THESIS

CHARACTERIZING NITROGEN DEFICIENCY OF MAIZE AT EARLY GROWTH

STAGES USING FLUORESCENCE MEASUREMENTS

Submitted by

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ABSTRACT

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Among all nutrients that are important for crop production, nitrogen (N) is one of the

least efficiently utilized, mainly due to its high mobility characteristics in soil. The possibility of

using crop sensing in real-time to detect variability in N deficiency within a field has the

potential to enhance N efficiency, increase crop yield, and reduce potential environmental risks

and crop production costs. Potassium (K), another important crop nutrient, can also lead to

higher yield when applied in the right amount and manner. Real-time fluoro-sensing is a new

technology for crop sensing and studies have shown that it could enable variable rate nutrient

management for precision agriculture practices. The objective of this study was (1) to evaluate if

fluorescence sensing can detect variability of N and K in crop canopy at early growth stages of

maize (prior to V6 crop growth stage) under controlled condition (greenhouse), (2) to evaluate

the effect of different fertilization dosages of N over the plant growth, and (3) to verify if

induced fluorescence can detect *in situ* N variability at early growth stages of maize. Research

was conducted in two stages, first in a greenhouse condition and later in field spread over three

site-years. The greenhouse research was conduct in year 2011 and plants were grown in plant-

pots with silica sand and supplied with modified Hoagland solution with different rates of N and

K. Field trials were conducted in year 2012 and 2013 in northern Colorado. For the greenhouse

study, data collected via fluorescence sensor (Multiplex[®]3) were analyzed using ANOVA and

Tukey's HSD to test significant differences among treatments in each experiment. For the N

experiment, regression analysis between the seven fluorescence indices and N uptake was performed for the 12 days of data acquisition at five different growth stages (i.e. 2-leaf to 6-leaf growth stages) and coefficient of determination was used to identify the best fluorescence indices to detect N status. Also, root mean square error (RMSE) was used to test the precision of the estimates for each index. Results of this study indicated that all fluorescence indices were able to detect N variability in maize canopy prior to V2 growth stage. However, the fluorescence indices failed to identify K deficiency as the maize plants with K treatments showed small variability at early crop growth stages. For the field study, two site-years had 5 N rate treatments applied as UAN 32% (urea and ammonium nitrate; 32-0-0), while one site-year had 6 N treatments applied pre-planting. Sensors used in this study were the Multiplex®3 for fluorescence sensing and the GreenSeeker® for reflectance sensing (NDVI). Sensor measurements were correlated with aboveground biomass, N content, and N uptake measured at two growth stages (V6 and V9 maize growth stage). The aboveground biomass, N content, N uptake, yield, and sensors readings were analyzed using ANOVA and Tukey's HSD to test test significant differences among the N treatments. Also, a regression tree between N uptake and the fluorescence indices was fitted along with the coefficient of determination (R^2). The N rates had no effect on aboveground biomass, N content and N uptake (for both sampled growth stages). Under field conditions, fluorescence indices failed to detect N variability in maize at early growth stages for all three site-years. This finding may require further investigation, as most of the N treatment plots, maize plants had sufficient N levels and another biotic or abiotic stress may be responsible for unexplained differences in N variability as measured by fluorescence sensor. Contrasting findings under greenhouse conditions versus field conditions limits the application of

fluorosensing sensor. Further field studies are be needed to evaluate the potential of this sensor to detect N variability *in situ*.

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SUMMARY OF CHAPTER 1

Real-time fluoro-sensing is a new technology for crop sensing and studies have shown that it could enable variable rate nutrient management for precision agricultural practices. The objective of this study was to evaluate if fluorescence sensing can detect variability of nitrogen (N) and Potassium (K) in crop canopy at early growth stages of maize (prior to V6 crop growth stage). Research was conducted under greenhouse conditions and plants were grown in plant pots with silica sand and supplied with modified Hoagland solution with different rates of N and K. Sensor readings were analyzed using ANOVA and Tukey's HSD to test differences in crop response to nutrient rates. Regression analysis were used to assess the precision of sensor indices in estimating N and K in the crop canopy. Results of this study indicate that all fluorescence indices enabled the detection of N variability in maize canopy prior to V2 growth stage. However, the fluorescence indices failed to identify K deficiency as the maize plants with K treatments showed little to no variability of this nutrient at early crop growth stages as measured with plant tissue analysis.

INTRODUCTION

Precision farming, or site-specific management, assists growers in making precise management decisions for different cropping systems throughout the world (Koch and Khosla, 2003). One of the most important tools used for this crop management system is variable-rate technology, which consists of application of specific inputs, such as nutrients, water, pesticides, for specific soil and crop conditions (Moran et al., 1997). Understanding the spatial and temporal variability that occurs within a field is a key factor when working with variable-rate application. Although most commercial products of variable-rate applications have been map based with the help of soil test, yield maps, and other spatial information, several real-time sensor systems for nitrogen (N) management are being marketed (Lowenberg-DeBoer, 2004). Using real-time crop sensing to accomplish variable-rate fertilizer applications can help reduce the amount of fertilizer needed and thus improve nutrient efficiency and perhaps cost efficiency.

Nitrogen is a key input in maximizing yields and economic return to farmers , with high plant uptake it is the most limiting nutrient for crop production (Khosla et al., 2002; Fageria and Baligar, 2005; Bender et al., 2013). A mismatch between N supply and crop N requirement can potentially hamper crop growth or harm the environment when N is under or over applied respectively. Either situation may result in low N use efficiency (NUE), hence may result in agronomic and economic loss. Too much N often leads to a greater risk of groundwater contamination as a result of NO_3-N leaching (Carpenter et al. 1998). Even though worldwide use of N is increasing, NUE is about 50% for maize (*Zea mays* L.), and around 30% for agricultural crops in general (Baligar et al., 2001; Cassman et al., 2002).

Biomass sampling is a destructive method that can provide accurate information about NUE, but it is time consuming and economically inefficient when large amounts of data is required to characterize spatial variability of field crops (Agati et al., 2013). In contrast, non-destructive sampling methods such as remote sensing techniques based on reflectance can generate large amounts of data at relatively low cost (Evan, 1983). Since the optical properties of leaves are affected by leaf chlorophyll concentrations, reflectance measurements have been widely used to predict N variability in plants. Along with reflectance, leaf transmittance and fluorescence are also influenced by N deficiency (Blackmer et al., 1996; Bilger et al., 1997).

Reflectance for the purpose of crop sensing has been widely studied and previous research has shown good correlation of reflectance data with plant biomass and yield (Ma et al., 1996; Shanahan et al., 2003; Solari et al., 2008). Some reflectance-based vegetation indices use a combination of wavebands. The normalized difference vegetation index (NDVI) is a ratio based on near-infrared (NIR) and red wavebands [(NIR – red)/(NIR + red)] (Rouse et al. 1973). Commercially available reflectance sensors such as the GreenSeeker [TM] (Trimble, Sunnyvale, USA) use an active light source to measure reflectance from crop canopies and an algorithm to determine N rates by comparing it to an N-rich strip within the field (Lowenberg-DeBoer, 2004).

The timing at which an input such as fertilizer is applied can influence final crop yield, as crop production may decrease if crop conditions at early stages of growth are not satisfactory. It is thus advised to provide farmers with the N status of the crop at an early growth stage that would allow the grower to apply the appropriate N rates based on an assessment of plant requirement and crop N deficiencies (Haboudane et al. 2001). The NDVI index has been stated to be one of the best indicators for N status in maize (Ma et al. 1996). However, commercial NDVI sensors such as the GreenSeeker[TM], has been reported to provide reliable measurements

between the V8 to V12 maize growth stages (Martin et. al., 2007, Shaver et al., 2010, 2011, and 2014). While such a finding is scientifically significant, most farmers complete their side-dress N application by or before the V6 maize growth stage to minimize tractor damage and prior to plants starting to show nutrient deficiency. A new sensing device based on fluorescence has enabled the detection of N variability prior to V5 maize growth stage in greenhouse conditions (Longchamps and Khosla, 2014).

Fluorescence sensors have been widely used for ecophysiological studies (Maxwell et al., 2000), but its application in precision agriculture is fairly new. The Multiplex®3 (Force-A, Orsay, France) is a new commercially available active sensor that acquires *in situ* fluorescence measurements over crop canopies. Active fluorescence measurements have been available for laboratory use while mobile platforms were limited by both, the power of the excitation energy source and the weakness of the fluorescence signal itself (Barnes et al., 2003). With the advent of more powerful light emitting diodes (LED) and more sensitive optical sensors, fluorescence can be acquired in the field with more reliable outcomes. Similar to chlorophyll, leaf flavonoids are compounds related to N content in plants (Norbaek et al., 2003) that can be detected *in situ* by a screening method called the ABC fluorescence method proposed and validated in laboratory spectroscopy studies (Bilger et al., 1997; Cerovic et al., 2002; Agati et al., 2007; Agati et al., 2011). This method uses the epidermal flavonoids fluorescence that has an inverse relationship with biomass N content. Cartelat (2005) reported that by combining epidermal flavonoids fluorescence with chlorophyll fluorescence (ChlF-Flav) it is possible to predict N content in wheat production. Different variations of this method lead to the development of the Nitrogen Balance Index (NBI) which has been reported to be a good predictor of N status in plant.

Other nutrient deficiencies, water stress or phytopathologic conditions could potentially interfere with the plant fluorescence emission which could lead to misinterpretation of the data. Fluorescence sensing has been reported as capable of detecting N deficiency among other stresses such as K deficiency (Muñoz-Huerta et al., 2013). In North America, Potassium (K) has not been considered as a major maize yield limiting nutrient (personal communication with Dr. Robert Miller). However, studies from International Plant Nutrition Institute (IPNI, 2010) reported a constant decrease of K in U.S. soils, especially in the Corn Belt where agriculture have been practiced intensively for decades. The IPNI (2010) report also suggested that for the Corn Belt region and areas east of Mississippi River, 50% or more of the sampled areas will likely require annual K application to avoid yield losses.

Both N and K are important nutrients that exhibit similar biotic stress symptoms in crop canopies. Early detection of variability of these two important nutrients in crop and the ability to distinguish the biotic stresses caused by the deficiency of the two nutrients from each other could significantly benefit farmers and their crop production practices. The hypothesis of this study was that induced fluorescence acquired by Multiplex$^{\circledR}$ 3 fluorescence sensor (FORCE-A, Orsay, France) can be used to distinguish and predict N and K deficiency in maize. The specific objectives were: (1) to investigate the relationship between induced fluorescence indices, plant growth, nitrogen content, nitrogen uptake, potassium content, and potassium uptake; and (2) to verify if induced fluorescence may be used to accurately characterize N and K uptake in maize at early growth stages.

Study site and plants

Maize (*Zea mays* L.) was planted in a greenhouse located in Fort Collins (CO) on 26th of October, 2012. Maize emergence was observed on 2nd of November. Plant-pots having a volume of 11 liters (23 cm in diameter and 21 cm in height) were filled with 6 kg of silica sand. Ambient light was provided by 430W high intensity discharged lamps for 16 hours a day, and the temperature variation ranged from 25° C to 20° C (day/night) in the greenhouse. Prior to planting, 400 ml of water was applied per pot. After the soil was wet, each pot was planted with 5 seeds (variety Dekalb DKC45-79) in a cross pattern at 2 cm depth. Each pot had drainage holes and as water drains easily in silica sand, a plastic saucer was placed under the pots to prevent any water drainage that could cause nutrient leaching. Pots were supplemented with a daily irrigation of 80 ml, which did not result in leaching of nutrients as no water or leaches were observed during the course of this entire study.

Treatments and replicates

Two experiments were setup based on nutrient variability. First experiment consisted of a randomized block design of four different N rates (0%, 25%, 50% and 100% of N recommended in Hoagland solution) (Hoagland and Arnon, 1950) with six replications. Second experiment consisted of a randomized block design of four different K rates (0%, 25%, 50% and 100% of K recommended in Hoagland solution) also with six replications. The N and K treatment with 100% N and K rates was the same for the two experiments and corresponded to the original Hoagland solution. Other N and K treatments (0%, 25% and 50%) were established

by modifying Hoagland Solution for each nutrient. Nutrient modifications for all the treatments

and original Hoagland solution are shown in Table 1.1.

Table 1.1 Nutrient treatments used for both experiments. Treatments consisted of different plant nutritive solution based on Hoagland Solution.

Ingredients	Stock solution (g/L water)	Hoagland 100%	Nitrogen 50%	25%	0%	Potassium 50%	25%	0%
		----------------------ml / L----------------------						
KH_2PO_4 (pH to 6.0 with 3M KOH)	136.09	1	1	1	1	-	-	-
KNO_3	101.11	5	2.5	1.25	-	-	-	-
$Ca(NO_3)_2$ x $4H_2O$	236.16	5	2.5	1.25	-	5	5	5
$MgSO_4$ x $7H_2O$	247.47	2	2	2	2	2	2	2
KCL	74.56	-	5	5	5	2.5	1.25	0
$CaCl_2$ x $2H_2O$	147.02	-	5	5	5	-	-	-
$NH_4H_2PO_4$	115.31	-	-	-	-	1	1	1
NH_4NO_3	80.04	-	-	-	-	2	2	2
NaH_2PO_4	119.98	-	-	-	-	1	1	1
Minors:	*	1	1	1	1	1	1	1
Fe-EDTA:	**	1	1	1	1	1	1	1

*Minors: micronutrients. Stock solution was prepared by H_3BO_3 (2.86 g/L), $MnCl_2$ x $4H_2O$ (1.81 g/L), $ZnCL_2$ x $7H_2O$ (0.1g/L), $CuCl_2$ (0.04g/L) and $H_2MoO4.H_2O$ (0.02g/L)

**Fe-EDTA: Ferric ethylenediaminetetra acetic acid. Stock solution was prepared by FeSO4 x $7H_2O$ (24.9g/L), EDTA-Na (33.2g/L) and NAOH 1N (89ml/L)

The amount of solution applied for all the treatments was 300 ml at 4 days after emergence (DAE), and a second application of the same amount at 28 DAE, when the maize plants were at V4 (4-leaf) growth stage.

Fluorescence Sensor and indices

The Mutiplex[®]3 multi-parameter fluorescence sensor has 4 excitation channels: UV (around 375 nm), blue (around 470 nm), green (around 515 nm) and red (around 625 nm). Excitation light pulses (20 µs per flash) were delivered by high-power light emitting diode arrays

located around the detectors and pointing in the direction of the sensed area. The three detection channels (filters) are yellow (590 nm ± 40 nm; YF), red (678 nm ± 22 nm; RF) and far-red (750 nm ± 65 nm; FRF). The detectors consist of three silicon photodiodes (20 mm x 20 mm), each having an optical band pass filter allowing only yellow, red or far-red light to reach the photodiode. The flash induces the emission of fluorescence and the filters allow the selection of the wavebands of interest. A firmware synchronizes the light pulses and the detectors in order to acquire each combination (12 in total) of excitation wavebands and detection channels for up to 476 readings of all parameter per second. The field-of-view is about 10 cm diameter (FORCE-A, Orsay, France). More details about the sensor hardware can be found in Cerovic et al. (2009). In this study, 7 indices were used based on ratios of band combinations: four N balance indices, two chlorophyll indices and one flavonoid index. These indices were chosen based on previous studies conducted in greenhouse (Longchamps et. al., 2012) and on a spectroscopy study (Cerovic et al, 2002). The equations for each index along with its description are presented in Table 1.2.

Table 1.2 Indices used for this study along with their description and formula

Parameter	Description	Formula
NBI_R	Nitrogen balance index (red)	$NBI_R = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{uv_i}}{FRF_{r_i}}$
NBI_G	Nitrogen Balance index (green)	$NBI_G = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{uv_i}}{FRF_{g_i}}$
NBI_B	Nitrogen balance index (blue)	$NBI_B = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{uv_i}}{RF_{b_i}}$
NBI1	Nitrogen balance index (green/red)	$NBI_1 = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{uv_i} + FRF_{g_i}}{FRF_{r}^{2}{}_i}$
CHL	Chlorophyll index (red)	$CHL = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{r_i}}{RF_{r_i}}$
CHL1	Chlorophyll index (green)	$CHL_1 = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\dfrac{FRF_{g_i}}{RF_{r_i}}$
FLAV	Flavonoid index	$FLAV = \dfrac{1}{n}\displaystyle\sum_{i=1}^{n}\log\!\left(\dfrac{FRF_{r_i}}{FRF_{UV_i}}\right)$

Data Acquisition

The Multiplex®3 sensor acquires data at 70 Hz, (i.e.) 70 cycles per second of induction and detection (12 different readings from 4 light sources and 3 unique filters for each cycle). The data acquisitions were made at 10 cm height from the plant's uppermost leaves. To ensure that the entire crop canopy was sensed and not just one specific leaf or plant, slow circular

movements of the sensor were performed across the canopy in each pot during fluorescence data acquisition. Each sample data acquisition lasted for about 4 seconds, which resulted in approximately 250 fluorescence readings. Data were acquired 3 times a week, between 11:00 and 13:00hrs. The first reading was made on the 10th DAE at V2 (2-leaf crop growth stage) and last set of readings were acquired on the 56th DAE atV8 (8-leaf crop growth stage). Complete set of readings took about 20 minutes to acquire at each data collection date.

Biomass sample and tissue analysis

Biomass samples were collected at the 36th DAE, at the V6 (6-leaf crop growth stage). Samples consisted of three plants from each pot and two plants were left behind to continue with sensing until V8 growth stage. Biomass samples were air dried, weighed and sent for tissue analysis to a commercial lab, Harris Laboratory, Lincoln, NE. Total N content (%) was analyzed by Kjeldahl digestion method (wet digestion in H_2SO_4-H_2O_2) and total K content (%) by Nitric Acid/Hydrogen Peroxide digestion (Association of Official Analytical Chemists, 1990).

Statistical analysis

The N uptake, expressed as the percentage of N in the plant tissue (N content) multiplied by the dried biomass, was used to indicate N variability in plants for the N experiment. Similarly K uptake was estimated as well. Analysis of variance (ANOVA) and Tukey's HSD test ($\alpha =$ 0.05) were used to test significant differences among treatments in each experiment. For the N experiment, regression analysis between the seven fluorescence indices and N uptake was performed for the 12 days of data acquisition at five different growth stages (i.e. 2-leaf to 6-leaf growth stages) and coefficient of determination was used to identify the best fluorescence indices to detect N status. Also, root mean square error (RMSE) was used to assess the precision of the estimates for each index. Statistical software R was used for all statistical analyses. The functions

used for those tests were "aov", "TukeyHSD", "lm" and "rmse" (Mangan et. al., 2011; R Development Core Team, 2012; Seither et al., 2012).

