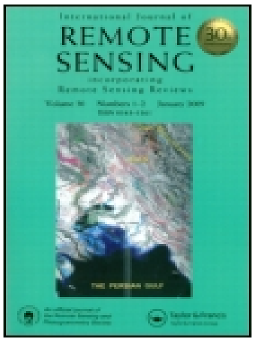

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Effects of leaf structure on reflectance estimates of chlorophyll content

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Effects of leaf structure on reflectance estimates of chlorophyll content

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Assessment of chlorophyll content is a valuable tool for agricultural and non-managed ecosystem studies, since it provides information on key vegetation properties that are, in turn, linked to net primary production. The effects of varying leaf structure (leaf thickness [LT], leaf mass area [LMA] and leaf mass density [LMD]) on reflectance-based chlorophyll indices were assessed using regression and correlation analyses for seven Mediterranean species. The chlorophyll indices used were: (1) corrected for differences in internal scattering, (2) corrected for differences in surface scattering and (3) based on first reflectance first derivatives. Within species, chlorophyll indices showed similar correlation with chlorophyll content (r^2 values larger than 0.80, $p<0.001$) while, across species, indices corrected for surface scattering and first reflectance derivative indices were more closely related to chlorophyll content ($r^2=0.78$ and $r^2=0.75$, respectively, $p<0.001$) than reflectance simple ratio indices ($r^2=0.70$, $p<0.001$). Nonetheless, species with thicker leaves showed lower index values at a similar chlorophyll content than species with thinner leaves. In species with thicker leaves, the increases in chlorophyll content were associated with increases in LMD rather than to changes in LT and were accompanied by significant reductions in NIR radiation scattering at 800 nm. The contribution of LT and LMD to changes in LMA, and their effects on NIR scattering, might promote deviation from the relationship between reflectance based chlorophyll indices and chlorophyll content.

1. Introduction

Assessment of leaf chlorophyll content may provide information on plant physiological status (Lichtenhaler 1998, Peñuelas and Filella 1998) and might be a valuable tool for agricultural and non-managed ecosystem studies, since it is closely linked to nitrogen content and, hence, to photosynthesis (Field and Mooney 1986).

Assessment of pigment composition through optical non-intrusive methods is of great interest because these methods are non-destructive and rapid. In particular, measurements of reflectance spectroscopy at the leaf level have been widely used to estimate pigment composition and content (Gitelson and Merzlyak 1994, Peñuelas and Filella 1998, Gamon and Surfus 1999, Sims and Gamon 2002). Moreover, they provide a tool for assessing pigment at the landscape level (Gamon and Qiu 1999, Sims and Gamon 2002) with potential application on ecosystem studies (Ustin *et al.* 2004) as well as in precision agriculture (Zarco-Tejada *et al.* 2004, Curran and Steele 2005).

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The prospect of reflectance based chlorophyll indices has triggered abundant research. A straightforward approach stems from the fact that absorption of biochemicals is highly wavelength-dependent and several studies estimated chlorophyll content from reflectance at various individual narrow wavelengths located at or close to where the absorption coefficients of chlorophyll pigments are high (e.g. Filella *et al.* 1995, Lichtenhaler *et al.* 1996, Blackburn 1998).

However, since differences in scattering properties among leaves produce additive offsets (baseline shifts) and multiplicative effects to the reflectance spectra, most reflectance-based chlorophyll indices are formulated using ratios of wavelengths where chlorophyll absorbs to regions where scattering is mainly driven by leaf internal structure (Chappelle *et al.* 1992, Gitelson and Merzlyak 1994, Lichtenhaler *et al.* 1996, Datt 1999, Sims and Gamon 2002, among others). In addition, and whenever hyperspectral data are available, methods based on the use of first reflectance derivatives provide chlorophyll estimates that are corrected for variation in leaf surface scattering (Horler *et al.* 1983, Vogelman *et al.* 1993, Datt 1999, Dash and Curran 2004). Nonetheless, although these indices are largely reliable within a species, their ability to estimate chlorophyll concentration across species or in other data sets is reduced and varies according to pigment content (Sims and Gamon 2002).

