

# Uncertainty in the evaluation of photosynthetic canopy traits using the green leaf area index

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## ABSTRACT

The green leaf area index (GLAI) has been widely used in agriculture, forestry, and environmental sciences for the analysis and modeling of many biophysical processes of vegetation, including the attenuation of light through the canopy, transpiration, photosynthesis, and carbon and nutrient cycles. Nevertheless, its usefulness is hampered by the uncertainty introduced through the lack of quantitative information on leaf biochemistry, particularly leaf chlorophyll content, in its computation. Thus far, this uncertainty has not been properly recognized nor quantified. The main goal of this study was to quantify the uncertainty of GLAI as used in the estimation of key photosynthetic canopy traits, namely canopy chlorophyll content (CCC). This uncertainty was assessed through the evaluation of the relationship between GLAI and CCC in structurally and functionally contrasting crop species (*Zea mays* L., *Glycine max* (L.) Merr., and *Oryza sativa* L.). Results show that for the same GLAI value, CCC varied 2- to 3-fold due mainly to the variability of leaf chlorophyll content. Therefore, we suggest using the absorption coefficient in the red-edge region of the electromagnetic spectrum as an alternative to GLAI for the evaluation of CCC and other important photosynthetic canopy traits. The absorption coefficient in this spectral region is particularly suitable as it has been successfully related with the gross primary productivity of vegetation canopies, the quantum yield of photosynthesis, and is sensitive to the repositioning of chloroplasts within leaf cells in response to water stress.

## 1. Introduction

Biophysical characteristics that allow estimation of the amount of solar radiation absorbed by vegetation and its conversion into sugars permit one to infer not only the potential for primary productivity of a vegetated area, but also its physiological status. The ratio of leaf surface area to unit ground surface area, called leaf area index (LAI) is one of these characteristics. Note that LAI has also been defined as the one-sided area of leaves per unit of ground surface area (Watson, 1947), or as the maximum projected leaf area per unit of ground surface area to account for different leaf types (Myneni et al., 1997). This metric describes the potential surface area available for the interception of photosynthetically active radiation (Oker-Blom et al., 1989) and water

(Ghilain et al., 2020), for evapotranspiration (Yan et al., 2012), for assessing gas exchanges between terrestrial vegetation and the atmosphere (Cowling and Field, 2003), and for many other canopy traits. Accurate and timely information on LAI is, therefore, important for many applications in agriculture [e.g., yield estimation (Doraiswamy et al., 2003; Johnson, 2016)]; stress evaluation (Avetisyan et al., 2021), ecosystem ecology [e.g., estimation of primary productivity (Clark et al., 2008)], and environmental change [e.g., changes in vegetation (Su et al., 2021)], among several others. This has induced the development of various techniques for obtaining accurate non-destructive and *in situ* estimations of LAI, combined with estimations of leaf angle distribution (Weiss et al., 2004). However, such techniques exhibit large uncertainties due to the clumped nature of leaves, together with the large

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**Table 1**  
Summary of experimental datasets.

Crops	Year	Sensor, spectral range	Distance, FOV	Leaf [Chl]determination	Total N-fertilization, $g\ m^{-2}$
Rice ( <i>Oryza sativa</i> L. japonica variety)	2009	ASD 350–2500 nm	2 m, 25°	Analytical	2, 6, 10, 14, 16, standard 10
Maize ( <i>Zea mays</i> L.) Mead, NE, USA	2002–2005	USB2000 400–1040 nm	5.5 m, 25°	Non-destructive & Analytical	10.6
Maize ( <i>Zea mays</i> L.) Shelton, NE, USA	2006	USB2000 400–900 nm	1 m, 22°	Analytical	0, 5, 10, 15, 20
Soybean ( <i>Glycine max</i> (L) Merr.)	2002, 2004	USB2000 400–1040 nm	5.5 m, 25°	Non-destructive & Analytical	12.8

variability in the amount of photosynthetic vs. non-photosynthetic canopy components.

Successful procedures that address leaf clumpiness are based on the estimation of the gap fraction (the probability that a beam of light penetrating inside a plant canopy will have no contact with any vegetative element) (Weiss et al., 2004). However, the separation of LAI into its non-photosynthetic and photosynthetic components [the latter termed green LAI (GLAI)] has not been properly addressed. This separation is commonly performed through a visual inspection of the “greenness” of leaves (Boegh et al., 2002; Curran, 1983; Viña et al., 2011), and without proper quantitative information about leaf biochemistry. Such visual inspection fails to account for the large variability of what constitutes “green”, as it depends on the canopy chlorophyll content (CCC) according to different species, leaf structures (e.g., monocots, dicots), biochemical pathways (e.g., C3, C4), growth stages, and stress levels (e.g., water and nitrogen deficiencies), among others (Broge and Leblanc, 2001; Curran, 1983; Viña et al., 2011).

