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Chlorophyll Fluorescence Emissions of Vegetation Canopies From High Resolution Field Reflectance Spectra

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Abstract—A two-year experiment was performed on corn (*Zea mays* L.) crops under nitrogen (N) fertilization regimes to examine the use of hyperspectral canopy reflectance information for estimating chlorophyll fluorescence (ChlF) and vegetation production. Fluorescence of foliage in the laboratory has proven more rigorous than reflectance for correlation to plant physiology. Especially useful are emissions produced from two stable red and far-red chlorophyll ChlF peaks centered at 685±10 nm and 735±5 nm. Methods have been developed elsewhere to extract steady state solar induced fluorescence (SIF) from apparent reflectance of vegetation canopies/landscapes using the Fraunhofer Line Depth (FLD) principal. Our study utilized these methods in conjunction with field-acquired high spectral resolution canopy reflectance spectra obtained in 2004 and 2005 over corn crops, as part of an ongoing multi-year experiment at the USDA/Agriculture Research Service in Beltsville, MD. SIF intensities for ChlF were derived directly from canopy reflectance spectra in specific narrow-band regions associated with atmospheric oxygen absorption features centered at 688 and 760 nm. The N treatments accounted for 80% of the variation in the foliar C/N ratios, which declined from ~23 in the lowest N treatment to ~14 at the highest N treatment. A leaf-level steady state fluorescence ratio, F_s/Chl , was positively related to foliar C/N ratio ($r^2 = 0.84$, $n=102$). Similarly, the red/far-red SIF ratio derived from the field reflectance spectra was positively related to the foliar C/N ratio ($r^2 = 0.64$, $n = 109$). Both fluorescence ratios were inversely, and non-linearly correlated with crop grain yield (Kg / ha) determined at harvest a month later (F_s/Chl ratio, $r = 0.92$; SIF Red/Far-Red ratio, $r = 0.85$). This study has relevance to future passive satellite remote sensing approaches to monitoring C dynamics from space.

Keywords- chlorophyll fluorescence; solar induced fluorescence; corn; carbon/nitrogen ratio.

I. INTRODUCTION

Biological carbon dioxide (CO₂) sequestration is driven by availability of nitrogen (N), a primary resource regulating photochemical processes and plant growth. A rigorous satellite-based spectral method is needed to monitor gross primary production (GPP) and/or related carbon (C) parameters for terrestrial ecosystems having variable species and age class composition and for seasonally changing natural and agricultural ecosystems at local spatial scales. Currently, most remote sensing of the Earth's vegetated surfaces is done using reflected light in the solar domain, or reflectance. While a number of narrow band reflectance indices are correlated to total chlorophyll (Chl) content, it has been difficult to consistently relate them to CO₂ uptake directly. However,

actively induced chlorophyll fluorescence (ChlF) is a well-documented indicator of photosynthetic function at the leaf and plant level [1-2] and has been used to differentiate N status of foliage in laboratory and field studies [3-5]. In terrestrial vegetation, ChlF occurs in the red and far-red spectrum, with peaks at 685±10 and 735±5 nm. ChlF represents energy that was absorbed by chlorophyll but then discarded as fluorescence (emitted photons at longer wavelengths) when it could not be used for C fixation. A common laboratory and field method is to use lasers to actively induce ChlF [6]. However, lasers cannot be used from aircraft and satellite platforms to gather GPP estimates across landscapes, due primarily to eye safety issues. ChlF is naturally and passively induced in vegetation by sunlight, referred to here as solar induced fluorescence (SIF). Although a relatively weak signal compared to reflected radiation, ChlF can be measured above vegetation using an established passive technique in narrow spectral regions of the apparent reflected solar spectrum [7-9]. This passive technique applies the Fraunhofer Line Depth (FLD) principle [10-11] to high spectral resolution canopy reflectance in spectral regions associated with solar Fraunhofer bands, and in oxygen (O₂) atmospheric (telluric) absorption bands. This approach differentiates fluorescence from reflectance in the narrow "dark" regions of the reflected vegetation spectra, potentially with sufficient resolution for low earth orbit observations by interferometer-type passive satellite systems [11]. The two appropriate narrow, telluric O₂ absorption bands (≤5 nm) for extracting SIF for ChlF included in canopy reflectance spectra are centered at 760.5 nm (O₂-A) and 688 nm (O₂-B). The 688 band falls directly on the red ChlF peak. The 760 nm band falls on the outside shoulder of the far-red ChlF peak but potentially provides a much greater signal at the top-of-atmosphere due to the greater depth and width of the absorption feature. These telluric O₂ features have Full-Width at Half Maxima (FWHM) bands of 4 nm (O₂-B) and 7 nm (O₂-A), formed from the merger of a series of narrow molecular O₂ atmospheric absorption bands. As part of our research program to identify spectral indices for monitoring C and N dynamics in vegetation, we examined the SIF from high spectral resolution reflectance obtained above mature plots provided N augmentation regimes in two years. Recently, we reported our initial first year SIF results [12-13]. Here, we report results from both years. An evaluation is made for corn foliage and whole plants under a range of N supply treatments for the passive FLD principle applied to telluric O₂ bands from field-acquired canopy reflectance spectra and foliar ChlF versus C/N measurements and crop yields.

