PREDICTING AND ENHANCING SPRING WHEAT GRAIN PROTEIN CONTENT THROUGH SENSING AND IN-SEASON NITROGEN FERTILIZATION

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Matthew John Rellaford

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

April 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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MASTER OF SCIENCE

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ABSTRACT

Grain protein content is an essential component to producing a profitable Hard Red Spring Wheat (HRSW) (*Triticum aestivum* L.) crop in the northern Great Plains. Growers can increase grain protein content through in-season N fertilization; however, the cost of these applications may outweigh the benefits. Predicting the grain protein content of early-season HRSW would give growers crucial information as they decide whether to apply in-season fertilizer to boost grain protein content. This research encompasses three studies; two of which aim to predict grain protein content with hand-held and aerial sensors respectively, and a third, which investigates the optimal rate, timing, and source of N fertilizer to boost grain protein content. Results of these experiments seemed to be greatly influenced by environmental factors. Findings of this research suggest that an in-season N application should be used for ameliorative purposes and not as a regular practice.

ACKNOWLEDGEMENTS

I would first like to acknowledge Dr. Joel Ransom for his support of me as a student. Having only taken one agriculturally related class prior to enrolling at North Dakota State, I felt that he took a risk taking me on. I am grateful that he took a chance on me, and I have learned incredible amount scientific skills from him. I’m grateful for his patience and understanding; Dr. Ransom is someone that you know cares about you. One highlight of my studies here at NDSU has been travelling with Dr. Ransom to East Africa where I gained a deeper understanding in the complexities and challenges relating to food security issues in that region. Joel has also supported my travel to several conferences, all of which have been a great experience for me.

I also want to take the time to thank Dr. Hans Kandel. Although Dr. Kandel is technically a committee member, he was much more than that. He has given me great advice both regarding my current research and my future. I’m grateful for his detailed comments and critiques that have helped me succeed in this research. I’m also grateful for John Nowatzki and Dr. Stephanie Day for serving on my graduate thesis committee. Dr. Day taught me everything I needed to know in ArcGIS to carry out this project.

None of this research would be possible without the help of the research specialists in the NDSU extension program. I want to give a special thanks to Chad Deplazes, Grant Mehring, and Darin Eisinger for their support and suggestions. Darin has helped me a lot with learning the complicated system behind flying drones. In addition, I want to thank the fellow graduate students in our program who helped carry out this research: Calli Feland, Tracy Hillenbrand, Lizzy Lovering, Nick Schimek, and Missy Geiszler. I further wish to acknowledge and thank the extension team, led by John Rickertsen, for planting and maintaining the Hettinger trial of this project.

This research also would not have been possible without the generous support from the Minnesota Research and Promotion Council and the Minnesota Association of Wheat Growers. As part of my graduate studies I was privileged to take part in an inaugural Ag Peer Group run through the Minnesota Wheat Growers that has enabled me to interact and learn from like-minded young farmers and other agricultural professionals. Special thanks goes to Lauren Proulx of the Minnesota Wheat Growers who helped coordinate much of the drone research in 2017. I'm also very grateful for Dave Torgerson for his support.

Finally, I'm grateful to God and my wife, Maja, and son, Henrie. God clearly led me to this program here at North Dakota State, and Maja and Henrie have supported me 110% of the way.

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LIST OF ABBREVIATIONS

HRSW	Hard Red Spring Wheat (*Triticum aestivum* L).
N	Nitrogen.
NDVI	Normalized Difference Vegetation Index.
NDRE	Normalized Difference Red Edge Index.
UAV	Unmanned Aerial Vehicle.
NIR	Near infrared reflectance.
UAN	Urea ammonium nitrate.
ZGS	Zadoks growth stage.
NUE	Nitrogen use efficiency.
NH_3	Ammonia.

INTRODUCTION

In the northern Great Plains of the United States and Canada, grain protein content can significantly impact the price that growers of hard red spring wheat (HRSW) (*Triticum aestivum* L.) receive. Grain protein content determines the price premiums and discounts, which, depending on the year, can have a marked impact on a farm's profitability (Brown et al., 2005; Jones and Olson-Rutz, 2012). Although growers are unable to predict price premiums and discounts placed on grain protein, decades of research have shown that they can increase the protein level in their crop using in-season nitrogen fertilization (Alkier et al., 1972; Bly and Woodard, 2003; Brown and Petrie, 2005; Fowler et al., 1990; and Otteson, et al. 2007). In-season N applications come at a cost though, and growers are resigned to make crucial economic and agronomic decisions with limited data on the crop's protein status. In addition, growers are coming under increasing public pressure to better manage fertilization as nutrient losses carry negative environmental side-effects (Johnston and Bruulsema, 2014).

Predicting HRSW grain protein content early in the growing season would give producers crucial data to determine whether they should fertilize to boost protein. Spectral indices such as the Normalized Difference Vegetation Index (NDVI) and, more recently, the Normalized Difference Red Edge Index (NDRE) are a fundamental component of sensing that can gauge status of the N supply to the grain of the early-season crop. These spectral indices have shown promise at predicting yield, but their ability to predict protein has been limited (Li-Hong et al., 2007). Furthermore, the limited research using early-season sensors to predict grain protein content has shown the most predictive sensing timings to be close to the heading stage (MacNack et al., 2014). Using sensors to predict grain protein content even earlier in the season would be more beneficial for growers as they would have more time to make decisions on in-season fertilization; and, considering the large farm size in the northern plains, producers would have a longer time window to apply in-season fertilizer. In addition, better methods for sensing need to be investigated further, as ground-based sensors are limited in their spatial and temporal ability to gather data.

This research explores the utility of ground-sensed NDVI and NDRE at predicting grain protein content in HRSW. In doing so, it examines whether these spectral indices are predictive of grain protein content, and if so, the strength of their predictive relationship. It examines whether NDVI and NDRE can predict grain protein content at the earliest growth stage when an in-season N application might first be applied. In addition to using ground-based sensors, this research includes a component of testing aerial sensors on a larger scale. Finally, it explores how in-season nitrogen applications at different rates and timings can influence grain protein content. These results will provide a greater foundation in the quest to develop decision support tools to help growers maximize economic returns while minimizing environmental damages.

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ARTICLE I: PREDICTING GRAIN PROTEIN CONTENT OF SPRING WHEAT WITH HAND-HELD AND AERIAL SENSORS

Abstract

Predicting the grain protein content of early-season Hard Red Spring Wheat (HRSW) (*Triticum aestivum* L.) would give growers crucial information as they decide whether to apply in-season fertilizer to boost grain protein content. This article looks at two experiments investigating the utility of using the normalized difference vegetation index (NDVI) and the normalized difference red edge index (NDRE) at predicting grain protein content. In the first experiment, NDVI was measured with a hand-held sensor on small plot trials in North Dakota and Minnesota at three wheat growth stages: the four-leaf, flag-leaf, and boot stages. NDVI results were then regressed with grain protein content to determine whether there was a predictive relationship. The second experiment used a sensor fixed to an unmanned aerial vehicle (UAV) to collect NDVI and NDRE at on-farm trials that included an N-rich strip and a check with fertilizer applied based on the management objectives of the farm. The change in protein and change in NDVI from the N-rich strip to the normal strip were also analyzed through linear regression. NDVI and NDRE were moderately predictive of grain protein content; however, there was great variability in the predictiveness based on location. Furthermore, it was not possible to distinguish between mid and high protein values thus limiting the utility of these indices under current management practices.

Introduction

Grain protein content plays an important role in the production of HRSW which is renowned for having superior protein content and quality. Increased grain protein content is directly tied to flour baking quality (Barneix, 2006), driving the demand for high-protein HRSW, much of which is exported to countries with a shortage of high quality wheat (Barneix, 2006). The advantageous quality of HRSW understandably warrants a higher price at grain markets; but it also vaults a high threshold of 140 g kg^{-1} grain protein content, below which a price discount often applies. Although producers typically receive a price premium above this threshold, the price discount can have a greater magnitude. For this reason, producers seek to mitigate financial losses by increasing their crop's grain protein content, especially as price premiums and discounts can be very fickle to predict (Brown et al., 2005).

Much of the effort to boost grain protein revolves around nitrogen (N), which is crucial to protein formation in the kernel. Grain protein is mostly derived from remobilized N originating from senesced plant tissues that then free up amino acids, from which N is eventually deposited into the kernel (Barneix, 2006; Clarke et al., 1990; Dalling et al., 2005). However, the plant's demand for nitrogen exceeds the amount provided by N remobilization, and it uses additional N from root reserves and the surrounding soil (Clarke et al., 1990). Additional soil-N will not always boost protein though, as other limiting environmental factors may compromise its efficacy (Fischer et al., 1993; Johansson et al., 2001). Yield and grain protein content are often inversely related; crops with low yield due to environmental restriction tend to have high protein, and vice versa (Cox et al., 1985). This inverse relationship comes about from the grain protein and starch deposition dynamics during grain filling. Early in the grain filling stage an increase in starch will often lead to a dilution effect on grain protein content, because starch at this time is deposited at a faster rate than grain protein (Jenner et al., 1991). Furthermore, a higher yield will require a greater amount of N uptake to proportionately increase grain protein content (Fowler et al., 1990). However, once a crop reaches its yield potential during grain filling, added N is much more likely to boost grain protein content (Jenner et al., 1991). Given these dynamics, fertilization to boost crop yield is best as a pre-plant fall or early spring application (Rehm and Franzen, 2005); whereas, fertilization aimed at enhancing protein should be applied in-season and is most effective between the boot stage (ZGS 45, Zadoks et al., 1974) and post-pollination (ZGS 69) when yield potential is much closer to being met (Brown and Petrie, 2005). Additional N applied at this stage has been proven to boost grain protein, typically between 5-10 g kg^{-1} (Kaiser et al., 2013; Otteson et al., 2007). Grain protein content increases of this range could be very profitable to growers in years with high discounts and premiums for grain protein, but it can be a challenge to visually assess whether in-season N would have the desired effect. As growers must make these decisions in-season, within a limited timeframe, decision support tools for predicting protein could be helpful (Bly and Woodard, 2003).

Remote sensing has arisen as a potential solution to help producers gain in-season crop health data across a large area; especially as the accessibility, usability and affordability of these tools has greatly increased (Nowatzki et al., 2017; Ortiz et al., 2011). Traditionally, remote sensing relied on man-guided aircraft or satellite imagery; however, these technologies are limited in usefulness because they often don’t provide adequate spatial and temporal resolution (Candiago et al., 2015). Recent developments in ground-based and aerial sensors have alleviated this concern, as they can output resolution at the cm level, as opposed to the 30 m resolution of Landsat Imagery (USGS, 2017). Ground-based sensors can be used as small, hand-held devices; but they are typically mounted on ground-based vehicles to cover a larger area. Aerial sensors mounted on unmanned aerial vehicles (UAV) may be a better alternative, as they are able to cover a larger area in a quicker timeframe while still providing superior spatial and temporal resolution. These advantages currently make UAVs the most cost-effective means of remote-sensing (Candiago et al., 2015).

Remote sensing techniques most commonly utilize red and near infrared reflectance (NIR) bands to create an index that measures a given parameter. Various indices have been explored, but the normalized difference vegetation index (NDVI) remains the most commonly used index to measure plant vigor (Nowatzki et al., 2017; Wright et al., 2004). Sensors that output NDVI values measure the reflected red and NIR bands from a surface and use a formula to indicate the greenness of a given area on a 0-1 scale. This index has been shown to be highly significant with the N status of the plant (Freeman et al., 2003) and thus overall plant health and vegetation cover (Elmore et al., 2000); yet it does possess some inherent limitations. Background soil can skew NDVI values downwards when there is limited canopy coverage (MacNack et al., 2014), and dense foliage can result in decreased sensitivity, partly due to its lack of sensitivity to high chlorophyll content (Kanke et al., 2012). Because dense foliage can cause NDVI values to saturate, it becomes increasingly difficult for this index to differentiate between plant N status.

In addressing this concern, the Normalized Difference Red Edge Index (NDRE), known colloquially as “Red Edge”, has been recently explored as an alternative index. This index utilizes a similar calculation as NDVI but is more precise because it narrows in on a “Red Edge Position” between the red (671±10 nm) and NIR (780±10 nm) wavebands making it less susceptible to saturation under high red absorption (Kanke et al., 2012). However, NDVI continues to be the go-to index, perhaps due to the relative novelty and limited availability of products compatible with NDRE sensors.

There is increasing use of NDVI in agriculture as there is strong evidence of its predictability of yield (Li-Hong et al., 2007; Shoch, 2013) and N uptake (Freeman et al., 2003). However, discovering how to use NDVI to predict grain protein content has been much more of a challenge, possibly because NDVI is inefficient at determining N translocation to the grain

(Freeman et al., 2003, Li-Hong et al., 2007). Wright et al. (2004) did find a modest relationship between NDVI taken at the boot stage (ZGS 45) and grain protein content of wheat grown in an irrigated loess soil using a hand-held active sensor (r^2=0.63), but the data were taken for only a single season and location. MacNack et al. (2014) found a decent relationship between NDVI taken at flag leaf (ZGS 37) and grain protein content ($R^2 = 0.56$), yet grain protein data was also only taken during a single growing season and location. Researchers in the Northern Great Plains also found modest relationships between post-boot NDVI and grain protein, but there was high variability between locations, with the best locations only having a modest r^2 values (Qualm et al., 2010; Shoch et al., 2013).

A limitation of many of these studies is that measurements were taken close to or at anthesis. Whilst this may be the timing when NDVI is most predictive of grain protein content, its utility at this stage is limited as it gives producers limited time to decide whether to apply an in-season N application considering the average farm size in the Northern Plains is 526 ha. MacNack et al. (2014) and Shoch et al. (2013) included earlier sensing dates in their studies but found poor relationships between NDVI at those stages and grain protein content. This research seeks to bridge the gap between previous studies by including early and mid-season sensing dates with multiple methods of sensing in order to pinpoint the most feasible and accurate timing when NDVI is predictive of grain protein content. In addition, it investigates the utility of ground-based and aerial NDVI sensors at predicting grain protein content.

Materials and Methods

Experimental Overview

This study utilized data collected from eight small-plots (see Figure 1.1) and four on-farm trials (see Figure 1.2) in North Dakota and Minnesota during the 2016 and 2017 growing seasons. All locations were in the Red River Valley, except for Hettinger, which is in southwestern North Dakota. The small-plot trials investigated the main hypothesis of the project; that is, whether NDVI is predictive of grain protein content. The on-farm trials served as an additional investigation of whether NDVI is predictive of grain protein content on a field scale. Soils in these locations are mollisols with low to high sand content and moderately high organic matter.

Small plot Trials

Weather data were collected from the closest North Dakota Agricultural Weather Network (NDAWN) Station. The 2016-2017 experimental locations with their proximity to the nearest NDAWN station are as follows: Ada, MN (8.7 km); Gentilly, MN (35.2 km); Red Lake Falls, MN (40.6 km); Casselton, ND (16.1 km); Prosper, ND (0.2 km); and Hettinger, ND (0.2 km). Soil characteristics from each research site can be found in Table 1.1. Previous crops were either soybean [*Glycine max* (L.) Merrill.] or HRSW (See Table 1.4). Residual NO_3-N for each location was determined from soil samples taken in the fall prior to planting (see Table 1.2). (Soil testing for Gentilly was conducted in the spring prior to planting). Treatments consisted of an incomplete factorial of N rates and timings and were arranged in a randomized complete (rate) from the North Dakota Wheat Nitrogen Calculator (Franzen and Kariluoma, 2009), which subtracts residual soil NO3-N and previous cover crop credits. Actual amounts of N applied by location can be found in Table 1.4. The rates used were representative of realistic amounts that producers would apply to attain profitable yields and grain protein. An unfertilized, 0 N check was included, and granular urea was hand-broadcast pre-plant for all treatments receiving fertilizer at either the 70% or 100% rate. Six of the treatments received additional N as granular urea or urea-ammonium-nitrate (UAN) at the four-leaf stage. These in-season N rates were added as part of a separate experiment, more of which will be discussed in the statistical analysis of this section. Experimental plots were seeded at a rate of 3 million viable seeds ha-1 with a GreatPlains 35605NT drill (Great Plains Mfg Inc., Salina, KS). In both years all plots consisted of seven rows with 18 cm spacing and spanned 5.9 m in length, with except for the 2016 Casselton plots, which were 3.7 m in length. Planting took place between April 14 and May 1 of each year for all locations except for the 2016 Casselton location, which was seeded on May 19. The late planting at Casselton was intended to provide an additional, lower-yielding environment. Border plots were included on the outside column of experimental plots to ensure similar plot-to-plot competition as interior plots. Weeds were controlled chemically and by hand-hoeing when needed. In 2016 the fungicide combination of propioconazole and trifloxystrobin was applied with a flat fan nozzle during anthesis at a rate of 64 g and 64 g ai ha-1 respectively.

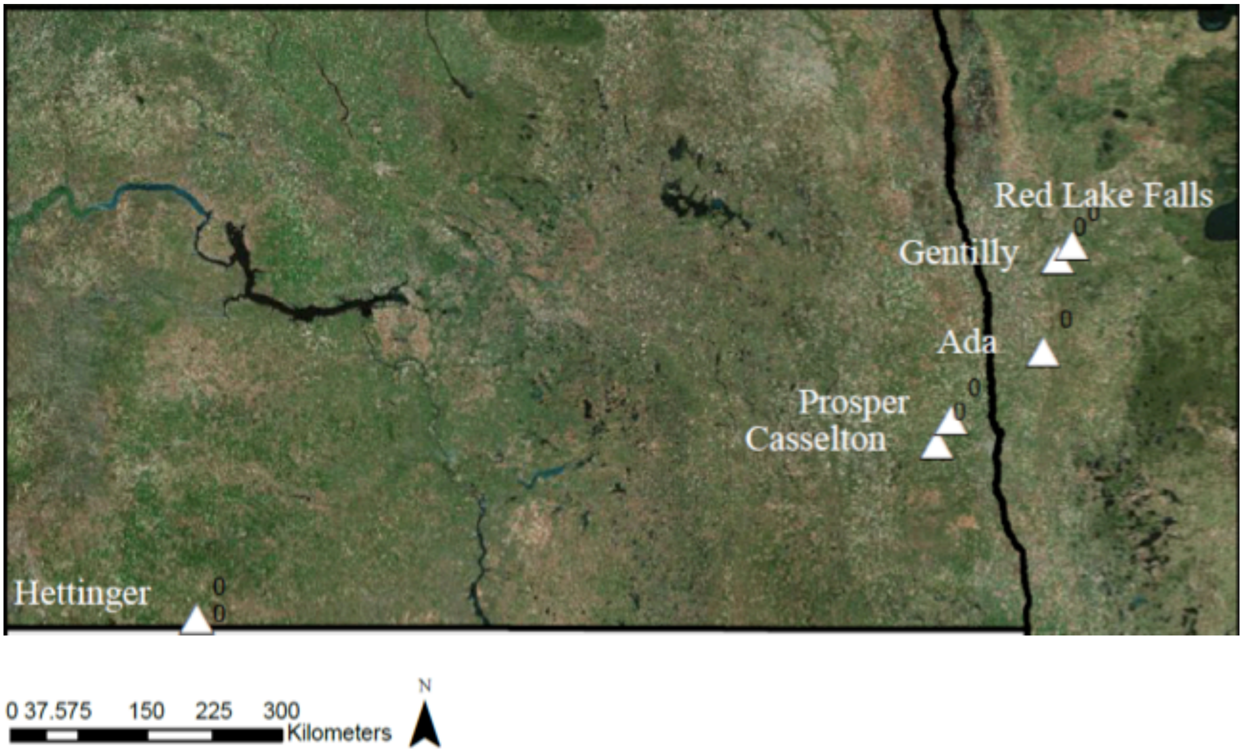

Figure 1.1. Experimental locations for 2016-2017 small-plot trials.

