Solar Induced Fluorescence and Reflectance Sensing Techniques for Monitoring Nitrogen Utilization in Corn

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Abstract- Remote sensing systems using either passive reflectance (R) or actively induced fluorescence (F) have long been explored as a means to monitor species composition and vegetative productivity. Passive F techniques using the Fraunhofer Line Depth (FLD) principle to isolate solar induced F (SIF) from the high resolution R continuum have also been suggested for the large-scale remote assessment of vegetation. The FLD principle was applied to both canopy R spectra and AISA multi-spectral imagery to discriminate the relatively weak *in situ* vegetation F in-fill of the telluric O_2 bands located at 688 nm and 760 nm. The magnitudes of SIF retrieved from R ranged from 7 to 36 $mW/m^2/nm/sr$ and the ratio of the two spectral bands successfully discriminated the four N treatment levels. In addition, a number of R indices including but not limited to the physiological reflectance index (PRI), R_{550}/R_{515} and R_{750}/R_{800} were calculated from the AISA aircraft imagery and the high-resolution canopy R spectra. These indexes were then evaluated against geo-referenced ground measurements of leaf area index (LAI), pigment contents, grain yields, and light use efficiency (LUE). A number of significant relationships were evident in both R and SIF indices to the biophysical changes in corn induced by N application rates. From this investigation we conclude that valuable SIF information can be extracted from high-resolution canopy R data and indices calculated from both data types can supply useful information for modeling N use for carbon sequestration by vegetation.

Keywords- Reflectance (R); Fluorescnece (F); Fraunhofer Line Depth (FLD); Physiological Reflectance Index (PRI)

I. INTRODUCTION

A major goal of the U.S. Carbon Cycle Science Program is to monitor the vegetation processes related to carbon dioxide (CO_2) uptake. The correct interpretation and implementation of spectral remote sensing bio-indices and associated modeling activities depends on understanding the underlying physiology, so that environmentally induced stress conditions that limit carbon (C) uptake can be adequately taken into account. Biological C sequestration is driven by nitrogen (N) availability since N is involved in photochemical processes and is one of the primary resources regulating plant growth. Large scale monitoring of these processes are currently possible only with remote sensing systems that rely heavily on passive reflectance (R) information. While a number of narrow band indices are correlated to total chlorophyll (Chl) content, it has been difficult to consistently relate them to C uptake. Fluorescence (F) emitted from Chl, or ChlF, is directly related to photochemical reactions and has been extensively used for the elucidation of the photosynthetic pathways. Recent studies have shown that ChlF can be extracted from high-resolution reflectance spectra of vegetation [1,2,3]. This approach has not yet been operationally implemented, in part because the relationship between these two remotely sensed parameters remains to be fully resolved. This study was designed to investigate relationships between R and solar induced F (SIF) techniques to provide information that can be incorporated into prescription algorithms for site-specific variable applications of N fertilizer for crop production.

II. METHODS AND MATERIALS

A. Plant Material

The field site is located at the USDA Beltsville Agricultural Research Center and is part of an intensive test site for a multi-disciplinary project entitled Optimizing Production Inputs for Economic and Environmental Enhancement (OPE). A total of 12 N treatment plots of field corn (*Zea mays* L. '*Pioneer 33A14*') were established within the OPE field site each measuring 18.2 m wide (containing 24 rows) by 28.3 m long. The experimental design was a randomized complete block with treatment groups of 210, 140, 70, and 0 kg N/ha, which provided plant growth conditions ranging from classical symptoms of N deficiency to physiological conditions consistent with an excess N supply.

B. Measurements

Biophysical measurements were obtained in 2001, 2004, and 2005 over from the corn crop at the grain fill (R3) reproductive stage. Measurements occurred *in situ* where possible, otherwise uppermost fully expanded leaves or 3rd leaf from terminal were excised from the plant canopy, immediately placed in water filled sample holders, and transported to the laboratory for further analysis. These measurements included pigment contents, leaf optical properties, and total leaf C:N. *In situ* georeferenced canopy measurements were comprised of; leaf area index (LAI), light use effcency (LUE, $\mu mol\ CO_2\ m^{-2}\ s^{-1}\ aPAR^{-1}$), grain yield (kg/ha), and canopy R. Refer to [1] for further details regarding these measurements.

