Winter Wheat Crop Reflectance and Nitrogen Sufficiency Index Values are Influenced by Nitrogen and Water Stress

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ABSTRACT

In-season N applications to winter wheat (*Triticum aestivum* L.) may increase profits and improve N fertilizer accuracy. The objectives of this experiment were to determine the impact of N and water stress on crop reflectance and N sufficiency index (SI) values. The experiment contained five N rates, two water treatments, and four blocks. Crop reflectance was measured at the stem extension and flag leaf growth stages, sufficiency index (SI)-NDVIw$_f$ was ratio between the underfertilized normalized difference vegetation index value {NDVI = [near infrared (NIR)-red]/[NIR+red]} and the NDVI value from well fertilized and well watered treatments, while SI-NDVI$_{mz}$ was ratio between underfertilized NDVI values and NDVI values from well fertilized plots within a water stress treatment. Yield losses due to water and N stress were determined using ^{13}C isotopic discrimination. This research shows that: (i) at the stem extension and flag leaf growth stages, water stress and N stress increased, green, red, and red-edge reflectance and reduced NDVI values (ii) following the economic optimum nitrogen rate (EONR) produced grain with greater than 120 g kg^{-1} protein and <10 min stability; (iii) at stem elongation and flag leaf, N fertilizer induced yield gains had a stronger relationship with SI-NDVI$_{mz}$ (stem extension, $r = 0.49^*$; flag leaf, $r = 0.51^{**}$) than SI-NDVI$_{wf}$ (stem extension, $r = 0.29$; flag leaf, $r = 0.33$); and (iv) SI-NDVI$_{mz}$ had greater fertilizer recommendation accuracy than SI-NDVI$_{wf}$. These findings suggest that in wheat production, SI should be referenced to well fertilized areas within a management zone.

Summit areas typically have less available water than lower backslope areas in northern Great Plains landscapes (Reese et al., 2002; Clay et al., 2005a; Mishra et al., 2008). Due to these differences, wheat growing in lower-backslope areas may have a minimal response to additional water and a large response to N, whereas plants in shoulder areas may have a moderate response to both N and water (Kim et al., 2008). Applying a single N rate designed for lower-backslope positions to wheat growing in summit/shoulder areas can produce conditions where N supply exceeds demand. Under these conditions, yields could be reduced, while nitrate leaching or N_2O emissions could be increased (Reese et al., 2002).

Remote sensing-based in-season N applications have been proposed as a technique to manage climate and soil variability. However, in dryland agriculture, in-season techniques must have the ability to separate water and N stress. Separating these stresses is complicated by both stresses having similar impacts on crop reflectance (Schepers et al., 1996; Clay et al., 2006). Attempts to separate the combined impacts of N and water stress on reflectance have linked remote sensing data with air/plant temperatures (Barnes et al., 2000), used multiple indices (Clay et al., 2006; Eitel et al., 2008), or used reflectance-based simulation models (Mishra et al., 2008). However, none of these solutions has been widely adopted. An alternative approach is to use well fertilized reference strips, from which N sufficiency indexes can be calculated (Raun et al., 2001, 2002; Flowers et al., 2003; Holland and Schepers, 2010). The sufficiency index is the ratio of the N status of underfertilized plants and well fertilized plants. For in-season N applications, the plant N status has been defined using the chlorophyll meter or remote sensing. However, sufficiency index protocols do not clearly define how many or where to place well-fertilized control areas. For example, Murdock and Jones (1997) and Francis and Piekielek (1999) suggested that relatively few well fertilized control areas are needed and that within a large field the number could be as little as one.

The sufficiency index approach is believed to improve fertilizer recommendation accuracy (Raun et al., 2001, 2002, 2005; Flowers et al., 2003; Shanahan et al., 2008; Holland and Schepers, 2010; Kitchen et al., 2010; Roberts et al., 2010; Solari et al., 2010; Scharf et al., 2011). However questions still exist on the well fertilized control area protocols (Bausch and Brodahl, 2011). The objectives of this experiment were to determine the impact of N and water stress on crop reflectance and the relationship between the SI calculation approach and winter wheat response to N fertilizer.

