

**CAN LEAF AREA INDEX AND PLANT HEIGHT MEASUREMENT  
IMPROVE SENSOR-BASED NITROGEN RECOMMENDATIONS AND  
YIELD PREDICTION FOR CORN?**

by

Jeremy Newswanger

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Jeremy Newswanger

Approved: \_\_\_\_\_  
Amy L. Shober, Ph.D.  
Professor in charge of thesis on behalf of the Advisory Committee

Approved: \_\_\_\_\_  
Erik H. Ervin, Ph.D.  
Chair of the Department of Plant and Soil Sciences

Approved: \_\_\_\_\_  
Calvin L. Keeler Jr., Ph.D.  
Dean of the College of Agriculture and Natural Resources

Approved: \_\_\_\_\_  
Louis F. Rossi, Ph.D.  
Vice Provost for Graduate and Professional Education and  
Dean of the Graduate College

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## ABSTRACT

Nitrogen (N) management remains a significant challenge for corn growers due to the unpredictability and influence of weather conditions, soil properties, and soil biological activity on N transformations in the soil. Innovative technology is needed to assist farmers in making accurate in-season N recommendations to improve N use efficiency (NUE) and reduce the environmental impacts of N losses. Sensor-based aerial imagery can be collected using unmanned aerial vehicles (UAVs) to assist with N management decisions and help improve NUE. However, there are limitations associated with vegetative indices from aerial imagery in guiding N decisions because the indices can reach a “saturation point” once the corn canopy closes. We hypothesized that adding leaf area index (LAI) data and plant height measurements could improve our understanding of how plant biomass is related to the vegetative indices for predicting corn N response by adding in a third dimension to the analysis. Corn N rate trials were established in Delaware, Maryland, and Pennsylvania (0, 30, 60, 90, 120, and 150% of university-based N rates; DE and MD did not have a 0 N rate) and four replicates in a randomized complete block design. In-season UAV-multispectral imagery, LAI, and plant height measurements were obtained at the V6 and R2 corn growth stages. Plant height was also derived from UAV imagery using structure from motion (UAV-SFM) using Pix4D photogrammetry software. Drone-derived vegetative indices, UAV-SFM, and LAI were used to predict the sidedress N rates and grain yields, which were compared to yield data at harvest.

The PA and DE sites exhibited a yield response to the N rate; however, the MD site was non-responsive. At the PA location, the N rate at the yield plateau occurred at 166 kg ha<sup>-1</sup>, with an in-season sensor-based N recommendation of 159 kg

ha<sup>-1</sup> and an economic optimum N rate (EONR) of 180 kg ha<sup>-1</sup>. At the DE site, the N rate at the yield plateau was 315 kg ha<sup>-1</sup>, with rates of 81 and 219 kg ha<sup>-1</sup> for the in-season recommendation and EONR, respectively. We saw a significant LAI response to N rate only at the PA site ( $P$ -value = .0027), whereas there was a significant plant height response to N rate at the DE site ( $P$ -value = .0029). The in-field plant height measurements and the UAV-SFM did not correlate 1:1 across all three sites and relationships were inconsistent across sites. As such, UAV-SFM derived plant height was deemed as an unsuitable proxy for the in-field plant height measurements across sites. The normalized difference red edge index (NDRE) was the best predictor of corn grain yield at the R2 stage ( $r^2 = .951$ ,  $P$ -value < .0001); the addition of LAI and plant height to the predictive model marginally improved upon the yield prediction of NDRE ( $r^2 = .9621$  and  $.9708$ , respectively). Therefore, NDRE alone is still the recommended vegetative index for predicting corn yield. Further studies are needed to explore the contribution of biomass estimates on in-season N recommendations and yield prediction.

## Chapter 1

### LITERATURE REVIEW

#### 1.1 Background on the Nitrogen Cycle and Soil Nitrogen Loss Pathways

Nitrogen (N) is the nutrient applied in the highest quantity in corn (*Zea mays* L.) production. The Haber-Bosch process, which is used to produce N fertilizers from atmospheric N<sub>2</sub> gas, has more than doubled the amount of fixed N since it was developed in the early 1900s (Morris et al., 2018). Increased N fertilizer availability and use resulted in increased crop yields (Cassman et al., 2002), which allowed for the rise in global population over the past century. However, this increase in N fertilizer availability and use has also contributed to agricultural N losses that can significantly impact surface and groundwater. Improving N management is the best way to reduce the environmental impact of agricultural N use while maintaining yield objectives and farmer profitability (Morris et al., 2018). Increasing N use efficiency (NUE) will increase plant N uptake and reduce the amount of N lost to the environment allowing agricultural production to be more sustainable for the future.

Plant available N (PAN) exists in the soil as ammonium (NH<sub>4</sub><sup>+</sup>) or nitrate (NO<sub>3</sub><sup>-</sup>). Ammonium and NO<sub>3</sub><sup>-</sup> have different chemical properties, causing them to behave differently in the soil environment. The positive charge associated with NH<sub>4</sub><sup>+</sup> allows it to bind to the clay or organic matter particles in the soil making NH<sub>4</sub><sup>+</sup> stability related to the soil's cation exchange capacity (CEC). Once bound, NH<sub>4</sub><sup>+</sup> will remain in the soil until it is assimilated by plant roots or soil microorganisms or converted to NO<sub>3</sub><sup>-</sup> (Weil and Brady, 2019). In contrast, NO<sub>3</sub><sup>-</sup>, has a negative charge

making it unable to bind to soil particles; therefore,  $\text{NO}_3^-$  can move with the flow of water in the soil (Weil and Brady, 2019). Soil organic matter (SOM) from manures or plant debris contains N, which is utilized by soil microorganisms and converted to PAN forms over time (White et al., 2020).

Knowing the three main soil N pools, their sources, and how they can be transformed from one to the other by the soil microbial community can help us to understand and control the main loss pathways of N from the soil. First, ammonium can volatilize from the soil surface as ammonia gas. Volatilization mainly occurs soon after manure or N fertilizer applications that are not incorporated into the soil when it is hot and humid. Volatilization is not a major environmental concern because ammonia is a weak greenhouse gas; however, ammonia can be converted to nitrous oxide ( $\text{N}_2\text{O}$ ), which is a more potent greenhouse gas (Woodward et al., 2021). Plus, volatilization represents an economic loss for farmers since applied N fertilizer that volatilizes is not being taken up by crops. The second major N loss pathway is denitrification, which occurs when  $\text{NO}_3^-$  in the soil is converted to  $\text{N}_2$  gas; denitrification occurs under anaerobic conditions and returns N to the atmosphere. However, incomplete denitrification can be detrimental as  $\text{N}_2\text{O}$ , is released instead of  $\text{N}_2$  (Weil and Brady, 2019).

The last major soil N loss pathway is leaching, or the movement of  $\text{NO}_3^-$  downward through the soil profile (Weil and Brady, 2019). Leaching of  $\text{NO}_3^-$  from the soil profile is the loss pathway that poses the greatest environmental threat and becomes even more of an issue with overapplication of N fertilizer. During leaching events,  $\text{NO}_3^-$  that is not intercepted by plant roots will eventually end up in the groundwater where it can impact the environment when it is discharged into surface

waters (McLellan et al., 2015; Pennino et al., 2017). Nitrate leaching can also lead to health risks for humans and animals (Pennino et al., 2017; Pan et al., 2021). Nitrate contamination of ground water and surface water can result in concentrations above the national limit of  $10 \text{ mg L}^{-1}$  for drinking water systems (Pennino et al., 2017). Pennino et al. (2017) showed that areas of the US that are dominated by agricultural production (e.g., Midwest states as well as California, Texas, and Pennsylvania) often have  $\text{NO}_3^-$  concentrations in drinking water that exceed the federal drinking water standard. High levels of  $\text{NO}_3^-$  in drinking water can cause methemoglobinemia (blue baby syndrome) in infants, and although the national limit for nitrate concentration is set at  $10 \text{ mg L}^{-1}$ , chronic exposure to  $\text{NO}_3^-$  in drinking water at lower levels has been linked to some cancers and birth defects (Pennino et al., 2017).

Aquatic ecosystems are also greatly impacted by eutrophication, which can be caused by algal blooms that are fueled by increased N concentration in receiving waters. Nitrogen and phosphorus (P) are the two most common limiting nutrients in aquatic ecosystems. When N or P levels increase the ecosystem becomes more productive and can lead to excessive algal growth. As the algae die off and get decomposed by microorganisms the oxygen becomes depleted at the bottom of the aquatic system creating hypoxic zones (Weil and Brady, 2019). There have been over 400 hypoxic zones identified worldwide in coastal waters, with the hypoxic area in the Gulf of Mexico being one of the largest (McLellan et al., 2015). Estuarine and coastal ecosystems, which are valuable ecosystems that local communities rely on, are particularly vulnerable to eutrophication because they receive the nutrient load from the entire upland watershed. Protecting the environment is key to sustaining

agricultural production for the future and through improved N management soil N losses can be reduced.

Though there is extensive understanding and knowledge of the N cycle, N management for crop production remains a challenge. In a recent review, Dobermann and Cassman (2002) stated the question, “are our current nutrient recommendations adequate to sustain and continue to meet food demands while holding to environmental quality standards?” Agronomists need to continually ask this question due to their current inability to predict the N cycle because soil N transformations are subject to a variety of environmental factors (Cassman et al., 2002). The microbial processes responsible for soil N transformations are largely controlled by the soil microbial community dynamics and weather (Weil and Brady, 2019). Our inability to accurately predict the weather effectively makes the N cycle also very difficult to predict. Dobermann and Cassman (2002) explained that novel strategies and technologies are needed to identify and account for more of the dynamic environmental factors that affect nutrient management.

