

# Comparison of measurements and FluorMOD simulations for solar induced chlorophyll fluorescence and reflectance of a corn crop under nitrogen treatments [SIF and reflectance for corn]

ELIZABETH M. MIDDLETON\*†, LAWRENCE A. CORP‡, and  
PETYA K. E. CAMPBELL§

†Hydrospheric & Biospheric Sciences Laboratory (Code 614.4)  
NASA/Goddard Space Flight Center, Greenbelt, MD 20771, USA

‡Science Systems and Applications, Inc. (SSAI), Lanham, MD 20706, USA

§University of Maryland Baltimore Co. (UMBC), Catonsville, MD, USA

\*Corresponding author. Email: Elizabeth.M.Middleton@nasa.gov

The FLuorescence EXplorer (FLEX) satellite concept is one of six semi-finalist mission proposals selected in 2006 for pre-Phase studies by the European Space Agency (ESA). The FLEX concept proposes to measure passive solar induced chlorophyll fluorescence (SIF) of terrestrial ecosystems. A new spectral vegetation Fluorescence Model (FluorMOD) was developed to include the effects of steady state SIF on canopy reflectance. We used our laboratory and field measurements previously acquired from foliage and canopies of corn (*Zea mays* L.) under controlled nitrogen (N) fertilization to parameterize and evaluate FluorMOD. Our data included biophysical properties, fluorescence (F) and reflectance spectra for leaves; reflectance spectra of canopies and soil; solar irradiance; plot-level leaf area index; and canopy SIF emissions determined using the Fraunhofer Line Depth principal for the atmospheric telluric oxygen absorption features at 688 nm ( $O_2\beta$ ) and 760 nm ( $O_2\alpha$ ). FluorMOD simulations implemented in the default "look-up-table" mode did not reproduce the observed magnitudes of leaf F, canopy SIF, or canopy reflectance. However, simulations for all of these parameters agreed with observations when the default FluorMOD information was replaced with measurements, although N treatment responses were underestimated. Recommendations were provided to enhance FluorMOD's potential utility in support of SIF field experiments and studies of agriculture and ecosystems.

*Keywords:* reflectance, chlorophyll fluorescence, solar induced fluorescence, FluorMOD, corn, nitrogen, abaxial foliar surface

## 1 Introduction

Solar induced chlorophyll fluorescence (SIF) from vegetation canopies is indicative of plant physiological function and allows early detection of environmentally induced stress.

However, the relation between steady state fluorescence (F) produced at the leaf level and the signal detected at the top of the canopy, or above the atmosphere by remote sensing techniques, is very complex. Therefore, models have been developed since the early 1990s to better quantify the relationships affecting the top-of-canopy F by consideration of factors influencing F measurements from single leaves. Rosema *et al.* (1991) developed the FLSAIL model, an extension of the SAIL (Scattering by Arbitrarily Inclined Leaves) model (Verhoef 1984) that included canopy architecture parameters. The FluoroMOD canopy F model builds on these earlier efforts. It includes the dependence of F emissions on the photosynthetically active radiation (PAR) conditions (Verhoef 2005), especially as influenced by leaf orientation with respect to the sun's position and changes in light quality with depth inside the canopy for the incident downward and diffuse upward fluxes.

An objective of the FLuorescence EXplorer (FLEX) Mission (Moreno 2005), a concept under development through the European Space Agency, is to utilize FluorMOD for simulating realistic SIF above vegetation surfaces and to estimate its detection from space. The FLEX mission concept relies on retrieval of steady state SIF from the apparent vegetation canopy reflectance spectra using the Fraunhofer Line Depth (FLD) principal (Plascyk 1975), for two major atmospheric telluric oxygen absorption features: O<sub>2</sub>∓, centered at 688 nm; and O<sub>2</sub>∕, centered at 760 nm. SIF varies with vegetation status (species, phenology, vigor, etc.) and is linearly dependent on PAR illumination intensity (Liu *et al.* 2005, XXXXXXXX). The intensities of SIF expected from vegetation across landscapes have yet to be fully characterized, and a consensus has not yet been reached on the optimal methods for SIF measurements or their expected SIF magnitudes. Currently, estimates made with different instruments and species under different illumination conditions vary for canopy red SIF from 1.5 to 10.5 mW m<sup>-2</sup> nm<sup>-1</sup> sr<sup>-1</sup>, while canopy far-red SIF estimates range from 0.5 to 12.0 mW m<sup>-2</sup> nm<sup>-1</sup> sr<sup>-1</sup> (Theisen 2000, Moya *et al.* 2003, Liu *et al.* 2005, Dobrowski *et al.*