MATERIALS AND METHODS

The experimental protocols were previously reported by Kharel et al. (2011). Selected details are summarized below. The plot widths were 9.1 and 8.5 m in 2007 and 2008, respectively. In both years, the experiment contained four blocks. A line source irrigation system was placed in the center of the experiment. This irrigation system produced a water gradient, and the well water and water stress treatments were located 2.3 and 16 m

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Abbreviations: mz, management zone; NDVI, normalized different vegetation indices; SI, sufficiency index; wf, whole field; YLWS, yield loss due to water stress.

from the line source, respectively. These two water treatments were designed to simulate management zones (mz) located in the lower back slope (2.3 m from line source, well watered) and the shoulder/summit (16 m from line source, water stressed) areas. The row spacing was 20.3 cm. Water, N budgets, and yields were previously reported by Kharel et al. (2011).

The experiment was conducted at the South Dakota, Dakota Lakes Research Farm (44°17' N, 99°59' W, elevation approximately 560 m). The soil at the site was a Lowry silt loam (coarse-silty, mixed, superactive, mesic Typic Haplustoll). Hard red winter wheat Overley was planted at 145 kg ha^{-1} on 21 Sept. 2006 and hard white winter wheat 'Alice' was seeded at 145 kg ha^{-1} on 8 Sept. 2007. An offset double disc opener was used for seeding. In 2007, 89.4 kg of 18–5.7–12.5–6 (%N, %P, %K, %S) was applied with the seed and in 2008, 89.4 kg of 11–11.4–12.5–6 was applied with the seed. The average temperature between flowering and grain fill (May to July) was 21.3 in 2007 and 18.3°C in 2008. Total precipitation during April, May, June, and July was 24.4 and 23.3 cm in 2007 and 2008, respectively.

The experimental treatments were five (0.0, 0.25, 0.50, 1.0, and 1.5 times the recommended rate) urea ammonia nitrate (28–0–0, UAN) rates and two water stressed environments (well-watered and water-stressed). The recommended N rates were based on yield goal, soil test NO_3–N, and previous legume N credit (Gerwing and Gelderman, 2005). The N rates for 2006–2007 were 0, 50, 100, 200, and 300 kg N ha^{-1}, whereas the N rates for 2007–2008 were 0, 40, 80, 160, and 240 kg N ha^{-1}. Fertilizer treatments were applied in the spring at Feekes 3.0.

Rain gauges were used to measure the irrigation amounts 2.3 (well-watered) and 16 m (water-stressed) from the line source irrigation system. Irrigation, applied in May, June, and July, was based on tensiometers placed at the 45- and 90-cm soil depths in the well-watered treatment. The irrigation plus natural rainfall amounts in the well-watered and water-stressed treatments were 68.7 and 51.9 cm in 2007 and 60.9 and 52.8 cm in 2008, respectively.

CROP REFLECTANCE MEASUREMENTS

Crop reflectance was measured at the erect growth/stem extension (Feekes 5–6, 21 May 2007 and 24 May 2008) and flag leaf (Feekes 8; 1 June 2007 and 2 June 2008) growth stages with a multispectral radiometer (Crop Scan, MSR16R Crop Scan unit, MultiSpectral Radiometer, Crop Scan Inc., Rochester, MN). The Crop Scan simultaneously measured reflected and incoming energy in the blue (506–514 nm), green (563–573 nm), red (605–615 nm), red edge (704–716 nm), and NIR (755–716 nm) bands. Reflectance was measured 2 m above the ground with field of view of 1 m^{2} between 1100 and 1400 h (central time). In each plot, three readings were collected and averaged. Of the multitude of reflectance indexes tested, the most commonly used for evaluated biomass production and N stress is NDVI (Clay et al., 2006; Reese et al., 2010). This index uses reflectance information from the red and near infrared bands. A limitation of NDVI is that it saturates at high biomass concentration. Based on reflectance measurements, NDVI and SI values were calculated using the equations,