Few studies have addressed the applicability of optical indices across a wide range of species, and even fewer have addressed the effect of leaf anatomical characteristics on reflectance estimates of chlorophyll content. Datt (1998) developed an index to correct for leaf differential surface reflectance and scattering in 21 Eucalyptus species. More recently, Le Maire *et al.* (2004) compared several chlorophyll indices combining an experimental approach and a simulated data set, and concluded that, across species, the best index was a modified one for surface reflectance. In addition, Sims and Gamon (2002) quantified the effects of structural variations on the relationship between pigment content and spectral indices on more than 50 different species and proposed an index [mSR_{705}] that successfully corrected for surface scattering. Nonetheless, Sims and Gamon (2002) reported that, at a similar chlorophyll content, leaves with a high water content showed lower mSR_{705} values than leaves with low water content.

This study attempts to further explore the effects of leaf structure on reflectance-based chlorophyll indices by comparing different chlorophyll indices and by determining variation in chlorophyll content vs. reflectance indices with relation to structural characteristics (leaf thickness, leaf density, and leaf mass per area).

2. Material and Methods

2.1 *Plant material*

Leaves of seven Mediterranean species were collected from the Barcelona Botanical Garden (41°21′ N, 2°09′ E) and included riparian (*Cornus sanguinea* and *Sambucus nigra*), sclerophyllous (*Arbutus unedo*, *Nerium oleander*, *Pistacea lentiscus*, and *Viburnum tinus*), and a drought deciduous species (*Cistus ladanifer*). Leaves were selected in order to obtain a wide range of chlorophyll contents (spanning from yellow or pale green to very dark green) and structural characteristics by measuring leaves at different developmental stage and canopy positions.

2.2 *Leaf reflectance*

Reflectance measurements were carried out with a field portable spectrometer (Unispec, PP Systems, Havervill, MA) using a 2.1-mm-diameter bifurcated fibre optic and a leaf clip (models UNI410 and UNI501, PP Systems, Havervill) on attached leaves (n=16 for each species) under field conditions. Two scans were taken on each leaf and corrected for the instrument's dark current. Each leaf scan represented the average of three passes internally averaged. A Spectralon reflectance standard was measured before each species data-set measurements. Once measurements were finished, leaves were clipped and placed with their petioles sink in vials with water and carried to the laboratory in an ice chest.

2.3 *Leaf structural parameters*

Leaf thickness [LT] was measured with a spring-loaded micrometer. Three replicates were measured and averaged for each leaf. Afterwards, leaves (excluding the petiole) were weighed before (initial fresh weight, [${W_{fresh}}^{i}$]) and immediately after disk punching (final fresh weight, [${W_{fresh}}^{f}$]). The remaining leaf portion was oven-dried at 70°C and the dry weight determined [${W_{dry}}^{f}$]. These values were used to calculate the corresponding fresh weight [W_{fresh}] and dry weight [W_{dry}] of leaf disks as follows:

$$W_{\mathrm{fresh}} = \left(W^{\mathrm{i}}_{\mathrm{fresh}} - W^{\mathrm{f}}_{\mathrm{fresh}}\right), \tag{1}$$

$$W_{\mathrm{dry}} = W_{\mathrm{fresh}} {}^{*} \left(W^{\mathrm{f}}_{\mathrm{dry}} \Big/ W^{\mathrm{f}}_{\mathrm{fresh}} \right). \tag{2}$$

The total area of leaf disks [A] was calculated by multiplying disk area by the number of disks used for chlorophyll extraction. Subsequently, leaf mass area [LMA] and leaf mass density [LMD] were derived as follows:

$$[\mathrm{LMA}] = W_{\mathrm{dry}} / A, \tag{3}$$

$$[\mathrm{LMD}] = [\mathrm{LMA}] / [\mathrm{LT}]. \tag{4}$$

Finally, Equivalent Water Thickness [EWT] ($\mathrm{g\,H_2O\,m^{-2}}$) was determined as:

$$[\mathrm{EWT}] = \left(W_{\mathrm{fresh}} - W_{\mathrm{dry}}\right) / A.$$

In addition, as in Sims and Gamon (2002), reflectance values at 800 nm [R_{800}] and 445 nm [R_{445}] were used as surrogates for internal and surface radiation scattering, respectively.

