



Water and Nitrogen Effects on Active Canopy Sensor Vegetation Indices

Luciano Shiratsuchi,* Richard Ferguson, John Shanahan, Viacheslav Adamchuk, Donald Rundquist, David Marx, and Glen Slater

ABSTRACT

Much of the previous evaluation of active crop canopy sensors for in-season assessment of crop N status has occurred in environments without water stress. The impact of concurrent water and N stress on the use of active crop canopy sensors for in-season N management is unknown. The objective of this study was to evaluate the performance of various spectral indices for sensing N status of corn (*Zea mays* L.), where spectral variability might be confounded by water-induced variations in crop reflectance. The study was conducted in 2009 and 2010 with experimental treatments of irrigation level (100 and 70% evapotranspiration [ET]), previous crop {corn–corn or soybean [*Glycine max* (L.) Merr.]–corn} and N fertilizer rate (0, 75, 150, and 225 kg N ha⁻¹). Crop canopy reflectance was measured from V11 to R4 stage using two active sensors—a two band (880 and 590 nm) and a three band (760, 720, and 670 nm). Among the indices, the vegetation index described by near infrared minus red edge divided by near infrared minus red (DATT) and Meris terrestrial chlorophyll index (MTCI) were the least affected by water stress, with good ability to differentiate N rate with both previous crops. The chlorophyll index using amber band (CI), normalized difference vegetation index using red edge band (NDVI_RE) and the normalized vegetation index using the red band (NDVI_Red) showed more variation due to water supply, and had only moderate ability to differentiate N rates.

IN-SEASON N MANAGEMENT for corn using active crop canopy sensors (ACS) relies on the use of algorithms that can trigger on-the-go N fertilization in the field based on crop canopy reflectance. Optical sensing equipment that employs this approach is commercially available and these sensors rely on some version of a vegetation index to express crop reflectance (Shanahan et al., 2008; Eitel et al., 2008) and prescribe N rate application.

There are different approaches and vegetation indices used to determine N rate based on these sensors, but the majority of algorithms use the nitrogen sufficiency index (NSI) approach previously proposed for chlorophyll meter readings (Varvel et al., 1997). For example, when the ratio between a targeted region in the field and a well-fertilized reference in the same field reaches a certain level, N fertilizer is needed according to a function that describes the relationship between yield and NSI readings (Bausch and Duke, 1996). Some N rate recommendation algorithms use yield potential that is determined by

growing degree days and an estimate of biomass at the day of sensing (Raun et al., 2002). Several additional vegetation indices have been used to calculate N rate for corn and wheat using active canopy sensors, such as the green normalized difference vegetation index (GNDVI) (Dellinger et al., 2008), and the CI (Solari et al., 2008).

Regardless of the approach used, an understanding of how these indices may be influenced by water stress and previous crop is needed. Previous work by Eitel et al. (2008) investigated the impact of water availability and N stress on leaf area index (LAI) in wheat using a multispectral radiometer and a chlorophyll meter. They showed that the ratio of the modified chlorophyll absorption ratio index to the second modified triangular vegetation index (MCARI/MTVI2) is sensitive to N and less susceptible to variable LAI caused by water stress. Another example of interaction between water and N stress in corn using remote sensing was the work done by Clay et al. (2006), where broad band widths were used to calculate different indices (NDVI, GNDVI, normalized difference water index [NDWI], and nitrogen reflectance index [NRI]), with the major conclusion being that water and N had additive effects on yield and optimum N rates (100–120 kg N ha⁻¹) were similar across different water levels. There are other examples of indices used specifically to detect water stress (Zygielbaum et al., 2009), to determine chlorophyll content, and to estimate gross primary productivity (Lemaire et al.,

L. Shiratsuchi and R. Ferguson, Univ. of Nebraska, Agronomy and Horticulture, 361 Keim Hall, Lincoln, NE, 68583; J. Shanahan, Pioneer Hi-Bred International Inc., Johnston, IA 50131; V. Adamchuk, McGill Univ., Bioresource Engineering, 21111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC H9X 3V9 Canada; D. Rundquist, Univ. of Nebraska, School of Natural Resources, 307 Hardin Hall, Lincoln, NE 68583; D. Marx, Univ. of Nebraska, Dep. of Statistics, 342C Hardin Hall North, Lincoln, NE 68583; G. Slater, Univ. of Nebraska-South Central Agriculture Lab., 1322 Hwy. 41, Clay Center, NE 68933. Received 23 June 2011. *Corresponding author (shozo@huskers.unl.edu).

Published in *Agron. J.* 103:1815–1826 (2011)

Posted online 28 Sept 2011

doi:10.2134/agronj2011.0199

Copyright © 2011 by the American Society of Agronomy, 5585 Guilford Road, Madison, WI 53711. All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Abbreviations: ACS, active crop canopy sensors; CC, irrigated corn after corn; CI, chlorophyll index vegetation index using amber and near infrared; CIRE, chlorophyll index vegetation index using red edge and near infrared; CS, irrigated corn after soybean; DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); ET, evapotranspiration; MTCI, Meris terrestrial chlorophyll index; NDVI_RE, normalized difference vegetation index using the red edge band; NDVI_Red, normalized difference vegetation index using the red band; NIR, near infrared; NSI, nitrogen sufficiency index.



Fig. 1. Platform for data acquisition (bicycle equipped with two optical sensors, DGPS, laptop computer, and batteries).

Table 1. Planting date and crop characteristics.

	2009	2010
Planting date	6 May	29 April
Hybrid	Pioneer 33H29	Pioneer 1395XR
Plant population	72,610 plants ha ⁻¹	72,610 plants ha ⁻¹
Row spacing	76.2 cm	76.2 cm

Table 2. Soil test analysis results for the study sites in 2009 and 2010.

Soil parameter	2009	2010
	0–20 cm	0–20 cm
Soil pH	6.6	6.6
Organic matter, %	3	3.3
Nitrate-N, mg kg ⁻¹	6.7	4.5
Bray-1 P, mg kg ⁻¹	25.5	24.3
K, mg kg ⁻¹	364	405
Cation exchange capacity	13.3	15.3
Fe, mg kg ⁻¹	52	58.3
S, mg kg ⁻¹	6.6	8.2
Mn, mg kg ⁻¹	7.8	15.4
Ca, mg kg ⁻¹	1838	2156
Mg, mg kg ⁻¹	210	227
Na, mg kg ⁻¹	12	25

2004; Inoue et al., 2008, Wu et al., 2009). All these indices were developed using spectral radiometers or other passive sensors. The same approaches can be used with active crop canopy sensors to calculate vegetation indices for in-season N management. However, the degree they are influenced by water stress and previous crop in corn production is unknown.

The objectives of this study were: (i) to compare the performance of various spectral indices for measuring N status in corn at different irrigation levels and previous crop; (ii) determine the potential of these indices to differentiate N rate at different crop stages; and (iii) compare the correlation of indices collected during vegetative growth stages with grain yield.

MATERIALS AND METHODS

Experimental Design and Site Description

The experimental site was located at the University of Nebraska South Central Agricultural Laboratory (40°34'12" N, -98°8'36" W, 558 m above mean sea level, Map Datum WGS 84) near Clay Center during the 2009 and 2010 growing seasons. The soil at this site is predominantly Crete silt loam (fine, smectitic, mesic Pachic Argiustolls), 0 to 1% slope and previously for 3 yr in continuous corn. Experimental treatments consisted of two irrigation levels (70 and 100% of estimated ET), two previous crops (corn after corn [CC] and corn after soybean [CS]), and four N rates (0, 75, 150, and 225 kg N ha⁻¹). The experimental design was a randomized complete block split split plot, with irrigation level as the main plot, previous crop as the subplot, and fertilizer N rate as sub-subplots. The irrigation treatments were delivered using a linear-move sprinkler system that varied travel speed to change water application rate. Climatological data were recorded on-site for both growing seasons using an automated weather station. Planting dates, plant population, and row spacing were similar both years (Table 1). Soil sampling and analysis was done each spring to characterize soil fertility where the experiments were conducted (Table 2). Soil pH was determined according to Watson and Brown (1998); extractable P and K were determined by Mehlich I (Sims, 1989), organic matter was estimated by loss-on-ignition method (Nelson and Sommers, 1996) and the micronutrients by routine certified laboratory procedures. The previous soybean crop was planted during the 2008 growing season to start the crop sequence. Crops were planted and managed using best management practices for high yielding corn, optimizing the supply of all crop nutrients other than N (Table 2).

