

Retrieving seasonal variation in chlorophyll content of overstory and understory sugar maple leaves from leaf-level hyperspectral data

Yongqin Zhang, Jing M. Chen, and Sean C. Thomas

Abstract. Leaf chlorophyll content is a useful parameter for assessing vegetation physiological status and dominates the spectral signal of leaf and canopy reflectance at visible wavelengths. Using hyperspectral instruments, we quantified leaf chlorophyll content and optical properties for 255 overstory and understory leaf samples through the growing season in a mature sugar maple (*Acer saccharum*) stand. Strong seasonal and canopy-height-related differences were observed in both leaf chlorophyll content and leaf reflectance and transmittance spectra. Seasonal and canopy-height-related variation in leaf spectra were closely related to leaf chlorophyll content. We estimated leaf chlorophyll content using two approaches, namely empirical spectral indices, and a mathematical inversion of the leaf optical model PROSPECT. Both estimates were highly correlated with the measured leaf chlorophyll content; however, the spectral indices resulted in greater accuracy, with the best-performing index (modified simple ratio) showing an accuracy of $R^2 = 0.88$ and $RMSE = 3.94 \mu\text{g}/\text{cm}^2$. A leaf thickness factor was introduced in the PROSPECT model to take into account the effects of changes in leaf structure on light absorption. The model inversion was improved after incorporating leaf thickness factors based on observed seasonal and canopy-height-related variation in leaf thickness. The improved model had the best performance, with an accuracy of $R^2 = 0.93$ and $RMSE = 3.09 \mu\text{g}/\text{cm}^2$ in retrieved leaf chlorophyll concentration in comparison with laboratory measurements.

Résumé. La teneur en chlorophylle des feuilles est un paramètre utile pour l'évaluation de l'état physiologique de la végétation et cette dernière domine le signal spectral de la réflectance des feuilles et du couvert dans les longueurs d'onde du visible. À l'aide d'instruments oeuvrant dans l'hyperspectral, nous avons quantifié la teneur en chlorophylle des feuilles ainsi que les propriétés optiques de 255 échantillons de feuilles de l'étage supérieur et inférieur du couvert au cours de la saison de croissance dans un peuplement d'érables à sucre (*Acer saccharum*). Des différences considérables ont été observées selon les saisons et au niveau de la hauteur du couvert à la fois dans la teneur en chlorophylle des feuilles et les spectres de réflectance et de transmittance des feuilles. Les variations saisonnières et de la hauteur du couvert dans les spectres des feuilles étaient étroitement reliées à la teneur en chlorophylle des feuilles. Nous avons estimé la teneur en chlorophylle des feuilles en utilisant deux approches : les indices spectraux empiriques et une inversion mathématique du modèle optique des feuilles PROSPECT. Les deux estimations étaient fortement corrélées avec la teneur en chlorophylle des feuilles mesurée; toutefois, les indices spectraux ont donné une plus grande précision, l'indice le plus performant (ratio simple modifié) affichant une précision de $R^2 = 0,88$ et $RMSE = 3,94 \mu\text{g}/\text{cm}^2$. Nous avons introduit un facteur pour l'épaisseur des feuilles dans le modèle PROSPECT pour tenir compte des effets des changements dans la structure des feuilles sur l'absorption de la lumière. L'inversion du modèle a été améliorée grâce à l'intégration de facteurs pour l'épaisseur des feuilles basés sur les variations saisonnières et de la hauteur du couvert observées au niveau de l'épaisseur des feuilles. Le modèle amélioré affichait la meilleure performance, avec une précision de $R^2 = 0,93$ et $RMSE = 3,09 \mu\text{g}/\text{cm}^2$ dans l'extraction de la concentration de la chlorophylle des feuilles comparativement aux mesures en laboratoire.

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Introduction

The physiological state of a plant is governed by its biochemical constituents, including photosynthetic and other enzyme systems, structural and nonstructural carbohydrates, chlorophyll and associated light-harvesting complexes, and photoprotective and ancillary pigments. Of these, leaf chlorophyll content stands out as

being both sensitive to environmental conditions and having a very strong influence on leaf optical properties and canopy albedo (Blackburn and Pitman, 1999; Baltzer and Thomas, 2005). Leaf chlorophyll content also serves as an input to photosynthesis and carbon-cycle models. Changes in leaf optical properties and chlorophyll content, including responses to rising atmospheric CO_2 and other global change variables, may have important

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implications to climate forcing as well (Thomas, 2005). Quantitative estimates of leaf chlorophyll content may thus provide a useful indicator of important physiological processes in forest canopies that can be readily assessed via remote sensing.

Leaf optical properties are influenced by the concentrations of chlorophyll and other chromophore-containing biochemical constituents, water content, and leaf structure. All green leaves have major absorption features in the 400–700 nm range caused by electron transitions in chlorophyll and carotenoid pigments (Belward, 1991). Most green vegetation shows absorption peaks near 420, 490, and 670 nm due to the strong absorption peaks of chlorophyll a and b. Differences in leaf and canopy reflectance between healthy and stressed vegetation due to changes in chlorophyll levels have been detected in the green peaks and along the red edge (690–750 nm) (Rock et al., 1988; Vogelmann et al., 1993; Carter, 1994; Belanger et al., 1995; Gitelson et al., 1996). Spectrally continuous hyperspectral data can detect subtle absorption features in foliar spectra. Hyperspectral measurements in very narrow bands are helpful to study the correlations of these minor absorption features with biochemical parameters. Information on vegetation structure and biochemistry can be estimated from hyperspectral data and is important for studying nutrient cycling, productivity, and vegetation stress and for driving ecosystem models (Curran, 1994; Jacquemoud et al., 1996; Noland et al., 2003). For example, hyperspectral data of the compact airborne spectrographic imager (CASI) are capable of land classification (Zarco-Tejada and Miller, 1999), plant discrimination (Lewis et al., 2001), mapping biophysical parameters (Chen et al., 1999), inventorying forests (Davidson et al., 1999a; 1999b), and assessing forest management practices (Sampson et al., 2001). The CASI data have also been used to map chlorophyll content by estimating leaf-level chlorophyll and scaling up to canopy level for closed canopy (Sampson et al., 2003).