$$\mathrm{NDVI} = (\mathrm{NIR\text{-}red})/(\mathrm{NIR{+}red}) \qquad [1]$$

$$\mathrm{SI\text{-}NDVI}_{wf} = \mathrm{NDVI}/\mathrm{NDVI}_{wf} \qquad [2]$$

$$\mathrm{SI\text{-}NDVI}_{mz} = \mathrm{NDVI}/\mathrm{NDVI}_{mz} \qquad [3]$$

In these calculations, NDVI is the index value from each plot, NDVI_{wf} is the reference NDVI value from well-fertilized and well-watered plots (irrigated plots fertilized at 1 and 1.5 times the recommended N rate) within each block; NDVI_{mz} is the reference NDVI value from the well-fertilized plots (average of the 1 and 1.5 times the recommended N rate) within each of the blocks water treatment; and $\mathrm{SI\text{-}NDVI}_{wf}$ and $\mathrm{SI\text{-}NDVI}_{mz}$ are standardized NDVI values for the whole field and mz reference approaches.

Grain Yield and Quality

Grain was harvested following crop maturity with a plot combine with a 1.52 m head. Grain was weighed, dried, and yields per plot were adjusted to 135 g kg^{-1} moisture content. Ground grain samples were analyzed for total N, total C, δ^{15} N and δ^{13} C on an Sercon 20–20 continuous flow ratio mass spectrometer (Sercon Ltd, UK).

Yield loss due to nitrogen stress (YLNS) and yield loss due to water stress (YLWS) were calculated simultaneously based on: (i) measured yields, (ii) grain δ^{13}C values, (iii) the equation defining the relationship between yield and δ^{13}C for well-fertilized wheat, and (iv) the equation defining the relationship between yield and δ^{13}C for different N rate (Clay et al., 2001b, 2005b; Kim et al., 2008; Kharel et al., 2011). This calculation is based on: (i) ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) preferentially fixing $^{12}CO_2$ over $^{13}CO_2$ into 3-phosphoglycerate (3-PGA); (ii) wheat partially closing its stomata when water stressed, which increases $^{13}CO_2$ fixation; and (iii) water and N stress having opposite impact on the relative amount of $^{13}CO_2$ fixed during photosynthesis. More complete details of this approach are available in Clay et al. (2001b).

Cleaned grain samples were analyzed for moisture, protein content, and a variety of dough quality characteristics on a Farinograph (Kharel et al., 2011;AACC International, 2011a, 2011b, 2011c, 2011d). Kernel protein (g protein kg^{-1} wet grain), which was measured using NIR analysis, is reported at 12% moisture. Measured protein contents were compared with total N concentrations. This moisture content was selected to simplify economic optimum N rate calculations. Different countries use different protein moisture standards for calculating wheat selling prices. For example, in the United States protein premiums or discounts are based on 12% moisture, while in Canada protein premium or discounts are based on 13.5% moisture.

Economically Optimum Nitrogen Rate

The average South Dakota hard red wheat price from 1973 to 2006 was \$129 Mg^{-1} grain (USDA-NASS, 2009). In 2007, 2008, and 2009, commodity values increased to \$257 Mg^{-1} grain (USDA-NASS, 2009). Based on the protein content, grain selling prices, protein-based discounts (selling price adjusted down) and premium (selling price adjusted up) the economic optimum nitrogen rate (EONR, $\delta\$/\delta N = 0$) were determined. For these calculations, the discount was-\$0.03 for each 10 g kg^{-1} less than 120 g kg^{-1}, the premium was +\$0.015

for each 10 greater than 120 g kg^{-1}, the N purchase price was \$2.05 (kg N) $^{-1}$, and the grain selling price was \$222 Mg^{-1} (South Dakota Wheat Growers, 2008). In this analysis, the relationship between yield and N was described using a quadratic [yield = $a + b$(N rate) + c(N rate)^2] model and the relationship between protein and N was described using a linear [protein = $a + b$(N rate)] model. Additional details about the economic analysis are available in Reese (2009). Maximum yields per unit N (δ yield/$\delta N = 0$) were determined.