2.4 *Chlorophyll extraction*

Chlorophyll concentration was determined on leaf punches for each individual leaf. Destructive spectrometric determination of chlorophylls a and b was made using *N*,*N*-dimethylformamide extraction procedure (Moran and Porath 1980). A total of five to eight disks (0.34 mm diameter) were cut using a cork borer and placed in a vial covered with aluminium foil containing 5 mL of *N*,*N*-dimethylformamide. Care was taken in order to punch the holes into the portion used for optical measurements. Afterwards, vials were kept in the dark and refrigerated (at ca

4°C) for 48 h when extraction was complete. The absorbance of the extracts was measured at 647 nm and 664.5 nm with a spectrophotometer (model UV-160, Shimadzu, Tokyo). Chlorophyll concentration was determined with the extinction coefficients of Inskeep and Bloom (1985). Chlorophyll content was calculated by using disk leaf area instead of disk leaf mass.

2.5 *Reflectance indices*

Several reflectance indices were calculated from each reflectance scan and averaged for each leaf sample. The chlorophyll indices considered in this study are listed in table 1 and can be grouped into three categories:

1. red-NIR indices: indices based on simple arithmetics of reflectance values at two different wavelengths that correspond to absorption by chlorophyll and internal scattering;
2. modified Red-NIR indices: these indices are an extension of Red-NIR indices and include reflectance at a certain wavelength that accounts for differences in surface reflectance; and
3. indices based on derivative spectrum analysis: these indices are related to the long wavelength absorption wing of the red chlorophyll pigment absorption band.

Table 1. List of chlorophyll indices tested in this study along with their formulation and reference.

	Index	Reference
(1) Red-NIR indices		
Pigment specific simple ratio for Chl *a*	R_{800}/R_{675}	Blackburn (1998)
Pigment Specific normalized difference	$(R_{800}–R_{680})/(R_{800}+R_{680})$	Blackburn (1998)
Normalized difference index [NDI]	$(R_{750}–R_{705})/(R_{750}+R_{705})$	Gitelson and Merzlyak (1994)
	R_{750}/R_{550}	Gitelson and Merzlyak (1994)
(2) Scatter- adjusted indices		
mSR_{705}	$(R_{750}–R_{445})/(R_{705}–R_{445})$	Sims and Gamon (2002)
mND_{705}	$(R_{750}–R_{705})/(R_{750}+R_{705}–2\times R_{445})$	Sims and Gamon (2002)
	$R_{672}/(R_{550}\times R_{708})$	Datt (1998)
	R_{672}/R_{550}	Datt (1998)
(3) First derivative indices		
Red edge amplitude	A_{RE}	Peñuelas *et al.* (1993a)
Red edge position	λ_{RE}	Horler *et al.* (1983)
Red edge index [REI]	DR_{715}/DR_{705}	Vogelman *et al.* (1993)

R and DR denote, respectively, reflectance and first derivative reflectance values at the wavelength indicated by subscripts.

2.6 *Data analysis*

Correlation (Pearson coefficient) and regression analyses (SPSS 12.0, SPSS, Chicago) were used to investigate the relations between reflectance indices and chlorophyll content. The effects of leaf structure were assessed by analysing the correlation between the residuals from regression of reflectance indices against chlorophyll content and the structural characteristics.