RESULTS AND DISCUSSION

Nitrogen and Potassium variability

Nitrogen experiment

As anticipated, the N rate treatments generated variability in dried maize aboveground biomass, which ranged from an average of 3 grams per pot for 0% N treatment to 16 grams per pot for 100% N treatment. As anticipated, dried maize biomass weight were significantly different ($\alpha = 0.05$) from each other for all N treatments. Additionally, maize showed small variability in N content at V6 growth stage, ranging from 0.22 to 0.40% of N in the plant dried tissue. The different N rates did not have a significant ($\alpha = 0.05$) effect on N content for all the treatments. Nitrogen uptake was significantly different ($\alpha = 0.05$) for all N rates. The relationships between N rates and that of N uptake, N content or dry biomass are illustrated in Fig. 1.1.

Results from this study indicate that different rates of N had a significant effect on aboveground biomass and N uptake, but did not show significant difference in N content. Furthermore, the N uptake was highly correlated with the N rates ($R^2 = 0.98$), demonstrating the efficiency of the treatments imposed on the plants to create N status variability and thus creating appropriate conditions for verifying fluorescence measurements response to N uptake and crop growth. This result also indicates that prior to V6 growth stage maize growth has already been affected by N deficiency.

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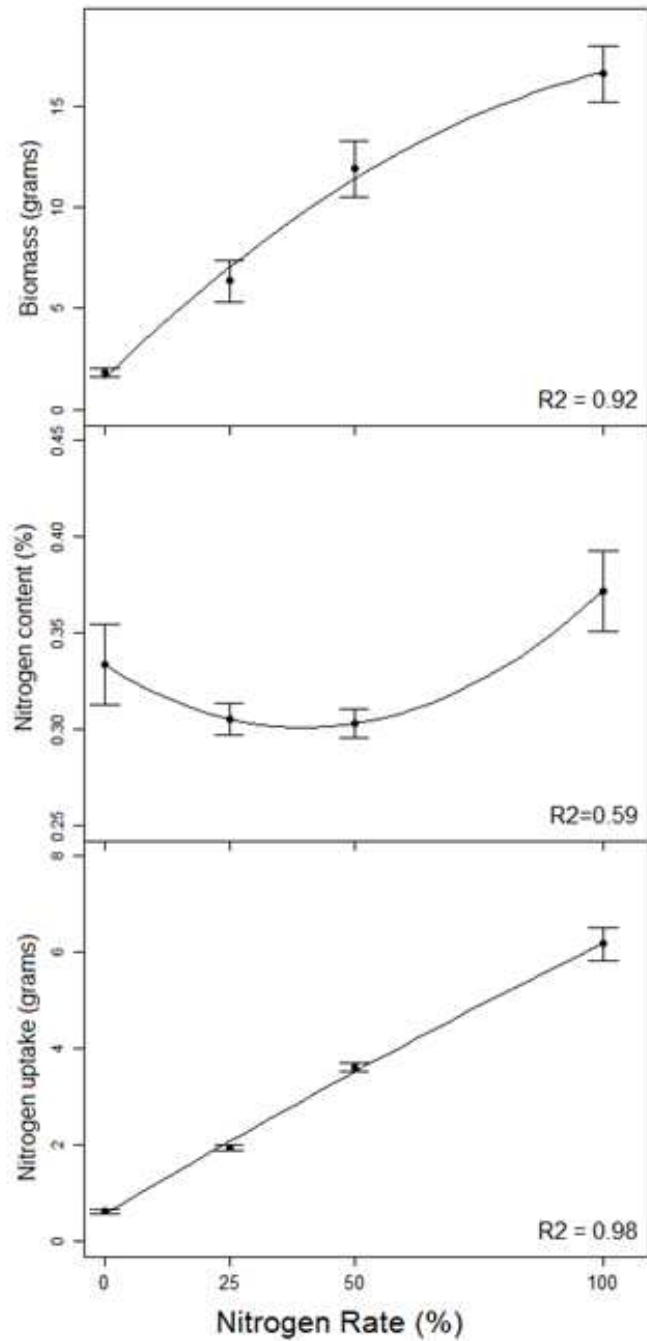
Figure 1.1 Dried maize aboveground biomass, N content, and N uptake as a function of different N rates based on a modified Hoagland Solution. Samples were collected 36 days after emergence (V6 growth stage). Error bars represent confidence intervals (α = 0.05) and fitted curves are quadratic polynomial. Coefficients of determination (R2) are indicated in each graph.

Potassium experiment

At the V6 growth stage of maize, the different rates of K imposed on the plants did not generate variability in dried maize biomass. The dried biomass values ranged from 14 to 16 grams per plot (Fig. 1.2). The K content of the 0% K treatment was significantly lower ($\alpha = 0.05$) than the 25 and 100% K treatments, but not different from the 50% K treatment that showed a wider confidence interval. Treatments 25%, 50% and 100% K were not significantly different from each other at the V6 growth stage of maize. For K uptake, treatments 0%, 25% and 100% K were significantly different ($\alpha = 0.05$) from each other, but treatment 50% was not significantly different from the others.

For K experiment, different rates of potassium at V6 growth stage did not have a significant effect on dried biomass, resulting in weights similar to Hoagland solution (100% treatment). On the other hand, the K treatments generated variability in K content, but only with the extreme treatments (i.e. 0% and 100% treatments). Confidence interval for the 25% and 50% K treatments were wide, resulting in a low coefficient of determination ($R^2 = 0.28$). Similar results were observed for K uptake, resulting in a significant difference for 0%, 25% and 100% K treatments, and ranging from an average of 6 milligrams per pot of K for treatment 0% to 15 grams of K per pot for treatment 100%. Although K uptake significantly differed among three of the four treatments, coefficient of determination were low, but slightly higher than that for the K content ($R^2 = 0.35$). This can possibly be explained by the wide confidence interval of the 25% and 50% treatments (Fig. 1.2).
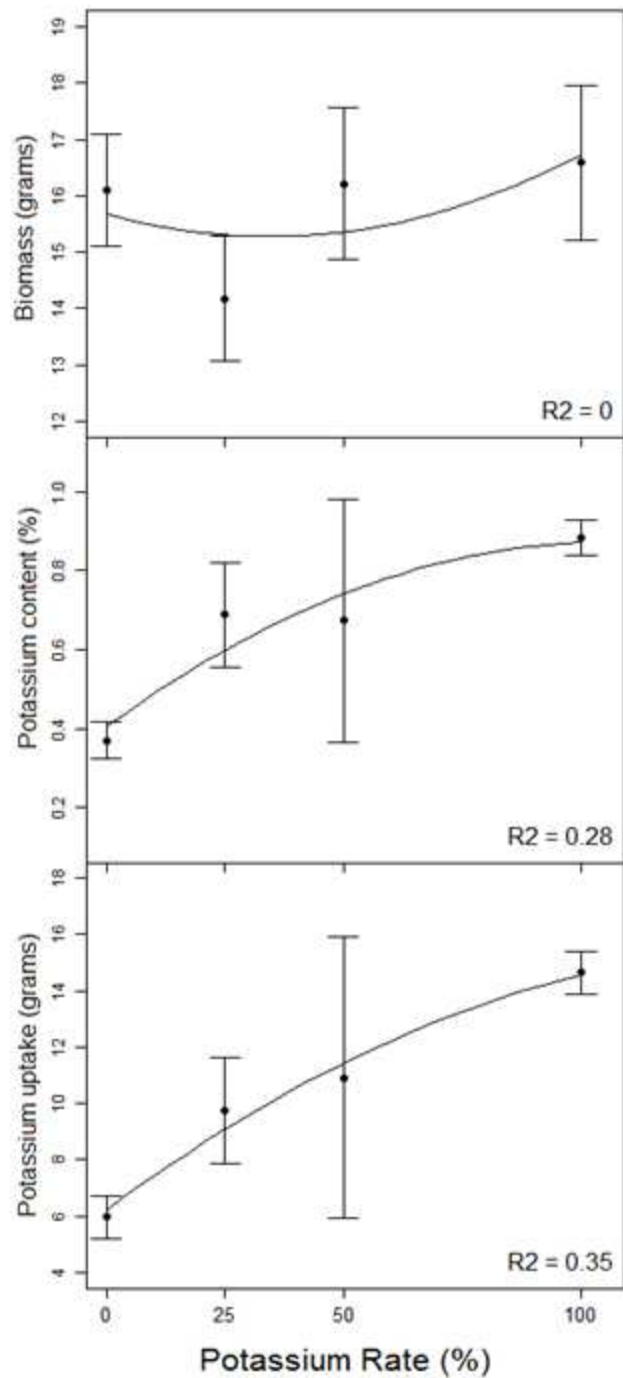
Figure 1.2 Dried Maize biomass, K content, and K uptake as a function of different K rates based on a modified Hoagland Solution. Samples were collected 36 days after emergence (V6 growth stage). Error bars represent confidence intervals ($\alpha = 0.05$) and fitted curves are quadratic polynomial. Coefficients of determination (R^2) are indicated in each graph.

Differences between N and K

Dried biomass values indicate that maize growth up to V6 growth stage was more influenced by N content than K content. Variability caused by N treatments generated a wide range of aboveground biomass, while K treatments and Hoagland solution (100% treatment) generated similar biomass values.

For the nutrient content measurements, study results indicated that K experiment generated a higher variability, ranging from an average of 0.4 to 0.8 grams of K per pot and treatments 0%, 25% and 100% K being significantly different ($\alpha = 0.05$) from each other. The N content varied from 0.32 to 0.37 grams of N per pot (fig. 1.2).

For the N experiment, the N uptake of all the treatments were significantly different ($\alpha = 0.05$) from each other, while for the K experiment, only treatments 0%, 25% and 100% were different. It appeared that at the V6 growth stage, different N rates had higher effect on N uptake than the K rates affected the K uptake. It was observed that the N uptake variability resulted from biomass weight, showing plant growth deficiency, whereas K uptake variability resulted from the plant K content. Benders et al. (2012) reported that by the V6 growth stage, N uptake accumulation represents around 20% of the total N requirement for the entire life-cycle of the maize plant, and only 10% of the total K requirement.

Characterizing nutrient uptake using fluoro-sensing

Fluoro-sensing and nitrogen uptake

The seven fluorescence indices in this study successfully estimated N uptake prior to the 18th DAE (V3 growth stage). For the seven indices, the lowest R^2 was 0.84 (from an average of the 12 reading dates). Three indices (NBI_B, NBI_R and CHL) consistently enabled the detection of N variability in maize canopy throughout the growth stages [all treatments were

significantly different ($\alpha = 0.05$) from each other prior to V2]. Based on the highest scores of coefficient of determination (R^2) and the lowest root mean square error (RMSE), the fluorescence indices NBI_B, NBI_R and CHL resulted in the highest correlation with N uptake. Regression analysis was used to model the relationship between N uptake and the three indices for the four different growth stages (Fig. 1.3).

The nitrogen balance index induced by red light (NBI_R) performed the best throughout all the reading dates when compared to the six other indices. Over the 12 reading dates, between V2 to V6 growth stages, the lowest coefficient of determination (R^2) for the relationship between N uptake and any NBI_R was 0.90, with an average value of 0.94 across all the 12 reading dates. The nitrogen balance index induced by blue light (NBI_B) provided similar detection capacity for N uptake in maize at early growth stages. For all 12 readings between V2 and V6 growth stages, the average coefficient of determination (R^2) for the relationship between the index and N uptake was 0.94 with the lowest score at V4 stage ($R^2 = 0.89$), indicating a very strong correlation between both indices and maize N uptake.

The Chlorophyll fluorescence index (CHL) efficiently enabled the detection of N uptake for maize at early stages as well. The CHL index generated the lowest coefficient of determination ($R^2 = 0.37$) at the 15th DAE reading date. Besides this reading date, all other readings had good relationship with N uptake. The average R^2 for the relationship between CHL and N uptake was 0.93 for the other 11 reading dates and the lowest R^2 was 0.86.

Figure 1.3 Fluorescence indices as a function of N uptake generated by different nutrient solutions with N variability in corn at 4 different growth stages under greenhouse condition. The V3 refers to the 18th day after emergence (DAE) and V4, V5, and V6 refers to the growth stages at the 26th, 35th and 38th DAE respectively. Bars represent confidence intervals ($\alpha = 0.05$) and fitted curves are quadratic polynomial with their respective coefficient of determination (R^2). The precision of the estimates for each index and dates are expressed by root mean square error (RSME).

In general, NBI_B and NBI_R indices had similar performance with slightly higher R^2 than that of the CHL index. These findings are consistent with Longchamps and Khosla (2014) who also detected N variability prior to V4 between the zero and the full N rate treatments. They also observed that NBI_B could detect variability of all treatments prior to V5 growth stage of maize. For the current study, the improvement observed in distinguishing different N rates at earlier crop growth stages as compared to the results reported by Longchamps and Khosla (2014) may be attributed to many reason (i) the higher level of control and the precision with which the current study was conducted, i.e., precision in the amounts of nutrients applied and (ii) the silica sand based sterile systems for growth of maize plants with no residual nutrients compared to field soil used in planting-pots by Laongchamps and Khosla (2014).

Root mean square error (RMSE) was used (i) to compare the error of N uptake to the N uptake predicted by the indices and (ii) to understand the precision of the characterization of each index (Table 1.3). The NBI_R index showed the most precise prediction at V5 growth stage with a RMSE of 10. However, the NBI_B was more consistent in predicting N uptake (i.e. averaging at 0.47 for all the reading dates) than NBI_R (i.e. an average of 0.54 for all reading dates). Both NBI indices showed better estimation than CHL index. Both NBI indices are induced by UV light, while CHL is only induced by red light. Studies in grape vines have shown that plants grown in greenhouse condition and brought to the field had reduction of chlorophyll fluorescence induced by UV-A and UV-B light over time, while chlorophyll fluorescence induced by blue-green light were not affected by the environmental change (Kolb et al., 2001). In the field, those plants created a protection against UV radiation that can also screens out the UV emission from the sensor (Kolb et al., 2001). Theoretically, this can be a concern for field trial

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when analyzing indices induced by UV light, such as NBI_R and NBI_B, but not as much to the

CHL index that is induced by visible wavebands.

Table 1.3 Precision of estimate (RMSE) and coefficient of determination (R^2) measured for nitrogen uptake as a function of various fluorescence indices.

	Nitrogen uptake as a function of[††]			DAE[†]	Growth stage	Date
	NBI_B	CHL	NBI_R			
R^2	0.98	0.93	0.98	10	V2	11/12/2012
RMSE	0.31	0.54	0.26			
R^2	0.93	0.89	0.91	13	V2	11/15/2012
RMSE	0.55	0.67	0.60			
R^2	0.93	0.37	0.97	15	V3	11/17/2012
RMSE	0.55	1.66	0.39			
R^2	0.98	0.96	0.97	18	V3	11/20/2012
RMSE	0.30	0.40	0.35			
R^2	0.92	0.90	0.92	24	V4	11/26/2012
RMSE	0.56	0.64	0.57			
R^2	0.96	0.98	0.91	26	V4	11/28/2012
RMSE	0.41	0.32	0.66			
R^2	0.89	0.99	0.90	28	V4	11/30/2012
RMSE	0.63	0.07	0.61			
R^2	0.93	0.92	0.90	31	V5	12/3/2012
RMSE	0.56	0.59	0.65			
R^2	0.93	0.91	0.95	33	V5	12/5/2012
RMSE	0.54	0.64	0.45			
R^2	0.99	0.86	0.99	35	V5	12/7/2012
RMSE	0.18	0.76	0.10			
R^2	0.96	0.98	0.95	38	V6	12/10/2012
RMSE	0.44	0.32	0.50			
R^2	0.96	0.93	0.97	41	V6	12/13/2012
RMSE	0.45	0.59	0.39			

† DAE: days after emergence of the plant.
†† All curve fitting were significant at p < 0.0001.

Fluoro-sensing and potassium uptake

The estimation of K uptake by the nitrogen balance index induced by blue light (NBI_B) had a low coefficient of determination (R^2) for all reading dates. At 28 DAE (V4 growth stage) the relationship between NBI_B and K uptake had the highest R^2 of 0.17 (Fig. 1.4). This reading

was just before the second application of the fertilizer solution, and possibly when K uptake differences among the treatments were the most pronounced. The NBI_B readings ranged from 0.8 to 1.4, with small variation between treatments. Figure 1.4 shows that the other treatment dates had notably higher reading values than reading values acquired at the V4 growth stage, which may indicate that all treatments sufficiently supplied with potassium. The NBI_B values acquired from plants treated with different levels of K were similar to NBI_B values acquired from plants treated with full Hoagland solution (100% treatment).

The NBI_R had similar performance as the NBI_B for K uptake prediction. The best coefficient of determination ($R^2 = 0.34$) based on the relationship between NBI_R and K uptake was obtained on the 28th DAE (V4 growth stage; Fig. 1.4). In general, the variability in NBI_R readings was low, ranging from 2.0 to 3.5. Readings acquired at the V4 growth stage had the best results over the other reading events, but coefficient of determination was still low ($R^2 = 0.31$) for detecting K variability at early stage of maize.