Statistical Design and Analysis

The experiment contained four blocks, 2 yr, and two factors, N and water. The five N treatments were randomly assigned to plots within each block. A line source irrigation system running through the center of each plot was established, making N rate and water treatment perpendicular to each other. Since water treatments were not randomized and two sides of the lines source "direction" could have an impact on the analysis, a special technique was applied to analyze the data (Stroup, 1989). In this analysis, a first-order autoregressive model under PROC MIXED in SAS 9.1 (SAS Institute, 2008) was used to determine the treatment differences. Block and its interaction with all treatments were random factors, whereas N, year, and water were fixed factors. Significant year × treatment interactions on reflectance were not detected.

RESULTS AND DISCUSSIONS

At the erect growth/stem extension growth stage, N fertilizer reduced reflectance in the green, red, and red edge bands and increased reflectance in the NIR band (Table 1). These results were attributed to the N fertilizer stimulating biomass production (Kharel et al., 2011). The NDVI values were greater for well-fertilized (averaged over water-stress) and well-watered (averaged over N stress) plants than the under-fertilized and water-stressed plants. The NDVI values were also impacted by year (Table 1).

To reduce the diagnosis of water stress as N stress or N stress as water stress two referencing approaches were tested. In the first approach, SI-NDVI$_{wf}$ was referenced to well-fertilized and well-watered plots (NDVI$_{wf}$). This approach referenced the underfertilized NDVI values to the highest yielding area in the field. The resulting SI-NDVI$_{wf}$ values were influenced by N rate, year, and water environment. In the second approach, NDVI was referenced to the well-fertilized treatment (NDVI$_{mz}$) within a water treatment (Table 1). When this approach was used, the resulting SI-NDVI$_{mz}$ values were only influenced by N rate and not year or water environment. In addition, changing from SI-NDVI$_{wf}$ to SI-NDVI$_{mz}$ reduced the strength of the relationship between the SI and yield loss due to water stress (YLWS) from a correlation coefficient (r) of −0.80** to −0.44*. These findings suggest that at this early growth stage, the importance of water stress on the NDVI value can be reduced by referencing the NDVI values to well-fertilized plots (i.e., SI-NDVI$_{mz}$) within a water treatment.

At the flag leaf growth stage, reflectance values in the visible range and red-edge were reduced by N fertilizer, whereas reflectance values in the NIR band were increased by N fertilizer (Table 2). The calculated NDVI values were lowest in the unfertilized and 0.25X N treatments. The NDVI and SI-NDVI$_{wf}$ index values were slightly higher in 2007 than 2008 and were lower in the water-stressed than well-watered treatment. When NDVI was standardized with NDVI values from the well-fertilized and well-watered treatments, the resulting SI-NDVI$_{wf}$ values were influenced by N rate, year, and water. Different results were observed for SI-NDVI$_{mz}$, where differences between years and water environments could not be detected. The relationship between SI-NDVI$_{wf}$ and YLWS was stronger (−0.76**) than the relationship betweenYLWS and SI-NDVI$_{mz}$ (−0.35) Associated with these results was a weaker relationship between SI-NDVI$_{wf}$ and YLNS (r=−0.22) than SI-NDVI$_{mz}$ and YLNS (r=−0.51**).

Table 1. The influence of N and water rates on crop reflectance at the erect growth/stem extension growth stage.

