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Deriving Chlorophyll Fluorescence Emissions of Vegetation Canopies from High Resolution Field Reflectance Spectra

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ABSTRACT

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 fluorescence (ChlF) peaks centered at 685 nm and 735 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 and small tree plots of three deciduous species (red maple, tulip poplar, sweet gum). Leaf level measurements were also made of foliage which included ChlF, photosynthesis, and leaf constituents (photosynthetic pigment, carbon (C), and nitrogen (N) contents). As part of ongoing experiments, measurements were made on N application plots within corn (280, 140, 70, and 0 kg N/ha) and tree (0, 37.5, 75, 112.5, 150 kg N/ha) sites 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 red/far-red SIF ratio (SIFratio) derived from these field reflectance spectra successfully discriminated foliar pigment ratios altered by N application rates in both corn crops. This ratio was also positively correlated to the C/N ratio at leaf and canopy levels, for the available corn data (e.g., 2004). No consistent N treatment or species differences in SIF were detected in the tree foliage, but additional 2005 data are forthcoming. This study has relevance to future passive satellite remote sensing approaches to monitoring C dynamics from space.

Keywords: Chlorophyll fluorescence; Fraunhofer Line Depth Method; solar induced fluorescence; carbon and nitrogen dynamics; corn; red maple; tulip poplar.

Abbreviations: C, carbon; Chl, chlorophyll; ChlF, chlorophyll fluorescence; FLD, Fraunhofer Line Depth; FRF, far-red fluorescence; N, nitrogen; RF, red fluorescence; SIF, solar induced fluorescence.

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1. INTRODUCTION

A major goal of the U.S. Carbon Cycle Science Program is to monitor the carbon dioxide (CO₂) uptake of ecosystems. Biological CO₂ sequestration is driven by availability of nitrogen (N), a primary resource regulating photochemical processes and plant growth. A rigorous satellite-based spectral method, integrated through appropriate models, is needed to monitor net ecosystem exchange (NEE) 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. Such an approach should be based on successful ground-based retrievals at experimental/validation sites. The improved accuracy of gross primary productivity (GPP) estimation might enable better quantification of seasonal and inter-annual variability among different ecosystems per biome, so that impacts and/or recovery from human and environmental disturbances and climate changes can be assessed.

The correct interpretation and implementation of spectral indices via remote sensing and modeling activities depends on understanding the underlying physiology, so that environmentally induced stress conditions that limit CO₂

uptake can be adequately taken into account. 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.¹⁻⁴ and has been used to differentiate N status of foliage in laboratory and field studies.⁵⁻⁸ In terrestrial vegetation, ChlF occurs in the red and far-red spectrum, with peaks at 685 and 735 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; energy is also discarded as heat. Plant physiological stress has been captured in ChlF in many studies.⁹⁻¹³

Remotely obtained ChlF from aircraft and satellite platforms might improve GPP estimates. But, use of lasers to actively induce ChlF over landscapes is not a feasible technology due primarily to eye safety issues. Therefore, the possibility of extracting ChlF from high spectral resolution reflectance spectra obtained under ambient solar conditions has been explored.¹⁴⁻²³ ChlF is naturally and passively induced in vegetation, and is referred to here as solar induced fluorescence (SIF). However, determination of SIF for canopies and landscapes has proven challenging because it is a relatively weak signal compared to reflected radiation. Moreover, few instruments are capable of remotely detecting the SIF signal directly,^{17, 24-25} and fewer still that have successfully ascribed the signal to vegetation stress.^{14, 21}

ChlF, as well as blue and green fluorescence, can be measured above vegetation using an established passive technique in narrow spectral regions of the apparent reflected solar spectrum. This passive technique applies the Fraunhofer Line Depth (FLD) principle²⁴⁻²⁵ 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.²⁶⁻²⁷

Extraction of SIF for ChlF assessments from canopy/landscape reflectance spectra has focused on two narrow, telluric oxygen (O₂) absorption bands (less than 5 nm) that 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 (Figure 1). 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. Recently, field studies conducted in Europe under the auspices of the European Space Agency (ESA), such as the Solar Induced Fluorescence Experiment (SIFLEX, 2003), the Airborne Fluorescence Experiment (AirFLEX, 2004), and the Sentinel-2 Fluorescence Experiment (SENT2FLEX, 2005), have shown that SIF from landscapes can be measured at these two O₂ bands, using above-canopy sensors.²⁶ It has also been recently demonstrated that the relatively weak ChlF (compared to reflectance) signal can be detected as SIF in the O₂ bands from satellite altitude with the MERIS instrument, at sufficient accuracy and relevant spatial resolution (300 m) for ecosystem models.²⁷