Foliar optical properties are expected to vary both seasonally and with respect to canopy position. For example, it has been found that seasonal changes in conifer species have a dramatic influence on spectral features such as the green peak reflectance and red-edge and near-infrared plateau reflectances (Rock et al., 1993). Seasonal changes in leaf optical properties in temperate deciduous species are expected to be even more pronounced, but detailed examinations are scarce (e.g., Demarez et al., 1999; Qi et al., 2003). Leaf optical properties are also expected to vary as a function of vertical gradients in light availability through forest canopies and associated changes in leaf pigmentation (e.g., Demarez et al., 1999; O'Neill et al., 2002; Leal and Thomas, 2003).

To derive chlorophyll content accurately from radiometric measurements and remote sensing imagery, an important step is to estimate leaf chlorophyll from leaf-level hyperspectral data. Empirical relationships between the leaf reflectance and biochemical contents have been widely used to estimate leaf chlorophyll content (Gitelson and Merzlyak, 1997; Gitelson et al., 2003; Datt, 1998; 1999). However, such empirical methods lack a clear mechanistic basis and thus may be expected to lack temporal and spatial continuity. Relatively robust radiative

transfer models have been developed and applied to assess leaf physiological conditions. These include the K-M model (Allen and Richardson, 1968), the plate model (Allen et al., 1969), the ray tracing model (Allen et al., 1973; Yamada and Fujimura, 1988), PROSPECT (Jacquemoud and Baret, 1990), and LEAFMOD (Ganapol et al., 1998). Numerical inversions of these models allow nondestructive estimations of leaf biochemical constituents from leaf spectral measurements (Jacquemoud et al., 1996; Demarez et al., 1999; Zarco-Tejada et al., 2001; Sampson et al., 2003). Although such model inversions have a clear theoretical basis, some recent studies indicate that the parameterization of the PROSPECT model is insufficient in terms of spectral resolution and the influence of fluorescence. The absorption coefficient of chlorophyll needs to be recalibrated (le Maire et al., 2004).

In this paper we (i) present data on seasonal and height-related variation in leaf chlorophyll content and leaf optical properties in a mature *Acer saccharum* stand from hyperspectral measurements; (ii) examine leaf chlorophyll content in relation to leaf optical properties; and (iii) estimate leaf chlorophyll content using empirical indices and an inversion of the PROSPECT model, and specifically examine the stability of model inversion in relation to temporal and canopy-height-related variation in leaf reflectance and transmittance spectra.

Materials and methods

Study site

Sampling was conducted in a mature stand of sugar maple (*Acer saccharum* Marsh) located at Haliburton Forest, Ontario, Canada (45°14'15.5"N, 78°32'18.0"W). The study site is 30 m × 50 m in size. A mobile canopy lift was used for canopy access, allowing repeated sampling from the same canopy locations. Three trees, one dominant, one codominant, and one suppressed, were selected for measuring leaf biochemical constituents and leaf optical properties. The diameter at breast height (DBH) for the dominant, codominant, and suppressed trees was 51.9, 35.0, and 20.4 cm, respectively.

Leaf sampling and measurements of leaf optical properties

Leaf sampling was carried out eight times during the growing season, starting from leaf expansion through the growing season to the onset of leaf senescence (on 27 May, 10 June, 1 July, 27 July, 16 August, 30 August, 10 September, and 30 September 2004). Three branches from the upper, middle, and lower positions of each tree were selected and marked using flagging tape. For each measurement, three leaves were sampled randomly from each marked branch. Six leaves from young understory trees were also collected for measurements. Altogether, 255 leaf samples were collected and measured through the growing season. After leaf sampling, leaves were immediately placed into zip-lock plastic bags, sealed, and kept in a cooler with ice (0 °C) for transport to a field laboratory at Haliburton Forest. After arrival, leaf adaxial

reflectance and transmittance were measured from 350 to 2500 nm at a 1 nm interval using the portable field spectroradiometer FieldSpec Pro FR (Analytical Spectral Devices, Inc. Boulder, Colo.) attached via a fibre optic to the LI-COR 1800 integrating sphere (LI-COR 1800-12S, Li-COR, Inc., Lincoln, Nebr.). Leaves were clamped into the sample port on the sphere wall. Reflectance and transmittance spectra were measured using methods described by Harron (2000).

Leaf chlorophyll extraction

Leaf chlorophyll content and optical properties were measured on the same leaves. A circle was marked around the leaf area that was against the sample port. Following the leaf optical measurements, a 1.9 cm² round leaf sample was punched from the marked area for measuring leaf chlorophyll content. Each leaf sample was immersed in a vial with *N,N*-dimethylformamide (DMF) (Spectralanalyzed grade, Fisher Scientific, Tustin, Calif.) and stored in a dark refrigerator (the interior temperature was 4 °C) until leaf chlorophylls were completely extracted. Chlorophyll extracts in DMF have been tested stable for up to 20 days while stored in a dark refrigerator (Moran and Porath, 1980). For new leaves collected in May, the samples were bleached in 3–4 days. With increasing leaf maturity, more time (2–3 weeks) was needed to completely extract chlorophyllous pigments. The spectral properties of the solvent were measured using the FieldSpec Pro FR spectroradiometer by illuminating the solvent with the LS-1 tungsten halogen light source connected to a 400 μm fibre (Ocean Optics, Inc., Dunedin, Fla.). The absorbance at 664, 647, and 480 nm was calculated to estimate chlorophyll a and b content using the extinction coefficients derived by Wellburn (1994).