N rate	Blue	Green	Red	Red edge	NIR†	NDVI	SI-NDVI$_{wf}$	SI-NDVI$_{mz}$
	506–514	563–573	605–515	704–716	753–765			
	% Reflectance							
0	4.73	7.2	6.59	12.4	35.2	0.71	0.89	0.95
0.25	4.65	7.05	6.36	12.2	37.6	0.74	0.93	0.98
0.5	4.64	7.04	6.36	12.1	38.1	0.74	0.92	0.97
1	4.45	6.77	6.10	11.7	37.6	0.76	0.94	1.00
1.5	4.46	6.75	6.09	11.7	38.3	0.76	0.95	1.00
p	0.13	0.005	0.03	0.004	0.001	0.001	0.001	0.03
LSD	ns‡	0.28	0.35	0.40	1.32	0.02	0.03	0.04
Year								
2007	3.55	5.83	4.9	11.2	42.9	0.86	0.98	1.00
2008	5.62	8.09	7.2	12.8	31.8	0.62	0.87	0.97
p value	0.001	0.001	0.001	0.008	0.001	0.001	0.02	0.08
Water								
Well watered	4.17	6.54	5.7	11.5	38.6	0.78	0.98	0.98
Stressed	5	7.38	5.9	12.5	26.1	0.70	0.87	0.98
p value	0.002	0.003	0.002	0.005	0.008	0.002	0.001	0.8

† NIR, near infrared; NDVI, normalized different vegetation index; SI-NDVI$_{wf}$, ratio between the underfertilized normalized difference vegetation index value and the NDVI value from well fertilized and well watered treatments; SI-NDVI$_{mz}$, ratio between underfertilized normalized difference vegetation index values and NDVI values from well fertilized plots within a water stress treatment.

‡ ns, not significant.

Relationship between Sufficiency Index and Wheat Responses to Nitrogen

For SI-NDVI$_{wf}$ there were two distinct relationships between N rate and SI-NDVI$_{wf}$ (Fig. 1). Reflectance values from 2007 and the well-watered plots in 2008 could be fit to one curve, while NDVI$_{wf}$ values from the 2008 water stress plots fit a different curve. Based on these findings, the 2008 SI-NDVI$_{wf}$ calculation approach would have diagnosed water stress as N stress, which would have increased the N rate to water stressed plants. In dryland wheat production, this error can reduce yields (Reese et al., 2002).

If the denominator in the whole field SI calculation is obtained from a water stress area, it is possible that the well-watered areas can be underfertilized. For example, if the reference value was obtained from the 1X N rate and water stressed irrigation treatment, then the NDVI for the 0N well-watered treatment would have been 1.07. Based on this value, N would not have been applied. This error would have reduced the yields in the well-watered 0N treatment 904 kg ha^{-1} (Table 3, Fig. 2). Associated with this yield decrease was dough that did not meet the stability goal of 10 min (Table 3) (www.theartisan.net/flour_criteria_judging.htm). In bread production, dough stability is important and provides information needed to determine how long dough can be mixed (Park, 2012). In addition, the underfertilization error also reduced water absorbance, protein, and the mixing tolerance index (MTI) rating, which decreased with increasing N. The 0N rate was also less than the economic optimum nitrogen rates (EONR) which was approximately 60 kg N ha^{-1}.

In summary, wheat grown in many northern Great Plains wheat fields is impacted by water and N stress simultaneously. For remote sensing-based recommendations, understanding

Table 2. The influence of N and water treatment on reflectance at the flag leaf growth stage in winter wheat.