Table 1.1. Soil series, texture, taxonomy, and slope for Ada, Red Lake Falls, Gentilly, MN and Casselton, Prosper, Hettinger ND for 2016 and 2017.

Location	Series†	Texture	Taxonomy	Slope
				%
Ada, 2016	Rockwell	sandy clay loam	Coarse-loamy, mixed, superactive, frigid Typic Calciaquolls	0-2
Ada, 2017	Glyndon	loam	Coarse-silty, mixed, superactive, frigid, Aeric Calciaquolls	0-3
Casselton	Kindred	silty clay loam	Fine-silty, mixed, superactive, frigid Typic Endoaquolls	0-2
Gentilly	Foxlake	silty clay loam	Fine, smectitic, frigid Vertic Epiaquolls	0-2
Hettinger	Belfield	silty clay loam	Fine, smectitic, frigid Glossic Natrustolls	0-1
Prosper	Bearden	silty clay loam	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-3
Red Lake Falls	Wheatville	fine sandy loam	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	0- 3

† Soil data obtained from Web Soil Survey (USDA-NRCS, 2018).

block replicated four times (see Table 1.3). Nitrogen rates were based on recommended rates.

Table 1.2. Nitrate-N,P,K and organic matter at 60 cm depth for small-plot trials in 2016-2017.

Location	NO_3-N	P	K	OM†
Cropping Season 2016	----------------------mg kg^{-1}	--------------------		%
Ada, MN	4	4	26	2.2
Red Lake Falls, MN	15	15	70	3.6
Casselton, ND	5	17	285	4.0
Cropping Season 2017				
Ada, MN	7	11	107	1.9
Gentilly, MN	26	6	196	3.2
Casselton, ND	37	11	368	3.3
Prosper, ND	48	17	222	2.4
Hettinger, ND	33	21	530	3.4

† OM = organic matter.

Table 1.3. Rate and timing of N treatments in 2016-2017.

	N Application Amount and Timing	
Treatment	Pre-plant	4-5 Leaf
		kg ha^{-1}
1	70% rate†	--
2	100% rate	--
3	70% rate	33.6 urea‡
4	70% rate	67.3 urea
5	100% rate	33.6 urea
6	100% rate	67.3 urea
7	70% rate	33.6 UAN§
8	70% rate	67.3 UAN
9	224 kg ha^{-1} rate	--
10	0	--

† Percentage of the recommended rate based on the North Dakota Wheat Nitrogen Calculator.
‡ 46-0-0.
§ UAN = urea ammonium nitrate (28-0-0).

Table 1.4. Previous crop and actual N amounts applied based on North Dakota Wheat Nitrogen Recommendation Calculator for 2016-2017.

		Amount of N applied at planting		
Location	Previous crop	70% rate	100% rate	N-rich
Cropping Season 2016		-------------------- kg ha^{-1}	-------------------	
Ada, MN	soybean†	86	123	224
Red Lake Falls, MN	soybean	86	123	224
Casselton, ND	HRSW‡	86	123	224
Cropping Season 2017				
Ada, MN	soybean	106	152	224
Gentilly, MN	soybean	106	152	224
Casselton, ND	HRSW	86	123	224
Prosper, ND	HRSW	86	123	224
Hettinger, ND	HRSW	86	123	224

The NDVI measurements were collected at the four-leaf (Zadoks, ZGS 15), flag leaf (ZGS 37), and boot growth stages (ZGS 45). In 2016 these measurements were taken with the hand-held GreenSeeker^{TM} sensor (Trimble, Sunnyvale, CA). In 2017 NDVI data were collected using a CS RapidScan (Holland Scientific, Lincoln, ND), which measures NDVI that is highly correlated to NDVI measurements from the GreenSeeker and also measures NDRE. Both are active-optical sensors, in that they provide their own light source enabling them to be used in variable light conditions. They beam multiple flashes of red (650 ± 10 nm) and NIR(770 ± 15 nm) light and average the reflectance readings into a single NDVI value using the following formula:

$$\frac{\rho 770 - \rho 650}{\rho 770 + \rho 650}$$

where $\rho 770$ and $\rho 650$ are the fractions of NIR and red light respectively emitted from the canopy and background soil (MacNack et al., 2013). Red Edge measurements were taken in trials during the 2017 growing season. The NDRE calculation is nearly identical to NDVI with a precisely determined Red Edge Position (REP) of $\rho 730$ used instead of the $\rho 650$. The sensors were held approximately 50 cm above the canopy (100 cm at the 4-5 Leaf Stage) and held across the middle of the plots at a walking speed of approximately 1.8 m s^{-1}.

Each plot was harvested with a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria); and yield, moisture, and test weight were immediately measured with a HarvestMaster Classic Grain Gage (Juniper Systems, Logan, UT). In calculating yield, it was decided to not measure plot lengths of each range and instead use the respective 5.9 m or 3.7 m for all plots in trial. A subsample was taken from each plot at harvest**,** processed, cleaned and analyzed for grain protein content adjusted for 12% moisture with a DA 7250 NIR analyzer (Perten Instruments, Hagersten, Sweden). Total protein harvested, which accounts for the total mass of protein in a given field, was calculated with the following formula:

$$\text{TPH (kg ha}^{-1}\text{) = GPC (g kg}^{-1}\text{) x Yield (Mg ha}^{-1}\text{)}$$

where TPH = total protein harvested, GPC = grain protein content fixed at 12% moisture, and yield measured at 13.5% moisture.

Statistical Analysis

All treatments were considered in this statistical analysis, which includes the six treatments receiving in-season N applied at the four-leaf stage. Although these treatments were applied primarily as part of a different experiment, they were included in this analysis as no further fertilization was made after sensing took place.

The relationship between NDVI at each recorded growth stage and grain protein content was analyzed through simple linear regression, with NDVI and grain protein content serving as the independent and dependent variables respectively. Coefficients of determination to quantify the regression tightness of fit were calculated with SAS Proc Reg (SAS Institute, Cary, NC).

Treatment means and least significant differences (LSD) were determined using Proc GLM in SAS. Statistical significance was determined at the 95% and 99% levels of confidence ($\alpha=0.05$, $\alpha=0.01$).

On-farm Trials

Weather data were collected using data from the nearest NDAWN Station. The 2016-2017 experimental locations with their proximity to the nearest NDAWN station are as follows: Ada, MN (8.7 km); Wendell, MN (11.6 km); and Perley, MN (7.4 km). Soil classification can be found in Table 1.5. The growers at each of these farms planted, harvested, and managed the crops throughout the season. Fertilizer rates were applied based on farm-specific management objectives and thus differed somewhat between locations (see Table 1.6). All fields included an N-rich strip on which an increased amount of N was added to the fertilizer application (see Table 1.6). Each N-rich strip was at least 100 m long by 21 m wide. The N-rich strip was included to provide a non N-limited environment, that when compared to the normal fertilizer amounts applied, could allow for estimation of the crop's potential response to unlimited N. Similar procedures were used by Johnson and Raun (2003) and Schoch (2013).

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 1.2. Locations of on-farm trials in 2017.

Table 1.5. Soil series, texture, taxonomy, and slope for Wendell, Perley, and Ada, MN on-farm trials, 2017.

Location	Series†	Texture	Taxonomy‡	Slope
				%
Wendell, MN,	Antler	Clay loam	Fine-loamy, mixed, superactive, frigid Aeric Calciaquolls	0-1
Perley, MN	Fargo	Silty clay	Fine, smectitic, frigid Typic Epiaquerts	0-2
Ada, MN, 2017	Glyndon	Loam	Coarse-silty, mixed, superactive, frigid, Aeric Calciaquolls	0-3

Sensing was done using a MicaSense RedEdge^{TM} camera (MicaSense, Seattle, WA) affixed to an AgBot UAV (Aerial Technology International, Oregon City, OR). The RedEdge camera has a multispectral sensor with filters to capture blue, green, red, red edge, and NIR light bands. The UAV was flown between 50 and 60 m above ground level at a speed of 21 km h^{-1}, which resulted in an image with pixel sizes ranging from 13-23 cm^{2}. Each field was flown at least once between the flag leaf and boot stages.

Table 1.6. HRSW cultivar and N fertilizer application for on-farm trials in 2017.

Location	Cultivar	Pre-plant N	N-rich strip
		----------------------kg ha^{-1}	----------------------
Wendell, MN 1	Croplan 3361	191	213
Wendell, MN 2	Bolles	191	213
Perley, MN	Shelly	135	269
Ada, MN	SY Valda	187	254

Prior to harvest, a total of four to six paired samples were taken each from the N-rich strip and an adjacent section of the field receiving no additional N. These samples were taken from 2 m diameter circular hand-harvested sections that were threshed, cleaned, processed, and analyzed for grain protein content with NIR spectroscopy.

Spatial Statistical Analysis

Imagery from the RedEdge camera was stitched using the MicaSense Atlas service, which uses the Pix4D software (Pix4D SA, Lausanne, Switzerland). The stitched images were then analyzed using ArcMap 10.4.1 (Esri, Redlands, CA). Because the RedEdge camera captured red, NIR, and red edge (RE) light bands; NDVI and NDRE were manually calculated and then output as a map. For each location a to-scale 2 m diameter shapefile was created on the NDVI/NDRE maps to represent the locations where protein samples were taken. Spectral indices for each pixel were then calculated and output as a mean value for the entire shapefile. As a final analysis, a larger shapefile that incorporated the protein sampling areas was created for the N-rich strip and the adjacent section receiving no additional N. Both NDVI and NDRE were calculated for this shapefile along with mean, standard deviation, and range of these respective indices.

Statistical Analysis

A paired t-test was performed using PROC TTEST in SAS to determine whether there was statistical significance at the 95% confidence level between grain protein contents in the N-rich strip and the strip receiving no additional N. Each location was treated as a replicate and field samples were treated as samples. The difference between both grain protein contents and spectral indices between the N-rich strip and area receiving no additional N were taken and regressed with simple linear regression. PROC REG in SAS was used to determine the statistical significance of the relationship between the change in NDVI/NDRE and the change in grain protein content.

Results and Discussion

Weather Information

Both the 2016 and 2017 growing seasons were drier than the 30-year norm with the 2017 being notably drier than 2016. As there was a statistically significant difference between locations in both growing seasons, weather information will be discussed by year and location.

2016 Growing Season

Temperatures during the early spring of 2016 were roughly three degrees C above average; however mean temperature was normal for the rest of the growing season (see Figure 1.3) (NDAWN, 2018). Total precipitation for the growing season was roughly normal, with June being drier but July and August being wetter than normal (with a few exceptions). Total precipitation for Ada was 403 mm, 402 at Red Lake Falls, and 277 at Casselton.

2017 Growing Season

The 2017 growing season made headlines throughout the country, as most of the state of North Dakota and parts of north west Minnesota were engulphed in a drought. Western North Dakota and Eastern Montana were the hardest hit, with Hettinger being one of the driest locations in the state (NDAWN, 2018). Growing season precipitation totals for the experimental sites reflect the drastic differences between 2016 and 2017 (see Figures 1.3-1.4). From April to August total rainfall for each location was: Ada, 236 mm; Gentilly, 195 mm; Casselton and Prosper, 224 mm; and Hettinger,142 mm. Precipitation during the month of May, when wheat rapidly accumulates biomass, was notably low. As Figure 1.2 shows, the drought hit the North Dakota locations hardest with Hettinger being the most severe. Rainfall was much higher in June; however, due to the dry spring, yield and protein were already affected.

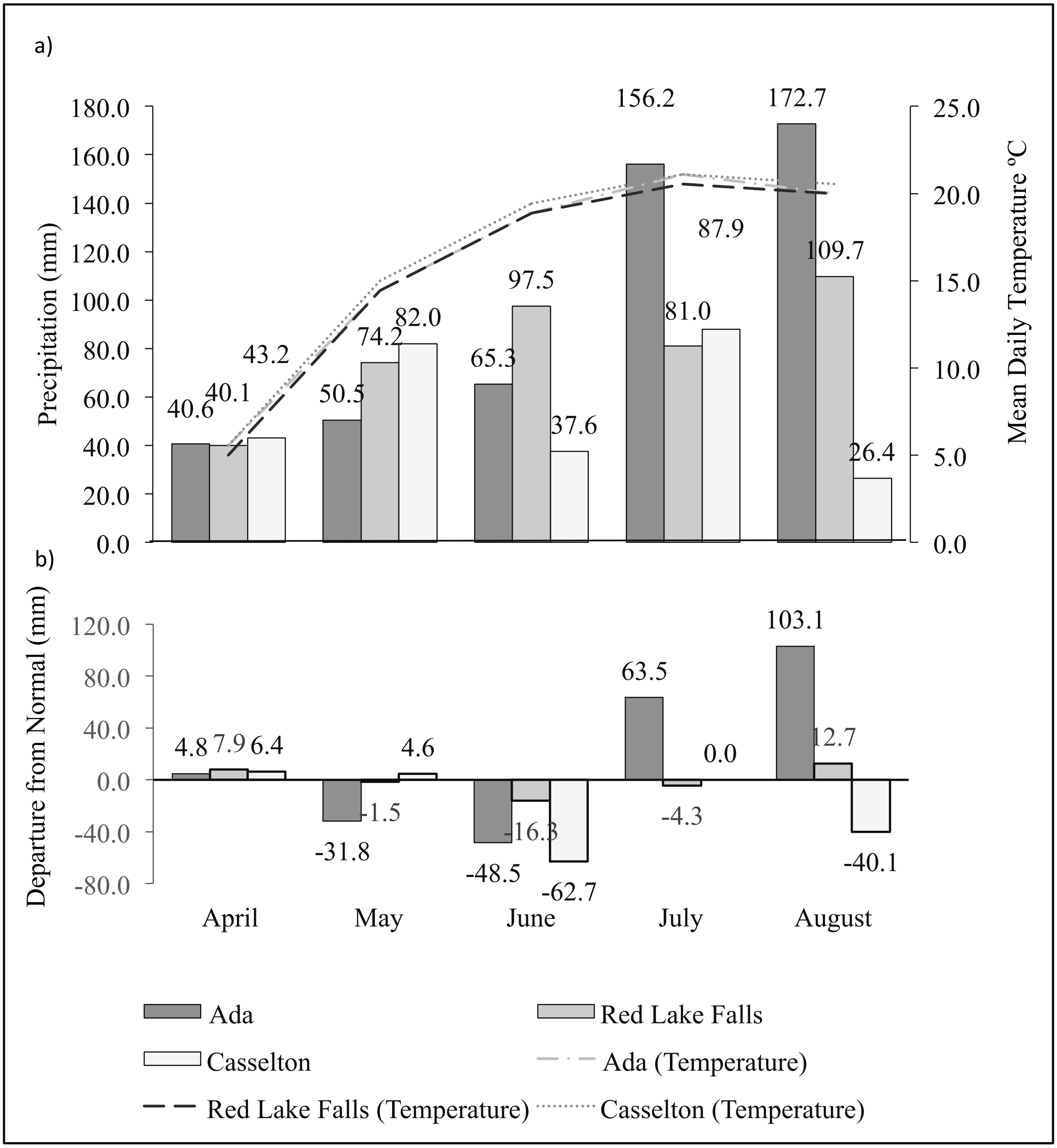

Figure1.3. a) Total precipitation and mean temperatures for 2016 growing season at Ada, MN; Red Lake Falls, MN; and Casselton, ND. b) Departure from normal precipitation for each experimental site.

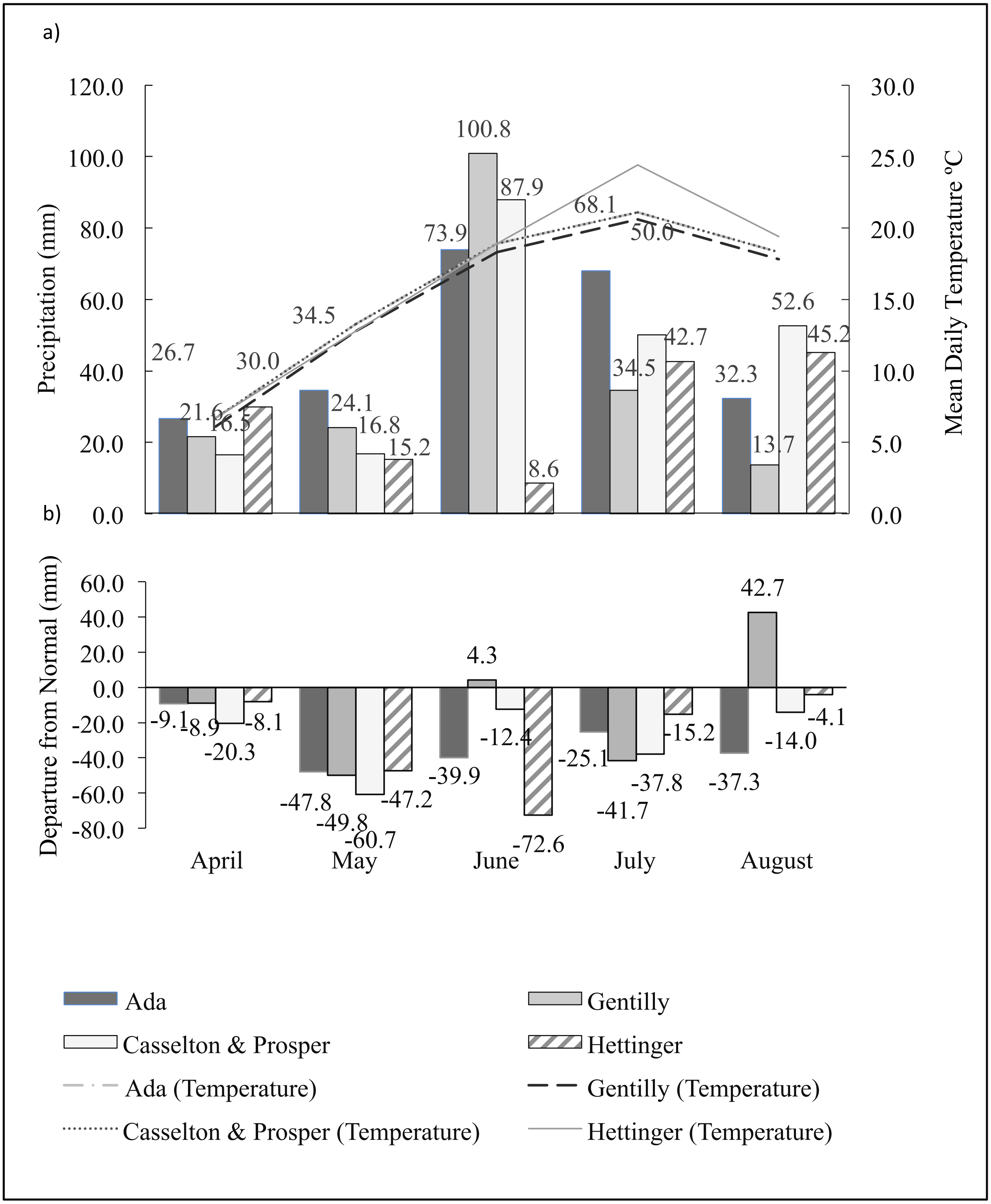

Figure 1.4. a) Total precipitation and mean temperatures for 2017 growing season at Ada, MN; Gentilly, MN; Casselton, ND; Prosper, ND; and Hettinger, ND. b) Departure from normal precipitation for each experimental site. Both Casselton and Prosper utilize the same NDAWN weather station.