The inefficiencies related to N management have led researchers to develop strategies to improve NUE, particularly in corn production where most of the N fertilizer is used (Cassman et al., 2002). According to Sharma and Bali (2018), NUE is defined as either plant N use efficiency or the ratio of N that is applied to the soil to the amount of N taken up by the plant. Low NUE occurs with poor N management practices, such as applying too much N fertilizer pre-plant or during in the fall before corn is planted. As much as 75% of N applied in the US corn belt over the past twenty years was applied before corn planting (Sharma and Bali, 2018). Sharma and Bali (2018) go on to explain that the N demand of corn is highest between three weeks

after planting until early tasseling; as such, fertilizer applications that correspond better to crop N uptake will improve NUE and can also limit N loss to the environment.

Improved N management is needed globally to improve NUE in agronomic production. Overapplication or inefficient application of fertilizers and manures is linked to N losses in runoff and leachate that fuel eutrophication in sensitive water bodies such as the Chesapeake Bay (Pan et al., 2021). The current environmental quality goal for the Chesapeake Bay is removal from the Environmental Protection Agency's impaired waters list by 2025 (Majsztrik and Lea-Cox, 2013). Improved N management and NUE can lead to reduced N losses from agricultural production, thereby reducing nutrient loading on the Chesapeake Bay (and other nutrient impacted water bodies) while providing an economic advantage to regional farmers.

## **1.2 Development of Nitrogen Rate Recommendations for Corn**

The first N fertilizer rate recommendations for corn were based on the classic yield-based algorithms developed by George Stanford's work in the 1960s-70s (Morris et al., 2018; Rodriguez et al., 2019; Stanford, 1973). Stanford's main goal was to develop an equation that related corn yield to N fertilizer that considered all the soil dynamics that impact N uptake (e.g., soil biological activity, weather, legumes, etc.). While Stanford was able to develop a full equation (eq. 1) to account for all factors affecting corn N uptake, the resulting equation cannot be used to determine N rate recommendations because we are unable to determine appropriate values to account for all the interactions between the soil, weather, manure, and legumes (Morris et al., 2018).

$$N_f = [U - (Q_{LN} \times E_{LN}) - (Q_{RLN} \times E_{RLN}) - (Q_{MIN} \times E_{MIN}) - (Q_{MON} \times E_{MON}) - (Q_{RMON} \times E_{RMON}) - (Q_{SNO_3} \times E_{SNO_3}) - (Q_{SOM} \times E_{SOM}) - (Q_{RNO_3} \times E_{RNO_3})] / RE_N$$

(eq. 1)

Where  $N_f$  is the N application rate, U is the crop N uptake, Q and E are the plant available and the efficiency parameter, respectively, for each of the sources of soil N [i.e., legume N (LN), residual legume N (RLN), manure inorganic N (MIN), manure organic N (MON), residual MON (RMON), soil nitrate ( $SNO_3$ ), soil organic matter (SOM), and residual  $SNO_3$  ( $RNO_3$ )], and  $RE_N$  is an approximation for the N recovery efficiency. Stanford determined an approximation, initially referred to as the “1.2 rule,” that simplified the N recommendation process to overcome issues populating variables in the full rate equation. The Stanford approximation infers that the amount of N fertilizer needed to meet the corn crop requirement is 1.2 times the expected yield goal ( $Y_{goal}$ ) after taking into account all N credits from manure and previous leguminous crops ( $N_{credits}$ ; Rodriguez et al., 2019), and is represented by following equation:

$$N_f = n(Y_{goal}) - N_{credits} \text{ (eq. 2)}$$

While the constant n was traditionally set at 1.2 lb bu<sup>-1</sup> of N (15.4 kg m<sup>-3</sup>), there have been adjustments to this value to account for regional differences in growing conditions, soil properties, and management practices (Rodriguez et al., 2019).

From 1970 until the early 2000s, all N recommendations for corn were based off Stanford’s ideas and equations with some regional variation in the methods used to determine the components of the yield-based equations (Morris et al. 2018). For example, there were several methods for determining corn yield expectations, a value that is vital to applying Stanford’s equations because resulting N recommendation is

directly related to yield. Methods for determining yield goal ranged from very direct methods (e.g., using a five-year average) to very open-ended methods (e.g., “a realistic target yield that is achievable in favorable growing conditions,” Morris et al., 2018). Similarly, there was regional variability in  $n$  (i.e., physiological N need). While most states used Stanford’s original value of  $15.4 \text{ kg m}^{-3} \text{ N}$  ( $1.2 \text{ lb bu}^{-1}$ ), some eastern states such as Pennsylvania, Maryland, and Virginia have historically used a physiological N rate of  $12.9 \text{ kg m}^{-3}$  ( $1.0 \text{ lb bu}^{-1}$ ), and states such as Florida and Vermont use a lower N value of  $10.3\text{--}11.6 \text{ kg m}^{-3}$  ( $.8\text{--}.9 \text{ lb bu}^{-1}$ ; Morris et al., 2018). Finally, there is variability in the N credits that are taken for previous legume crops and the organic N portion of manure applications, with different methods for determining various legume crops (based on stand quality or yield) and allocating different percentages of manures organic N fractions (Morris et al., 2018). While N recommendations are expected to vary somewhat between regions, the significant regional inconsistencies between values used in Stanford’s equation caused some regions to search for a better N recommendation method.

In the early 2000s, the upper midwestern states developed a new N recommendation system (maximum return to N or MRTN) to address inefficiencies and issues with the yield-based methods (e.g., regional variability, inaccuracies in yield-based rates, lack of economics, etc.; Vanotti and Bundy, 1994a, 1994b). Long-term research from the Midwest Corn Belt showed that the optimal N rate had not changed from the 1970s to the 1990s, even though corn yields increased over this same period (Sawyer et al., 2006). Regional corn N response trials were conducted across multiple locations and crop rotations to generate data for the Corn N Rate

Calculator, which determines the economically optimal N rate based on the return to N and the price of corn grain and N fertilizer (Sawyer et al., 2006).

While the MRTN approach improves upon the yield-based approach by using real field and economic data, there are still similar limitations to the MRTN method due to temporal and spatial field variability (Morris et al., 2018). Plus, many growers and agronomic consultants still have the preconceived notion that more N fertilizer is needed to increase yield and feel that the methods used to determine N rate recommendation under MRTN do not allow for yield improvements over time (Morris et al., 2018). Another limitation of MRTN is that the database only includes trials conducted in the Midwest; as such, MRTN cannot be applied to other regions without conducting additional N response trials and creating regional corn N rate calculators (Morris et al., 2018). Therefore, N rate recommendations for corn in the northeast, and other regions of the country, are widely still based on general yield goal recommendations (Morris et al., 2018).

Both Morris et al. (2018) and Rodriguez et al. (2019) proposed the need for a site-specific approach to N recommendations. On-farm trials will allow growers to see for themselves the optimum N rate for their location, soil types, and management practices (Rodriguez et al., 2019). Cumulative on-farm trials will allow for the creation of a comprehensive database that could result in more reliable N recommendations for a variety of different regions, soil characteristics, corn growth stages, and management practices (Morris et al., 2018). More efficient N recommendations for corn production will ensure that crops take up more applied N and will reduce the potential for overapplication of N fertilizers that can cause N losses from the soil through runoff and leaching.

### **1.3 Approaches to Refine Nitrogen Rate Recommendations**

Improvements in technology have allowed for better management (e.g., rate, placement, and timing) of N fertilizer, allowing farmers who adopt best management practices (BMPs) to get the greatest return on investment while reducing the risk of N pollution. Morris et al. (2018) describes several technologies [e.g., pre-sidedress nitrate test (PSNT), plant tissue tests, computer simulation N models, and aerial imagery and sensors] that when used to guide in-season N decisions may increase farmer NUE. In fact, majority of the new N management tools have already been shown to improve NUE in corn production (Sharma and Bali, 2018); however, each of these tools has limitations and some tools require further research to ensure their accuracy before widespread adoption by farmers can occur (Morris et al., 2018).

The first N management tools that were developed required direct in-field collection of soil or plant tissue sample. The most common soil measurement (especially in the eastern US) is the PSNT (Andraski and Bundy, 2002). The PSNT was developed in the 1980s to guide in-season N rate adjustments for corn grown in fields with a history of manure applications by predicting the potential for additional organic N mineralization (Magdoff et al., 1984). Proper use of the PSNT typically results in a reduction in the sidedress N recommendation, resulting in lower N application rates than the more general yield-based or MRTN methods, which do not account for any in-season adjustments (Magdoff et al., 1984). The PSNT has strict procedural guidelines that need to be followed to ensure accurate results. For example, soil samples need to be taken to a depth of 30 cm when the corn is between 15–45 cm tall (Magdoff et al., 1984). The PSNT also relies on a quick turnaround from the analytical lab to get the information back to the grower to limit the effect weather has on the results (Magdoff et al., 1984).

Another common in-field method used to make in-season N rate adjustments in corn (and other crops) is plant tissue testing. Scharf (2001) showed that plant measurements (whole plant tissue N content and chlorophyll readings) taken at V6 have better correlation to the optimal N fertilizer requirement than soil N measurements since they directly measuring plant N uptake rather than measuring the available N pool in the soil. Like soil N tests, a quick turnaround from the lab is required as growers using tractor-based application equipment face a logistical issue the taller corn gets (Morris et al., 2018), although if irrigation is present fertigation methods can be used for N application at later corn stages. An additional corn tissue test is the corn stalk nitrate test (CSNT), which measures the  $\text{NO}_3^-$  concentrations in the corn stalk at the end of the growing season. Excess levels of stalk  $\text{NO}_3^-$  at the end of the season indicate overapplication of N (Binford et al., 1992). Therefore, the CSNT provides a post-mortem analysis of N management decisions for that cropping year; the CSNT cannot be used as a tool for in-season decisions (Morris et al., 2018).

In general, plant and soil N measurements can be useful tools for guiding in-season N management to improve NUE, but they have limitations that hinder their widespread adoption as N management tools. The major drawbacks for in field plant and soil measurements are that they are limited in accounting for spatial variabilities within fields, the unpredictability of weather before and after sampling influences the results, and quick lab turnaround is required for the best N application results (Sharma and Bali, 2018; Morris et al., 2018).

