-PCU	0.83	0.83	0.83
Oct.	75	SU	0.81	0.82	0.81
Oct.	100	SU	0.82	0.83	0.83
Oct.	75	Urea+ENP	0.81	0.79	0.80
Oct.	100	Urea+ENP	0.84	0.84	0.84
Nov.	75	Urea	0.83	0.82	0.83
Nov.	100	Urea	0.82	0.82	0.82
Nov.	75	PCU	0.83	0.80	0.82
Nov.	100	PCU	0.85	0.82	0.83
Nov.	75	Urea-PCU	0.83	0.83	0.83
Nov.	100	Urea-PCU	0.82	0.83	0.83
Nov.	75	SU	0.82	0.83	0.83
Nov.	100	SU	0.81	0.83	0.82
Nov.	75	Urea+ENP	0.83	0.83	0.83
Nov.	100	Urea+ENP	0.84	0.84	0.84
LSD (0.05)‡			NS	0.03	NS

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is encapsulated nitrapyrin coated urea.
‡ LSD values are to be used to compare all numbers within the same column.

In the combined ANOVA, type of fertilizer applied performed differently at the two locations with respect to RNDVI readings recorded with the Crop Circle at V8. This interaction was a crossover type interaction (Table 24). A lower RNDVI reading occurred at Casselton when PCU was used compared to SU. However, the SU at Steele County had a higher average RNDVI reading than when PCU was applied. Variations are mostly likely caused by increased rainfall in Casselton compared to Steele County or variations in soil type. Time of day that the sensor was used should not have caused RNDVI readings to vary (Barker and Sawyer, 2013).

Spring Factorial Comparisons

A second factorial analysis of the data in 2016 was conducted for balanced treatments of N rate (75% and 100%), application timing (spring, October, and November), and fertilizer types (urea, PCU, and Urea-PCU). There was no significance between treatments in the Folded F test. Therefore, locations have been combined.

The combined ANOVA did not show any significant interactions or main effects for yield (Table 25). When grain yields were analyzed separately, Casselton showed a significant interaction between rate and type of fertilizer applied. The type of fertilizer applied main effect was significant in Steele County.

The significant N rate by fertilizer type applied interaction in Casselton grain yield was caused by a crossover interaction (Fig. 3). Grain yield increased when a 75% rate of urea was used instead of a 100% rate, while decreased yield occurred with the 75% rate PCU compared to the 100% rate. It is unclear why the 75% rate of urea resulted in greater yield than the 100% rate, while the additional N applied as PCU increased grain yield. This differs from the converging rate by type of fertilizer interaction for corn grain yield in a non-irrigated and non-drained production system by Nelson et al. (2009). In their study, a smaller yield decrease occurred when PCU was applied at 140 kg ha^{-1} and 280 kg ha^{-1} than when urea was applied at the same rates.

Table 19. Yield averages for the 2015 corn locations and combined analysis.

Application Timing	Rate	Fertilizer Type †	Fargo	Steele County	Combined
	---%---		----------------kg ha^{-1}----------------		
-	-	Check	7 741	8 321	8 031
Spring	75	Urea	10 194	11 326	10 760
Spring	100	Urea	9 850	11 028	10 439
Spring	75	Urea-UAN	8 947	10 566	9 756
Spring	100	Urea-UAN	9 577	9 995	9 786
Oct.	75	Urea	9 480	10 771	10 126
Oct.	100	Urea	10 381	11 225	10 803
Oct.	75	PCU	10 127	8 853	9 490
Oct.	100	PCU	9 633	10 368	10 001
Oct.	75	Urea-PCU	9 708	9 850	9 779
Oct.	100	Urea-PCU	9 778	9 910	9 844
Oct.	75	SU	10 059	10 429	10 244
Oct.	100	SU	10 382	10 449	10 416
Oct.	75	Urea+ENP	9 902	10 771	10 336
Oct.	100	Urea+ENP	9 656	11 609	10 633
Nov.	75	Urea	9 935	9 690	9 813
Nov.	100	Urea	9 216	10 394	9 805
Nov.	75	PCU	9 080	9 876	9 478
Nov.	100	PCU	9 714	10 282	9 998
Nov.	75	Urea-PCU	9 535	10 980	10 257
Nov.	100	Urea-PCU	9 383	11 102	10 243
Nov.	75	SU	9 253	10 194	9 723
Nov.	100	SU	9 876	10 063	9 969
Nov.	75	Urea+ENP	9 612	9 524	9 568
Nov.	100	Urea+ENP	10 531	9 928	10 229
LSD (0.05)‡			1 130	NS	1 100

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is urea coated with encapsulated nitrapyrin.

‡ LSD values are to be used to compare all numbers within the same column.

Table 20. Yield averages for the 2016 corn locations and combined analysis.

Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined
	--%--		------------------kg ha^{-1}	-----------------	
-	-	Check	14890	15552	15221
Spring	75	Urea	15780	15861	15820
Spring	100	Urea	14932	15871	15401
Spring	75	PCU	15468	15790	15629
Spring	100	PCU	16271	16408	16339
Spring	75	Urea-PCU	15580	16004	15792
Spring	100	Urea-PCU	16222	16453	16338
Spring	75	Urea-UAN	15585	15692	15638
Spring	100	Urea-UAN	14690	16070	15380
Spring	150	Urea	15317	15779	15548
Oct.	75	Urea	15588	15639	15614
Oct.	100	Urea	15256	15655	15455
Oct.	75	PCU	15239	15855	15547
Oct.	100	PCU	15789	15800	15795
Oct.	75	Urea-PCU	15765	15758	15762
Oct.	100	Urea-PCU	15166	15584	15375
Oct.	75	SU	15633	15764	15699
Oct.	100	SU	15998	15968	15983
Oct.	75	Urea+ENP	16261	15712	15986
Oct.	100	Urea+ENP	15796	15650	15723
Nov.	75	Urea	15689	15915	15802
Nov.	100	Urea	15596	15759	15678
Nov.	75	PCU	15257	15699	15478
Nov.	100	PCU	16596	16158	16377
Nov.	75	Urea-PCU	15494	16188	15841
Nov.	100	Urea-PCU	14870	16190	15530
Nov.	75	SU	15658	15516	15587
Nov.	100	SU	16144	15889	16017
Nov.	75	Urea+ENP	15293	15995	15644
Nov.	100	Urea+ENP	15166	16129	15648
LSD (0.05)‡			NS	NS	NS

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is urea coated with encapsulated nitrapyrin.

‡ LSD compares all numbers within the same column.

Table 21. Correlation coefficients between red-normalized difference vegetative index (RNDVI) readings taken with the GreenSeeker at V6 and yield, and the RNDVI readings taken at V8 with the Crop Circle and yield at two locations in North Dakota, 2016.

RNDVI	Casselton	Steele County
GreenSeeker	-0.15	-0.06
Crop Circle	-0.03	0.35*

* Significant at the 0.05 probability level

Yield was significantly affected by application timing in Steele County (Table 25). Treatments applied in the spring and November yielded more than treatments applied in October, supporting findings by Randall et al. (2003). This seven year study conducted in Minnesota showed N applied in October yielded 0.45 Mg ha^{-1} less than treatments applied in November. Temperatures fluctuations above 10 °C occurred after the October fertilizer application at Steele County in 2015. Fall applied N can be lost through leaching and denitrification if temperatures remain above 10 °C for extended periods of time as a result of NH_4^+-N fall nitrification to NO_3^--N (Randall et al., 2003). The Steele County site had the lightest soil texture and the least amount of organic matter compared to other sites, which may be the reason that differences in treatments due to timing were recorded there and not at other locations.

The combined ANOVA showed the main effect of rate was significant for RNDVI (GS) readings at the V6 growth stage (Table 26). A three way interaction between rate, timing, and fertilizer type was significant for RNDVI readings taken with the Crop Circle at the V8 growth stage (Table 26). The location by rate and location by fertilizer type interactions were both significant for protein levels in the combined ANOVA, as well (Table 26).

Table 22. Mean grain protein for 2016 corn locations and combined analysis expressed at 12.5% moisture.

Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined
	---%---			---%---	
-	-	Check	8.9	8.7	8.8
Spring	75	Urea	9.1	9.4	9.3
Spring	100	Urea	9.4	9.5	9.4
Spring	75	PCU	9.6	9.1	9.4
Spring	100	PCU	9.5	9.4	9.4
Spring	75	Urea-PCU	9.3	9.2	9.3
Spring	100	Urea-PCU	9.5	9.1	9.3
Spring	75	Urea-UAN	9.4	9.3	9.3
Spring	100	Urea-UAN	9.5	9.3	9.4
Spring	150	Urea	9.8	9.5	9.6
Oct.	75	Urea	9.5	9.5	9.5
Oct.	100	Urea	9.4	9.2	9.3
Oct.	75	PCU	9.5	9.3	9.4
Oct.	100	PCU	9.7	9.4	9.5
Oct.	75	Urea-PCU	9.3	8.9	9.1
Oct.	100	Urea-PCU	9.6	9.0	9.3
Oct.	75	SU	9.3	9.1	9.2
Oct.	100	SU	9.3	9.1	9.2
Oct.	75	Urea+ENP	9.3	9.3	9.3
Oct.	100	Urea+ENP	9.4	9.5	9.4
Nov.	75	Urea	9.0	9.3	9.2
Nov.	100	Urea	9.6	9.1	9.4
Nov.	75	PCU	9.4	9.3	9.4
Nov.	100	PCU	9.5	9.3	9.4
Nov.	75	Urea-PCU	9.5	9.5	9.5
Nov.	100	Urea-PCU	9.7	9.5	9.6
Nov.	75	SU	9.4	9.2	9.3
Nov.	100	SU	9.4	9.5	9.4
Nov.	75	Urea+ENP	9.5	9.3	9.4
Nov.	100	Urea+ENP	9.7	9.4	9.5
LSD (0.05)‡			0.4	0.4	0.3

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is urea coated with encapsulated nitrapyrin.

‡ LSD values are to be used to compare all numbers within the same column.

Table 23. Effect of N rate, time of N fertilizer application, and fertilizer source on corn yield at four environments, 2015 and 2016.

		2015		2016		2015-16
		Fargo	Steele County	Casselton	Steele County	Combined
		-----------------------------------kg ha^{-1}	-----------------------------------			
Rate	75	9669	10094	15588	15804	12789
	100	9855	10533	15638	15878	12976
Timing	Oct.	9911	10424	15649	15738	12931
	Nov.	9614	10203	15576	15944	12834
Fertilizer†	Urea	9753	10520	15532	15742	12887
	PCU	9639	9845	15720	15878	12770
	Urea-PCU	9601	10460	15324	15930	12829
	SU	9893	10284	15858	15784	12955
	Urea+ENP	9925	10458	15629	15871	12971
LSD (0.05) ‡		NS	NS	NS	NS	NS

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, SU is Super-U, Urea+ENP is encapsulated nitrapyin applied to the rate of urea.

‡LSD compares types of N fertilizer in the same column. The means for rate, timing and fertilizer type factors were found to not be statically different using an F-test.

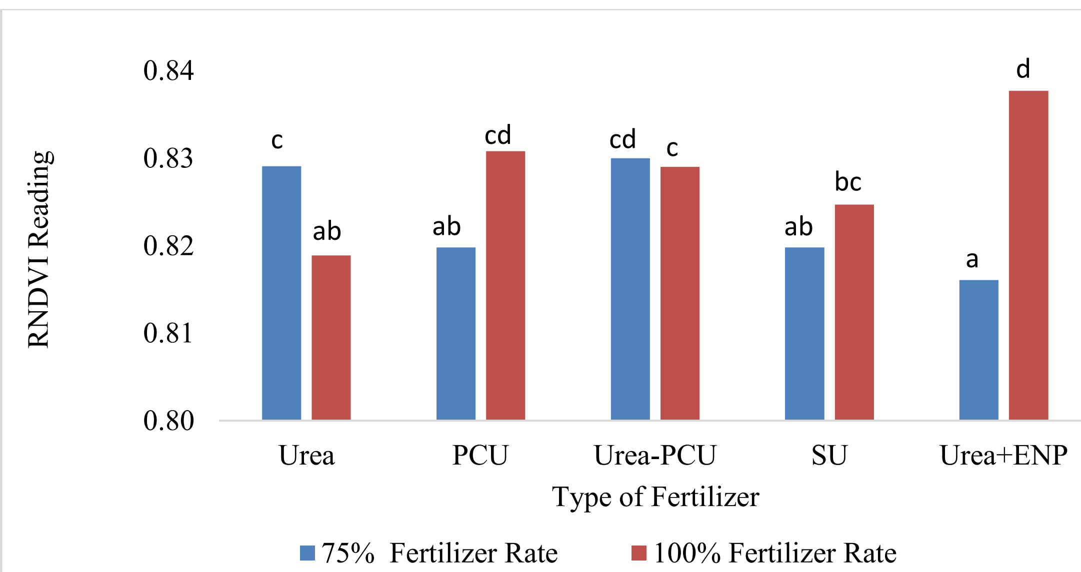

Fig. 2. The rate by fertilizer interaction in 2016 corn combined analysis across environments found statistical differences in Crop Circle RNDVI readings taken at the V8 growth stage in corn (LSD used to compare all means=0.008).

Table 24. The interaction between location and type of fertilizer for RNDVI measurements obtained with a Crop Circle handheld device at the V8 growth stage in corn.

Type of Fertilizer †	Casselton	Steele County
Urea	0.8249	0.8231
PCU	0.8375	0.8131
Urea-PCU	0.8306	0.8285
SU	0.8186	0.8259
Urea+ENP	0.8291	0.8247
LSD (0.05)	0.0143	

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the nitrogen rate applied as urea and 50% applied as PCU, SU is Super-U, Urea+ENP is encapsulated nitrapyrin applied to the rate of urea.

Table 25. Effect of N rate, time of N fertilizer application, and fertilizer source on corn yield at two locations and combined across locations in 2016.

		Casselton	Steele County	Combined
		----------------------kg ha^{-1}--------------------		
Rate †	75	15 540	15 857	15 698
	100	15 633	15 986	15 810
Timing	Spring	15 709	16 064	15 887
	Oct.	15 467	15 715	15 591
	Nov.	15 584	15 984	15 784
LSD(0.05) ‡		NS	237	NS
Fertilizer	Urea	15 474	15 783	15 628
	PCU	15 770	15 952	15 861
	Urea-PCU	15 516	16 030	15 773
LSD (0.05) ‡		NS	NS	NS

† The main effect of rate was not significant
‡ LSD values are to be used to compare yield averages within the same column.

The 75% rate of N had a statistically lower GreenSeeker reading of 0.594 compared to 0.606 seen with the 100% rate of N. Work by Barker and Sawyer (2013) showed N applied at

135 kg ha^{-1} resulted in higher RNDVI values than when 67 kg N ha^{-1} was applied to corn. Measurements were taken with a GS during the day similar to our study.

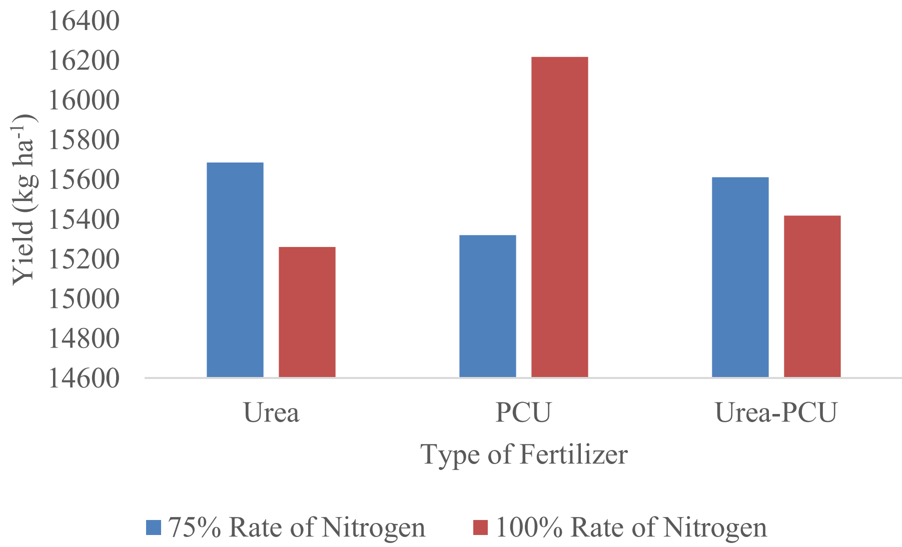

Fig. 3. Rate by type of applied fertilizer interaction for grain yield in 2016 at the Casselton corn location (LSD used to compare all means=644).

The rate by timing by fertilizer type interaction for RNDVI readings taken with the Crop Circle at V8 growth stage in corn were statistically different in the combined ANOVA (Table 27). The 75% rate of October applied urea, 75% rate of October applied Urea-PCU, 100% rate of spring applied Urea-PCU, and 100% rate of November applied PCU had significantly higher RNDVI readings than the 100% rate of October applied urea and 75% rate of November applied PCU.