Empirical methods for estimating leaf chlorophyll content

Many spectral indices have been developed and proved effective for nondestructive estimations of leaf chlorophyll content from leaf spectral reflectance (Gitelson and Merzlyak, 1997; Datt, 1999; Maccioni et al., 2001). In the red and blue regions of the spectrum, chlorophylls have strong absorbance peaks. To avoid the saturation of indices under low chlorophyll content, reflectances near instead of exactly at the maximum absorption wavelengths are generally selected to develop spectral indices. Indices that are not sensitive to leaf structure and species have been investigated for chlorophyll estimation (Sims and Gamon, 2002; le Maire et al., 2004). Le Maire et al. (2004) concluded that at leaf level simple spectral indices give better estimations than indices related to the red-edge inflection point, derivative-based indices, or indices based on neural network analysis of empirical hyperspectral data. In this paper, we analyzed a number of chlorophyll indices that have previously been shown to produce low deviation from empirical measurements. These indices include the modified simple ratio index mSR $((R_{728} - R_{434})/(R_{720} - R_{434}))$, where R is the reflectance and the subscript denotes the wavelength) and the modified normalized difference index mND

$((R_{728} - R_{720})/(R_{728} + R_{720} - 2R_{434}))$ developed by Sims and Gamon (2002); the double difference index DD $((R_{749} - R_{720}) - (R_{701} - R_{672}))$, the first derivative based index BmSR $((\delta R_{722} - \delta R_{502})/(\delta R_{701} - \delta R_{502}))$, and the first derivative based index BmND $((\delta R_{722} - \delta R_{699})/(\delta R_{722} + \delta R_{699} - 2\delta R_{502}))$ from le Maire et al. (2004); and the red-edge normalized differences index NDI $((R_{750} - R_{705})/(R_{750} + R_{705}))$ from Gitelson and Merzlyak (1994).

Modeling approach and improvement

Among the leaf-level radiative transfer models, PROSPECT is simple but effective for estimating leaf reflectance and transmittance (Jacquemoud and Baret, 1990). The PROSPECT model is based on Allen's multiple layer plate model but adopts a solid angle of incident radiation instead of an isotropic direction. A leaf is taken as several absorbing plates with rough surfaces giving rise to scattering of light. Leaf optical spectra from 400 to 2500 nm are simulated through the upward and downward hemispherical radiation flux. Only a limited number of input parameters are needed to run the PROSPECT model for calculating leaf reflectance and transmittance. The model assumes the leaf is a stack of L identical elementary layers separated by $L - 1$ air spaces. The number of layers mimics the scattering within the leaf. Scattering is described by the refractive index (n) of leaf materials and by a parameter characterizing the leaf mesophyll structure (L). Layers are defined by their refractive index and absorption coefficient K_i . Absorption is the linear summation of the contents of the biochemicals and the corresponding specific absorption coefficients:

$$K(\lambda) = K_e(\lambda) + \sum K_i(\lambda)C_i/L \quad (1)$$

where λ is the wavelength; $K_e(\lambda)$ is the absorption coefficient of elementary albino and dry layer; C_i is the content of constituent i (chlorophyll_{a+b}, water, and dry matter) per unit area; $K_i(\lambda)$ is the corresponding specific absorption coefficients of the constituent i ; and L is the leaf structure parameter, which is the number of compact layers specifying the average number of air – cell wall interfaces within the mesophyll.

The model can be inverted by numerical iteration to derive the biochemical contents from the leaf spectra. First, an initial guess of the structure parameter L and the concentration of three constituents are input in the forward model to calculate the absorption coefficient $K(\lambda)$ and the hemispherical reflectance and transmittance. The estimated hemispherical reflectance and transmittance are then compared with the measured leaf reflectance and transmittance. Using an optimization algorithm, the i constituents can be numerically iterated by minimizing the merit function (Forsythe et al., 1976):

$$\Delta = \sum_{\lambda} \{ [R_{\text{mes}}(\lambda) - R_{\text{mod}}(\lambda)]^2 + [T_{\text{mes}}(\lambda) - T_{\text{mod}}(\lambda)]^2 \} \quad (2)$$

where R_{mes} and T_{mes} are the measured reflectance and transmittance, respectively; and R_{mod} and T_{mod} are the estimated reflectance and transmittance, respectively, from the model.

Leaf structure and chlorophyll content vary through the growing season, with leaf chlorophyll content generally reaching a maximum in summer. Leaf structural differences such as leaf thickness, density, and number of air–water interfaces influence the absorption of light. Light scattering is primarily determined by leaf internal structure, e.g., the number of air–water interfaces and cell sizes (Knippling, 1970; Grant, 1987). The PROSPECT model calculates the light scattering as a function of elementary layers separated by air spaces. This implies that light scattering increases with an increase in leaf thickness. In the visible bands, the path length of light through the leaf would increase with an increase in light scattering, which would result in increased absorption by pigments. However, as chlorophyll density increases, the efficiency of light capture by any given chlorophyll molecule decreases owing to the effects of internal shading. This relationship is found in a range of photosynthetic organisms from single-celled cyanobacteria to trees (Agustí et al., 1994). Light conditions also influence the leaf absorption. Leaf chlorophyll concentrations commonly decrease in response to increased ambient light conditions (Björkman, 1981; Givnish, 1988). Leaf spectral absorbance at visible wavelengths likewise generally increases in plants grown under low-light conditions compared with those grown under high-light conditions, although responses are quite variable among species (Lee et al., 2000; Baltzer and Thomas, 2005). Leaf absorption efficiency per unit biomass increases by approximately 40% owing to reduced leaf mass per unit area under low light (Baltzer and Thomas, 2005). Assume that the tissue density has no significant change; leaf thickness is the determinant factor. Leaves produced under high light are thicker than those produced under low light (Björkman, 1981; Givnish, 1988), and additional factors such as water transport limitation and proximity to reproductive sinks also contribute to large differences in leaf morphology between canopy and subcanopy leaves (Leal and Thomas, 2003; Koch et al., 2004). Prior measurements in temperate deciduous forest have found that the leaf thickness varies through the seasons and differs in sun and shaded leaves (Demarez et al., 1999). Leaf thickness has been proved to influence the extinction coefficient of leaf layers (Zarco-Tejada, 2000).