II. METHODS

In situ measurements were made and plant material collected in August 2004 and July 2005 at the grain fill (R3) reproductive stage. Crop yields were determined at harvest in September of both years. Micrometeorological information at the time of measurements can be summarized for these variables—the average photosynthetically active radiation (PAR); the average daily air temperature; and the accumulated rainfall. PAR and rainfall were higher in June-August during 2004 (PAR, 220 W/m²; rainfall, 393 mm) than in 2005 (PAR, 209 W/m²; rainfall, 245 mm), but it was almost 2 degrees C warmer in 2005 (22°C vs. 23.9°C).

A. Plant Material

Plant material was collected from a research field site established to examine N augmentation regimes on cornfields at the USDA Beltsville Agricultural Research Center, Beltsville, MD, US. The cornfield is an intensive test site included in a multi-disciplinary USDA project. N plots on the corn (*Zea mays* L.) field are large enough (515 m²) to capture the spatial variability of crop and soil parameters. The experimental design was a randomized complete block with treatment groups of 28, 70, 140, and 280 kg N / ha to provide plant growth conditions ranging from N deficiency to over-fertilization, or 20%, 50%, 100% and 200% of the recommended N levels for this soil. These are designated as N treatments 1-4, respectively, in figures. The N plots were planted in the same locations per N treatment in both years.

B. 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° field of view and a 0° nadir view zenith angle. A second cross-calibrated ASD radiometer was used in a similar viewing geometry over a Spectralon reference panel (Labsphere, North Sutton, NH) to simultaneously track changes in solar irradiance. Reflectance was calculated as the ratio of upwelling to downwelling radiances, expressed as a percent, and represented the total reflectance per corn plant. The ASD spectroradiometer uses a 512 channel silicon photodiode array overlaid with an order separation filter to provide spectral resolution of 3 nm FWHM at a 1.4 nm sampling resolution. This is sufficient for the quantification of SIF and reflectance within the major telluric O₂ features. Measurements were obtained on clear days in a two-hour window around solar noon. The FLD principle was applied to discriminate the relatively weak *in situ* SIF in-fill of the telluric O₂ bands that overlap the ChlF peaks. For the algebraic expressions of the FLD principle used to obtain reflectance and SIF from vegetated surfaces refer to [11] in this proceeding.

Photosynthetic capacity (A_{max}, μmol CO₂ m⁻²s⁻¹) and light-adapted steady-state ChlF (F_s) were determined simultaneously *in situ* on leaf #13, typically the 3rd leaf from terminal, with a Li-Cor 6400 photosynthetic system (Li-Cor, Lincoln, NE, USA) fitted with a leaf fluorometer chamber.

A_{max} and F_s were determined on the adaxial surfaces under controlled conditions of 1500-2000 μmol m⁻² s⁻¹ PAR, saturating CO₂ concentration (1000 ppm), controlled leaf temperature (22-24°C), and relative humidity (~35%). With the Li-Cor 6400, F_s is detected by a broadband sensor centered at 700 nm and provides a measure of total photons fluoresced per second from both the red and far-red ChlF bands. Leaves were then excised and immediately placed in water filled florist cuvettes for transport to the laboratory for further analysis. Leaf Chl *a*, Chl *b*, and total carotenoid concentrations were determined from freshly cut leaf disks (2.54 or 3.00 cm²) that were sealed in cuvettes with 3.5 ml dimethyl sulfoxide (DMSO), for 36 hours at 25°C before absorption spectra were obtained using a dual beam spectrophotometer (Lambda 40, Perkin-Elmer, Norwalk CT, USA), from which pigment concentrations were determined. For each leaf, the F_s/Chl ratio was determined using F_s from the Li-Cor fluorometer and total chlorophyll from the pigment determinations. The remainder of each leaf sample was oven dried at 50° C, and ground to pass a 1 mm mesh, from which total leaf C and N determinations were obtained by the Dumas combustion method by the University of Delaware Soil Testing Program, Newark DE, USA. Corn grain yields were based on hand harvested corn ears obtained from five plants per plot at locations where foliar samples were obtained. Grain samples were oven dried at 50° C prior to weighing.