As part of our research program to identify spectral indices for monitoring C and N dynamics in vegetation, we examined the SIF from high resolution reflectance spectra obtained above mature corn and tree sapling plots provided N augmentation regimes. Recently, we reported our initial first year SIF results.²¹ Here, we report additional results now available from the first year and preliminary results from the second year, at both sites. Comparisons are made among species and N supply treatments for the passive FLD principle applied to telluric O₂ bands from field-acquired canopy reflectance spectra and foliar measurements.

2. METHODS

2.1 Plant Material

Plant material was collected from two research field sites (corn, trees) established to examine N augmentation regimes on vegetation at the USDA Beltsville Agricultural Research Center, Beltsville, MD, USA. 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. The values for 2004 (June, July, August): PAR, 220 W/m²; temperature, 22°C; and rainfall, 393 mm. The values for 2005 (June, July, Aug TBD): PAR, 209 W/m²; temperature, 23.9°C (June, July); and rainfall, 245 mm.

For deciduous trees, nine and ten year old saplings were examined in 2004 and 2005, sampled from a multiyear experiment using tulip poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.) and sweet gum (*Liquidambar styraciflua* L.) planted in 2001. N was applied in the form of urea and concentrations were varied from (0, 37.5, 75, 112.5, 150 kg N/ha) to provide 4 N levels (None, Low, Medium, High), simulating a range of total seasonal atmospheric

N deposition. The design was six blocks comprised of four N plots, each plot having one individual per species, and another block comprised of groups of 3 individual per species in each of 4 N plots, replicated twice. After four seasons, there were ~96 living saplings. The leaf and canopy measurements acquired during September/October 2004 and in August 2005 were used for this study.

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 0, 70, 140, and 280 kg N / ha to provide plant growth conditions ranging from N deficiency to over-fertilization, or 0%, 50%, 100% and 200% of the recommended N levels for this soil. These are designated as N treatments 1-4, respectively, in figures. Canopy and leaf level measurements were acquired in August 2004 and July 2005 at the grain fill (R3) reproductive stage. The 2005 N plots were planted in the same locations per N treatment as in 2004.

2.2 Actively Induced Fluorescence Measurements

A spectrofluorometer (Fluorolog-II, Spex Industries, Edison NJ, USA) was used to collect emission spectra, induced by excitation at fixed wavelengths: 360, 420, 435, 460, 475, 495, and 530 nm. The red and far-red ChlF peaks at 680 and 740 nm were extracted from all emission spectra. The Fluorolog-II utilizes two 0.22 m double spectrometers with gratings of 1200 grooves/mm. A 450 W xenon lamp was attached to the excitation spectrometer, for which a 3.2 nm excitation bandpass (between 250 - 700 nm) resulted for entrance and exit slits set to 2 mm. Correction factors for equalizing fluctuations in lamp intensity as a function of wavelength were generated from data obtained when a beam splitter delivered a portion of the excitation radiation to a silicon photo-diode. The emission spectrometer was attached to a photon-counting photomultiplier tube, which was radiometrically calibrated to obtain linear measures of emission intensity (400 to 800 nm) using a 3.2 nm bandpass with the entrance and exit slits set to 2 mm. Leaf samples were held in place by a non-fluorescent anodized aluminum solid sample-holder. Tree leaves were retained in water-filled florist cuvettes during measurements. Ratios of emission peaks were determined, especially the red/far-red (R/FR) ChlF ratios. R/FR ChlF emission ratios were calculated for each excitation wavelength set, and denoted as R/FR(360EX), R/FR(420EX), R/FR(435EX), R/FR(460EX), R/FR(475EX), R/FR(495EX), and R/FR(530EX).

2.3 FLD determination of SIF in the atmospheric O₂ bands

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. 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 (Fig. 1). Measurements were obtained on clear days in a two-hour window around solar noon. The sample size for sweet gum canopies was much smaller than for red maple and poplar because its less dense late season foliage succumbed to environmentally induced leaf drop.

