



## Estimating leaf chlorophyll content in sugar beet canopies using millimeter- to centimeter-scale reflectance imagery



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### ABSTRACT

Accurate estimation of leaf chlorophyll content ( $C_{ab}$ ) from remote sensing is of tremendous significance to monitor the physiological status of vegetation or to estimate primary production. Many vegetation indices (VIs) have been developed to retrieve  $C_{ab}$  at the canopy level from meter- to decameter-scale reflectance observations. However, most of these VIs may be affected by the possible confounding influence of canopy structure. The objective of this study is to develop methods for  $C_{ab}$  estimation using millimeter to centimeter spatial resolution reflectance imagery acquired at the field level.

Hyperspectral images were acquired over sugar beet canopies from a ground-based platform in the 400–1000 nm range, concurrently to  $C_{ab}$ , green fraction (GF), green area index (GAI) ground measurements. The original image spatial resolution was successively degraded from 1 mm to 35 cm, resulting in eleven sets of hyperspectral images. Vegetation and soil pixels were discriminated, and for each spatial resolution, measured  $C_{ab}$  values were related to various VIs computed over four sets of reflectance spectra extracted from the images (soil and vegetation pixels, only vegetation pixels, 50% darkest and brightest vegetation pixels). The selected VIs included some classical VIs from the literature as well as optimal combinations of spectral bands, including simple ratio (SR), modified normalized difference (mND) and structure insensitive pigment index (SIPI). In the case of mND and SIPI, the use of a blue reference band instead of the classical near-infrared one was also investigated. For the eleven spatial resolutions, the four pixel selections and the five VI formats, similar band combinations are obtained when optimizing VI performances: the main bands of interest are generally located in the blue, red, red-edge and near-infrared domains. Overall, mND<sub>blue</sub>[728,850] defined as  $(R_{440} - R_{728}) / (R_{440} + R_{850})$  and computed over the brightest green pixels obtains the best correlations with  $C_{ab}$  for spatial resolutions finer than 8.8 cm with a root mean square error of prediction better than 2.6  $\mu\text{g}/\text{cm}^2$ . Conversely, mND<sub>blue</sub>[728,850] poorly correlates with variations in GF and GAI, thus reducing the risk of deriving non-causal relationships with  $C_{ab}$  that would actually be due to the covariance between  $C_{ab}$  and these canopy structure variables. As mND<sub>blue</sub>[728,850] can be calculated from most current multispectral sensors, it is therefore a promising VI to retrieve  $C_{ab}$  from millimeter- to centimeter-scale reflectance imagery.

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### 1. Introduction

Photosynthesis is one of the most important biological processes, allowing life on Earth through production of oxygen and organic matter (Ustin et al., 2009). Chlorophyll is one of the major plant pigments that contribute to the absorption of photosynthetically active radiation.

Quantifying chlorophyll temporal dynamics is therefore critical to monitor the vegetation physiological status or to estimate primary production (Blackburn, 2007, 1998). For this purpose, non-destructive estimation of leaf chlorophyll content (denoted  $C_{ab}$  hereafter) based on optical measurements has proven to be effective since  $C_{ab}$  drives most of the leaf reflectance and transmittance variabilities in the visible domain. A high  $C_{ab}$  retrieval accuracy is usually obtained at the leaf scale under controlled experimental conditions, e.g., using dedicated leaf clips measuring transmittance at a few wavelengths (Cerovic et al., 2012), or using hemispherical reflectance and/or transmittance

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measurements to invert physical models such as PROSPECT (Jacquemoud and Baret, 1990) or to apply spectral indices (Gitelson et al., 2003; Féret et al., 2011; le Maire et al., 2004). The estimation of  $C_{ab}$  is more challenging at the canopy scale: soil reflectance and canopy architecture interact with leaf scattering properties to generate canopy reflectance. As a consequence, the effect of leaf composition may be confounded with those of canopy structural properties, making the inversion of canopy reflectance models an ill-posed problem (Baret and Buis, 2008; Combal et al., 2003): several combinations of green area index (GAI) and  $C_{ab}$  values may indeed correspond to similar canopy reflectance spectra in the visible domain, which increases the uncertainty of  $C_{ab}$  retrieval (Baret and Buis, 2008). Further, non-causal relationships between canopy reflectance and the targeted variable may be observed when structural and biochemical variables are correlated as reported by Knyazikhin et al. (2013). Effects of canopy structure and leaf composition should therefore be disentangled with great care when relating remote-sensing observations to foliar biochemistry (Knyazikhin et al., 2013; Latorre-Carmona et al., 2014; Ustin, 2013).

A first approach has been proposed to improve the  $C_{ab}$  estimation performance at the canopy level by maximizing the spectral sensitivity to foliar biochemistry while minimizing the effects of soil and vegetation structure. It consists in using a ratio vegetation index (VI), where the numerator is a  $C_{ab}$ -sensitive VI such the Modified Chlorophyll Absorption Reflectance Index (MCARI) (Daughtry et al., 2000), and the denominator is a VI sensitive to canopy structure such as the Optimized Soil-Adjusted Vegetation Index (OSAVI) (Rondeaux et al., 1996). MCARI/OSAVI (Daughtry et al., 2000), TCARI/OSAVI (Haboudane et al., 2002) and derived versions of these two VIs (Wu et al., 2008) are examples of such combined indices, which have been demonstrated to provide accurate  $C_{ab}$  estimation results at the canopy level (Kooistra and Clevers, 2016).