Crop Canopy Sensing

Crop canopy reflectance was measured for corn during the following growth stages V11, V13, V15, R2, R3, and R4 (Abendroth et al., 2011) using two active canopy sensors—a two-band sensor (880 and 590 nm, Crop Circle 210), and a three band sensor (760, 720, and 670 nm, Crop Circle 470) (Holland Scientific, Lincoln, NE). The platform used for sensor data acquisition consisted of a bicycle modified to support two optical sensors, a GeoXT GPS receiver (Trimble Navigation, Ltd., Sunnyvale, CA) and a netbook computer (Fig. 1).

The platform provided the ability to maintain a distance of at least 60 cm between the sensors and the top of the crop canopy when acquiring readings throughout the growing

season and avoiding soil compaction near the row and additional damage that could occur if high clearance machinery were used. Each plot (9.14 by 6.09 m) consisted of eight rows, and rows 3 and 6 were sensed at each growth stage with about 30 sensor output mean values recorded per plot. Both optical sensors were mounted together to measure the crop reflectance at about the same target (sensors were mounted 0.3 m apart). To sense rows 3 and 6, two passes were made through each plot. Approximately 12 readings from each sensor were averaged to record with each geographical location. With the typical speed traveled through plots, and one GPS location recorded per second, approximately 30 geographic locations were recorded for each plot. Sensors measurements were collected and integrated (averaged) using customized LabView software (National Instruments, Austin, TX), filtered using MathLab (Mathworks, Natick, MA), Microsoft Excel and ArcGIS 9.3 (ESRI, Redlands, CA) to eliminate the plot-border effect and some GPS inaccuracies. Collected and filtered data were used for statistical data analysis (SAS 9.2) (SAS, Cary, NC).

Vegetation Indices

Six vegetation indices (CI, chlorophyll index vegetation index using amber and NIR, CIRE, chlorophyll index vegetation index using red-edge and NIR) were evaluated in terms of their potential to differentiate N rates with both irrigation levels and previous crop (Table 3). The criteria for index selection for N assessment was guided by previous successful use in cereal crops (CI and NDVI); possibility of use with satellite imagery (MTCI) and by the ranking proposed by Lemaire et al. (2004), where the root mean square error (RMSE) was minimized and the agreement with the PROSPECT Model (Jacquemoud and Baret, 1990) was maximized for chlorophyll estimation (it was the case for the DATT index).

All vegetation index values were normalized (actual index value divided by the index value of the highest N rate) to facilitate comparison among indices and to perform statistical analysis. The normalization was cited in previous work as the sufficiency index (SI) and is used to minimize factors that can affect vegetation indices, including N rate, hybrid, stages of growth, and environmental conditions. (Scheepers et al., 1992; Scheepers, 1994; Varvel et al., 1997).

Table 3. Vegetation index formulas and wavebands used in this study.

Indices	Wavebands† (nm)	Formula	Source
CI‡	880, 590	$CI = (R_{880}/R_{590}) - 1$	Gitelson et al., 2005
CIRE	760, 720	$CIRE = (R_{760}/R_{720}) - 1$	Gitelson et al., 2005
DATT	760, 720, 670	$DATT = (R_{760} - R_{720}) / (R_{760} - R_{670})$	Datt, 1999
NDVI_Red	760, 670	$NDVI_Red = (R_{760} - R_{670}) / (R_{760} + R_{670})$	Rouse et al., 1974
NDVI_RE	760, 720	$NDVI_RE = (R_{760} - R_{720}) / (R_{760} + R_{720})$	Rouse et al., 1974
MTCI	760, 720, 670	$MTCI = (R_{760} - R_{720}) / (R_{720} - R_{670})$	Dash & Curran, 2004

† For our calculation, we used the bands 880 and 590nm (Crop Circle, Model 210 sensor), 760, 720, 670 nm (Crop Circle, Model 470 sensor) because these were the wavebands collected by the respective sensors.

‡ CI, chlorophyll index vegetation index using amber and near infrared; CIRE, chlorophyll index vegetation index using red edge and near infrared; DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); NDVI_Red, normalized difference vegetation index using the red band; NDVI_RE, normalized difference vegetation index using the red edge band; MTCI, Meris terrestrial chlorophyll index.

Soil Moisture Measurement and Crop Yield Assessment

Soil moisture content was monitored hourly during the growing season by means of Watermark soil moisture sensors (Irrometer Co, Riverside, CA) installed at 30, 61, and 91 cm depths in plots with 225 kg N ha⁻¹ for the two different water levels (70 and 100% ET) and previous crop (CC and CS). For comparison between irrigation levels, the soil matric potential was averaged by day for each depth.

Grain yield for each plot was measured with a plot combine Gleaner K (two rows) using the Harvest Master System (Juniper Systems Inc., Logan, UT) and corrected to an average grain moisture content of 15.5 g kg⁻¹.

Statistical Analyses

To evaluate treatment effects on grain yield, the 2 yr of data were analyzed by the PROC MIXED procedure of SAS for ANOVA and means separation using the Duncan's Multiple Range Test ($p < 0.1$), Year, irrigation levels, previous crop, as well as replications were considered random effects. The effects of treatments on vegetation indices also used repeated measures ANOVA (time-repeated measures analysis) with PROC MIXED since several growth stages were measured for each of the six vegetation indices evaluated both years, with different previous crop, irrigation levels, and N rates. Again, only two levels of irrigation were tested, Year was included as a random effect and was considered a replication of irrigation level in the statistical analysis. To test the ability of the vegetation indices to differentiate N rates with different previous crop, the Duncan Multiple Range Test ($p < 0.10$) was used disregarding irrigation effects. The vegetation indices were tested for the effects of irrigation levels comparing the variance between the vegetation indices considering variation caused by two irrigation levels (70 and 100% ET) using the Bartlett's test. The vegetation indices were ranked by pairwise *F* test comparison from the least to the most affected by irrigation levels, considering the variation caused by irrigation levels for each index during 2 yr. Lastly to measure the relationship between vegetation indices, chlorophyll meter, and grain yield PROC GLM and MANOVA were used to obtain partial correlations adjusting for irrigation levels and previous crop.

RESULTS AND DISCUSSION

Rainfall and temperature history, along with application amounts for the 100% ET irrigation treatment, are shown in Fig. 2 for both growing seasons. Overall climatic conditions were near normal for this location, although 2009 was slightly warmer and drier in the early season than the same period in 2010. Consequently, irrigation was initiated earlier in 2009 (around V10) than in 2010 (around V13) (Fig. 2). The soil-moisture content at V11 and V13 was lower for the 70% ET treatment compared to 100% ET, even without the irrigation which was implemented in 2010 (Fig. 3), likely due to irrigation limitation imposed in the previous season.

Treatment Effects on Grain Yield

Irrigation Level

There was no effect of irrigation levels (70 and 100% ET) on corn grain yield, and neither of the two-way interactions of interest (Previous Crop \times Irrigation and N \times Irrigation) were significant. Al-Kaisi and Yin (2003), studying the effects of irrigation, plant population and N rate on corn yield, observed similar results where application of water at 80 and 100% ET had no difference in water extraction from the soil profile and also no yield advantage for 100% ET. Such results suggest that reducing irrigation level (e.g., 80% ET) can save water with little impact on grain yield.

In 2009, grain yield for irrigation levels were significantly different, with the 100% ET treatment yielding 591 kg ha⁻¹ more than the 70% ET treatment. However, in 2010 there were no statistically significant differences in grain yield with irrigation level, though the difference was still 487 kg ha⁻¹. In 2009, the average grain yield was higher and optimized by irrigation. Yield differences due to water levels can vary due to several factors, such as irrigation timing. Payero et al. (2009) showed that corn yield with the same level of water supply can vary with different timing of irrigation application.

During both years, and by grouping irrigation levels (disregarding the previous crop), the difference between 70 and 100% ET treatment yields were 538 kg ha⁻¹, with 10,846 and 11,385 kg ha⁻¹ for 70 and 100% ET, respectively (Fig. 4). For the CC treatment, yields were 9322 kg ha⁻¹ and 9323 kg ha⁻¹ for 70 and 100% ET respectively, showing no yield advantage due to the higher irrigation level (Fig. 5). For the CS treatment, yields were 12,370 and 13,447 kg ha⁻¹ for 70 and 100% ET with a difference of 1077 kg ha⁻¹ (significant at $p < 0.1$, Duncan's Multiple Range Test) (Fig. 5).