2005, Louis *et al.* 2005, Meroni *et al.* 2006, Corp *et al.* 2006). This range in values could be attributed to the large variation in the illumination conditions during data acquisition and/or to uncertainties in the units of measure.

Our research group has been investigating several aspects of F properties of foliage and canopies, including SIF. Using the FLD principle applied to spectra spanning the two oxygen bands, we estimated canopy average red (at 688 nm) SIF values of  $4.3 \pm 1.0 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$  and far-red (at 760 nm) SIF values of  $2.0 \pm 0.8 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$  for a cornfield, under a relative PAR intensity of  $1660 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (Middleton *et al.* 2006). These *in situ* canopy SIF levels, which fall within the range suggested by other investigations, were utilized in the present study along with contemporaneously acquired supporting field and laboratory measurements for a rigorous preliminary evaluation of FluorMOD. The plausibility of using actively induced F information gained from laboratory measurements on individual leaves for estimating the SIF that occurs in nature under ambient illumination and other environmental conditions for vegetation canopies, via scaling up methods based on 1-D radiative transfer models is addressed.

## **2 Materials and methods**

### ***2.1 Plant material, field and leaf measurements***

A corn (*Zea mays* L.) crop was arranged in plots within three complete blocks at the USDA Agricultural Research Service in Beltsville, MD, USA. Each plot received a controlled N fertilization regime at one of four dosages (280, 140, 70, and 0 kg N/ha) in experiments conducted in August of 2004. These treatments augmented existing soil N levels by 200%, 100%, 50% or 0% of the recommended rate. Measurements were made when the crop had achieved the grain filling reproductive (R3) growth stage and had attained a height of ~3 m.

Leaf-level measurements were obtained on leaf #13 (the ear leaf) and included optical properties (reflectance, transmittance, absorptance), chlorophyll content, specific leaf mass and other physiological measurements (Middleton *et al.* 2006a,b). Plot-level leaf area index (LAI) was measured contemporaneously using the Li-Cor 2000 (Li-Cor, Lincoln, NE, USA).

The canopy spectra were acquired using a spectroradiometer (ASD-FR FieldSpec Pro, Analytical Spectral Devices, Inc., Boulder, CO, USA). The ASD spectroradiometer uses a 512-channel array of silicon photodiodes overlaid with an order separation filter to provide a 3 nm full width at half maximum (FWHM) spectral resolution, sampled at 1.4 nm. Nadir radiances were measured at 1 m above plant canopies (4 m above the soil surface) with an ASD within a 22° field of view, providing a ~1.2 m ground resolution. A second ASD radiometer simultaneously acquired solar irradiance spectra over a stationary Spectralon reference panel (Labsphere, North Sutton, NH, USA). Canopy ASD measurements were obtained on a clear day at noon  $\forall$  2 hours (photosynthetic photon flux density,  $\sim 1660 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Nadir canopy spectral reflectance was calculated as the ratio of canopy radiance to panel irradiance for each sample, and presented as per cent reflectance per waveband.

## ***2.2 Laboratory fluorescence measurements***

Actively induced F spectra were obtained in the laboratory for both adaxial and abaxial surfaces at selected discrete excitation (EX) wavelengths, including 420 nm (420EX) and 532 nm (532EX), using a spectrofluorometer (Fluorolog-II, Spex Industries, Edison NJ, USA). Excitation spectra were also obtained for emissions at the peak chlorophyll fluorescence (ChlF) wavelengths, F680 and F740. for the adaxial leaf surfaces only, excitation-emission matrices (EEMs) were produced at a spectral resolution of  $\leq 5$ , for EX wavelengths between 400-750 nm and emission wavelengths between 600-800 nm. A standard, calibrated EEM was constructed from these measurements on leaves from each N