A spectroradiometer (ASD-FR FieldSpec Pro, Analytical Spectral Devices, Inc., Boulder, CO) was used to measure canopy radiance 1 m above plant canopies with a 22^o field of view and a 0^o nadir view zenith angle. The spectroradiometer has 3 nm Full-Width at Half Maximum (FWHM) spectral

TABLE 1. N Treatment Effects on Biophysical Measures of Field Corn Growth

Treatment (kg N/ha)	Chl a+b ($\mu g/cm^2$)	Chl a ($\mu g/cm^2$)	Chl b ($\mu g/cm^2$)	Chl a:b	Chl a+b/ carotene	C:N	LUE‡	LAI	Yield (kg/ha)
280	63.9 a†	44.3 a	19.7 a	0.098 a	11.83 a	13.7 a	0.021 a	3.14 a	9833 a
140	59.3 b	42.8 a	16.6 b	0.106 a	10.37 b	14.2 a	0.022 a	2.74 a	8320 b
70	52.6 c	38.6 b	14.1 c	0.121 b	8.89 c	16.4 b	0.020 a	2.45 b	7420 c
0	31.8 d	23.9 c	8.0 d	0.156 c	7.02 d	22.9 c	0.016 b	2.05 c	4808 d
$LSD_{.05}$ (n=180)	4.5	2.8	1.7	0.019	1.23	0.5	0.0011	0.269	1178

†Column-wise mean values represent a combined analysis of three years (2001, 2004, 2005) of corn growth parameters at the R3 (grain fill) development stage. Means with the same letter are not separable by a repeated measures mixed model $ANOVA_{LSD.05}$.
‡LUE is expressed as maximum photosynthesis per μmole of absorbed photosynthetically active radiation.

resolution at a 1.4 nm sampling resolution. Concomitantly, aircraft multispectral R imagery was acquired with the Airborne Imaging Spectrometer for Applications (AISA, flown by 3DI LLC, Easton MD). The AISA imaging spectrometer was configured with 25 bands ranging from 520 nm to 884 nm each with a spectral resolution of 1.6 nm FWHM. The instrument was flown at 2500 m with an instantaneous field of view of 1 mrad yielding a 2.5 m per pixel ground resolution.

C. Spectral Indices

Several R indices were calculated from canopy R spectra and AISA imagery. Results were reported for the top three indices that exhibited the strongest relationship to N supply in corn. These included two R ratios (R_{550} / R_{515}, R_{750} / R_{800}) and (1) the physiological R index [5].

$$PRI = (R_{550}-R_{530})/(R_{550}+R_{530}) \quad (1)$$

The FLD principle was applied to canopy R spectra and AISA imagery to discriminate the relatively weak *in situ* vegetation F in-fill of the telluric O_2 bands that fall within the ChlF region. The major telluric O_2 features are located at 688 nm and 760 nm and have a FWHM of 4 nm and 7 nm, respectively. The following algebraic expressions of the FLD principle adapted from [4] were used to obtain canopy R at wavelength 'd' (1) and F at wavelength 'd' (2).

$$R_d = (c-d)/(a-b) \quad (2)$$

$$F_d = d - Rb = (ad - cb)/(a-b) \quad (3)$$

Here 'a' and 'b' represent the reference panel radiance in and out of each O_2 feature, respectively, and 'c' and 'd' represent the target radiance (Fig. 1). F within a Fraunhofer feature can also be expressed as the relative stationary yield (f = F/a) a dimensionless number representing the degree to which radiance within a relatively dark Fraunhofer line is augmented. A mixed model analysis of variance (SAS Inc., Cary NC, USA) with repeated measures was used to assess the separation of plant parameters, R, and F features with respect to treatment effects. LSD mean separations were deemed significant at $p \leq 0.05$.

III. RESULTS

A. Analysis of Plant Growth

The multi-year analysis indicated that leaf parameters (Chl, C:N, Amax, LUE) and crop parameters (LAI, grain yield) increased with N level (Table 1). LAI, C:N, Chl *a*, Chl *a:b* and Chl *a+b*/carotene were similar for the two N fertilization rates ≥ 140 kg N/ha, whereas significant decreases were obtained for treatments ≤ 70 kg N/ha. The Chl *a+b*, Chl *b* and grain yields exhibited decreases with increasing levels of N supply, with minimum significant differences of 4.5 $\mu g/cm^2$, 1.7 $\mu g/cm^2$ and 1178 kg/ha ($LSD_{.05}$), respectively. Although, the LUE parameter did not exhibit significant change for treatments ≥ 70 kg N/ha, the data trended toward higher values as N level increased. Overall, the analysis of corn growth and condition at lower N availability was consistent with classical symptoms of N deficiency.