Previous Crop and Nitrogen Rate

The N \times Previous Crop was statistically significant, indicating that yield responses to N were different between the two previous crops (Table 4 and Fig. 4).

Average yield differences between previous crops were considerable (3585 kg ha⁻¹). This shows how legumes as a previous crop can improve crop productivity, with greater access to mineralized soil N due to the low C/N ratio of the soybean residue. In 2009, at the 70% ET irrigation level, yield differences between CC and CS were 2924 kg ha⁻¹ ($p < 0.01$), with yields of 10,763 kg ha⁻¹ and 13,688 kg ha⁻¹ respectively. For the 100% ET irrigation level, the differences were similar (2963 kg ha⁻¹), but yield levels were higher (11,334 and 14,294 kg ha⁻¹).

All N fertilization rates significantly increased corn yield with corn as the preceding crop, showing almost linear response to N. On the other hand, fertilizer N rate higher than 150 kg N ha⁻¹ did not increase grain yield when the previous crop was soybean in 2009 (Fig. 4).

For the CS treatment in 2010, there were higher yields with the 100% ET treatment compared to the 70% ET treatment, and greater yield response to N at lower N rates (Fig. 5).

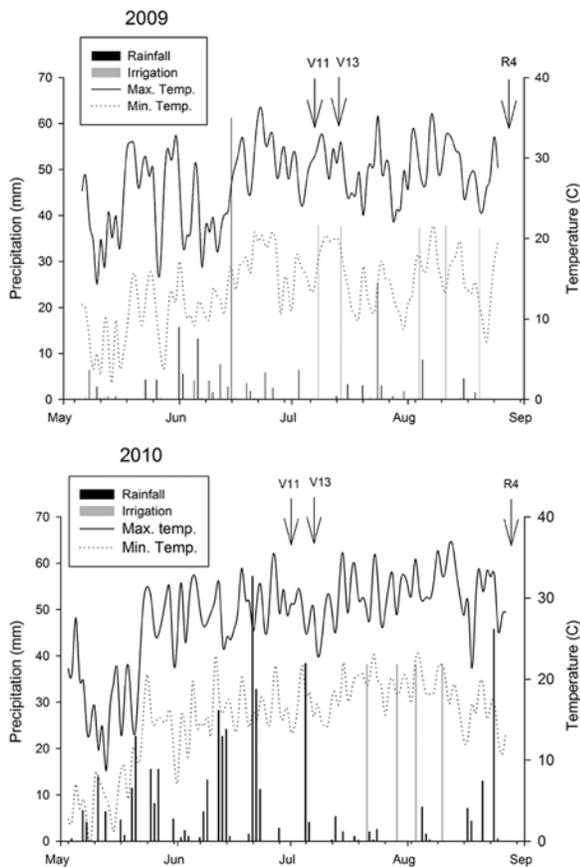


Fig. 2. Daily rainfall, irrigation, and air temperatures for the 2009 and 2010 growing seasons at the South Central Agricultural Laboratory.

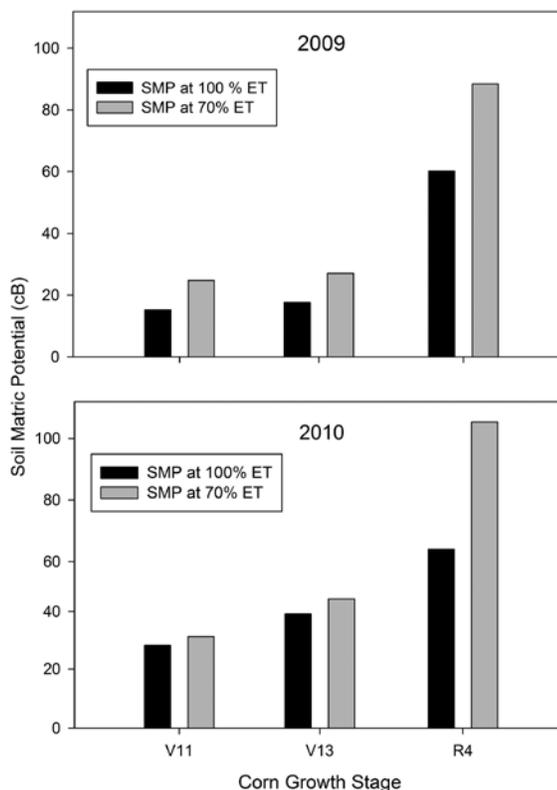


Fig. 3. Soil matrix potential (SMP) measured by Watermark sensors at V11, V13, and R4 growth stages at 61-cm soil depth.

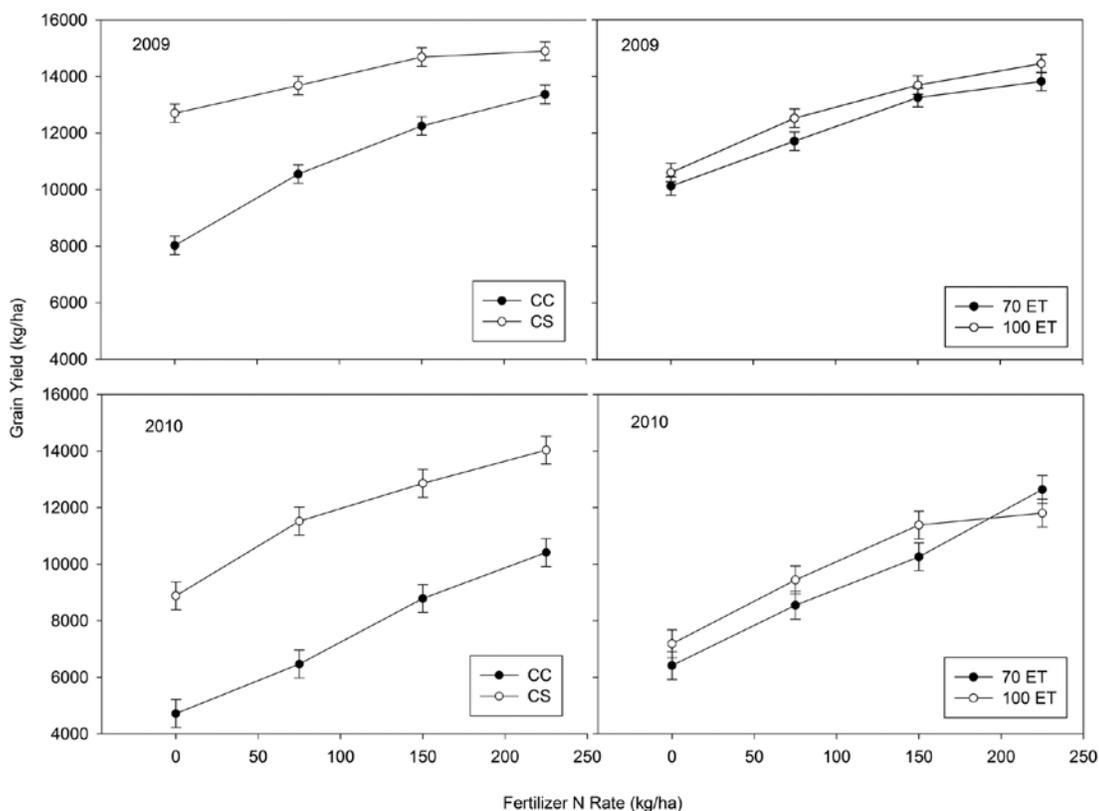


Fig. 4. Grain yield as influenced by N rate, previous crop and water levels during 2009 and 2010. Errors bars represent standard error.

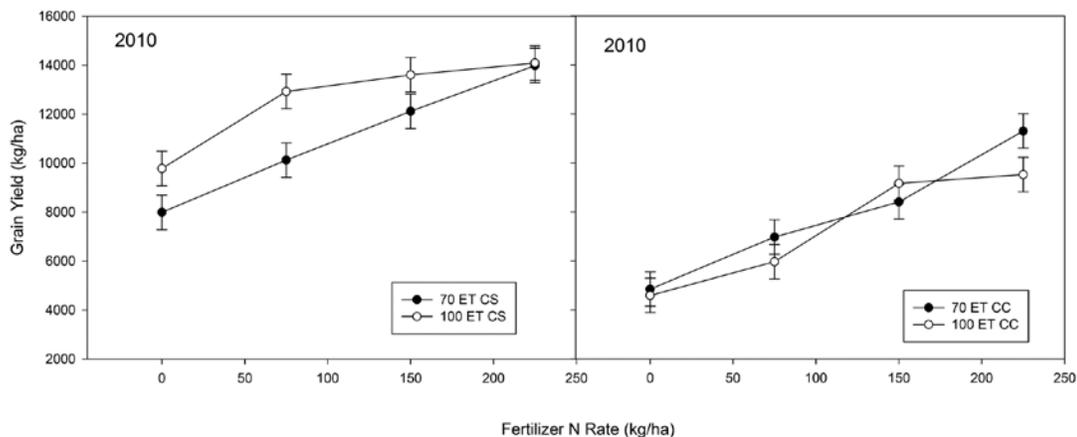


Fig. 5. Grain yield as influenced by N rate, under different water levels with different previous crop (CC and CS). Errors bars represent standard error.