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. The following algebraic expressions of the FLD principle adapted from Plascyk²⁵ were used to obtain reflectance and SIF from vegetated surfaces:

$$\text{Reflectance} = [(c - d) / (a - b)] \quad (1)$$

$$\text{Fluorescence, as SIF} = (d - Rb) = [(ad - cb) / (a - b)] \quad (2)$$

Here 'a' and 'b' represent the reference panel radiance in and out of each O₂ feature, respectively, and 'c' and 'd' represent the target radiance. SIF within a Fraunhofer feature can also be expressed as the relative stationary yield ($f = \text{SIF}/a$) a dimensionless number representing the degree to which radiance within a relatively dark Fraunhofer line is augmented. The steady state SIF variables obtained were SIF₆₈₈ and SIF₇₆₀. The ratio of these two SIF intensities is denoted as the red/far-red SIF ratio (SIFratio) or the FLD red + far-red total (SIFsum).

2.4 Biophysical Measurements

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

controlled conditions of 1500-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, saturating CO_2 concentration (1000 ppm), controlled leaf temperature (22-24°C), and relative humidity (~35%). The Li-Cor 6400 steady state ChlF parameter (Fs) 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. After *in situ* measurements, leaves were excised from the plant canopy, immediately placed in water filled florist cuvettes, and transported 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 cm^2) that were placed in 3.5 ml of dimethyl sulfoxide (DMSO) and sealed for 36 hours at 25°C. Absorption spectra were obtained using a dual beam spectrophotometer (Lambda 40, Perkin-Elmer, Norwalk CT, USA) and pigment concentrations were determined by procedures outlined by Wellburn²⁸ and expressed as mass per unit leaf area ($\mu\text{g}/\text{cm}^2$). The remainder of each leaf sample was oven dried at 50°C, and ground to pass a 1 mm mesh. Pigment variables were calculated for the Chl *a/b* ratio and the Chl/Carotenoid ratio. The Li-Cor steady state ChlF was expressed per unit total Chl, as the Fs/Chl ratio ($\text{photons/s}/\text{Chl}$). Total leaf C and N determinations were obtained by the Dumas combustion method²⁹ by the University of Delaware Soil Testing Program, Newark DE, USA.

The leaf area index (LAI, m^2/m^2) of the corn canopy was measured with the LAI 2000 Plant Canopy Analyzer (Li-Cor Inc., Lincoln NE, USA). Five sets of LAI (a single above canopy and four below canopy) measurements were acquired at predetermined sample locations throughout each treatment plot. 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 7.0 (Jandel Scientific, San Rafael, CA, USA). LSD mean separations were deemed significant at $p = 0.05$.

3. RESULTS AND DISCUSSION

As was expected, increases with N availability occurred in both years for Chl *a*, Chl *b*, and Amax. Also, the SIF760 intensity, the SIFsum, the fraction of absorbed PAR, and the Chl/Carotenoid ratio increased with N availability. Decreases with N availability occurred in both years for the Chl *a/b* ratio and the SIFratio. Some crop growth variables exhibited saturation responses at the 100% application rate, such as LAI. The observed changes to these variables associated with dose-related N effects were more pronounced in 2004 than in 2005. Several variables (Chl *a* content, Chl/Carotenoid ratio, Chl *a/b* ratio, and LAI) are shown from the corn experiments in Figure 2 (A-D). The crop planting was delayed in 2004 to late May by a wet spring, followed by favorable growth conditions during June-August. In contrast, the 2005 was planted in early May and reached maturity in early August. We sampled these crops at comparable growth stages in the two years, but the 2005 crop may have had the benefit of residual N left in the soil from the previous favorable summer. The SIFratio obtained above the corn canopy was related to photosynthetic pigment content, especially Chl *a* ($r = 0.75$) and the Chl/Carotenoid ratio ($r = 0.86$) (Figure 3 A,B).