For the chlorophyll fluorescence index (CHL), readings varied from 3.5 to 4.0. Only readings acquired at V4 growth stage presented relationship between CHL and K uptake, although the coefficient of determination was too low ($R^2 = 0.18$) to claim that the index was a good tool for detecting K variability in maize.
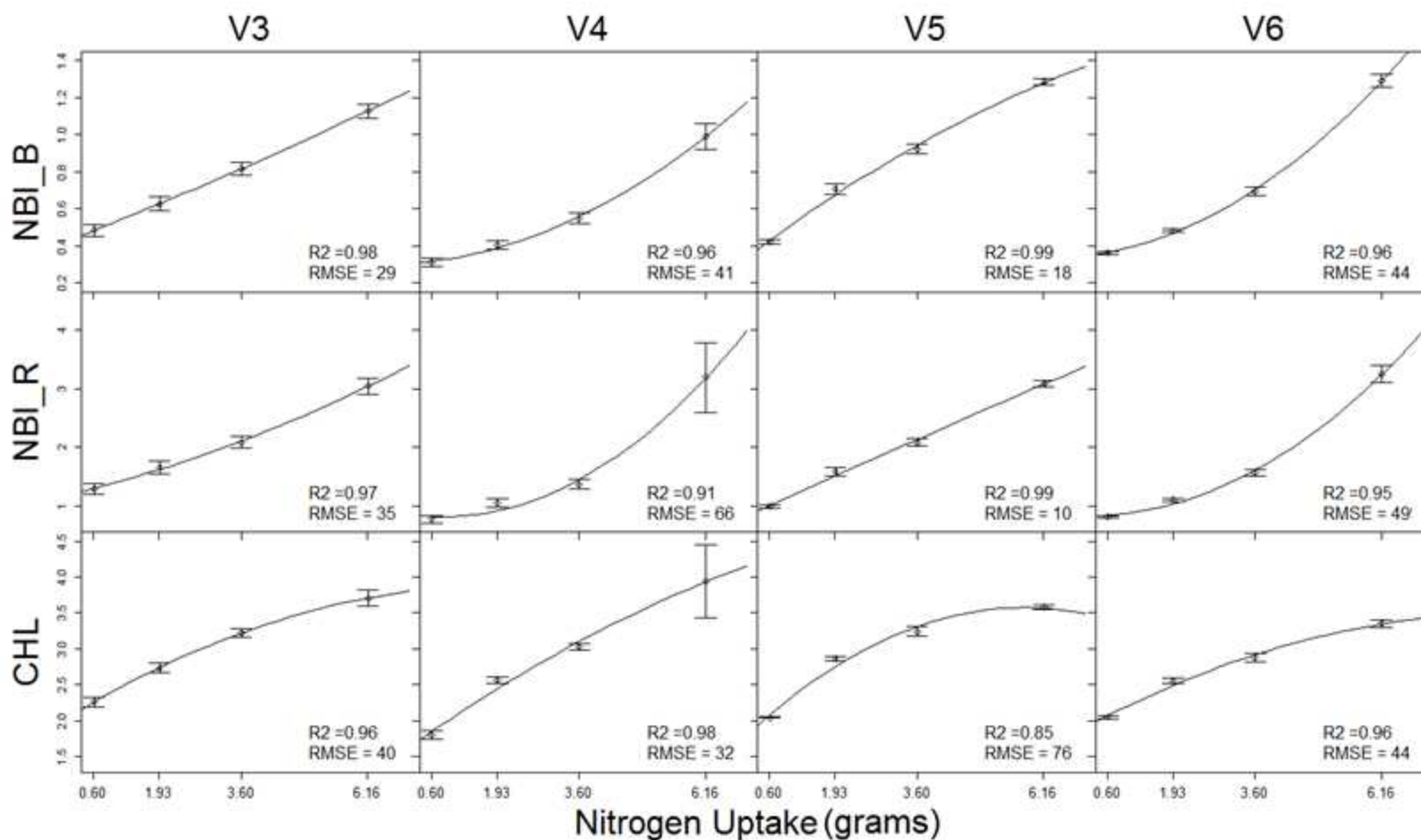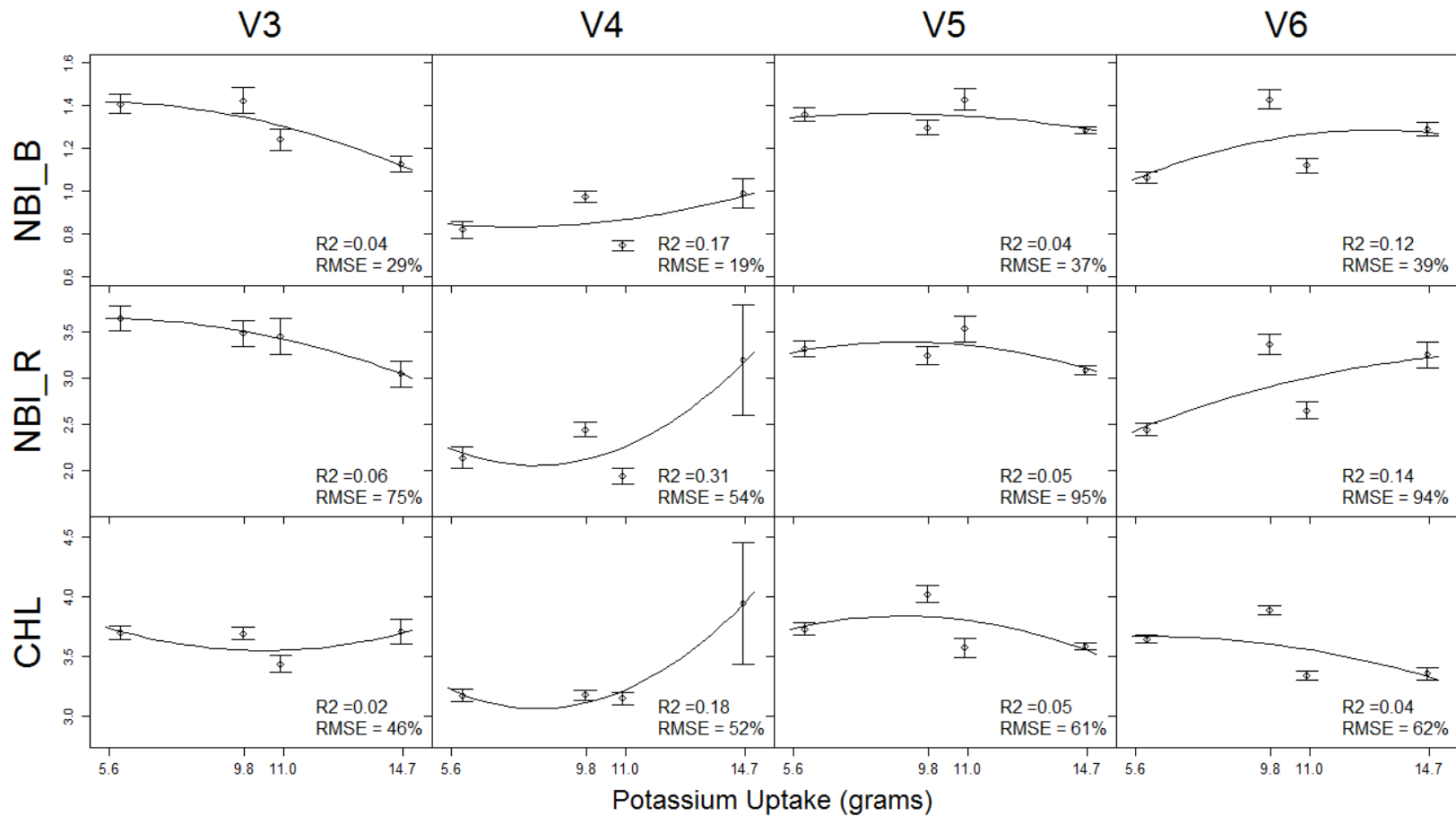
Figure 1.4 Fluorescence indices as a function of K uptake generated by different nutrient solutions with K variability in corn at 4 different growth stages under greenhouse condition. The V3 refers to the 18th day after emergence (DAE) and V4, V5, and V6 refers to the 26th, 35th and 38th DAE respectively. Bars represent the confidence intervals ($\alpha = 0.05$) and fitted curves are quadratic polynomial with their respective coefficient of determination (R^2) and the precision of the estimates for each index and dates are expressed by root mean square error (RSME).

The variability of the readings from all three indices was low, which can be explained by the low variability induced by the K treatments. Even the 0% K treatment did not create considerably lower amounts of K uptake at 28 DAE. The NBI_B index ranged from 0.4 to 1.4 for the N uptake while for the K uptake it ranged from 1.2 to 1.4. The NBI_R index ranged from 0.7 to 3.2 for the N uptake while for the K uptake it ranged from 1.9 to 3.2. The CHL index ranged from 1.7 to 3.8 for the N uptake while for the K uptake it ranged from 3.5 to 4.0. Comparing the variability in indices values between the two experiments, it appeared that the N experiment generated higher variability in the fluorescence readings. The K experiment generated fluorescence readings in the same range as readings acquired from plant treated with full Hoagland solution. As we could not create low amount of K uptake at early maize growth stage, we cannot conclude that those indices are good estimator to detect K uptake using Multiplex fluorescence sensor.

The different amounts of K applied for each treatment did not significantly affect maize biomass, but did affect K uptake, where 0%, 25% and 100% K were significantly different from one another. Nevertheless, K uptake variability was not detected by the fluorescence indices. The indices studied in this research have been cited showing good correlation with chlorophyll content and other molecules that contain N (Cerovic, 2002), while for other nutrients deficiency there is no specific index. This could be the reason that the indices used in this study perhaps have not been able to detect potassium variability among the treatments.

The absence of a significant effect of K treatments on fluorescence indices clearly indicate that low values of fluorescence indices prior to V6 is related to low maize N uptake but not to K uptake. Therefore, measurements taken by the Multiplex® sensor prior to V6 can detect N variability in maize independently of the K availability in the growth medium.

CONCLUSION

Chlorophyll fluorescence sensor enabled the detection of N variability in maize under greenhouse condition prior to V2 growth stage. The indices NBI_B and NBI_R were the most accurate in characterizing the different N rates throughout the growth stages (from V2 to V6).

Even though different rates of K have generated K uptake variability, the indices used were not able to detect that variability in maize at any growth stage. Different indices that are more specific for potassium deficiency might be able to characterize K uptake in maize at early growth stages. Also, it is possible to conclude that K deficiency might not interfere on N uptake characterization by the indices at early growth stages, as the indices showed low variability across K treatments.

Findings of this study result are promising for field studies, as this sensor was able to detect N variability in maize at earlier growth stages than any other commercially available remote sensor reported in literature. Testing the applicability of fluorescence sensor on-the-go, for mobile measurements *in situ* can potentially make this sensor an excellent tool for maize precision N fertilization management.

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CHAPTER 2

EARLY IDENTIFICATION OF NITROGEN VARIABILITY IN MAIZE BY AN *IN SITU*

FLUORESCENCE SENSOR

SUMMARY CHAPTER 2

The idea of a plant sensor that can characterize nutrient variability in situ has been aimed for decades. In order to apply the right amount of fertilizer at every location of the field, knowing nutrient variability across the field is key information. Fluorescence sensors have been shown to be good indicators of nitrogen status for maize under greenhouse conditions, but few field experiments have been conducted to assess their potential. The objectives of this study were (1) to evaluate the effect of different rates of N over the plant health related to plant N nutrition, and (2) to verify if induced fluorescence can detect N variability in maize at early growth stages under field conditions. This study was conducted over three site-years between 2012 and 2013 in northern Colorado. At each site, treatments consisted of various amounts of N (applied as Urea Ammonium Nitrate 32%) fertilization. Sensors used in this study were the Multiplex® 3 for fluorescence sensing and the GreenSeeker® for reflectance sensing. Those measurements were correlated with aboveground biomass, N content, and N uptake collected at two different dates (corresponding to the V6 and V9 maize growth stage). The aboveground biomass, N content, N uptake, yield and the sensors readings were analyzed using ANOVA and Tukey's HSD to test differences among nutrient rates. Also, a regression tree between N uptake and the fluorescence indices was fitted. Results of this study showed that grain yield responded to the N application

until it reached a plateau. The N rates had no effect on aboveground biomass, N content, and N uptake for both sampled dates. Fluorescence indices could not detect N variability in maize at early stage. Further studies may be required to draw conclusions, as for most of the N treatment, plants were sufficient in N and drought stress may have interfered on plant growth and yield.

INTRODUCTION

Maize (*Zea mays* L.), one of the most important cereal crop to achieve global food security, is responsible for a large proportion of world fertilizer consumption. Heffer (2009) pointed that maize production accounted for 16.8% of global nitrogen (N) use, while maize, wheat (*Triticum spp.*) and rice (*Oryza spp.*) together were responsible for 49.7% of global N use in 2007-2008. Over the last decades, maize breeders have targeted high yield as the most desirable trait. Improved genetics capable of reaching higher yield along with higher N fertilization led to highest crop productivity in history. In other words, maize has been bred for yield optimization along with fertilizations in high amounts. Erisman et al. (2008) estimated that almost half of the world's population food relies on inorganic N fertilizers.

Producing a crop with lower amounts of fertilizers, water and energy has been characterized as the key point for sustainable agriculture. In that matter, spatial management using on-the-go crop sensors can potentially reduce the amount of natural resources required for plant growth, making a more environmentally and cost efficient system. These sensors can contribute to a better understanding of the field spatial variability, leading to a more precise variable-rate management of inputs (e.g. water and fertilizers).

Variable-rate fertilization stands as a potent way to avoid waste and increase nutrient use efficiency. While enough nutrients should be made available to the crop to attain the yield potential of the recent varieties that require high amount of nutrients, unneeded fertilizer applications should be avoided. Cassman et al. (2002) reported that only 30-50% of the world's N applications are recovered by the plants, which means that considerable amounts of N could be conserved. This translates in a less cost efficient product, increase in energy consumed to produce food and higher environmental pollution (Ladha et al. 2005). It is important to consider that N losses cannot be avoided completely, but Gupta and Khosla (2012) pointed that an increase of 10% in Nitrogen Use Efficiency (NUE) for maize, wheat and rice crops could save US$ 5 billion per year based on 2012 N fertilizer price of US$ 1,000 per metric ton.

The NUE can be defined by the equation: NUE = [(total cereal N removed from harvest) - (N coming from the soil + N deposited in the rainfall)] / (fertilizer N applied to cereal) (Raun and Johnson, 1999). This definition has been studied to understand not only the maximum yield that a plant can produce, but also how efficient the plant is with the inputs applied in different environments. This approach can account for total yield, as well as for the cost efficacy of the grain. Lately, input prices have been rising annually and accounting for NUE is gaining interest among farmers.

New tools and techniques are available to help farmers characterize and manage the spatial variability of their field for better cost efficient crop production. A category of tools that has gains popularity among farmers include reflectance based sensors. In the last three decades, different approaches of reflectance sensors based on remote and proximal platform have been used to monitor crop growth (Bauer, 1975; Walburg et al., 1982) and understand N variability in maize with successful field trials (Shaver et al., 2011) and commercial usage. Variation in plant

pigmentation can be caused by many factors, but nutrient stress is generally the primary

consideration (Schepers et al., 1996). Also, Blackmer et al. (1996) concluded that there are

relatively strong correlations between light reflectance readings and relative grain yield,

chlorophyll content and leaf N content, which indicate that reflectance procedures are promising

for assessing crop N status. Different wavebands and indices have been tested. Commercially,

the most tested and used index is the Normalized Difference Vegetation Index (NDVI) (Guyot,

1988), which has shown good correlation with aboveground biomass and N status. The index is

calculated by the following equation: (NIR-RED) / (NIR+RED) (Rouse et al., 1973), where NIR

is the Near Infrared wavelength. Shaver et al. (2011) observed that NDVI showed positive

correlation with N applied in Colorado soils and optimal correlations were made when plants

were between V12 to V14 maize growth stage. Best management practices advices farmers to

apply nitrogen before V6 growth stage, for which NDVI index may not be a reliable measure for

farmers to characterize maize N variability at early growth stages.

Another remote sensing approach that has shown strong relationship with plant N status

is chlorophyll fluorescence sensing. When a light spectrum reaches the leaf tissue, it can use

three different pathways: reflection, transmission or absorption. Fluorescence is part of the

absorption pathway. Leaf pigments absorb photons from a light source that are involved in

photosynthesis and other photochemical processes (McMurtrey III et al., 1994). Photons that

were absorbed by fluorescent pigments can be reemitted as fluorescence at longer wavebands.

While only a small portion of the absorbed photon is reemitted as chlorophyll fluorescence, most

of the light absorbed will generate heat and produce energy by photochemical processes

(Chappelle et al., 1985; Stober and Lichtenthaler, 1993; Buschmann et al., 2001). Chlorophyll

fluorescence emission is inversely related to plant photosynthesis efficiency, and the intensity of

UV induced chlorophyll fluorescence signal has an inverse relationship with UV-absorbing compounds (Burchard et al., 2000; Bilger et al., 2001).

The idea of a mobile fluorescence sensor has been discussed lately utilizing a sensor called Multiplex®3 (Force-A, Orsay, France). Longchamps and Khosla (2014) showed that this sensor has the capacity to differentiate maize N status under greenhouse condition prior to V5 growth stage. Agati et al. (2013) were able to discriminate different N rates of soil fertilization for two different turf grass varieties and they found linear correlation between fluorescence indices and leaf N content. While the potential of this sensor has been proven in greenhouse conditions, it's capability to discriminate N status in maize under field conditions and in motion has not been tested. The hypothesis of this study was that maize N variability may be detected by an active fluorescence sensor prior to V6 maize growth stage. The specific objectives were (1) to evaluate the effect of different rates of N over the physiological factors related to plant N nutrition, and (2) to verify if induced fluorescence can detect N variability in maize at early growth stages.

MATERIAL AND METHODS

Study site

The experiment consisted of three site-years. The first site was set over two consecutive years and the second site over one year. The first site was located at Colorado State University's Agricultural Research Development & Education Center (ARDEC) on field number 3100 (40°39'57.4"N, 104°59'53.1"W). The experiment at this site was performed over two years, notably 2012 and 2013. These two site-years will be referred to as ARDEC12 and ARDEC13

respectively for the scope of this paper. The second site was located in the city of Iliff, Colorado (40°46'05.2"N, 103°02'32.7"W) during agricultural season 2012 and will be referred to as Iliff.

Soil at ARDEC site is classified as Kim loam (Fine-loamy, mixed, active, calcareous, mesic Ustic Torriorthents) and Nunn clay loam (Fine, smectitic, mesic Aridic Argiustolls) (Soil Survey Staff, 1980). At Iliff, soil is classified as Loveland clay loam (fine-loamy over sandy, mixed, superactive, calcareous, mesic Fluvaquentic Endoaquolls) and Nunn clay loam (Fine, smectitic, mesic Aridic Argiustolls) (Soil Survey Staff, 1977).