3. Results

3.1 *Species chlorophyll content and structural parameters*

Total chlorophyll content [Chl T] largely varied among species ranging from 0.04 in *A. unedo* to 1.05 mmol m^{-2} in *P. lentiscus* (table 2). A similar variation was recorded in chlorophyll *a* content (from 0.027 to 0.741 mmol m^{-2}) while chlorophyll b varied by ~15-fold (0.015–0.236 mmol m^{-2}). Leaf structural characteristics varied to a lesser extent than chlorophyll content among the species studied: LT ranged from 86.7 µm in *C. sanguinea* to 603.3 µm in *C. ladanifer*, while LMD showed a lower range of variation (from 0.233 g dm^{-3} to 0.853 g dm^{-3}). EWT varied from 117.6 g m^{-2} to 507.5 g m^{-2} (fivefold). Similarly, LMA ranged from 70.5 to 350.6 g m^{-2} (tenfold). Across species, increased LMA was related to either increased LT (R=0.86, p<0.001) and LMD (R=0.49, p<0.001).

There was no significant correlation between structural characteristics and chlorophyll content for the whole data set: the highest correlation coefficient was between LMA and Chl T (R=0.17, p=0.08). However, when considering species separately, the degree of correlation between Chl T and structural characteristics varied. In winter deciduous species (i.e. *C. sanguinea* and *S. nigra*), Chl T was significantly related in each species with both LT and LMA (R values ranging from R=0.62 to R=0.91, p<0.001, depending on the species), while these correlations were not significant in the remaining species. On the other hand, in species with thicker leaves (i.e. *C. ladanifer* and *N. oleander*), Chl T was found to be correlated to LMD (R=0.73, p<0.01 and R=0.62, p<0.05, respectively).

3.2 *Chlorophyll estimates from reflectance indices*

For the sake of clarity, results are reported only for the best performing index of each category (i.e. NDI, mSR_{705} and REI). Across species, chlorophyll content was significantly correlated with reflectance indices (figure 1), whereas these correlations

Table 2. Minimum [Min], maximum [Max], average [Avg] and coefficient of variation [CV] (%) of leaf total chlorophyll content [Chl T], leaf thickness [LT], leaf mass density [LMD], leaf mass area [LMA], equivalent water thickness [EWT], fresh weight per unit area [FW/A], and dry-to-fresh weight ratio [DW/FW] for the species studied (n=112).

	Min	Max	Average	CV (%)
Chl T (mmol m^{-2})	0.042	1.053	0.563	36.4
LT (µm)	160	603	359	32.6
LMD (g dm^{-3})	0.233	0.853	0.505	20.3
LMA (g m^{-2})	70.5	350.6	177.9	33.7
EWT (g H_2O m^{-2})	117.6	507.5	258.8	38.0
FW/A (g m^{-2})	195.6	705.9	436.6	30.0
DW/FW (g g^{-1})	0.189	0.609	0.413	17.7