treatment, and values near the F peaks were interpolated (referred to below as the standard, solar uncorrected EEM). A solar-corrected EEM was developed from the standard EEM by applying correction factors to achieve a spectral profile and intensity for the xenon illumination source that closely resembled that of the solar spectrum, as determined from mid-day summer irradiance spectra collected with an ASD over a Spectralon panel (Campbell *et al.* 2006) for PAR at  $1660 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Simulated adaxial leaf SIF intensities ( $\text{mW m}^{-2} \text{nm}^{-1} \text{sr}^{-1}$ ) were then obtained through integration of monochromatically acquired excitation spectra (Corp *et al.* 2006b). Other details on the instrumentation and methodologies for F measurements can be found in Corp *et al.* (2006b) and Middleton *et al.* (2005, 2006a,b).

Leaf-level SIF was directly determined using full solar spectrum, simultaneously available polychromatic radiation from a 250 W solar simulator (Oriel 91160A, Newport Stratford Inc., Stratford, CT, USA) outfitted with a Global Air Mass Filter (Oriel 81080, Newport Stratford Inc., Stratford, CT, USA). The system simulated mid-day solar irradiation typical for the Washington, DC (USA) area in mid July and was used to illuminate leaves held in special frames. Apparent foliar spectral reflectance measurements of these leaves were acquired for leaf areas of  $\sim 2 \text{ cm}^2$  using an ASD spectroradiometer with a sensor foreoptic view angle of  $8^\circ$ , with a Spectralon panel serving as a reference. To determine the F contribution, a Schott RG 665 long pass filter was used to prevent F induction by wavelengths below 665 nm. This combined solar simulator and ASD system was used to separately induce and measure ChlF and apparent reflectance across the 650 – 800 nm region, for both adaxial and abaxial surfaces of the same leaves used for the previously described laboratory F measurements. Before illumination, leaves were dark-adapted for five minutes, and steady state F was achieved within  $\sim 25 \text{ s}$  illumination, as described in Campbell *et al.* (2002, 2006).

### 2.3 FLD determination of SIF over the cornfield

SIF was extracted from the apparent canopy reflectance spectra at 688 and 760 nm using the Fraunhofer Line Depth (FLD) principal (Plascyk 1975, Corp *et al.* 2006b). The FWHM for these two major telluric features is 4 nm for O<sub>2</sub>∩ (centered at 688 nm) and 7 nm for O<sub>2</sub>∨ (centered at 760 nm). The following algebraic expressions of the FLD principle adapted from Plascyk (1975) were used to obtain canopy reflectance and F from vegetated surfaces in each of these SIF bands:

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

$$\text{Fluorescence} = d - Rb = (ad - cb) / (a - b) \quad (2)$$

Here 'a' and 'b' represent the reference panel radiances, within and outside each O<sub>2</sub> feature, respectively; 'c' and 'd' represent the comparable target radiances. This method was applied to the above-canopy ASD upwelling and downwelling spectra acquired at marked locations with the N plots; the irradiance spectra were normalized to 1660 μmol m<sup>-2</sup> s<sup>-1</sup> since SIF is correlated with PAR intensity (Liu *et al.* 2005), to enable comparison across N treatments acquired throughout the mid-day period. The ASD's 3 nm FWHM spectral resolution sampled at 1.4 nm was assumed to have sufficient resolution for the quantification of SIF within these major telluric O<sub>2</sub> features.

### 2.4 FluorMOD

The FluorMOD combines plant F, leaf and canopy radiative transfer equations, and atmospheric correction algorithms to predict the spectral responses of vegetation, including SIF. The FluorMOD V3.1 graphic user interface (Zarco-Tejada 2005) was used to link the inputs and outputs from both FluorMODleaf and FluorSAIL modules to provide realistic top-of-canopy SIF. Leaf input parameters include total chlorophyll (*a+b*), water equivalent thickness, dry matter content, F quantum efficiency, leaf temperature, species temperature

dependence, and stoichiometry of PSII to PSI reaction centers, in addition to soil reflectance and solar irradiance. Other inputs of the model describe the canopy architecture, given by LAI, two leaf inclination distribution function parameters and the hot spot parameter, the illumination and viewing geometries, and two parameters describing the dependence of leaf F on the PAR intensity.