During the time of the first study year (from March to July of 2012), Colorado registered temperatures slightly above average (as compare with 20 years of climate dataset), and for 2013, temperatures were slightly below average. The 20 year average (1993-2013) annual precipitation at ARDEC was 263.5 mm. For ARDEC12, which was considered a dry agricultural season, annual precipitation average was 148.6 mm, with monthly averages considerably below historical records during measured months. The ARDEC13 site had also low rainfall events early in the cropping season, and also averaged under overall monthly average for measured dates (between June and July). Due to a high and unusual rainfall event that caused flooding in the state of Colorado in September, the year of 2013 had annual precipitation higher than historic averages with 302 mm. Precipitation and temperatures for both years at ARDEC site can be seen in Figure 2.1 and Figure 2.2. Overall, for the months when experiment was conducted, those two years had precipitations below historical averages, with ARDEC12 being slightly dryer. Historical average (2009 to 2013) annual precipitation for Iliff site is 365.6 mm. During the experimental year, rainfall was 275.59 mm (Fig. 2.3, Fig. 2.4; CoAgMet, 2014).
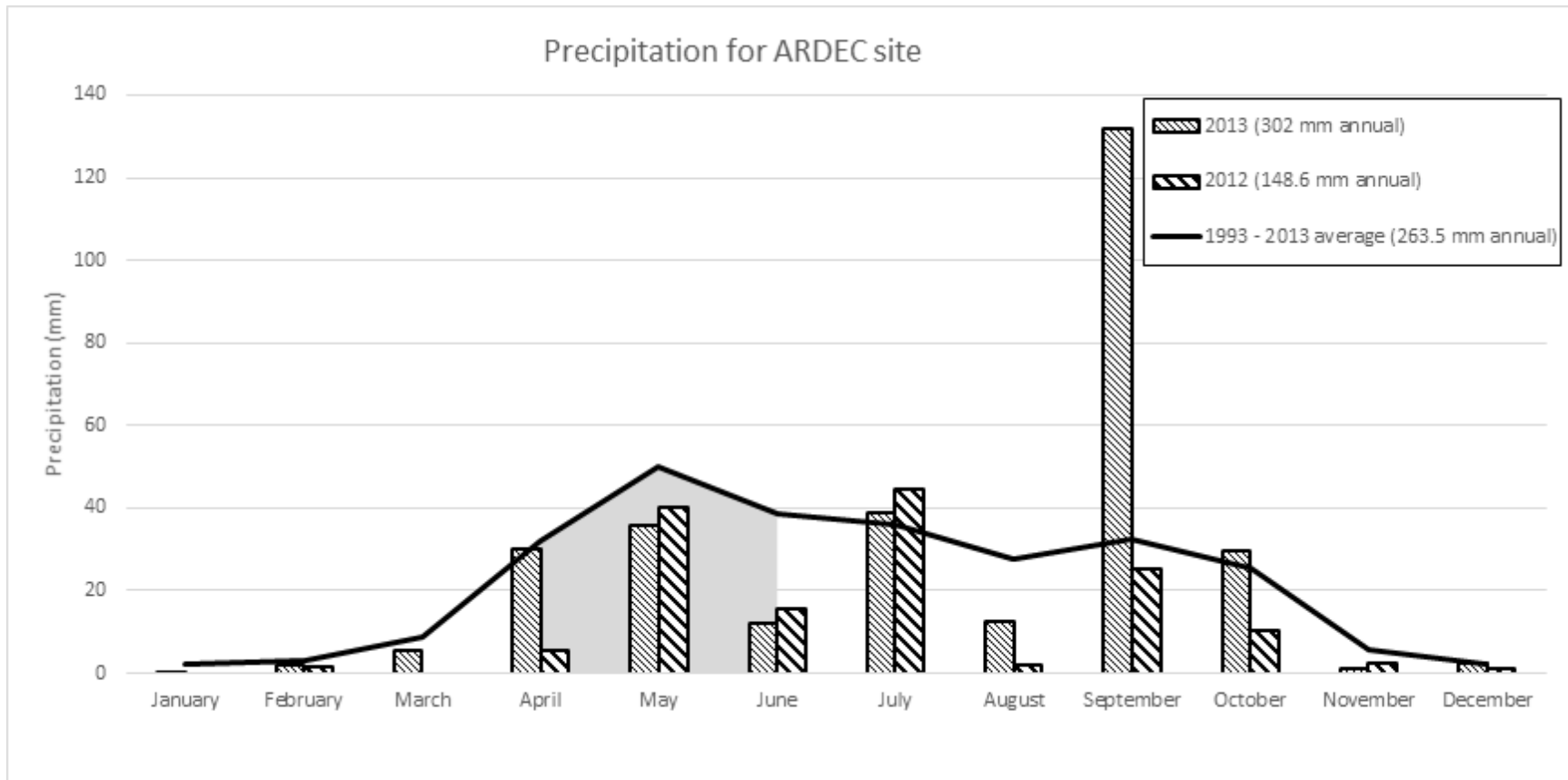
Figure 2.1 Monthly precipitation (mm) registered at ARDEC location for the year of 2012 and 2013 (bar plots). Line shows the mean of 20 years record from 1993 to 2013. Gray area represents the dates that precipitation influenced crop growth for the present study.
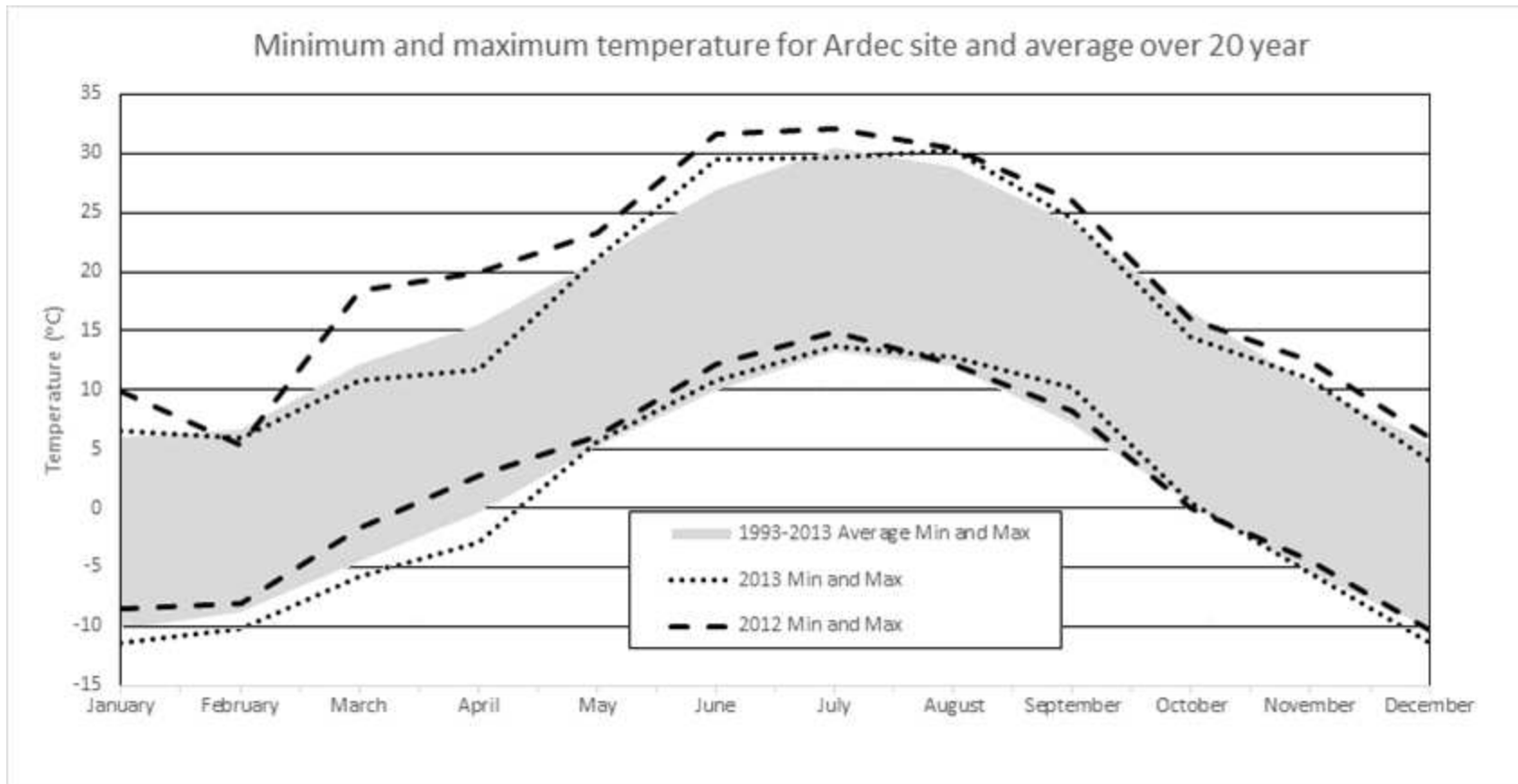
Figure 2.2 ARDEC location: Monthly minimum and maximum temperature (°C) for the years of 2012 and 2013 (lines) and mean of minimum and maximum on background (gray area) from 1993 to 2013.
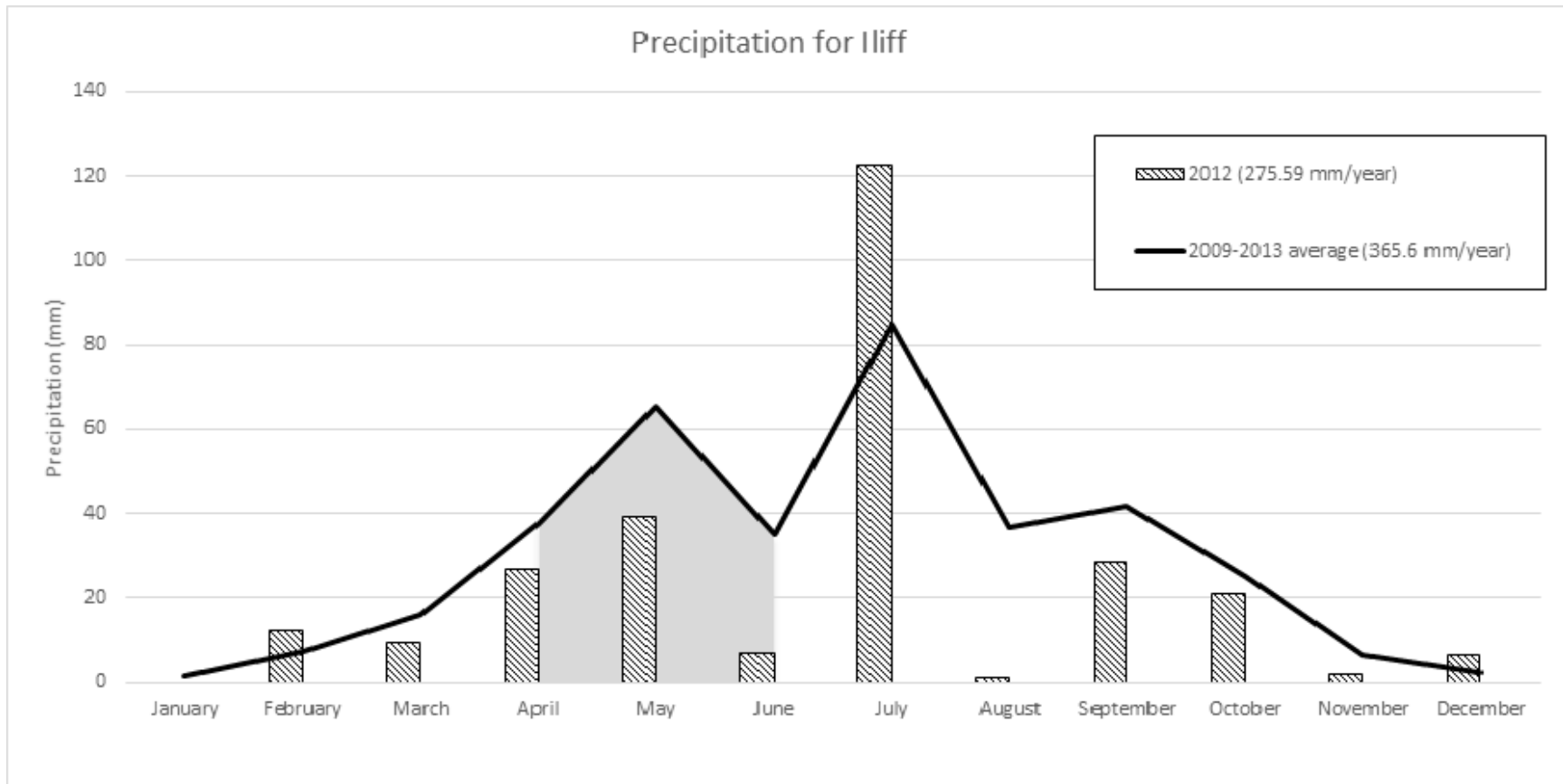
Figure 2.3 Monthly precipitation (mm) registered at Iliff location for the year of 2012 (bar plots). Line shows the mean of 5 years record from 2009 to 2013. Gray area represents the dates that precipitation influenced crop growth for the present study.
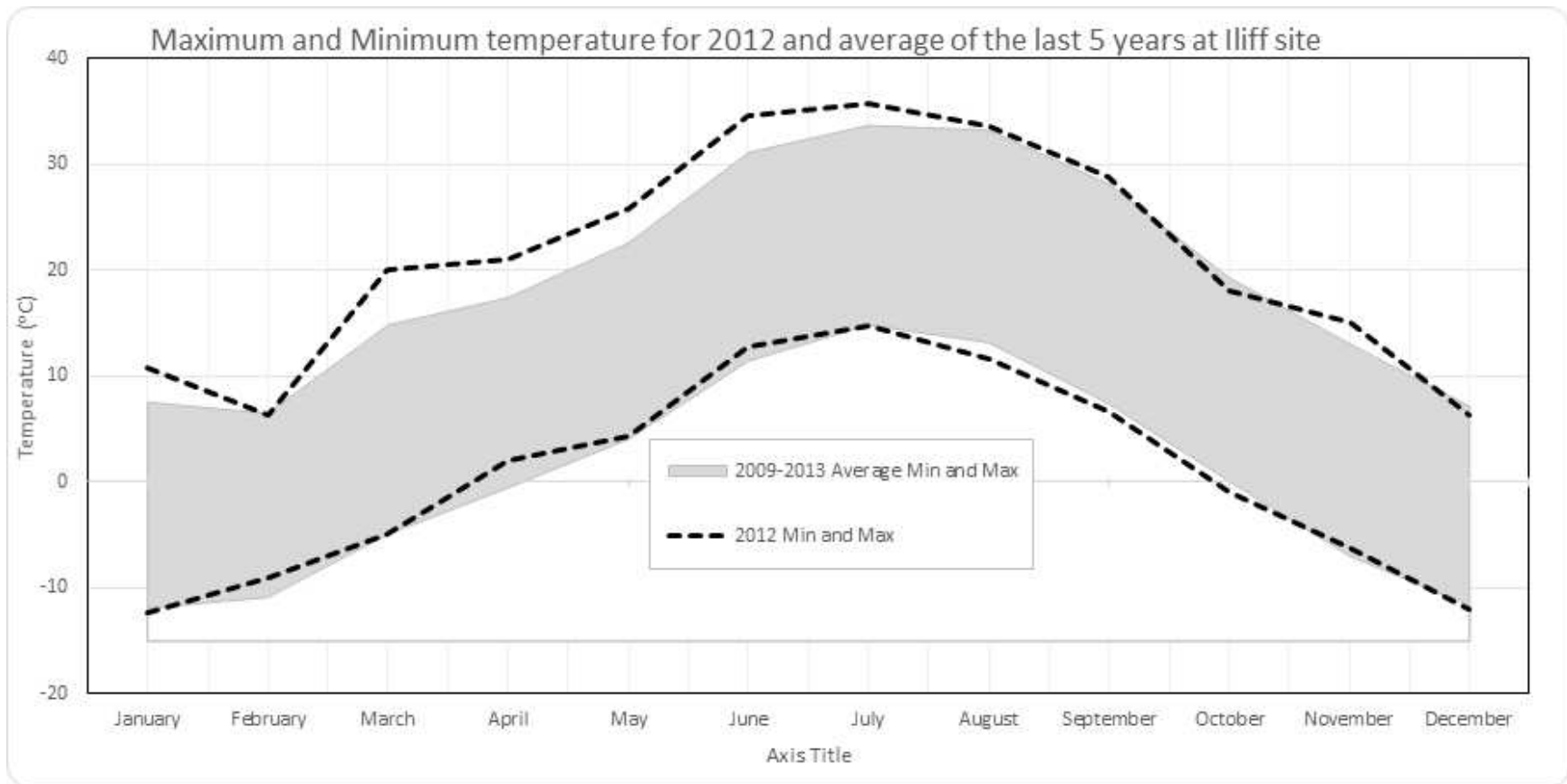
Figure 2.4 Iliff location: Monthly minimum and maximum temperature (^{o}C) for the years of 2012 (lines) and mean of minimum and maximum on background (gray area) from 2009 to 2013.

Plants and agricultural practices

During 2012, maize (*Zea mays* L.) variety Dekalb DKC45-79VT3 was planted on May 5th for ARDEC12.The variety planted at Iliff was Dekalb DKC52-59VT3 on May 14th. For 2013, maize variety Dekalb DKC46-20RIB was planted at ARDEC13 on May 16th. For ARDEC location, the planter used was a Monosem (NG+3 Series) with six row precision vacuum system, and inter-row width of 76.2 cm. Plant population rate was 81,500 and 84,000 seeds per hectare, for ARDEC12 and ARDEC13 respectively. At Iliff site, a 16 rows Case planter was used at a plant population of 84,000 seeds per hectare and an inter-row width of 76.2cm.

A systematic unaligned sampling design was used for soil sampling at all three site-years, collecting soil from 0-20 cm and 20-60 cm deep at each sampling location. Each sample consisted of five cores collected randomly within a 2.0 m radius around each geo-referenced location. At ARDEC12, 84 soil samples (density of 8.67 samples ha^{-1}) were collected at two depths from April 8th to 14th, while for Iliff site, 20 soil samples (density of 33.94 samples ha^{-1}) at two depths were collected on April 30th. For ARDEC13, 27 soil samples (density of 25.71 samples ha^{-1}) were collected at two depths on May 25th. Soil samples were air dried, and sent to a laboratory for chemical and physical analysis. In 2012, soil samples were sent for routine analysis to Agvise laboratories (Benson, MN), while 2013 samples were sent to Sevi-tech laboratories (Hastin, NE). Minimum, maximum and average of chemical and physical properties analyzed in each site are shown in Table 2.1.