Combined Analysis

Combining locations for statistical analysis allows for greater inferences about the effect of a given treatment across a wider geographical area or in future seasons; however, if there is a significant treatment by location interaction then locations should be discussed on an individual, and not combined, basis (Moore and Dixon, 2015). Combined analyses were explored for the following combinations: a) all experiments spanning both years, b) 2016 experiments only, and c) 2016 experiments plus Ada in 2017. In each of these combined analyses the treatment by location interaction was significant at the $\alpha = 0.05$ level. Therefore, it was decided to present and discuss each experimental location individually.

Nitrogen Treatment Effects

Nitrogen treatment had a statistically significant effect on NDVI at five of the locations. The three locations that showed limited or no significance were likely adversely affected by drought and will be discussed further. At the Red River Valley locations NDVI values ranged from 0.28 to 0.52 at the four-leaf stage, 0.65 to 0.87 at the flag-leaf stage, and 0.55 to 0.90 at the boot stage (see Tables 1.13-1.19). Values for Hettinger will be presented and discussed later (see Table 1.20).

Predictive Ability of Spectral Indices

Early Sensing Timing

In each location, NDVI and NDRE (when taken) were the least predictive of grain protein content at the four-leaf stage with R^2 values ranging between 0.00 to 0.18 for NDVI and 0.00 to 0.14 for NDRE (see Tables 1.7 and 1.10). This markedly lower predictability is consistent with results reported by MacNack et al. (2014) where NDVI taken before jointing

(ZGS 25) was also much more poorly correlated with grain protein content (R^2 =0.30) than when taken at later stages.

For both total protein harvest and yield, Both NDVI and NDRE had a much weaker relationship at the four-leaf stage than at later sensing timings (see Tables 1.8 -1.9, and 1.11-1.12). Although the relationships between NDVI/NDRE taken at the four-leaf stage were generally statistically significant with increased grain protein content, coefficients of variation were larger when compared with sensing timing at later stages (see Tables 1.13-1.20).

Table 1.7. Coefficients of determination for the relationship between NDVI taken at three growth stages and grain protein content for the 2016 growing season.

	Ada	Red Lake Falls	Casselton
		R^2	
NDVI† ZGS‡ 15	0.27**	0.04	0.04
NDVI ZGS 37	0.41**	0.31**	--
NDVI ZGS 45	0.39**	0.48**	0.39**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
-- indicates sites where data was not taken or is not available.
† NDVI = Normalized Difference Vegetation Index.

Table 1.8. Coefficients of determination for the relationship between normalized NDVI taken at three growth stages and total protein harvest for the 2016 growing season.

	Ada	Red Lake Falls	Casselton
		R^2	
NDVI ZGS† 15	0.34*	0.05	0.09*
NDVI ZGS 37	0.63**	0.38**	--
NDVI ZGS 45	0.49**	0.54**	0.37**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
-- indicates sites where data was not taken or is not available.
† ZGS = Zadoks growth stage.

Table 1.9. Coefficients of determination for the relationship between normalized NDVI taken at three growth stages and yield for 2016 growing season.

	Ada	Red Lake Falls	Casselton
	----------------------------------	R^2	------------------------------------
NDVI ZGS† 15	0..28**	0.05	0..05*
NDVI ZGS 37	0.61**	0.33**	--
NDVI ZGS 45	0..47	0.45**	0.25*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
-- indicates sites where data was not taken or is not available.
† ZGS = Zadoks growth stage.

Table 1.10. Coefficients of determination for the relationship between normalized NDVI and NDRE taken at three growth stages and yield for 2017 growing season.

	Ada	Gentilly	Casselton	Prosper	Hettinger
	----------------------------------		R^2		------------------------------------
NDVI ZGS† 15	0.18*	0.00	0.08*	0.04	0.01
NDVI ZGS 37	0.43**	0.07	0.00	0.03	--
NDVI ZGS 45	0.43**	0.02	0.06	0.04	0.17*
NDRE ZGS15	0.14	--	0.00	--	--
NDRE ZGS 37	0.37**	0.00	0.03	--	--
NDRE ZGS 45	0.44**	0.01	0.11*	--	0.20*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
-- indicates sites where data was not taken or is not available.
† ZGS = Zadoks growth stage.

Table 1.11. Coefficients of determination for the relationship between normalized NDVI and NDRE taken at three growth stages and total protein harvest for 2017 growing season.

	Ada	Gentilly	Casselton	Prosper	Hettinger
	----------------------------------		R^2		------------------------------------
NDVI ZGS†15	0.36**	0.00	0.00	0.04	0.18*
NDVI ZGS 37	0.82**	0.04	0.00	0.02	--
NDVI ZGS 45	0.86**	0.16*	0.35**	0.04	0.06
NDRE ZGS15	0.13*	--	0.00	--	--
NDRE ZGS 37	0.58**	0.12*	0.05	--	--
NDRE ZGS 45	0.66**	0.11*	0.16*	--	0.02

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
-- indicates sites where data was not taken or is not available.
† ZGS = Zadoks growth stage.

Table 1.12. Coefficients of determination for the relationship between normalized NDVI and NDRE taken at three growth stages and yield for 2016 growing season.

	Ada	Gentilly	Casselton	Prosper	Hettinger
			---------- R^2 ----------		
NDVI† ZGS 15‡	0.38**	0.00	0.03	0	0.15*
NDVI ZGS 37	0.86**	0.06	0.00	0.03	--
NDVI ZGS 45	0.90**	0.15*	0.21*	0.17	--
NDRE† ZGS15	0.35**	--	0.01	--	--
NDRE ZGS 37	0.84**	0.10*	0.09	--	--
NDRE ZGS 45	0.85**	0.10*	0.06	--	0.00

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

-- indicates sites where data was not taken or is not available.

† ZGS = Zadoks growth stage.

Later Sensing Timings

Spectral indices were much more predictive of grain protein content, total protein harvest, and yield when taken later in the season. This is consistent with findings from MacNack et al. (2014), as higher plant biomass decreased the amount of background soil visible to sensors. Spectral indices taken at the boot stage were generally more predictive of grain protein content, total protein harvest, and yield than when taken at the flag-leaf stage; However, this was not always the case and will be discussed on a location by location basis.

Although the highest coefficients of determination of spectral indices were found with yield and then total protein harvest, these were often poorly correlated with NDVI or NDRE. This deviates from results reported by Schoch (2013), Feland (2018), and Li-Hong et al. (2007) where yield was highly correlated with NDVI.

Values for NDVI congregated closer to a maximum of 0.9 as the plant progressed from the four-leaf to the boot stage (see Figures 1.6 – 1.9). This seemed to exhibit some of the saturation under high biomass that may be a limitation for the utility of NDVI (Kanke et al., 2012).

Spectral Index Comparison

Under higher biomass, previous studies have found NDRE to be more predictive than NDVI (Kanke et al., 2012); however, at most locations NDRE was slightly less predictive of grain protein content, total protein harvest, and yield when compared with NDVI. It is possible that due to the drought, the advantages of NDRE in detecting differences in high biomass were negated due to the lower than normal biomass accumulation.

Predictive Power of Spectral Indices

Overall, the relationship between NDVI and NDRE and grain protein content was statistically significant but demonstrated weak predictive ability. Because the treatment by location interaction was significant, results on the predictive capability of these spectral indices will be discussed on a location by location basis. Locations that had very similar effects will be discussed together.

Ada and Red Lake Falls, MN – 2016

In both locations, nitrogen treatment was statistically significant for NDVI at all growth stages (see Tables 1.13-1.14). The only significance in later-season sensing timings was between the check and added N. In Ada, the predictive ability of NDVI on grain protein content increased from an R^2 of 0.27 at the four-leaf stage to an R^2 value of 0.41 at the flag-leaf stage and $R^2 = 0.39$ at the boot stage. A similar increase happened at Red Lake Falls with R^2 values of 0.04 at the four-leaf stage, 0.31 at the flag-leaf stage, and 0.48 at the boot stage. Data were plotted with NDVI from each sensing timing as the independent variable and grain protein content as the dependent variable (see Figures 1.5-1.6). Graphical presentation of linear regression data from Red Lake Falls are not shown as the distribution was similar to Ada. As the season progressed, NDVI values clustered closer to 1.0, with a few lower points at 0.95. Even with a higher R^2

value, this clustering makes it difficult to decipher the difference between NDVI values and grain protein content. This concept plays a key role in the utility of NDVI as a predictive measurement for grain protein content and will be discussed further at the end of this chapter.

Table 1.13. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment at Ada, 2016.

N treatment	NDVI†					
	ZGS‡ 15	ZGS 37	ZGS 45	GPC§	TPH¶	Yield
kg ha^{-1}				g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.34	0.70	0.74	113.8	460	4.04
70% rate PP#	0.33	0.84	0.84	121.3	630	5.19
100% rate PP	0.38	0.85	0.87	127.1	758	5.96
224 kg ha^{-1} PP	0.33	0.84	0.85	130.1	761	5.85
70% rate + 34 kg	0.38	0.87	0.87	128.8	774	6.01
70% rate + 67 urea ZGS 15	0.40	0.86	0.87	132.0	832	6.31
100% rate + 34 urea ZGS 15	0.36	0.86	0.87	131.9	823	6.24
100% rate + 67 urea ZGS 15	0.36	0.85	0.87	134.3	803	5.98
70% rate + 34 UAN†† ZGS 15	0.39	0.85	0.87	129.4	787	6.08
70% rate + 34 UAN ZGS 45	0.37	0.84	0.87	130.0	793	6.10
CV %	7.90	2.40	4.00	2.2	14.0	5.06
LSD (0.05)††	0.04	0.05	0.05	4.1	143.0	0.50

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.

Table 1.14. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment at Red Lake Falls, 2016.

N treatment	NDVI†					
	ZGS‡ 15	ZGS 37	ZGS 45	GPC§	TPH¶	Yield
kg ha^{-1}				g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.45	0.83	0.82	109.7	585	5.31
70% rate PP#	0.45	0.86	0.88	127.5	783	6.28
100% rate PP	0.45	0.87	0.88	130.6	826	6.33
224 kg ha^{-1} rate PP	0.39	0.86	0.87	136.2	879	6.59
70% rate + 34 kg	0.46	0.87	0.88	133.9	857	6.40
70% rate + 67 urea ZGS 15	0.47	0.87	0.87	135.1	863	6.39
100% rate + 34 urea ZGS 15	0.45	0.86	0.87	135.7	871	6.42
100% rate + 67 urea ZGS 15	0.44	0.86	0.88	135.1	860	6.37
70% rate + 34 UAN†† ZGS 15	0.42	0.86	0.86	123.9	778	6.28
70% rate + 34 UAN ZGS 45	0.43	0.87	0.88	135.1	842	6.32
CV %	7.08	1.75	1.37	3.0	6.2	5.6
LSD (0.05)‡‡	0.05	0.02	0.02	5.6	72.1	0.49

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.

At Ada and Red Lake Falls, NDVI was more predictive of yield, and consequently total protein harvest, than grain protein content at all timings except at the boot stage for Red Lake Falls (see Table 1.7). This supports findings by Schoch (2014), Feland (2018) and Li-Hong et al. (2007). In Ada, the most predictive NDVI timing for yield (R^2=0.63) and total protein harvest (R^2 = 0.63) was at the flag-leaf stage. However, the most predictive timing at Red Lake Falls for yield and total protein harvest (R^2 = 0.45 and 0.54 respectively) was at the boot stage.

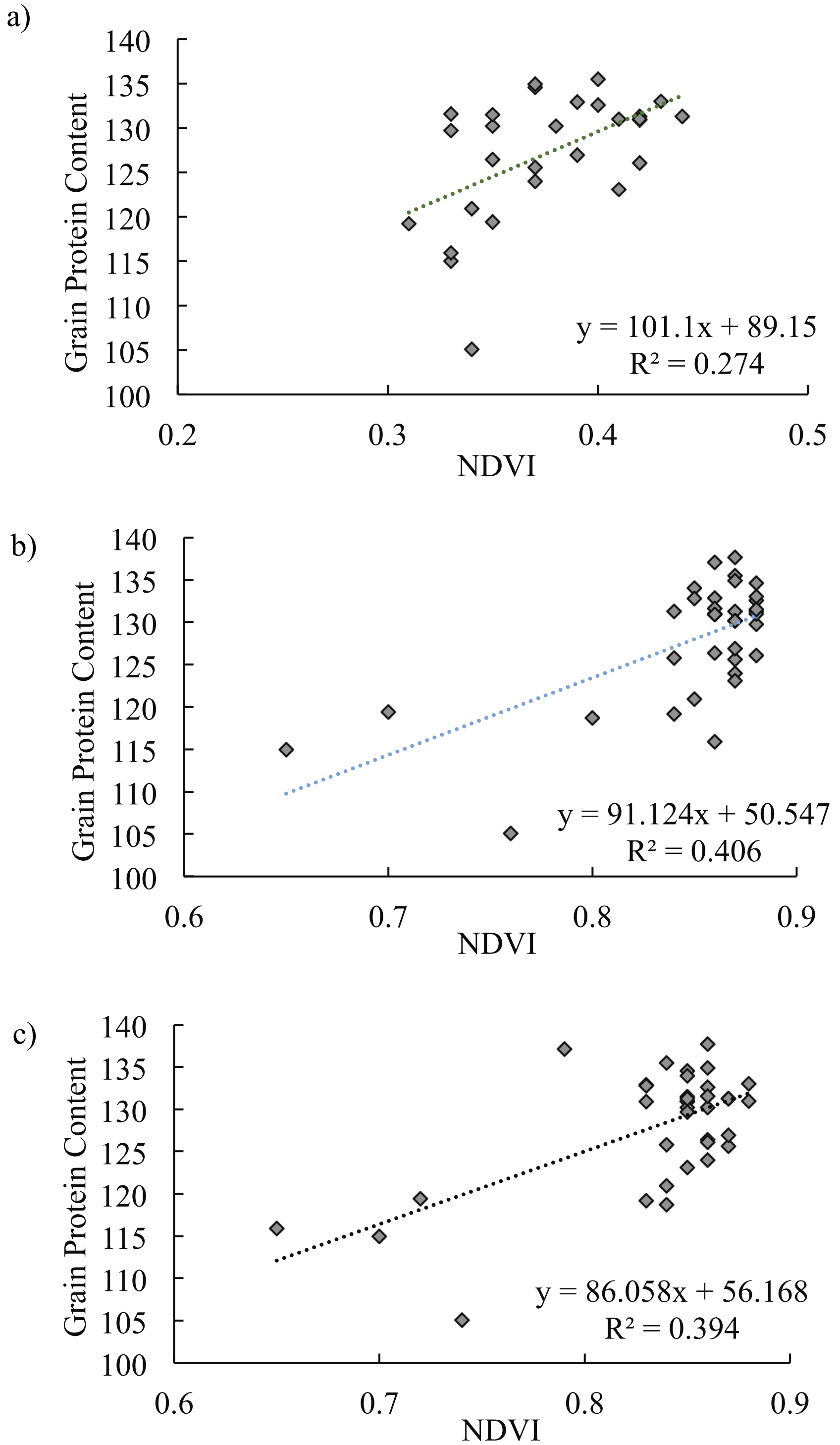

Figure 1.5. Linear Regression of grain protein content (dependent variable) by NDVI (independent variable) taken at a) four-leaf, b) flag-leaf, and c) boot at Ada, MN, 2016.

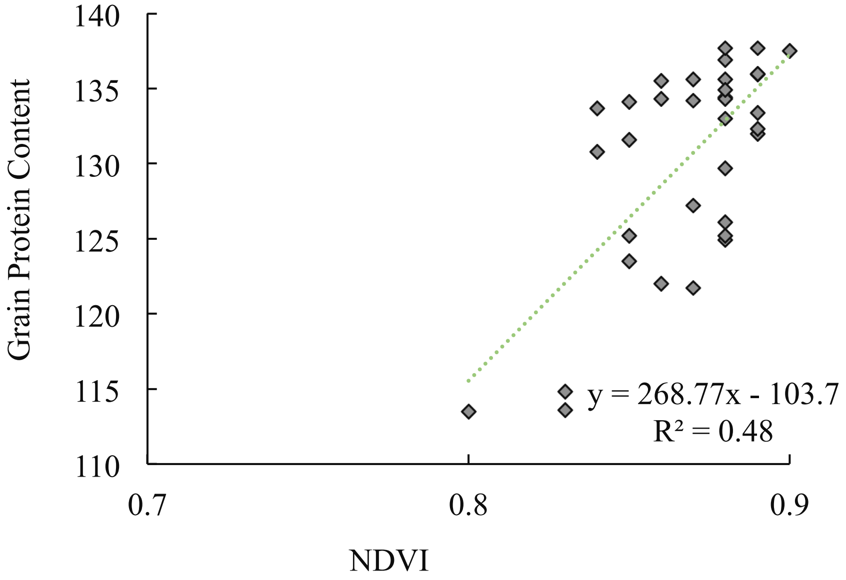

Figure 1.6. Linear Regression of grain protein content (dependent variable) by NDVI (independent variable) taken at a) four-leaf, b) flag-leaf, and c) boot stages at Red Lake Falls, MN, 2016.

Casselton, ND – 2016

The data presented for Casselton represent those of a late planting date (19 May, 2016). The later planting date resulted in a high grain protein content and a low yield. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment for Casselton in 2016 can be found in Table 1.15.

Table 1.15. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment at Casselton, ND, 2016.

N treatment	NDVI† ZGS‡ 15	NDVI† ZGS 45	GPC§	TPH¶	Yield
kg ha^{-1}			g kg^{-1}	Kg ha^{-1}	Mg ha^{-1}
0	0.33	0.78	150.8	544	3.61
70% rate PP#	0.38	0.78	153.2	579	3.78
100% rate PP	0.37	0.83	153.4	609	3.97
224 kg ha^{-1} PP	0.47	0.84	154.6	652	4.22
70% rate + 34 kg	0.43	0.84	153.2	634	4.14
70% rate + 67 urea ZGS 15	0.37	0.83	157.4	623	3.96
100% rate + 34 urea ZGS 15	0.42	0.84	156.0	580	3.72
100% rate + 67 urea ZGS 15	0.42	0.83	154.6	642	4.15
70% rate + 34 UAN††ZGS 15	0.40	0.84	154.3	608	3.94
70% rate + 34 UAN ZGS 45	0.37	0.85	154.3	571	3.70
CV %	10.80	2.00	1.8	6.1	6.8
LSD (0.05) ‡‡	0.06	0.02	5.8	82.2	0.34

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.

Simple linear regression results demonstrate that the relationship between NDVI and grain protein content was statistically significant at the flag-leaf stage but less predictive at Casselton than at Ada and Red Lake Falls (see Table 1.7 and Figure 1.7). Data points also begin to congregate around a maximum NDVI value of 1.00 but show more horizontal distribution than at Ada and Red Lake Falls. As was the case at Red Lake Falls, the predictive ability of NDVI on yield (R^2 = 0.25) and total protein harvest (R^2 = 0.37) increased to a maximum at the boot stage.