While there is a significant relationship between canopy chlorophyll content (CCC) and GLAI, such a relationship often exhibits a large variation in CCC values for the same GLAI value. Moreover, there is often hysteresis in this relationship when separately considering the vegetative and senescence periods of canopy development (Peng et al., 2017). Such large variation is the result of having leaves with quite different chlorophyll contents to be assessed as “green”, thus making the GLAI obtained to be less sensitive to the variability of CCC. This lack of sensitivity constitutes a source of uncertainty in the estimation of many photosynthetic canopy traits, including gross primary productivity (Gitelson et al., 2014). Yet, this source of uncertainty has not been properly recognized, or even quantified, in the scientific literature.

In this study we describe a procedure, based on the relationship between GLAI and CCC, to quantify the uncertainty in the use of GLAI for assessing photosynthetic traits of contrasting crop canopies and under variable management practices (e.g., different watering regimes, soil nutrient applications). Being a function of LAI and leaf chlorophyll content (leaf [Chl]), CCC is sensitive to changes in plant physiology and constitutes a measure of potential plant productivity (Gitelson et al., 2016). Therefore, it can be used to evaluate how sensitive GLAI is to changes in LAI and leaf [Chl]. To assess the reduction in uncertainty introduced when leaf [Chl] is incorporated into the assessment of photosynthetic canopy traits, we also evaluated how GLAI relates to the light absorption coefficient of plant canopies, which has been successfully used as a measure of CCC (Gitelson et al., 2021, 2019).

## 2. Methods

This study utilized datasets of three contrasting crop species (maize – *Zea mays* L.; soybean – *Glycine max* (L.) Merr.; rice – *Oryza sativa* L.), with different leaf structures (i.e., monocot, dicot), plant physiognomies (i.e., plagiphile, planophile, erectophile), photosynthetic pathways (i.e., C3, C4), in a wide range of agricultural conditions (e.g., different cultivars and N-fertilization regimes, rainfed, irrigated), acquired in different locations, and across different years. These datasets have been analyzed in multiple studies for different purposes (Ciganda et al., 2009; Gitelson et al., 2019, 2018, 2016; Gitelson et al., 2005, 2008, 2006;

Inoue et al., 2016; Peng et al., 2017; Viña et al., 2011). A summary of the procedures employed for their acquisition follows. More details on the data collection protocols are found in the cited papers.

### 2.1. Study sites

Data collection campaigns for soybean and maize were carried out during the growing seasons (from May to September) of 2002 to 2005 in three AmeriFlux sites located near Mead, Nebraska, USA. Sites 1 and 2 were equipped with a center-pivot irrigation system. Site 1 was continuously under maize, while site 2 was under a maize-soybean rotation (maize in odd and soybean in even years). Site 3 was also under a maize-soybean rotation but relied entirely on rainfall (Verma et al., 2005). In irrigated and rainfed sites nitrogen (N) fertilizer (Urea Ammonium Nitrate; UAN) was applied a few days before planting (128  $kg\ N\ ha^{-1}$  and 106  $kg\ N\ ha^{-1}$  in irrigated and rainfed sites, respectively) through coulters tied to tractors. In addition to this application, 34  $kg\ N\ ha^{-1}$  (UAN) were also applied in the irrigated sites through the irrigation system in two different occasions throughout the growing season. An additional dataset on maize was collected during the growing season of 2006 in a sprinkler irrigated site near Shelton, Nebraska, USA under five N treatments (0, 50, 100, 150, and 200  $kg\ N\ ha^{-1}$ ). The data collection campaign for rice was performed in 2009 at experimental fields of the National Institute for Agro-Environmental Studies (NIAES) in Tsukuba, Japan (Inoue et al., 2016). In addition to the standard level of N application (10  $g\ m^{-2}$ ), four different N levels (2, 6, 14 and 16  $g\ m^{-2}$ ) were further applied to induce a wide range of LAI and CCC. According to the usual cultivation practices, a bundle of 3 to 4 rice seedlings was transplanted as a hill at a 30 cm x 16 cm spacing.