Table 4. Analysis of variance of corn yield (2009 and 2010) for 70 and 100% evapotranspiration (ET) under different previous crop (irrigated corn after corn [CC] and irrigated corn after soybean [CS]).

Source of variation	Num DF	Den DF	F value	P > F
Irrigation	1	9	2.19	0.1733
Previous crop	1	10	123.26	<0.0001
Previous crop × Irrigation	1	10	2.78	0.1264
N	3	60	106.43	<0.0001
N × Irrigation	3	60	1.28	0.2903
N × Previous crop	3	60	4.6	0.0058
N × Previous crop × Irrigation	3	60	0.26	0.8575

Treatments Effects on Vegetation Indices Water Effects on Vegetation Indices

The amount of water available to plants began to be limiting around V11 in 2009 and after V13 in 2010, when irrigation commenced and there were differences in soil-moisture levels between irrigation treatments (Fig. 2 and 3). Due to rainfall patterns, the effect of irrigation level on vegetation indices were evaluated at later growth stages than the time window recommended for N application. Treatments were evaluated when soil moisture levels were different between irrigation levels (V11 and R4 growth stages in 2009 and R4 in 2010). Only spectral reflectance data collected at the V11 through R4 growth stages were included in the ANOVA (Table 5). The ANOVA

Table 5. Analysis of variance of six vegetation indices calculated from active canopy sensor reflectance at different irrigation levels (70 and 100% evapotranspiration [ET]) and different previous crop (irrigated corn after corn [CC] and irrigated corn after soybean [CS]) between growth stages V11 and R4.

Source of variation	Num DF	CI†	CIRE	DATT	MTCI	NDVI_RE	NDVI Red
Effect							
Irrigation	1	0.179	0.2972	0.5847	0.5341	0.3894	0.4446
Previous crop	1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Previous crop × Irrigation	1	0.104	0.0924	0.0755	0.0621	0.1373	0.5467
N	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
N × Irrigation	3	0.749	0.6557	0.8788	0.8425	0.7532	0.899
N × Previous crop	3	<0.0001	<0.0001	0.0008	0.0041	<0.0001	0.0011
N × Previous crop × Irrigation	3	0.9535	0.7933	0.8833	0.8268	0.8548	0.9711
Stage	4	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Stage × Irrigation	4	0.5524	0.6846	0.191	0.1626	0.4712	0.4473
Stage × Previous crop	4	<0.0001	<0.0001	0.3835	0.043	0.0008	<0.0001
Stage × Previous crop × Irrigation	4	0.6904	0.836	0.469	0.5019	0.934	0.8897
Stage × N	12	0.0038	0.1772	0.0974	0.3744	0.5884	0.0004
Stage × N × Irrigation	12	0.9748	0.9997	0.9994	0.9999	0.9986	0.9985
Stage × N × Previous crop	12	0.0209	0.0271	0.7407	0.6584	0.0146	0.0009
Stage × N × Previous crop × Irrigation	12	0.9999	0.9979	0.9999	0.9998	0.9996	0.9783

† CI, chlorophyll index vegetation index using amber and near infrared; CIRE, chlorophyll index vegetation index using red edge and near infrared; DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); MTCI, Meris terrestrial chlorophyll index; NDVI_RE, normalized difference vegetation index using the red edge band; NDVI_Red, normalized difference vegetation index using the red band.

for vegetation indices indicated that during the period of V11 through R4, there were no incidences of statistically significant four- and three-way interactions of treatment effects on vegetation indices. Of primary interest then are the significant two-way interactions involving growth stage, previous crop and N rate. For example, the N × Previous Crop interaction was significant for all indices. The effect of irrigation level on vegetation indices varied between the 2 yr, as expected.

Vegetation index values were normalized (actual index value divided by the index value of the highest N rate) to facilitate comparison among indices (Fig. 6 and 7).

Before testing the response of vegetation indices to irrigation level, we evaluated the impacts of site characteristics (background soil fertility, historical management) on vegetation indices early in the growing season when there was no water stress. For both years, there was no influence of site characteristics (soil organic matter differences, soil texture, residual soil N, residue) on canopy reflectance (data not shown), so we can assume that the variations in vegetation indices were influenced primarily by irrigation water level and fertilizer N rate.

The Barlett's Test showed significant differences due to the variance caused by irrigation levels on the six vegetation analyzing all vegetation indices together (degrees of freedom = 5; chi square = 26.71; $p < 0.05$). Using a *F* test to separate pairwise variances the vegetation indices were ranked accordingly to the variance caused by irrigation level (Table 6).

Among all indices tested, the DATT index was least influenced by irrigation level and showed the lowest mean square error and standard error (Table 6, Fig. 6 and 7). This may be particularly important in environments where water stress is

likely to be confounded with N stress, but its response to N rates was smaller during the window for in-season N management for both years compared to MTCI or CIRE. The DATT vegetation index was first validated for sensitivity to chlorophyll content in uncorrected as well as scatter-corrected spectra, showing that this index can enhance the chlorophyll absorption in reflectance by removing the interferences caused by variations in leaf scatter that can be affected by water stress and leaf and architecture (Datt, 1999).

The MTCI was the second least influenced by irrigation level, but it had a higher standard error for fertilizer N rate than the DATT index (Fig. 6 and 7). However, the N response was better in the sense of showing less saturation with an increase of N rate, and it displayed better distinguishing ability with regard to differences in N supply. The vegetation indices CI and CIRE showed good responses to N rates but they were the most affected by water level at V11 and R4 (Table 6, Fig. 6 and 7).

The NDVI_RE was the third best index in terms of identifying N stress independent of irrigation level, but it plateaued beyond 75 kg ha⁻¹ ha of fertilizer N in 2009, limiting its utility for N fertilizer application and compromising its ability to differentiate N rates in that particular year. It is important to point out that the slope of response for N rates was smaller (as expected) when NDVI was used to estimate chlorophyll content or biomass due to saturation at high leaf area index (LAI) at those corn stages (Gitelson and Merzlyak, 1996). So, the expected saturation of the NDVI (Gitelson, 2004) also occurred in this experiment at these growth stages (Fig. 6K and 6L, and 7K and 7L).