Computer technology has greatly increased in the last decade allowing electronic software and models to be integrated into agriculture production and assist in management decisions. Precision agriculture has become commonplace making

information readily available to growers. Computer simulation tools are used as a precision agriculture tool, some of which are used to guide in-season N management. The first comprehensive N models were developed at Cornell University (Adapt-N) and the University of Nebraska (Maize-N; Morris et al., 2018). For the most part, these N models have similar functionality and information, but there are key differences in their function. For example, Adapt-N uses high-resolution weather data allowing for more stability with in-season N decisions in areas with the potential for significant in-season weather variability. In contrast, Maize-N uses long-term weather data, and typically performs better in drier or more irrigated growing conditions where weather variability is not as big of a factor (Thompson et al., 2015).

In-field evaluations of N models show how they compare to other N management tools or previous recommendation methods. Sela et al. (2017) conducted an N rate response trial in New York from 2011-2015 to compare N rates generated by the Adapt-N model to the state's current yield-based N recommendations for corn. The Adapt-N model more accurately predicted the EONR than the yield-based recommendations and increased farmer profits, while also reducing the N application leading to predict much less N loss to the environment (Sela et al., 2017). In another study by Sela et al. (2018), the Adapt-N model was used to predict the N rates of 127 on-farm field trials from seven different states from 2011–2016 and compare them to the static N recommendations at each location. Overall, the dynamic N model simulation allowed for a 32% reduction in N rate compared to the static N recommendations, while still maintaining yield; these results indicated that NUE can be improved using modeling tools (Sela et al., 2018). The reduced N rate resulted in a 36% reduction in N surplus, which was calculated by eq. 3.

$$N_{surpl} = N_{input} - N_{output} \text{ (eq. 3)}$$

Where  $N_{input}$  is the sum of the N fertilizer and N credits from manure applications and previous legumes for each treatment, and  $N_{output}$  is the amount of N removed from each treatment at harvest (Sela et al., 2018). Nitrogen response rate trials in Michigan from two different years (2015–2016) compared recommendations from Adapt-N, MRTN, and the GreenSeeker crop sensor (Rutan and Steinke, 2017). In the first year, the Adapt-N model increased the sidedress N rate  $35 \text{ kg ha}^{-1}$  compared to the MRTN method to account for a wet growing season, although there was no statistical yield difference. The GreenSeeker crop sensor resulted in lower N rates and corn yield than MRTN and Adapt-N. In the second year of the study when the growing season was drier, all three methods resulted in similar N application rates and yield responses (Rutan and Steinke, 2017).

Sensing technologies [e.g., the use of satellites, handheld sensors, unmanned aerial vehicles (UAVs), etc.] have also become available for farmer use. The greatest advantage of sensing technology is its ability to provide non-destructive in-season information about crop conditions. The different sensing technologies are categorized by their distance from the plant canopy (i.e., proximal vs. remote) and whether the sensor emits its own light source (i.e., active vs. passive; Mulla, 2013). Most sensors measure the light reflectance from plants, which is inversely related to the light absorbed by the chlorophyll pigments and therefore, gives indication of plant health (Mulla, 2013). Aase and Siddoway (1980; 1981) were among the first researchers to use light reflectance as an in-season tool to measure the stand density and dry matter content of wheat. Further development of reflectance measurements resulted in different vegetative indices, such as the normalized difference vegetation index

(NDVI) and the normalized difference red edge index (NDRE), and are the major tools utilized today for analyzing in-season plant nutrition, such as N content (Mulla, 2013).

Sensor-based images from UAVs provide a nondestructive method to identify crop stress factors. Stressors such as weeds, N deficiency, or water stress can be detected by NDVI and NDRE; the indices can also be used to estimate plant biomass (Sapkota et al., 2020; Corti et al., 2019). Both NDVI and NDRE were proven to improve in-season N management decisions and increase NUE when compared to farmers traditional N management practices (Thompson and Puntel, 2020). The vegetative indices have been used successfully to reduce N applications in corn and small grains systems while still maintaining yield (Ali et al., 2017; Butchee et al., 2011). Yet, the algorithms used to make N recommendations from NDVI/NDRE require further refinement to ensure farmers receive accurate N recommendations, particularly when these vegetative indices are collected by UAVs because prior research focused mainly on collection of data using handheld sensors or satellite imagery (Mulla, 2013; Thompson and Puntel, 2020).

A limitation of using vegetative indices to drive N management decisions is that they only depict a field as a two-dimensional (2D) image. Once the crop canopy closes (typically between the V8 and V10 growth stages for corn), these vegetative indices can become “saturated,” meaning they reach a maximum value. When NDVI or NDRE saturation occurs, these indices are no longer able to determine differences in crop growth or biomass accumulation, thereby decreasing their effectiveness as a decision N management tool as the season progresses (Baret and Guyot, 1991).

#### **1.4 Potential Improvements to Sensor-Based Nitrogen Recommendations**

To date, research related to the use of UAVs and vegetative indices (NDVI or NDRE) has focused in two main areas: 1) comparing UAV-based imagery to imagery collected from handheld or equipment-mounted devices or satellites (Gabriel et al., 2017) and 2) determining the best timing for collection of imagery using UAVs for in-season recommendations or predicting end of season yield (Maresma et al., 2020). Both NDVI and NDRE can be effective tools for guiding in-season N management in young corn crops (White et al., 2019; Thompson and Puntel, 2020) but these measurements are less useful for making in-season N rate recommendations after canopy closure. In addition, timing of sensing can influence the measurements using vegetative indices. For instance, yield was found to be strongly correlated with NDVI at R2 (Maresma et al., 2020), which is after most in-season N applications are made (Ali et al., 2017). Vegetative indices must be used earlier in the growing season to best guide N applications. Yet, interpreting vegetative indices too early in the season can be difficult, as the crop is less developed, and soil can contribute to the light reflectance measured at low crop densities (Mulla, 2013). However, vegetative indices can become “saturated” later in the season due to canopy closure, which may make N-rate prescriptions based on vegetative indices less useful for those applying N through fertigation at the V9 stage, when crop height and leaf area index (LAI) will be greater (Mulla, 2013). We seek to improve sensor-based vegetation indices to guide in-season N management for corn after canopy closure with the inclusion of a plant biomass estimate, such as LAI or plant height.

Leaf area index is a common agronomic measurement used to determine the amount of leaf area per unit ground area. Light reflectance was first used by Aase and Siddoway (1980; 1981) in their work to measure winter wheat stand density and

biomass production and can be related to LAI. In corn, the LAI may vary depending on the plant canopy structure and row spacing (Lacasa et al., 2022). Lacasa et al. (2022) explained that the intercepted radiation from the sun and LAI may influence the light, water, and N efficiency, and needs to be considered to improve crop simulation models. Recently, Zhao et al. (2018) showed LAI to be linearly proportional with N-uptake in corn during the vegetative growth stage. The authors also used LAI data to create N-dilution curves based on the measurements taken from the V6 to R1 corn growth stages, indicating that LAI can be used as a tool to guide N management (Zhao et al., 2018). Therefore, LAI seems play a role in improving N recommendations produced from vegetative indices. To our knowledge, no researchers have identified a relationship between LAI and vegetative indices (NDVI and NDRE) to improve imagery-based in-season N recommendations.

The addition of LAI measurements of the canopy after “saturation” may make it possible to achieve a three-dimensional (3D) analysis with UAV-based imagery. Structure from motion (SFM, i.e., UAV-plant height) can also be measured with UAVs. If LAI can improve the relationship between the vegetative indices and N fertilizer rates, then it may be possible to use structure from motion to also guide in-season N management decisions, thereby reducing the need to measure LAI (which can be labor intensive as it currently must be collected on foot). Improving NDVI and NDRE imagery after canopy closure can guide better in-season N fertilizer rates, allowing for greater NUE that ultimately can help private nutrient consultants, local extension agents, conservation district planners, and other technical service providers make more efficient N rate recommendations to corn growers. Plus, results from this

research will apply to many other agronomic crops and could be used to evaluate N dynamics and performance of cover crops.

## **1.5 Research Objectives**

The goal of this study is to use LAI and plant height to improve UAV-mounted, sensor-based imagery (i.e., NDVI, NDRE) estimates of in-season crop biomass production and crop N demand using corn as a model crop.

### **1.5.1 Objective 1: Determine the N rate yield response for each on-farm site.**

Each on-farm site location will contain an N rate study with N rates ranging from 0-150% of the university-based yield goal N recommendations. All the plots, except any 0 N plots, will receive 30% of the N at planting. The remainder for the N for each corresponding rate will be applied as a sidedress at the V6 corn growth stage. The individual strip plots will be harvested and the yield will be fit against the different N rates using a quadratic-plateau model for each site to produce the yield response curves. The N rate that occurs at each site's yield plateau will be compared to the in-season sensor-based recommendation and the EONR at each site.

### **1.5.2 Objective 2: Produce in-season N recommendations from NDVI and compare to yield response and EONR for each site.**

The UAV will be flown at the V6 growth stage to produce the NDVI orthomosaic for each site. The average NDVI values from each plot, along with the NDVI values from the low and high N reference areas, will be used in the Virginia Tech N algorithm to produce the in-season N recommendation. The EONR will be calculated based on the average price of corn and cost of N fertilizer to produce the profit obtained from each N rate. Then the in-season N recommendation will be

compared to the N rate at the yield plateau and the EONR within each site to determine how accurate the in-season N recommendation corresponded to each site's yield response and EONR.

**1.5.3 Objective 3: Evaluate any N rate responses from LAI and plant height.**

The LAI and plant height measurements will be taken at both the V6 and R2 growth stages. The R2 measurements will be used to evaluate any N rate responses detected by either the LAI or plant height measurements. An ANOVA statistical test will be run to determine if either LAI or plant height are variables that can be used to detect the N rate response at each site.