The output from FluorMODleaf is passed to FluorSAIL for which output parameters include the solar irradiance, sky irradiance, total irradiance, radiance without F, radiance with F included, total radiance, reference reflectance, total reflectance, reflectance (from SAIL), the ratio SAIL/FluorSAIL, and reference and total top-of-atmosphere radiances. Initially, we generated FluorSAIL canopy outputs based on FluorMODleaf look-up table information, in conjunction with measured vegetation parameters summarized in Table 1, to simulate leaf reflectance and F spectral properties (the 'default' mode). Subsequently, the FluorSAIL canopy component of the model was run using our measurements for vegetation parameters, leaf and field spectra, and upward EEMs for the adaxial foliar surfaces (referred to below as the 'revised' mode). Unfortunately, we did not have measured downward EEMs of the abaxial surfaces to replace the 'default' downwelling matrices in FluorMOD.

### **3 Results and Discussion**

The 2004 biophysical data utilized here for the FluorMOD parameterization/comparison is summarized in Table 1. A full description of the leaf, canopy, and crop measurements acquired in two growing seasons (2004, 2005) at the USDA cornfield appears elsewhere (Middleton *et al.* 2005, 2006a,b; Corp *et al.* 2006a).

#### ***3.1 Leaf Chlorophyll fluorescence***

A comparison of ChlF emission spectra (650-850 nm) of corn leaves obtained from measurements versus estimates from FluorMOD (Figure 1) reveals that the model simulations produced much lower F values than the measurements at two selected excitation wavelengths (420EX, 532EX). Model underestimates were approximately one-third that of measured values for emissions produced with 420EX, and more than an order of magnitude lower for those produced with 532EX (Fig. 1, A vs. B). For the 420EX case, the relative magnitudes of the red and far-red ChlF peaks were about equal, as expressed in both the measured and simulated spectra (Fig. 1A). However, for 532EX, the measured far-red ChlF peaks were 2-3 times greater than the red peaks, but they were approximately equal in the simulated spectra (Fig. 1B). For measured emissions, the far-red ChlF peaks were similar ( $\sim 0.07 - 0.11 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) from both EX wavelengths, although N treatments were better captured with 532EX (Fig. 1A,B). These ChlF values appear small because they represent emissions resulting from sequential monochromatic wavelength EX.

In excitation (Figure 2) spectra, as for emission spectra above, N treatment differences were well expressed in the leaf-level F measurements, but only weakly expressed in the simulations. As in the emission spectra, underestimates occurred in excitation spectra for the simulated emissions at the ChlF peaks (680 and 740 nm) when compared to the measured, solar corrected (normalized to measured solar irradiance) ChlF. Fig. 2 also shows that the measured emission intensities at the ChlF peaks resulting from monochromatic excitation in the blue-green region were higher ( $\leq 0.22 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) than those shown in Fig. 1 for 420EX and 532EX, which bracket this region.

There appears to be a shift in the modeled red ChlF (F680) resulting from blue excitation, with peaks produced at 420 and 440 nm instead of 430 and 470 nm as observed in the measured excitation spectra, and as would be expected to match established chlorophyll *b* and carotene absorption features (Fig. 2A). The underestimate of the simulated far-red ChlF

(F740) emissions as a function of EX wavelength is much greater, and especially deficient throughout the green region (Fig. 2B). Furthermore, it appears that the same EX curves were utilized to create these F680 and F740 responses (e.g., note the feature at 675 nm in Figs. 2 A&B). The F740 emission peak centered at 675 nm excitation (675EX, Fig. 2B) is too narrow, whereas the same feature in Fig. 2A physically cannot exist at that position (since emissions result from EX at shorter wavelengths), and may be intended to represent the emission peak for excitation at 650 nm that appears in the measured spectra.