The PROSPECT model has been deemed to have insufficient details for general applications because it requires recalibration of the specific absorption coefficients and refractive index (le Maire et al., 2004). The specific absorption coefficients of the biochemical constituents in the PROSPECT model were calibrated using the data from the LOPEX campaign collected in summer (Hosgood et al., 1995; Jacquemoud et al., 1996). Assuming that leaf structural variables were across-season averages, the specific absorption coefficient of chlorophyll in summer or at upper canopies would tend to be low. Using the specific absorption coefficient calibrated in summer (overstory) would thus result in overestimation of leaf chlorophyll content for leaves in other seasons (understory). To simulate the seasonal and canopy-height-related variation of leaf chlorophyll content, we defined and incorporated a leaf thickness factor in

the PROSPECT model to consider the influence of seasonal and canopy-height-related variability in leaf structure on light absorption. In the visible bands, chlorophyll is the dominant absorber. The specific absorption coefficients of all constituents were adjusted using the same leaf thickness factor. The absorption of nonpigment cellular constituents is related to leaf thickness, so the absorption coefficient of elementary albino and dry layer was adjusted accordingly:

$$K(\lambda) = K_c(\lambda)/T + [\sum K_i(\lambda)C_i]/LT \quad (3)$$

where T is the thickness factor, which is the ratio of overstory leaf thickness in summer to overstory and the understory leaf thickness in other growing seasons.

We measured the leaf thickness for 33 leaf samples through the growing season using a digital micrometer following leaf spectra measurements. Leaf thickness varies through the growing season, reaching a maximum in summer (on 16 August 2004). Our measurements showed that the thickness of overstory leaves was 1.00–1.28 times that of understory leaves. The ratio of the average overstory leaf thickness in mid-summer (August 16) to the average thickness of the overstory and the understory leaves on other dates were used to estimate T in Equation (3) (Table 1).

Results and discussion

Seasonal and canopy-height-related variation in leaf chlorophyll content

Strong seasonal variation was observed in average leaf chlorophyll_{a+b} concentration (Figure 1a). The leaf chlorophyll_{a+b} concentration ranged from 20.5 to 47.8 µg/cm² over the growing season. In the early growing season during leaf expansion, an increase in chlorophyll concentration was observed, with chlorophyll_{a+b} concentration increasing from 20.5 µg/cm² on 27 May to 33.5 µg/cm² on 10 June. After this rapid increase, leaf chlorophyll_{a+b} concentration reached a plateau and remained stable from July to mid-September, with a maximum chlorophyll_{a+b} concentration of 47.8 µg/cm² on 30 August. From 10 to 30 September, chlorophyll_{a+b} concentration decreased dramatically from 42.9 to 28.9 µg/cm², a 35% decrease in 20 days.

Leaf chlorophyll_{a+b} concentration also demonstrated differences between the overstory and understory leaves (Figure 1a). Though understory leaves showed a seasonal trend similar to that of overstory leaves in chlorophyll_{a+b} concentration, the average chlorophyll_{a+b} concentration of understory leaves was lower through the growing season. The difference in chlorophyll_{a+b} concentration was large in summer, with the largest difference of 16.9 µg/cm² observed on 27 July, and smaller in fall, with a difference of 2.2 µg/cm² on 30 September. Average chlorophyll_{a+b} concentration of both understory and overstory leaves reached the maximum on 30 August.

Figure 1b shows the seasonal profile of chlorophyll content per unit volume for overstory and understory leaves, which is the chlorophyll concentration in Figure 1a divided by the

Table 1. Leaf thickness factor used in the model for overstory and understory leaves through the growing season.

	27 May	10 June	1 July	27 July	16 Aug.	30 Aug.	11 Sept.	30 Sept.
Overstory	1.45	1.07	1.02	1.00	1.00	1.00	1.05	1.30
Understory	1.55	1.25	1.20	1.18	1.05	1.05	1.12	1.20

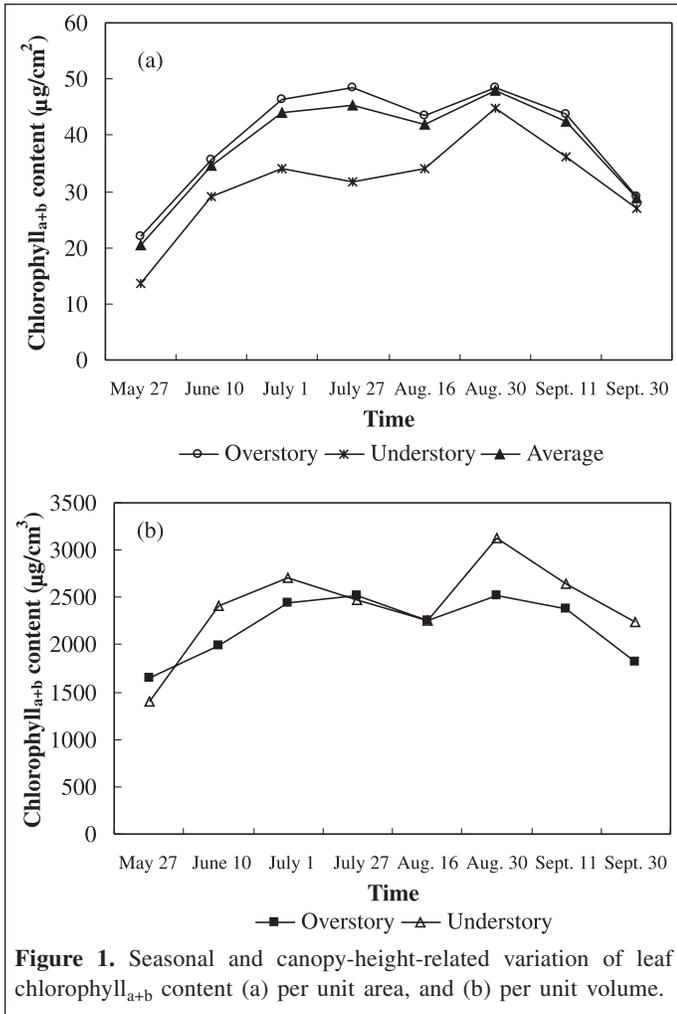


Figure 1. Seasonal and canopy-height-related variation of leaf chlorophyll_{a+b} content (a) per unit area, and (b) per unit volume.

corresponding average leaf thickness during the growing season. The original PROSPECT model assumes that, during the growing season, the specific absorption coefficients of pigments do not depend on time and leaf species. **Figure 1b** demonstrates that leaf chlorophyll content per unit volume varies during the growing season. After considering the seasonal variation of leaf thickness, the model can capture the seasonal and canopy-height-related variation of chlorophyll absorption.