N rate	Blue 506–514	Green 563–573	Red 605–615	Red edge 704–716	NIR† 753–765	NDVI	SI-NDVI$_{wf}$	SI-NDVI$_{mz}$
	% reflectance							
0	3.24	5.68	4.62	9.95	36.3	0.81	0.91	0.94
0.25	3.01	5.35	4.20	9.43	38.7	0.85	0.96	0.98
0.5	2.96	5.25	4.08	9.28	40.3	0.86	0.97	0.99
1	2.95	5.19	4.03	9.16	40.0	0.87	0.97	1.00
1.5	2.92	5.06	3.91	8.90	41.3	0.87	0.97	1.00
p value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD	0.16	0.23	0.26	0.40	1.87	0.01	0.01	0.02
Year								
2007	2.81	4.69	3.62	8.42	39.7	0.88	0.98	0.99
2008	3.22	5.93	4.71	10.30	38.9	0.83	0.94	0.98
p value	0.008	0.001	0.001	0.001	0.24	0.01	0.01	0.64
Water								
Well watered	2.64	4.91	3.84	9.13	39.9	0.88	0.99	0.99
Stressed	3.39	5.71	4.49	9.56	38.8	0.83	0.93	0.98
p value	0.001	0.001	0.001	0.03	0.09	0.002	0.002	0.36

† NIR, near infrared; NDVI, normalizeddifferent vegetation index; SI-NDVI$_{wf}$, ratio between the underfertilized normalized difference vegetation index value and the NDVI value from well fertilized and well watered treatments; SI-NDVI$_{mz}$, ratio between underfertilized normalized difference vegetation index values and NDVI values from well fertilized plots within a water stress treatment.

Table 3. The influence of N and water treatments on grain yields, yield loss due to water stress (YLWS), yield loss due to nitrogen stress (YLNS), protein, water absorbance, dough stability, and mixing tolerance index (MTI).

N rate	Yield	YLWS	YLNS	Protein	Water absorbance	Stability	MTI†
	kg ha^{-1}			g kg^{-1}		min	B.U.
0	4087	910	1162	120	540	6.7	29
0.25	4594	622	951	129	543	8.2	22.1
0.5	4662	699	821	136	545	10.0	18.0
1	4991	628	556	143	549	10.2	17.3
1.5	5019	675	488	149	555	11.3	16.1
p value	0.001	0.001	0.001	0.001	0.001	0.001	0.001
LSD	180	133	194	3	4	1.6	5.4
Year							
2007	4174	522	814	143	560	8.8	20.8
2008	5167	892	777	127	534	9.7	20.2
	0.01	0.01	0.70	0.004	0.002	0.4	0.8
Water							
Well watered	4922	593	658	134	547	9.1	20.7
Stressed	4419	820	933	137	547	9.5	20.3
p value	0.007	0.01	0.04	0.1	0.96	0.4	0.79

† B.U., Brabender units.

the impact of stress on reflectance is needed to optimize management. This research suggests that: (i) standardization of underfertilized NDVI values with NDVI values from well-fertilized reference areas located within each management zone can be used to improve N recommendations; (ii) diagnosing N as water stress can result in applying less N than the EONR, while diagnosing water as N stress can result in the overapplication of N; (iii) N and water stress had similar impacts on reflectance; (iv) SI-NDVI$_{wf}$ was impacted by both N and water stress, while NDVI$_{mz}$ was primarily impacted by N stress; (v) SI-NDVI$_{mz}$ had a stronger relationship with N fertilizer induced yield gains than SI-NDVI$_{wf}$; and (vi) both standardizing approaches (SI-NDVI$_{wf}$ and SI-NDVI$_{mz}$) provide valuable information that should not be used interchangeably.