### 2.2. Reflectance measurements at canopy scale

In the maize and soybean fields located near Mead, NE, U.S.A., time series of canopy reflectance measurements were obtained using two inter-calibrated radiometers (USB2000, Ocean Optics, Dunedin, FL; Table 1). One radiometer was equipped with a 25° field-of-view optical fiber pointing downward to measure upwelling radiance within a 2.4  $m^2$  sampling area (by placing the fiber at a height of approximately 5.5 m above the top of the canopy, which in maize had a maximum height of 3 m). The other sensor was equipped with an optical fiber and a cosine diffuser pointing upward to measure downwelling irradiance. Percent reflectance was calculated as the ratio of upwelling radiance to downwelling irradiance (Rundquist et al., 2004; Viña et al., 2011). Data collection in the maize fields located near Shelton, NE, U.S.A., followed the same protocols as those in Mead, NE, U.S.A., except placing the upward fiber at a height of one meter above the top of the canopy (Table 1). In the rice fields located in Tsukuba, Japan, time series of canopy reflectance factors were obtained using a portable spectroradiometer (ASD FieldSpec-Pro, Analytical Spectral Devices, Inc., Longmont, CO) with a 25° field-of-view at a nadir-looking angle from 2-m above the plants. Canopy height in rice ranged from 0.35 to 0.81 m during the study period. The placement of the upward optical fiber above of the crop canopies varied according to the uniformity of the spatial distribution of canopy elements within the Instantaneous Field of

**Table 2**

Minimum (Min), maximum (Max), mean (Mn), and median (Md) leaf chlorophyll ( $a + b$ ) content (in  $\mu\text{g cm}^{-2}$ ), canopy chlorophyll content (in  $\text{g m}^{-2}$ ), and green leaf area index (in  $\text{m}^2 \text{m}^{-2}$ ) in the maize [Mead (M;  $n = 124$  samples) and Shelton (S;  $n = 60$  samples)], soybean ( $n = 73$ ), and rice ( $n = 64$ ) sites.

Sites	Leaf Chl content				Canopy Chl content				Green LAI			
	Min	Max	Mn	Md	Min	Max	Mn	Md	Min	Max	Mn	Md
Maize (M)	231	800	567	581	0.07	3.61	2.04	2.13	0.17	5.52	3.49	4.04
Maize (S)	164	654	412	411	0.15	2.59	1.26	1.07	0.43	5.23	2.91	3.08
Soybean	80	623	358	362	0.03	2.69	1.07	0.97	0.16	5.45	2.59	2.62
Rice	152	584	322	294	0.01	2.13	0.63	0.53	0.08	6.73	2.13	2.14

View (IFOV). This allows obtaining representative measurements of canopy reflectance. Canopy reflectance factors were calculated as the ratio of the upwelling radiance to that of a Spectralon-Labsphere white reference to account for differences in atmospheric conditions (Inoue et al., 2016) (Table 1).

### 2.3. Green leaf area index (GLAI)

Time series of GLAI estimation in maize and soybean sites in Mead and Shelton, NE, U.S.A. (Table 2) were obtained destructively from samples collected in plots (20 m x 20 m) representing major soil and crop production zones within each site (see Verma et al., 2005 for additional details). The GLAI plots were located adjacent to the footprint of the flux towers to minimize disturbance within these footprints. Green leaf samples obtained destructively in each plot were run through an area meter (LI-3100, Li-Cor, Inc., Lincoln, NE) to calculate the leaf area per plant. This area was then multiplied by the plant population (assessed in each plot) to obtain a plot-level GLAI, which were then averaged across plots within each sampling site to obtain a site-level GLAI (Viña et al., 2011). Time series of GLAI estimation in the rice sites (Table 2) were obtained destructively in five randomly sampled hills per plot using an area meter (LI-3100, Li-Cor, Inc., Lincoln, NE), after carefully removing all senescent leaf parts (Inoue et al., 2016).