The combined ANOVA showed location by rate and location by fertilizer type interactions were significant for protein. Thus, locations will be discussed separately for these variables. Protein content was not significantly different in Steele County for rate averaged across timing and fertilizer type or fertilizer type averaged across rate and timing. However, both interactions were significant in Casselton (Table 28). The 75% rate of fertilizer averaged 9.35% protein compared to the significantly higher 100% rate of fertilizer averaging 9.53%. The increased N applied may have provided additional N to be used for protein production. The type of fertilizer applied in Casselton resulted in discernable differences in protein. The 50:50 ratio of urea and PCU resulted in the same protein level as urea and PCU. However, the protein content in urea was significantly less than that of PCU. The polymer coating may have prevented N loss or supplied a greater quantity of N when needed. This allowed for additional N to be utilized for protein production by the plant after yield was established.

Table 26. F Values and their significance for RNDVI readings taken with the GreenSeeker at V6 growth stage, RNDVI readings taken with the Crop Circle at the V8 growth stage, protein levels, and yield for 2016 combined corn locations.

Sources	GreenSeeker	Crop Circle	Protein	Yield
Rate (R)	841.00*	0.68	0.78	37.12
Timing (T)	1.25	0.05	0.41	14.52
Fertilizer Type (F)	8.12	0.56	0.36	1.97
RxT	1.07	1.24	0.28	8.33
RxF	1.06	4.23	0.05	3.02
TxF	2.32	0.27	3.37	0.69
RxTxF	1.62	7.69*	1.77	2.54
Location (L)xR	0.00	0.19	4.23*	0.03
LxT	1.91	5.80**	1.15	0.19
LxF	0.49	2.27	4.12*	0.86
LxRxT	2.51	0.71	1.18	0.14
LxRxF	2.45	0.38	1.97	2.05
LxTxF	0.67	1.46	1.2	1.15
LxRxTxF	1.49	0.08	0.96	0.59

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table 27. Average RNDVI readings obtained with a Crop Circle device at the V8 growth stage in corn when combined across 2016 locations.

Rate	Timing	Fertilizer Type †	Crop Circle
---%---			
75	Spring	Urea	0.8274
75	Spring	PCU	0.8195
75	Spring	Urea-PCU	0.8268
75	Oct.	Urea	0.8332
75	Oct.	PCU	0.8239
75	Oct.	Urea-PCU	0.8332
75	Nov.	Urea	0.8251
75	Nov.	PCU	0.8157
75	Nov.	Urea-PCU	0.8269
100	Spring	Urea	0.8302
100	Spring	PCU	0.8212
100	Spring	Urea-PCU	0.8332
100	Oct.	Urea	0.8162
100	Oct.	PCU	0.8289
100	Oct.	Urea-PCU	0.8310
100	Nov.	Urea	0.8215
100	Nov.	PCU	0.8327
100	Nov.	Urea-PCU	0.8270
LSD (0.05)			0.0073

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU.

Table 28. Mean protein for main effects at Casselton and Steele County 2016 corn locations.

		Casselton	Steele County
		--------------%--------------	
Rate (%) †	75	9.35	9.28
	100	9.53	9.27
Timing	Spring	9.38	9.28
	Oct.	9.48	9.22
	Nov.	9.45	9.32
LSD (0.05) ‡		0.15	NS
Fertilizer Type §	Urea	9.33	9.35
	PCU	9.53	9.29
	Urea-PCU	9.46	9.18
LSD (0.05) ‡		0.15	NS

† The main effect of rate was not significant.
‡ LSD value used to compare averages of the same main effect in the same column.
§ PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the nitrogen rate applied as urea and 50% applied as PCU.

Wheat

<u>All Treatment Comparisons</u>

Bartlett's test for homogeneity of variance was conducted on wheat locations for each growing season. The test was found not significant, thereby allowing combination of locations prior to analysis.

Normalized difference vegetative index measurements were not collected with a GreenSeeker or Crop Circle handheld device in 2015. In 2016, RNDVI measurements collected with the GreenSeeker at the Feekes 2, 7, and 10 growth stages and the Feekes 10.51 growth stage with the Crop Circle were significant in Ada and when combined across locations, but not at Casselton (Table 29). Active-optical sensor readings were not significant due to treatment at

Casselton in 2016 probably because there were no yield differences due to treatment at Casselton in 2016. Lack of differences due to treatment with sensor readings predicted lack of yield differences due to treatment.

Table 29. Dependent variable F values and significance levels for all treatments in 2015-16 wheat locations and locations combined within the same growing season.

	2015		2016		
	Casselton	Combined	Casselton	Ada	Combined
GreenSeeker Feekes 2	-	-	1.53	2.42***	2.46**
GreenSeeker Feekes 7	-	-	1.24	4.03***	2.90**
GreenSeeker Feekes 10	-	-	1.39	5.06***	2.84**
Crop Circle Feekes 10.51	-	-	1.46	4.92***	1.86*
Yield	2.57**	1.34	1.59	2.92***	1.49
Protein	1.00	1.54	1.59	1.75*	1.62
Total Protein	1.70*	1.71	1.91*	3.72***	2.61**

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

The combined ANOVA showed GreenSeeker readings taken at Feekes 2 were significant for treatment. Greater RNDVI readings were seen with the 75% rate of November applied urea than the untreated check, the 75% and 100% rates of spring applied urea, the 100% rate of spring applied Urea-UAN, and the 150% rate of spring applied urea (Table 30). Plant roots are able to absorb increased rates of NO_3^-- N compared to NH_4^+- N due to an additional high affinity NO_3^-- N transport system (Glass et al., 2002). Elevated NO_3^-- N rates were seen in the November applied urea compared to spring applied urea in Ada in 2016, which may explain the additional greenness seen with the 75% rate of November applied urea.

The combined location treatment means varied for the Feekes 7 RNDVI measurements (Table 31). The untreated check had statistically lower RNDVI readings than all other treatments. The RNDVI readings taken at Feekes 10 with a GreenSeeker (Table 32) and Feekes

10.51 with the Crop Circle (Table 33) showed the untreated check had lower RNDVI readings compared to other treatments. A similar difference in yield or protein to these RNDVI readings were not detected, however.

NDVI readings at all growth stages correlated significantly with yield at both locations and the mid- to late-season NDVI readings at Ada correlated with protein (Table 35). These data suggest that the color difference detected by the GS and CC were predictive of yield at the end of the season and in more limited cases, protein.

No spring wheat yield differences were measured in Steele County in 2015, at Casselton in 2016, or in the combined data from 2015 and 2016 (Table 34). Insignificance in 2015 data could be attributed to environmental conditions favoring mineralization. Thus, the lack of yield differences is supported by the in season RNDVI measurements.

Differences in yield were seen when treatment means were compared at Casselton in 2015 (Table 34). The 100% N rate of November applied SU out-yielded the 75% rate of urea applied in the spring, October, and November, as well as the 100% rate of urea applied in the spring and November. The DCD and NBPT in SU apparently protected the N from soil losses, while yield from unprotected urea was lower than protected urea treatments. The 100% rate of November applied SU yielded more than the 100% rate of SU applied in October. The DCD and NBPT may not have prevented N losses in the October applied SU until the N was protected from loss when temperatures fall below 4˚C. In 2016, yield varied with treatment at Ada (Table 34). The 100% rate of PCU applied in October yielded less than the 100% rate of urea applied in October. It is unclear why this occurred.

Table 30. Mean RNDVI values obtained using a GreenSeeker in spring wheat at Feekes 2 in 2016.

Application timing	Rate	Fertilizer Type †	Casselton	Ada	Combined
	---%---				
-	-	Check	0.23	0.36	0.30
Spring	75	Urea	0.26	0.41	0.34
Spring	100	Urea	0.25	0.41	0.33
Spring	75	PCU	0.28	0.43	0.36
Spring	100	PCU	0.29	0.41	0.35
Spring	75	Urea-PCU	0.28	0.43	0.36
Spring	100	Urea-PCU	0.31	0.39	0.35
Spring	75	Urea-UAN	0.28	0.44	0.36
Spring	100	Urea-UAN	0.27	0.40	0.33
Spring	150	Urea	0.25	0.37	0.31
Oct.	75	Urea	0.32	0.41	0.36
Oct.	100	Urea	0.29	0.45	0.37
Oct.	75	PCU	0.32	0.41	0.37
Oct.	100	PCU	0.32	0.42	0.37
Oct.	75	Urea-PCU	0.28	0.40	0.34
Oct.	100	Urea-PCU	0.30	0.45	0.37
Oct.	75	SU	0.30	0.43	0.36
Oct.	100	SU	0.29	0.47	0.38
Oct.	75	Urea+ENP	0.31	0.45	0.38
Oct.	100	Urea+ENP	0.32	0.44	0.38
Nov.	75	Urea	0.33	0.46	0.39
Nov.	100	Urea	0.30	0.45	0.37
Nov.	75	PCU	0.30	0.47	0.38
Nov.	100	PCU	0.31	0.42	0.36
Nov.	75	Urea-PCU	0.28	0.46	0.37
Nov.	100	Urea-PCU	0.27	0.46	0.36
Nov.	75	SU	0.31	0.42	0.36
Nov.	100	SU	0.28	0.44	0.36
Nov.	75	Urea+ENP	0.27	0.44	0.35
Nov.	100	Urea+ENP	0.33	0.45	0.39
LSD (0.05)‡			NS	0.04	0.04

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is urea coated with encapsulated nitrapyrin.