The red/far-red emission ratio (F680/F740) was determined from the information given in Figs. 2 A&B (Fig. 2C). The excitation spectra produced for this F680/F740 ratio by FluorMOD show values close to 1.0 ( $\forall$  20%) except for a  $\sim$ 40% decrease centered at  $\sim$ 530 nm (Fig. 2C). However, this ratio should fall to zero beyond 680 nm in concert with F680 emissions, as observed in the measured ratio spectra (Fig. 2C). The measured F680/F740 ratio spectra exhibit a similar but more pronounced shape, with most values of the ratio falling well below 1.0 and with a  $\sim$ 70% decrease centered at  $\sim$ 530 nm (Fig. 2C), beyond which the measured F680/F740 ratios are  $\leq$ 0.6 at green through red EX wavelengths.

One reason for the FluorMOD underestimates of corn leaf F is shown in 3-D EEM plots (Figure 3), where the solar-corrected EEM (normalized to solar irradiance, Fig. 3A) was compared with the EEM created from FluorMOD (Fig. 3B). The correction for the total solar PAR intensity of  $1660 \mu\text{mol m}^{-2} \text{s}^{-1}$  and for the actual spectral variations in intensity across the visible (VIS) solar irradiance spectrum, relative to the flat irradiance spectrum of the lamp source, enhanced F intensities— especially in the green region ( $\sim$ 500-600 nm) where solar irradiance is higher relative to the far-red region. The standard EEM (not shown) is similar to Fig. 3A.

The relative difference between the solar-corrected versus the FluorMOD EEM for the 200% N group, presented as a 2-D contour plot (Figure 4A), indicates that the greatest

discrepancies ( $\leq 0.12 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) occurred for far-red ChlF emissions across most EX wavelengths. The impact of performing the solar-correction on the standard EEM is highlighted for the 200% N group (Fig. 4B); here, the subtle differences between the solar corrected and standard EEMS are apparent, whereas they are difficult to see in a 3-D plot. Fig. 4B shows that solar correction augmented ChlF (especially in the peaks) emanating from blue EX wavelengths; the far-red ChlF emissions were also augmented when produced from green EX wavelengths; reductions in F occurred for the ChlF peaks when they resulted from longer wavelength EX ( $> 650 \text{ nm}$ ) or EX  $< 430 \text{ nm}$ . The impact of the experimental range of N treatments (0 – 200%) on the solar-corrected, measured EEMs is shown for a similar 2-D difference plot group (Fig. 4 C), as F for the high (200% N) group minus F for the lowest (0%) N. The greatest differences ( $\leq 0.02 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) due to N availability occurred in both the red and far-red ChlF peaks resulting from EX centered around  $\sim 470 \text{ nm}$ , and for far-red ChlF from green ( $\sim 550 \text{ nm}$ ) EX wavelengths.

### ***3.2 Leaf-level SIF***

Fig. 2 (A, B) showed that F680 emanated largely from excitation by the blue and green wavelengths, whereas F740 resulted from contributions that were more distributed across the visible (VIS, 400-700 nm) excitation spectrum. This is shown more clearly in Figure 5, where the red (F680) and far-red (F740) ChlF peak emissions were solar-corrected and integrated across EX wavelengths in the VIS spectrum to obtain estimates of leaf adaxial SIF resulting from polychromatic EX, as occurs in natural ambient solar environments. The 200% N treatment produced an adaxial red SIF of  $4.7 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ , while the far-red SIF was higher at  $8.2 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ , and the F685/F740 ratio equaled 0.57. Both red and far-red peak SIFs were significantly greater (by  $\sim 2 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) for the 200% N group than for the lower N treatments (0-100%). Somewhat lower adaxial SIF values were obtained

from the second approach that provided polychromatic induction of ChlF using the solar simulator, but this is expected at a lower lamp PAR intensity ( $\sim 1275$  vs.  $1660 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). These SIF results for the adaxial 200% N treatment were: F685,  $\sim 1.7 \pm 0.5 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ; F740,  $\sim 4.0 \pm 1.2 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ; and F685/F740 ratio, 0.46 (Figure 6 A,B). This compares favorably with the ‘polychromatic’ adaxial red/far-red SIF ratio (0.57) obtained above for the 200% N group in Fig. 5. The adaxial red/far-red ratio (F685/F740, Fig. 6C) was significantly smaller (0.35) for the lower two N groups (0-50%).