### 2.4. Leaf and canopy chlorophyll content

Leaf [Chl] of maize and soybean (ear leaf in maize plants and the top-most fully expanded leaf in soybean plants) in the Mead sites (Table 2) was measured in the laboratory (Richardson et al., 2002; Wellburn, 1994), concurrently with spectral reflectance measurements of the same leaves using an Ocean Optics radiometer equipped with a leaf clip (Ciganda et al., 2009). Foliar reflectance measurements were used to calculate the red edge chlorophyll index, a surrogate of leaf [Chl] (Gitelson et al., 2005):

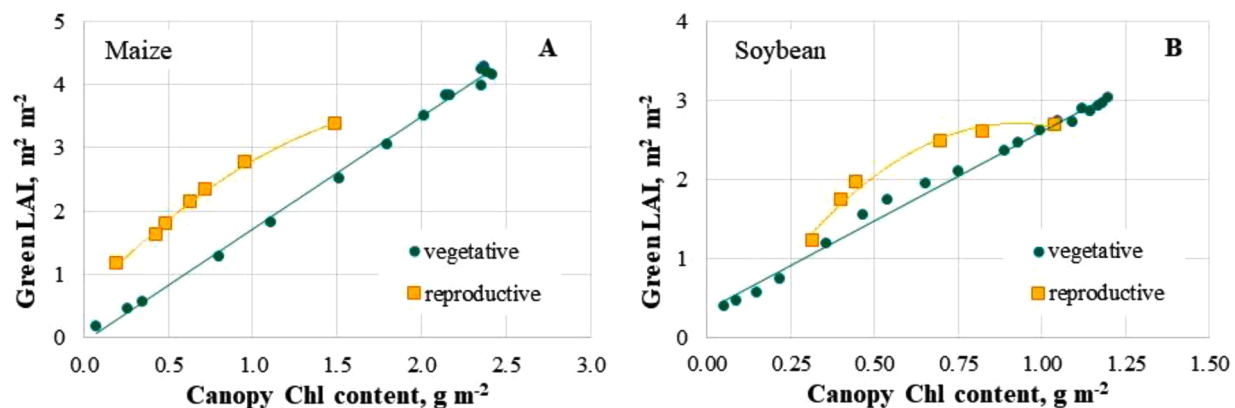
$$CI_{rededge} = [(\rho_{NIR} / \rho_{720-730}) - 1] \quad (1)$$

where  $\rho_{NIR}$  is reflectance in the NIR range 780–800 nm and  $\rho_{720-730}$  is reflectance in the red edge range 720–730 nm.  $CI_{red edge}$  was linearly related with the destructive lab measurements of leaf [Chl]. The relationship leaf [Chl] vs.  $CI_{red edge}$ , calibrated on a per-year basis, was used to retrieve leaf [Chl] (Ciganda et al., 2009; Gitelson et al., 2005, 2006) throughout the entire time series using canopy reflectance data. CCC was then calculated as the product of leaf [Chl] and GLAI. Maize leaf [Chl] in the Shelton sites was obtained destructively in the laboratory (Richardson et al., 2002; Wellburn, 1994) from the leaves of harvested plants in 1.0 m<sup>2</sup> sampling areas. CCC was then obtained by adding the leaf [Chl] of all leaves per plot (Table 2). In rice, five hills per plot were randomly sampled to obtain a measure of leaf [Chl]. CCC was then obtained by multiplying leaf [Chl] by the biomass of green leaves per m<sup>2</sup> of ground area (Inoue et al., 2016) (Table 2). All these procedures assumed that the destructively sampled measurements of leaf [Chl] were representative of the crop canopies, as the variability in leaf [Chl] is driven more by the physiological status of the crop, which affects the entire canopy, than by other characteristics. This assumption is reasonable, as it was shown for maize that the leaf [Chl] of the ear leaf relates closely ( $R^2 > 0.85$ ) to the total CCC (Ciganda et al., 2008).