**1.5.4 Objective 4: Predict yield at R2 and include LAI and plant height along with the other vegetative indices.**

Each of the vegetative indices (i.e., NDVI, NDRE, GNDVI, and ExG) will be produced from the R2 UAV-imagery. The values from the vegetative indices along with the LAI and plant height measurements will be regressed against the yield from each plot using a stepwise regression in JMP. The stepwise regression will provide the best single variable predictors of yield as well as any significant multivariate models.

## Chapter 2

### MATERIALS AND METHODS

#### 2.1 2022 On-Farm N Rate Experiment

The experiment took place at three different locations in the Mid-Atlantic region. The field sites were near Seaford, DE, Federalsburg, MD, and Mt. Joy, PA (Table 1). The project is part of a larger USDA-NRCS on-farm research project that is focused on improving NUE in the Mid-Atlantic and identifying barriers to the adoption of in-season N management tools by farmers. At each “on-farm” field site, grower cooperators planted corn in strips that received four replications of five N rate treatments (30, 60, 90, 120, and 150% of total yield-based N recommendation from each corresponding university; PA had an additional 0% treatment); strips were arranged in a randomized complete block design. All treatments received 30% of total N pre-plant except for the 0 N treatment at PA (control). The remainder of the total N fertilizer was applied as a UAN sidedress application around growth stage V6. At the DE and PA locations, university researchers applied the sidedress N with a calibrated sprayer. The grower in MD used their own calibrated sprayer to sidedress at 50% of each plots N rate, and the other half was applied through fertigation at V9.

Table 1: The soil data, planting and sidedress dates, and N rates based on each sites yield goal for the 2022 on-farm N rate trial site locations.

<b>Site Location</b>	<b>Mt. Joy, PA</b>	<b>Seaford, DE</b>	<b>Federalburg, MD</b>
<b>County</b>	Lancaster	Sussex	Caroline
<b>Soil Series</b>	Hagerstown	Pepperbox-Rockawalkin	Rosedale
<b>Soil Texture</b>	Silty Loam	Loamy Sand	Loamy Sand
<b>Yield Goal (Mg ha<sup>-1</sup>)</b>	12.6	15.7	17.6
<b>Planting Date</b>	5/13/2022	5/5/2022	5/15/2022
<b>Sidedress Date</b>	6/30/2022	6/8/2022	6/15/2022 and 7/9/2022
<b>Total N Rates (kg ha<sup>-1</sup>)</b>	0, 37, 73, 146, 219, and 292	84, 168, 252, 336, and 420	96, 188, 282, 377, and 424

The three field sites were flown with either a DJI Matrice 210 V2 or DJI Inspire 2 UAV at the V6 and R2 corn growth stages, with an additional flight at the MD location at V9. A Micasense RedEdge MX multispectral camera attached to the UAV captures the images to create the orthomosaics of the plots. All flights were flown at 120 m above ground level with an image overlap of 75%. Ground control points for DE and MD were georeferenced with real time kinematic (RTK) positioning to improve the accuracy of the structure from motion (SFM) measurements with the UAV. For PA, Google Earth was used to get the GPS coordinates of several telephone poles along the road and the corner of the barn next to the plots to be used for ground control points. The image data obtained from the UAV flights was stitched together using Pix4D photogrammetric software and uploaded into ArcGIS Pro, where the vegetative index and plant height data were extracted to be analyzed. The RTK positioning unit used in DE and MD also marked plot borders to create a polygon data layer for extracting index data produced from the UAV imagery. To extract the data

from the drone imagery in PA, a polygon layer was created for the plots in the ArcGIS Pro software.

The in-field plant measurements were collected the same day as the UAV flights (at V6 and R2, with an additional sample time at V9 in MD). Leaf area index measurements were conducted using the AccuPAR LAI ceptometer, model LP-80. For each plot, 10 LAI measurements distributed along the whole plot length were obtained across the center 2 rows by walking the length of each plot. Each LAI sample point consists of one measurement taken above the corn canopy and a corresponding measurement below the corn leaves near the ground surface to obtain the amount of light absorbed by the canopy. The LP-80 produces an LAI value using those two measurements. The LAI data was transferred to a computer with the File Viewer 2200 software and exported as an Excel spreadsheet for future analysis. Plant height from the ground to the highest point of the top leaf was measured at 5 locations within each plot and was recorded using a tape measure the same day as the LAI measurements.

Structure from motion can be determined by the UAV imagery when ground control points are used, allowing for plant height to be estimated. A digital surface model (DSM) is created by the Pix4Dmapper software and represents the canopy layer seen in the UAV images. Then to obtain the plant height the ground level can be subtracted out by using the digital elevation model (DEM) for each site. State lidar data, downloaded from ArcGIS Online, was used for each site's DEM (USDA: NRCS Geospatial Data Gateway, n.d.; FirstMapDE, 2022; MD iMap Data Catalog, 2019).

## **2.2 Yield Response to Nitrogen Rate**

At harvest, yield data was collected from each strip. For the DE and PA locations a calibrated weigh wagon was used to weigh the grain from each plot.

However, at the MD site the plot yields were determined by the farmers calibrated combine yield monitor. Relative yield was calculated by dividing the yield in each plot by the maximum plot yield achieved at the site (Pearce et al., 2022). The relative yield-based N response curve for each site was created using the NLIN function in SAS to fit the appropriate non-linear model (i.e., quadratic plateau).

### **2.3 Determination of NDVI-based In-Season Nitrogen Rate and Comparison to Yield Response Curves and EONR**

The sidedress N rate predictions based on NDVI at V6 were derived using the Virginia Tech N algorithm (Reiter et al., 2014) and these values were compared to the yield-based N response curves developed from the harvested yield. The inputs needed for the Virginia Tech N algorithm are the amount of N applied at planting, how many days from planting, the NDVI values from a low N and high N reference plot, and the NDVI value of each individual strip (Reiter et al., 2014). The Virginia Tech algorithm assumes a NUE of 60%. The N rate at the yield plateau was compared to the in-season NDVI-based N recommendation using the percent change. The EONR for each site was calculated using the October 2022 corn price from the records listed by the USDA National Agricultural Statistics Service (USDA NASS, 2023) and the June 2022 price for UAN28 N fertilizer (Quinn, 2023) to be compared to both the in-season N rate developed from the Virginia Tech N algorithm to determine the accuracy of the in-season recommendations and the N rate from the yield plateau.

### **2.4 LAI and Plant Height Analysis**

The relationship between LAI and NDVI/NDRE was determined by graphing the vegetative index values against the LAI data at the two different corn growing stages (V6 and R2). Either a quadratic-plateau or quadratic model was fit to the data to

describe the relationship between LAI and vegetative indices, as appropriate. The plant height estimates derived from the UAV-SFM for each of the flights were plotted against the in-field plant height measurements to assess the accuracy of the SFM method. The plant height measurements were plotted against NDVI and NDRE to determine if the SFM and LAI data produce the same relationships with the vegetative indices.

## **2.5 Yield Prediction by Vegetative Indices**

The vegetative indices (Table 2), LAI values, and plant height measurements (i.e., both the in-field measurements and heights from the UAV-SFM) were compared to the yield data from each site. Linear regressions between the various indices and measurements at R2 and grain yield were performed. Where regressions were significant, they were compared based on fit of the line ( $r^2$ ), with the highest values representing the best predictor of yield. A stepwise regression in JMP was used to determine the best relationships between yield and the different vegetative indices and other in-season measurements from R2. The yield prediction analysis was performed for each of the three sites individually, and then on all the data combined to identify the best model for the whole region.

Table 2: Vegetative indices and corresponding formula used in calculating in-season N recommendations and yield prediction for 2022 on-farm corn N rate trials. The index formulas refer to red (R), green (G), blue (B), red edge (RE), and near infrared (NIR) wavebands of light.

4 <b>Vegetative Index</b>	5 <b>Formula</b>
6 Normalized Difference Vegetative Index (NDVI)	7 $\frac{NIR-R}{NIR+R}$
8 Normalized Difference Red Edge Index (NDRE)	9 $\frac{NIR-RE}{NIR+RE}$
10 Green Normalized Difference Vegetative Index (GNDVI)	11 $\frac{NIR-G}{NIR+G}$
12 Excess Green Index (ExG)	13 $2G - (R + B)$

## Chapter 3

### RESULTS AND DISCUSSION

#### 3.1 Yield Response to Nitrogen Rate

The Mt. Joy, PA (Figure 1) and Seaford, DE (Figure 2) sites exhibited corn yield response to N rate that could be fit with a quadratic-plateau model. Scharf et al. (2005) showed that the quadratic-plateau model produced the best fit for corn yield response to N rates over other commonly used models (e.g., linear, quadratic, and linear-plateau). At the PA site, the yield plateau occurred at a 94% relative yield, which corresponded to an N rate of 166 kg ha<sup>-1</sup>. The yields ranged from 6.14-12.5 Mg ha<sup>-1</sup>, with the plateau occurring at 11.75 Mg ha<sup>-1</sup> for the PA site. The yield plateau at Seaford, DE also occurred at a relative yield of 94%, but this yield plateau corresponded to an N rate of 315 kg ha<sup>-1</sup>. Yields at the DE site ranged from 13.9–18.1 Mg ha<sup>-1</sup>, with the yield plateau occurring at 17.0 Mg ha<sup>-1</sup>. The Federalsburg, MD site (Figure 3) did not exhibit a yield response to N rate; relative yield was 94% or higher regardless of N rate. Therefore, we were unable to fit the MD yield data to any model that is typically used to describe yield response (e.g., linear plateau, Mitcherling, etc.). The yields at the MD site ranged from 18.0–19.2 Mg ha<sup>-1</sup>, with an average of 18.5 Mg ha<sup>-1</sup>. The average corn yields in 2022 were 11.50, 10.57 (13.13 irrigated), and 10.92 Mg ha<sup>-1</sup> for Lancaster County, PA, Sussex County, DE, and Caroline County, MD, respectively (USDA-NASS). The PA site produced yields in range with the county average, but the DE and MD sites had much higher yields compared to their county averages, which was due to both the DE and MD sites being irrigated.