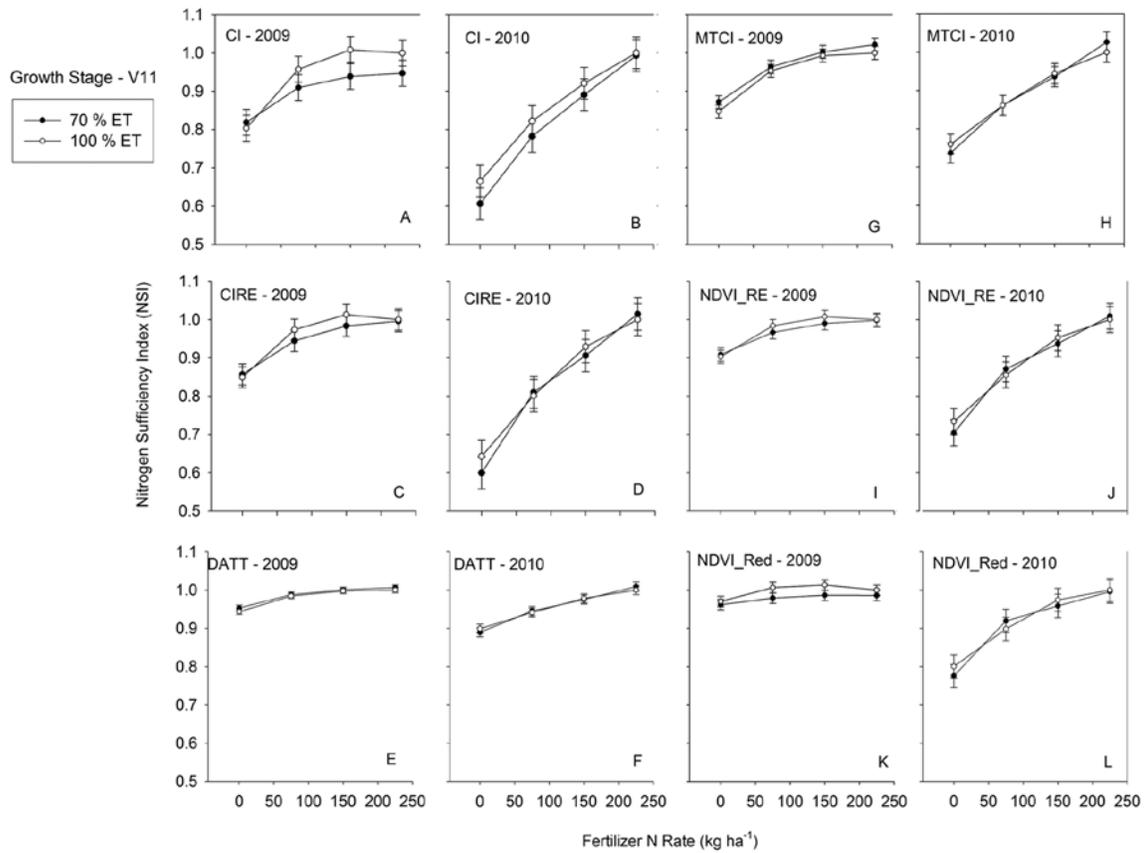


Fig. 6. Vegetation indices response to fertilizer N rate and irrigation level, at the V11 growth stage during 2009 and 2010 growing seasons. Error bars represent standard error.

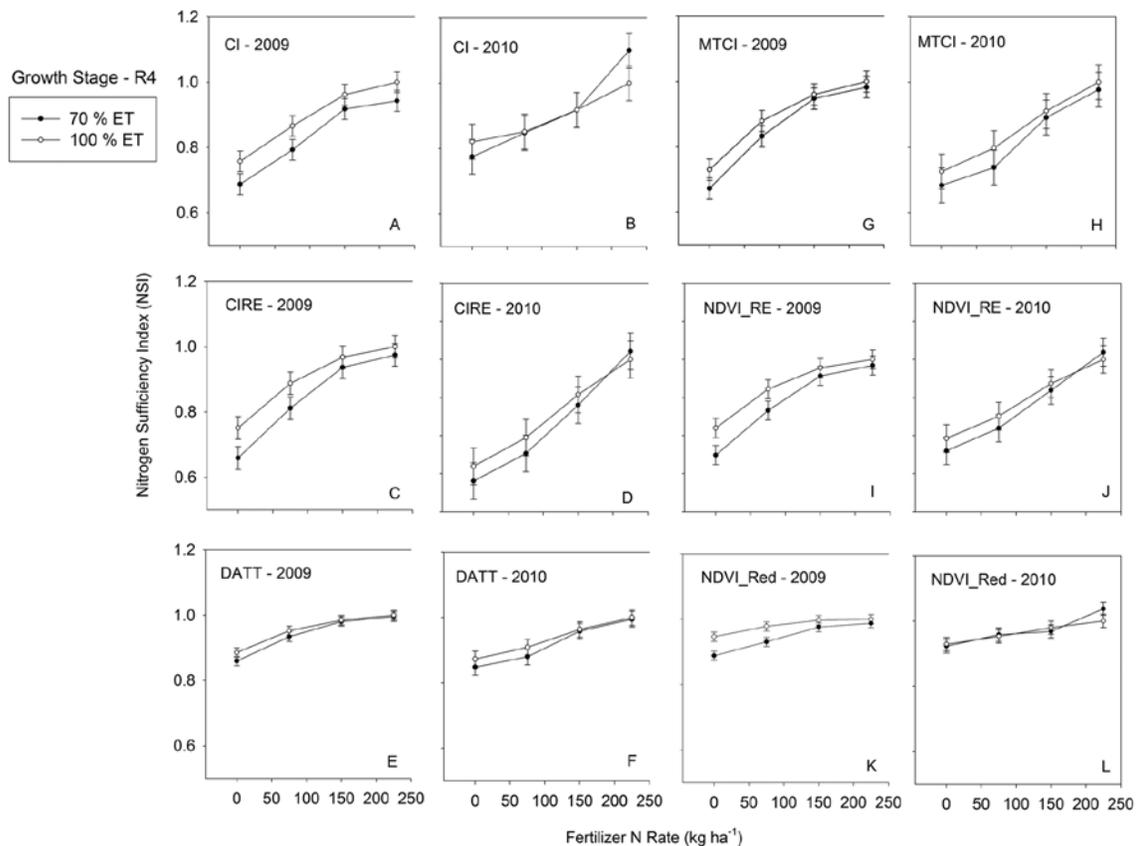


Fig. 7. Vegetation indices response to fertilizer N rate and irrigation level, at the R4 growth stage during 2009 and 2010 growing seasons. Error bars represent standard error.

Table 6. Ranking of variation to irrigation levels analyzed during 2009 and 2010 growing seasons. Values followed by the same letter are not significantly different ($p < 0.10$).

Rank	Vegetation index	Mean squared error	F test $p < 0.10$
Least affected			
1	DATT†	0.00006199	c
2	MTCI	0.00017699	b
3	NDVI_RE	0.00036718	b
4	NDVI_Red	0.00044026	b
5	CIRE	0.00058541	ab
6	CI	0.00163116	a
Most affected			

† DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); MTCI, Meris terrestrial chlorophyll index; NDVI_RE, normalized difference vegetation index using the red edge band; NDVI_Red, normalized difference vegetation index using the red band; CIRE, chlorophyll index vegetation index using red edge and near infrared; CI, chlorophyll index vegetation index using amber and near infrared.

In general, we observed that corn plants under water stress (70% ET) had changes in leaf structure rather than LAI, but only at later stages (after VT). As reported earlier, impacts of water stress will vary with growth stage, but water stress at early growth stages will affect LAI the most (Çakir, 2004). Normally, in this region of the Great Plains, irrigation commences between V14 and VT growth stages, depending on stored

soil water and precipitation. Consequently, irrigation effects on vegetation indices may not be evident until after the VT growth stage (Fig. 7). During vegetative stages, there were more pronounced effects of irrigation level only at (V11) in 2009, as it was a drier season than 2010.

Ability of the Vegetation Indices to Differentiate Nitrogen Rates

Among the indices proposed for assessment of crop N status, CIRE, MTCI, and NDVI_RE did not have a significant N × stage interaction, indicating that these indices did not require a specific growth stage within this window (V11 until R4) to differentiate N rates. All other N management indices need adjustments for specific growth stage to be used for managing N within the window studied. All indices had similar responses, but CI, CIRE, and NDVI_Red had significant N × Previous Crop × stage interactions, indicating that those indices may vary in their ability to differentiate the impact of N rate at these growth stages.

In 2009 the CI, CIRE, DATT, and MTCI indices could differentiate fertilizer rates of 0, 75, and 150 kg N ha⁻¹ for the CC at V11 until R6, while the NDVI_RE index was only able to differentiate 0 kg ha⁻¹ from the other N rates at the R6 growth stage (all averaged over irrigation level). The NDVI_Red index could differentiate N rates at V13, R2 and R4. The DATT and MTCI indices could differentiate fertilizer rates of 0, 75, and 150 kg N ha⁻¹ with the CS at V15 (Table 7). The CIRE

Table 7. Nitrogen sufficiency index (NSI) for the vegetation indices during several growth stages and N rates with corn-soybean (CS). Means followed by the same letter are not significantly different ($p < 0.10$).