Statistical analysis was performed using Systat 9.0 (Jandel Scientific, San Rafael, CA, USA). LSD mean separations were deemed significant at $p = 0.05$.

III. RESULTS AND DISCUSSION

Decreases with N availability occurred in both years for the foliar C/N ratio, the foliar F_s/Chl ratio, and the whole plant SIF Red/Far-red ratio (Figure 1 A,B,C). The N treatments accounted for 80% of the variation in the foliar C/N ratios, which declined from ~23 in the lowest N treatment to ~14 at the highest N treatment. The observed changes to these variables associated with dose-related N effects were more pronounced in 2004 than in 2005 when environmental conditions were less favorable due to lower rainfall and PAR under warmer conditions. The SIF Red/Far-red ratio obtained above the corn canopy was related to photosynthetic pigment content (data not shown), especially Chl *a* ($r = 0.75$) and the Chl/Carotenoid ratio ($r = 0.86$). The leaf-level steady state ChlF normalized to the total Chl content, expressed as the F_s/Chl ratio was positively and linearly related to the foliar C/N ratio ($r^2 = 0.84$, Figure 2A). The SIF Red/Far-red ratios obtained above the corn crops in both years were also positively related to the foliar C/N ratio ($r^2 = 0.64$, Figure 2B). A lower association to the foliar C/N for the canopy SIF Red/Far-red ratio (vs. F_s/Chl ratio of that leaf) occurred because all of the leaves contribute to the observed SIF, and contributions also unavoidably come from adjacent plants. These ChlF ratios were also negatively correlated with the grain yield (kg / ha) for both corn crops (Figure 3 A,B), which were determined at harvest six weeks later ($r = 0.85-0.92$).