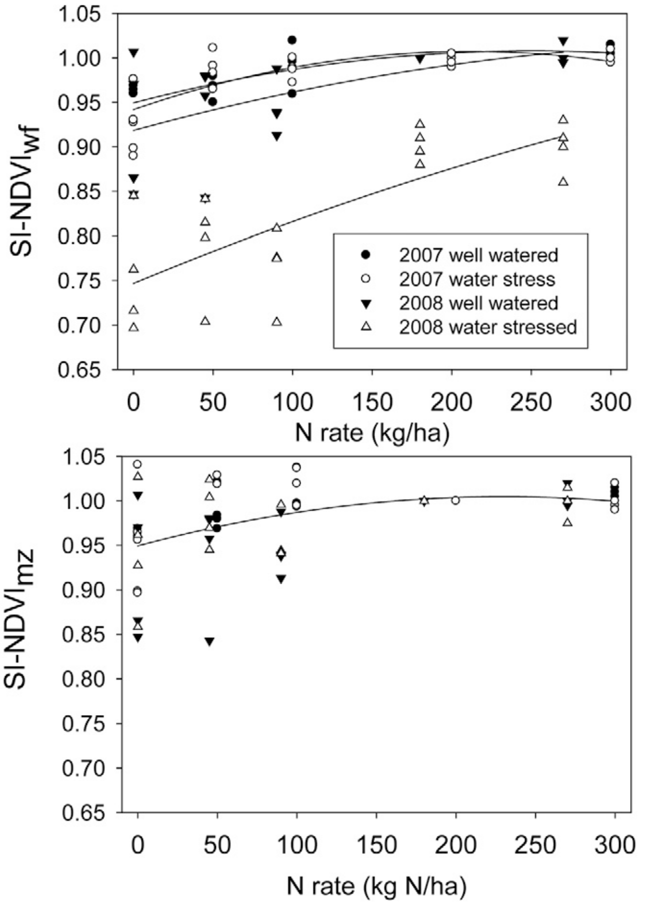

Fig. 1. The influence of N rate, year, and water stress on the whole field (SI-NDVI$_{wf}$) and management zone (SI-NDVI$_{mz}$) sufficiency indexes at the erect/stem extension growth.

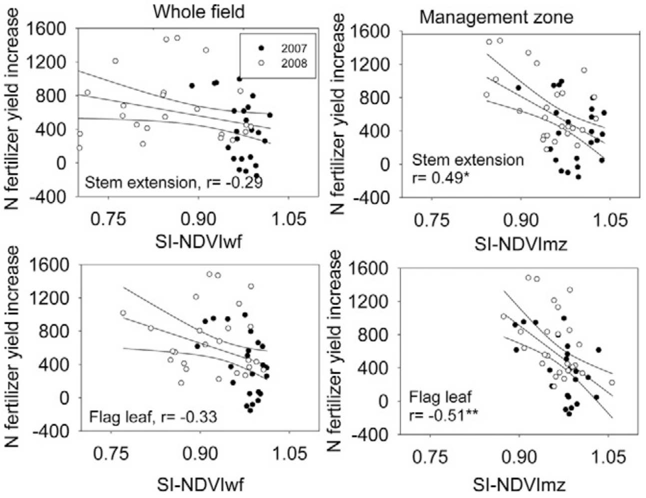

Fig. 2. The relationship between N induced yield increases (yield in well fertilized plots – yield in underfertilized plots) and sufficiency index-normalized different vegetation indices (SI-NDVI) calculated using the whole field and management zone approaches at the erect/stem extension and flag leaf growth stages.

Well-fertilized reference areas can be implemented in northern Great Plains landscapes by placing well-fertilized N strips at locations where they cross as many elevation zones as possible. In many northern Great Plains areas, elevation zones frequently account for spatial variability in salts and soil moisture (Clay et al., 2001a; Sudduth et al., 2002; Heiniger et al., 2003). These zones can be further differentiated by using apparent electrical conductivity, yield maps, or remote sensing (Kleinjan et al., 2007). Bausch and Brodahl (2011) compared two approaches (order 2 soil map and apparent electrical conductivity) for identifying mzs in irrigated Colorado corn fields. They reported that mzs based on an order 2 soil survey map resulted in water stress being diagnosed as N stress in lighter textured soils This diagnosis error was reduced by using apparent electrical conductivity to identify management zone boundaries.

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