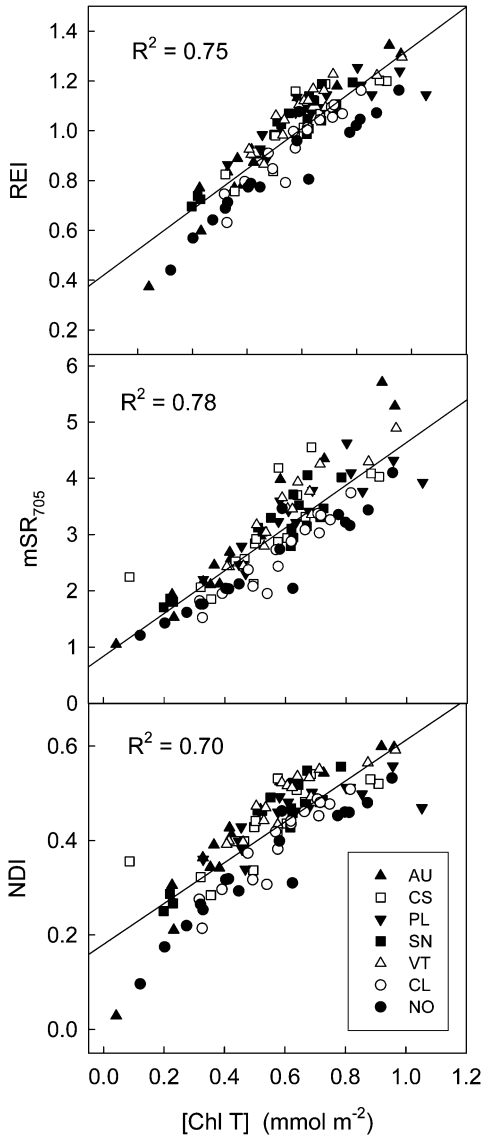

Figure 1. Relationship between the Normalized Difference Index [NDI], modified Simple Ratio index [mSR_{705}] and Red Edge Index [REI] (see table 1 for details on the formulation of these indices) and total chlorophyll content [Chl T] for seven Mediterranean species (AU, *A. unedo*; CS, *C. sanguinea*; PL, *P. lentiscus*; SN, *S. nigra*; VT, *V. tinus*; CL, *C. ladanifer*, and NO, *N. oleander*) (n=112).

were not significant when chlorophyll was expressed as concentration (data not shown). Even though there was a close correlation among NDI, mSR_{705} and REI (R=0.98, p<0.001), mSR_{705} provided better estimates of chlorophyll content than either NDI or REI. Nonetheless, species with LT>350 µm (particularly, *C. ladanifer* and *N. oleander*) showed consistently lower index values than species with thinner leaves.

3.3 *Effects of leaf structure on chlorophyll estimates*

To study the effects of leaf structure on the performance of chlorophyll indices, linear regressions were carried out using Chl T as the independent variable and the reflectance indices as the dependent variables, and the degree of correlation between the resulting residuals and structural parameters was determined (figure 2). For the whole data set, the residuals of the Chl T vs. NDI regression [Z_{NDI}] were significantly (p<0.001) correlated with LT (R=−0.60) and LMA (R=−0.44). A similar response was observed when the residuals from the Chl T vs. REI regression [Z_{REI}] were analysed (R=−0.55, and R=−0.39, p<0.001, for LT and LMA, respectively), whereas the degree of correlation between the residuals from the Chl T vs. mSR$_{705}$ regression [Z_{mSR705}] and structural parameters was lower than for the above-mentioned indices (R=−0.44 and R=−0.25, p<0.001, for LT and LMA, respectively). Due to the close correlation between LT and EWT (R=0.899, p<0.001), Z_{NDI} and Z_{REI} showed, in turn, a significant correlation with EWT (R=−0.60 for both indices, p<0.001). Similarly, although to a lesser extent, Z_{mSR705} was related to EWT (R=−0.49, p<0.001).