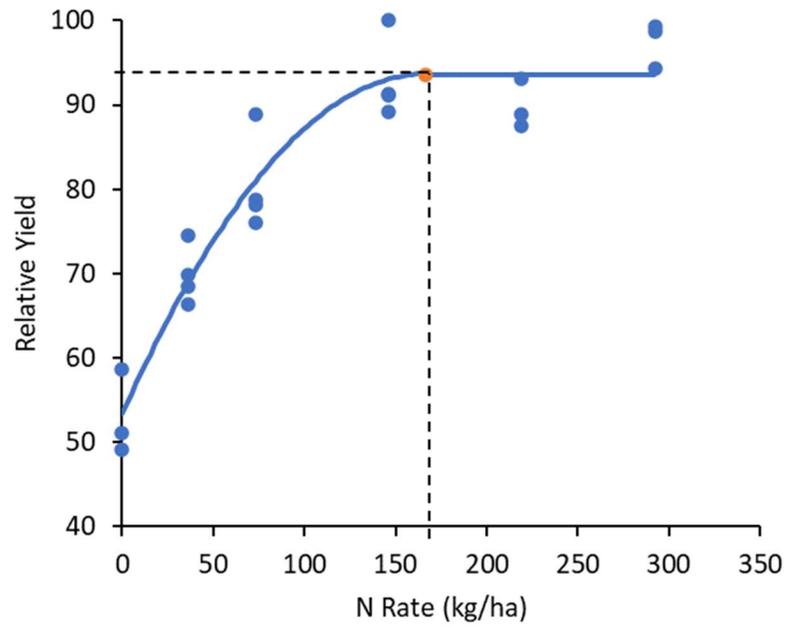


Figure 1: Quadratic plateau yield response curve for corn fertilized at six N rates in a replicated strip trial conducted in 2022 near Mt. Joy, PA. The yield plateau occurred at a relative yield of 94% and N rate of 166 kg N ha<sup>-1</sup>.

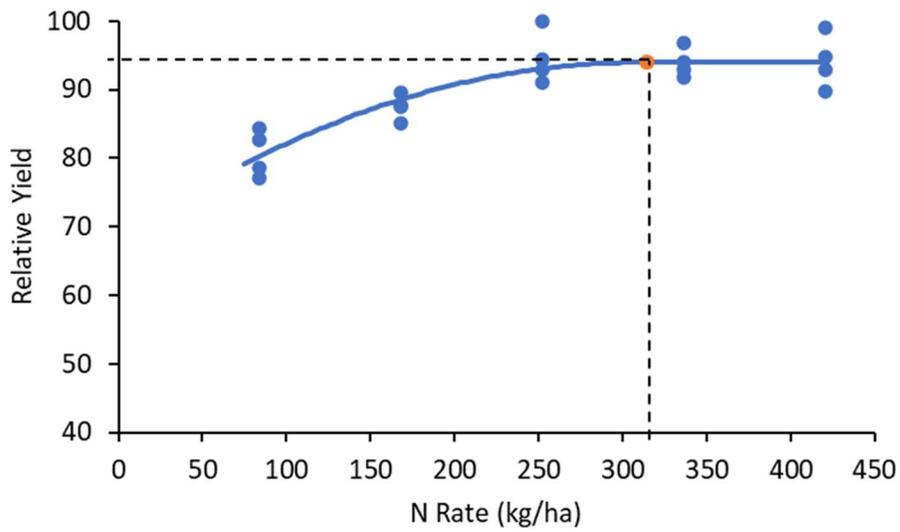


Figure 2: Quadratic plateau yield response curve for corn fertilized at five N rates in a replicated strip trial conducted in 2022 near Seaford, DE. The yield plateau occurred at a relative yield of 94% and N rate of 315 kg N ha<sup>-1</sup>.

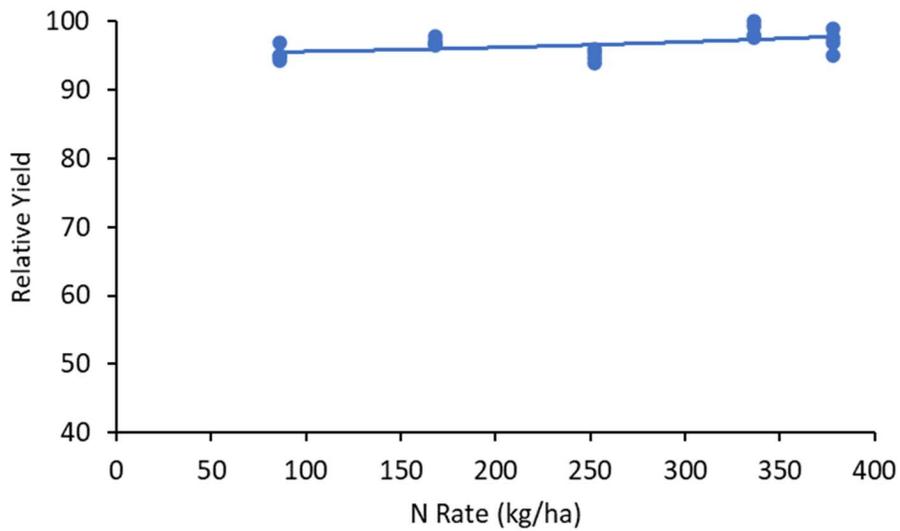


Figure 3: Yield response for corn fertilized at five N rates in a replicated strip trial conducted in 2022 near Federalsburg, MD. Data from this site could not be fit to any yield response function because there was no yield response with the N rates that were applied at this site.

The EONR for the PA site was  $180 \text{ kg ha}^{-1}$ , which was unexpectedly higher than the N rate at the yield plateau (Table 3). The increase in EONR compared to the N rate at the yield plateau can be explained by the two different statistical models that were applied to calculate these values. The quadratic plateau fit used in the yield response curve (Figure 1) becomes linear once the plateau is reached. In contrast, a true quadratic curve was fit to calculate the EONR (Figure 4). The vertex of the quadratic, which is where the EONR occurs, is forced to the right more with the quadratic fit to account for the slight increase in yield of the highest N rate at the PA site over the second highest. The EONR for the DE site was found to be  $219 \text{ kg ha}^{-1}$ . The EONR was lower than the N rate at the yield plateau for DE (Table 3). Oglesby et al. (2022) calculated the EONR and compared it to the N rate at the yield plateau and the traditional yield goal method based on studies by Stanford (1973) across two years at four sites in Mississippi. The authors reported that EONR was lower or not significantly different than the N rate at the yield plateau for all but one site-year during their study. Based on the yield response curve at our DE site (Figure 2), fertilizing at the EONR should result in a relative yield of 92%. Therefore, increasing the fertilizer rate to reach the yield plateau would not result in an increased profit for the grower. At our MD site, the EONR was estimated to be  $0 \text{ kg ha}^{-1}$  because the site was not responsive to N applications. The growers at this site applied  $96 \text{ kg ha}^{-1}$  pre-plant N, rather than the expected  $72 \text{ kg ha}^{-1}$ . The extra N applied at planting and a build-up of N in the soil from previous years of fertilization may explain why there was no N response at the MD site.

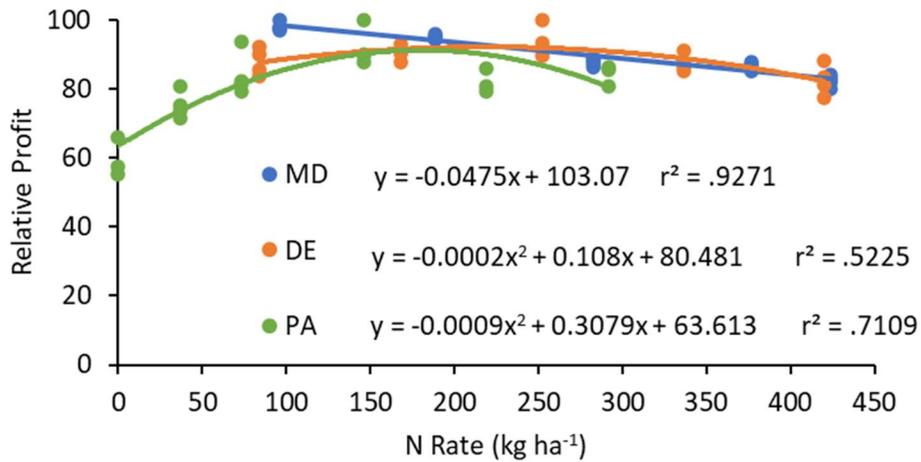


Figure 4: Calculation of EONR for corn fertilized at five N rates in a replicated strip trial conducted in 2022 at field sites in DE, PA, and MD using a corn price of \$256 Mg<sup>-1</sup> and an N fertilizer price of \$694 metric ton<sup>-1</sup>.

Previously, researchers reported variability in N response across sites and regions (Raza and Farmaha, 2022; Scharf et al. 2005), which they attributed to several factors (e.g., soil type, pH, soil inorganic N, precipitation, and management practices) that can impact corn yield response to N fertilizer. We expected that higher N rates would be needed for the DE and MD sites when compared to the PA site because these sites have sandy Coastal Plains soils with low levels of organic matter and increased nitrate leaching potential due to low water holding capacity (Raza and Farmaha, 2022). The DE site exhibited this trend; however, the MD site was non-responsive and did not need any N at sidedress. In contrast, the PA site consists of Piedmont soils with higher organic matter and water holding capacity.