Seasonal and canopy-height-related variation in leaf optical properties

Leaf optical properties showed pronounced seasonal variation. **Figure 2** illustrates the average leaf spectra in spring, summer, and fall (average spectra of all overstory and understory leaf samples on 27 May, 27 July, and 30 September 2004). In the

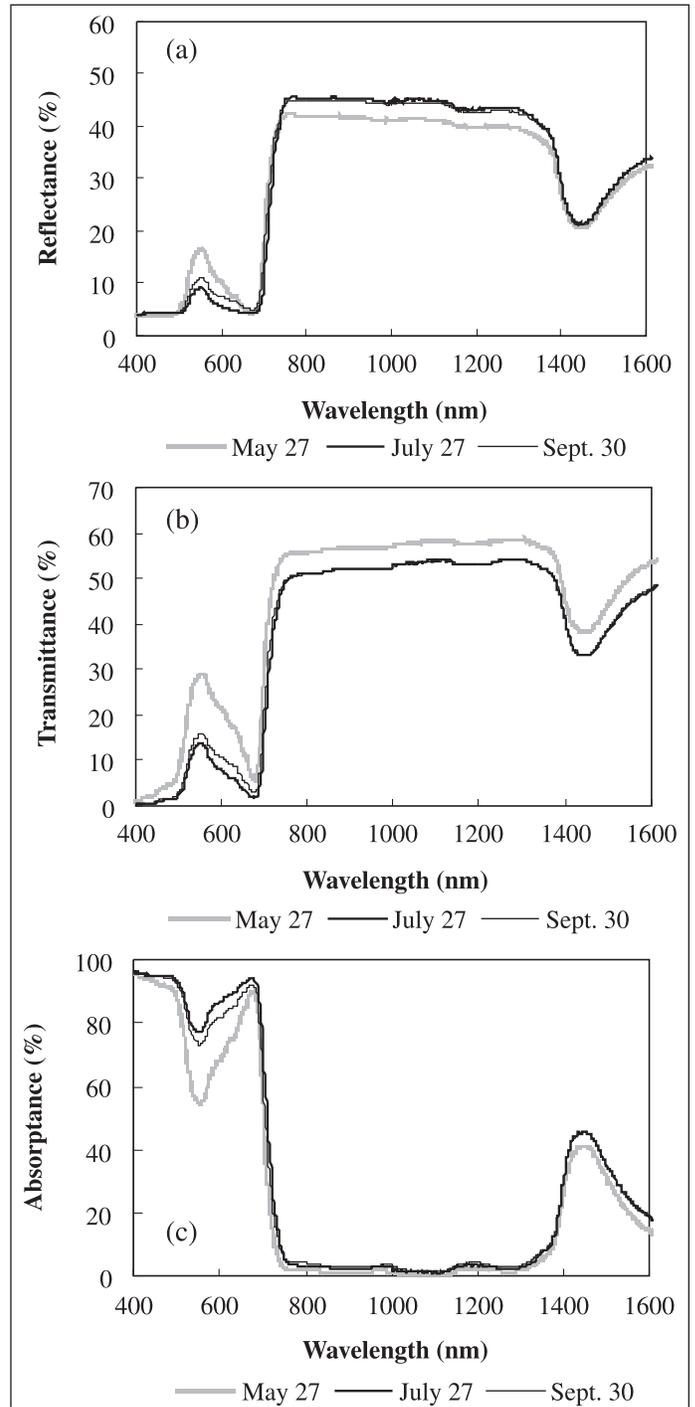
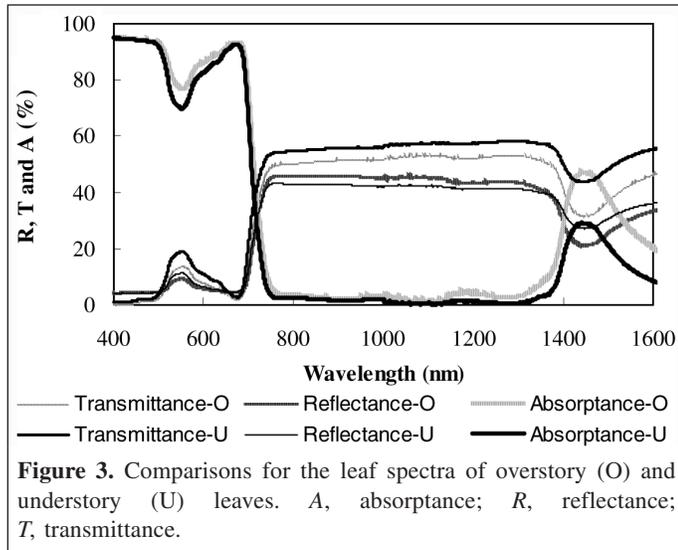


Figure 2. Comparison of seasonal variation of leaf optical properties measured in spring (27 May), summer (27 July), and fall (30 Sept.) of 2004: (a) leaf reflectance; (b) leaf transmittance; (c) leaf absorbance.