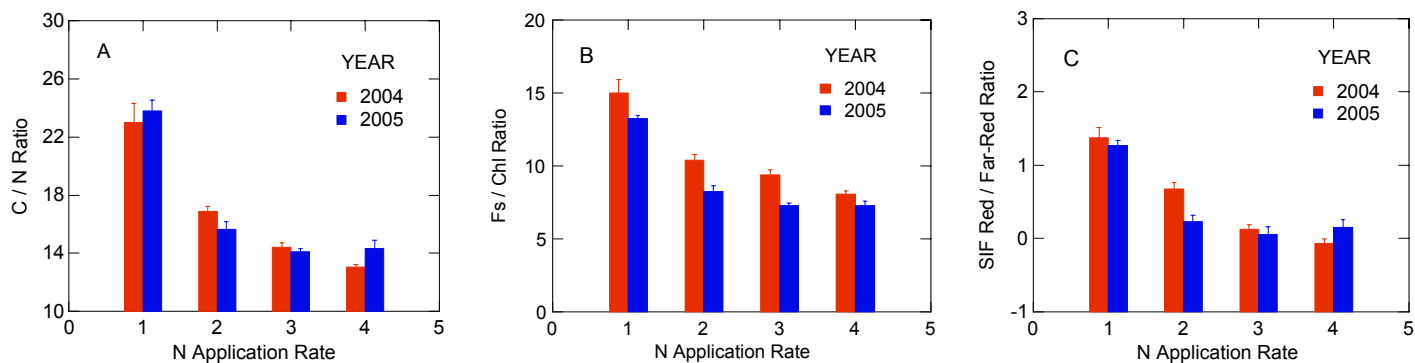


Figure 1. The response of corn leaves to nitrogen application is shown for three variables: A] the foliar C/N ratio ($r^2 = 0.80$, $P < 0.001$ for N treatment); B] the steady state foliar chlorophyll fluorescence (Fs) normalized to the total chlorophyll content (Chl), expressed as the Fs/Chl ratio ($r^2 = 0.80$, $P < 0.001$ for N treatment & year); and C] the steady state whole plant solar passively induced chlorophyll fluorescence (SIF), expressed as the Red/Far-red ratio ($r^2 = 0.72$, $P < 0.001$ for N treatment & the treatment * year interaction). Nitrogen application rates for treatments 1-4 were: 28, 70, 140 and 240 kg N / ha, corresponding to 20%, 50%, 100%, and 200% of optimal fertilizer regimes for this soil type and climate zone. Measurements ($n = 115$ -120) were acquired at the same grain fill (R3) growth stage in two years.

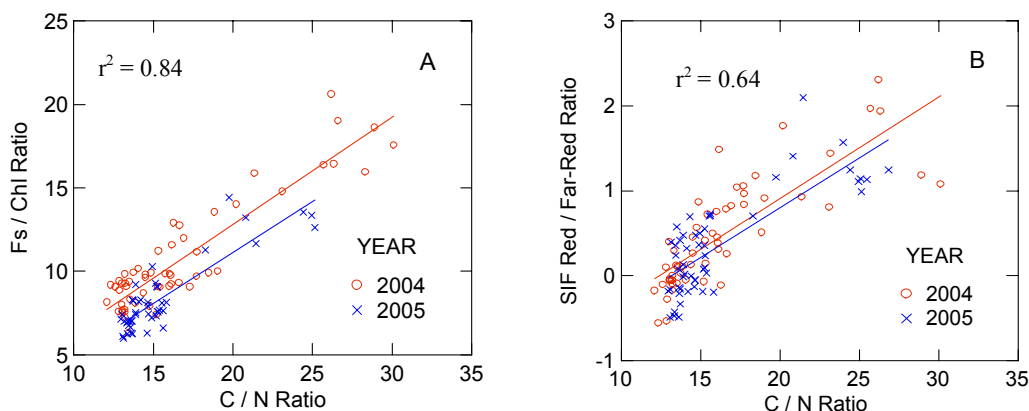


Figure 2. The linear relationships for the two chlorophyll fluorescence ratios to the foliar C/N ratio, from foliage measured at the grain fill (R3) growth stage in two years: A] the foliar Fs/Chl ratio ($r^2 = 0.84$, $n=102$); and B] the whole plant solar passively induced chlorophyll fluorescence (SIF), as the Red/Far-red ratio ($r^2 = 0.64$, $n=109$).

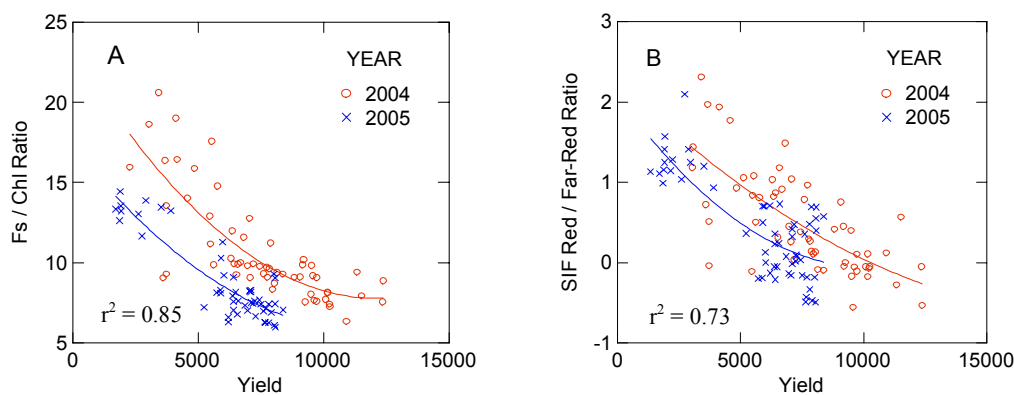


Figure 3. The non-linear, inverse relationships of steady state ChlF obtained at the R3 growth stage (August 2004 and July 2005) to grain yield (Kg / ha) determined at harvest in September (2004, 2005) are shown: A] for the foliar Fs/Chl ratio ($r = 0.92$, $n = 110$); and B] for the whole plant solar passively induced chlorophyll fluorescence (SIF), as the Red/Far-Red ratio ($r = 0.85$, $n=116$).

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