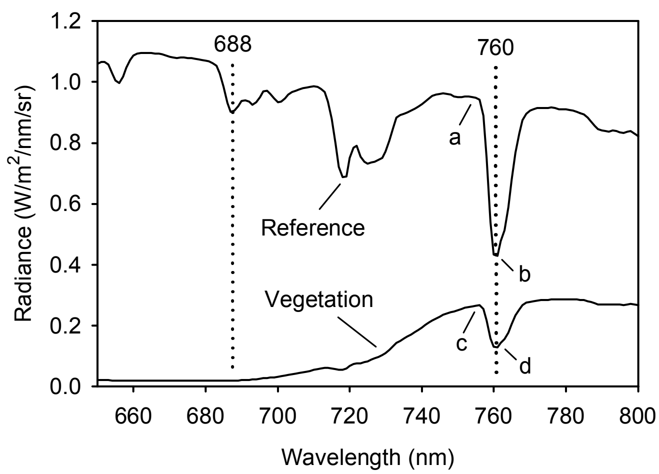

Figure 1. Radiance measured in the red through far-red spectral region. Telluric oxygen bands are denoted with dotted lines, while a, b, c, and d correspond to the points used for Fraunhofer line depth determination of far-red solar induced fluorescence.

TABLE 2. Spectral remotely sensed assessment of N treatment effects on field corn growth.

Treatment (kg N/ha)	Canopy R Spectra[a]					AISA Imagery[b]				
	R_{550} / R_{515}	PRI	R_{750} /R_{800}	F_{760}‡	$F_{688/760}$	R_{550} / R_{515}	PRI	R_{750}/R_{800}	F_{760}[d]	F_{688} / F_{760}
280[c]	1.82 a	0.082 a	0.864 a	15.7 a	1.48 a	1.147 a	0.0339 a	0.989 a	12.0 a	0.178 a
140	1.86 a	0.084 a	0.845 b	14.8 a	2.38 b	1.160 b	0.0354 a	0.998 b	11.6 a	0.197 b
70	1.91 b	0.087 b	0.851 b	13.3 b	1.72 ab	1.191 c	0.0470 b	1.014 c	10.1 b	0.273 c
0	2.22 c	0.103 c	0.907 c	7.8 c	4.17 c	1.231 d	0.0558 c	1.031 d	7.8 c	0.433 d
$LSD_{.05}$	0.04	0.002	0.006	0.9	0.38	0.003	0.0016	0.001	0.15	0.007

[a]R indices were calculated from spectra obtained over the crop canopy (2004, n=60; 2005,n=144).
[b]Georeferenced pixel intensities extracted from AISA imagery (2001, n=60; 2004, n=60).
[c]Within group column-wise means with the same letter are not separable by a repeated measures mixed model $ANOVA_{LSD.05}$.
[d]FLD derived fluorescence values are expressed in $mW/m^2/nm/sr$.

B. Spectral Assessment of Plant Growth

Spectral R indices were calculated from canopy R spectra obtained with the ASD spectroradiometer over the corn crop (Table 2). The top performing indices differentiated three of the four N application rates in a combined ANOVA analysis using two years of canopy near-field spectral measurements. Aircraft multi-spectral AISA imagery was obtained in 2001 and 2004. R indices were calculated from aircraft imagery at the R3 grain fill growth stage and are displayed with a vector overlay of the OPE corn N experiment site (Fig. 2). Variations that can be attributed to N fertilization are apparent in the false-color AISA R index images. Geo-referenced mean values from AISA R indices that corresponded to the ground observations are shown in Table 2. The two R ratio indices R_{550} / R_{515} and R_{750} / R_{800} outperformed the PIR index and were able to differentiate the four N treatment regimes.

The FLD principle was applied to R measurements made over the corn canopies in two telluric O_2 bands centered at 688 and 760 nm that occur in the ChlF region. The magnitudes of F retrieved from R ranged from 7 to 36 $mW/m^2/nm/sr$. SIF retrieved for F_{688} was greater than for the F_{760}, but the two F bands had opposing trends with respect to N supply. The FLD technique applied to AISA aircraft data provided slightly better sensitivity to corn N supply, as compared to the R indices calculated from canopy spectral measurements. For information regarding the biophysical correlations of these parameters refer to a related article [7] in this proceeding.

IV. DISCUSSION

Here we have successfully demonstrated the extraction of ChlF information from canopy R and established relationships among R and F features to crop growth and condition. The four N groups were discriminated for field corn using either passive solar induced $F_{688/760}$ or R ratio indices. The reported F intensities in the O_2 bands are for near-ground observation. Absorption of ChlF by atmospheric O_2 is expected to have a significant attenuation on the signal by the time it reaches orbital altitudes. Solar-induced ChlF intensities retrieved from the telluric O_2 absorption features at 688 and 760 nm were on