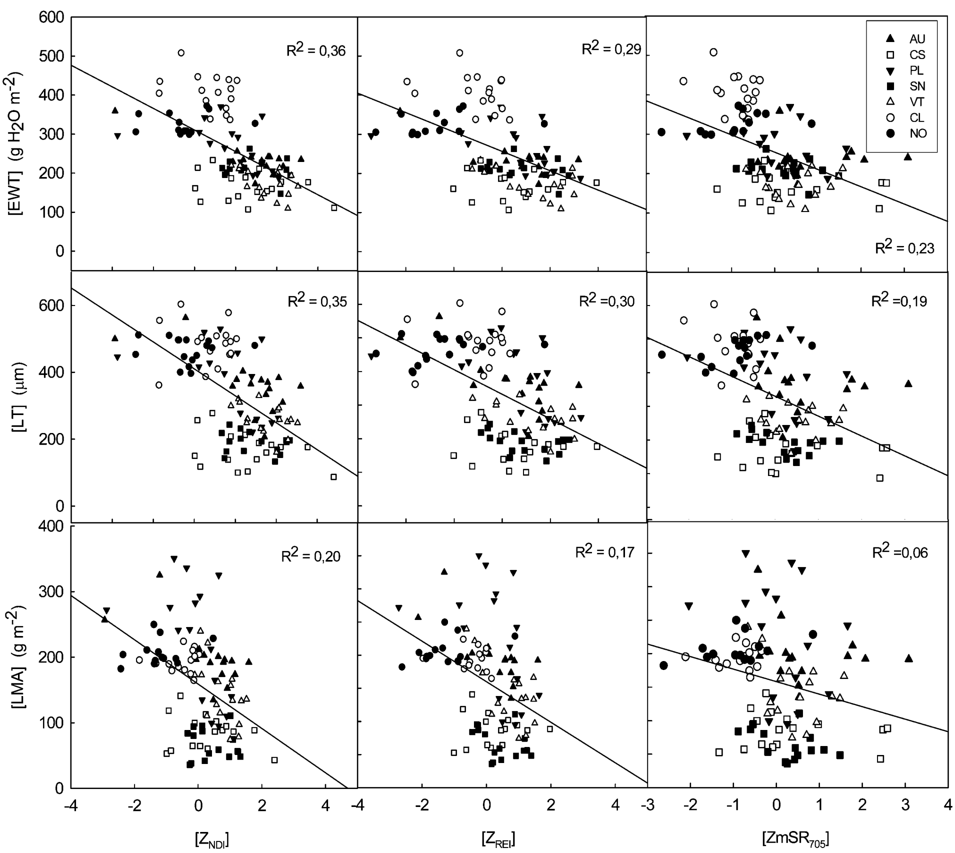

Figure 2. Relationship between the residuals of total chlorophyll content vs. Normalized Difference Index [Z_{NDI}], modified Simple Ratio index [Z_{mSR705}] and Red Edge Index [Z_{REI}] regression and leaf mass area [LMA], leaf thickness and [LT], and equivalent water thickness [EWT] for seven Mediterranean species (AU, *A. unedo*; CS, *C. sanguinea*; PL, *P. lentiscus*; SN, *S. nigra*; VT, *V. tinus*; CL, *C. ladanifer*, and NO, *N. oleander*) (n=112).

4. Discussion

In agreement with previously reported results (Sims and Gamon 2002), mSR_{705}, an index that corrects for surface scattering, yielded the best correlation with Chl T across species. Nonetheless, as indicated by the correlation of regression residuals with leaf structural characteristics, variation in thickness resulted in error estimates of chlorophyll content with contrasting effects among the species studied. In species with thicker leaves (i.e. in *C. ladanifer* and *N. oleander*), the residuals had mostly negative values, indicating that, in these species, chlorophyll content was underestimated. In addition, and due to the close correlation between LT and EWT, chlorophyll content was thus found to be underestimated in leaves with higher water content. Sims and Gamon (2002) reported that, although mSR_{705} eliminated the effect of specular and internal light scattering, there was still a large variability in the mSR_{705} vs. chlorophyll content relationships associated with water content and attempted to correct for these differences by using information from the 970 nm water band. In a data set similar to that in the present study, it was found that the Water Index [WI] (Peñuelas *et al.* 1993b) vs. EWT relationship was downshifted in species with thicker leaves (Serrano, unpublished data), which might explain why attempts to correct for variation in mSR_{705} using the WI have not been very successful.

In the present study, R_{445} was related to LT ($R=0.38$, $p<0.001$) and LMA ($R=0.37$, $p<0.001$) as well as to EWT ($R=0.28$, $p<0.001$). LMA is particularly sensitive to the prevailing light conditions, and it increases in sun leaves as a result of more parenchyma layers and, thus, in leaf thickness. Since plants exposed to high light invest more resources in photoprotective pigments (Thayer and Björkman 1990, Demmig-Adams and Adams 1996), correction for surface scattering, which includes reflectance at 445 nm where carotenoids absorb, led to partial adjustments for variation in LMA and LT as indicated by the lower correlation of regression residuals with LT and EWT in mSR_{705} when compared with NDI or REI.