Table 2.1 Samples collected at 0-20 and 20-60 cm soil depth with the maximum, minimum and mean values for Sand, Silt, Clay, pH, Nitrogen, Phosphorus, Potassium, Calcium, Magnesium and Organic matter for all three site-years

| Site | Depth cm | Descriptive statistics | Sand | Silt | Clay | pH | N (Nitrate) | P (Olsen) | K | Ca | Mg | OM |
			------------- % ------------				--------------------------------- ppm ---------------------					%
ARDEC13	0-20	min.	42.50	15.00	30.00	8.00	2.00	26.00	214.00	4363.00	530.00	1.20
		mean	47.84	20.26	31.90	8.28	4.52	52.52	314.07	4656.97	616.03	1.49
		max.	52.50	25.00	37.50	8.40	17.00	193.00	617.00	5001.00	724.00	2.20
	20-60	min.	37.50	17.50	30.00	8.00	1.00	9.00	156.00	4463.00	584.00	0.70
		mean	43.10	22.84	34.05	8.23	6.17	16.55	195.52	4864.55	653.72	0.97
		max.	47.50	27.50	37.50	8.40	41.00	42.00	338.00	5322.00	758.00	1.50
ARDEC12	0-20	min.	47.00	8.00	25.00	7.80	4.00	5.00	238.00	4362.00	554.00	1.00
		mean	52.88	14.48	32.65	8.04	13.54	19.15	317.72	4992.89	630.63	1.76
		max.	63.00	20.00	35.00	8.20	38.50	73.00	496.00	5428.00	736.00	2.20
	20-60	min.	39.00	12.00	29.00	7.90	1.50	2.00	162.00	4300.00	471.00	0.80
		mean	47.13	17.84	35.02	8.12	8.83	8.94	245.02	5117.48	646.68	1.41
		max.	57.00	30.00	39.00	8.30	32.50	51.00	377.00	5492.00	747.00	1.90
Iliff	0-20	min.	39.00	20.00	33.00	7.80	11.50	14.00	597.00	3530.00	724.00	3.00
		mean	42.00	22.70	35.30	8.09	15.60	19.35	671.00	4190.15	767.75	3.40
		max.	45.00	24.00	37.00	8.20	27.00	29.00	745.00	4488.00	838.00	3.70
	20-60	min.	29.00	20.00	31.00	7.90	3.50	6.00	433.00	4346.00	670.00	2.20
		mean	35.60	24.20	40.20	8.05	6.90	13.95	499.40	4956.00	931.40	2.85
		max.	47.00	26.00	47.00	8.30	10.50	18.00	597.00	5384.00	1077.00	3.30

Soil analysis, biomass sampling and yield

For ARDEC and Iliff sites, soil samples were analyzed for pH (1:1; soil to water), organic matter content (loss on ignition), nitrate-nitrogen content (0.01 M KCl analyzed by Cd reduction), phosphorus content (Olsen - Bicarbonate phosphorus test), potassium, calcium, and magnesium content via (Ammonium Acetate Exchangeable using inductively coupled plasma), and sand, silt and clay content (Particle-size distribution was determined by using the hydrometer method).

Aboveground biomass samples were harvested from one linear meter in length randomly selected within each plot from which all plants were harvested. Paper bags were used to store the plants which were immediately sent to a drier maintained at 60^oC. After air drying, plants were weighted and ground and were sent for tissue analysis at Harris Laboratories, Lincoln, NE. Analysis of total N content (%) was performed using the Kjeldahl digestion method (wet digestion in H_2SO_4-H_2O_2). Biomass samples were collected at ARDEC12 on June 26th (41 DAP; days after planted) when plants were at V6 growth stage and on July 10th (55 DAP) when plant were at V9 growth stage. For ARDEC13, biomass samples were collected on June 22nd when plants were at V9 growth stage. Biomass samples were collected at Iliff site on June 25th and July 11th, at V6 and V9 growth stages respectively.

Grain harvest was performed by hand. For all three site-years, yield samples consisted of 10 samples collected in a regular pattern within each plot. Each sample consisted of harvesting one linear meter on the crop row. After separating kernels from the cob, weight and grain moisture were measured. Further, all grain weights were normalized at 15.5% moisture content and converted to metric ton per hectares.

Nitrogen treatments

At all three site-years, N treatments were imposed to the crop in a completely randomized design. For both years at ARDEC location, 5 treatments and 12 replicates were laid out in plots of 6 rows wide (4.57 m) by 6 m long (27.42 m^2). For both ARDEC12 and ARDEC13, N rates applied were 0, 56, 112, 168 and 224 kg ha^{-1} of UAN 32% (urea and ammonium nitrate; 32-0-0). At Iliff site, 4 N treatments and 6 replicates were laid out in study plots that were 6 rows wide (4.57 m) by 12 m long (54.8 m^2). Nitrogen rates applied at Iliff site were 0, 34, 67, 101, 135 and 168 kg ha^{-1} of UAN 32%.

Plant height and growth stage

Plant height and growth stage were measured between V3 and V8 growth stages. For ARDEC12, measurements were done on 44, 47, 51, 54, 58 and 65 DAP. For Iliff, six measurements were acquired on the following dates: 30, 35, 42, 45, 49 and 58 DAP. For ARDEC13, measurements were done on 19, 27, 29, 31, 33 and 38 DAP.

Crop sensing measurements were done between 11:00 to 14:00hrs on each date. Ten plants selected in a regular pattern along the third row (center row) of each study plot was marked at its base using "zip ties" and inspected weekly for height and growth stage. Height measurements were taken with a ruler from the ground to the tip of the highest leaf lifted up by hand. Growth stage considers the number of leaves (excluding the cotyledon leaf) that are not within the whorl and that are fully expanded with visible leaf collar (e.g. maize plants with two expended leaf with visible collar was marked as being in the V2 growth stage).

Reflectance sensor

The GreenSeeker® (Trimble, USA) sensor was used for the acquisition of NDVI index data. This active optical sensor generates red and near infrared light through light emitting diodes (LED) and measures the amount of light reflected by the plant with a photodiode. This sensor was used to acquire NDVI data on six dates at each site-year. Measurements were taken from a distance of 0.8 m from the plant canopy. At ARDEC locations it took an average of 10 to 12 seconds to record data from each plot and 20 to 25 seconds at Iliff. The GreenSeeker® sensor measures the data at a frequency of 10 hertz. At ARDEC, 100-120 readings were acquired within each plot, while for Iliff an average of 250 readings were acquired within each plot.

Fluorescence sensor

The Multiplex®3 (FORCE-A) was used to collect maize fluorescence information. This active hand-held fluorescence sensor allow for *in situ* measurements under daylight. Principle behind this sensor is based on the chlorophyll fluorescence screening method (Bilger et al., 1997; Cerovic et al., 2001; Agati et al., 2007). Four different emission bands (UV-A: 375nm, blue: 470nm, green: 516nm, red: 625 nm) generated by LEDs induce plant fluorescence is detected by three photodiodes [yellow (YF), red (RF) and far-red (FRF)]. Combining the four induction bands and the three detecting bands, the sensor acquires 12 different signals at every reading. Every signal was generated 70 times per second, and measurements where collected at 10 cm above plant canopy as per manufacturer's recommendation. Based on previous research (Agati et al., 2013; Longchamps and Khosla, 2014) seven indices were used for this research: Four N balance indices (NBI_R, NBI_B, NBI_B and NBI1), two chlorophyll indices (CHL and CHL1) and one flavonoid index (FLAV). All the indices are shown in Table 2.2.

Table 2.2   Indices used for this study along with their description and formula

Parameter	Description	Formula
NBI_R	Nitrogen balance index (red)	$NBI_R = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{uv_i}}{FRF_{r_i}}$
NBI_G	Nitrogen Balance index (green)	$NBI_G = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{uv_i}}{FRF_{g_i}}$
NBI_B	Nitrogen balance index (blue)	$NBI_B = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{uv_i}}{RF_{b_i}}$
NBI1	Nitrogen balance index (green/red)	$NBI_1 = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{uv_i} + FRF_{g_i}}{FRF_{r\ i}^{2}}$
CHL	Chlorophyll index (red)	$CHL = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{r_i}}{RF_{r_i}}$
CHL1	Chlorophyll index (green)	$CHL_1 = \dfrac{1}{n}\sum\limits_{i=1}^{n} \dfrac{FRF_{g_i}}{RF_{r_i}}$
FLAV	Flavonoid index	$FLAV = \dfrac{1}{n}\sum\limits_{i=1}^{n} \log(\dfrac{FRF_{r_i}}{FRF_{UV_i}})$

Nitrogen critical zone and nutrition index

Relationship between N and yield can be variable across experiments due to the confounding influences of soil N supply from non-fertilizer sources, such as variations in weather, crop variety and cropping practices (Muchow, 1994). Lemaire and Salette (1984) developed two methods to diagnose N status in winter oilseed rape. The first one is called critical

N curve (N_c) and accounts for the N content of the aboveground plant biomass to define what is the minimum amount to reach maximum yield. This method has been successfully used with several crops including maize (Plénet and Lemaire, 2000; Herrmann and Taube, 2004; Ziadi et al., 2008). The N_c is calculated by equation 1(Plénet and Lemaire, 2000):

$$N_c = 34.1\,W^{-0.37} \qquad\qquad\qquad\qquad Eq.1$$

where W is the total plant biomass (Mg ha^{-1}). For W values below 1 Mg ha^{-1}, N_c takes the value of 34.1 Mg ha^{-1}. The second index relates the critical N curve and the N content from plants of the same plotIs called nitrogen nutrition index (NNI). The NNI is a ratio between N_c and N content (NNI = $N_{content}/N_c$). It characterizes N deficiency based on N content and aboveground biomass creating a critical N curve. Ideally, values of NNI that are below the curve (NNI < 1) can be translated as plants with N deficiency status, while values higher than 1 indicate sufficient N nutrition (Plénet and Lemaire, 2000). Studies suggest that when NNI is higher than 0.93, N does not impact yield (Ziadi et al., 2008).

Filtering method

A filtering method was necessary to discern fluorescence reading acquired on the plant from readings acquired on non-vegetative objects and for other errors. The data filtering method was performed in three steps.

Step 1: Removing non-vegetative reading

This first step was employed to remove non-vegetative fluorescence readings. For each set of readings, the far-red fluorescence induced by red light signal (FRF_R) generated two peaks of voltage. One peak was between 0 to 20 mV and another one above 100 mV. Readings for which the FRF_R had a value below 20mV were not considered as leaf fluorescence and thus were removed from the data set.

Step 2: Removing sensor errors

This step was employed to remove any data that were erroneous due to sensor malfunction. A linear regression was performed between the signals FRF_R and RF_R. Data were filtered to eliminate the prediction error outliers. Any residuals that were outside of the interval of the average plus or minus three times the standard deviation were deleted from the original data set. Iteratively, new data set were created without the data removed in the previous iteration and the filtering process was repeated for 10 times. The same cleaning method was also performed on the linear regression between YF_R and RF_G and between FRF_G and FRF_R.

Step 3: Removing outliers

With the data set downsized using Step 1 (that removed non-vegetative readings) and Step 2 (that removed sensor malfunction errors), every single index used in this study to predict N status in maize were filtered in order to remove the outliers. Cleaning method to remove the outliers consisted of removing any data that would not be within the interval of the average plus or minus three times the standard deviation. Iteratively, new data set were created without the data removed in the previous iteration and the filtering process was repeated for 10 times. After completing this filtering method individually for each index, data from each study plot was divided into 10 subsamples by splitting into 10 groups based on their time of acquisition and averaging each group.

Statistical analysis

The ANOVA and Tukey's HSD ($\alpha = 0.05$) test were used to detect differences among treatments in each experiment. Those tests were performed for aboveground biomass, N content (%), N uptake, yield (hand harvest), NDVI and the chlorophyll fluorescence indices. Also, for the indices, a regression tree analysis was performed to verify the potential of fluorescence

indices to detect crop N status. A regression tree is grown by binary recursive partitioning using the response in a specified formula and choosing splits from the terms of the fluorescence indices. Over-fitted data were avoided by running a *10*-fold cross-validation to find the number of terminal nodes that minimized the prediction sum of squares. The regression tree was pruned to remove the least significant terminal nodes identified in the cross-validation. For the regression tree, the coefficient of determination was calculated by:

$$R^2 = 1 - \text{variance (residuals)/variance(data)} \hspace{3cm} \text{Eq.2}$$

where the residuals are the difference between the observed values and those predicted from the regression tree and data is the growth parameter to be estimated (e.g. N uptake).

All the statistical analysis were performed by using the statistical software R with the functions "aov", "TukeyHSD", and package "tree" with the functions "tree", "cv.tree" and "prune.tree" at (Ripley, 2014; R Development Core Team, 2014).

RESULTS AND DISCUSSION

Results of filtering process

Filtering process employed in step 1 removed 2.6% to 6.4% of the sensor data over the six measurement dates of ARDEC12. For ARDEC13, the percentage of deleted readings was 8.6% to 20.0% while for Iliff the deletion ranged from 19.0% to 30.0%. This step cleaned the data from non-vegetation (e.g. soil) readings. For the step 2 filtering process that dealt with sensor error, the percentage of deleted data points over the six measurement dates ranged from 4.4% to 6.3% for ARDEC12, from 3.3% to 6.8% for ARDEC13 and from 5.5% to 8.0% for Iliff. The outlier removal performed in step 3 eliminated less than 1% of data for the three site-years. A larger amount of data was deleted in step 1 process as compared to the other steps because, at

early crop growth stage, the sensor captured greater amount of soil background noise in between the plants. The capability of the sensor to differentiate soil noise and stepwise removal of noise it is advancement when compared to reflectance based vegetation indices (e.g. NDVI), where soil color and moisture can influence the results (Liu and Huete, 1994). Also, step 1 process removed more data from Iliff than the other two sites, which can be attributed due to plant density, where Iliff average 5.3 plants m[-1] while ARDEC12 and ARDEC13 average 5.9 and 5.7 plants m[-1], respectively. In other words, plant emergence was lower and inter-row spacing was larger for Iliff site, which resulted in more background noise and hence the removal of data points.

Aboveground biomass samples and yield

ARDEC12 site

Nitrogen applied at five rates did not have significant effect ($\alpha = 0.05$) on maize aboveground biomass values at the V6 and V9 growth stages. The aboveground biomass samples ranged from 4 to 30 grams at V6 growth stage and from 35 to 133 grams at V9 growth stage. Similar results were observed for N uptake, for which all five treatments were not significantly different ($\alpha = 0.05$). Measurements of N uptake ranged from 12 to 74 milligrams of N for V6 growth stage, while for V9 growth stage N uptake ranged from 106 to 403 milligrams of N per plant. There was a significant difference in leaf N content between 0 kg N ha^{-1} treatment and 168 and 224 kg N ha^{-1} treatments at the V6 growth stage, but not for V9 growth stage (fig. 2.5). Total N concentration in the leaf at the V6 growth stage ranged from 1.66% to 3.38%, and for V9 growth stage ranged from 1.72% to 4.20%.
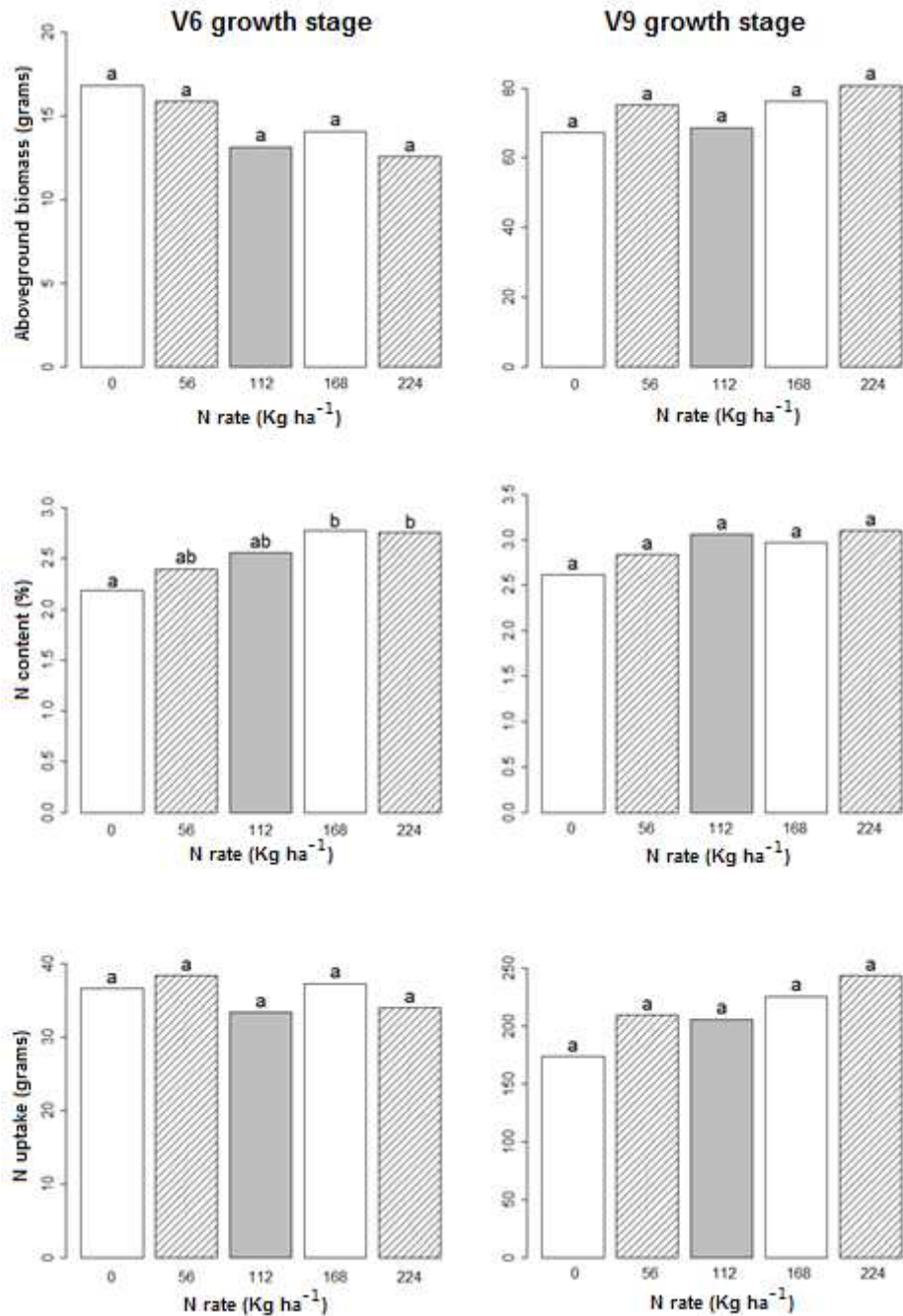
Figure 2.5 Bar plots of the different plant parameters measured on tissue samples for ARDEC12 site. Two different dates of biomass sample. On the left at V6 growth stage and on the right at V9 growth stage. Barplot are aboveground dried maize (Top), N content of the plant biomass (middle) and N uptake (bottom) for the five N treatments. Different letters indicate significant difference between treatments (Tukey's HSD, $\alpha = 0.05$)

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For ARDEC12, the yield ranged from 4.6 to 12.8 Mg ha^{-1} with an overall average of 7.7

Mg ha^{-1}, which was consistent with the N content response from V6 growth stage aboveground

biomass samples. The yield values from the N treatments 0, 56 and 112 kg ha^{-1} were

significantly different. Beyond the N rate of 112 kg of N ha^{-1}, the yield reached a plateau and

consequently, the N rate treatments of 112, 168, and 224 kg N ha^{-1} were not significantly

different (Fig. 2.6). This suggests that the treatments generated three distinct yield groups, where

the lowest group (treatment 0 kg N ha^{-1}) had a yield 22% lower than the highest group yield (i.e.,