‡ LSD values are to be used to compare all numbers within the same column.

Table 31. Red-normalized difference vegetative index values for spring wheat obtained using a GreenSeeker at Feekes 7 in 2016.

Application Timing	Rate	Fertilizer Type †	Casselton	Ada	Combined
	---%---				
-	-	Check	0.51	0.69	0.60
Spring	75	Urea	0.63	0.82	0.73
Spring	100	Urea	0.64	0.84	0.74
Spring	75	PCU	0.64	0.82	0.73
Spring	100	PCU	0.61	0.83	0.72
Spring	75	Urea-PCU	0.65	0.84	0.75
Spring	100	Urea-PCU	0.71	0.84	0.78
Spring	75	Urea-UAN	0.65	0.83	0.74
Spring	100	Urea-UAN	0.64	0.84	0.74
Spring	150	Urea	0.65	0.83	0.74
Oct.	75	Urea	0.70	0.77	0.73
Oct.	100	Urea	0.66	0.83	0.75
Oct.	75	PCU	0.65	0.81	0.73
Oct.	100	PCU	0.70	0.82	0.76
Oct.	75	Urea-PCU	0.64	0.80	0.72
Oct.	100	Urea-PCU	0.63	0.82	0.73
Oct.	75	SU	0.63	0.83	0.73
Oct.	100	SU	0.62	0.82	0.72
Oct.	75	Urea+ENP	0.67	0.83	0.75
Oct.	100	Urea+ENP	0.71	0.82	0.76
Nov.	75	Urea	0.69	0.83	0.76
Nov.	100	Urea	0.67	0.83	0.75
Nov.	75	PCU	0.65	0.82	0.74
Nov.	100	PCU	0.67	0.83	0.75
Nov.	75	Urea-PCU	0.61	0.83	0.72
Nov.	100	Urea-PCU	0.66	0.82	0.74
Nov.	75	SU	0.62	0.81	0.71
Nov.	100	SU	0.59	0.84	0.72
Nov.	75	Urea+ENP	0.60	0.82	0.71
Nov.	100	Urea+ENP	0.70	0.86	0.78
LSD (0.05)‡			NS	0.03	0.05

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is urea coated with encapsulated nitrapyrin.

‡ LSD values are to be used to compare all numbers within the same column.

Table 32. Red-normalized difference vegetative index values for wheat obtained with a GreenSeeker at Feekes 10 in 2016.

Application Timing	Rate	Fertilizer Type †	Casselton	Ada	Combined
	---%---				
-	-	Check	0.79	0.72	0.75
Spring	75	Urea	0.83	0.83	0.83
Spring	100	Urea	0.83	0.84	0.84
Spring	75	PCU	0.83	0.83	0.83
Spring	100	PCU	0.83	0.84	0.84
Spring	75	Urea-PCU	0.82	0.83	0.83
Spring	100	Urea-PCU	0.84	0.85	0.84
Spring	75	Urea-UAN	0.84	0.84	0.84
Spring	100	Urea-UAN	0.83	0.84	0.84
Spring	150	Urea	0.83	0.85	0.84
Oct.	75	Urea	0.84	0.81	0.83
Oct.	100	Urea	0.83	0.83	0.83
Oct.	75	PCU	0.84	0.82	0.83
Oct.	100	PCU	0.83	0.82	0.83
Oct.	75	Urea-PCU	0.83	0.81	0.82
Oct.	100	Urea-PCU	0.83	0.84	0.84
Oct.	75	SU	0.82	0.84	0.83
Oct.	100	SU	0.83	0.84	0.83
Oct.	75	Urea+ENP	0.83	0.83	0.83
Oct.	100	Urea+ENP	0.84	0.82	0.83
Nov.	75	Urea	0.84	0.83	0.84
Nov.	100	Urea	0.84	0.83	0.83
Nov.	75	PCU	0.83	0.83	0.83
Nov.	100	PCU	0.82	0.84	0.83
Nov.	75	Urea-PCU	0.83	0.84	0.83
Nov.	100	Urea-PCU	0.83	0.83	0.83
Nov.	75	SU	0.83	0.83	0.83
Nov.	100	SU	0.83	0.84	0.84
Nov.	75	Urea+ENP	0.82	0.83	0.83
Nov.	100	Urea+ENP	0.84	0.84	0.84
LSD (0.05)‡			NS	0.02	0.03

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is encapsulated nitrapyrin coated urea.
‡ LSD values are to be used to compare all numbers within the same column

Table 33. The treatment average for RNDVI readings taken with a Crop Circle ground-based active-optical sensor at the Feekes 10.51 spring wheat growth stage in 2016 at Casselton and Ada and combined across locations.

Application Timing	Rate	Fertilizer Type †	Casselton	Ada	Combined
	---%---				
-	-	Check	0.79	0.72	0.76
Spring	75	Urea	0.81	0.79	0.80
Spring	100	Urea	0.81	0.81	0.81
Spring	75	PCU	0.81	0.80	0.80
Spring	100	PCU	0.82	0.81	0.81
Spring	75	Urea-PCU	0.81	0.80	0.81
Spring	100	Urea-PCU	0.82	0.81	0.82
Spring	75	Urea-UAN	0.82	0.80	0.81
Spring	100	Urea-UAN	0.82	0.82	0.82
Spring	150	Urea	0.82	0.81	0.81
Oct.	75	Urea	0.83	0.78	0.80
Oct.	100	Urea	0.82	0.80	0.81
Oct.	75	PCU	0.83	0.78	0.81
Oct.	100	PCU	0.82	0.79	0.81
Oct.	75	Urea-PCU	0.82	0.79	0.80
Oct.	100	Urea-PCU	0.82	0.80	0.81
Oct.	75	SU	0.81	0.80	0.81
Oct.	100	SU	0.81	0.81	0.81
Oct.	75	Urea+ENP	0.82	0.80	0.81
Oct.	100	Urea+ENP	0.83	0.79	0.81
Nov.	75	Urea	0.82	0.80	0.81
Nov.	100	Urea	0.82	0.80	0.81
Nov.	75	PCU	0.82	0.80	0.81
Nov.	100	PCU	0.81	0.81	0.81
Nov.	75	Urea-PCU	0.82	0.80	0.81
Nov.	100	Urea-PCU	0.82	0.80	0.81
Nov.	75	SU	0.81	0.79	0.80
Nov.	100	SU	0.81	0.81	0.81
Nov.	75	Urea+ENP	0.82	0.80	0.81
Nov.	100	Urea+ENP	0.83	0.81	0.82
LSD (0.05)‡			NS	0.02	0.02

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is encapsulated nitrapyrin coated urea.
‡ LSD values are to be used to compare all numbers within the same column.

Protein levels differed significantly in Steele County in 2015 and at Ada in 2016 (Table 36). Apparently, fertility levels were adequate for spring wheat yield, but not for maximum protein. Mineralization may have caused the lack of protein differences seen in the combined ANOVA tables. Protein levels varied when treatment means were compared at the 2015 Steele County location (Table 36). Greater protein levels were seen with the 75% rate of urea-UAN application in the spring than all application timings and rates of urea. Protein levels also varied when treatment means from Ada in 2016 were compared (Table 36). The protein concentration of the 75% rate of November applied PCU was 13.1%, which was statistically greater than the protein concentrations of the 75% rates of urea applied in the spring and October at 12.9 and 13.0%, respectively. It is difficult to hypothesize what may have caused varying protein levels due to the complexity of protein establishment. That being said, the increased protein levels seen in Steele County in 2015 with the 75% rate of urea-UAN application in the spring may be aided by N applied later in the plant's life cycle (Brown et al., 2005).

Because protein and yield are related inversely, total protein was collected and evaluated to determine treatment effects across the two variables. Total protein levels were significant only in Casselton in 2015, while Casselton, Ada and data combined across locations were significant in 2016 (Table 37). Differences in treatment means were seen at Casselton in 2015 (Table 37). The 100% rate of November applied SU provided more total protein than the 75% rates of urea applied in the spring, October, and November and the 100% rate of November applied urea. The greater total protein levels seen in the 100% rate of November applied urea can be attributed to increased yields and elevated protein concentrations seen with this treatment. Discrepancies in treatment means for total protein were seen in Casselton in 2016 (Table 37). The 100% rate of Urea+ENP applied in November had more total protein than the 100% rate October applied urea at 727 kg ha^{-1} and 610 kg ha^{-1}, respectively. The averaged treatment means found in Ada in 2016 showed the total protein levels of the 100% rate of PCU applied in the spring was statistically similar to the 100% rate of urea applied in the spring, October, and November. The combined across location treatment means in 2016 showed the 100% rate of Urea+ENP applied in October or November had similar total protein levels to the 75% rate of urea applied in October and November, as well as the 100% rate of urea applied in the spring, October, and November (Table 37). Similarities between these treatments are caused by comparable protein and yield levels.