In agreement with previous studies, there was no correlation between leaf thickness and R_{800} across species (Slaton et al 2001). Nonetheless, R_{800} decreased with increased leaf thickness in species with thicker leaves (i.e. *C. ladanifer* and *N. oleander*). These relationships were tighter when differences for surface reflectance were taken into account. Thus, R_{800}–R_{445} significantly decreased with increased leaf thickness in species where LT>350 μm ($R=-0.785$, $p<0.001$, and $R=-0.496$, $p<0.05$, $n=16$, for *C. ladanifer* and *N. oleander*, respectively). In addition, in these above-mentioned species, increased LMD was correlated to increased chlorophyll content, which might indicate a thicker layer of palisade parenchyma as well as lower fraction of mesophyll as intercellular spaces (Niinemets 1999). In these leaves, thus, a lower fraction of spongy mesophyll in relation to lamina thickness might be expected. Palisade parenchyma cells propagate radiation deeper into the leaf interior (Vogelman and Martin 1993, Smith *et al.* 1997), whereas the more spongy mesophyll cells tend to scatter radiation (DeLucia and Nelson 1993). Thus, leaves with a greater palisade to spongy parenchyma thickness ratio may trap a greater amount of NIR radiation and have lower reflectance values from the adaxial surface (Slaton *et al.* 2001), which is consistent with the decrease observed in R_{800} along with increases in LT in these two species. Since R_{800} and R_{750} (used in the formulation of NDI and mSR_{705} indices) were closely related ($r^2=0.97$, $p<0.001$), NDI and mSR_{705} might present lower index values at a similar chlorophyll content in species where variations in LMD and chlorophyll content are closely related.

5. Conclusions

In agreement with previous studies, a modified index for surface reflectance provided the best correlation with chlorophyll content across species. Although thicker leaves (and, thus, higher EWT) showed lower index values at a similar chlorophyll content than thinner leaves, the deviation from the general relationship appears to be related to the different contribution of LMD and LT to changes in LMA. The results obtained in this study are in agreement with those reported by Slaton *et al.* (2001), indicating that light scattering is more closely related to internal structure (proportions of parenchyma) rather than to thickness per se. More studies are needed in order to confirm these results in a wider range of species and functional groups.

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References

BLACKBURN, G.A., 1998, Quantifying chlorophylls and carotenoids at leaf and canopy scales: an evaluation of some hyperspectral approaches. *Remote Sensing of Environment*, **66**, pp. 273–285.

CHAPPELLE, E.W., KIM, M.S. and MCMURTRIE, J.E., 1992, Ratio analysis of reflectance spectra RARS: an algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sensing of Environment*, **39**, pp. 239–247.

CURRAN, P.J. and STEELE, C.M., 2005, MERIS: the re-branding of an ocean sensor. Imaging spectrometry for ecological applications. *International Journal of Remote Sensing*, **26**, pp. 1781–1798.

DASH, J. and CURRAN, P.J., 2004, The MERIS Terrestrial Chlorophyll Index. *International Journal of Remote Sensing*, **25**, pp. 5003–5013.

DATT, B., 1998, Remote sensing of chlorophyll a, chlorophyll b, chlorophyll a + b, and total carotenoid content in Eucalyptus leaves. *Remote Sensing of Environment*, **66**, pp. 111–121.

DATT, B., 1999, Visible/near infrared reflectance and chlorophyll content in Eucalyptus leaves. *International Journal of Remote Sensing*, **20**, pp. 2741–2759.

DELUCIA, E.H. and NELSON, K.P., 1993, Contribution of internal reflectance to light absorption and photosynthesis of shade leaves. *Bulletin of the Ecological Society of America*, **74**, pp. 211–212.

DEMMIG-ADAMS, B. and ADAMS III, W.W., 1996, The role of xantophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, **1**, pp. 21–27.

FIELD, C. and MOONEY, H.A., 1986, The photosynthesis-nitrogen relationship in wild plants. In On the economy of plant form and function, edited by T.D., Givnish (Cambridge University Press, Cambridge) 25–55.

FILELLA, I., SERRANO, L., SERRA, J. and PEÑUELAS, J., 1995, Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop Science*, **35**, pp. 1400–1405.