CS NSI	N rate	2009						2010						
		V11	V13	V15	R2	R3	R4	V11	V13	V15	R2	R3	R4	
	kg ha ⁻¹													
CI†	0	0.99a	0.98a	0.92a	0.88c	0.84c	0.83c	0.75c	0.78c	0.91c	0.89b	0.85c	0.69b	
	75	1.02a	1.00a	0.96a	0.96b	0.93b	0.94b	0.92b	0.90b	1.01b	0.97a	0.93b	0.77a	
	150	1.03a	1.01a	0.98a	0.99a	0.97a	1.02a	0.97a	0.96a	1.18a	1.05a	1.00a	0.83a	
CIRE	0	0.97b	0.96b	0.91b	0.86c	0.84b	0.81c	0.74c	0.76c	0.92c	0.81c	0.80c	0.66c	
	75	1.02a	1.00a	0.98a	0.95b	0.95a	0.95b	0.91b	0.90b	1.00b	0.91b	0.90b	0.76b	
	150	1.02a	1.02a	1.00a	1.00a	0.98a	1.02a	0.96a	0.96a	1.08a	0.98a	0.96a	0.86a	
DATT	0	0.98b	0.98b	0.97c	0.95c	0.95c	0.93c	0.92c	0.91c	0.97c	0.92c	0.91c	0.85c	
	75	0.99a	0.99a	0.99b	0.98b	0.98b	0.99b	0.97b	0.97b	0.99b	0.96b	0.95b	0.90b	
	150	1.00a	1.00a	1.00a	1.00a	1.00a	1.01a	0.99a	0.99a	1.01a	0.99a	0.98a	0.97a	
MTCI	0	0.94b	0.94b	0.91c	0.85c	0.83c	0.80c	0.79c	0.76c	0.92c	0.79c	0.77c	0.68c	
	75	0.98a	0.98a	0.97b	0.94b	0.94b	0.96b	0.92b	0.90b	0.98b	0.90b	0.88b	0.78b	
	150	1.00a	1.00a	0.99a	0.99a	0.99a	1.02a	0.96a	0.96a	1.03a	0.96a	0.94a	0.92a	
NDVI_RE	0	0.98b	0.98b	0.95b	0.92c	0.90b	0.87b	0.84c	0.85c	0.95c	0.88c	0.87c	0.75c	
	75	1.01a	1.00a	0.99a	0.97b	0.97a	0.97a	0.95b	0.94b	1.00b	0.94b	0.94b	0.82b	
	150	1.01a	1.01a	1.00a	1.00a	0.99a	1.01a	0.98a	0.98a	1.04a	0.99a	0.97a	0.90a	
NDVI_Red	0	1.02a	1.01a	0.99b	0.99b	0.97b	0.95b	0.93b	0.96c	0.99b	0.98b	0.99a	0.90a	
	75	1.02a	1.01a	1.00a	1.00a	1.00ab	0.98b	0.98a	0.99b	1.01b	0.99b	1.00a	0.93a	
	150	1.02a	1.01a	1.00a	1.00a	0.99ab	1.00a	1.00a	1.00a	1.04a	1.01a	1.00a	0.93a	

† CI, chlorophyll index vegetation index using amber and near infrared; CIRE, chlorophyll index vegetation index using red edge and near infrared; DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); MTCI, Meris terrestrial chlorophyll index; NDVI_RE, normalized difference vegetation index using the red edge band; NDVI_Red, normalized difference vegetation index using the red band.

Table 8. Nitrogen sufficiency index (NSI) for the vegetation indices during several growth stages and N rates with irrigated corn after corn (CC). Means followed by the same letter are not significantly different ($p < 0.10$).

CC NSI	N rate	2009						2010					
		V11	V13	V15	R2	R3	R4	V11	V13	V15	R2	R3	R4
	kg ha ⁻¹												
CI†	0	0.68c	0.67c	0.66b	0.65c	0.65c	0.65c	0.50c	0.58c	0.61c	0.71c	0.75c	0.85b
	75	0.89b	0.87b	0.86a	0.83b	0.81b	0.76b	0.67b	0.72b	0.72b	0.84b	0.85b	0.86b
	150	0.97a	0.94a	0.92a	0.95a	0.91a	0.91a	0.84a	0.86a	1.02a	0.93a	0.95a	0.93a
CIRE	0	0.73c	0.70c	0.63c	0.63c	0.58c	0.60c	0.46c	0.54c	0.60c	0.69c	0.69c	0.73b
	75	0.90b	0.87b	0.83b	0.78b	0.77b	0.76b	0.66b	0.70b	0.73b	0.81b	0.82b	0.78b
	150	0.98a	0.96a	0.94a	0.87a	0.90a	0.91a	0.85a	0.87a	1.00a	0.93a	0.92a	0.91a
DATT	0	0.91c	0.89c	0.87c	0.85c	0.83c	0.82c	0.86c	0.85c	0.87c	0.88c	0.87c	0.88b
	75	0.97b	0.96b	0.95b	0.94b	0.91b	0.90b	0.90b	0.90b	0.92b	0.92b	0.92b	0.89b
	150	0.99a	0.99a	0.98a	0.98a	0.96a	0.96a	0.96a	0.96a	0.99a	0.97a	0.96a	0.96a
MTCI	0	0.76c	0.72c	0.66c	0.63c	0.59c	0.59c	0.68c	0.66c	0.72c	0.73c	0.72c	0.75b
	75	0.91b	0.88b	0.84b	0.82b	0.76b	0.75b	0.77b	0.76b	0.81b	0.81b	0.81b	0.78b
	150	0.98a	0.96a	0.94a	0.94a	0.89a	0.90a	0.89a	0.89a	0.98a	0.91a	0.90a	0.91a
NDVI_RE	0	0.89b	0.87b	0.83b	0.84b	0.80c	0.81a	0.58c	0.66c	0.71c	0.78c	0.77c	0.79b
	75	0.98b	0.97b	0.95b	0.95b	0.94b	0.92a	0.76b	0.79b	0.81b	0.87b	0.88b	0.83b
	150	1.01a	1.01a	1.01a	1.01a	0.99a	1.00a	0.90a	0.92a	1.00a	0.95a	0.95a	0.94a
NDVI_Red	0	0.93b	0.92c	0.90b	0.92c	0.89b	0.89c	0.64c	0.78c	0.81c	0.91c	0.90b	0.92c
	75	0.98a	0.97b	0.97a	0.97b	0.95a	0.94b	0.83b	0.89b	0.89b	0.97b	0.98a	0.95b
	150	1.00a	0.99a	0.99a	1.00a	0.98a	0.98a	0.94a	0.96a	1.02a	1.00a	1.00a	0.98a

† CI, chlorophyll index vegetation index using amber and near infrared; CIRE, chlorophyll index vegetation index using red edge and near infrared; DATT, vegetation index calculated using near infrared, red edge, and red bands published by Datt (1999); MTCI, Meris terrestrial chlorophyll index; NDVI_RE, normalized difference vegetation index using the red edge band; NDVI_Red, normalized difference vegetation index using the red band.

and NDVI_RE indices could only differentiate zero from the other N rates at vegetative stages between V11 and V15 with CS. The CI, DATT, and MTCI indices were able to separate N rates after tasseling until R4 but NDVI_RE and NDVI_Red separated N rates only at R6 with CS in 2009.

In 2010, with the CC, all indices could differentiate N rates from V7 until R3. The NDVI_Red could not differentiate 75 kg N ha⁻¹ from 150 kg N ha⁻¹ during R3 (Table 8). With CS, the CI, CIRE, MTCI and DATT indices could differentiate N rates during most of the vegetative growth stages. The NDVI_Red index could not differentiate N rates after V15. Except for R4 all indices could at least separate 0 kg ha⁻¹ from the other N rates. Similar results were found in another long-term CC experiment where a two band sensor (Crop Circle 210) was used to differentiate N rates in small plots, where in a growing season with high N response the sensor could differentiate most N rates (0, 75, 150, and 300 kg N ha⁻¹), and in another growing season they could distinguish only 0 kg ha⁻¹ from other N rates using CI (Shiratsuchi et al., 2009).

Among all indices tested, the MTCI and DATT indices were found to have the best ability to differentiate the effect of fertilizer N rate on crop canopy status, across different levels of irrigation and previous crop. For this reason, these indices were used to illustrate the difference in vegetation-index response with different previous crops (Fig. 8). It is important to stress that MTCI and DATT were minimally affected by irrigation

level and therefore could be better indices to sense for N variances in situations where N deficiency and water stress occurs simultaneously.

In dry environments where irrigation is imposed and the water management is near optimal, the DATT and MTCI could perform better for site-specific N management than the other vegetation indices tested, because they showed better sensitivity to N rate and less variability due to irrigation levels. If the previous crop was soybean in a rainfed environment, these indices are preferable preferred due to their ability to separate N rates with soybeans as the preceding crop.