Although differences in dependent variables were found, complex treatment analyses do not clearly reveal reasons for data dissimilarities. Therefore, the data were further analyzed to see if date of application, rate of fertilizer applied, type of fertilizer used, and any interactions between these factors affected the GreenSeeker RNDVI readings, Crop Circle RNDVI readings, yield, protein, or total protein.

Fall Factorial Comparisons

In 2015 and 2016, select treatments that were balanced for the number of levels for the factors that were used to develop the full treatment list, were analyzed as a factorial to look closer at the effect of the main factors of rate, timing, and fertilizer type, as well as their interactions. The factors analyzed were N rate (75 and 100%), fertilizer application timing (October and November), and fertilizer type (urea, PCU, Urea-PCU, SU, and Urea+ENP). Bartlett's test for homogeneity error of variance was not significant when testing the experiments at each environment; therefore, locations across growing seasons were also combined in the ANOVA for yield, protein, and total protein. Only in 2016 were RNDVI measurements taken. Therefore, Bartlett's test for homogeneity of error variance was conducted for RNDVI measurements across 2016 growing locations. No significance between locations was found, and therefore experiments conducted in 2016 locations were combined.

The combined ANOVA showed N rates behaved differently between locations for yield (Table 38). The 2015 locations yielded less than 2016 locations (Fig. 4). One factor that affected the yield differences between years was caused by the applied rate determination structure of N rate treatments in 2015 compared to 2016 when N rates accounted for the fall N soil test and previous crop credit. In 2015 in Steele County there were no differences in yield between the 75% and 100% rate of N. In 2015 and 2016 in Casselton and in 2016 in Ada yield increases varied when the 100% rates of N was applied compared to the 75% rate (Fig. 4). The greatest yield increase occurred at Casselton in 2015. Wheat was the previous crop grown at this location and its previous production in a field does not provide a crop credit, which results in low initial soil N levels. In addition, current N rate determination recognizes that greater than a 907 kg ha^{-1} straw residue results in greater tie-up of N in the soil the subsequent year. This modification of N rate was not considered in 2015. This, along with poorly drained soils and heavy rainfall in May allowed for preponderant yield differences.

The rate by timing by fertilizer type interaction affected yield at Casselton in 2015. The causes of this interaction cannot easily be understood given its complexity. Rate also impacted yield at Casselton in 2015 (Table 39). The 100% rate of N produced more yield than the 75% rate of N. Yield was not statistically different for all interactions and main effects at Steele County in 2015 (Table 38). High levels of mineralization and excellent soil fertility may have prevented discernable yield differences.

Table 34. Spring wheat yield means in 2015 and 2016 at locations and combined across locations by year.

			2015			2016		
Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined	Casselton	Ada	Combined
	-%-		----------------------------------kg ha^{-1}-------------------------------					
-	-	Check	2 538	3 198	2 868	4 354	3 660	4 007
Spring	75	Urea	3 381	3 408	3 394	4 546	5 014	4 780
Spring	100	Urea	3 665	3 302	3 484	4 786	5 386	5 086
Spring	75	PCU	-	-	-	4 663	5 422	5 043
Spring	100	PCU	-	-	-	4 751	5 578	5 165
Spring	75	Urea-PCU	-	-	-	4 848	5 132	4 990
Spring	100	Urea-PCU	-	-	-	4 995	5 218	5 106
Spring	75	Urea-UAN	3 714	3 028	3 371	4 593	5 088	4 841
Spring	100	Urea-UAN	3 794	3 272	3 533	4 812	5 518	5 165
Spring	150	Urea	-	-	-	4 976	5 071	5 023
Oct.	75	Urea	3 255	3 326	3 290	5 154	5 020	5 087
Oct.	100	Urea	3 883	3 309	3 596	4 712	5 495	5 104
Oct.	75	PCU	3 454	3 091	3 272	5 349	4 996	5 172
Oct.	100	PCU	3 884	3 293	3 588	5 251	4 940	5 095
Oct.	75	Urea-PCU	3 227	2 887	3 057	5 049	4 741	4 895
Oct.	100	Urea-PCU	3 745	3 160	3 452	4 958	5 436	5 197
Oct.	75	SU	3 415	3 093	3 254	5 077	5 053	5 065
Oct.	100	SU	3 354	3 343	3 349	5 094	5 437	5 265
Oct.	75	Urea+ENP	3 364	3 116	3 240	4 930	5 365	5 147
Oct.	100	Urea+ENP	3 759	3 445	3 602	5 214	5 306	5 260
Nov.	75	Urea	3 619	3 355	3 487	5 251	5 068	5 160
Nov.	100	Urea	3 560	3 381	3 471	4 832	5 356	5 094
Nov.	75	PCU	3 742	3 060	3 401	5 149	5 159	5 154
Nov.	100	PCU	3 802	3 638	3 720	4 849	5 483	5 166
Nov.	75	Urea-PCU	3 436	3 329	3 382	5 000	5 404	5 202
Nov.	100	Urea-PCU	3 686	3 285	3 486	4 916	5 420	5 168
Nov.	75	SU	3 060	3 440	3 250	5 019	4 915	4 967
Nov.	100	SU	4 250	3 325	3 787	4 917	5 293	5 105
Nov.	75	Urea+ENP	3 399	2 967	3 183	4 967	5 058	5 012
Nov.	100	Urea+ENP	3 521	3 325	3 423	5 307	5 444	5 375
LSD (0.05)‡			583	NS	NS	NS	495	NS

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is encapsulated nitrapyrin coated urea.

‡ LSD values are to be used to compare all numbers within the same column.

Table 35. Correlation coefficients (r) for RNDVI values and yield and protein at two locations in North Dakota, 2016.

	Casselton		Ada	
RNDVI	Yield	Protein	Yield	Protein
GreenSeeker Feekes 2	0.74**	0.74**	0.55**	0.15
GreenSeeker Feekes 7	0.57**	0.33	0.84**	0.57**
GreenSeeker Feekes 10	0.53**	0.36*	0.85**	0.53**
Crop Circle Feekes 10.51	0.66**	0.17	0.88**	0.61**

** Significant at the 0.01 probability level
* Significant at the 0.05 probability level

The rate by fertilizer type interaction was significant for yield at Casselton in 2016 (Fig. 5). An increase in fertilizer rate from 75% to 100% did not result in a statistical difference in yield for Urea-PCU and SU. Despite this, a crossover interaction was seen between Urea+ENP, urea, and PCU (Fig. 5). The crossover interaction resulted from Urea+ENP applied at the 100% rate yielded more than the 75% rate, while yield decreased when urea or PCU were applied at the higher rate. A diverging response occurred when the 100% rates of PCU and urea were compared. Although differences between yields decreased with both types of fertilizer when additional N was applied, reduced yield loss was seen with PCU application. This differed from work by Geng et al. (2016). In this study, yield showed a converging rate by fertilizer type interaction when comparing the 70% and 100% rates of PCU and urea. Both urea and PCU yielded more when the 100% rate of N was applied, but a greater yield increase was seen with PCU use. It is unclear why the 100% rates of urea, PCU, Urea-PCU, and SU yielded less than the 75% rates in this study.

The main effect of rate was significant in Ada in 2016 (Table 39). The additional N applied with the 100% rate resulted in an increase of 283 kg ha^{-1} compared with the 75% rate of N. A field study by Ayoub et al. (1994) also found increased N rates produced more spring wheat yield when averaged across four wheat varieties. Nitrogen was applied at 60, 120, and 180 kg ha^{-1}; the wheat yielded 2.9, 3.0, and 3.1 t ha^{-1}, respectively. The increase in yield was attributed to greater kernel per spike counts. Kernel per spike counts were not collected in our research, therefore, it is unclear if the additional N provided in the 100% rate yielded more due to additional kernels per spike.

Differences in protein were not significant in the combined ANOVA for the main effects and all interactions (Table 38); similar results were seen at Casselton in 2015 and 2016 (Table 40). However, significant differences were recorded at Steele County in 2015 and at Ada in 2016 (Table 40).