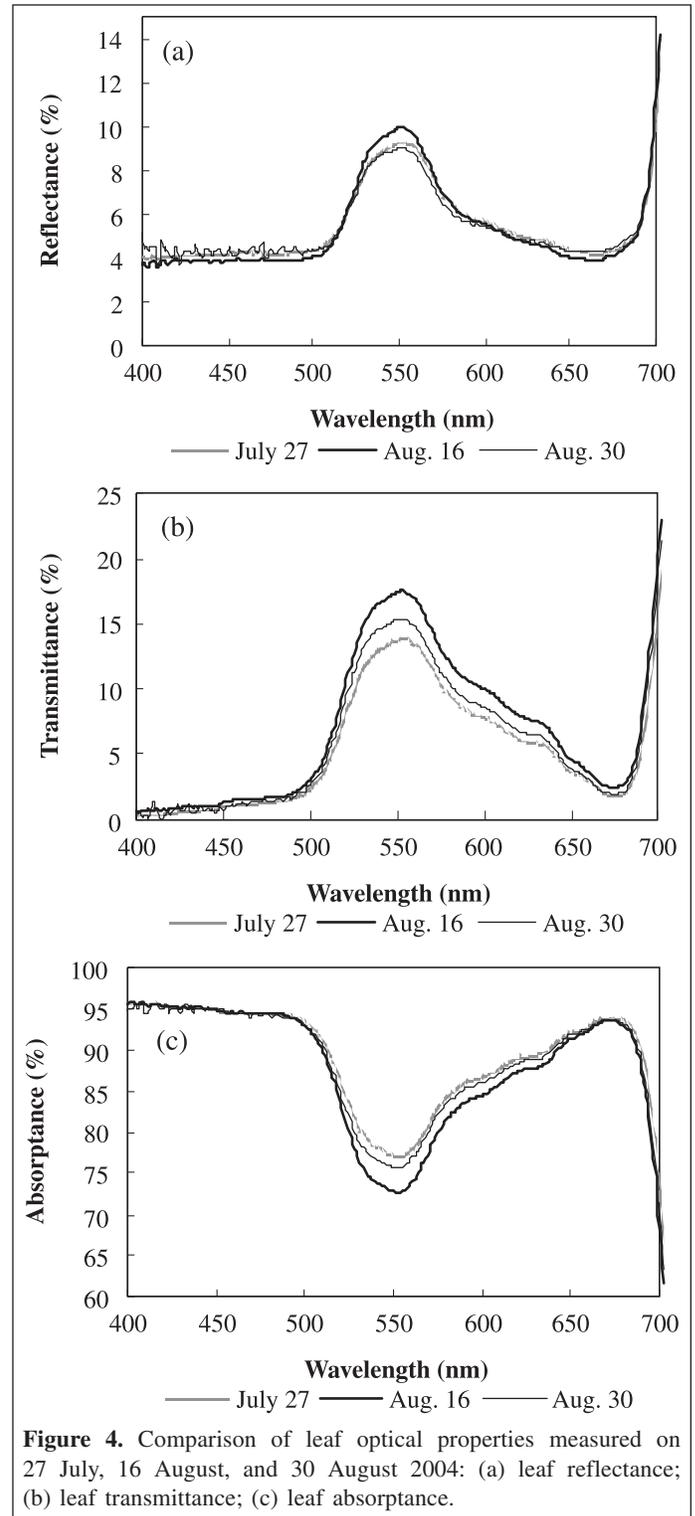


visible band (400–700 nm), leaf reflectance was higher in spring than in summer and fall, with the largest differences observed in the green and red bands (**Figure 2a**). Leaf reflectances showed the lowest values in the green and red bands in summer, and in the near-infrared region from 750 to 1350 nm leaf reflectance was the smallest in spring. We speculate that with increasing leaf maturity, the amount of light intercepting leaf tissues and air-water interfaces between intercellular spaces and cell walls may increase and thus result in higher near-infrared reflectance in summer and fall than in spring. Little change was observed in leaf reflectance spectra from summer to fall.

Leaf transmittance decreased with an increase in the content of absorbing pigments and intercepting leaf tissues. In spring, leaves showed much higher transmittance in the visible, near-infrared, and shortwave infrared bands than in summer and fall (**Figure 2b**). In the visible region, leaf transmittance reached the highest values in spring and the lowest in summer. At the green peak (wavelength = 554 nm), the leaf transmittance on 27 May was 2.2 times that on 27 July. In fall, leaf transmittance increased slightly in the region of 400–540 nm. But from 540 to 680 nm, leaf transmittance was higher in fall than in summer. In the near-infrared region and longer wavelengths, leaf transmittances in fall remained nearly the same as those in summer.

Seasonal trends were also apparent in leaf absorbance spectra (1-reflectance-transmittance) (**Figure 2c**). The absorption features were in accordance with observed changes in leaf chlorophyll content. In the visible band, leaves presented the lowest absorption in spring and the highest absorption in summer. Leaf absorption decreased from summer to fall. In the shortwave infrared region, leaf absorption spectra were high in summer and fall, which is likely caused by increased leaf liquid water during these periods.

Differences were observed in the spectra of overstory and understory leaves through the growing season (**Figure 3**). In the green and red bands, the average reflectance spectrum of understory leaves was higher than that of overstory leaves, whereas the opposite trend was found in the near-infrared



region. In the range of the green, red, and near-infrared bands, understory leaves demonstrated higher transmittance than overstory leaves, corresponding to differences in both leaf chlorophyll_{a+b} content and leaf thickness. The overstory leaves had systematically higher absorption than understory leaves in the visible to near-infrared bands. The differences in absorption spectra were particularly large in the green and red

Table 2. Comparison of empirical spectral indices used for estimation of leaf chlorophyll content.

Index	Formula	RMSE ($\mu\text{g}/\text{cm}^2$)	R^2
mSR	$(R_{728} - R_{434})/(R_{720} - R_{434})$	3.94	0.875
mND	$(R_{728} - R_{720})/(R_{728} + R_{720} - 2R_{434})$	3.97	0.872
BmSR	$(\delta R_{722} - \delta R_{502})/(\delta R_{701} - \delta R_{502})$	3.98	0.872
NDI	$(R_{750} - R_{705})/(R_{750} + R_{705})$	4.11	0.864
DD	$(R_{749} - R_{720}) - (R_{701} - R_{672})$	4.15	0.861
BmND	$(\delta R_{722} - \delta R_{699})/(\delta R_{722} + \delta R_{699} - 2\delta R_{502})$	4.27	0.853

owing to pigment absorption and in the 1350–1600 nm region because of water absorption.