N rate treatment of 224 kg N ha-1).
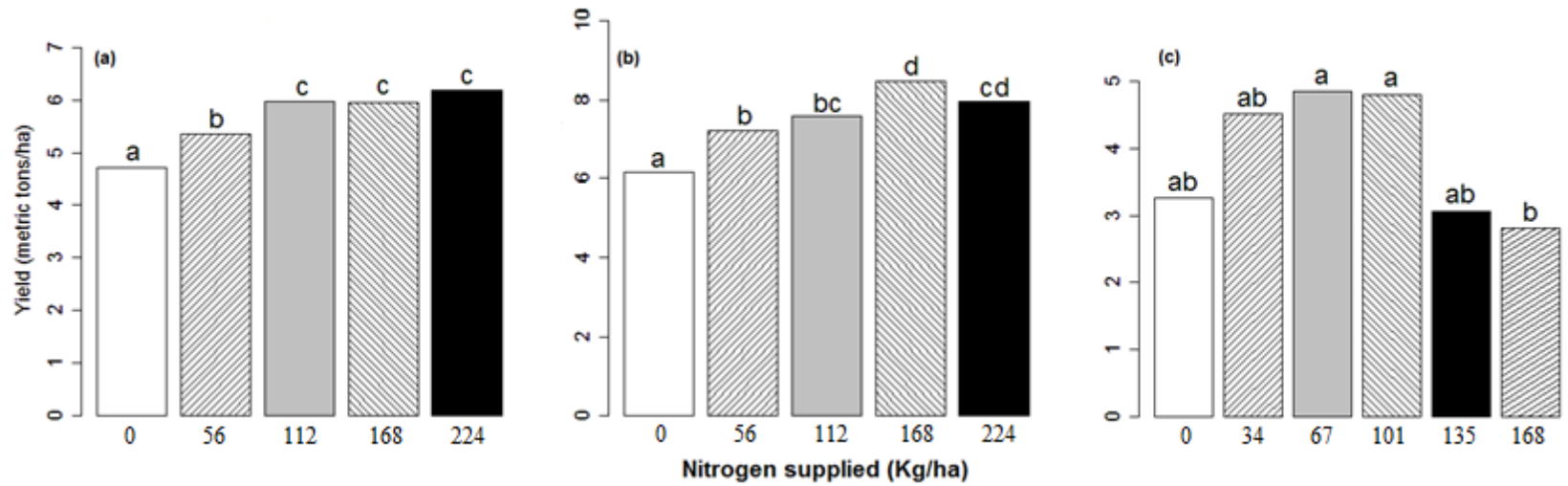
Figure 2.6 Barplots of the grain yield for the five N treatments (metric ton/ha) for site-years: ARDEC12 (a), ARDEC13 (b) and Iliff (c). Different letters indicate significant difference between treatments (Tukey's HSD, α = 0.05.

For plant measurements acquired at early growth stages, the variability caused by the N rates did not impact aboveground biomass and N uptake for both sampled dates (V6 and V9 growth stage) as the variability was found to be high for all treatments. The N rates had no significant effect on N content from V9 growth stage either, while for V6 growth stage the N content for the 0 kg N ha^{-1} treatment was significantly lower than that of the 168 and 224 kg N ha^{-1} treatments. Overall, only N content measured at V6 growth stage was positively correlated with the N rates and yield. All other measurements had no correlation with either the N rates or yield.

Comparing different N rates to create a critical N curve and N nutrition index for maize (NNI), Ziadi et al. (2008) concluded that NNI values above 0.93 indicate that N is not limiting the yield, and that a NNI ranging from 0.60 to 0.93 can result in 25 to 35% variability in grain yield. For the ARDEC12 site, NNI varied from 0.62 to 1.10 at the V6 growth stage, and from 0.49 to 1.02 at the V9 growth stage (Fig. 2.7). Plénet and Lemaire (2000) obtained NNI values ranging from 0.4 to 1.7 while Ziadi et al. (2008) observed values from 0.30 to 1.35. In comparison with these studies, the NNI variability for ARDEC12 was small, although the maize showed some N deficiency based on NNI values.

The absence of a significant effect of N rates on N uptake for the V6 growth stage sampled date may be attributed to the residual N in the soil at the beginning of the season. There was a positive correlation between N rates and N uptake mean values measured on plant tissue at V9 growth stage. However, because the confidence intervals were high, the effect of N rates was not significant. This may be explained by external factors influencing plant development such as drought stress. This site is part of a large research farm where water is not readily available.

Water availability in conjunction with a particularly dry cropping season may have resulted in

sub-optimal water supply to the crop.

Figure 2.7 ARDEC12 site: Scatter plots between nitrogen nutrition index (NNI) and yield at two different growth stages (a and b; V6 and V9 respectively). Dotted line represents the critical point below which yield could be explained by the N deficiency.

Iliff site

None of the six N rate treatments had a significant effect ($\alpha = 0.05$) on aboveground biomass, N content and N uptake. Aboveground biomass ranged from 1.63 to 25.82 grams at the V6 growth stage and from 8.55 to 187.53 grams at the V9 growth stage. Nitrogen content ranged from 2.92% to 3.68% at the V6 growth stage and from 2.29% to 3.15% at the V9 growth stage. Nitrogen uptake ranged from 4.95 to 84.60 and from 25.56 to 532.20 milligrams of N at the V6 and V9 growth stages respectively (Fig. 2.8). Based on the NNI, N deficiency was observed only for three plots at the V6 growth stage. At the V6 growth stage, the NNI ranged from 0.86 to 1.08. At the V9 growth stage, the NNI remained mostly in the N critical zone of 0.82 to 1.00 (Fig. 2.9).

The relationship between grain yield and NNI shows that the yield was independent from NNI and that N rates did not influence the grain yield of the study plots. Figure 2.6 illustrates the average yield for each treatment. The highest yield observed were for treatments 67 and 101 kg N ha^{-1}, while treatments that received 135 and 168 kg N ha^{-1} had the lowest yield. Surprisingly, the yield from the 168 kg N ha^{-1} rate was significantly lower ($\alpha = 0.05$) than the yield from the 67 and 101 kg N ha^{-1} rates. The yield ranged from 2.9 to 5.4 Mg ha^{-1}, with an overall mean of 4.0 Mg ha^{-1}. These yield results are low as compared with the other site-years of this study.
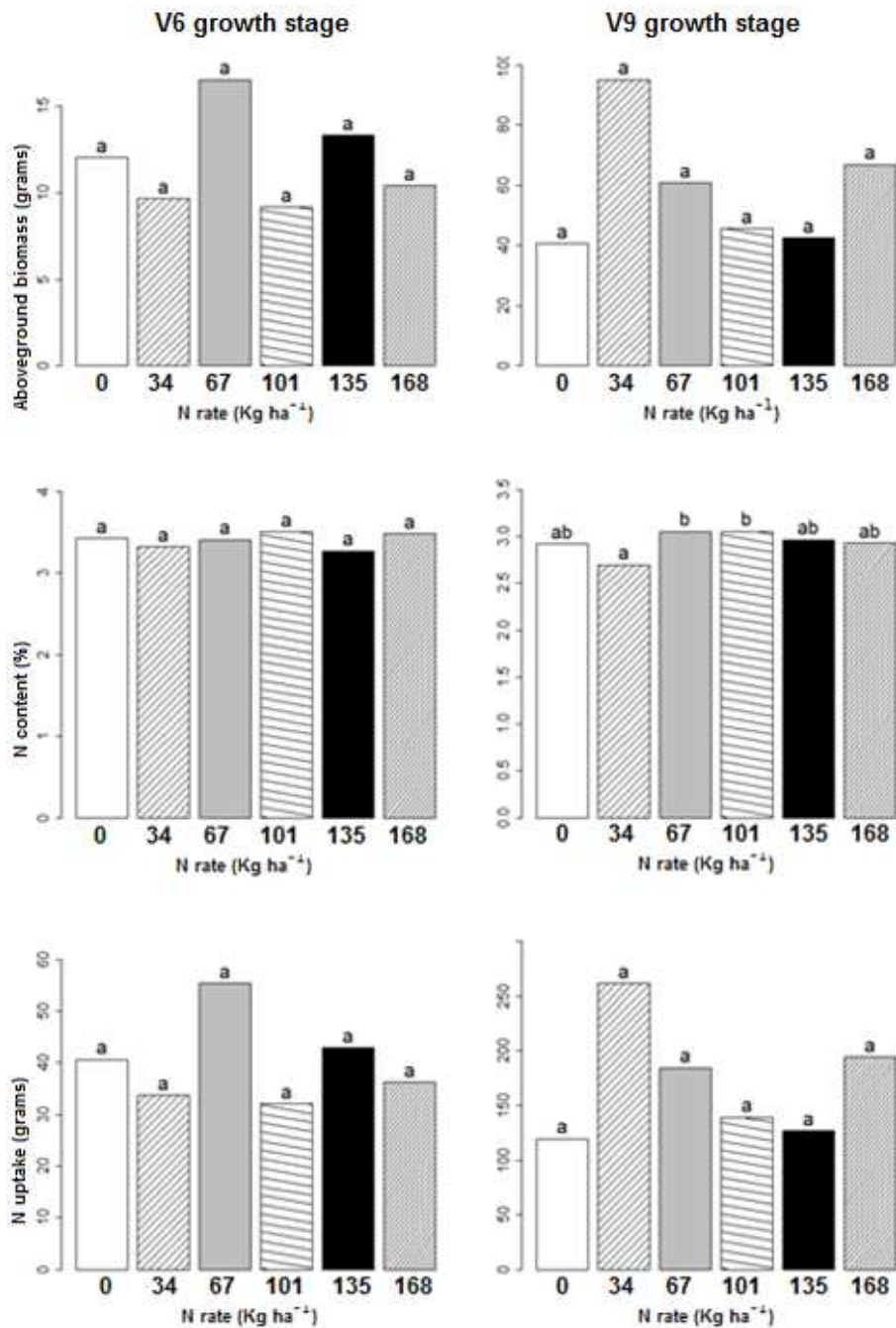
Figure 2.8 Bar plots of the different parameters measure on tissue samples for Iliff site. Two different dates of biomass sample. On the left at V6 growth stage and on the right at V9 growth stage. Barplot are aboveground dried maize (Top), N content of the plant biomass (middle) and N uptake (bottom) for the five N treatments. Different letters indicate significant difference between treatments (Tukey's HSD, $\alpha = 0.05$)
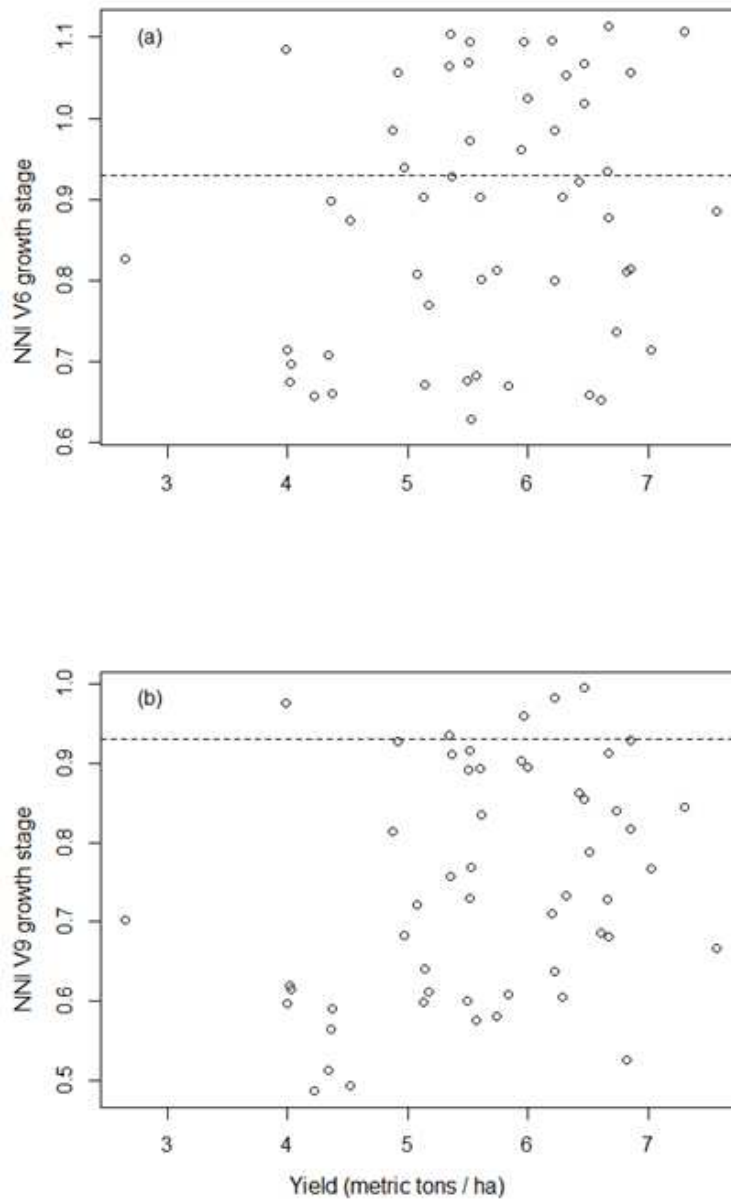
Figure 2.9 Iliff site: Scatter plots between nitrogen nutrition index (NNI) and yield at two different growth stage (a and b; V6 and V9 respectively). Dotted line represents critical point of were yield could be explained by nitrogen deficiency.

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The results described above indicate that the soil environmental conditions had more influence on the yield variability than the N rates applied. Indeed, treatments did not follow a logical pattern (e.g. the higher the N rates, the higher the yield). The high variance among treatments may explain the absence of significant differences, as Iliff had higher variability in biomass, N content, N uptake and yield when compared with the other sites.