Differences in protein levels were seen at Steele County in 2015 and at Ada in 2016 when comparing the rate by timing by fertilizer type interaction. Differences in protein levels occurred in the rate by application timing interaction at Ada in 2016 (P=0.0498). Differences between protein levels increased when October application rates increased from 75 to 100% compared to the November application timing (Fig. 6). The delayed application timing seen with N applied in November reduced the amount of time N was exposed to nitrification that fall. Differences between years may have been caused by discrepancies in N rate calculations. Rate was also significant at Steele County in 2015 and Ada in 2016 (Table 40). Greater protein levels were seen when the 100% rate of fertilizer was used instead of the 75% rate. This is caused by greater N availability to the plant after yield establishment.

Spring Factorial Comparisons

A second factorial in 2016 was analyzed to look closer at two N rates (75% and 100%), three application timings (spring, October, and November), and three fertilizer types (urea, PCU, and Urea-PCU) and their possible interactions. Bartlett's homogeneity of variance test was conducted with no significance.

Table 36. Mean protein (at 12.5% moisture) as a result of treatment, 2015 and 2016 locations and combined across location within year.

			2015			2016		
Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined	Casselton	Ada	Combined
	-%-		--------------------------------------			%-----------------------------------		
-	-	Check	11.2	12.8	12.0	13.0	12.0	12.5
Spring	75	Urea	11.6	13.2	12.4	14.0	12.4	13.2
Spring	100	Urea	11.9	13.2	12.6	14.2	12.9	13.6
Spring	75	PCU	-	-	-	14.5	12.5	13.5
Spring	100	PCU	-	-	-	14.3	13.1	13.7
Spring	75	Urea-PCU	-	-	-	13.9	13.0	13.5
Spring	100	Urea-PCU	-	-	-	14.1	13.0	13.5
Spring	75	Urea-UAN	11.0	13.8	12.4	14.2	12.5	13.4
Spring	100	Urea-UAN	12.2	13.5	12.8	14.4	13.0	13.7
Spring	150	Urea	-	-	-	14.7	13.2	13.9
Oct.	75	Urea	10.5	12.9	11.7	14.0	12.4	13.2
Oct.	100	Urea	11.5	13.1	12.3	13.3	13.1	13.2
Oct.	75	PCU	11.9	13.2	12.5	13.2	12.3	12.8
Oct.	100	PCU	11.8	13.3	12.5	13.3	13.1	13.2
Oct.	75	Urea-PCU	11.1	13.2	12.2	13.5	12.6	13.1
Oct.	100	Urea-PCU	11.2	13.3	12.2	13.8	12.5	13.2
Oct.	75	SU	11.1	12.9	12.0	14.1	12.7	13.4
Oct.	100	SU	11.9	13.1	12.5	13.6	12.8	13.2
Oct.	75	Urea+ENP	11.7	13.1	12.4	14.2	12.2	13.2
Oct.	100	Urea+ENP	11.1	13.0	12.1	13.9	12.9	13.4
Nov.	75	Urea	11.5	13.2	12.3	13.9	13.0	13.4
Nov.	100	Urea	11.1	12.9	12.0	14.0	12.8	13.4
Nov.	75	PCU	11.2	13.2	12.2	13.8	13.1	13.5
Nov.	100	PCU	11.9	13.2	12.5	13.9	12.9	13.4
Nov.	75	Urea-PCU	11.5	12.9	12.2	13.7	12.5	13.1
Nov.	100	Urea-PCU	11.5	13.3	12.4	13.9	13.1	13.5
Nov.	75	SU	10.4	13.1	11.8	13.2	12.9	13.0
Nov.	100	SU	12.9	13.4	13.1	13.9	12.7	13.3
Nov.	75	Urea+ENP	10.7	13.1	11.9	13.1	12.6	12.8
Nov.	100	Urea+ENP	11.1	13.4	12.3	14.1	12.9	13.5
LSD (0.05)‡			NS	0.4	NS	NS	0.5	NS

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of the rate applied as urea and 50% applied as PCU, Urea-UAN is 50% urea applied /incorporated spring plus 50% as UAN streamed at 4 leaf stage, SU is Super-U, Urea+ENP is encapsulated nitrapyrin coated urea.

‡ LSD values are to be used to compare all numbers within the same column.

Table 37. Mean total protein yield due to treatment for spring wheat locations and locations combined within year, 2015 and 2016.

			2015			2016		
Application Timing	Rate	Fertilizer Type †	Casselton	Steele County	Combined	Casselton	Ada	Combined
	-%-		---------------------------------kg ha^{-1}------------------------------					
-	-	Check	284	398	341	552	427	490
Spring	75	Urea	383	437	410	618	602	610
Spring	100	Urea	426	423	424	660	676	668
Spring	75	PCU	-	-	-	655	657	656
Spring	100	PCU	-	-	-	659	711	685
Spring	75	Urea-PCU	-	-	-	656	649	652
Spring	100	Urea-PCU	-	-	-	686	653	669
Spring	75	Urea-UAN	401	402	401	636	615	626
Spring	100	Urea-UAN	449	427	438	674	695	684
Spring	150	Urea	-	-	-	711	649	680
Oct.	75	Urea	334	417	376	703	607	655
Oct.	100	Urea	436	421	429	610	697	654
Oct.	75	PCU	403	395	399	703	596	650
Oct.	100	PCU	448	424	436	679	627	653
Oct.	75	Urea-PCU	352	370	361	660	582	621
Oct.	100	Urea-PCU	412	406	409	664	663	663
Oct.	75	SU	369	386	377	697	626	661
Oct.	100	SU	389	427	408	671	677	674
Oct.	75	Urea+ENP	386	395	390	679	639	659
Oct.	100	Urea+ENP	405	436	421	707	666	686
Nov.	75	Urea	405	430	418	709	638	674
Nov.	100	Urea	383	423	403	657	665	661
Nov.	75	PCU	409	392	400	692	659	676
Nov.	100	PCU	442	466	454	656	687	672
Nov.	75	Urea-PCU	384	418	401	667	658	662
Nov.	100	Urea-PCU	412	424	418	665	687	676
Nov.	75	SU	307	438	373	644	616	630
Nov.	100	SU	531	431	481	688	654	671
Nov.	75	Urea+ENP	356	376	366	631	619	625
Nov.	100	Urea+ENP	384	432	408	727	682	705
LSD (0.05)‡			107	NS	NS	71	76	73

† PCU is Environmentally Smart Nitrogen, Urea-PCU is 50% of rate is urea and 50% applied as PCU, Urea-UAN is 50% urea and 50% is UAN, SU is Super-U, Urea+ENP is urea+encapsulated nitrapyrin.

‡ LSD values are to be used to compare all numbers within the same column.

The location by timing by fertilizer type interaction statistically impacted the yield response in the combined ANOVA (Table 41). However, the complexity of the interaction makes it difficult to know the cause of significance. The location by rate and location by application timing interactions differences were significant for yield in the combined ANOVA (Table 41).

Table 38. The combined ANOVA F values and significance for the main effects of rate, timing, and fertilizer type and their interactions for yield and protein when averaged across four environments.

Sources	Yield	Protein
Rate (R)	3.66	8.08
Timing (T)	2.04	3.96
Fertilizer Type (F)	0.78	0.61
RxT	11.24	0.61
RxF	0.78	0.72
TxF	0.77	0.77
RxTxF	0.70	1.87
Location (L)xR	5.12**	0.99
LxT	0.70	0.20
LxF	0.64	0.78
LxRxT	0.02	2.15
LxRxF	1.03	1.28
LxTxF	0.93	0.82
LxRxTxF	1.72	1.01

** Significant at the 0.01 probability level.
* Significant at the 0.05 probability level.

A crossover interaction occurred in the location by rate interaction in the combined ANOVA (Fig. 7). Yield decreased in Casselton when the rate of N increased from 75 to 100%. In Ada, yield increased when the 100% rate of N was applied compared to the 75% rate. It is unclear why yield decreased in Casselton at the elevated rate.

Data converged in the location by application timing interaction (Fig. 8). When N was applied in October instead of in the spring at Casselton yield increased, but decreased in Ada.

Casselton yielded more when N was applied in October than it did in November. In contrast, Ada yielded more with November N application than October. The Ada location had sandier soil that was more prone to leaching than the soil type at Casselton, which may explain why November and October applications caused greater yield differences at their respective locations. A converging yield response was also seen when spring application was compared to November N application (Fig. 8). Smaller yield differences between the two locations were seen with the November application timing than with N applied in the spring. Nearly all N taken up and used by plants is in the NO_3^--N form (Glass et al., 2002). Nitrogen applied in November at Casselton would have additional time for nitrification to occur to compensate for the warmer soil temperature and soil moisture and aeration balance in Ada that favors nitrification. Lower soil temperatures in the spring and waterlogged soil in Casselton may have inhibited the nitrification process compared to Ada. Therefore, less NO_3^--N was available for plant uptake at Casselton.