It should be noticed that the average leaf chlorophyll_{a+b} content measured on 16 August was lower than those on 27 July and 30 August. Leaf spectra on these three dates agreed with the fluctuation of the leaf chlorophyll_{a+b} content (Figure 4). The leaf spectra measured on 16 August showed higher reflectance and transmittance, particularly in the green and red bands, leading to lower absorptance on 16 August than on 27 July and 30 August 30. This variation suggests that trees were under some stress in mid-August.

Estimation of leaf chlorophyll content

The previous analysis indicates that seasonal and canopy-height-related patterns of leaf optical properties in visible bands were primarily driven by the chlorophyll content, which has been confirmed by other findings (Thomas and Gausman, 1977; Agustí et al., 1994; Gitelson and Merzlyak, 1994). Leaf absorption spectra, particularly in the pigment absorption bands, are closely related to the chlorophyll content. Using the two approaches introduced previously, we estimated leaf chlorophyll content from the leaf spectra. The root mean square error (RMSE) was calculated to estimate the deviation between the simulated and measured chlorophyll content:

$$\text{RMSE} = \sqrt{\frac{1}{m} \sum_{i=1}^m (\hat{y}_i - y_i)^2} \quad (4)$$

where \hat{y}_i is the chlorophyll content from the estimations based on empirical spectral indices or model inversion, y_i is the measured chlorophyll content, and m is the number of leaf samples.

The spectral indices examined gave good estimations of leaf chlorophyll content (Table 2). The modified simple ratio mSR produced the best estimation, with RMSE = 3.94 $\mu\text{g}/\text{cm}^2$ and correlation $R^2 = 0.875$, and the modified normalized difference index mND performed nearly as well. The performances of these spectral indices agree with the conclusions of le Maire et al. (2004).

Using the reflectance and transmittance spectra of 255 leaf samples as inputs, seasonal and canopy-height-related variation in the leaf chlorophyll content were estimated. We compared the performance of the original PROSPECT model and the model after incorporating the leaf thickness factor. The original PROSPECT model performed well for the overstory leaf

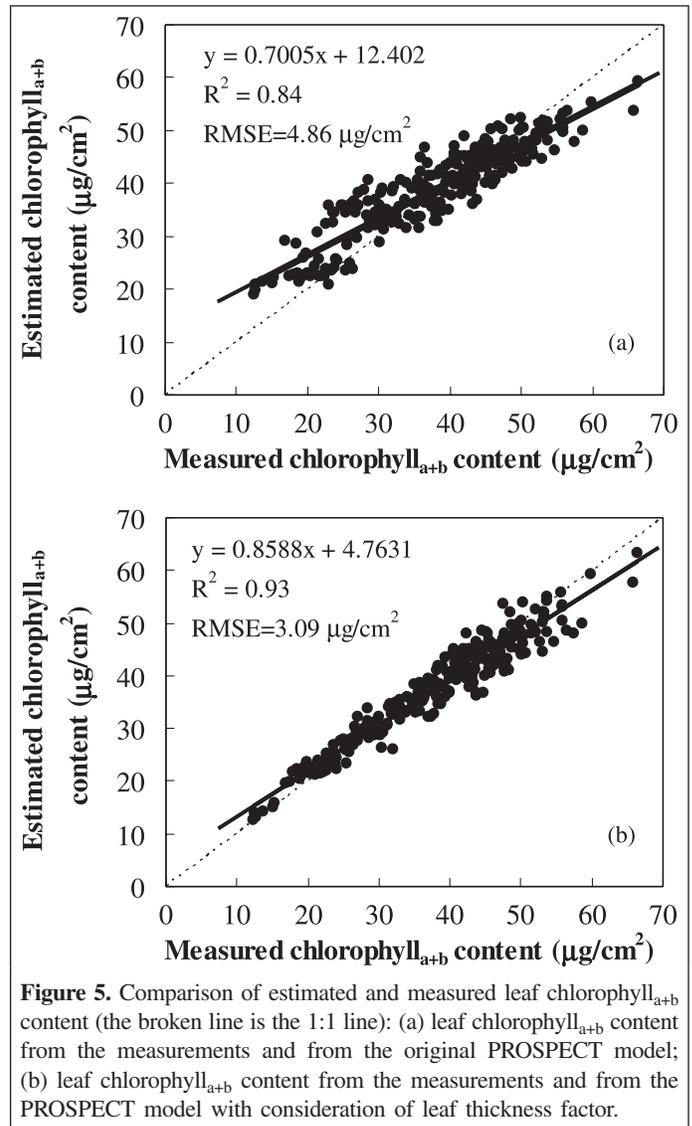
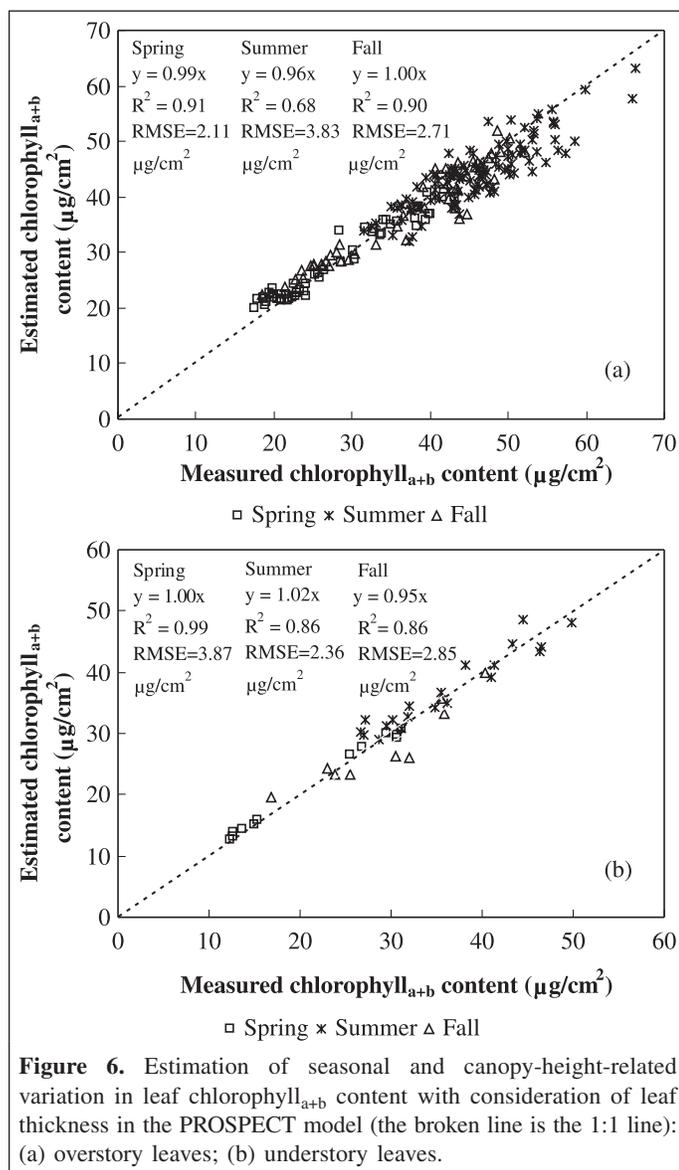