We are still processing 2005 data from both the corn and tree measurements, and the 2005 leaf chemistry data are not yet available. We verified that steady state ChlF can be measured successfully at the leaf level for both corn and tree foliage. However, it is evident that the SIF results for the perennial trees are not as easy to interpret as the annual corn crop data, after only 4 years of N manipulation. Whereas the corn crops are subject to conditions over a single growing season, the trees must integrate their responses to environmental conditions over multiple seasons. For our tree plots, the different experimental years have provided a drought year in 2002, wet/cloudy weather in 2003, and a cicada (*Cicadidae*) insect infestation in early June 2004. However, we are able to control N and nutrient availability and water was provided as needed. The tree data for both years were combined here to show that the SIF variables (SIFsum, SIFratio) did exhibit N treatment and species differences, as did the Chl/Carotenoid foliar ratio (Figure 4 A,B,C). However, these differences were not consistently related to N availability. The SIFsum captured the species differences over all N groups (Fig. 4A).

We achieved intriguing results from the 2004 corn crop (Figure 5 A,B), where the SIFratio above the canopy was positively correlated to the foliar C/N ratio (Fig. 5A). Furthermore, this result was consistent with the leaf-level steady state fluorescence normalized to the total Chl content, as the Fs/Chl ratio (Fig. 5B). This ratio was also negatively correlated with the grain yield (kg / ha) for this 2004 corn crop (Figure 6), which was determined at harvest six weeks later ($r = 0.92$). Consequently, grain yields in late September were negatively related to the foliar C/N ratio as determined at the R3 growth stage in mid-August ($r^2 = 0.82$, including N treatment effects, not shown). Although agreement of canopy and leaf fluorescence was not yet observed in the tree saplings, the actively induced R/FR(530EX) ChlF ratio was positively correlated with the C/Chl ratio, varying with year and season. We combined the results for trees (Figure 7) from three years (2002-2004) that spanned spring through autumn seasons, to demonstrate that species

and seasonal differences in the C/Chl ratio were captured by the actively induced R/RF(530EX) ChlF ratio. The C/Chl ratio may be a useful and important carbon cycle parameter, especially if direct measures of carbon uptake (e.g., A_{max}) cannot be strongly related to spectral fluorescence or reflectance indices.

4. SUMMARY AND CONCLUSIONS

We evaluated the success of above-canopy SIF measurements of ChlF to track pigment, carbon, and growth characters at the foliar and/or canopy levels for vegetation provided N augmentation regimes, in two corn crops (2004, 2005) and in tree plots examined in two years (2004, 2005). We found that the 2004 corn experiment produced good correspondence of SIF and leaf-level ChlF, and these were related to the vegetation variables examined. In particular, the Chl/Carotenoid foliar ratio, the C/Chl foliar ratio, and the grain yield were related to foliar C/N ratio, and N treatment effects on these variables were captured in the SIFratio. When the leaf chemistry and harvest data are available for the 2005 corn crop, we will evaluate similar trends for that year. Similarly, we will further examine the 2005 tree results when leaf chemistry data are available. However, based on the available 2004-2005 data, SIF observations were too variable to attribute to either N or species differences, although leaf-level relationships to ChlF could be established.

These results support further examination of the FLD technique to derive SIF for ChlF from high resolution reflectance spectra of canopies/landscapes. Model simulations should also be performed to estimate SIF at satellite altitudes. Thus far, vegetation GPP has only been estimated in the optical and near-infrared wavelength range (e.g., using the near infrared (NIR) and red satellite reflectance bands with the Normalized Difference Vegetation Index, $(NIR - Red)/(NIR + Red)$). GPP and related carbon parameters estimated from SIF of terrestrial vegetation, whose source is the ChlF emissions of physiologically active and carbon-assimilating leaves, have not yet been exploited by any satellite mission. Our research program complements that of ESA-sponsored projects to evaluate SIF for that purpose, with the ultimate goal of defining an appropriate satellite mission based on SIF to monitor carbon dynamics across ecosystems.