The most impressive new information is that F685 from the leaf undersides (the abaxial surfaces) was significantly larger than for adaxial surfaces in all N groups. For example, the 200% N group produced abaxial F685 and F740 of 3.2 and  $4.1 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ , respectively (Fig. 6 A,B). F740 was greater for abaxial surfaces only in the higher (100-200%) N treatments. However, the F685/F740 ratio for the lower, abaxial surfaces was substantially larger (0.6-0.8) across all N treatments relative to ratio values for their upper surfaces (0.3-0.5, Fig. 6C).

### **3.3 Canopy SIF**

The leaf-level SIF characterizations were utilized to estimate canopy SIF emissions with FluorMOD (Figure 7). The modeled canopy SIF was very low ( $< 1 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) when executed in the ‘default’ mode, and did not express N treatment effects. After replacing the default FluorMOD information with our own measurements for leaf and environmental spectra, including solar-corrected SIF (i.e., the ‘revised’ mode), the simulated canopy SIF was enhanced by a factor of  $\sim 5$ , the far-red SIF peak exceeded the red peak intensity, and N treatment effects on SIF were captured (Fig. 7).

When the FluorMOD simulation was run in this adaptive, revised mode, canopy SIF at the red and far-red ChlF peaks was  $\sim 3.4 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$  and  $\sim 5.6 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ,

respectively, for the high (200%) N group. These modeled canopy SIF intensities correspond reasonably well with our leaf-level measurements presented in Figs. 5 & 6. In addition, the estimated red/far-red SIF ratio (F688/F760) changed from ~0.95 in 'default' simulations to 0.61 in 'revised' simulations, consistent with the leaf-level values presented above.

Consequently, replacing the 'default' leaf spectra and EEMs with measurements greatly enhanced and improved characterization of the corn canopy SIF responses, as estimated from actively induced laboratory F measurements. However, we might have achieved a better correspondence to measured canopy SIF (by producing higher red canopy SIF) as discussed below, if we had been able to provide abaxial EEMs for the 'revised' FluorMOD simulations. It must be noted that diurnal changes in leaf and canopy SIF have not been addressed in these examinations, although mid-day reductions in ChlF and/or SIF have been reported (Dobrowski *et al.* 2005, Jouis *et al.* 2005).

In the revised mode, FluorMOD's estimate of canopy SIF for F688 in the 200% N group ( $3.7 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) was only slightly lower than our *in situ* field SIF values for F688 ( $4.3 \pm 1.0 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ), but the comparable SIF for F760 was twice as high as our field values ( $5.6$  vs.  $2.0 \pm 0.8 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ). Although both the 'default' and 'revised' FluorMOD simulations indicate a canopy red/far-red SIF ratio (F688/F760)  $\leq 1.0$  (Fig. 7), we actually obtained values  $> 1.0$  in our ASD field determinations of this ratio: ~1.4 for the higher (100 - 200%) N groups and 3.9 for the two lower (0 - 50%) N groups (Middleton *et al.* 2006). The discrepancy between canopy SIF observed in the field and SIF estimates generated from leaf-level adaxial laboratory F information might be partially explained by the higher ChlF emissions of abaxial leaf surfaces that influence canopy SIF, responses that are not sufficiently described in FluorMOD. Where red SIF is 2-3 times greater for abaxial than adaxial surfaces of healthy, vigorous vegetation (as shown in Fig. 6), and subject to multiple scattering within the canopy, the resulting canopy red SIF would be enhanced over leaf-level

values. A red/far-red ratio  $> 1.0$  has also been reported by other researchers. For example, Campbell *et al.* (2002, 2006), Meroni and Colombo (2006), and Liu *et al.* (2005).

### ***3.4 Leaf-Level Reflectance***

At the leaf level (Figure 8), FluorMOD simulations produced reflectance spectra that generally agreed with measured spectra. These spectra show apparent leaf reflectance (reflectance + SIF). In both measured (Fig. 8A) and modeled (Fig. 8B) leaf spectra, the 0% N treatment produced a higher green reflectance than other groups, although the model simulations underestimated the measured value (10% vs. 15%). In the near-infrared region (NIR, 800-1000 nm), the 200% N treatment generated a higher measured reflectance (at ~46%, Fig. 8A) than other N treatments, but the FluorMOD reflectances were lower (at ~37%, Fig. 8B) and did not exhibit N treatment effects.