### **3.2 In-season NDVI-based N Recommendations**

The in-season N rate recommendation based on NDVI measurements at V6 was 159, 81, and 0 kg N ha<sup>-1</sup> for the PA, DE, and MD fields, respectively (Table 3). Fertilization at the NDVI-based N rate was predicted to achieve 94% relative yield at the PA site based on the field specific yield response curve (Figure 1). The NDVI-based N recommendation of 159 kg ha<sup>-1</sup> was 4.5% less than the N rate needed to reach the yield plateau of 166 kg ha<sup>-1</sup>, however, both were predicted to achieve relative yields of approximately 94%. At the DE site, both the NDVI-based in-season N rate recommendation at V6 and the EONR (81 and 219 kg N ha<sup>-1</sup>, respectively) were 74 and 30% lower, respectively than the N rate at the yield plateau (315 kg ha<sup>-1</sup>). However, fertilizing based on the NDVI-based in-season N rate recommendation was predicted to produce 80% relative yield and result in only \$69 of economic loss based on the field specific yield response and EONR curves for the DE site (Figure 2, Figure 3). The NDVI-based in-season N rate recommendation suggested no in-season N at the MD site. The NDVI-based N rate recommendation corresponds with the yield response data from the trials, which showed that the site was non-responsive to N fertilizer.

Table 3: In-season, sensor-based N recommendations at V6 as predicted by the Virginia Tech model and compared to the N rate at the yield plateau and EONR for corn fertilized at five N rates in 2022 replicated strip trials conducted in PA, DE, and MD. The MD site was non-responsive to the N rates, and so no yield plateau observed.

Site	Sensor-Based N Recommendation (kg ha <sup>-1</sup> )	N Rate at Yield Plateau (kg ha <sup>-1</sup> )	Economic Optimum N Rate (kg ha <sup>-1</sup> )
<b>Mt. Joy, PA</b>	159	166	180
<b>Seaford, DE</b>	81	315	219
<b>Federalburg, MD</b>	0	N/A	0

The in-season sensor-based N recommendation matched well with the N response and EONR curves generated for the PA and MD sites. In contrast, the sensor-based N recommendation did not result in the same level of accuracy at the DE site. The Virginia Tech model used to develop the in-season NDVI-based N recommendations requires specific information about the amount of N applied at planting, the days since planting, and NDVI readings from the corn at V6 from the field, as well as low- and high-N reference strips. The difference between the NDVI values from the low- and high-N reference plots at the DE and MD field sites was much smaller (.01) compared to the NDVI values at the PA site (.15; Table 4). The lack of variability in the NDVI values in the reference plots accurately reflected the crop status at the MD site; in contrast, the similarity of the NDVI readings in the reference areas, thereby, limited the ability of the Virginia Tech in-season model to accurately predict sidedress N needs at the DE site. We suspect that similarities in NDVI values between the low- and high-N reference areas at DE may have resulted from the plant growth in the high-N reference being limited by a factor other than N (e.g., water, micronutrients, etc.) at V6. As such, the crop in the high-reference areas

was not responding to applications of N at a higher rate. Also, the NDVI values measured at the DE site at V6 were about half the values measured at both the PA and MD sites (Table 4). The NDVI values at the DE site were also below the NDVI threshold of .27 reported by Thomason et al. (2007); values below this threshold do not correlate to corn grain yield. As such, our work corroborates the findings of Thomason et al. (2007) that low NDVI values cannot be utilized to make in-season N management decisions for corn. The NDVI-based in-season N recommendation would likely have improved if spectral measurements were collected at a later growth stage or at a lower UAV flight height to achieve higher resolution (Thomason et al., 2007). In fact, one challenge of using in-season NDVI measurements to predict sidedress N needs is the need to balance the corn growth needed to detect the variability in NDVI readings due to N status, while allowing enough time for the grower to make a fertilizer application with their equipment in time to address the in-season N deficiencies.

Table 4: Virginia Tech algorithm inputs used to produce the in-season sensor-based N recommendation for 2022 N rate strip trials in PA, DE, and MD. The difference in the low- and high-N reference NDVI was much lower at the DE and MD sites compared to at PA.

<b>Site</b>	<b>N Applied at Planting (kg ha<sup>-1</sup>)</b>	<b>Days from Planting</b>	<b>NDVI of Low N Reference</b>	<b>NDVI of High N Reference</b>	<b>Average V6 NDVI</b>
<b>Mt. Joy, PA</b>	0	48	.42	.57	.43
<b>Seaford, DE</b>	34	34	.25	.26	.20
<b>Federalsburg, MD</b>	96	25	.48	.49	.49

### **3.3 Plant Height Analysis and Leaf Area Index (LAI)**

The PA site exhibited a significant LAI response to N rate at R2 (P-value = .0027, Figure 5); no LAI response was noted at the DE or MD sites (P-value = .15 and .38, respectively, data not shown). The lack of N response at MD and the smaller variation in yield between the different N rates at DE may have limited the LAI response to N rate compared to the PA site. Unlike at the DE and MD sites, the variability in color, structure, and overall biomass between corn receiving the N rate treatments was visually distinguishable at the PA site even though the range in LAI values was similar at all three sites (2.97–5.46 at PA, 3.76–6.06 at DE, and 4.70–7.40 at MD, respectively). Corn growing in plots receiving the 0 and 36 kg ha<sup>-1</sup> N rates at the PA site showed visible N deficiencies and appeared to have fewer leaves compared to the higher N rates (219 and 292 kg ha<sup>-1</sup>) at R2. In comparison, there were no obvious visual differences in corn plant health at the DE or MD sites at R2.

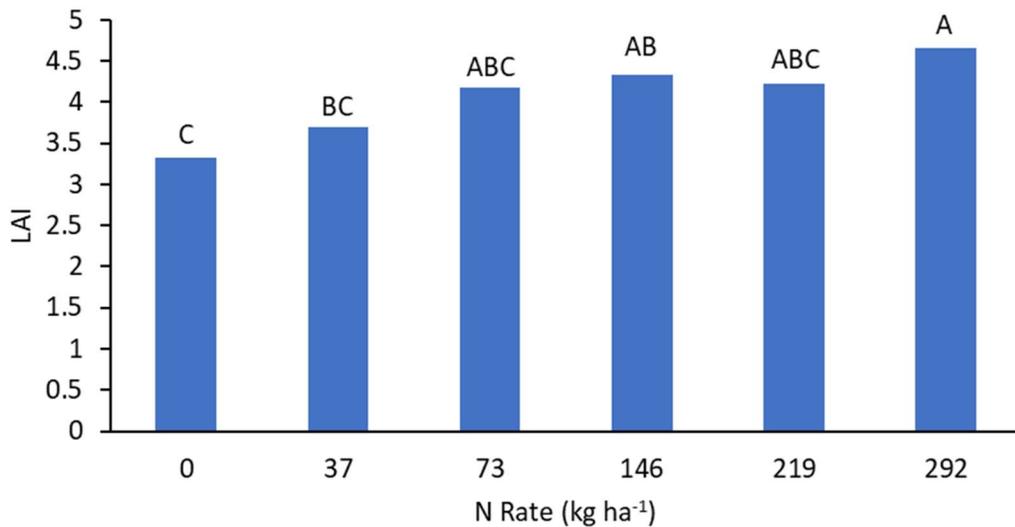


Figure 5: Leaf Area Index (LAI) response to N rate at R2 in a replicated strip trial with four replications of six N rates conducted at Mt. Joy, PA in 2022. Bars with the same letter are not statistically different by Tukey's honestly significant test at  $\alpha = .05$ .

There was a plant height response to the N rate at the DE site (P-value = .0029, Figure 6), but not at the PA or MD sites (P-value = .28 and .26, respectively; data not shown). Similarly, to LAI, the lack of N response at the MD site inhibited a plant height response to the N rate. The lack of a significant plant response to N at the PA site was likely due to the large standard error among height measurements within each N rate treatment. For example, the average standard error for plant height measurements at PA was .058 m, compared to .014 m at the DE site. The larger variation in height among plants within a single N rate treatment could be a result of the topography at the PA site. The DEM of the PA site shows how the topography changes within the plots (Figure 7). These changes in topography may have caused a plant height response based on the physiological pathway of shade avoidance rather

than being N related (Dubois and Brutnell, 2011). The corn plants in the depression area needed to grow taller to collect enough sunlight and not be shaded out by the neighboring rows at higher elevation.

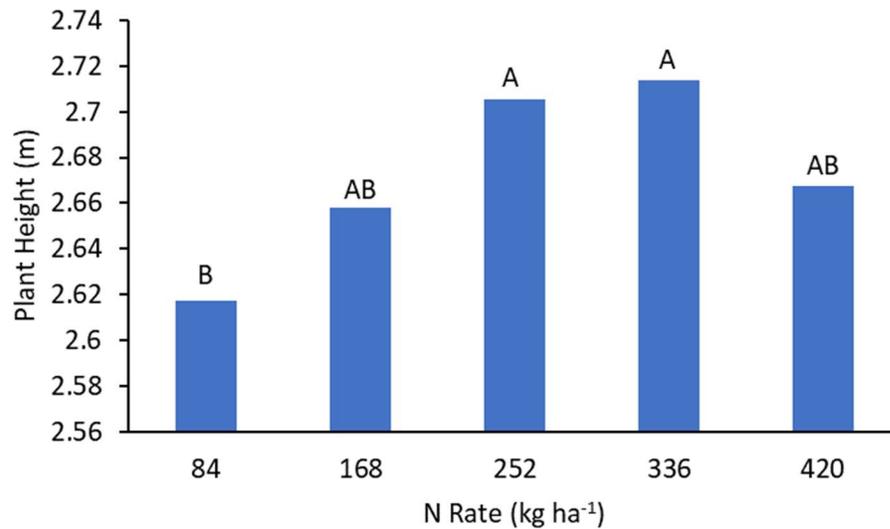


Figure 6: In-field plant height response to N rate at R2 in a replicated strip trial with four replications of five N rates conducted at Seaford, DE in 2022. Bars with the same letter are not statistically different by Tukey's honestly significant test at  $\alpha = .05$ .