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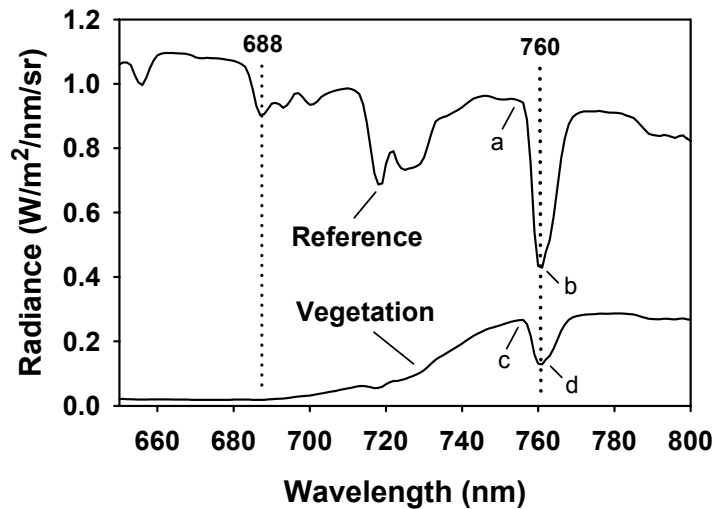


Figure 1. Radiance measured in the red through far-red spectral region. Telluric O₂ bands centered at 688 and 760 nm are denoted with dotted lines, while a, b, c, and d correspond to points in Eqns. 1 & 2 for the FLD determination of far-red SIF.

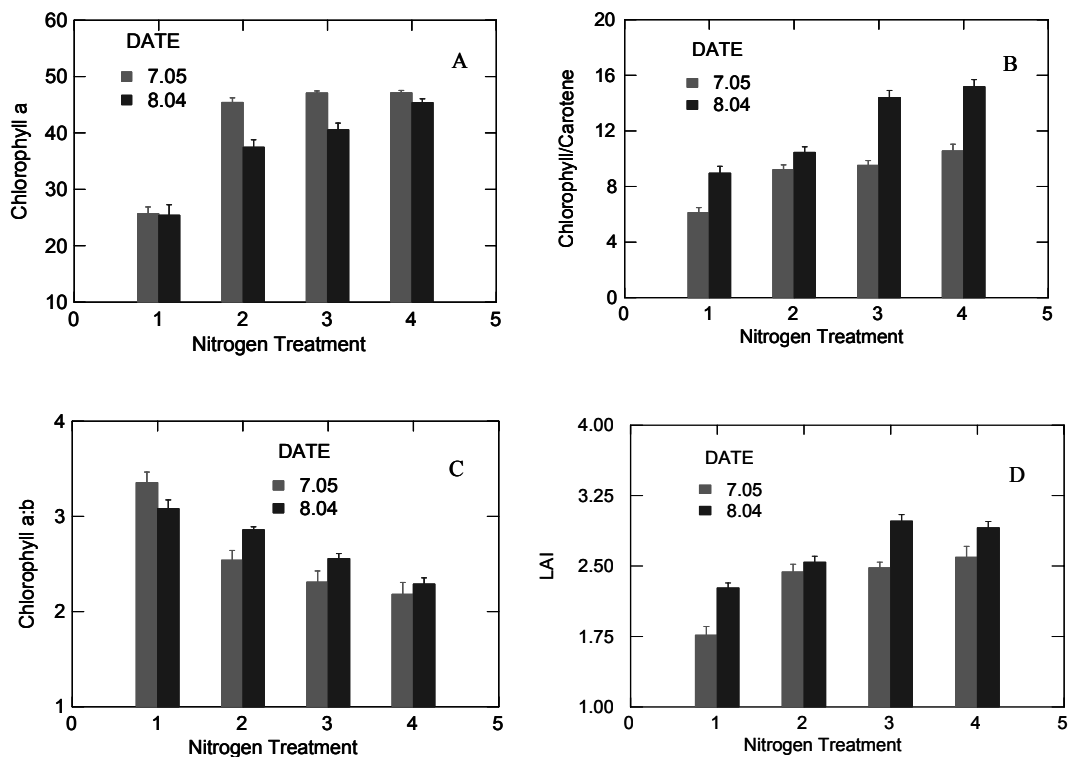


Figure 2. The means and standard errors (SE) for three variables are shown for measurements made for the two corn crops (2004, 2005). The experiment-wide coefficient of determination is given for a 3-factor model (N treatment, date, and N*date differences, $n = 119-120$): **A]** Chl *a* (microg/cm²), $r^2 = 0.82$; **B]** Chl/Carotenoid ratio, $r^2 = 0.75$; **C]** Chl *a/b* ratio, $r^2 = 0.75$; and **D]** LAI (m²/m²), $r^2 = 0.71$. Nitrogen augmentation treatments 1 - 4 are 0, 70, 140, and 240 Kg N / ha, respectively.