The difference spectra (observed minus modeled, Fig. 8C) indicate that reflectance was underestimated at all wavelengths (450-1000 nm) in the model simulations. There were no N treatment effects in the VIS region, where measurements were up to 5% higher than FluorMOD estimates (with the greatest difference at 560 nm). However, discrepancies between FluorMOD and measured spectra were most apparent in the NIR region, where differences increased with N treatments (0 – 200%) and the largest (6-10%) occurred at 760 nm – the location of the  $O_2\text{V}$  feature. A small shift in the red edge for modeled vs. measured leaf spectra was also expressed in the difference spectra near 700 nm.

### ***3.5 Canopy Reflectance***

Much better correspondence was achieved between FluorMOD simulations and canopy measurements for apparent canopy reflectance spectra (Figure 9) than for canopy SIF. FluorMOD simulations made in the ‘revised’ mode with our spectral measurements produced

good correspondence to ASD field reflectance measurements (Fig. 9A, B), especially for the 0% N group. However, canopy reflectance was underestimated by a few percent in the other N groups (e.g., -1.5% at 570 nm, 50-200% N; -6% at 930 nm, for 200% N). Both the measured and FluorMOD canopy spectra expressed additional responses to N deficiency, captured in the greater blue-ward shift of the red edge and lower NIR coupled with enhanced green reflectance. Further examination of the red edge demonstrates that N treatment effects were more pronounced in the measured canopy spectra. For example, an 8 nm shift (738 to 730, high to low N treatment) was observed in the measured canopy red edge, whereas only a 4 nm shift (733 to 729 nm) was seen in modeled canopy spectra.

The measured ASD canopy reflectance spectra were lower in intensity than measured leaf spectra (Figs. 9A vs. 8A), but exhibited greater N treatment effects in the green and NIR regions. For example, canopy reflectance in the green (at 570 nm) was 5-7%, compared with leaf reflectance of 11-15%; canopy NIR reflectance ranged between 27-42%, compared with leaf reflectance of 43-46%. One notable feature of the measured canopy spectra, not reproduced in FluorMOD simulations, was an increase in reflectance from the NIR shoulder (~760 nm, 0%N; ~770 nm, 200% N) that reached maximum values at 930 nm. These effects could be due to a combination of several factors, including: the influence of exposed soil and dead leaves in the lower parts of canopies (especially in the 0 and 50% N treatments); representation of the canopy as a single homogeneous layer comprised of adaxial leaf surfaces by FluorMOD; differences in leaf display in response to N treatment that affected the projected LAI of adaxial vs. abaxial leaf surfaces; and differences in leaf morphology throughout the vertical canopy profile that were not captured in the measurements of each plants' representative (#13) leaf nor in FluorMOD canopy characterizations.

The canopy apparent reflectance difference spectra (Fig. 9C) revealed that FluorMOD estimates agreed with measurements in the VIS region (#2%). However, FluorMOD

overestimated (by #3.5%) the NIR reflectance of the two high N treatments, especially around the 760 nm feature ( $O_2V$ ). FluorMOD overestimates were greater (#6.5%) for the two low N treatments. Separation of the high and low N groups in the difference spectra occurred about 710 nm. A small spike at 760 nm indicates some residual differences for modeled and measured SIF.

In general, FluorMOD fairly successfully modeled the canopy reflectance for the high N “unstressed” treatments. This indicates that the FluorMOD default information based on the physiology and morphology of  $C_3$  bean leaves is more relevant to the conditions associated with the thicker, healthy corn leaves rather than the thinner, N deficient  $C_4$  monocot leaves, and perhaps the proportion of standing green/brown vegetation in the canopy. In the two low (0, 50%) N groups, canopies were up to 0.25 m shorter and their lower one-third to one-half portions were comprised entirely of dead, attached leaves at the time of measurements. In contrast, the thicker, larger, and wider leaves of the high (100, 200%) N groups produced more ChlF and had larger scattering and absorbing surfaces in a taller canopy. Evidently, more information is needed to supplement green LAI in describing the canopy foliage density and distribution for stressed vegetation.