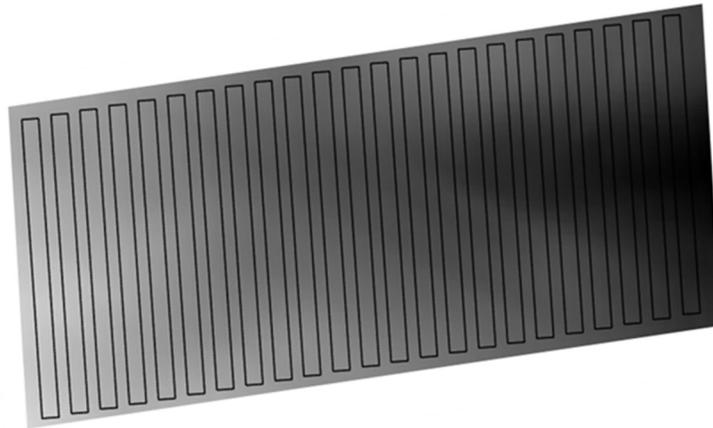


Figure 7: The digital elevation model (DEM) of the site location of the 2022 corn N rate strip trial conducted near Mt. Joy, PA. The strips go in order from right to left. Higher elevation is shown as the lighter areas. The darker area on the right side of the plot area is the depression that alters the topography across the strips and may have caused a physiological shade avoidance response rather than a plant height response to N rate.

The correlation between the two plant height measurements, the UAV-SFM and actual in-field measurements, did not result in a 1:1 linear response among all sites (Figure 8). We did see a significant correlation between these two plant height measurements at the DE site (P-value < .0001); however, the UAV-SFM heights were on average .68 m taller (25% increase) than the in-field measurement at the site. For the other two sites the difference between the UAV-SFM and the in-field height was about 1 m. Also, the UAV-SFM height at the DE site did not exhibit the same significant plant height N rate response as the in-field measurement (P-value = .18; data not shown). These results differ from previous studies that showed that there was correlation between in-field height measurements and the UAV-SFM plant height (Sarkar et al., 2020). In their study, Sarkar et al. (2020) measured the height of peanut

plants with a UAV using the DSM from Pix4D at a height of 20 m above ground level. Our UAV was flown at 120 m above ground level, which results in lower resolution that could impact the accuracy of the UAV-SFM measurement. Relationships between measured plant height and UAV-SFM heights were strongest at the DE site because the differences in plant height were more prominent compared to our other sites. Plus, the DE site lacked significant topography changes that limited the plant height response to shade avoidance. As discussed previously, half the corn at the PA site was planted across a depression. The presence of this depression may have impacted the alignment of the DSM and DEM needed to produce the UAV-SFM plant height measurements in PA. In addition, the in-field ground control points at the PA and the MD site were hard to distinguish at R2 due to corn growth, which also may have impacted the DSM produced by Pix4D. Currently, we feel that the UAV-SFM method of measuring plant height (as described for this experiment) may not be able to be utilized as a substitute for in-field corn plant height measurements across a widespread region, such as the Mid-Atlantic, because of the flight-specific nature of the measurements. Further method refinements, such as clearing more area to see the ground control points and lower flights, may improve the UAV-SFM plant height measurements in the future.

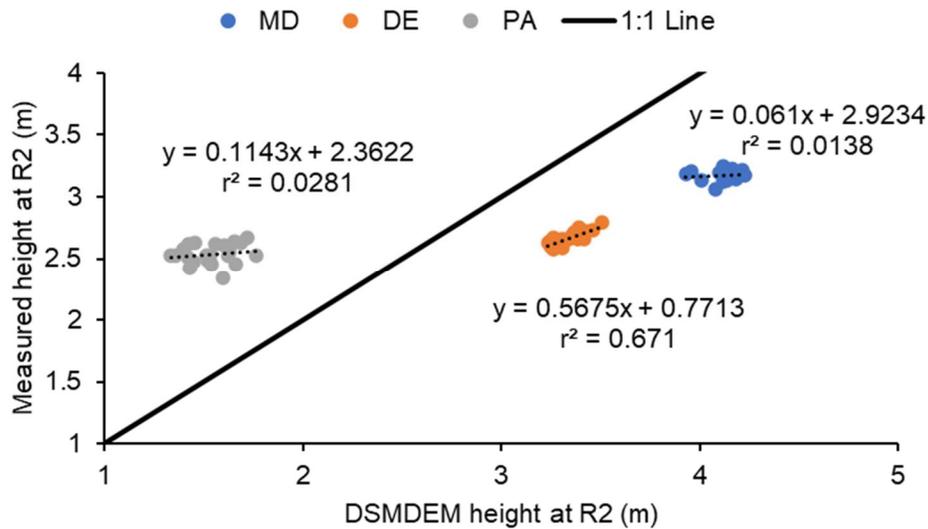


Figure 8: The relationship between the measured plant height and the UAV-based calculated plant height (UAV-SFM) for replicated corn N rate trials located near Mt. Joy, PA, Seaford, DE, and Federalsburg, MD in 2022. The linear relationship between these two height measurements was strongest at the DE site (P-value < .0001). Overall, there was little correlation between the actual field measurements and UAV-SFM and was site- and UAV flight-specific.

### 3.4 Yield Prediction by Vegetative Indices

In general, the NDRE and ExG indices had the strongest relationships with corn yield when evaluated at individual site locations; measures of plant height also exhibited relatively strong relationships with corn yields (Table 5). Other researchers also reported that NDRE was a better predictor of corn yield than NDVI at R2 (Kelly et al. 2015; Olson et al., 2019). Crop height measurements can also be related to corn grain yield. For example, Olson et al. (2019) used UAVs to obtain NDVI and NDRE at several corn growth stages in Minnesota across three site years to track both the corn N response and to produce a yield prediction. The researchers estimated plant height using the DSM created from Pix4D (as described above for this study) and

found that combining plant height by DSM with NDVI improved some of the grain yield predictions (Olson et al., 2019). Similarly, Kelly et al. (2015) collected NDVI and in-field plant height measurements in Oklahoma from five site years for corn at V12 and reported that both plant height and NDVI  $\times$  height resulted in the highest correlation to yield compared to NDVI alone ( $r^2 = .50$ ).

Table 5: The linear relationships and multivariate regressions between yield and each R2 measurement and vegetative index for the 2022 N rate strip trials in PA, DE, and MD. The MD site had much lower relationships due to the lack of N response at the site.

Site		Vegetative Index or Measurement at R2	r <sup>2</sup> Value	P-Value of Model
Mt. Joy, PA	Single Variable	NDRE	.9250	<.0001
		GNDVI	.9148	<.0001
		ExG	.9082	<.0001
		NDVI	.8722	<.0001
		LAI	.5580	<.0001
		Height	.0279	.4692
	Two Variables	NDRE, GNDVI	.9342	<.0001
		NDRE, Height	.9288	<.0001
		NDRE, NDVI	.9283	<.0001
		NDRE, ExG	.9283	<.0001
		NDRE, LAI	.9272	<.0001
Seaford, DE	Single Variable	ExG	.7945	<.0001
		NDRE	.7588	<.0001
		GNDVI	.7347	<.0001
		Height	.6282	<.0001
		NDVI	.3485	.0061
		LAI	.0739	.2462
	Two Variable	ExG, Height	.8713	<.0001
		GNDVI, Height	.8559	<.0001
		NDRE, Height	.8553	<.0001
		ExG, LAI	.8484	<.0001
Federalsburg, MD	Single Variable	NDRE	.2409	.028
		ExG	.2238	.0352
		LAI	.1521	.0891
		GNDVI	.1507	.0907
		Height	.0318	.4522
		NDVI	.0025	.833
	Two Variables	NDRE, GNDVI	.3328	.0321
		ExG, LAI	.3314	.0327
		NDRE, NDVI	.3249	.0355
		NDVI, GNDVI	.2891	.055
		NDRE, LAI	.2737	.066
		ExG, GNDVI	.2584	.0788
		ExG, Height	.2524	.0844
		ExG, NDVI	.2446	.0922

The relationships between corn yield and vegetative indices and measures of plant height or LAI were strongest for the PA site ( $r^2 = .558-.9342$ ; Table 5). Strong relationships between these measurements and corn yields were also observed for the DE site, although  $r^2$  values were somewhat lower ( $r^2 = .3167-.8713$ ) than reported for the PA site. Relationships between the vegetative indices or plant height measurements and corn yield were much weaker for the MD site ( $r^2 = .0025-.2409$ ); only relationships between NDRE or ExG and corn yield were statistically significant (Table 5). The weak relationships at MD were likely due to the lack of response to N rate at the site. Accurate yield prediction seems to require variability in yield across a field to produce a good relationship. For all three site locations the yield prediction improved with the addition of a second variable to the regression models (Table 5). However, the impact of adding a second variable to the yield prediction model (particularly plant height) was greatest for the DE site, where there was also a height response to N rates. Similarly, Yin and McClure (2013) reported an increase in corn yield prediction when including V12 corn plant height along with NDVI in their study across three site years in Tennessee. They found that including plant height (NDVI\*plant height or NDVI+plant height) improved the  $r^2$  for the regression of yield at V12 compared to NDVI alone. However, they concluded that combining NDVI and plant height was not any different than using plant height alone as a yield predictor at V12, which is different than what was found in this study.

The relationships between yield and vegetative indices were much stronger when evaluated across all three sites because of the increased sample size and range of data. Overall, corn yields across all sites were best predicted using the NDRE data collected at R2, followed by, GNDVI, NDVI, ExG, LAI, and plant height ( $r^2 = .9517$ ,

.8994, .8783, .864, .661, and .6216, respectively; Figure 9). As mentioned previously, the UAV-SFM plant heights showed a site-specific pattern, which was evidenced in the residual plots for regression models that contained this variable. Therefore, the UAV-SFM plant heights were not used in the yield prediction.

The two variable regression models (Figure 10) including NDRE and plant height ( $r^2 = .9708$ ) or NDRE and LAI ( $r^2 = .9621$ ) improved yield prediction by 2.01% and 1.09%, respectively when compared to NDRE alone. The in-field measurements of plant height and LAI showed that the use of biomass metrics can be used to improve the yield prediction at R2 across the Mid-Atlantic region, as was suggested by Kelly et al. (2015), Olsen et al. (2019), and Yin and McClure (2013).