Fig. 4. Location by N rate interaction for spring wheat yield across four locations, 2015 and 2016 (LSD used to compare all means=163).

Table 39. The mean spring wheat yield for the main effects of fertilizer rate, application timing, and fertilizer type within 2015 and 2016 locations and combined across locations and years.

		2015		2016		2015-16
		Casselton	Steele County	Casselton	Ada	Combined
		---------------------------------kg ha^{-1}				------------------------------
Rate	75	3 397	3 170	5 093	5 078	4 184
	100	3 744***	3 350	5 006	5 361**	4 365
Timing	Oct.	3 534	3 209	5 077	5 179	4 250
	Nov.	3 608	3 310	5 022	5 260	4 300
Fertilizer	Urea	3 579	3 343	4 987	5 235	4 286
	PCU	3 720	3 270	5 145	5 145	4 320
	Urea-PCU	3 523	3 173	4 981	5 250	4 232
	SU	3 520	3 300	5 028	5 174	4 256
	Urea+ENP	3 511	3 213	5 106	5 293	4 281
LSD (0.05)†		NS	NS	NS	NS	NS

† LSD compares yield averages of the same main effect in the same column.

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

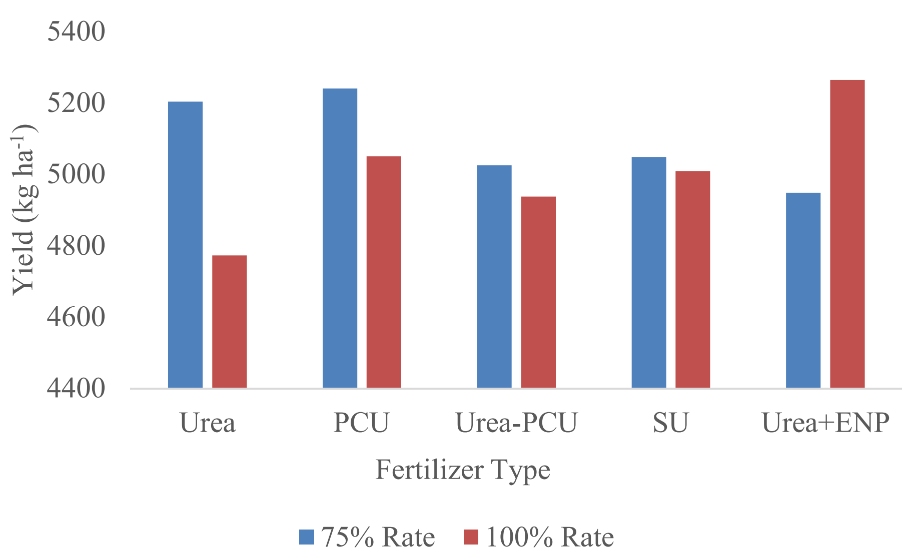

Fig. 5. Spring wheat yield response to the fertilizer rate by fertilizer type interaction at Casselton in 2016 (LSD used to compare all means=93).

Table 40. Mean wheat protein and significance for the main effects of rate, timing, and fertilizer type by location and combined across locations and years.

		2015		2016		2015-16
		Casselton	Steele County	Casselton	Ada	Combined
		--------------------------------%----------------------------------				
Rate	75	11.2	13.1	13.7	12.6	12.6
	100	11.6	13.2*	13.8	12.9*	12.9
Timing	Oct.	11.4	13.1	13.7	12.7	12.7
	Nov.	11.4	13.2	13.7	12.8	12.8
Fertilizer	Urea	11.1	13.0	13.8	12.8	12.7
	PCU	11.7	13.2	13.6	12.8	12.8
	Urea-PCU	11.3	13.2	13.7	12.7	12.7
	SU	11.6	13.1	13.7	12.8	12.8
	Urea+ENP	11.1	13.1	13.8	12.6	12.7
LSD (0.05)†		NS	NS	NS	NS	NS

† LSD compares protein averages for the fertilizer type main effect in the same column.
* Significant at the 0.05 probability level for rate effect between sites within a year.

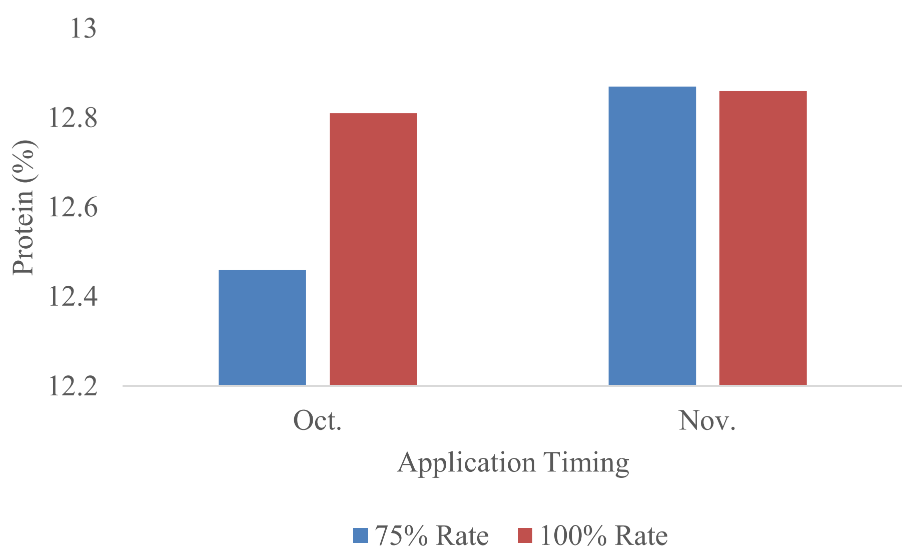

Fig. 6. Application timing by rate interaction for protein in spring wheat at Ada in 2016 (LSD used to compare all means=0.26).

Differences in yield levels were seen at Casselton in 2016 when N rate by application timing interactions were compared (Fig. 9). A converging response occurred between spring applied rates and rates applied in November. The additional N applied with the 100% rate in the spring increased yield by 158 kg ha^{-1}, while the additional N applied with the 100% rate in November decreased yield levels by 268 kg ha^{-1}. It is unclear why the additional N applied in November decreased yield levels at Casselton. The 100% rate of N was well within acceptable application rates, therefore, it is highly unlikely that NH_4^+ toxicity or excessive salts would have reduced yield levels. Stand counts were not taken on an entire plot basis, thus, differences in plant stand may have affected yield levels.

Table 41. F values and significance for the main effects and interactions of wheat yield and protein from the combined ANOVA table.

Sources	Yield	Protein
Rate (R)	0.18	1.48
Timing (T)	0.27	2.53
Fertilizer Type (F)	2.98	0.27
RxT	0.55	0.20
RxF	0.34	0.13
TxF	0.11	0.98
RxTxF	0.76	0.63
Location (L)xR	8.73**	1.67
LxT	5.39**	2.32
LxF	0.19	0.17
LxRxT	1.73	0.89
LxRxF	0.76	0.91
LxTxF	2.78*	0.76
LxRxTxF	0.81	1.33

** Significant at the 0.01 probability level.
* Significant at the 0.05 probability level.

There was a significant difference in yield production levels seen at Ada in 2016 when comparing the main effect of rate when averaged across application timing and fertilizer type ($P<0.05$). When a 75% rate of N was applied, the average yield was 5106 kg ha^{-1}, the additional N applied with the 100% rate increased yield levels by 262 kg ha^{-1}. A similar yield increase was seen when elevated N levels were applied in a North Dakota field study by Otteson et al. (2007). The increased N levels allowed additional tillers and increased spikes to be produced. Therefore, the increased yield in this study may be explained by the elevated N levels allowing wheat plants that produced more tillers and spikes.

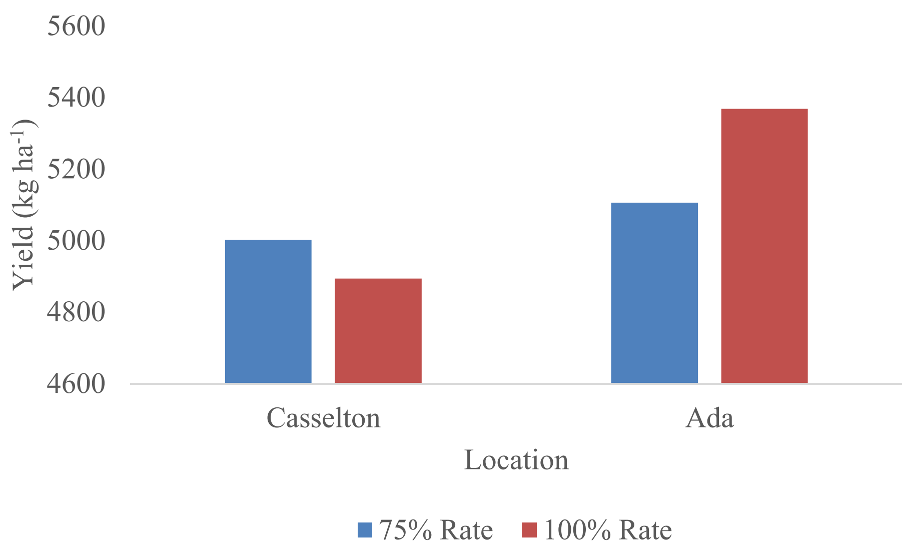

Fig. 7. The location by rate interaction for yield levels was significant for the 2016 wheat combined ANOVA (LSD used to compare all means=123).