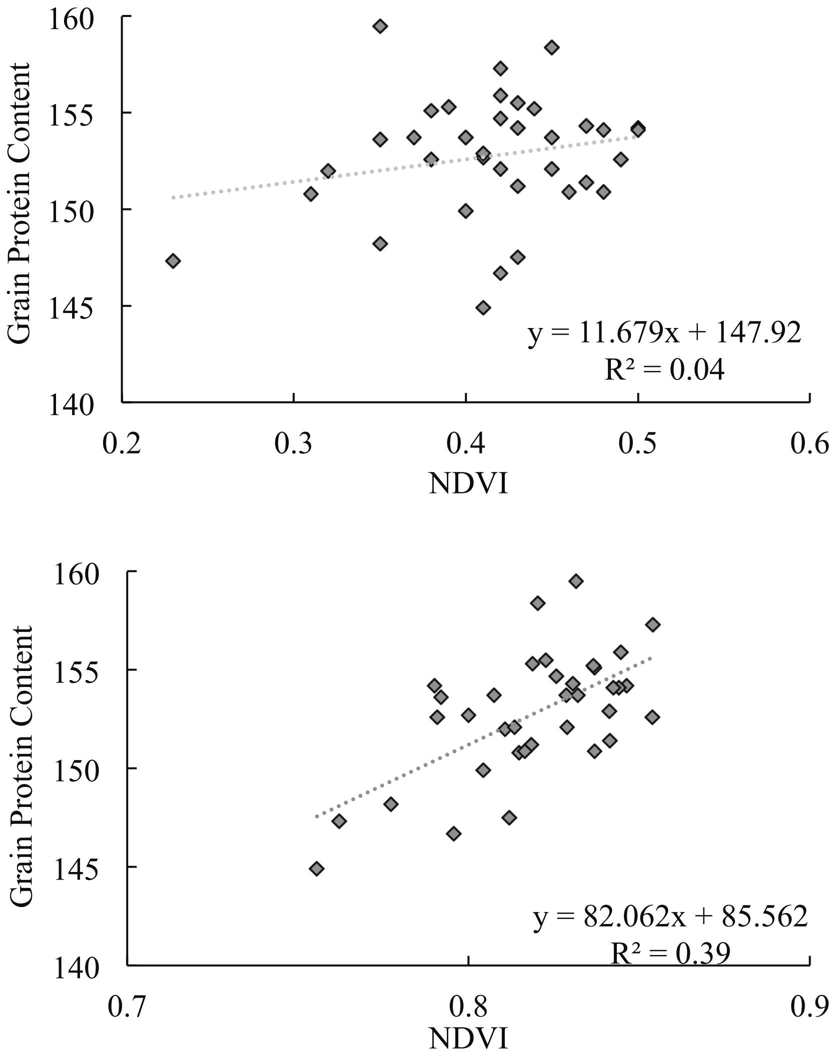

Figure 1.7. Linear Regression of grain protein content (dependent variable) by normalized NDVI taken at a) Zadoks growth stage (ZGS) 15 and b) ZGS 45 at Casselton, ND, 2016.

2017 Growing Season

As mentioned previously, drought was the dominating weather during this growing season. Although the number of geographical locations increased to five, several of them seem to show water limitation which seemed to detrimentally impact the predictive ability between NDVI/NDRE and grain protein content.

Ada, MN – 2017

The experimental site at Ada was the only 2017 site where the response to N treatment was statistically significant (See Table 1.16). F-protected LSD values indicate that N treatment on spectral indices was only statistically significant when compared with the check. The experimental site at Ada was notably more responsive to N treatment and spectral indices than the other locations in 2017 (See tables. 1.17 – 1.19). The relationships between NDVI and grain protein content, yield, and total protein were all statistically significant at all sensing timings. Relationships between NDRE and grain protein content, yield, and total protein were statistically significant at the flag-leaf and boot stages but not at the four-leaf stage. Similar to results from Red Lake Falls and Casselton in 2016, NDVI increased in its predictive ability of grain protein content with each additional sensing timing and reached its maximum at the boot stage (R^2 =0.43). NDRE data also followed a similar pattern with a maximum at the boot stage (R^2 = 0.41). The biggest difference between the 2017 Ada experiment and all other sites was the superior predictive ability of spectral indices on yield and total protein harvest. NDVI was highly predictive of yield at the flag-leaf stage (R^2 = 0.86) and ZGS 45 (R^2 = 0.90), and NDRE was close behind with R^2 values of 0.84 and 0.85 at the flag-leaf and boot stages respectively.

Table 1.16. Means for NDVI, NDRE, grain protein content, total protein harvest, and yield by N treatment at Ada, MN, 2017.

N treatment	NDVI†			NDRE			GPC §	TPH ¶	Yield
	ZGS ‡ 15	ZGS 37	ZGS 45	ZGS † 15	ZGS 37	ZGS 45			
kg ha^{-1}							g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.40	0.65	0.55	0.16	0.21	0.21	128.7	436	3.37
70% rate PP#	0.44	0.83	0.82	0.18	0.31	0.31	134.5	730	5.83
100% rate PP	0.44	0.85	0.84	0.18	0.32	0.34	143.5	879	6.15
224 kg ha^{-1} PP	0.43	0.84	0.83	0.18	0.32	0.33	146.2	873	5.97
70% rate + 34 kg	0.47	0.85	0.84	0.18	0.33	0.34	137.0	809	5.90
70% rate + 67 urea ZGS 15	0.45	0.84	0.84	0.18	0.33	0.34	145.4	847	5.83
100% rate + 34 urea ZGS 15	0.47	0.85	0.83	0.18	0.33	0.34	143.1	847	5.93
100% rate + 67 urea ZGS 15	0.44	0.85	0.84	0.17	0.32	0.35	145.6	895	6.15
70% rate + 34 UAN†† ZGS 15	0.44	0.84	0.83	0.18	0.32	0.33	137.5	800	5.82
70% rate + 34 UAN ZGS 45	0.46	0.85	0.83	0.18	0.32	0.33	140.6	851	5.86
CV %	6.10	3.60	5.90	4.80	7.40	7.40	2.4	6.1	5.9
LSD (0.05) ‡‡	0.04	0.04	0.07	0.01	0.03	0.03	4.9	70.2	0.46

† Normalized Difference Vegetation Index.

‡ ZGS = Zadoks Growth Stage.

§ GPC = grain protein content.

¶ TPH = Total protein harvest = GPC x yield.

PP = pre-plant N applied according to the recommended rate.

†† UAN = urea ammonium nitrate (28-0-0).

‡‡ LSD = least significant difference, applies to columns only.

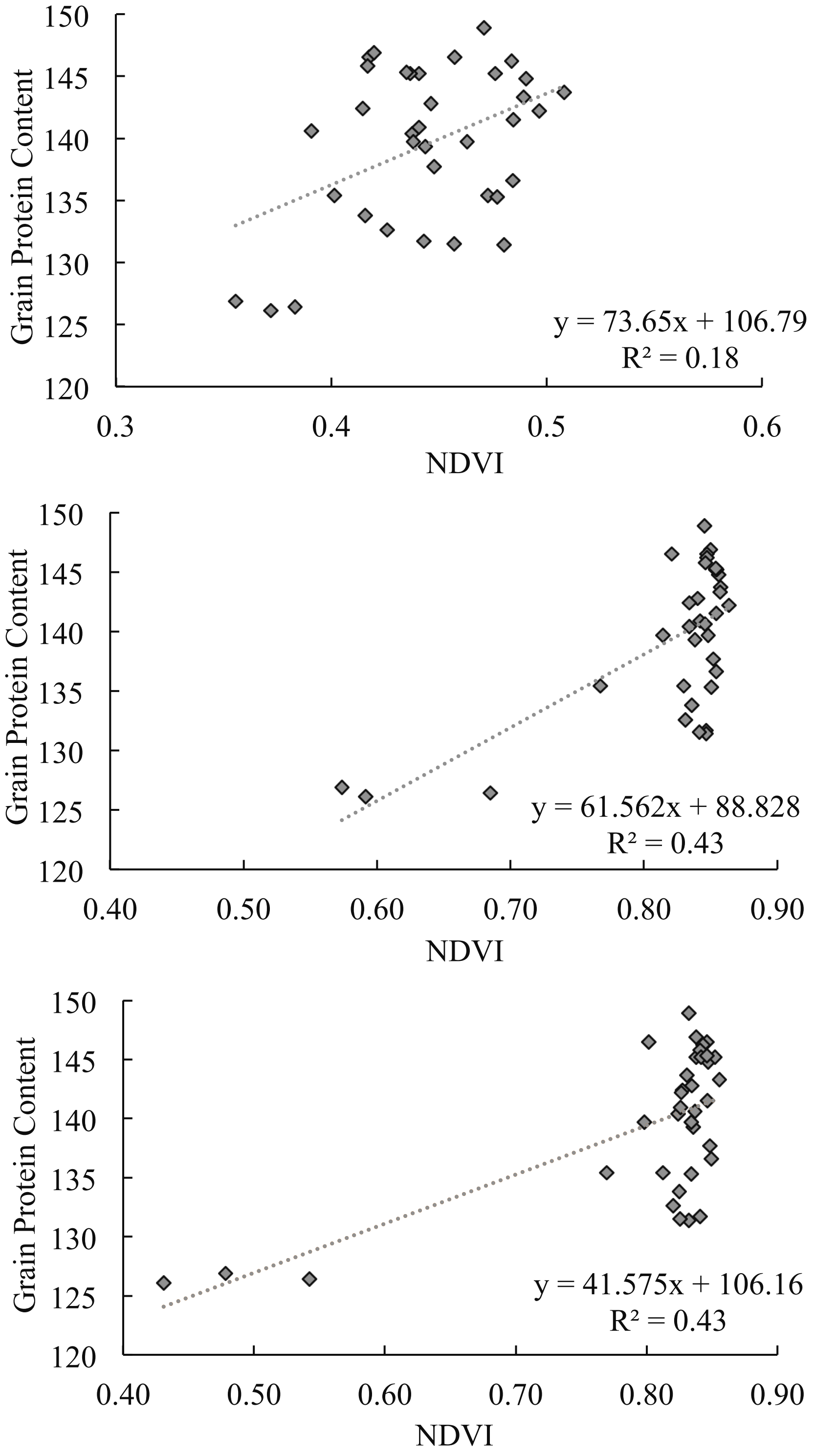

Figure 1.8. Linear Regression of grain protein content (dependent variable) by NDVI (independent variable) taken at a) four-leaf, b) flag-leaf, and c) boot stages at Ada, MN, 2017.

Gentilly, MN, Prosper, ND, and Casselton, ND - 2017

These locations responded similarly to N treatment in their ability to predict grain protein content. Overall, statistical significance between N treatment and NDVI/NDRE was limited (see Tables 1.17-1.19).

Table 1.17. Means for NDVI, NDRE, grain protein content, total protein harvest, and yield by N treatment at Gentilly, MN, 2017.

	NDVI†			NDRE				
N treatment	ZGS‡ 15	ZGS 37	ZGS 45	ZGS 37	ZGS 45	GPC§	TPH¶	Yield
kg ha^{-1}						g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.50	0.80	0.78	0.31	0.28	135.8	515	3.79
70% rate PP#	0.50	0.80	0.79	0.27	0.30	143.7	563	3.92
100% rate PP	0.49	0.81	0.80	0.28	0.31	145.7	596	4.09
224 kg ha^{-1} PP	0.52	0.80	0.79	0.27	0.30	148.7	662	4.45
70% rate + 34 kg	0.51	0.80	0.80	0.25	0.30	139.0	485	3.49
70% rate + 67 urea ZGS 15	0.44	0.79	0.78	0.29	0.27	153.7	635	4.13
100% rate + 34 urea ZGS 15	0.50	0.81	0.79	0.28	0.30	152.0	632	4.16
100% rate + 67 urea ZGS 15	0.49	0.80	0.80	0.30	0.30	151.7	605	3.99
70% rate + 34 UAN†† ZGS 15	0.47	0.77	0.79	0.28	0.28	147.4	569	3.86
70% rate + 34 UAN ZGS 45	0.49	0.80	0.78	0.27	0.28	150.7	609	4.04
CV %	9.00	2.00	2.60	6.80	2.20	2.2	8.9	10.2
LSD (0.05)‡‡	ns§§	0.04	ns	ns	ns	5.6	ns	1.07

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.
§§ ns = not statistically significant.

Table 1.18. Means for NDVI, NDRE, grain protein content, total protein harvest, and yield by N treatment at Casselton, ND, 2017.

N treatment	NDVI†			NDRE					
	ZGS ‡15	ZGS 37	ZGS 45	ZGS † 15	ZGS 37	ZGS 45	GPC §	TPH ¶	Yield
kg ha^{-1}							g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.32	0.76	0.75	0.15	0.29	0.29	120.2	543	4.52
70% rate PP#	0.31	0.75	0.78	0.14	0.29	0.31	137.4	589	4.29
100% rate PP	0.39	0.75	0.76	0.17	0.29	0.31	129.1	615	4.76
224 kg ha^{-1} PP	0.36	0.74	0.79	0.15	0.29	0.32	142.6	612	4.29
70% rate + 34 kg	0.34	0.75	0.79	0.16	0.29	0.31	135.9	680	5.00
70% rate + 67 urea ZGS 15	0.35	0.76	0.78	0.16	0.29	0.30	139.2	614	4.41
100% rate + 34 urea ZGS 15	0.35	0.76	0.78	0.16	0.29	0.32	144.3	635	4.40
100% rate + 67 urea ZGS 15	0.37	0.76	0.80	0.17	0.29	0.32	141.0	668	4.74
70% rate + 34 UAN††ZGS 15	0.35	0.74	0.79	0.16	0.28	0.31	134.0	659	4.92
70% rate + 34 UAN ZGS 45	0.35	0.76	0.76	0.15	0.30	0.30	142.6	704	4.94
CV %	8.30	3.90	3.40	10.00	6.30	4.60	7.2	12.2	13.0
LSD (0.05)††	ns§§	ns	ns	ns	ns	ns	14.3	ns	ns

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.
§§ ns = not statistically significant.

Spectral indices at these locations were limited at predicting grain protein content. The relationship between spectral indices and grain protein content was not significant at Gentilly, and at Prosper and Casselton was only significant at the four-leaf stage. The relationship between NDRE and grain protein content was statistically significant at the boot stage; however, the relationship between NDVI and grain protein content at this stage was not significant. Of these three sites, the highest predictive relationship between NDVI and grain protein content was at Casselton ($R^2 = 0.08$). At Prosper, there was an insignificant, negative R^2 value meaning that as NDVI increased grain protein content decreased (see Figure 1.9). The relationships between spectral indices and yield and total protein harvest were higher than with grain protein content, but also very weak (see Table 1.11-1.12. It is hypothesized that the poor results were due to the drought. Although not measured, anecdotal observation indicated that the wheat crop in each of these three locations was shorter and had much shorter flag leaves than the Ada experiment in 2017.

Table 1.19. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment at Prosper, ND, 2017.

N treatment	NDVI†					
	ZGS‡ 15	ZGS 37	ZGS 45	GPC§	TPH¶	Yield
kg ha^{-1}				g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.35	0.74	0.76	134.2	605	4.51
70% rate PP#	0.32	0.74	0.77	143.0	600	4.22
100% rate PP	0.28	0.68	0.73	142.6	644	4.52
224 kg ha^{-1} PP	0.32	0.75	0.76	147.6	670	4.55
70% rate + 34 kg	0.30	0.71	0.75	144.3	626	4.35
70% rate + 67 urea ZGS 15	0.30	0.72	0.75	142.1	638	4.49
100% rate + 34 urea ZGS 15	0.31	0.73	0.76	142.5	684	4.81
100% rate + 67 urea ZGS 15	0.34	0.75	0.78	145.1	694	4.82
70% rate + 34 UAN†† ZGS 15	0.33	0.74	0.76	141.9	619	4.38
70% rate + 34 UAN ZGS 45	0.32	0.71	0.75	143.7	670	4.45
CV %	7.60	3.70	3.30	2.0	6.6	7.5
LSD (0.05) ‡‡	0.03	0.03	ns§§	4.2	61.4	ns

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.
§§ ns = not statistically significant.

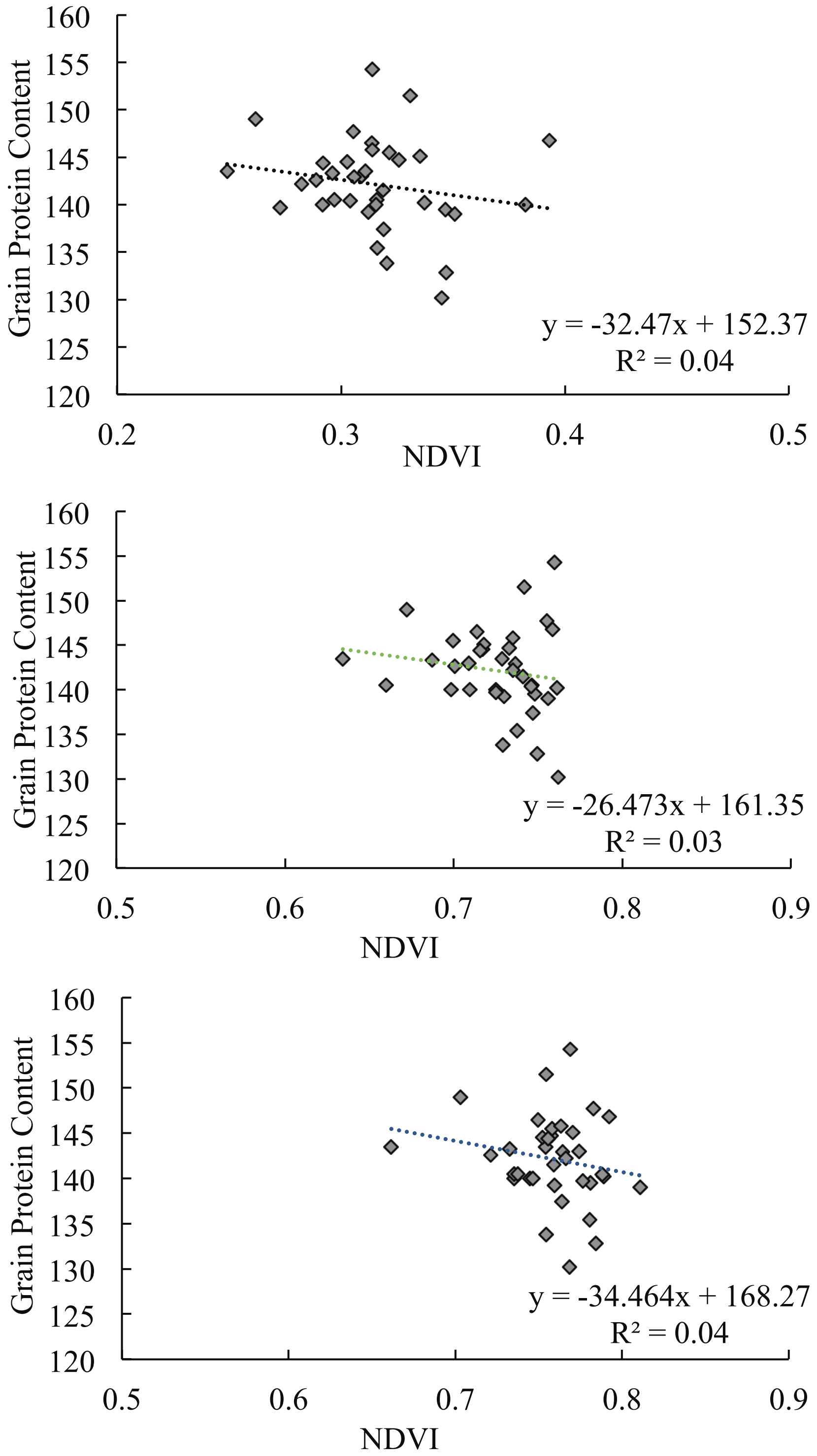

Figure 1.9. Linear Regression of grain protein content (dependent variable) by NDVI (independent variable) taken at a) four-leaf, b) flag-leaf, and c) boot stages at Prosper, ND, 2017.