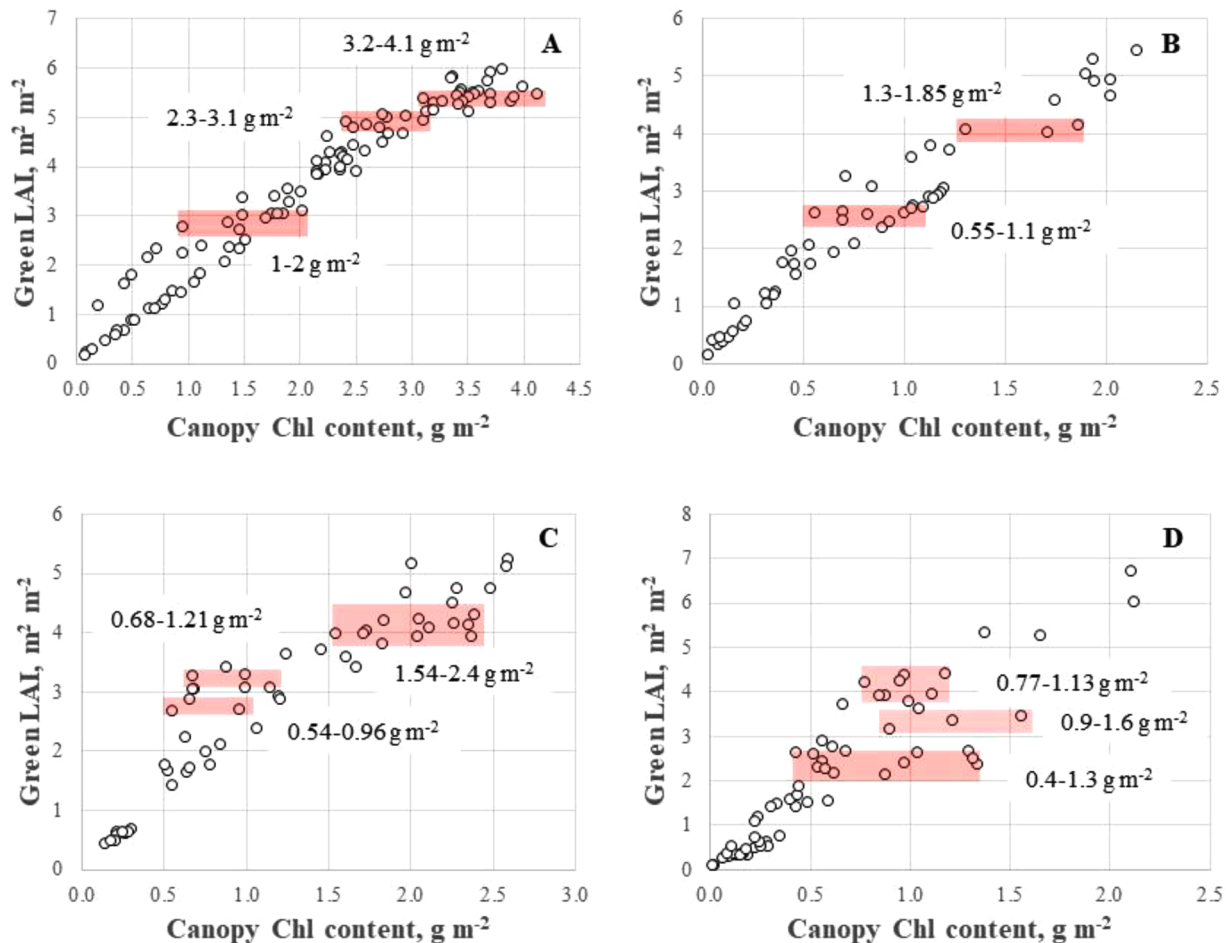
## 3. Results and discussion

Since CCC is a product of leaf [Chl] and GLAI, the slope of the GLAI vs. CCC relationship corresponds to  $(\text{leaf [Chl]})^{-1}$ . Thus, the relationship between GLAI and CCC can be used to assess the information content of both of these traits, while leaf [Chl] constitutes the main factor defining the form of this relationship (i.e., whether straight or curved).

The relationship between CCC and GLAI obtained using data from the Mead sites under a standard, non-variable N-fertilization (Table 1) shows pronounced hysteresis. During the vegetative stage, before the LAI reaches maximal values, the relationship is quite close to linear in both maize and soybean canopies. However, during the reproductive stage (when leaf [Chl] declines), CCC is lower for the same GLAI as compared to the vegetative stage (Fig. 1). This shows the effect of growth stage on the variability of CCC, which is not fully captured by the



**Fig. 1.** Relationship between GLAI and CCC in rainfed maize (A) and soybean (B) during vegetative and reproductive stages. These crop fields received standard, non-variable N-fertilization regimes.



**Fig. 2.** GLAI vs. CCC in irrigated and rainfed maize Mead sites in 2002–2005, with a standard, non-variable N-fertilization regime (A); soybean irrigated and rainfed sites in 2002 and 2004 with a standard, non-variable N-fertilization regime (B); maize Shelton sites in 2006 with a variable N-fertilization regime (C); rice sites in 2009 with a variable N-fertilization regime (D). The red boxes show minimal and maximal CCC values for selected GLAI values.

**Table 3**

Statistics of the relationship between CCC and GLAI among crops and sites. The root mean squared error (RMSE) of the CCC estimated from the Green LAI is expressed in  $\text{g m}^{-2}$ , the normalized RMSE in percent, the average CCC in  $\text{g m}^{-2}$ , and the average Green LAI in  $\text{m}^2 \text{m}^{-2}$ .