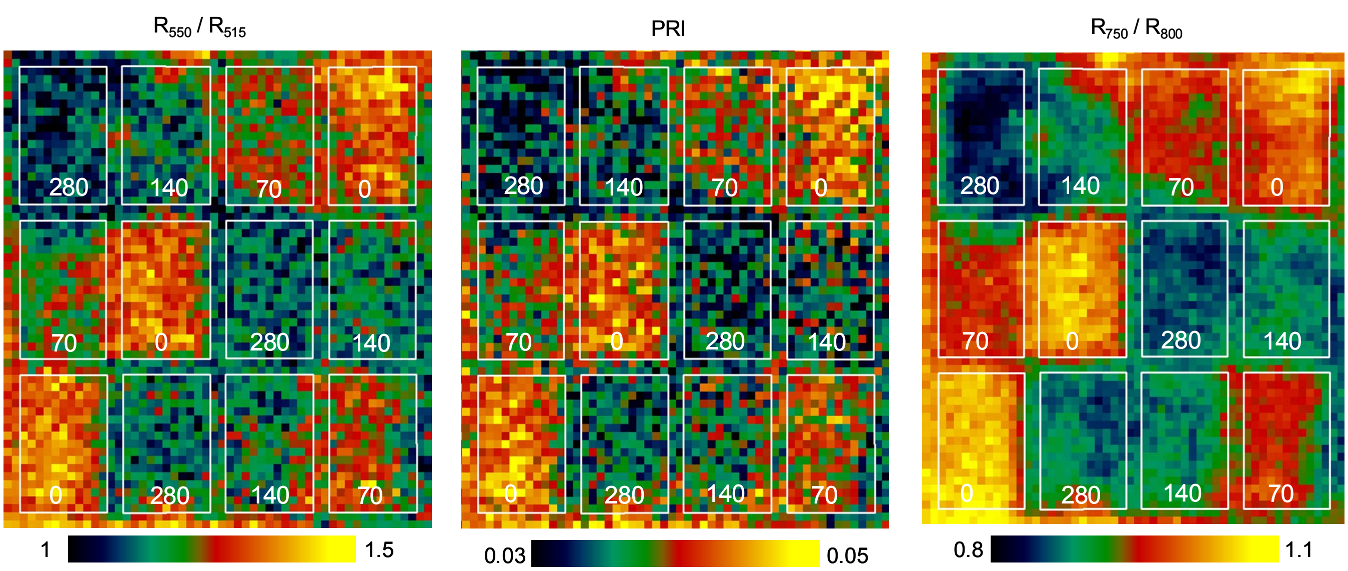

Figure 2. Reflectance indices from 2004 AISA multi-spectral imagery over the corn N experiment site.

the same orders of magnitude as those obtained in a similar fashion [2]. The emitted ChlF increased with Chl content for both F bands. However, the F_{680} band is subject to re-absorption by Chl, which can lead to decreasing intensities as Chl contents increase. Since F_{760} is outside the absorption features of Chl, it is not subject to re-absorption and its observed magnitude predominantly increases with Chl content [8]. Due to the greater bandwidth of the O_2 absorption features, retrieved near-ground ChlF is several orders of magnitude greater than F retrieved from solar Fraunhofer features. As a result, increased emphasis has been placed on the development of instrumentation explicitly for detecting near-field ChlF in these attenuated regions of the spectrum [9,10]. However, absorptions by atmospheric O_2 will increasingly attenuate the F signal with sensor altitude. At orbital observation levels, this could negate the advantages of broadband O_2 features and favor the narrowband Fraunhofer line discriminators [1]. The next logical step in these investigations is to use these summary parameters to project top-of-canopy SIF emissions to the top of atmosphere. Further studies are need with radiative transfer models to estimate the atmospheric effects on signal transmittance at orbital altitudes.

High spectral resolution reflectance data have been demonstrated to provide significant improvement over the broadband indices for detection of differences in vegetation physiology. A series of experiments in a controlled environment facility demonstrated that the R derivative double-peak feature is due primarily to ChlF [3]. Further relationships between R and vegetative growth parameters have also been achieved with several derivative leaf R indices [11]. In this study, the impact of chlorophyll fluorescence contributing to the red-edge R was apparent. Preliminary findings also suggest strong relationships exist between ASD canopy derivative R spectral indices and plant growth parameters. However, the spectral sampling resolution of the AISA instrument was not sufficient to calculate these derivative indices. The AISA sensor has been replaced with a new hyperspectral imaging sensor and over-flights of the OPE experimental area have been scheduled for 2006.

V. CONCLUSIONS

Corn crops are among the highest consumers of N fertilizers in the United States and a rapid quantitative measure of N status for this crop would prove useful to many farming systems where substantial investments are made in the application of N fertilizers. A rapid non-destructive assessment of leaf N would be useful in determining problem spots in fields where organic or chemical supplements may improve soil fertility and crop yield while reducing the potential for contamination of surface and ground waters. The findings from this study identified the several well performing spectral bio-indices, which could be calculated from the available AISA wavebands, for the assessment of leaf N. These studies are critical to define the optimal narrow band information required for monitoring ecosystem health from space.

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