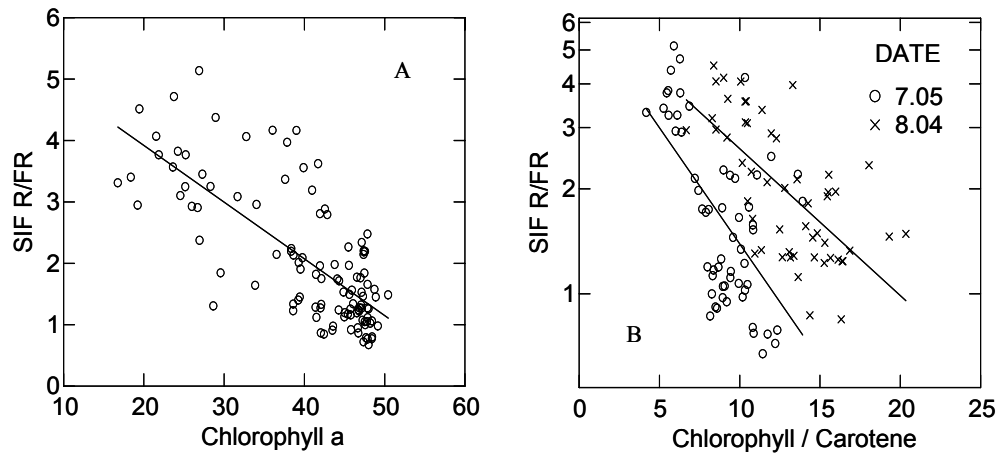


Figure 3. The SIF R/FR canopy ratio (SIFratio), expressed on a log scale, is correlated to two foliar pigment variables: **A]** Chl *a* (microg/cm²), both years combined ($r = 0.75$, $n = 119$); and **B]** log Chl/Carotenoid ratio, with separate lines per year-- 2004 & 2005 ($r = 0.86$, $n = 119$).