Sites	Mean CCC	Mean GLAI	RMSE	NRMSE,%
Maize (M)	1.96	3.49	0.45	23
Maize (S)	1.21	2.87	0.62	51
Soybean	1.05	2.59	0.6	57
Rice	0.63	2.13	0.8	127
All together	1.37	2.89	0.54	31

GLAI. In the particular case of maize, CCC variability is dominated by changes in N during the reproductive stage, as it is often relocated from the lower leaves to other plant parts. This N relocation occurs because N acquisition becomes more limited due to root die-off after anthesis.

Beyond growth stage, differences in irrigation (i.e., irrigated vs. rainfed fields), crop cultivars used across sites and years, and N-fertilization regimes, cause a significant variability in leaf [Chl], which is captured by the CCC but not completely by the GLAI (Fig. 2). A 2-fold variability of CCC for the same GLAI value is apparent, which is caused not only by different leaf [Chl] during the vegetative and reproductive stages, but also due to the variability in leaf [Chl] induced by the differences in irrigation and crop cultivars under standard non-variable N-fertilization regimes (Fig. 2A and B). Furthermore, up-to 3-fold variability in CCC for the same GLAI was observed under variable

N-fertilization regimes (Fig. 2C and D), which shows the effects of significant changes in leaf [Chl] not captured by the GLAI.

The relationship between CCC and GLAI varies widely among crops, and in different irrigation and N-fertilization regimes (Table 3, Fig. 3A). The slope of this relationship was maximal in maize, which has the highest leaf [Chl] per unit of area (Gitelson et al., 2021), and 50% smaller in rice. Scattering of the sampling points from the regression line was quite large (Fig. 3A), generating large RMSE values (Table 3) and residuals exceeding 150% (Fig. 3B).

The relationship presented in Fig. 3 constitutes a typical situation when multiple crop fields with different species, at different growth stages, and with different water and N treatments co-occur within the same scene obtained by airborne or spaceborne imaging remote sensors. Considering that CCC is a quantitative measure of the potential absorption of solar radiation in the photosynthetically active region of the electromagnetic spectrum, and thus directly related to gross primary production (Gitelson et al., 2006; Peng et al., 2011), this relationship (Fig. 3; Table 3) shows that plant productivity may vary up-to 3-fold in canopies exhibiting the same GLAI. Thus, it constitutes a quantitative assessment of the uncertainty of GLAI obtained using a visual assessment of leaf color.

While there are several destructive (e.g., using area meters) and non-destructive techniques for obtaining estimates of LAI in the field/lab (Jonckheere et al., 2004; Welles and Norman, 1991), the separation between the photosynthetic and non-photosynthetic canopy components, with few exceptions (Denison and Russotti, 1997), almost always follows a visual assessment of leaf color. Such assessment has been used

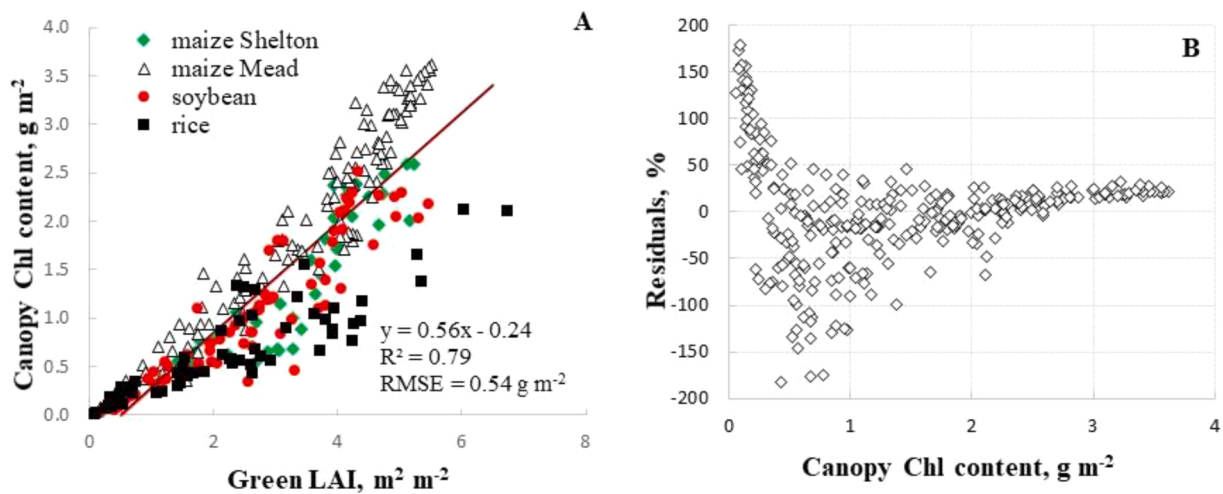


Fig. 3. (A) Relationship between CCC and GLAI for all crops and sites evaluated; (B) residuals of this relationship for all datasets combined.