Hettinger – 2017

Results and discussion of Hettinger are presented separately due to its geographic and ecological isolation from the rest of the trials. Nitrogen treatment was not statistically significant with NDVI at either timing; however, spectral indices at the boot stage were statistically significant and weakly predictive of grain protein content (R^2=0.17 for NDVI, R^2=0.20 for NDRE). An interesting observation is that the predictive ability of NDVI on yield (R^2 = 0.15) at Hettinger was the highest of all the 2017 locations save Ada. This may be due to the relative abundance of residual soil pore water from a heavy winter snowfall, which likely provided enough water for early-season growth. However, as the season progressed without adequate precipitation the plant could not further take up N.

Table 1.20. Means for NDVI, grain protein content, total protein harvest, and yield by N treatment at Prosper, ND, 2017.

	NDVI†				
N treatment	ZGS‡ 15	ZGS 45	GPC§	TPH¶	Yield
kg ha			g kg^{-1}	kg ha^{-1}	Mg ha^{-1}
0	0.37	0.50	136.5	252	1.85
70% rate PP#	0.38	0.49	136.2	251	1.84
100% rate PP	0.37	0.50	136.8	230	1.68
224 kg ha^{-1} PP	0.34	0.48	141.1	228	1.62
70% rate + 34 urea ZGS 15	0.38	0.51	134.8	249	1.85
70% rate + 34 UAN†† ZGS 15	0.35	0.49	135.3	261	1.93
CV %	12.60	9.30	1.80	8.0	7.8
LSD (0.05) ‡‡	ns§§	ns	3.60	ns	ns

† Normalized Difference Vegetation Index.
‡ ZGS = Zadoks Growth Stage.
§ GPC = grain protein content.
¶ TPH = Total protein harvest = GPC x yield.
# PP = pre-plant N applied according to the recommended rate.
†† UAN = urea ammonium nitrate (28-0-0).
‡‡ LSD = least significant difference, applies to columns only.
§§ ns = not statistically significant.

Discussion

Spectral indices were predictive of grain protein content at some but not all locations. This phenomenon is likely due to environmental factors such as precipitation and soil classification. As mentioned previously, locations where rainfall was lower had poor predictability between NDVI and grain protein content. Often, drought conditions result in a lower yield but higher grain protein content; because photosynthesis, and thus grain filling, is reduced, whereas, N remobilization to the kernel is less affected (Brown et al., 2005; Jenner et al., 1991; Gooding et al., 2003; and Ercoli et al., 2007). Because of this, estimating early-season N would be a poor predictor of grain protein content as water stress also greatly influences protein. It seems that in the 2017 Prosper, ND and Gentilly, MN experiments this may be a principal cause for such poor predictability, as yields were low and grain protein contents were high. Furthermore, severe drought has been shown to also curtail grain protein deposition (Ercoli et al., 2007). Results in Hettinger, where the drought was severe, support this finding as grain protein content was somewhat low despite very low yields of < 2 Mg ha^{-1}.

In effect, the positive linear relationship between NDVI/NDRE and grain protein content seems to break down at a certain threshold of drought stress. The Prosper 2017 experiment provides an interesting example of this theory (See Figure 1.9). Normalized NDVI taken at the three timings, particularly at the four leaf and flag leaf stages, indicated that several experimental plots had yellower plants than those plots with higher spectral index readings. Based on linear regression curves from 2016 data and Ada in 2017, these yellower plots would be expected to have lower grain protein contents than plots with higher early-season NDVI values. However, these yellower plots had slightly higher grain protein contents than plots with higher NDVI values. This may indicate that this positive linear relationship between normalized NDVI and grain protein content may break after a certain level of drought stress has been reached.

Determining the drought stress threshold behind the breakdown of the positive linear relationships between NDVI and grain protein content would provide a useful limitation of this model. Fowler et al. (1990) found that yield is a good indicator of the cumulative environmental influence on crop growth. Thus, lower than normal yields, after ruling other factors out, could indicate a potential breakdown in this model between NDVI and grain protein content.

The timing of rain events seems to have played a major role in the response of these experiments to N. Prosper and Casselton received less than 20 mm of rainfall each month in the spring of 2017. Although Ada was drier than normal during this period, it still received close to 40 mm of rainfall, which was enough to allow the crop to take up additional N where it was available. All the Red River Valley locations received significantly more rainfall in June than the previous two months with Prosper recording the most with over 100 mm. By the time this June precipitation came though, the plants were already at or near heading, past the time when the majority of N is taken up (Fowler et al., 1990).

Soil type may play an important role in the predictability of NDVI on grain protein content. Ada and Red Lake Falls sit on or near the former sandy beachline of glacial Lake Agassiz and thus have a higher sand content than the North Dakota Red River Valley locations. Because N is often in the NO_3-N form it is easily leached. Leaching potential in sandier soils is greater than clayey soils because of the reduced matric potential holding water particles within the profile. This may explain why Ada had the lowest organic matter content of the locations (see Table 1.2) and thus benefitted the most from added N. The N mineralization potential was much higher in the clayey Red River Valley soils at Casselton and Prosper, which decreased the effect that N treatments had on the crop.

A way of quantifying potential locational particularities that may explain why some sites responded more favorable to N treatment could be to compare the ranges of total protein harvest at each location. Table 1.21 shows the range in total protein harvest along with the R^2 value at the boot stage when NDVI was compared with grain protein content. When all locations are combined there is a statistically insignificant, weak negative relationship suggesting that this analysis doesn't hold for explaining the locational variability. However, when the four least drought-affected locations (all three 2016 locations plus Ada in 2017) are grouped together there is a statistically insignificant yet strong relationship between total protein harvest and the predictive ability of NDVI on grain protein content (R^2=0.74). Due to the statistically insignificant relationship, a graphical portrayal of linear regression between these variables is not portrayed.

Table 1.21. The range of total protein harvest and R^2 values of NDVI at ZGS 45 by grain protein content for each location in 2016-2017.

Location	R^2 at ZGS 45	TPH Range
Ada 2016	0.42	496
Red Lake Falls 2016	0.47	487
Casselton 2016	0.32	193
Ada 2017	0.43	597
Gentilly 2017	0.02	490
Casselton 2017	0.06	499
Prosper 2017	0.00	508
Hettinger 2017	0.17	514

Previously, when presenting the graphical linear regression at Ada 2016 data in Figure 1.5. it was noted that data points began to cluster at a normalized NDVI maximum of around 1.0. This phenomenon was even more evident in the 2017 Ada experiment (see Figure 1.8) At the same time, a few data points at lower grain protein contents and NDVI are conspicuously separated from the rest of the data points. The increasingly vertical nature of the high protein and high NDVI data points in these graphs makes it difficult to determine between higher grain protein contents and NDVI values. This limits the utility of NDVI at deciphering between crop areas with protein levels, i.e. between a grain protein content of 135 g kg^{-1} and the 140 g kg^{-1} threshold.

Aerial Spectral Indices

The locations of the on-farm trials were not adversely affected by the abnormally dry summer to the same degree as small plot locations in North Dakota (see Figure 1.10). Total precipitation from April to August in Ada, Wendell, and Perley was 485 mm, 319 mm, and 211 mm respectively. Mean spring temperatures from April-May were 10 ºC at Ada, 10.8 ºC at Wendell, and 10.3 ºC at Perley.

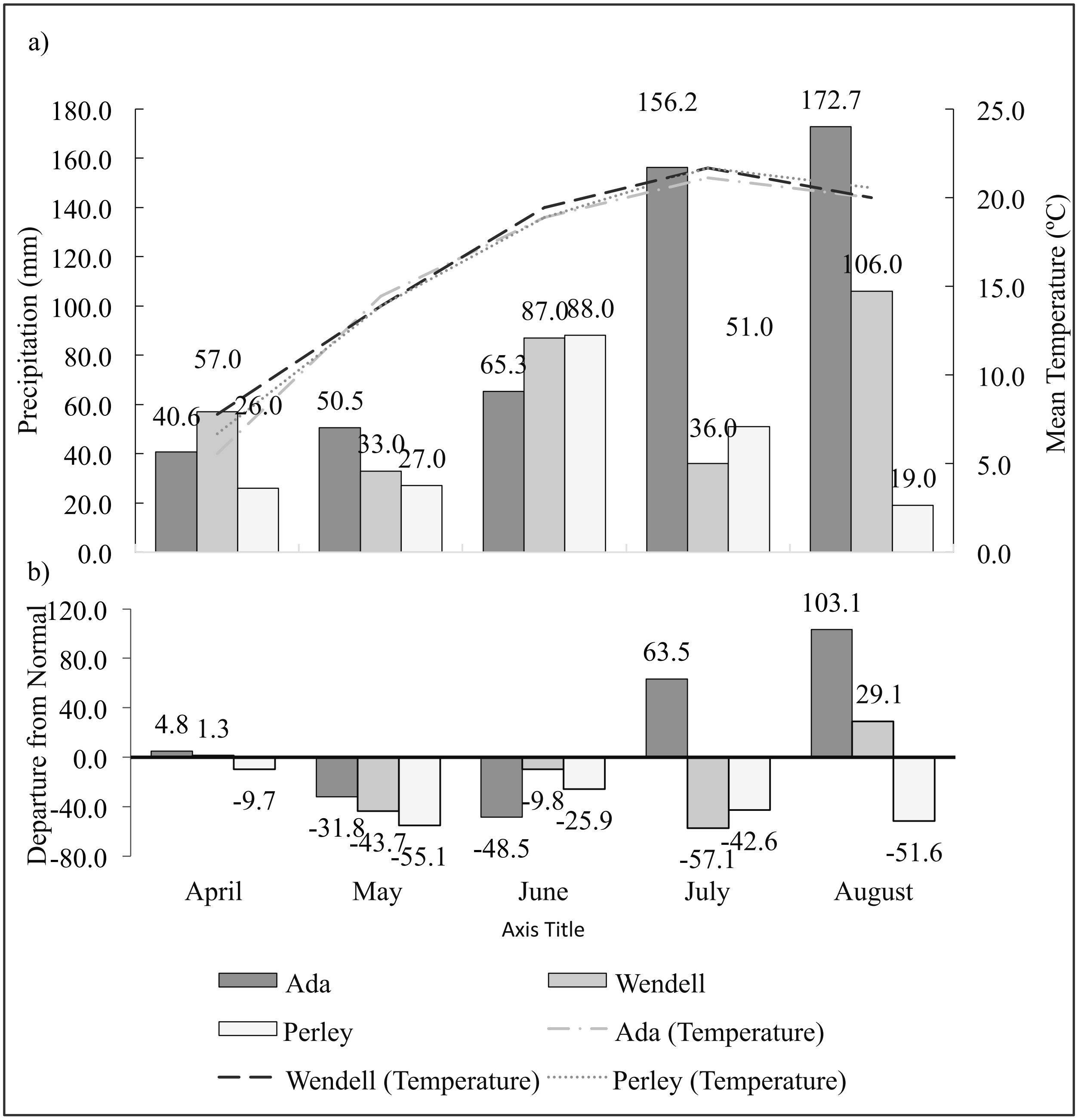

Figure 1.10. a) Total precipitation and mean temperatures for 2017 growing season at Ada, MN; Wendell, MN; and Perley, MN. b) Departure from normal precipitation for each experimental site.

A paired t-test was used to analyze whether grain protein content in the N-rich strip was statistically significant from the control strip, which received no extra N. This test was statistically significant at α=0.05 with a t value of -3.46 and the probability of a greater t at 0.0061. This indicates that the N-rich strip had increased grain protein content relative to the normal field. The change in grain protein content was relatively small, albeit positive in most cases (Table 1.22). There was generally an increase in spectral indices from the check to the N-rich strip. The change in protein is plotted against the change in both NDVI and NDRE in Figure 1.10 and illustrates the strength in the relationship between these variables. Proc Reg analysis in SAS failed to prove significance between the change in grain protein content and the change in NDVI (Pr > t = 0.19) and NDRE (Pr > t = 0.62) respectively. This may be due to the limited degree of freedom due to the small dataset.

Table 1.22. Change in grain protein content means, NDVI, and NDRE between the check and N-rich treatments for on-farm trials in Ada, Wendell, and Perley, MN in 2017.

Location	Δ† GPC‡ mean	Δ NDVI mean	Δ NDRE mean
	$g\ kg^{-1}$		
Ada	7.35	0.0104	0.0183
Wendell 1	6.20	0.0028	0.0079
Wendell 2	2.87	0.0011	0.0047
Perley	1.40	0.0055	0.0108

† Δ = change. The value of the check was subtracted from the value of the N-rich strip for each sample.

‡ GPC = grain protein content.

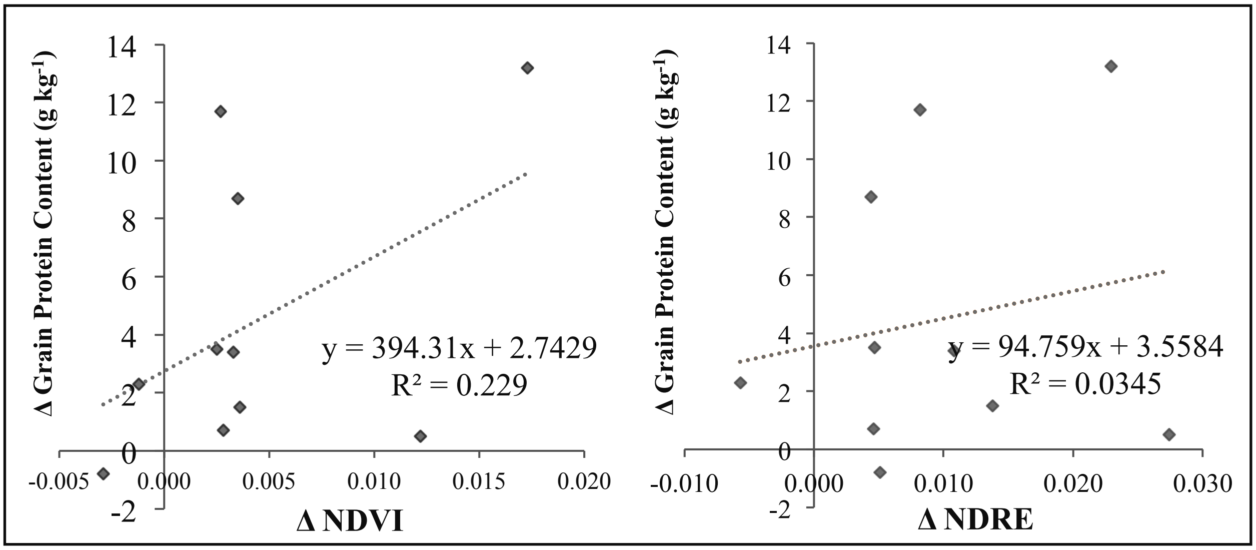

Figure 1.11. Change in grain protein content by change in spectral index based on comparison between the N-rich strip and the check for on-farm trials at Ada, Wendell, and Perley, MN in 2017. a) change in NDVI by change in grain protein content. b) change in NDRE by change in grain protein content.

A clear advantage of aerial imagery is that spectral data can be collected for a single pixel size. Although protein data points were limited in this study, hundreds of thousands of NDVI data were collected (see Tables 1.23-1.24). An example of a sample area for both the N-rich and check strips is delineated in Figure 1.12. The pixel number equals the number of sampling points for NDVI/NDRE, and thanks to the sheer number of samples, more confident conclusions can be made about the ability of an added in-season N rate to influence NDVI or NDRE. The mean for NDVI was slightly higher in the N-rich strip than in the check; but, with the exception Ada, differences were minimal. Interestingly, this wasn't the case for NDRE, where the check outperformed the N-rich strip in Ada and was roughly equal in the rest of the locations. Ranges and standard deviations were Included in these tables to illustrate an interesting difference between the N-rich strip and the check. With NDVI, the ranges and standard deviations in the check were greater than those of NDVI means from the N-rich strip. It's hypothesized that this may be due to greater N stress in the check. With NDRE however, this observation only held true for two locations.

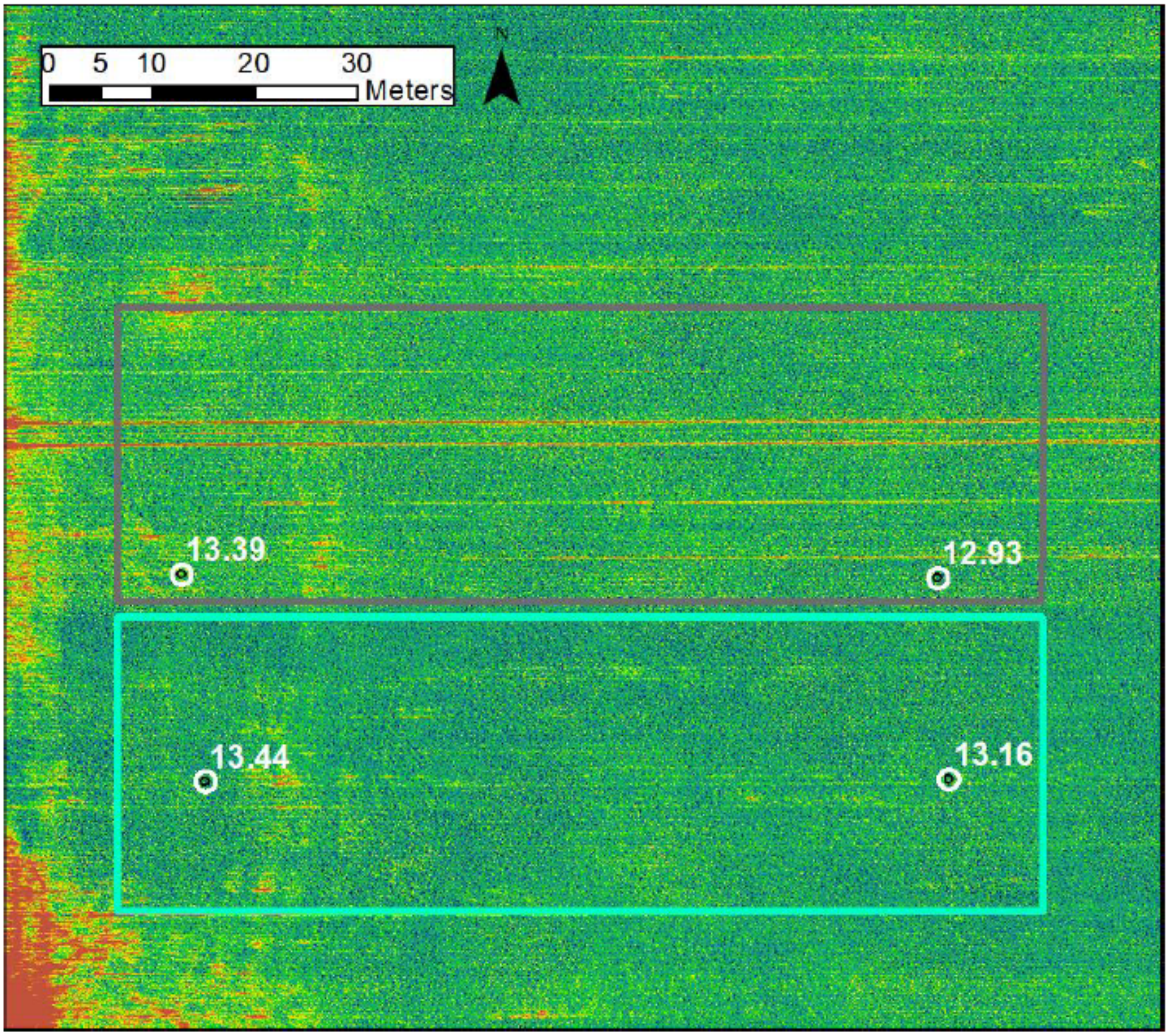

Figure 1.12. Sample location at Perley, MN. N-rich strip is highlighted in turquoise and the check is in gray. Protein sampling points are set to scale and values indicated in g $100g^{-1}$.