Relationships between Vegetation Indices and Grain Yield

In 2009, at the V11, V13, and V15 growth stages, all vegetation indices showed a high partial correlation with final grain yield (Fig. 9) adjusting for water and previous crop. The NDVI_RE, CIRE, MTCI, and DATT indices showed high, and similar, correlations at all growth stages studied, even higher than the chlorophyll meter (SPAD). The NDVI_Red index had a lower correlation with final grain yield at V11 and V13 growth stages compared to other indices. All correlations showed the same trend of stronger relationships at later growth stages except for SPAD. Due to sampling procedures (amount and method) and practicality, the chlorophyll meters may be biased due to human error during data gathering. Chlorophyll

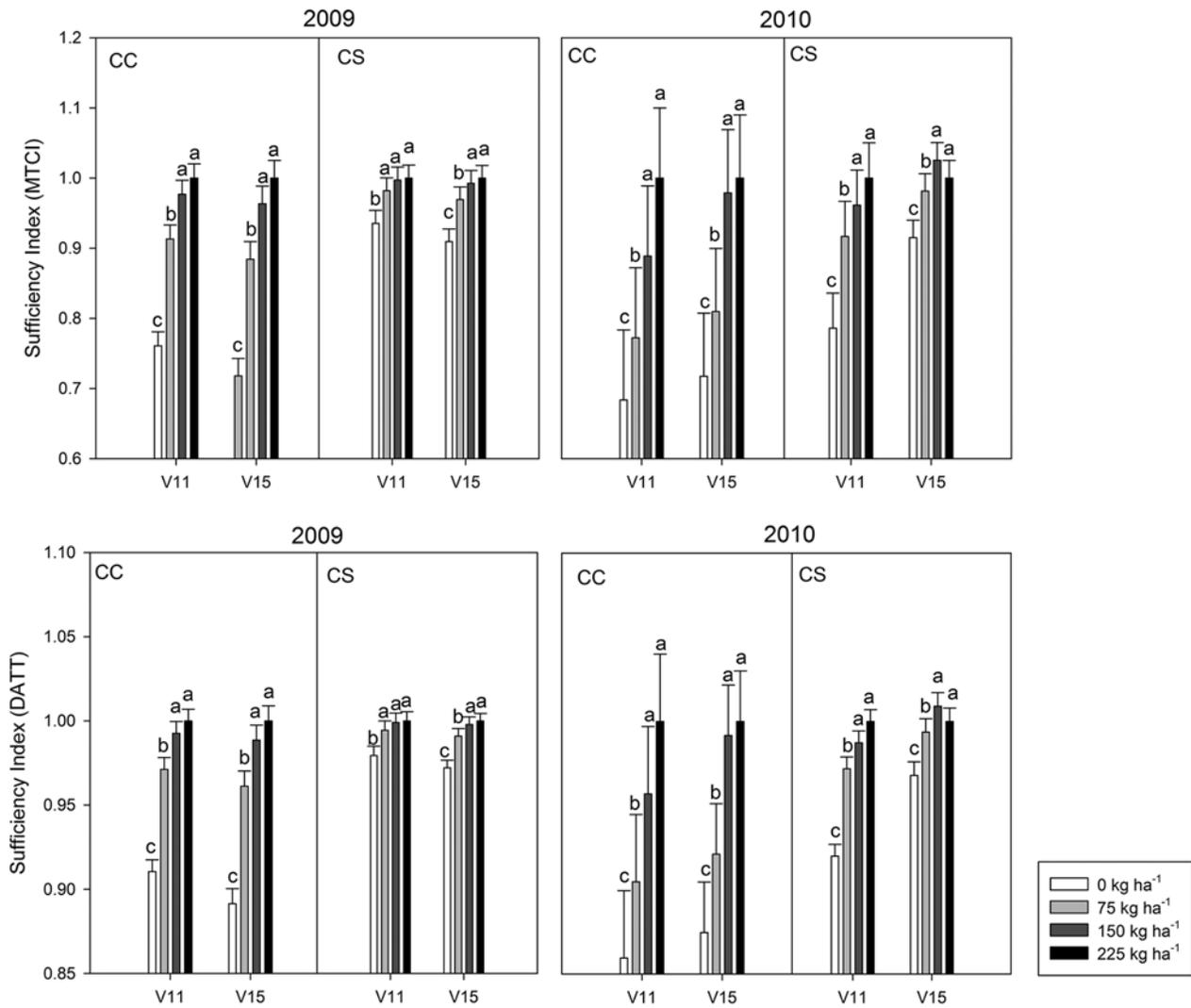


Fig. 8. The effect of previous crop (CC and CS) and growth stage on crop canopy reflectance using two vegetation indices, MTCI and DATT, averaged across water level for 2009 and 2010. Errors bars represent standard error and letters the statistically significant differences according to Duncan's Multiple Range Test ($p < 0.10$).

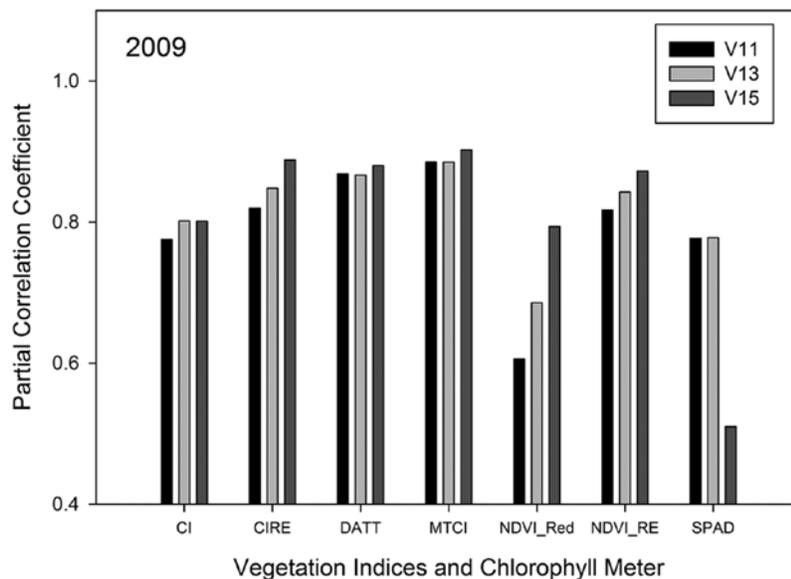


Fig. 9. Partial correlation coefficient values between vegetation indices and grain yield for three growth stages in 2009 accounting for irrigation levels and previous crop. All correlations were significant with $p < 0.01$.

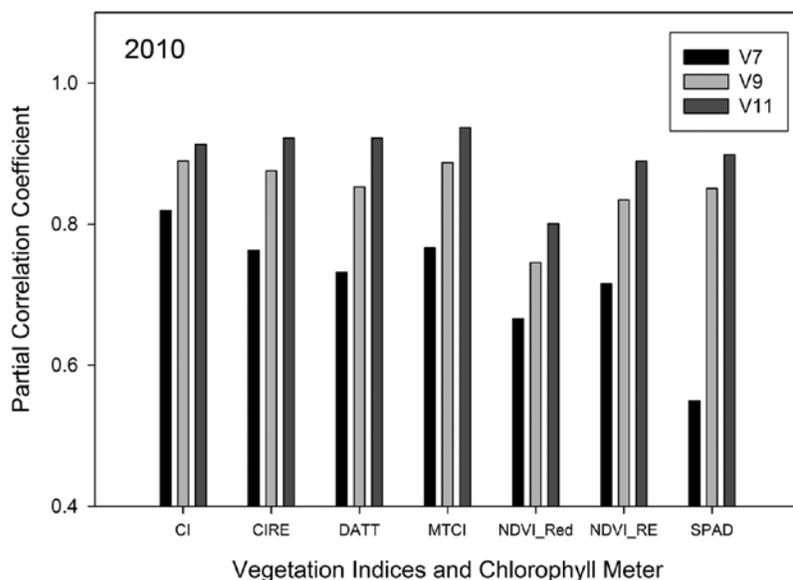


Fig. 10. Partial correlation coefficient values between vegetation indices and grain yield for three growth stages in 2010 accounting for irrigation levels and previous crop. All correlations were significant with $p < 0.01$.

meters may need a specific growth stage and should be sampled at the ear leaf (after silking) for best performance (Costa et al., 2001), but they are less sensitive to variations in canopy structure that causes changes in reflectance.

The same trend of increasing correlation between vegetation index and grain yield with growth stage was observed in 2010. All vegetation indices showed relatively high partial correlations with grain yield at V7, V9, and V11 growth stages (Fig. 10). The NDVI_Red and SPAD had the lowest correlations, though still relatively high and significant. The CIRE, CI, MTCI, and DATT indices had the highest correlation with grain yields. The good associations between grain yield and these types of indices at early growth stages (V7, V9, and V11) bode well for the many applications of this technology to better meet crop N demand in-season, reducing N loss and protecting the environment.