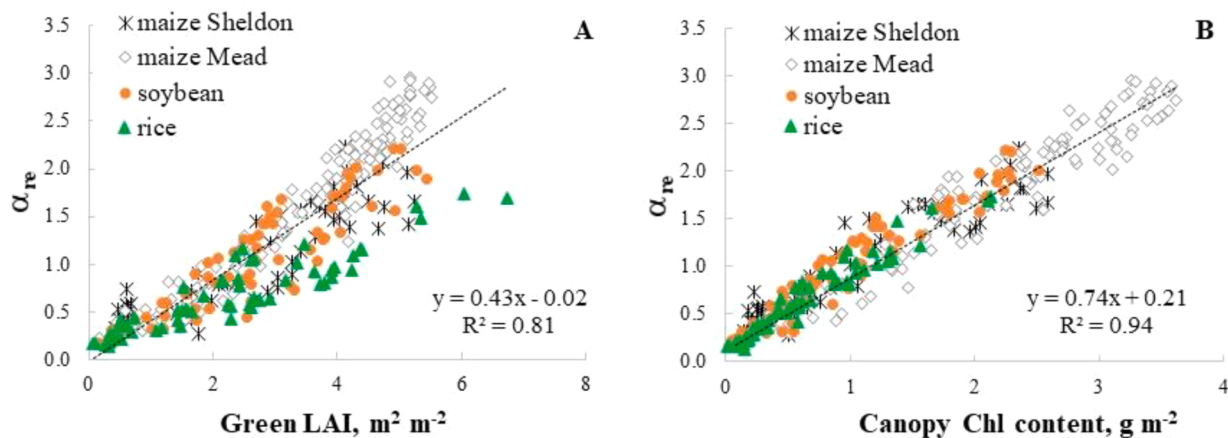


Fig. 4. Absorption coefficient of chlorophyll in the red-edge spectral region,  $\alpha_{re}$ , versus green LAI (A) and versus canopy chlorophyll content (B) for all datasets evaluated.

quite extensively in the calibration and validation of models based on remotely sensed data for the estimation of spatial and temporal variability of GLAI at multiple spatial scales (Broge and Leblanc, 2001; Guindin-Garcia et al., 2012; Haboudane et al., 2004; Nguy-Robertson et al., 2012). Yet, as our results show, these assessments may have quite high uncertainties, as they fail to incorporate the variability of leaf [Chl]. Therefore, alternative metrics, such as those based on remotely sensed data, are needed to properly incorporate such variability.

The theoretical background developed for satellite remote sensing of LAI (Myneni et al., 1995) establishes that the derivative of vegetation reflectance,  $\rho$ , with respect to wavelength,  $\lambda$  ( $d\rho/d\lambda$ ), common to all vegetation indices (VI), is indicative of the abundance and activity of absorbers in leaves. Thus, any model developed for the remote estimation of GLAI must incorporate leaf [Chl], together with LAI, for an accurate estimation of vegetation productivity and vigor. The absorption coefficient of pigments at leaf and canopy scales constitutes a suitable measure of the photosynthetic status of plants (Gitelson et al., 2022, 2021, 2020; Gitelson et al., 2019). The absorption coefficient of chlorophyll in the red-edge spectral region,  $\alpha_{re}$ , was introduced in the form (Gitelson et al., 2019):

$$\alpha_{re} = \rho_{NIR} / \rho_{710-740} - 1 \quad (2)$$

where  $\rho_{NIR}$  is canopy reflectance in the near-infrared spectral region beyond 780 nm, and  $\rho_{710-740}$  is canopy reflectance in the red-edge

spectral region between 710 and 740 nm.  $\alpha_{re}$  was shown to be an accurate and, perhaps more important, generic measure of CCC in plants with contrasting leaf structures, canopy architectures and photosynthetic pathways (Gitelson et al., 2019). Such feature of  $\alpha_{re}$  is particularly important given the significant effect of leaf and canopy structural properties on the absorption of photosynthetically active radiation by plants. Thus, it can be used to understand and quantify how GLAI and CCC are related to the absorption coefficient of chlorophyll in plant canopies.