#### **4 Conclusions**

In this paper, we conducted an evaluation of the performance of FluorMOD by utilizing field and laboratory measurements of reflectance, F, and biophysical properties of corn leaves and corn plots under N fertilization regimes. We pursued this endeavor in spite of uncertainties introduced by using different instruments and methods associated with estimating leaf-level SIF from actively induced, monochromatic data and with uncertainties associated with the application of the FLD approach to field data acquired with an ASD spectroradiometer.

We discovered that FluorMOD, when provided measurements specific to the vegetation to be modeled, produced canopy SIF simulations that were close to ‘reality’ for our cornfield under N treatments, especially for red SIF (e.g., F688). The agreement (within  $1 \text{ mW m}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ ) of red SIF from FluorMOD and those retrieved in the field from ASD reflectance spectra strengthens our confidence in the application of the FLD for this band. On the other hand, far-red canopy SIF (e.g., F760) was overestimated in simulations, since far-red > red SIF in revised FluorMOD runs, whereas  $F760 < F688$  in the field. We are confident that the ASD field estimate of F760 provides a reliable SIF determination. Thus, the ability to capture leaf to canopy shifts in the red/far-red SIF ratio, probably associated with the arrangement of foliar material in 3-D space, has not been demonstrated in these FluorMOD simulations. Inclusion of high quality abaxial matrices may improve the simulations in future simulations.

The satisfactory correspondence between the canopy-level FluorMOD simulations and the ASD canopy spectra was accomplished by substituting FluorMOD’s leaf-level ‘default’ characterizations with measured information. We did not obtain useful F estimates with FluorMOD by using the ‘default’ look-up table information plus our biophysical measurements (Tab. 1). In that mode, serious shortcomings were revealed in the fundamental leaf-level characterizations of the FluorMOD fluorescence matrices which produced underestimates for steady state ChlF and apparent reflectance, that were subsequently manifested as underestimated steady state SIF and apparent reflectance at the canopy level. Consequently, our study highlights the importance of verifying the integrity of F spectra (e.g., EEMs), then correcting or normalizing it with solar irradiance information, in order to estimate SIF at the leaf and canopy scales. This correction ensures that green F is adequately represented in SIF, which has an important impact on the far-red ChlF emissions, and consequently, the red/far-red SIF ratio.

Currently, FluorMOD does not include spectral reflectance or F information specific to corn (a  $C_4$  species), nor does it specifically address spectral responses to environmental stress such as drought, nutrient deficiencies, high light, and high/low temperature exposures, or the influence of SIF from leaf undersides. We suggest that future upgrades to FluorMOD might consider replacing existing default EEMs with new leaf-level matrices displaying corrected spectral characteristics; normalizing these leaf-level EEMS using measured solar irradiance to obtain ambient SIF; adding the capability to simulate spectral responses to environmental stresses, including influences on leaf display that affect the projected distribution of foliage within canopies (e.g., projected adaxial: abaxial leaf surface ratio); adding more species types, including corn; augmenting the user documentation; and providing prototype data assemblage sets (EEMS, leaf spectra, etc.) for the vegetation expected to be examined in upcoming field campaigns.

In addition, it is hoped that field SIF experiments will be comprehensive, to include extensive laboratory F measurements in support of field spectral observations, coupled with biophysical and physiological measurements of vegetation. Our intention is to assist the remote sensing and F community to improve SIF characterization and its impact on reflectance as examined in nature, and ultimately from space-based platforms.

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Abbreviations:

ASD, Analytical Spectral Devices; ChlF, chlorophyll fluorescence; EEM, excitation emission matrix; EM, emission; EX, excitation; F, fluorescence; FLEX, FLuorescence EXplorer Mission; FLD, Fraunhofer line depth; FWHM, full width at half maximum; LAI, leaf area index; N, nitrogen; NIR, near-infrared wavelengths (800-1000 nm); SIF, solar induced chlorophyll fluorescence; USDA, United States Dept. of Agriculture, VIS, visible wavelengths (400-700 nm).

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