Table 1.23. Descriptive Statistics for NDVI data over a N-rich strip and check at on-farm trials in Ada, Wendell, and Perley, MN in 2017.

				NDVI					
				N-rich			Check		
	Sample Area	Pixel Number	Pixel Size	Mean	Range	Std†	Mean	Range	Std†
Ada	267	115,582	23	0.8067	0.6565	0.080	0.7799	0.6991	0.106
Wendell 1	1418	864,958	16	0.9232	0.3432	0.015	0.9195	0.3558	0.017
Wendell 2	1196	616,378	19	0.9191	0.1362	0.010	0.9182	0.1420	0.001
Perley	1904	1518077	13	0.9191	0.3344	0.015	0.9135	0.3408	0.020

† std = standard deviation.

Table 1.24. Descriptive Statistics for NDRE data over a N-rich strip and check at on-farm trials in Ada, Wendell, and Perley, MN in 2017.

				NDRE					
				N-rich			Check		
	Area †	Pixel Number	Pixel Size	Mean	Range	Std	Mean	Range	Std
Ada	267	115,582	23	0.4054	0.3829	0.053	0.4220	0.3544	0.043
Wendell 1	1418	864,958	16	0.5686	0.4203	0.031	0.5540	0.4349	0.036
Wendell 2	1196	616,378	19	0.5783	0.2760	0.024	0.5794	0.2518	0.024
Perley	1904	1518077	13	0.5761	0.3922	0.036	0.5670	0.4301	0.040

† Area where NDVI and NDRE were taken.

Conclusion

Timing of sensing application clearly plays a role in the predictive ability of NDVI and NDRE of grain protein content, with sensing at the boot stage generally being the most predictive. Sensing at the four-leaf stage was not predictive of grain protein content with the highest positive R^2 at this timing being 0.27. Linear regression shows that even with the most predictive experiment and timing, normalized NDVI was only moderately predictive of grain protein content ($R^2 = 0.48$). Even under the most predictive situations, it was only possible to distinguish higher protein contents from lower ones; distinguishing between grain protein contents of 130 g kg^{-1} from 140 g kg^{-1} was not possible. This presents a challenge as producers will often need to distinguish between grain protein contents, such as 135 g kg^{-1} from 140 g kg^{-1}, that likely result from current fertilization practices. Thus, the utility of NDVI at predicting grain protein content given the current management practices is limited. However, in agreement with Wiersma (2017), NDVI could be useful as a "rescue option" during years when weather may indicate that low grain protein contents will result. Using a model such as the Decision Support System for Agrotechnology Transfer (DSSAT) could be a useful tool in this regards as it factors in future weather predictions (Schimek, 2018). NDVI and NDRE were generally more predictive of total protein harvest. Although total protein harvest is not factored into the price that growers receive for their grain, it may be a useful indicator because it accounts for the change in yield and protein from a given treatment. Attaching an economic value to a total protein harvest could allow NDVI to predict the economic return from a potential N application. These data also suggest that in these locations NDRE is less predictive of grain protein content. This may be due to the lower biomass accumulation likely resulting from drier-than-average growing conditions.

Environment seems to play a major role in the predictive power of spectral indices. Precipitation and soil classification could play a role in determining whether a location will have a higher NDVI predictive ability with grain protein content. The range in total protein harvest could also signify how predictive NDVI may be on grain protein content. The predictive ability of NDVI may be higher for a location with a larger range in total protein harvest.

Aerial sensors could play an important role in predicting grain protein content. As aerial imagery from this study demonstrated, collecting NDVI can be done on a much more massive yet precise scale than with hand-held sensors. This experiment showed a very weak, positive relationship between NDVI/NDRE and grain protein content; however, there were not enough protein samples for significance at the $\alpha=0.05$ probability level. To best evaluate whether sensors can effectively predict grain protein content, methods to take greater protein samples should be incorporated into the experimental procedure. One innovative method may be to use an on-combine protein analyzer in conjunction with aerial-sensed fields, as these protein analyzers are able to take measurements every few meters (Proulx and Proulx, 2017).

Finally, to best determine the predictive ability of spectral indices on grain protein content under given environmental factors, a multiple step-wise regression model that includes candidate explanatory variables such as total precipitation, growing degree days, and pre-plant N content should be applied, similar to what was done by MacNack et al. (2014).

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ARTICLE II: NITROGEN FERTILITY OPTIMIZATION FOR BOOSTING GRAIN PROTEIN CONTENT IN HARD RED SPRING WHEAT

Abstract

Optimizing nitrogen rate, timing, and source is important for boosting grain protein content of hard red spring wheat (HRSW) (*Triticum aestivum* L.) and for reducing negative environmental impacts. This study investigated the grain protein boosting potential of different N rates, timings, and sources on small plot trials in North Dakota and Minnesota. All treatments, were fertilized pre-plant at either 70% or 100% of the recommended rate. Experimental plots then received an in-season application of urea or urea ammonium nitrate (UAN, 28-0-0) at rates of 33 kg ha^{-1} or 67 kg ha^{-1} at the four-leaf, boot or post-anthesis growth stage. A post-anthesis UAN application generally boosted grain protein content above treatments not receiving in-season applications but was, with one exception, lower than boot-stage N applications. Nitrogen applied as urea the boot stage at a rate of 67 kg ha^{-1} generally resulted in the highest grain protein contents. Boot stage applications may also provide a greater economic return than an application at anthesis.

Introduction

Nitrogen fertility management is a key agronomic factor in growing a profitable crop of Hard Red Spring Wheat (HRSW, *Triticum aestivum* L.). Profitable HRSW production requires having a high yield and high grain protein content, both which require ample N; albeit at different plant growth stages. Furthermore, nitrogen management is crucial to reducing negative side-effects of fertilization to the environment (Johnston and Bruulsema, 2014). Recently, efforts to coordinate a global nutrient framework have centered around the “4R’s”, best management practices recommending the *r*ight source, *r*ight rate, *r*ight timing, and *r*ight place of fertilizer applications (Johnston and Bruulsema, 2014; Snyder, 2016). Properly applying these management practices to boost grain protein content begins with understanding the crop's lifespan.

As the wheat plant progresses through its lifespan, its demand for N follows a sigmoidal pattern. The N demand is low up to tillering, increases rapidly from stem elongation to anthesis, and then slows after anthesis (Brown et al., 2005; Clarke et al., 1990). Whether N increases yield and/or protein is highly dependent on when it is used by the plant and weather conditions. If moisture, P, and K are not limiting, sufficient N from emergence through heading encourages tillering and increases kernel number, thus boosting yield (Brown et al., 2005; Jones and Olson-Rutz, 2012). Under the same environmental conditions, after the number of tillers and kernels is fixed, an ample N supply lessens its effect on yield, and instead increases grain protein content (Alkier et al., 1972; Brown et al., 2005; Jones and Olson-Rutz, 2012). Weather can drastically affect these processes however, particularly during the grain filling stages.

During grain filling both starch and protein are deposited into the kernels, the starch coming from recently fixed CO_2 and the protein mainly from remobilized N (Jenner et al., 1991). Starch influences the plant's yield; whereas, deposited N makes up the grain protein content that wheat quality is graded upon. Hot, dry conditions favor protein deposition because starch accumulation ceases above 30º C (Jenner et al., 1991), and restricted moisture slows photosynthesis (Brown et al., 2005). Under extreme drought, both starch and protein deposition can suffer (Campbell et al., 1997; Jenner et al., 1991). In contrast, sufficient moisture increases both protein and starch in the grain; however, because ample moisture also often results in increased tillers and starch deposition, grain protein is generally diluted (Fowler et al., 1990; Gao et al., 2012). Although there is often a negative relationship between yield and grain protein

(Orloff et al., 2012), the functions that govern starch and protein deposition are independent, separate mechanisms and theoretically it is possible to have both a high yielding and high protein wheat crop (Jenner et al., 1991).

Understanding the mechanism of grain protein deposition is important to properly managing N. Grain protein deposition begins approximately 10 days after flowering (Jenner et al., 1991). This protein is derived from free amino acids originating from senesced tissue and storage proteins such as Rubisco (Barneix, 2006). The mechanisms governing rubisco degradation are relatively unknown, but the hormone cytokinin plays a major role in the degradation of senesced tissue (Barneix, 2006). High cytokinin activity represses senescence and when it is removed apoptotic cell expression is induced freeing up amino acids that are then transported to the grain (Barneix, 2006). Estimates range between 65-80% of grain protein originating from remobilized N (Dalling et al., 1976; Spiertz, 1983), leaving a need for additional N derived from soil NO_3-N (Clarke et al., 2003). Timing this additional N is also crucial to ensure that it boosts grain protein content.

Growers have several viable options to apply N with hopes of boosting their crop's grain protein content while maintaining yield. In the Northern Great Plains N is generally applied entirely before or at planting (Rehm and Franzen, 2005), either as granular urea or anhydrous ammonia (Schoch, 2013). Spring wheat growers are generally able to attain high yields when solely fertilizing at planting; however, N losses from denitrification and leaching can result in an inadequate N supply for the plant to reach the grain protein content market threshold of 140 g kg^{-1} (Woolfolk et al., 2002.

Consequently, there has been much focus on fertilizer application strategies to also boost grain protein content. One strategy employs a mixture of granular urea and a slow-release polymer-coated urea, and it has shown promise at boosting both yield and protein (Farmaha and Sims, 2013). This may be highly dependent on environment however, as other studies have found no added benefit of a urea-PCU mixture on yield and/or protein (Hillenbrand, 2017; McKenzie, 2006). Another strategy focuses on splitting N fertilizer between pre-plant and in-season applications. This can reduce N losses due to the temporal asynchrony between N supply and plant demand and has potential for boosting grain protein content (Alkier et al., 1972; Bly and Woodard, 2003; Brown and Petrie, 2005; Otteson, et al. 2007). In these split-application studies, additional N boosted grain protein content anywhere from 5 to 17 g kg^{-1} depending on the rate, timing, and environment. Nevertheless, for the additional N to boost grain protein the vegetative needs of the plant must have been met (Jones and Olson-Rutz, 2012). Gauer et al. (1992) demonstrated that if the N requirement for yield potential has not been met, adding N will, at best, only marginally increase grain protein. In some situations, added N may boost yield but decrease grain protein content (Fischer et al., 1993). It is also possible that abundant N can hamper grain protein by buttressing cytokinin; which results in a delay in senescence, and with that, N remobilization (Barneix, 2006).

A key component of a successful split application to boost grain protein content is to time fertilization so that assimilated N will result in an increase in grain protein. This narrow time window is roughly between the boot stage (ZGS 45, Zadoks et al., 1974) and post-anthesis (ZGS 69). More N is assimilated into the grain at the boot stage than at anthesis; however, because some N still goes towards yield at this stage, protein content may be diluted (Brown and Petrie, 2005). On the other hand, applications made at or post-anthesis have a “singular” effect of boosting grain protein content (Strong, 1982), because yield is marginally influenced after this time (Gooding and Davies, 1992). At this stage, a foliar application of UAN (28-0-0) has been shown to boost grain protein more than at other stages (Bly and Woodard, 2003; Finney et al., 1957; 2003; Strong, 1982). Minnesota Extension affirms that a foliar application of 34 kg ha^{-1} at anthesis will boost grain protein content by 5 to 10 g kg^{-1} 80% of the time (Kaiser et al., 2013).

Foliar N application has come into use because of the potential for more rapid nutrient absorption (Gamble and Emino, 1987; Fernandez et al., 2013), increased nitrogen use efficiency (NUE), and as a method for plant uptake in stressed conditions (Gooding and Davies, 1992). Because N is mostly taken up through the leaves with this method, NUE is improved as there are fewer losses to the environment in the short-term due to the minimal fertilizer contact with the soil. (Gooding and Davies, 1992). Foliar application is also advantageous under saline soils or dry weather conditions that limit root activity; although drier conditions may cause the foliar solution to crystallize, hampering uptake (Gooding and Davies, 1992). A common concern of foliar applications is that they can cause leaf burn, especially when tank mixed with fungicide and/or applied in hot temperatures (Schimek, 2017). Damage at this stage seems to be mainly aesthetic though, as yield is only marginally affected, if at all (Franzen, 2017).

A concern with both UAN and urea applications is their poor NUE (Jones and Olson-Rutz, 2012). Ammonia (NH_3) volatilization is a principal means of N loss with foliar and granular urea applications; because urea is readily hydrolyzed to NH_3 by ureases (Soares et al., 2012), especially when a light precipitation occurs after application. Mixing a urease inhibitor can be an effective way to potentially prevent significant N losses, although its potential for leaf-burn may be greater with N rates above 22 kg ha^{-1} (Jones and Olson-Rutz, 2012). Whether a urease inhibitor is added or not, rainfall is critical for proper in-season uptake of soil-applied N. Therefore, Brown and Petrie (2005) recommend coordinating an in-season application with a likely rain event anytime from boot to anthesis.

Although an additional in-season N application will likely boost grain protein content, it may not always be in the grower's best interest. Economic factors, namely protein premium/discounts and fertilizer prices, need to be carefully considered. Additional N applications often fail to reach a financial breakeven point (Gauer et al., 1992). Targeting 140 g kg^{-1} grain protein content may not always be economical either (Baker et al., 2004).

The objective of this experiment was to determine the optimal N application and timing at boosting grain protein content given a range of environmental conditions. In conjunction, it sought to establish the economic utility of additional fertilization.

Materials and Methods

Experimental Procedure

Experiments were conducted in six locations in the North Dakota and Minnesota Red during the 2016-2017 growing seasons. All locations were in the Red River Valley, except for Hettinger, which is in southwestern North Dakota. Weather data were collected from the closest North Dakota Agricultural Weather Network (NDAWN) Station. The 2016-2017 experimental stations with their proximity to the nearest NDAWN station are as follows: Ada, MN (8.7 km); Gentilly, MN (35.2 km); Red Lake Falls, MN (40.6 km); Casselton, ND (16.1 km); Prosper, ND (0.2 km); and Hettinger, ND (0.2 km). Soils in these locations are mollisols with relatively high organic matter and low to high sand contents (See Table 2.1). Residual NO_3-N for each location was determined from soil testing conducted in the fall prior to planting (See Table 2.2). (Soil testing for Gentilly was conducted in the spring prior to planting). Previous crops were either soybean or HRSW (See Table 2.2).

Table 2.1. Soil series, texture, taxonomy, and slope for Ada, Red Lake Falls, Gentilly, MN and Casselton, Prosper, Hettinger ND for 2016 and 2017.

Location	Series†	Texture	Taxonomy	Slope %
Ada, 2016	Rockwell	sandy clay loam	Coarse-loamy, mixed, superactive, frigid Typic Calciaquolls	0-2
Ada, 2017	Glyndon	loam	Coarse-silty, mixed, superactive, frigid, Aeric Calciaquolls	0-3
Casselton	Kindred	silty clay loam	Fine-silty, mixed, superactive, frigid Typic Endoaquolls	0-2
Gentilly	Foxlake	silty clay loam	Fine, smectitic, frigid Vertic Epiaquolls	0-2
Hettinger	Belfield	silty clay loam	Fine, smectitic, frigid Glossic Natrustolls	0-1
Prosper	Bearden	silty clay loam	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-3
Red Lake Falls	Wheatville	fine sandy loam	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	0- 3

† Soil data obtained from Web Soil Survey (USDA-NRCS, 2018).
‡ Soil taxonomy listed on individual lines based on hyphenated soil series name.

Table 2.2. Nitrate-N, P, K and organic matter content for experimental locations 2016-2017.

Location	NO_3-N	P	K	OM†
Cropping Season 2016	----------------------mg kg^{-1}--------------------			%
Ada, MN	4	4	26	2.2
Red Lake Falls, MN	15	15	70	3.6
Casselton, ND	5	17	285	4.0
Cropping Season 2017				
Ada, MN	7	11	107	1.9
Gentilly, MN	26	6	196	3.2
Casselton, ND	37	11	368	3.3
Prosper, ND	48	17	222	2.4
Hettinger, ND	33	21	530	3.4

† OM = organic matter.

All experimental locations were comprised of treatments derived from an incomplete factorial of N rates by timings arranged in a randomized complete block replicated four times (see Table 2.3). The N treatments were based on recommended rates (rate) from the North

Dakota Wheat Nitrogen Calculator (Franzen and Kariluoma, 2009), which subtracts residual soil NO_3-N and previous cover crop credits (see Table 2.4). The rates used were representative of realistic amounts that producers would apply to attain profitable yields and protein (see Table 2.3).

Table 2.3. Rate and timing of N treatments in 2016-2017.

		N application Amount and Timing		
Treatment†	Pre-plant	Four Leaf (ZGS¶ 15)	Boot (ZGS 45)	Anthesis (ZGS 69)
			----------------------------------Kg N ha^{-1} ---------------------------------	
1	70% rate‡			
2	100% rate			
3	70% rate	33.6 urea		
4	70% rate	67.3 urea		
5	100% rate	33.6 urea		
6	100% rate	67.3 urea		
7	70% rate		33.6 urea	
8	70% rate		67.3 urea	
9	100% rate		33.6 urea	
10	100% rate		67.3 urea	
11	70% rate			33.6 UAN
12	100% rate			33.6 UAN
13	70% rate	33.6 UAN#		
14§	70% rate	67.3 UAN		
15	70% rate		33.6 UAN	
16§	70% rate		67.3 UAN	
17	224 kg ha^{-1} rate			
18	0			

† At Hettinger only Treatments 1,2,3,7,11,13,15,17 and 18 were applied.
‡ Percentage of the recommended rate based on the North Dakota Wheat Nitrogen Calculator.
§Treatments 14 and 16 were applied in 2017 only.
¶ ZGS = Zadoks Growth Stage.
# UAN = urea ammonium nitrate (28-0-0).

Granular urea (46-0-0) was hand-broadcast pre-plant for all treatments receiving fertilizer at either the 70% or 100% rate, with one treatment at a rate of 224 kg ha^{-1}. Although some locations in 2017 received a different fertilizer amount for other treatments (see Table 2.4) based on the North Dakota wheat nitrogen recommendations, this 224 kg ha^{-1} treatment was applied at all locations. Because this treatment was intended to provide more than enough N for the HRSW crop at locations with little residual NO_3-N, it was assumed that locations with a higher residual NO_3-N would receive no added benefit from a higher rate comparative to other locations. Each location had a check of 0 N. All experimental locations had 18 treatments, except for Hettinger, which had nine. Fourteen treatments (five at Hettinger) received additional N as granular urea (46-0-0) or urea ammonium nitrate (UAN, 28-0-0) at the four-leaf stage, boot stage, or anthesis. Granular urea was broadcast by hand at a rate of either 34 kg ha^{-1} or 67 kg ha^{-1}. Application of UAN was done with streamer bars at the four-leaf and boot stages to reduce leaf burn and decrease drift; application at anthesis utilized a flat fan XR Teejet 8002 VS nozzle. Ratios of UAN to H_2O were 100:0 for the four-leaf and boot stage applications and a 50:50 mixture for the anthesis application. Application of UAN was done with a pack sprayer and walking speed was adjusted to align with the proper treatment rate. Foliar applications at the four-leaf and boot stages were applied at a rate of 33.6 kg ha^{-1} or 67 kg ha^{-1}; whereas, the two UAN treatments at anthesis were applied at a rate of33.6 kg ha^{-1}. In 2017 to compensate for the lack of rain Agrotain Advanced, a urease inhibitor (N-(n-butyl) thiophosphoric triamide, Koch Agronomic Services, LCC, Wichita, KS) was added to urea and UAN treatments at a rate of 2 ml kg^{-1} and 1 ml kg^{-1} respectively. Foliar applications were applied mid-morning when possible.