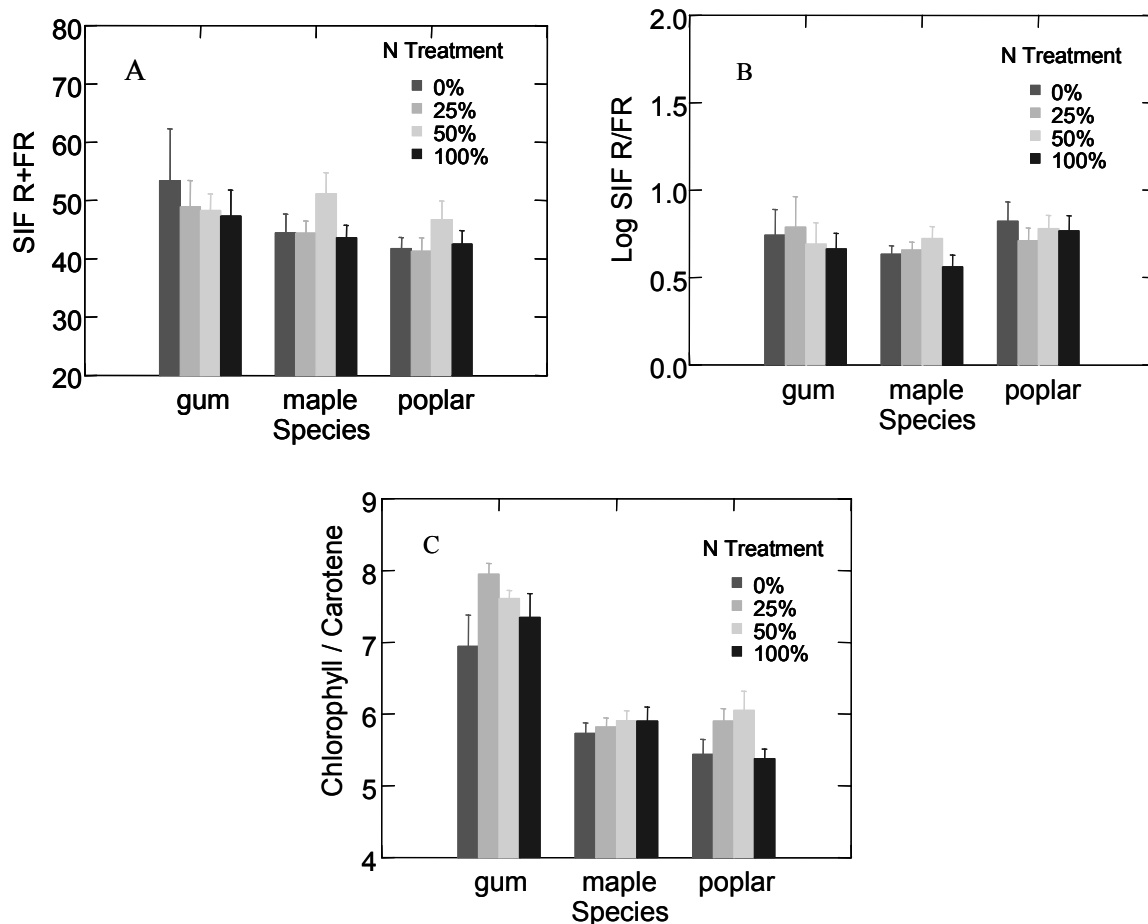


Figure 4. The means and standard errors (SE) are shown for two SIF canopy variables and a foliar pigment variable from measurements made in two years on three species of tree saplings (sweet gum, red maple, tulip poplar): **A]** SIF R+FR (SIFsum, W/m²); **B]** SIF R/FR, or SIFratio expressed in log relative units; and **C]** Chl/Carotenoid ratio. Nitrogen augmentation treatments 1 - 4 are None, Low, Medium, and High, respectively.

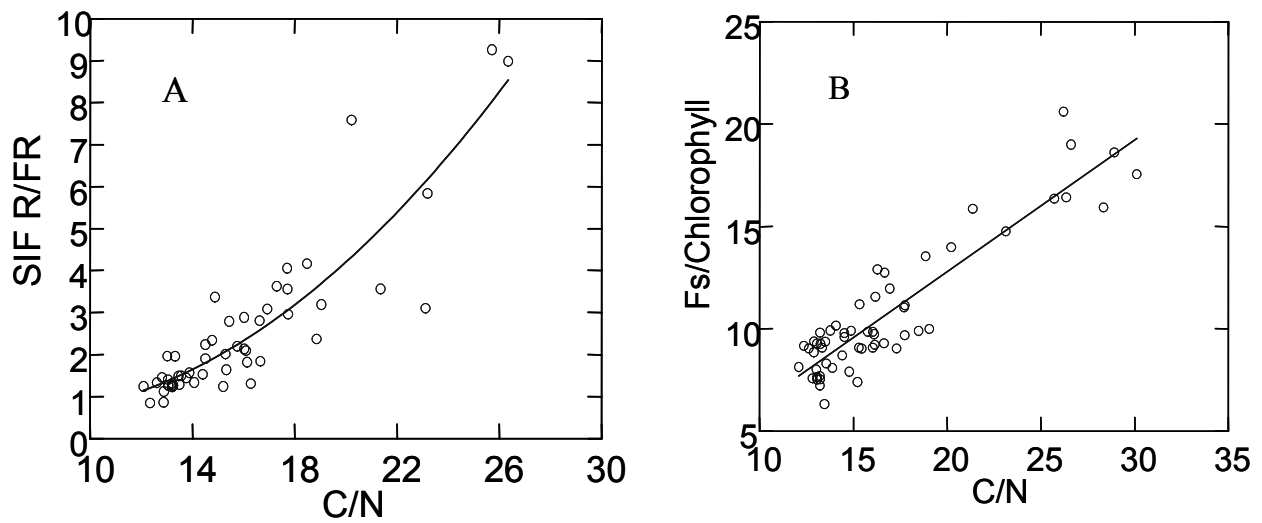


Figure 5. Agreement was achieved between canopy and foliar steady state ChlF variables (expressed in relative units) in the 2004 corn crop, since both were positively correlated to the foliar C/N content: **A]** the SIF R/FR ratio, or SIFratio ($r = 0.89$, $n = 53$); and **B]** the Fs/Chl ratio ($r = 0.92$, $n = 58$).