ARDEC13 site

For the year 2013, aboveground biomass samples were collected only at the V9 growth stage. The biomass ranged from 134 to 231 grams and none of the treatments had a significant effect ($\alpha = 0.05$) on the weight of the sample. Similar results were observed for the N content that ranged from 3.90 to 4.89% and N uptake that ranged from 622.4 to 1,039.5 milligrams of N (Fig. 2.10). Those results are relatively high when compared to the other two site-years and consistent with the NNI values that range from 1.5 to 2.2 (Fig. 2.11). As expected, a similar behavior was observed as the yield result for this location was higher than for the other two site-years for all treatments, ranging from 5.8 to 15.0 Mg ha^{-1}, with an overall average of 9.8 Mg ha^{-1} (Fig. 2.6). Ziadi (2008) concluded that maize has the ability to take up more N than required for maximum growth. The relationship between NNI and yield are expressed by a quadratic and linear-plateau. It was reported that for NNI values above 0.88 the grain yield reaches a plateau as was the case in this study (Ziadi, 2008). For this particular site, maize had sufficient N availability for all the plots and its relationship with aboveground biomass samples and yield are only representing where the relationship between N and those measurements reached asymptote.
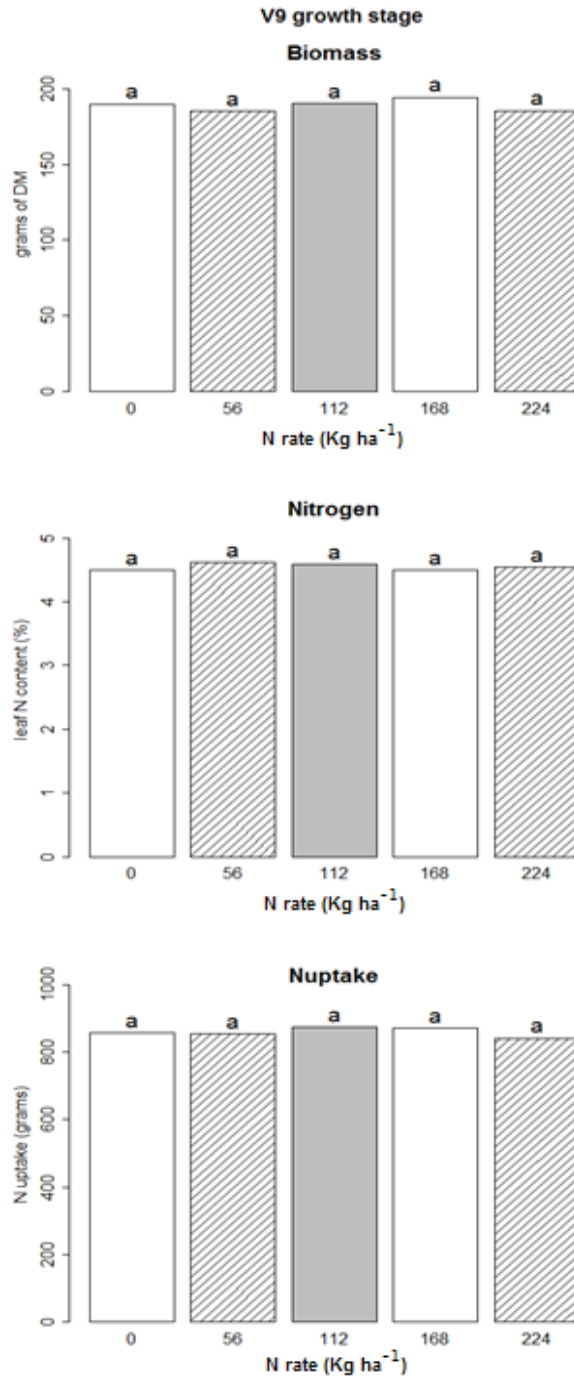
Figure 2.10 Bar plots of the different parameters measure on tissue samples for ARDEC13 site. Biomass sample at V9 growth stage. Barplot are aboveground dried maize (Top), N content of the plant biomass (middle) and N uptake (bottom) for the five N treatments. Different letters indicate significant difference between treatments (Tukey's HSD, α = 0.05)
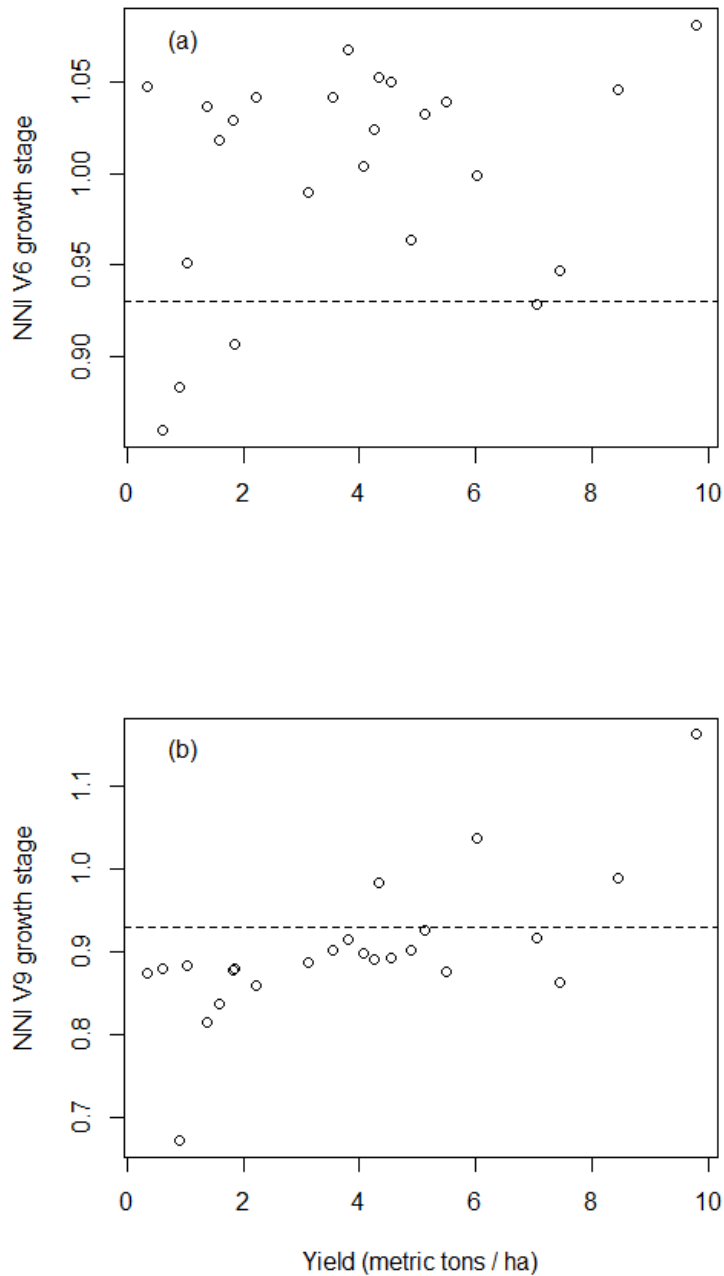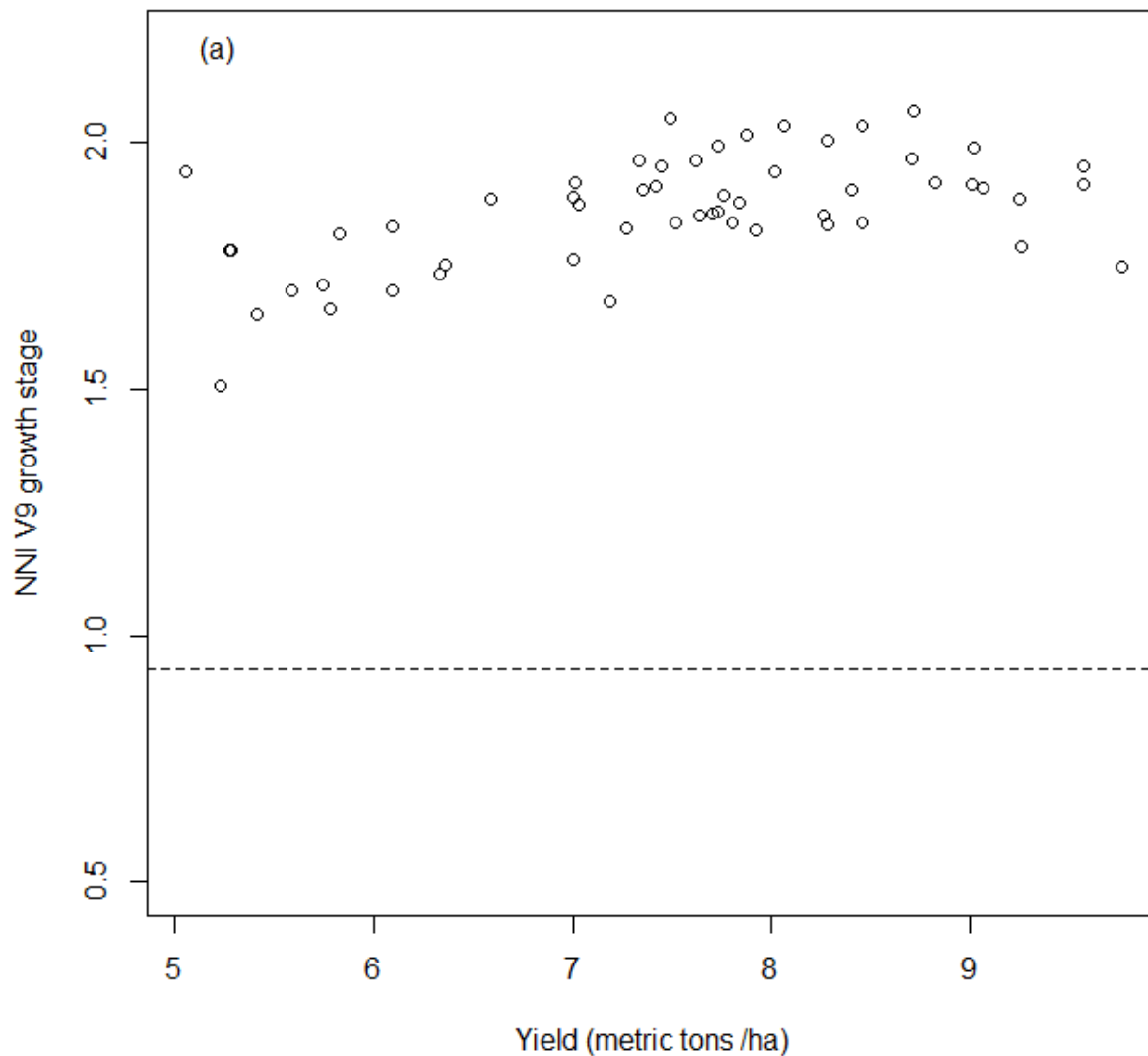
Figure 2.11 ARDEC13 site: Relationship between nitrogen nutrition index (NNI) and yield at V9 different growth stage. Dotted line represents critical point of were yield could be explained by nitrogen deficiency.

Reflectance sensing

       ARDEC12 site

The reflectance based NDVI index significantly ($\alpha = 0.05$) increased from one reading date to the other (Fig. 2.12), as leaf area index increased. The NDVI did not detect differences among N treatments throughout the six reading dates (Fig. 2.13). For the first three reading dates, when plants were small, the NDVI values for treatment 0 kg N ha^{-1} were significantly ($\alpha = 0.05$) higher than for the other N rates. For only one reading date (July 7th), NDVI followed the same pattern as the N rate treatments. On July 17th the NDVI values acquired from the low N rate plots (0 and 56 kg N ha^{-1}) were significantly lower ($\alpha = 0.05$) than the NDVI values from the high N rate plots (168 and 224 kg N ha^{-1}). There was a significant variability in NDVI across treatments for this site, but this variability did not follow a positive correlation with the N rates applied to the plots.
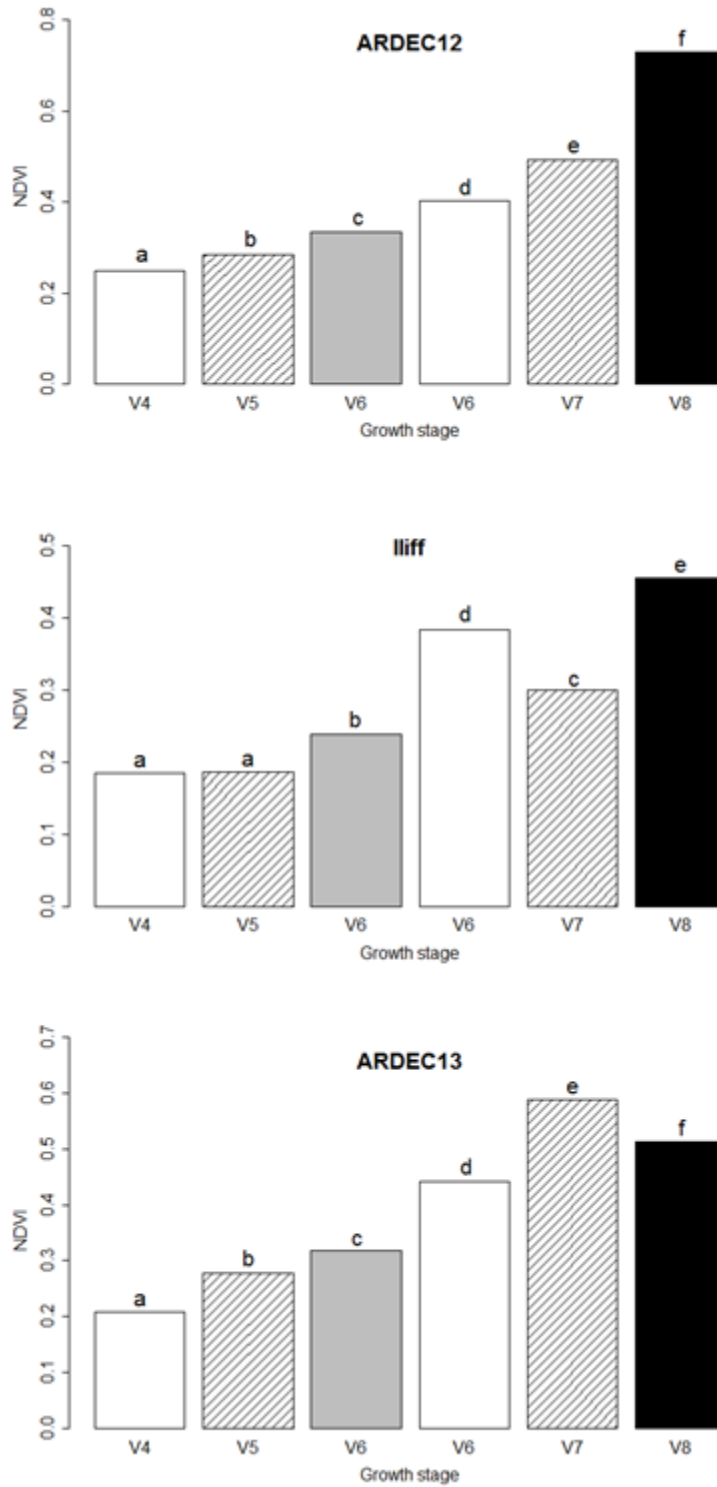
Figure 2.12 Barplots are average NDVI reading collected in each day (each bar represents one day) for the six reading dates for the three site-years. Different letters indicate significant difference between dates (Tukey's HSD, $\alpha = 0.05$).

Figure 2.13 ARDEC12 site: Barplots represent NDVI readings (y-axis) by different N treatments (x-axis) collected in six different days. Different letters indicate significant difference between dates (Tukey's HSD, $\alpha = 0.05$).

Iliff site

The NDVI acquired at Iliff location increased significantly with increasing growth stage except for a lower average NDVI acquired at V7 (Fig 2.14). The increase in NDVI mean values followed the same trend as the plant height measurements. For the first two reading dates (June 13th and June 18th), NDVI increased significantly from treatment 34 kg N ha^{-1} to treatment 168 kg N ha^{-1}, when N applied and biomass showed a positive correlation. However, for the control treatment, where no N was applied, the NDVI measurements were significantly ($\alpha = 0.05$) higher than the other treatments. This was not observed at subsequent reading dates, when treatments of 0 and 34 kg N ha^{-1} showed NDVI values lower than NDVI values acquired for treatments 67 and 101 kg N ha^{-1}. On the other hand, treatments 135 and 168 kg N ha^{-1} showed lower NDVI values than readings acquired from treatments 67 and 101 kg N ha^{-1} (Fig. 2.14). Based on the observations of NDVI values, which did not follow a constant pattern, it was concluded that NDVI did not respond to the N treatments prior to V8 maize growth stage. For this site, as yield did not respond to the N treatments, it was expected that NDVI reading would not show correlation with the N treatments.
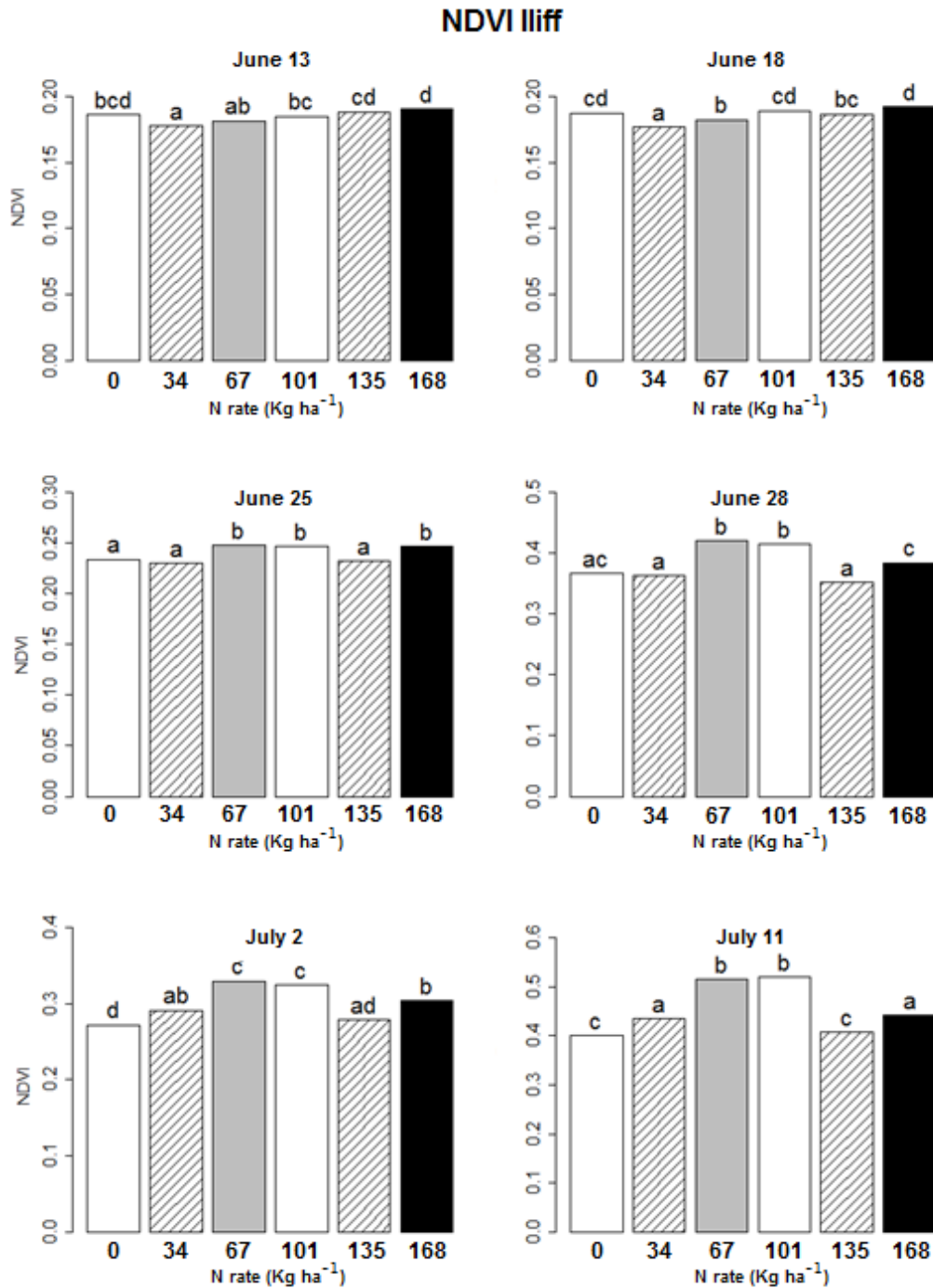
Figure 2.14 Iliff site: Barplots represent NDVI readings (y-axis) by different N treatments (x-axis) collected in six different days. Different letters indicate significant difference between dates (Tukey's HSD, $\alpha = 0.05$).

ARDEC13 site

In general, for the year 2013, the NDVI values showed an increase, which was similar to the observations made in the two other sites (Fig. 2.15). At ARDEC13 site, all plots had NNI above 1, which indicates that N availability to the plants was not a limiting factor as compared to the other two sites. Consistently, readings from this site had the lowest variability across treatments. Only the NDVI readings from the control treatments at early growth stages (before June 21st) were significantly ($\alpha = 0.05$) higher than the NDVI values from the other treatments, which were not significantly different from each other. Comparing the average NDVI values over time, there was a steady increase from V4 to V7 growth stage and a decrease at V8 growth stage.