Figure 5. Comparison of estimated and measured leaf chlorophyll_{a+b} content (the broken line is the 1:1 line): (a) leaf chlorophyll_{a+b} content from the measurements and from the original PROSPECT model; (b) leaf chlorophyll_{a+b} content from the measurements and from the PROSPECT model with consideration of leaf thickness factor.

samples collected in summer (July and August) (Figure 5a). However, it could not estimate the variation of leaf chlorophyll content across the season and with respect to canopy height. Specifically, for understory leaf samples and samples collected in the early (on 27 May and 10 June) and late (on 11 and 30 September) growing season that had low leaf chlorophyll content, the model overestimated the leaf chlorophyll content and performed worse than the spectral indices.

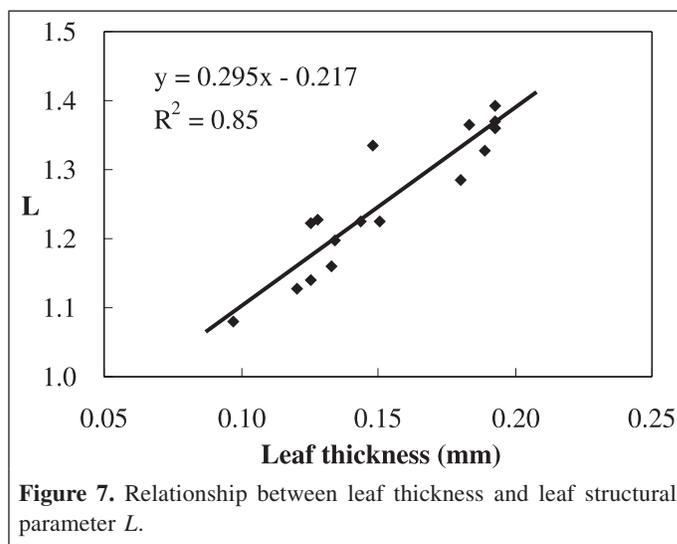
Table 3. Seasonal variation of leaf structure parameter L for overstory and understory leaves.

	27 May	10 June	1 July	27 July	16 Aug.	30 Aug.	11 Sept.	30 Sept.
Overstory	1.160	1.284	1.328	1.394	1.360	1.370	1.365	1.335
Understory	1.081	1.127	1.140	1.228	1.224	1.225	1.198	1.200

**Figure 6.** Estimation of seasonal and canopy-height-related variation in leaf chlorophyll_{a+b} content with consideration of leaf thickness in the PROSPECT model (the broken line is the 1:1 line): (a) overstory leaves; (b) understory leaves.

After incorporating the leaf thickness factor in the model for these samples, the estimation was improved from $\text{RMSE} = 4.86 \mu\text{g}/\text{cm}^2$ and $R^2 = 0.84$ to $\text{RMSE} = 3.09 \mu\text{g}/\text{cm}^2$ and $R^2 = 0.93$ (**Figure 5b**). With the consideration of average leaf thickness ratio, the model performed better in estimating the leaf chlorophyll content for understory leaves and overstory leaves in the early and late growing season (**Figure 6**), though there remains some bias, with low values of leaf chlorophyll content for spring canopy leaves being slightly but systematically overestimated by the model inversion.

Leaf structural parameter L derived from the PROSPECT model falls within the usual range of values (1.0–3.0). This

**Figure 7.** Relationship between leaf thickness and leaf structural parameter L .

parameter varies through the growing season (**Table 3**). L was large in the early growing season from 27 May to 1 July and did not vary much from July to the end of September when the leaf structure became stable. A good correlation ($R^2 = 0.85$) was found between the mean leaf thickness and the mean values of structural parameter L derived from the model (**Figure 7**). During the whole growing season, L was closely related to leaf thickness of both overstory and understory leaves. With the increase of leaf thickness, the number of equivalent horizontal layers that constitute the leaf thickness increases. Good correlations between these two parameters were found among different species (Demarez et al., 1999). Therefore, consideration of leaf structure appears necessary to accurately estimate chlorophyll content for different species and for different seasons and canopy heights.

Conclusion

Hyperspectral data reveal the subtle spectral responses of leaves to leaf chlorophyll content, which facilitates the radiometrical retrieval of leaf chlorophyll content from leaf optical properties. Empirical spectral indices produced better estimation of leaf chlorophyll content than the original PROSPECT inversion model. However, with an additional input of leaf thickness, as a surrogate to capture the seasonal and locational variation in leaf structure and non-chlorophyll light absorption, the PROSPECT model was improved. The improved model performed better than spectral indices and was capable of deriving the seasonal and canopy-height-related variation in leaf chlorophyll content from leaf reflectance and transmittance spectra. Our results suggest that accurate

estimation of leaf-level chlorophyll content should rely either on validated algorithms based on empirical indices or on model inversions that take into account leaf thickness change. This leaf-level radiometrical retrieval is a critical step for accurate mapping of the canopy-level chlorophyll content using hyperspectral remote sensing imagery.

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