Alternatively, a second approach consists in increasing the sensitivity to foliar biochemistry by optimizing the sun-sensor geometry: off-nadir measurements are generally more sensitive to leaf properties than nadir measurements (Baret et al., 2010; Comar et al., 2012; Dorigo, 2012; Jacquemoud et al., 2009; Jay et al., 2017). This is not only due to the higher proportion of vegetation seen by the sensor, but also to the large fraction of photons that have interacted with leaves before reaching the sensor (Jacquemoud et al., 2009). Further, the relative viewing azimuth angle affects the canopy reflectance sensitivity: measurements acquired in the backward direction, where shadows are minimized, generally exhibit a higher sensitivity to leaf biochemistry (Dorigo, 2012; Jacquemoud et al., 2009; Jay et al., 2017).

Finally, a third approach consists in focusing on the illuminated vegetation pixels when the spatial resolution is sufficient: this limits the detrimental influences of soil and canopy architecture and consequently strengthens the sensitivity to  $C_{ab}$  (Moorthy et al., 2008; Zarco-Tejada et al., 2004, 2001). Multi- and hyperspectral cameras operated from ground-based or low-altitude platforms provide a very high spatial resolution, ranging from a few millimeters to a few decimeters. However, most current retrieval methods do not fully exploit the new possibilities offered by such high spatial resolution imagery, thereby stimulating the need for new algorithms (Elarab et al., 2015; Houborg et al., 2015). The large variability of leaf orientation and illumination conditions observed at this scale induces strong variations in leaf radiance. For example, Jay et al. (2016) have proposed to invert the PROSPECT + COSINE (CLOSe-range Spectral Imaging of lEaves) model to map  $C_{ab}$  over individual leaves when the influence of surrounding elements is negligible. However, when individual leaves are submitted to the radiative transfer conditions that prevail in the canopy, the problem was not yet addressed. Most current VIs have been designed for leaf and canopy levels, and may therefore be suboptimal to handle the above-mentioned variations in leaf reflectance (Bánkestad and Wik, 2016).

This study focuses on  $C_{ab}$  estimation in sugar beet canopies using millimeter- to centimeter-resolution reflectance imagery. Hyperspectral images were acquired from a ground-based platform

and concurrent measurements of GAI and  $C_{ab}$  were completed. These data were used to design VIs dedicated to  $C_{ab}$  estimation that take advantage of such high spatial resolution imagery. Performances were compared to those obtained with several VIs of the literature for the range of spatial resolutions investigated.

## 2. Materials and methods

### 2.1. Field experiments

Field experiments were conducted in France in 2015 and 2016. Three study sites with different soil properties were considered as illustrated in Fig. 1. A chalky soil was present at the “Vaucogne” (48°31'N, 4°21'E, denoted site 2) and “Viapres” (48°35'N, 4°2'E, denoted site 3) sites, while the “La Selve” site (49°35'N, 4°01'E, denoted site 1) was characterized by a loamy soil. The details of these field experiments are summarized in Table 1. Seven sugar beet cultivars exhibiting differences in plant structure were submitted to variable levels of nitrogen fertilization. Rows were spaced 45 cm apart and plant population density was between 10 and 12 plants per square meter. So as to further increase the representativeness and heterogeneity of the data set, various phenological stages were considered during the 2015 and 2016 growing seasons, i.e., on June, 2–3 2015, June, 23–24 2015 and July, 26–27 2016. In particular, the crops considered in the 2016 experiment were carefully chosen so as to decorrelate  $C_{ab}$  and canopy structural properties. In total, the overall data set included 55 samples and encompassed a large variability due to differences in cultivars, nitrogen fertilizations, development stages, and soil and weather conditions.

### 2.2. Reflectance measurements

For each plot, an area corresponding to five consecutive plants along a row was imaged using a HySpex VNIR-1600 hyperspectral camera (Norsk Elektro Optikk, Norway) set up on a ground-based platform as shown in Fig. 2. The push-broom camera pointed vertically downward from a 1.15 m distance to the bare soil. It measured the reflected radiation in 160 spectral bands ranging from 415 to 994 nm with a 3.7 nm spectral sampling interval and 4.5 nm full width at half maximum, and acquired successive scans of 1600 pixels along the row. The across-track field of view (FOV) was about 35 cm per scan at the ground level, providing a 0.02 cm across-track sampling distance. A 40% diffuse reflectance reference panel (Spectralon®, Labsphere) was used to measure the incoming solar irradiance while limiting possible saturation of the sensor. The reference panel was placed horizontally above the canopy to reduce the influence of possible vicinity effects. The HDRF (Hemispherical-directional Reflectance Factor) was finally computed by dividing the signal measured for each band and each pixel over the target by that measured over the reference panel and multiplying it by the reflectance of the reference panel provided by Labsphere (assuming the panel to be Lambertian). Completion of the scans over the 5 plants took a few seconds during which the incoming radiation was supposed to be stable. Measurements were collected around solar noon with solar zenith angle always lower than 36°. Illumination conditions differed between experiments, ranging from a clear blue sky to a fully overcast sky (Table 1).

### 2.3. $C_{ab}$ and canopy structure measurements

The leaf chlorophyll content was estimated for each plot after image acquisition over the same five plants. Six measurements per plant were made using a Dualex scientific +™ (Force-A, Orsay, France). This leaf clip measures leaf transmittance in a few wavebands from which  $C_{ab}$  is estimated using the relationship proposed by Cerovic et al. (2012) for dicotyledons, achieving an accuracy of around  $4 \mu\text{g}/\text{cm}^{-2}$ . Measurements were performed at different leaf levels to better consider the possible  $C_{ab}$  vertical gradient between leaves of different age or differently

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