Table 2.4. Previous crop and actual N amounts applied based on North Dakota Wheat Nitrogen Recommendation Calculator for 2016-2017.

		Amount of N applied at planting		
Location	Previous crop	70% rate	100% rate	224 kg ha^{-1} rate
Cropping Season 2016		-------------------- kg ha^{-1}	--------------------	
Ada, MN	soybean†	86	123	224
Red Lake Falls, MN	soybean	86	123	224
Casselton, ND	HRSW‡	86	123	224
Cropping Season 2017				
Ada, MN	soybean	106	152	224
Gentilly, MN	soybean	106	152	224
Casselton, ND	HRSW	86	123	224
Prosper, ND	HRSW	86	123	224
Hettinger, ND	HRSW	86	123	224

Experimental plots were seeded at a rate of 3 million viable seeds ha^{-1} with a GreatPlains 35605NT drill (Great Plains Mfg. Inc., Salina, KS). In both years all plots consisted of seven rows with 18 cm spacing and spanned 5.9 m in length, except for the 2016 Casselton plots, which were 3.7 m in length. Planting took place between April 14 and May 1 of each year for all locations except the 2016 Casselton experiment, which was seeded on May 19. The late planting at Casselton was intended to provide an additional, lower yielding environment. Border plots were included on the outside column of experimental plots, to ensure similar plot-to-plot competition as interior plots. Weeds were controlled chemically and by hand-hoeing when needed. In 2016 the fungicide combination of propiconazole (1-((2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole) and trifloxystrobin (Benzeneacetic acid, (E,E)-alpha(methoxyimino)-2-[[[[1-[3- (trifluoromethyl)phenyl]ethylidene]amino] oxy]methyl]- ,methylester) was applied with a flat fan nozzle during anthesis at a rate of 64 g and 64 g ai ha^{-1} respectively. Each plot was harvested with a Wintersteiger Classic plot combine (Wintersteiger Ag, Ried, Austria); and yield, moisture, and test weight were immediately measured with a HarvestMaster Classic Grain Gage (Juniper Systems, Logan, UT). In calculating yield expressed at 13.5% moisture, it was decided to not measure plot lengths of each range and instead use the respective 5.9 m or 3.7 m for all plots in the respective trial. A subsample was taken from each plot at harvest, processed and cleaned and analyzed for grain protein content adjusted at 12% moisture with a DA 7250 NIR analyzer (Perten Instruments, Hagersten, Sweden).

Statistical Analysis

Data were analyzed using the PROC GLM procedure in SAS 9.4 (SAS Institute, Cary, NC). Replication was considered a random effect and treatment was considered a fixed effect. Statistical significance was determined at the 95% and 99% levels of confidence (α=0.05, α=0.01). In addition, mean separation was performed using Fischer's protected least significant difference (LSD) at the 95% confidence level (α=0.05).

Orthogonal contrasts were used to compare meaningfully grouped treatment means based on planned investigations of the experiment (See Table 2.5). The same contrasts were performed at each location, except for Hettinger where two of the contrasts were not performed as not all treatments were included at this location (see Table. 2.5).

Table 2.5. Orthogonal contrasts with treatment groups in 2016-2017.

Orthogonal Contrast	Treatment(s)†
100% rate vs. 70% rate + 34 kg ha^{-1} N in-season	2 vs. 3,5,7,15
70% rate vs. 100% rate ‡	1,3,4,7,8 vs 2,5,6,9,10
In-season urea vs. in-season UAN	7,8,3,4 vs. 13,14§,15,16§
N applications at ZGS 15 & 45 vs. ZGS 69	11,12, vs. 9,7,5,3
N applications at ZGS 15 vs. ZGS 45	3,4,5,6,13,14§ vs. 7,8,9,10,15,16§
0N (check) vs. added N	18 vs. 1-17
224 kg ha^{-1} rate vs. all other N rates	17 vs 1-16§
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1} ‡	3,5,7,9,13,15 vs. 4,6,8,10,14§,16§

† See Table 2.3 for treatment descriptions.
‡ These contrasts were not performed at Hettinger.

Results and Discussion

Weather Information

Weather can have a notable impact on crop performance, and thus will be included for both growing seasons. Both the 2016 and 2017 growing seasons were drier than the 30-year norm with the 2017 being significantly drier than 2016 (See Figure 2.1). As there was a statistically significant difference between locations in both growing seasons, weather information will be discussed by year and location.

Combined Analysis

Combining locations for statistical analysis allows for greater inferences about the effect of a given treatment across a wider geographical area or in future seasons; however, if there is a significant treatment by location interaction then locations should be discussed on an individual, and not combined, basis (Moore and Dixon, 2015). Combined analyses were explored for the following combinations: a) all experiments spanning both years, b) 2016 experiments only, and c) 2016 experiments plus Ada in 2017. In each of these combined analyses the treatment by location interaction was significant at the $\alpha = 0.05$ level. Therefore, it was decided to present and discuss each experimental location individually.

2016 Growing Season

Temperatures during the early spring of 2016 were roughly three degrees Celsius above average; however mean daily temperature was normal for the rest of the growing season. Mean temperatures from April-May were 10 ºC at Ada, 9.7 ºC at Red Lake Falls, and 10.3 ºC at Casselton. Mean temperatures from June-August were 20 ºC at Ada, 19.8 ºC at Red Lake Falls, and 20.4ºC at Casselton (NDAWN, 2018). Total precipitation for the growing seasons was roughly normal, with June being drier but July and August generally being wetter than normal.

Total precipitation for Ada was 403 mm, 402 at Red Lake Falls, and 277 at Casselton. Wetter-than-normal precipitation may have increased mineralizable N uptake into the plant as more water was available.

2017 Growing Season

The 2017 growing season made headlines throughout the country; most of the state of North Dakota and parts of northwest Minnesota were engulphed in a drought. Western North Dakota and Eastern Montana were the hardest hit, with Hettinger being one of the driest locations in the state (NDAWN, 2018). Growing season precipitation totals for the experimental sites reflect the drastic differences between 2016 and 2017 (see Figures 2.1 – 2.2). From April to August total rainfall for each location was 236 mm (Ada), 195 mm (Gentilly), 224 mm (Casselton & Prosper), and 142 mm (Hettinger). Precipitation during the month of May, when wheat rapidly accumulates biomass, was notably low. As Figure 2.2 shows, the drought hit the North Dakota locations hardest with Hettinger being the most severe. Rainfall was much higher in June; however, due to the dry spring, yield and protein were already significantly affected.

Average temperatures for experimental locations were normal. April-May mean temperatures for the experimental locations were 10 ºC (Ada), 6.1 ºC (Gentilly), 10 ºC (Casselton & Prosper), and 9.8 ºC (Hettinger). June-August mean temperatures were 19.4 ºC (Ada), 18.9 ºC (Gentilly), 19.4 ºC (Casselton & Prosper), and 20.9 (Hettinger).

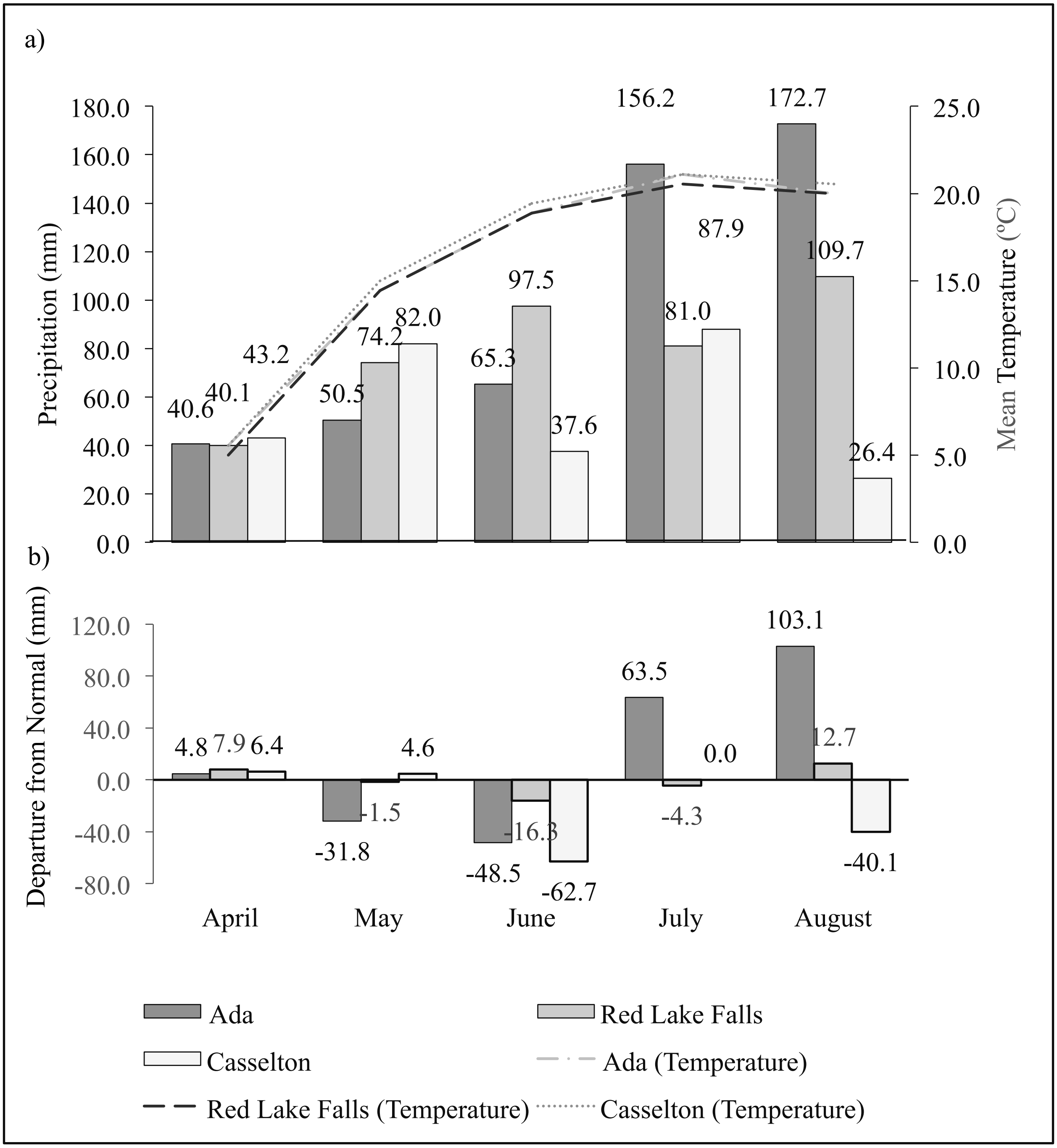

Figure 2.1. a) Total precipitation and mean temperatures for 2016 growing season at Ada, MN; Red Lake Falls, MN; and Casselton, ND. b) Departure from normal precipitation for each experimental site.

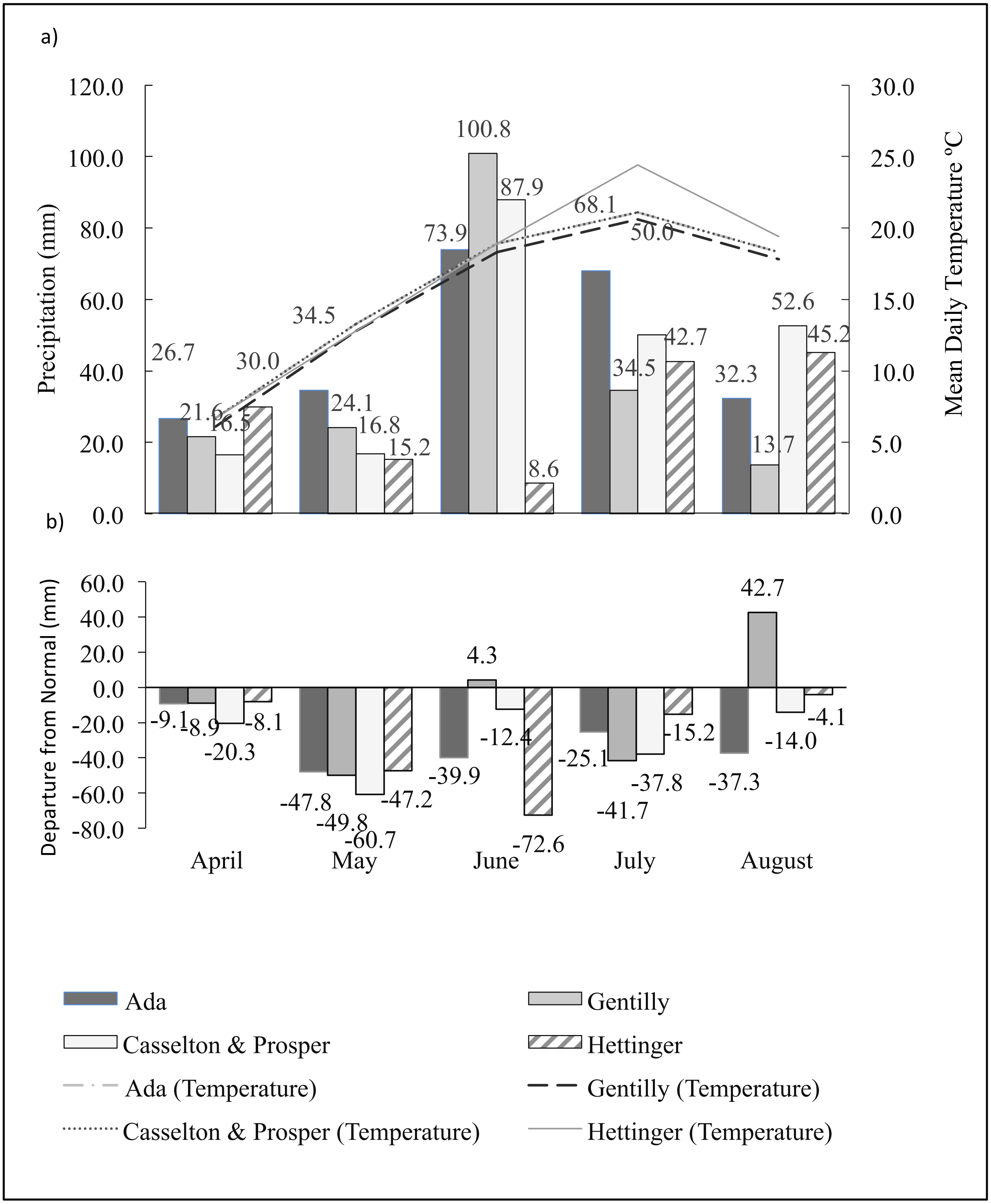

Figure 2.2. a) Total precipitation and mean temperatures for 2017 growing season at Ada, MN; Gentilly, MN; Casselton, ND; Prosper, ND; and Hettinger, ND. b) Departure from normal precipitation for each experimental site. Both Casselton and Prosper utilize the same NDAWN weather station.

2016 Growing Season

Nitrogen treatment was statistically significant for grain protein content at all locations and several treatments were statistically significant from one another based on the F-protected LSDs ranging from 2.8 to 4.2. Orthogonal contrasts were used to investigate and compare treatment timings, rates, and fertilizer types. These, along with other trends, will be addressed by each location.

Ada, MN

As was hypothesized, added N was statistically significant at the α=0.05 level at boosting grain protein content when compared to the 0 N treatment. On the other side of the spectrum, the 224 kg ha^{-1} recommended rate at pre-plant was also statistically significant from other N treatments. Contrasts between all treatments receiving 70% of the recommended N rate at pre-plant and 100% at pre-plant were not statistically significant at Ada and were not statistically significant in any location (See Table 2.6). Of the two in-season rates – 34 kg ha^{-1} and 67 kg ha^{-1} – the higher rate was statistically significant from the lower at the α=0.01 level of confidence

Timing between certain N applications was statistically significant at boosting grain protein content, with statistical significance between applications at the four-leaf stage compared to boot stage (See Table 2.6). The treatments with the highest grain protein content both had applications at the boot stage. It was hypothesized that the fertilization timing that would boost grain protein content the most would be the post-anthesis application at the boot stage; however, there was no significance between application at anthesis and the earlier in-season applications. This contradicts previous results that found UAN application at this stage to be the most effective at boosting grain protein content (Bly and Woodard, 2003; Brown et al., 2005; Fowler et al., 1990). Because the summer was drier than average and there was no significant rainfall over 1 cm soon after UAN application to bring N into the rooting zone, much of the N may have volatilized. This may explain why urea was significantly higher at boosting grain protein content when compared to UAN. Nitrogen rate and timing treatments were only statistically significant at boosting yield – and indirectly grain protein content – when compared with the check and the 70% pre-plant-only treatment (see Table 2.7).

Table 2.6. Means of grain protein content resulting from N fertilizer treatment at Ada, MN; Red Lake Falls, MN; and Casselton, ND during the 2016 Growing Season.

			Grain Protein Content		
N source	Pre-plant N	In-season N rate and timing	Ada	Red Lake Falls	Casselton
		kg ha^{-1}		-------- g kg^{-1} -------	
Urea†	0		113.9	109.7	149.3
Urea	70%‡		121.3	127.5	153.9
Urea	100%		127.1	130.6	152.7
Urea	224 kg ha^{-1}		130.1	136.2	153.5
Urea	70%	34 at ZGS 15	128.8	133.9	152.3
Urea	70%	67 at ZGS 15	132.0	135.1	155.1
Urea	100%	34 at ZGS 15	131.9	135.7	155.0
Urea	100%	67 at ZGS 15	134.3	135.1	152.9
Urea	70%	34 at ZGS 45	132.4	135.0	153.6
Urea	70%	67 at ZGS 45	137.3	137.1	156.0
Urea	100%	34 at ZGS 45	134.2	135.1	153.2
Urea	100%	67 at ZGS 45	137.2	137.5	153.4
UAN§	70%	34 at ZGS 69	132.4	133.4	163.6
UAN	100%	34 at ZGS 69	132.8	135.9	161.4
UAN	70%	34 at ZGS 15	129.4	123.9	151.5
UAN	70%	34 at ZGS 45	125.8	130.3	152.2
CV %			2.2	2.4	1.28
LSD (0.05) ¶			4.1	4.6	2.8
Contrasts					
100% rate vs. 70% rate + 34 kg ha^{-1} N in-season			ns	ns	ns
70% rate vs. 100% rate			ns	ns	ns
In-season Urea vs. in-season UAN			**	**	*
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns	**
N applications at ZGS 15 vs. ZGS 45			*	*	ns
0N (check) vs. added N			**	**	**
224 kg ha^{-} rate vs. all other N rates			*	ns	ns
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1}			**	**	*

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

¶ LSDs apply to columns.

Table 2.7. Means for yield and total protein harvest resulting from N fertilizer treatment at Ada,MN; Red Lake Falls, MN; and Casselton, ND for the 2016 growing season.