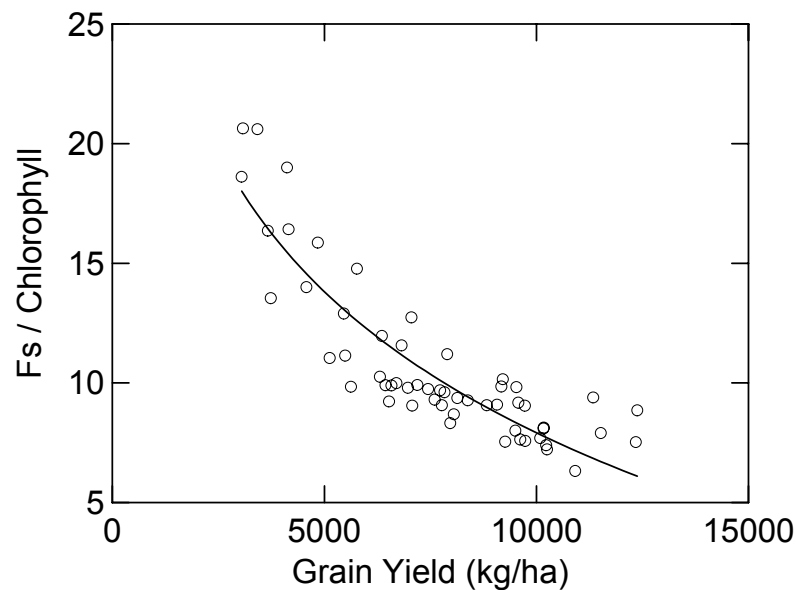


Figure 6. For the 2004 corn crop, the relationship of steady state ChlF obtained in August, expressed as the Fs/Chl ratio, to grain yield (Kg / ha) determined at harvest in September is shown ($r = 0.92$, $n = 56$).

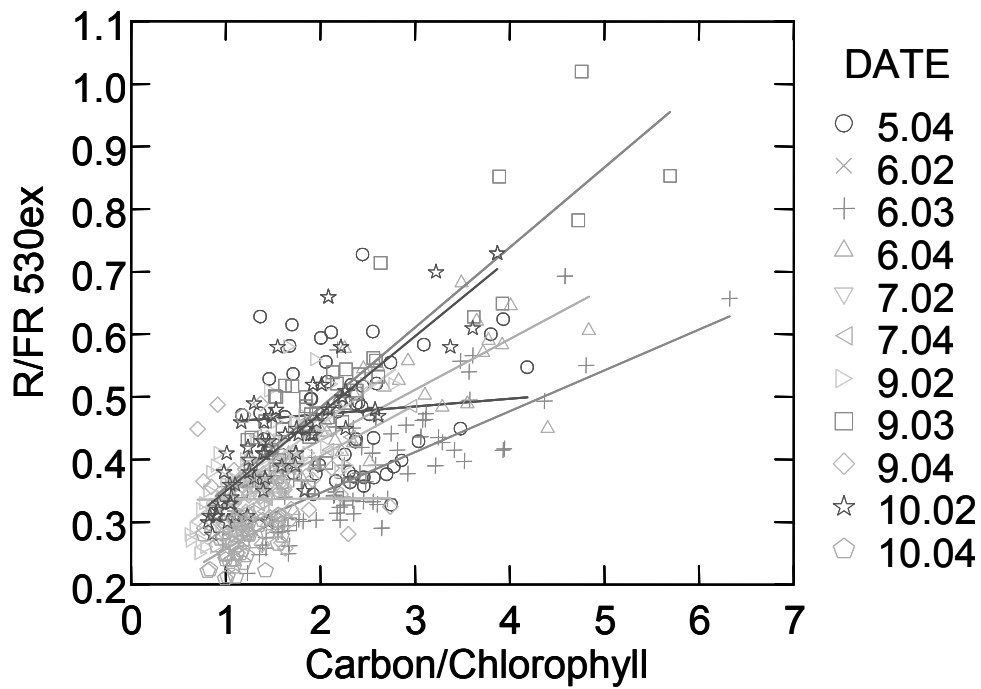


Figure 7. The actively induced foliar R/FR(530EX) ChlF ratio obtained from foliage of trees in several years (2002, 2003, 2004) and seasons (spring, summer, fall) is positively related overall to the C/Chl ratio ($r = 0.87$), a potentially important carbon parameter. Dates (month.year) of acquisitions appear on the right side of the figure.