Overall, it was not possible to conclude that NDVI was responsive to the N treatments for all site-years. Several studies, including sites in Colorado, have shown positive relationship between NDVI and different N rates applied on the field (Shaver et al., 2011).
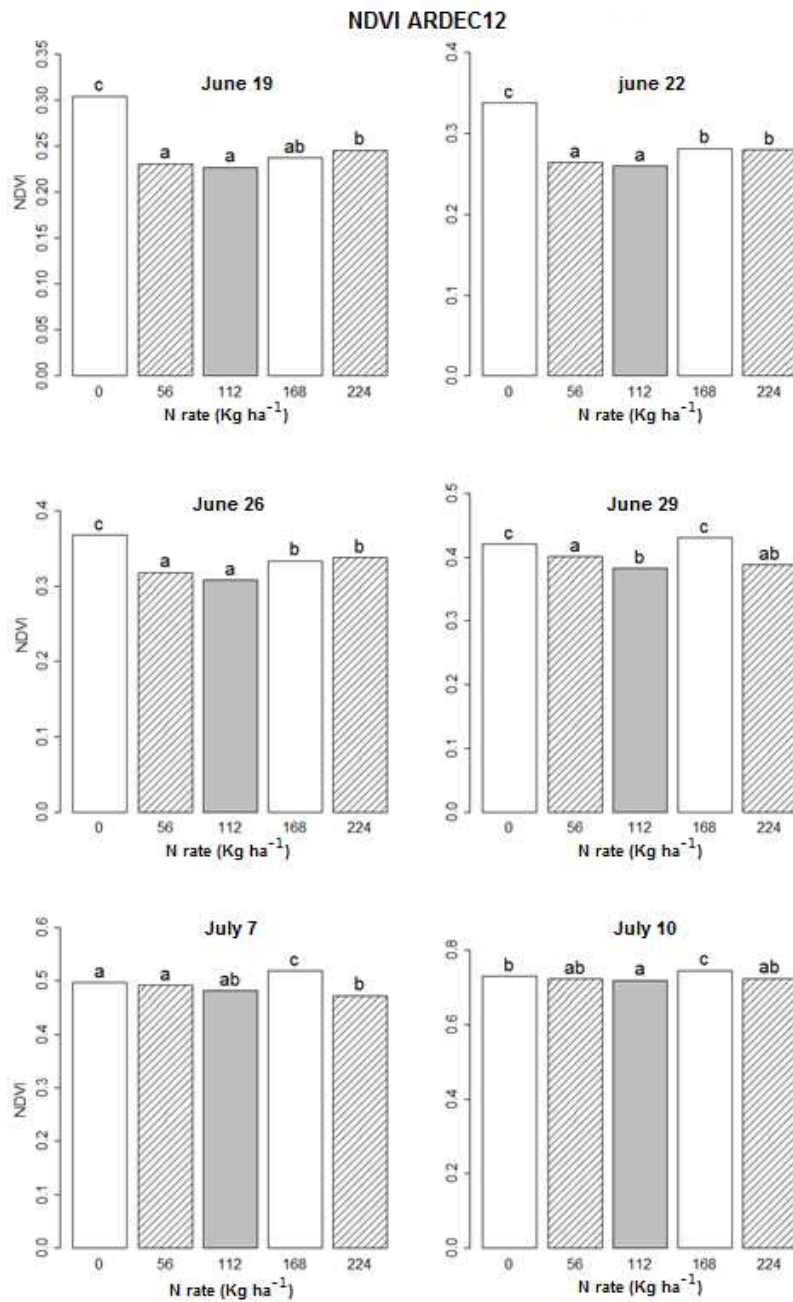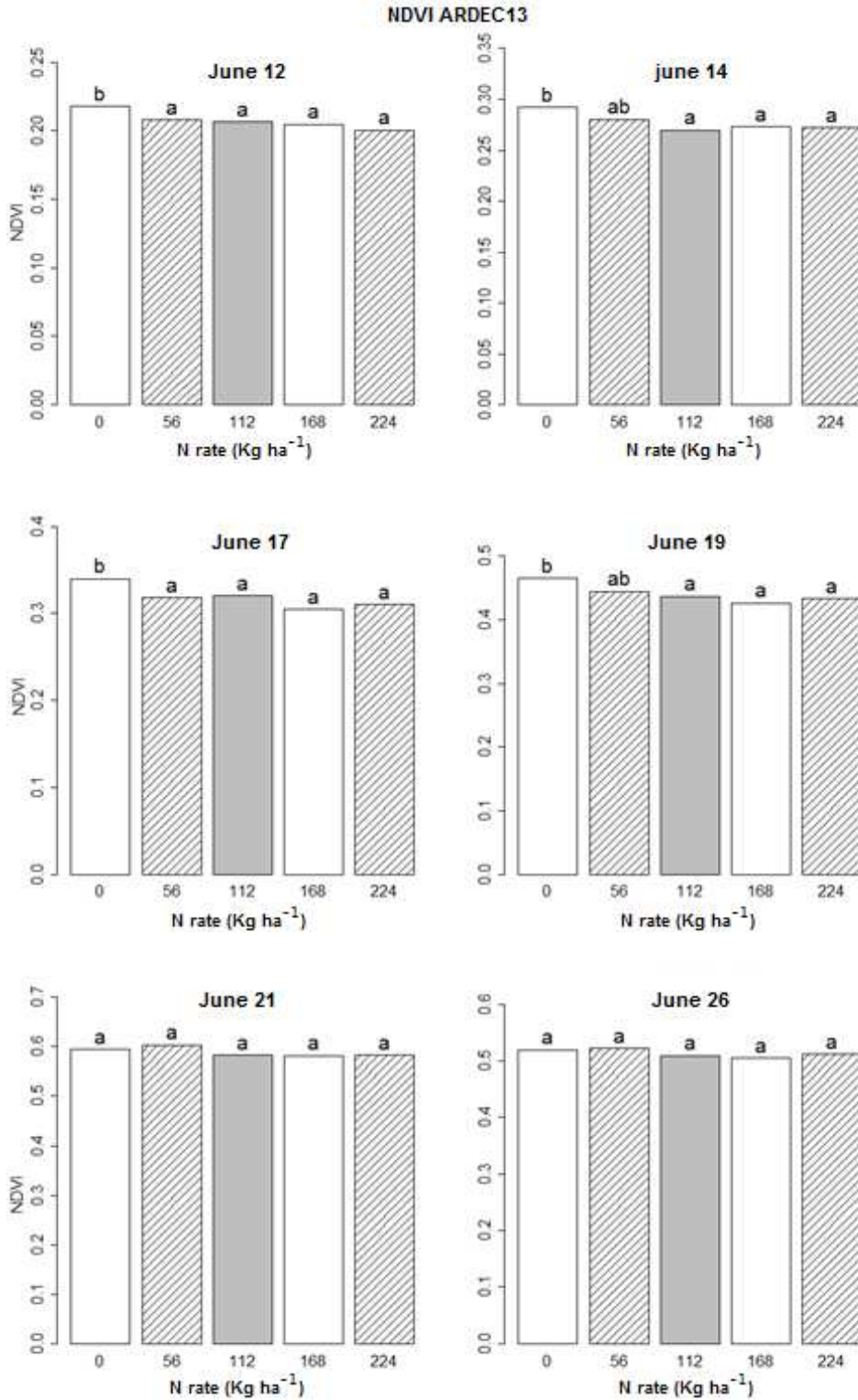
Figure 2.15 ARDEC13 site: Barplots represent NDVI readings (y-axis) by different N treatments (x-axis) collected in six different days. Different letters indicate significant difference between dates (Tukey's HSD, $\alpha = 0.05$).

Fluorescence sensing

ARDEC12 site

For the present research, within plot variability was high and the applied N rates did not show correlations with NDVI, height, weight, N content and N uptake of the aboveground biomass. In that matter, the fluorescence parameters were compared to the aboveground biomass observations (weight, N content and N uptake). Also, none of the fluorescence indices enabled the detection of N variability with precision. Furthermore, all seven fluorescence indices were used in a regression tree analysis to predict plant biomass, N content or N uptake for both sampling dates (i.e. V6 and V9 growth stage). The number of nodes used after pruning the regression tree was different for each set of readings (Table 2.3). Among the three growth parameters observed in this study (i.e. biomass, N content and N uptake), the best coefficient of determination (R^2) for the relationship between the actual and the predicted values using fluorescence indices was observed for the N content (Table 2.3). Among all measurement dates, the highest R^2 between the fluorescence indices and the N content was observed at the V5 growth stage ($R^2 = 0.81$). Throughout the six days of data acquisition, the average R^2 was 0.55. In general, the R^2 between N content of samples and the fluorescence indices was lower at the V6 than at the V9 growth stage. The average R^2 at V6 growth stage across the six reading dates was 0.38 with the highest value being 0.45 at V5 growth stage (Table 2.3).

Table 2.3 ARDEC12 site: Biomass, N content and N uptake collected at V6 and V9 growth stage. Cross-validated R-square from regression tree analysis. Number of prune for cross-validation was based on the smallest deviance

| | | | Biomass V6 | | Biomass V9 | |
Site	Date	Growth stage	Prune	R-square	Prune	R-square
ARDEC12	19-Jun	4	4	0.41	5	0.42
ARDEC12	22-Jun	5	3	0.33	7	0.49
ARDEC12	26-Jun	6	4	0.25	6	0.5
ARDEC12	29-Jun	6	3	0.34	7	0.56
ARDEC12	3-Jul	7	3	0.2	5	0.34
ARDEC12	10-Jul	8	3	0.23	3	0.36

| | | | N content V6 | | N content V9 | |
Site	Date	Growth stage	Prune	R-square	Prune	R-square
ARDEC12	19-Jun	4	4	0.29	8	0.73
ARDEC12	22-Jun	5	4	0.45	8	0.81
ARDEC12	26-Jun	6	5	0.36	10	0.64
ARDEC12	29-Jun	6	3	0.37	4	0.4
ARDEC12	3-Jul	7	3	0.27	7	0.55
ARDEC12	10-Jul	8	5	0.53	5	0.53

| | | | N uptake V6 | | N uptake V9 | |
Site	Date	Growth stage	Prune	R-square	Prune	R-square
ARDEC12	19-Jun	4	6	0.42	7	0.45
ARDEC12	22-Jun	5	8	0.43	5	0.39
ARDEC12	26-Jun	6	5	0.33	4	0.35
ARDEC12	29-Jun	6	5	0.44	5	0.62
ARDEC12	3-Jul	7	4	0.3	7	0.58
ARDEC12	10-Jul	8	4	0.44	4	0.43

The R^2 values of the regression tree between the aboveground biomass and the fluorescence indices collected at V9 was higher than for the aboveground biomass collected at V6, averaging at 0.37 and 0.26 respectively for the six reading dates. The R^2 of the relationship

between the biomass collected at V9 and the fluorescence indices peaked at the V6 growth stage ($R^2 = 0.56$), while for the biomass collected at V6, it peaked at the V4 growth stage ($R^2 = 0.41$).

The regression tree between the indices and the growth parameters characterized best the N content followed by the N uptake and the aboveground biomass. Over the six reading dates, for N uptake the samples collected at V9 growth stage showed higher R^2 values than the samples collected from V6 growth stage (V6: 0.35; V9: 0.47). The coefficient of determination between the N uptake and the fluorescence indices for both sampling dates peaked at the V6 reading date (V6: 0.44; V9: 0.62).

Overall, the samples collected at the V9 growth stage showed higher coefficient of determination between growth parameters (i.e. biomass, N content or N uptake) and fluorescence indices. The NNI of samples collected at the V9 growth stage were all below 1.00 and for most of the plots even below 0.93 (fig. 2.7), which is considered a critical zone where N deficiency may influence yield (Ziadi et al. 2008). Conversely, for the samples acquired at the V6 growth stage, the crop was sufficient in N for about 30% of the plots according to the NNI threshold of 0.93. Higher NNI from V6 growth stage samples may explain why the fluorescence indices performed better over the samples collected at the V9 growth stage (i.e. higher coefficient of determination) than the samples at the V6 growth stage, where a greater portion of the data was sufficient in N. This result indicates that maize began to show N deficiency over the time and the fluorescence indices improved the characterization with the plant N deficiency.

Fluorescence indices enabled a better detection of the N content variability imposed by the N treatments than biomass or N uptake. Coelho (2012) found similar results in potatoes under different N rates, where the indices CHL and NBI_R showed high correlation with N content and different rates of N applied to the crop.

Iliff site

None of the fluorescence indices enabled the detection of N variability with precision, which is similar to ARDEC12 results. A regression tree analysis with all seven indices against aboveground biomass, N content or N uptake was used for prediction. The coefficient of determination for the model with aboveground biomass across the six reading dates averaged at 0.50 and 0.53 for the V6 and V9 growth stages respectively (Table 2.4). The highest R^2 value for the samples collected at V6 growth stage was observed when maize was also at V6 growth stage ($R^2 = 0.72$), while for samples collected at V9 growth stage, the highest R^2 value (R2 = 0.60) was observed from maize at the V4 growth stage.

In terms of N content, maize sampled at the V6 growth stage had slightly lower R^2 values as compared with R^2 values from samples at the V9 growth stage. The average coefficient of determination across the six reading dates was 0.34 and 0.48 for V6 and V9 respectively. Nitrogen content sampled at the V9 growth stage showed the strongest relationship with the fluorescence indices acquired at the V5 growth stage ($R^2 = 0.61$), while N content sampled at V6 had the highest coefficient of determination with readings acquired at the V3 growth stage ($R^2 = 0.45$).

On average over the six reading dates, the R 2 of the regression tree analysis between fluorescence indices and the N uptake were 0.53 and 0.57, for the V6 and V9 growth stages respectively. The relationship between the fluorescence indices and the N uptake was better than with the other two growth parameters (i.e. biomass and N content). The fluorescence readings that showed the strongest relationship with N uptake were acquired at the V6 growth stage of maize, for which the R 2 were 0.72 and 0.74 for the V6 and V9 growth stages respectively.

Table 2.4 Iliff site: Biomass, N content and N uptake collected at V6 and V9 growth stage. Cross-validated R-square from regression tree analysis. Number of prune for cross-validation was based on the smallest deviance

Site	Date	Growth stage	Biomass V6		Biomass V9	
			Prune	R-square	Prune	R-square
Iliff	13-Jun	3	4	0.62	3	0.54
Iliff	18-Jun	4	4	0.44	3	0.6
Iliff	25-Jun	5	3	0.39	4	0.6
Iliff	28-Jun	6	4	0.72	4	0.53
Iliff	2-Jul	7	4	0.48	2	0.32
Iliff	11-Jul	8	2	0.32	4	0.58

Site	Date	Growth stage	N content V6		N content V9	
			Prune	R-square	Prune	R-square
Iliff	13-Jun	3	4	0.45	4	0.47
Iliff	18-Jun	4	3	0.26	3	0.51
Iliff	25-Jun	5	3	0.36	3	0.61
Iliff	28-Jun	6	3	0.34	3	0.42
Iliff	2-Jul	7	4	0.41	4	0.4
Iliff	11-Jul	8	3	0.22	3	0.48

Site	Date	Growth stage	N uptake V6		N uptake V9	
			Prune	R-square	Prune	R-square
Iliff	13-Jun	3	4	0.64	2	0.39
Iliff	18-Jun	4	3	0.41	3	0.57
Iliff	25-Jun	5	3	0.42	4	0.59
Iliff	28-Jun	6	4	0.72	4	0.74
Iliff	2-Jul	7	4	0.44	3	0.43
Iliff	11-Jul	8	4	0.54	4	0.69

A large number of plots showed a N nutrition index above the critical threshold of 0.93 for both sampled dates. Samples from V6 growth stage showed higher NNI values as compared

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with samples collected at V9 growth stage (Figure 2.9). This may explain why samples collected at V9 were slightly better in estimating maize aboveground biomass, N content or N uptake using the fluorescence indices.

ARDEC13 site

For ARDEC13 site, above ground biomass samples were collected only at the V9 growth stage. On average over six reading dates from V4 to V8 growth stages, the coefficient of determination between the predicted and the measured biomass values based on the indices was 0.29 (Table 2.5). The highest R^2 was observed with fluorescence readings acquired at the V4 growth stage ($R^2 = 0.41$). For N content, over the six reading dates, the average R^2 was 0.38, and the highest R^2 was obtained with fluorescence readings acquired at the V8 growth stage ($R^2 = 0.53$). The regression tree between N uptake and the fluorescence indices showed an average coefficient of determination of 0.39, and was fairly constant throughout all the reading dates with the highest value at V8 growth stage ($R^2 = 0.44$). Overall, for this site-year, the fluorescence indices performed better in estimating N content and N uptake than aboveground biomass. When comparing to the two other site-years, ARDEC13 indices performed poorly in estimating N status. Figure 2.11 shows that NNI for this location was very high for all plots indicating that the crop had sufficient amounts of N (lowest NNI = 1.5).

Nitrogen rates treatments were used to generate high variability of available N to the plant. However, based on NNI results, it seems like maize was sufficient in N at early growth stages, especially for ARDEC13 site. Nitrogen nutrition index showed that most of the plots for all three site-years did not have N deficiency and the range of N content did not represent maize with high deficiency of N (as the N content was higher than 2.29%), with a large majority of

plots showing a NNI above 1. This perhaps explains why the indices could not accurately detect

N variability in maize at early growth stages.

Table 2.5 ARDEC13 site: Biomass, N content and N uptake collected at V9 growth stage. Cross-validated R-square from regression tree analysis. Number of prune for cross-validation was based on the smallest deviance

| | | | Biomass V9 | |
Site	Date	Growth stage	Prune	R-square
ARDEC13	12-Jun	4	4	0.41
ARDEC13	14-Jun	5	3	0.33
ARDEC13	17-Jun	6	4	0.25
ARDEC13	19-Jun	6	3	0.34
ARDEC13	21-Jun	7	3	0.2
ARDEC13	26-Jun	8	3	0.23

| | | | N content V9 | |
Site	Date	Growth stage	Prune	R-square
ARDEC13	12-Jun	4	4	0.29
ARDEC13	14-Jun	5	4	0.45
ARDEC13	17-Jun	6	5	0.36
ARDEC13	19-Jun	6	3	0.37
ARDEC13	21-Jun	7	3	0.27
ARDEC13	26-Jun	8	5	0.53

| | | | N uptake V9 | |
Site	Date	Growth stage	Prune	R-square
ARDEC13	12-Jun	4	6	0.42
ARDEC13	14-Jun	5	8	0.43
ARDEC13	17-Jun	6	5	0.33
ARDEC13	19-Jun	6	5	0.44
ARDEC13	21-Jun	7	4	0.3
ARDEC13	26-Jun	8	4	0.44

CONCLUSION

For the ARDEC location, during both years, the yield was responsive to the different N rates applied following a linear upper plateau pattern. For Iliff site, yield was not responsive to the increasing N rates, reaching asymptote at 101 Kg/ha of N and decreasing at higher N rates. Iliff location did not show a positive relationship between N rates and yield. For both 2012 sites, drought stress was possibly an important external factor that impacted the yield, as that year suffered from severe drought. The year of 2013 had early season rainfall below the historical average, but not as drastic as the previous year and the overall yield were higher than both sites in 2012. Limited availability of water for the three site-years is reflected in the increase in N content in the plant contributing to a high NNI values.

For all three sites-years, the N variability at early growth stages did not respond as expected due to high variability of the residual N in the soil. Those externals factors contributed largely to the variability of height, weight and N content of plant aboveground biomass that were not responsive to the different N rates applied.

For all three sites-years, NDVI values increased over the reading dates as plants were growing. None of the site-years showed a positive correlation between NDVI and N rates. Many studies have shown positive correlation between NDVI measurements and applied N fertilizers prior to V8 (Yin and McClure, 2013; Shaver et al., 2011; Kitchen et al., 2010). The water availability as a limiting factor may be the reason for this study not being consistent with previous researches.

It has been reported that the Multiplex® sensor has a great potential in estimating N status in N deficient plants (Tremblay et al., 2012). As most of the plots did not show N deficiency, it

cannot be stated that the sensor failed in detecting N variability in maize at early growth stages. After an intensive data filtering and regression tree analysis accounting for all fluorescence indices, a prediction of N uptake was generated, but this estimation was site dependable and not accurate enough for estimating maize N status (R^2 varied largely across reading dates and site-years).

Similarly to N deficiency, water deficit also impacts on plant fluorescence emissions and might need to be accounted for future studies (Shangguan et al., 2000). These results show that the sensor may be able to detect N variability in maize at early growth stages, but further studies in environments that contain lower amounts of N would be more appropriate to evaluate maize early stage N deficiency. Also, combining different N rates with well-watered and drought treatments might be important to interpret if water stress influences maize N characterization by a fluorescence sensor at early stage.

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