			Ada		Red Lake Falls		Casselton	
N source	Pre-plant N	In-season N rate and timing	Yield	TPH†	Yield	TPH	Yield	TPH
		kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	Mg ha^{1}	kg ha^{1}
urea‡	0		4.04	658	5.31	585	3.61	538
urea	70%§		5.19	719	6.28	783	3.78	581
urea	100%		5.96	725	6.33	826	3.97	605
urea	224 kg ha^{-1}		5.85	685	6.59	879	4.22	648
urea	70%	34 at ZGS 15	6.01	742	6.40	857	4.14	629
urea	70%	67 at ZGS 15	6.31	722	6.39	863	4.00	613
urea	100%	34 at ZGS 15	6.24	765	6.42	871	3.72	577
urea	100%	67 at ZGS 15	5.98	723	6.37	860	4.15	635
urea	70%	34 at ZGS 45	5.97	724	6.23	840	3.95	606.
urea	70%	67 at ZGS 45	5.95	758	6.16	844	4.12	642
urea	100%	34 at ZGS 45	5.95	730	6.28	849	4.25	651
urea	100%	67 at ZGS 45	6.41	761	6.32	869	3.83	586
UAN¶	70%	34 at ZGS 69	6.01	731	6.16	820	3.32	543
UAN	100%	34 at ZGS 69	5.91	721	6.28	860	3.67	592
UAN	70%	34 at ZGS 15	6.08	730	6.32	778	3.94	597
UAN	70%	34 at ZGS 45	6.10	719	6.	823	3.70	563
CV %			6.1	6.3	5.5	5.8	6.1	5.8
LSD (0.05)			0.51	ns	0.49	68.7	0.34	50
Contrasts								
100% rate§ pre-plant vs. 70% rate pre-plant + 34 kg ha^{-1} N in-season			ns	ns	ns	ns	ns	ns
70% rate vs. 100% rate			ns	ns	ns	ns	ns	ns
In-season Urea vs. in-season UAN			ns	ns	ns	*	*	*
N applications at ZGS 15 & 45 vs. 69			ns	ns	ns	ns	**	ns
N applications at ZGS 15 vs. ZGS 45			ns	ns	ns	ns	ns	ns
0N (check) vs. added N			ns	**	**	**	*	**
224 kg ha^{-1} rate vs. all other N rates			ns	ns	ns	*	*	*
In-season 34 kg ha^{-1} vs. 67 kg ha^{-1}			ns	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† TPH = Total Protein Harvest.

‡ (46-0-0).

§ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

¶ UAN = urea ammonium nitrate (28-0-0).

Red Lake Falls

The Red Lake Falls location had many similarities with Ada. The only statistical significance with pre-plant N rates was with the check compared to all other N rates (See Table 2.6). Unlike at Ada, the 224 kg ha^{-1} rate pre-plant treatment was not statistically significant from others. Similar to the Ada experiment, timing of application was statistically significant between the four-leaf and boot stages but was not statistically significant for anthesis applications. Also like at Ada, top-dress urea applications of 67 kg ha^{-1} at the boot stage were the top treatments for boosting grain protein content. Some of the UAN treatments had the lowest grain protein contents, particularly a treatment of 34 kg ha^{-1} that had a lower grain protein content than if no in-season N had of been applied.

Similar to the Ada location, yield and total protein harvest were statistically significant with rate and timing treatments but only when compared with the check.

Casselton

Nitrogen rate and timing treatments had different responses at the 2016 Casselton site than were observed at the 2016 Ada and Red Lake Falls sites. This may be due to the fact that the Casselton site was planted five weeks after the Ada and Red Lake Fall locations. Concerning pre-plant N rate, only the check was statistically significantly from other treatments. Similar to Ada and Red Lake Falls, in-season applications of 67 kg ha^{-1} boosted grain protein content significantly better than in-season applications of 34 kg ha^{-1}. Unlike at Ada and Red Lake Falls, in-season treatments at four-leaf and boot stages were not statistically significant from one another. Perhaps the most distinguishing point of the Casselton experiment is that the post-anthesis UAN application significantly boosted grain protein content. In fact, this UAN treatment boosted grain protein content over 10 g kg^{-1} when compared to the 67 kg ha^{-1} application of urea at the boot stage. This boost is even more impressive when considering the grain protein content was nearly at or above 150 g kg^{-1} for all treatments. This individual experiment seems to contradict previous research that found it much more difficult to increase grain protein content above 140 g kg^{-1} (Brown et al., 2005).

Despite the boost in grain protein content with a UAN application at post-anthesis, yield was much lower at Casselton, not surprisingly given the later planting date. A quick comparison of total protein harvest at Casselton with Ada and Red Lake Falls demonstrates that this high grain protein content does not make up for the loss of yield as total protein harvest is much lower at Casselton. Earlier it was pointed out that a post-anthesis application of UAN at 33 kg ha^{-1} boosted grain protein content an entire grain protein content percentage point above a 67 kg ha^{-1} urea application at the boot stage. Even with that additional protein boost, the post-anthesis treatment had a lower total protein harvest (543 kg ha^{-1}) than the previously mentioned one at the boot stage (563 kg ha^{-1}) (See Table 2.7). Casselton was the only location where N source was statistically significant for yield, and UAN applications seemed to negatively affect yield. This could be due to leaf burn but is more likely be attributed to the grain protein content boost.

2017 Growing Season

Ada, MN

The Ada 2017 experiment was the most responsive to N treatment of all the trials spanning both years (see Table 2.8). Similar to the 2016 experiments, pre-plant N rate was statistically significant from the check. The only other pre-plant rate that was statistically significant from other N treatments was the 224 kg ha^{-1} pre-plant rate, similar to results from the Ada, 2016 experiment. There was no statistical significance between the 70% pre-plant and the 100% pre-plant treatments; however, in-season applications of 67 kg ha^{-1} were better at boosting grain protein content than the in-season 34 kg ha^{-1} application.

Table 2.8. Grain protein content by N fertilizer treatment at Minnesota locations in 2017.

N source	Pre-plant N	In-season N rate and timing	Grain Protein Content Ada,MN	 Gentilly,MN
		kg ha^{-1}	------------ g kg^{-1}	------------
urea†	0		128.7	135.5
urea	70%‡		134.6	146.8
urea	100%		143.5	147.9
urea	224 kg ha^{-1}		146.3	146.8
urea	70%	34 at ZGS 15	137.0	144.3
urea	70%	67 at ZGS 15	145.4	151.3
urea	100%	34 at ZGS 15	143.1	148.0
urea	100%	67 at ZGS 15	145.7	149.7
urea	70%	34 at ZGS 45	144.2	150.1
urea	70%	67 at ZGS 45	149.2	153.7
urea	100%	34 at ZGS 45	145.6	153.8
urea	100%	67 at ZGS 45	147.7	148.1
UAN§	70%	34 at ZGS 69	137.3	148.9
UAN	70%	34 at ZGS 69	145.2	149.1
UAN	70%	34 at ZGS 15	137.5	150.1
UAN	70%	34 at ZGS 15	140.6	149.7
UAN	70%	34 at ZGS 45	138.6	147.8
UAN	70%	67 at ZGS 45	142.8	149.2
CV %			2.3	2.6
LSD (0.05)			4.5	5.5
Contrasts				
100% RR vs. 70% RR + 34 kg ha^{-1} N in-season			ns	ns
70% RR vs. 100% RR			ns	ns
In-season Urea vs. in-season UAN			***	ns
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns
N applications at ZGS 15 vs. ZGS 45			**	ns
0N (check) vs. added N			***	***
224 kg ha^{-1} rate vs. all other N rates			*	ns
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1}			***	ns

* Significant at the 0.05 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

Table 2.9. Means for yield and total protein harvest resulting from N treatment at Minnesota sites in 2017.

			Ada		Gentilly	
N treatment	Pre-plant N	In-season N Rate and Timing	Yield	TPH	Yield	TPH
		kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}
urea‡	0		3.34	436	3.79	513
urea	70%§		5.44	729	3.92	570
urea	100%		6.13	879	4.09	601
urea	224 kg ha^{-1}		5.97	873	4.35	635
urea	70%	34 at ZGS 15	5.90	809	4.45	636
urea	70%	67 at ZGS 15	5.83	847	3.49	528
urea	100%	34 at ZGS 15	5.93	847	4.13	605
urea	100%	67 at ZGS 15	6.15	895	4.16	619
urea	70%	34 at ZGS 45	5.90	849	4.01	600
urea	70%	67 at ZGS 45	5.94	886	3.74	572
urea	100%	34 at ZGS 45	6.15	894	3.89	594
urea	100%	67 at ZGS 45	6.29	928	4.70	687
UAN¶	70%	34 at ZGS 69	5.86	805	4.04	599
UAN	70%	34 at ZGS 69	6.21	900	4.12	609
UAN	70%	34 at ZGS 15	5.86	800	3.86	575
UAN	70%	67 at ZGS 15	6.05	851	4.04	600
UAN	70%	34 at ZGS 45	5.86	812	3.90	572
UAN	70%	67 at ZGS 45	5.90	843	3.74	555
CV %			5.4	5.7	18.7	16.1
LSD (0.05)			0.44	60.0	ns	ns
Contrasts						
100% RR vs. 70% RR + 34 kg ha^{-1} N in-season			ns	ns	ns	ns
70% RR vs. 100% RR			*	*	ns	ns
In-season Urea vs. in-season UAN			ns	ns	ns	ns
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns	ns	ns
N applications at ZGS 15 vs. ZGS 45			ns	*	ns	ns
0N (check) vs. added N			**	**	ns	ns
224 kg ha^{-1} rate vs. all other N rates			ns	ns	ns	ns
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1}			ns	*	ns	ns

* Significant at the 0.05 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

Similar to the 2016 experiments, timing between certain N applications was statistically significant at boosting yield when N-fertilized treatments were compared with the check; however, unlike all other locations included in 2016 and 2017, the 70% pre-plant recommended rate was statistically significant for yield and total protein harvest (see Table 2.9). Apart from Hettinger, Ada was the only location in 2017 that had any statistical significance for yield.

Gentilly, Casselton, and Prosper

Due to a statistically significant Bartlett's test for homogeneity error of variance, these sites were statistically analyzed separately; however, due to their similar lack of response to N treatment they will be discussed together. As was mentioned in Article I, drought was likely the driving force behind the lack of response in these sites.

In each of these three locations grain protein content was only statistically significant when N treatments were compared with the check, with the exception of Prosper where the 224 kg ha^{-1} pre-plant N rate was also statistically significant from other treatments (see Tables 2.8 and 2.10). This suggests that N treatment had some effect, even with the limited rainfall. Yield was not statistically significant for any of the N treatments, including the check (see Tables 2.9 and 2.11). Total protein harvest only had significance at Casselton when N treatments were compared with the 0 and 224 kg ha^{-1} pre-plant recommended rates.

Table 2.10. Grain protein content by N fertilizer treatment at Casselton and Prosper, 2017.

			Grain Protein Content	
N source	Pre-plant N	In-season N rate and timing	Casselton	Prosper
		kg ha^{-1}	---------------g kg ------------	
urea†	0		120.2	134.2
urea	70%‡		137.4	143.6
urea	100%		129.1	142.6
urea	224 kg ha^{-1}		142.6	147.6
urea	70%	34 at ZGS 15	136.0	144.3
urea	70%	67 at ZGS 15	139.2	142.1
urea	100%	34 at ZGS 15	144.3	142.5
urea	100%	67 at ZGS 15	141.0	145.1
urea	70%	34 at ZGS 45	133.8	143.3
urea	70%	67 at ZGS 45	139.9	143.8
urea	100%	34 at ZGS 45	141.5	143.7
urea	100%	67 at ZGS 45	139.5	143.9
UAN§	70%	34 at ZGS 69	135.8	142.3
UAN	70%	34 at ZGS 69	138.3	141.4
UAN	70%	34 at ZGS 15	134.7	141.9
UAN	70%	34 at ZGS 15	142.7	143.7
UAN	70%	34 at ZGS 45	131.8	138.2
UAN	70%	67 at ZGS 45	132.3	142.6
CV %			6.2	2.0
LSD (0.05			12.1	4.0
Contrasts				
100% RR vs. 70% RR + 34 kg ha^{-1} N in-season			ns	ns
70% RR vs. 100% RR			ns	ns
In-season Urea vs. in-season UAN			ns	ns
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns
N applications at ZGS 15 vs. ZGS 45			ns	ns
0N (check) vs. added N			**	**
224 kg ha^{-1} rate vs. all other N rates			ns	*
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1}			ns	ns

* Significant at the 0.05 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

Table 2.11. Yield and total protein harvest by N fertilizer treatment at Casselton and Prosper, 2017.

N source	Pre-plant N	In-season N rate and timing	Casselton		Prosper	
			Yield	TPH†	Yield	TPH
		kg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	Mg ha^{1}	kg ha^{1}
urea‡	0		4.52	548	4.51	605
urea	70%§		4.29	586	4.22	600
urea	100%		4.75	611	4.52	644
urea	224 kg ha^{-1}		4.95	703	4.55	670
urea	70%	34 at ZGS 15	5.03	681	4.35	626
urea	70%	67 at ZGS 15	5.00	696	4.49	638
urea	100%	34 at ZGS 15	4.41	633	4.81	684
urea	100%	67 at ZGS 15	4.74	661	4.79	694
urea	70%	34 at ZGS 45	4.83	644	4.46	636
urea	70%	67 at ZGS 45	4.39	612	4.79	687
urea	100%	34 at ZGS 45	4.47	627	4.56	654
urea	100%	67 at ZGS 45	4.81	668	4.38	635
UAN¶	70%	34 at ZGS 69	4.99	675	4.57	649
UAN	70%	34 at ZGS 69	5.15	710	4.45	617
UAN	70%	34 at ZGS 15	4.92	659	4.56	618
UAN	70%	67 at ZGS 15	4.35	616	4.45	639
UAN	70%	34 at ZGS 45	4.70	616	4.56	630
UAN	70%	67 at ZGS 45	4.94	652	4.26	607
CV %			12.9	11.9	7.8	6.8
LSD (0.05)			ns	ns	ns	ns
Contrasts						
100% RR vs. 70% RR + 34 kg ha^{-1} N in-season			ns	ns	ns	ns
70% RR vs. 100% RR			ns	ns	ns	ns
In-season Urea vs. in-season UAN			ns	ns	ns	ns
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns	ns	ns
N applications at ZGS 15 vs. ZGS 45			ns	**	ns	ns
0N (check) vs. added N			ns	ns	ns	ns
224 kg ha^{-1} rate vs. all other N rates			ns	*	ns	ns
In-season applications of 34 kg ha^{-1} vs. 67 kg ha^{-1}						

* Significant at the 0.05 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

Hettinger

Hettinger was included separately in the presentation and discussion of results as it was geographically and ecological separated from the rest of these locations. As mentioned in Article I, Hettinger was severely impacted by the drought. This is likely the reason behind an interesting case of N treatment significance that differed from other locations. The only N treatment that was statistically significant at Hettinger was for the 224 kg ha^{-1} recommended rate pre- plant treatment, which was statistically significant at $\alpha = 0.0001$ (see Table 2.12). It's hypothesized that this significance was due to residual moisture from a higher than normal snowfall the previous winter allowed for enough of the N to be taken up to later boost protein. Later in-season treatments were not able to boost grain protein because the moisture wasn't available for the mineralized N to be taken up into the plant. Although treatment was not statistically significant for yield, the orthogonal contrast comparing 224 kg ha^{-1} against other N treatments was statistically significant for yield. This again, supports the theory that moisture availability in the spring was enough for the plant to boost yield and protein but that moisture limitation during the rest of the season effectively prevented N from being taken up.

Table 2.12. Grain protein content, yield, and total protein harvest by N fertilizer treatment at Hettinger, ND in 2017.

N source	Pre-plant N	In-season N rate and timing	Grain Protein Content	Yield	Total Protein Harvest
		kg ha^{-1}	g kg^{-1}	Mg ha^{-1}	kg ha^{-1}
	0		136.5	1.85	252
urea†	70%‡		136.2	1.84	251
urea	100%		136.8	1.68	230
urea	224 kg ha^{-1}		141.1	1.62	228
urea	70%	34 urea ZGS 15	134.8	1.85	249
urea	70%	34 urea ZGS 45	135.1	1.93	260
UAN§	70%	34 UAN‡ ZGS 69	135.8	1.91	259
UAN	70%	34 UAN ZGS 15	135.3	1.93	261
UAN	70%	34 UAN ZGS 45	133.4	1.80	240
	CV %		2.6	7.8	122
LSD (0.05)			5.5	ns	ns
Contrasts					
100% rate vs. 70% RR + 34 kg ha^{-1} N in-season			ns	*	ns
In-season urea vs. in-season UAN			ns	ns	ns
N applications at ZGS 15 & 45 vs. ZGS 69			ns	ns	ns
N applications at ZGS 15 vs. ZGS 45			ns	ns	ns
0N (check) vs. added N			ns	ns	ns
224 kg ha^{-1} rate vs. all other N rates			**	*	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† (46-0-0).

‡ Percentage of recommended rate based on the North Dakota Wheat Nitrogen Recommendation Calculator.

§ UAN = urea ammonium nitrate (28-0-0).

Discussion

Given the wealth of past research (Bly and Woodard, 2003; Finney et al., 1957; 2003; Strong, 1982), it was hypothesized that a foliar UAN application post-anthesis would boost grain protein content more than other applications. This was only true in the 2016 Casselton location. Air humidity may be a potential explanation. A common assumption of foliar fertilization is that high rates of foliar N uptake depend on high humidity (Gooding and Davies, 1992). This is supposed because dry conditions would likely cause foliar fertilizer to crystallize on the leaf surface. The late-planting of the 2016 Casselton location meant that the post-anthesis UAN application wasn’t applied until 22 July of that year. This happened to be at the time of the greatest humidity that summer as the dewpoint at Casselton that day was 25 ºC (NDAWN, 2017). Given the high relative humidity, this may be a plausible explanation for the success of the UAN application at this timing.

Conclusion

These data show that pre-season rate of N application is statistically significant in boosting grain protein content but is often limited to comparison between a given N application and a 0 N application. When an in-season N application is applied, a 67 kg ha^{-1} application is better than a 34 kg ha^{-1} one at boosting grain protein content. .

Application timing also plays a role in boosting grain protein content as the boot stage applications boosted grain protein content better when compared to applications at the four-leaf stage; however, the hypothesis that a post-anthesis N application would have the highest protein was only true in one location.

Drought seems to influence the ability of added N to boost grain protein content. This hypothesis is supported by the drought-stricken locations in North Dakota not responding to N treatment. In most of these locations grain protein content was high, while yield was low.

Furthermore, in most locations the post-anthesis foliar application had a lower total protein harvest than the earlier foliar application at the boot stage. Although this may be due to leaf burn, this suggest that unless a protein premium is very high the lower yield would outweigh the benefit to protein. These results support Rehm and Franzen’s (2005) advice that in-season applications be used for more ameliorative rather than regular practices.

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CONCLUSION

These results demonstrate that NDVI and NDRE are only modestly predictive of grain protein content. Furthermore, NDVI and NDRE are were found in our research to not be predictive before the wheat plant reaches the jointing stage. These spectral indices were most predictive at distinguishing between low and high grain protein contents (for example between 110 and 130 g kg^{-1} in 2016; 125 g kg^{-1} and 140 g kg^{-1} in 2017) and were not able to distinguish between mid-range and high grain protein contents, i.e.125 g kg^{-1} from 135 g kg^{-1} in 2016; 135 g kg^{-1} from 140 g kg^{-1} in 2017. There is also high environmental variability between the ability of these spectral indices to predict grain protein content. It is likely that drought stress detrimentally impacts the predictive ability of NDVI and NDRE of grain protein content. The spectral indices collected from aerial imagery were less predictive of grain protein content; however, due to limited protein sampling, further research needs to be conducted to gauge the utility of aerial sensors.

Nitrogen applications at the boot stage resulted in the highest grain protein contents in most locations. In addition, when an in-season N application was made, the higher N rate was better at boosting grain protein content. Contrary to previous studies, a foliar post-anthesis application was not effective at boosting grain protein content when compared with previous application timings in all environments. This may be due to environmental conditions such as low air humidity. Even when a foliar post-anthesis application did result in the highest grain protein content it likely would not result in higher revenue. Given the costs involved, these results support recommendations that foliar fertilization only be applied for ameliorative purposes.

Future research should include environmental, and potentially economic, factors in a multiple regression model aimed at gauging the ability of spectral indices to predict grain protein content. In addition, further investigating whether aerial sensors can assist in this effort would be beneficial.