While CCC explains 94% of the  $\alpha_{re}$  variance, GLAI explains only 81% of the of the  $\alpha_{re}$  variance (Fig. 4). The wide scattering of the sampling points from the best-fit function using GLAI (Fig. 4A) indicates a large variability in the absorption coefficient of canopies with the same GLAI. In contrast, a significant drop in this scattering of the sampling points is observed for the CCC (Fig. 4B). Considering that CCC is a function of leaf [Chl] and GLAI, this means that the incorporation of leaf [Chl] into the CCC (as in Fig. 4B) considerably reduces the uncertainty in the estimation of plant photosynthetic traits.

To quantify the effect of either GLAI or CCC on  $\alpha_{re}$ , we used a sensitivity metric (Gitelson, 2013; Viña and Gitelson, 2005). This metric, termed noise equivalent (NE), is defined as the ratio of the root mean square error (RMSE) of the GLAI vs.  $\alpha_{re}$  and the CCC vs.  $\alpha_{re}$  relationship, respectively, and the first derivative of the best-fit function relating either GLAI or CCC to the  $\alpha_{re}$ :

**Table 4**

Noise equivalent (NE) of the Green LAI vs.  $\alpha_{re}$  and NE of the canopy chlorophyll content (CCC) vs.  $\alpha_{re}$  relationships.  $\Delta, \% = (NE\Delta GLAI - NE\Delta CCC) / (NE\Delta GLAI) \times 100$  corresponds to the relative contribution of leaf [Chl] to the NE $\Delta$ GLAI, obtained through removal of uncertainty in the estimation of plant structural and biochemical traits using CCC instead of GLAI.

	Maize (Shelton)	Rice	Soybean	Maize (Mead)	All together
NE $\Delta$ Green LAI	0.31	0.16	0.26	0.25	0.37
NE $\Delta$ CCC	0.22	0.1	0.16	0.2	0.19
$\Delta, \%$	29	37.5	38.4	20	48.6

$$NE\Delta GLAI = RMSE[GLAI \text{ vs. } \alpha_{re}] / [dGLAI / d\alpha_{re}] \quad (3)$$

$$NE\Delta CCC = RMSE[CCC \text{ vs. } \alpha_{re}] / [dCCC / d\alpha_{re}] \quad (4)$$

This function reflects how effectively GLAI and CCC characterize the absorption of radiation by plants in the photosynthetically active spectral region and how close to linearity are the GLAI vs.  $\alpha_{re}$  and the CCC vs.  $\alpha_{re}$  relationships. The smaller the noise equivalent, the closer the GLAI and CCC relate to the absorption coefficient (i.e., smaller RMSE and/or higher  $dGLAI/d\alpha_{re}$  or  $dCCC/d\alpha_{re}$ ). For different crops and sites, the noise equivalent ranged from 0.1 to 0.31, with all values for the CCC being lower than those for the GLAI (Table 4). Considering all datasets together, the noise equivalent of CCC was around 50% that of GLAI (Table 4). The relative contribution of leaf [Chl] to the noise equivalent of GLAI [calculated as  $\Delta, \% = ((NE\Delta GLAI - NE\Delta CCC) / NE\Delta GLAI) \times 100$ ], was between 20 and 38.4% for the different crop and sites evaluated. The uncertainty reaches 48.6% when all datasets are considered together (Table 4). These uncertainties of the GLAI are due to the variability in leaf [Chl] not included in the GLAI formulation.

#### 4. Concluding remarks

Results of this study show that the incorporation of leaf [Chl] addresses the inaccuracy introduced by the visual assessment of leaf greenness in the determination of GLAI and allows the quantification of the uncertainties in the estimation of photosynthetic canopy traits using GLAI. In addition, metrics for the estimation of canopy chlorophyll content, such as the light absorption coefficient in the red edge region, which have also been successfully related with gross primary productivity of vegetation canopies (Gitelson et al., 2008, 2006; Hilker et al., 2011; Rossini et al., 2014), quantum yield of photosynthesis (Gitelson et al., 2022), and are sensitive to the repositioning of chloroplasts within leaf cells occurring in response to water stress (e.g., Zyguelbaum et al., 2012), provide a more accurate representation of the photosynthetic status of plant canopies. Therefore, the estimation of canopy chlorophyll content through remote sensing procedures constitutes an alternative to GLAI, as it is directly related to photosynthesis activities. Such estimation should become a standard practice in agriculture, forestry, and photosynthesis research and applications.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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