SUMMARY AND CONCLUSIONS

This study investigated how active crop canopy reflectance, measured by different vegetation indices (calculating NSI), was influenced with different levels of irrigation, fertilizer N rate, and previous crop. We investigated the ability of these indices to differentiate fertilizer N rate under various conditions, and the correlation of indices collected during various growth stages with grain yield. Among the indices studied, the MTCI and DATT indices were the least affected by irrigation level, and by inference, water stress, with ability to differentiate fertilizer N rates with both continuous corn and corn following soybean. The CI, CIRE, NDVI_RE, and NDVI_Red indices showed more variation due to irrigation level, and low ability to distinguish fertilizer N rate with corn following soybean. The ranking from the least affected by irrigation level to the most affected were DATT, MTCI, NDVI_RE, NDVI_Red, CIRE, and CI. Comparing the vegetation indices for N differentiation, DATT and MTCI had the best ability to separate N rates, so these two indices are more appropriate if low variance for water stress and high ability to distinguish N rates across previous crop is needed. All vegetation indices had good correlation

with final grain yield when sampled between V11 and V15, where MTCI and DATT again were stronger across years. The results suggest that careful attention should be given to how water stress and previous crop can affect the ability of these vegetation indices to determine crop N status.

REFERENCES

- Abendroth, L.J., R.W. Elmore, M.J. Boyer, and S.K. Marlay. 2011. Corn growth and development. PMR 1009. Iowa State Univ. Ext., Ames.
- Al-Kaisi, M.M., and X. Yin. 2003. Effects of nitrogen rate, irrigation rate, and plant population on corn yield and water use efficiency. *Agron. J.* 95:1475–1482. doi:10.2134/agronj2003.1475
- Bausch, W.C., and H.R. Duke. 1996. Remote sensing of plant nitrogen status in corn. *Trans. ASAE* 39:1869–1875.
- Cakir, R. 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crops Res.* 89:1–16. doi:10.1016/j.fcr.2004.01.005
- Clay, D.E., K.I. Kim, J. Chang, S. Clay, and K. Dalsted. 2006. Characterizing water and nitrogen stress in corn using remote sensing. *Agron. J.* 98:579–587. doi:10.2134/agronj2005.0204
- Costa, C., L. Dwyer, P. Dutilleul, D. Stewart, B. Ma, and D. Smith. 2001. Inter-relationships of applied nitrogen, spad, and yield of leafy and non-leafy maize genotypes. *J. Plant Nutr.* 24:1173–1194. doi:10.1081/PLN-100106974
- Dash, J., and P.J. Curran. 2004. The MERIS terrestrial chlorophyll index. *Int. J. Remote Sens.* 25:5403–5413. doi:10.1080/0143116042000274015
- Datt, B. 1999. Visible/near infrared reflectance and chlorophyll content in Eucalyptus leaves. *Int. J. Remote Sens.* 20:2741–2759. doi:10.1080/014311699211778
- Dellinger, A.E., J.P. Schmidt, and D.B. Beegle. 2008. Developing nitrogen fertilizer recommendations for corn using an active sensor. *Agron. J.* 100:1546–1552. doi:10.2134/agronj2007.0386
- Eitel, J.U.H., D.S. Long, P.E. Gessler, and E.R. Hunt. 2008. Combined spectral index to improve ground-based estimates of nitrogen status in dry land wheat. *Agron. J.* 100:1694–1702. doi:10.2134/agronj2007.0362
- Gitelson, A.A. 2004. Wide dynamic range vegetation index for remote quantification of biophysical characteristics of vegetation. *J. Plant Physiol.* 161:165–173. doi:10.1078/0176-1617-01176
- Gitelson, A.A., A. Viña, V. Ciganda, D.C. Rundquist, and T.J. Arkebauer. 2005. Remote estimation of canopy chlorophyll content in crops. *Geophys. Res. Lett.* 32:L08403. doi:10.1029/2005GL022688. doi:10.1029/2005GL022688

- Gitelson, A.A., and M.N. Merzlyak. 1996. Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing. *J. Plant Physiol.* 148:493–500.
- Inoue, Y., J. Penuelas, A. Miyata, and M. Mano. 2008. Normalized difference spectral indices for estimating photosynthetic efficiency and capacity at a canopy scale derived from hyperspectral and CO₂ flux measurements in rice. *Remote Sens. Environ.* 112:156–172. doi:10.1016/j.rse.2007.04.011
- Jacquemoud, S., and F. Baret. 1990. PROSPECT: A model of leaf optical properties. *Remote Sens. Environ.* 34:75–91. doi:10.1016/0034-4257(90)90100-Z
- Lemaire, G., C. François, and E. Dufrêne. 2004. Towards universal broad leaf chlorophyll indices using PROSPECT simulated database and hyperspectral reflectance measurements. *Remote Sens. Environ.* 89:1–28. doi:10.1016/j.rse.2003.09.004
- Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon and organic matter. p. 961–1010. *In* D.L. Sparks et al. (ed.) *Methods of soil analysis*. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, Madison, WI.
- Payero, J.O., D. Tarkalson, S. Irmak, D. Davison, and J.L. Petersen. 2009. Effect of timing of a deficit-irrigation allocation on corn evapotranspiration, yield, water use efficiency and dry mass. *Agric. Water Manage.* 96:1387–1397. doi:10.1016/j.agwat.2009.03.022
- Raun, W.R., J.B. Solie, G.V. Johnson, M.L. Stone, R.W. Mullen, K.W. Freeman, W.E. Thomason, and E.V. Lukina. 2002. Improving nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. *Agron. J.* 94:815–820. doi:10.2134/agronj2002.0815
- Rouse, J.W., R.H. Haas, J.A. Schell, D.W. Deering, and J.C. Harlan. 1974. Monitoring the vernal advancements and retrogradation of natural vegetation. Final rep. NASA/GSFC, Greenbelt, MD.
- Schepers, J.S. 1994. New diagnostic tools for tissue testing. *Commun. Soil Sci. Plant Anal.* 25:817–826. doi:10.1080/00103629409369082
- Schepers, J.S., D.D. Francis, M.F. Vigil, and F.E. Below. 1992. Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. *Commun. Soil Sci. Plant Anal.* 23:2173–2187. doi:10.1080/00103629209368733
- Shanahan, J.F., N.R. Kitchen, W.R. Raun, and J.S. Schepers. 2008. Responsive in-season nitrogen management for cereals. *Comput. Electron. Agric.* 61:51–62. doi:10.1016/j.compag.2007.06.006
- Shiratsuchi, L.S., R.B. Ferguson, V.I. Adamchuck, J.F. Shanahan, and G.P. Slater. 2009. Integration of ultrasonic and active canopy sensors to estimate the in-season nitrogen content for corn. p. 182–188. *In* Proc. 2009 North Central Extension-Industry Soil Fertility Conf., Des Moines, IA, 18–19 Nov. 2009. Int. Plant Nutrition Inst., Brookings, SD.
- Sims, J.T. 1989. Comparison of mehlich 1 and mehlich 3 extractants for P, K, Ca, Mg, Mn, Cu and Zn in Atlantic coastal plain soils. *Commun. Soil Sci. Plant Anal.* 20:1707–1726 doi:10.1080/00103628909368178
- Solari, F., J.F. Shanahan, R.B. Ferguson, J.S. Schepers, and A.A. Gitelson. 2008. Active sensor reflectance measurements of corn nitrogen status and yield potential. *Agron. J.* 100:571–579. doi:10.2134/agronj2007.0244
- Varvel, G.E., J.S. Schepers, and D.D. Francis. 1997. Ability for in-season correction of nitrogen deficiency in corn using chlorophyll meters. *Soil Sci. Soc. Am. J.* 61:1233–1239. doi:10.2136/sssaj1997.03615995006100040032x
- Watson, M.E., and J.R. Brown. 1998. pH and lime requirement. p. 13–16. *In* J.R. Brown (ed.) *Recommended chemical soil test procedures for the north central region*. NCR Res. Publ. 221. Univ. of Missouri, Columbia.
- Wu, C., Z. Niu, Q. Tang, W. Huang, B. Rivard, and J. Feng. 2009. Remote estimation of gross primary production in wheat using chlorophyll-related vegetation indices. *Agric. For. Meteorol.* 149:1015–1021. doi:10.1016/j.agrformet.2008.12.007
- Zygielbaum, A.I., A.A. Gitelson, T.J. Arkebauer, and D.C. Rundquist. 2009. Non-destructive detection of water stress and estimation of relative water content in maize. *Geophys. Res. Lett.* 36:L12403. doi:10.1029/2009GL038906