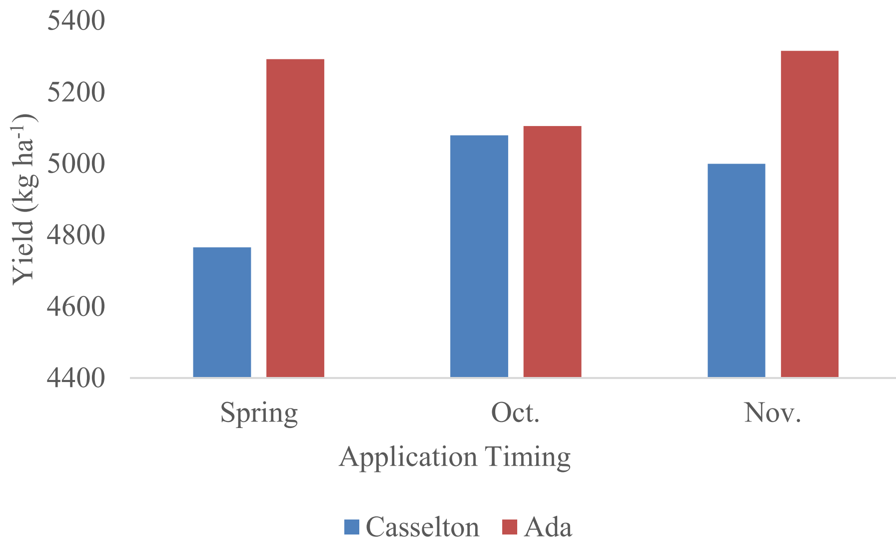

Fig. 8. The location by application timing for protein levels was significant for the 2016 wheat combined ANOVA (LSD used to compare all means=151).

There were no significant differences in protein levels seen with interactions or main effects in the combined ANOVA (Table 41). However, significant differences in protein levels were seen at Casselton and in Ada (Table 42). At Casselton in 2016, significant differences in protein levels were seen when the N was applied at different times. The protein levels when N was applied in October were statistically lower than when N was applied in the spring. The delayed fertilizer application shortened environmental exposure time that is conducive to N loss. Thus, more N was available for nutrient uptake by the plant. Differences in protein levels were also seen with varying N application rates at Ada in 2016 (Table 42). When the 75% rate of N was applied, the protein level was 12.6%. With the additional N applied with the 100% rate, protein increased 0.3%. The increased N availability seen with higher N rates may allow for greater amino acid production, which is essential for protein synthesis (Brown et al., 2005).

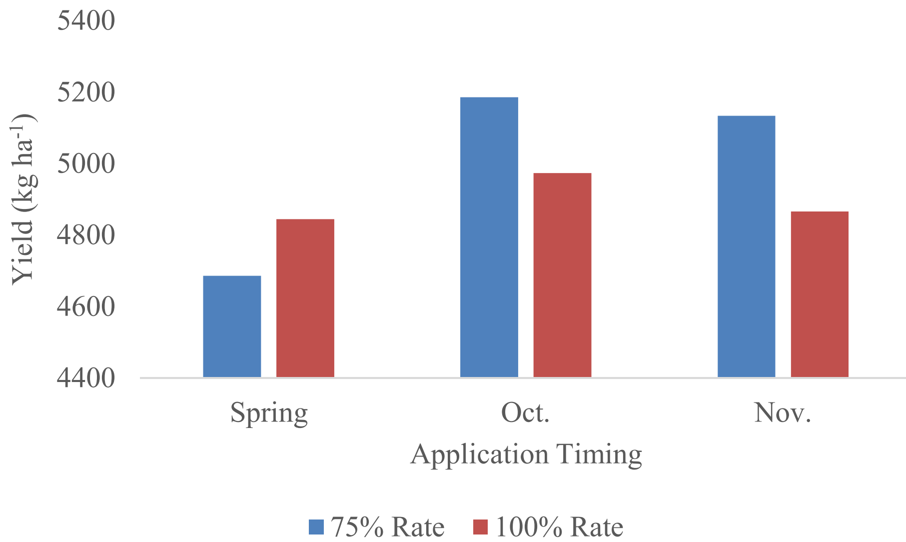

Fig. 9. The N rate by application timing interaction at Casselton in 2016 significantly affected yield levels when averaged across three fertilizer types (LSD used to compare all means=246).

Table 42. Wheat protein averages and significance for the main effects of nitrogen rate, timing of application, and fertilizer type at Casselton and Ada in 2016 and combined across the two locations.

		Casselton	Ada	Combined
			--------%-------	
Rate	75	13.8	12.6	13.2
	100	13.9	12.9*	13.4
Timing	Spring	14.2	12.8	13.5
	Oct.	13.5	12.7	13.1
	Nov.	13.9	12.9	13.4
LSD (0.05)†		0.4	NS	NS
Fertilizer	Urea	13.9	12.8	13.3
	PCU	13.8	12.8	13.3
	Urea-PCU	13.8	12.8	13.3
LSD (0.05)†		NS	NS	NS

† LSD compares protein averages of the same main effect in the same column
* Significant at the 0.05 probability level.

CONCLUSIONS

The data from the PCU release experiment, showed that regardless of depth of placement, PCU released nitrogen relatively slowly in the environment. Most, but not all of the urea was released from the PCU-N granules by the end of the six-week experiment. This release pattern more closely follows the N demand of crops than urea, which usually dissolves within a few hours of application if moisture is present and could be a very useful tool in reducing N losses in those environments where losses are high. From the field experiments where PCU was applied to either corn or wheat, there was only limited data to support a higher yield and protein level from the use PCU. This may have been due to limited N losses in the environments where these experiments were conducted.

Fertilizer NH_4^+-N and NO_3^--N levels varied due to environmental differences. The levels of NH_4^+-N were similar between the untreated check and all other fertilizers. This is most likely caused by the high levels of mineralization seen in 2016. Thus, the nitrification and urease inhibitors, as well as the CRF were not beneficial this growing season. Total N is calculated as the sum of NH_4^+-N and NO_3^--N, therefore, more plant available inorganic N is available with greater total N values. Greater total N content was seen with SU application than the other fertilizer types.

High levels of mineralization and excellent soil fertility in 2015 and 2016 made testing nitrification and urease inhibitors, as well as CRF in corn difficult. For the most part, RNDVI readings taken in corn with the GreenSeeker and Crop Circle hand held devices did not show differences for all treatment comparisons, particularly at sites with no yield response to N. In 2015, the addition of N at any rate, application timing, or fertilizer type yielded more than the untreated check. No differences in corn yield were seen between treatments, including the untreated check in 2016. Overall, the data from the past two years shows applying nitrogen to corn at any rate, application timing, or form did not increase yield. The past two growing seasons in North Dakota do not accurately reflect the additional N requirements required of corn. Further experiments should be conducted under diverse environments to see if varied rates, application timings, and/or fertilizer types impact corn yield in North Dakota. Differences in corn grain protein levels were noted in 2016 across all treatments, the fall factorial, and the spring factorial.

Similar to the corn trials, high levels of mineralization and excellent soil fertility in 2015 and 2016 caused few if any discernable differences in dependent traits in spring wheat. When all data are compared, applying N at any rate, timing, or form did not increase wheat yield. However, applying a 100% rate of N yields significantly more than when a 75% rate is applied to wheat, according to the fall factorial. The spring factorial demonstrated that significantly more yield can be obtained in sandier soils with lower organic matter content, like Ada, MN, with the additional N applied in a 100% rate of N compared to a 75% rate. Fertilizer applied closer to plant utilization increased wheat yield in these soil types, as well. Soils containing greater clay content produce more yield when N is applied in October. Nitrogen application did not increase wheat protein content in 2015 and 2016.

Overall, N was an unnecessary input for corn and wheat production systems in 2015 and 2016 due to high levels of mineralization. Continuing this research over a wider range of variable growing season climates that North Dakota growers are subjected to over their farming career may reveal fertilizer rate, application timing, and type of fertilizer effects on yield and protein in corn and wheat production systems.

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