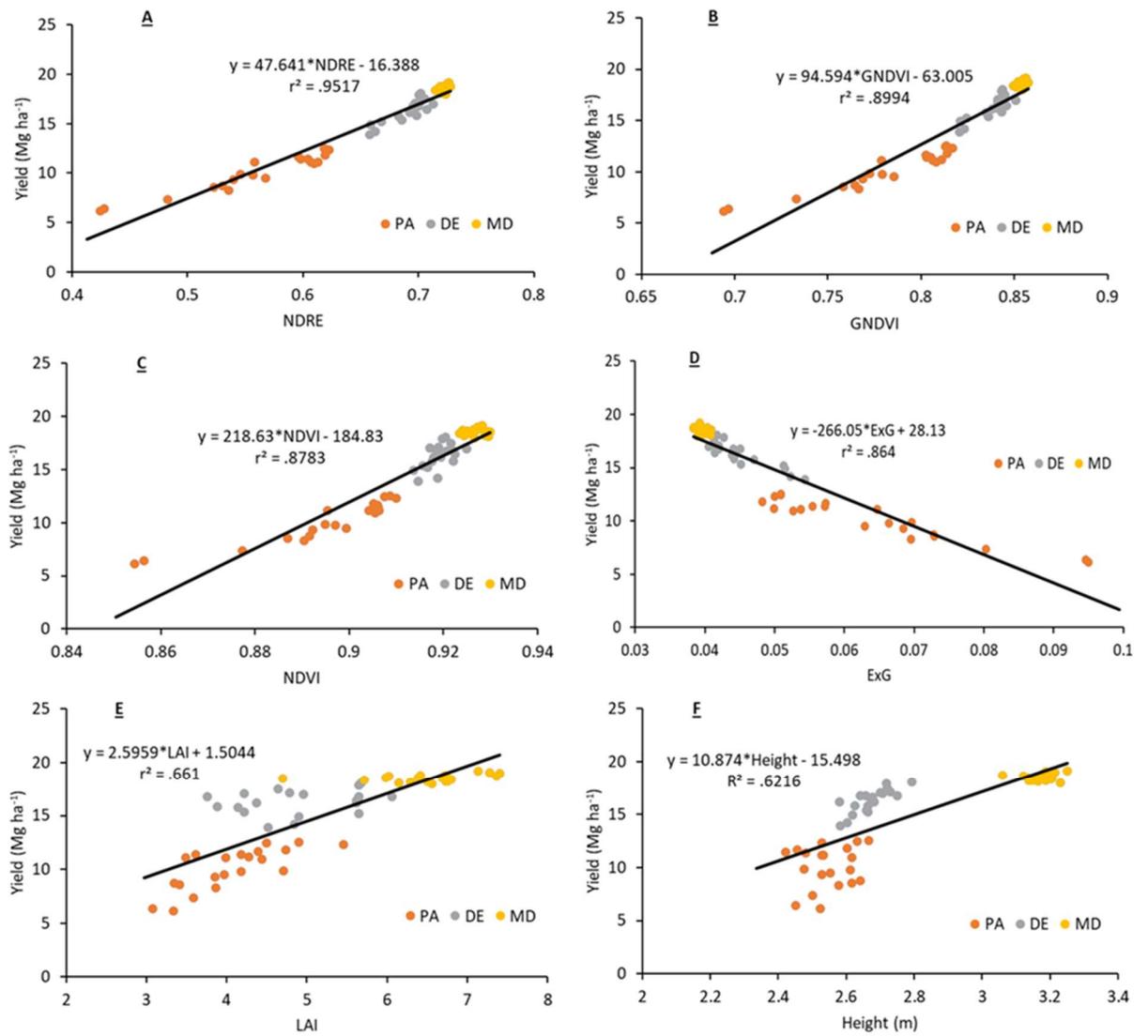


Figure 9: Yield prediction at R2 for the 2022 PA, DE, and MD N rate strip trials by NDRE (A), GNDVI (B), NDVI (C), ExG (D), LAI (E), and height (F). All are significant with a P-value < .0001.

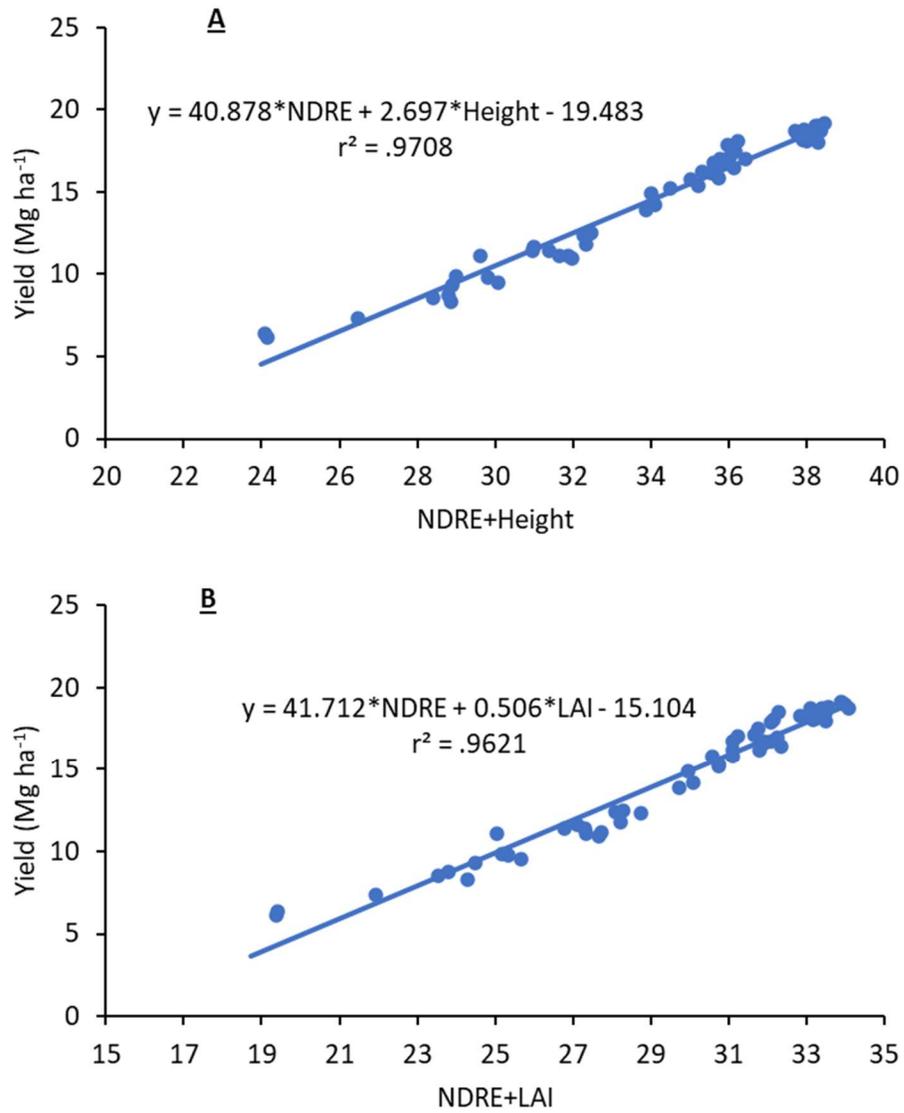


Figure 10: Multivariate yield prediction at R2 across all three sites (PA, DE, and MD) of 2022 N rate strip trials for NDRE+Height (A) and NDRE+LAI (B). Both models significant with a P-value < .0001.

### 3.5 Conclusion

The applications of spectral imagery for precision agriculture are numerous and include improving in-season N recommendations, estimating biomass, yield prediction for corn, as well as providing aerial imagery to showcase field variability throughout the year. However, the results of our study show that continued research needs to be done to further understand the contribution of biomass estimates to produce more accurate in-season sensor-based recommendations.

The NDRE index was determined to have the best correlation to yield in this study at both the individual sites and regional scale. The use of UAVs and multispectral cameras is increasing in agriculture, but their cost and the learning curve are a large barrier to their adoption. A multispectral camera is required to obtain the needed wavelengths for most vegetative indices (NDRE, NDVI, and GNDVI), but a cheaper RGB camera can produce the required wavelengths for the ExG index, which was shown to also correlate well to yield in this study. Therefore, the ExG index is a reliable option for corn grain yield prediction if a sunlight correction sensor is used on the UAV. Measurements of LAI and plant height at R2 resulted in slight improvements to the corn grain yield prediction models when combined with vegetative indices. However, collecting in-field biomass measurements is a time-consuming process, which limits incorporation of biomass estimates into yield prediction models. The continual development of obtaining plant height or other biomass metrics via UAV-imagery will allow for decreased sampling time, and if they correlate to in-field measurements, will provide the opportunity to improve both late season fertilizer applications and yield prediction models.

Our ability to make accurate in-season N recommendations using UAV-mounted sensors is necessary to increase precision fertilizer applications while also

limiting N losses to the environment. In our study, we noted vegetative indices at V6 were highly uniform across fields, likely because there was not enough biomass to influence the vegetative index measurements. Future research should focus on identifying the corn growth stage at which plant biomass begins to influence the vegetative indices obtained from spectral imagery. The in-season sensor-based N recommendations also relied heavily on the functionality of the high and low N reference areas and the growth stage of the corn. At the PA site, the in-season Virginia Tech N algorithm resulted in an accurate N recommendation compared to the end of season yield results. However, at the DE site, the in-season N recommendation was significantly lower than the N rate at the yield plateau. The non-functional high N reference and measuring the NDVI with the UAV before V6 (corn was at V4 when initial UAV flights were conducted) both impacted the accuracy of the in-season tool. These results show that sensor-based systems can be used to produce precision agriculture N recommendations, but they need to be performed at the optimal time and are reliant on the field's reference areas, which cannot always be obtained in each individual growers' situation or management system. This study showcases some of the advantages and disadvantages of using sensor technology as an N management tool and is an example of why more research is required before there is greater adoption of in-season sensor-based technology.

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