GAMON, J.A. and QIU, H., 1999, Ecological applications of remote sensing at multiple scales. In F.I. Pugnaire and F. Valladares (Eds). *Handbook of Functional Plant Ecology*, pp. 805–846 (New York: Marcel Dekker).

GAMON, J.A. and SURFUS, J.S., 1999, Assessing leaf pigment content and activity with a reflectometer. *New Phytologist*, **143**, pp. 105–117.

GITELSON, A.A. and MERZLYAK, M.N., 1994, Spectral reflectance changes associated with autumn senescence in *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. *Journal of Plant Physiology*, **143**, pp. 286–292.

HORLER, D.N.H., DOCKRAY, M. and BARBER, J., 1983, The red edge of plant leaf reflectance. *International Journal of Remote Sensing*, **4**, pp. 273–288.

INSKEEP, W.P. and BLOOM, P.R., 1985, Extinction coefficients of chlorophyll a and chlorophyll b in dimethylformamide and 80% acetone. *Plant Physiology*, **77**, pp. 483–485.

LE MAIRE, G., FRANÇOIS, C. and DUFRÊNE, E., 2004, Towards universal broad leaf chlorophyll indices using PROSPECT simulated database and hyperspectral reflectance measurements. *Remote Sensing of Environment*, **89**, pp. 1–28.

LICHTENHALER, H.K., 1998, The stress concept in plants: An introduction. *Annals of the New York Academy of Science*, **851**, pp. 187–198.

LICHTENHALER, H.K., GITELSON, A.A. and LANG, M., 1996, Non-destructive determination of chlorophyll content of leaves of a green and an aurea mutant of tobacco by reflectance measurements. *Journal of Plant Physiology*, **148**, pp. 483–493.

MORAN, R. and PORATH, D., 1980, Chlorophyll determination in intact tissues using *N,N*-dimethylformamide. *Plant Physiology*, **65**, pp. 478–479.

NIINEMETS, U., 1999, Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist*, **144**, pp. 35–57.

PEÑUELAS, J. and FILELLA, I., 1998, Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science*, **3**, pp. 151–156.

PEÑUELAS, J., FILELLA, I., BIEL, C., SERRANO, L. and SAVÉ, R., 1993b, The reflectance at the 950–970 nm region as an indicator of plant water status. *International Journal of Remote Sensing*, **14**, pp. 1887–1905.

PEÑUELAS, J., GAMON, J.A., FREDDEN, A., MERINO, J. and FIELD, C.B., 1993a, Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. *Remote Sensing of Environment*, **48**, pp. 135–146.

SIMS, D.A. and GAMON, J.A., 2002, Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, **81**, pp. 337–354.

SLATON, M.R., HUNT, E.R. and SMITH, W.K., 2001, Estimating near-infrared leaf reflectance from leaf structural characteristics. *American Journal of Botany*, **88**, pp. 278–284.

SMITH, W.K., VOGELMAN, T.C., DELUCIA, E.H., BELL, D.T. and SHEPHERD, K.A., 1997, Leaf forma and photosynthesis. *BioScience*, **47**, pp. 785–793.

USTIN, S.L., ROBERTS, D.A., GAMON, J.A., ASNER, G.P. and GREEN, R.O., 2004, Using imaging spectroscopy to study ecosystem processes and properties. *Bioscience*, **54**, pp. 523–534.

THAYER, S.S. and BJÖRKMAN, O., 1990, Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis Research*, **23**, pp. 331–343.

VOGELMAN, J.E., ROCK, B.N. and MOSS, D.M., 1993, Red edge spectral measurements from sugar maple leaves. *International Journal of Remote Sensing*, **14**, pp. 1563–1575.

VOGELMAN, T.C. and MARTIN, G., 1993, The functional significance of palisade tissue: penetration of directional versus diffuse light. *Plant, Cell and Environment*, **16**, pp. 65–72.

ZARCO-TEJADA, P.J., MILLER, J.R., MORALES, A., BERJÓN, A. and AGÜERA, J., 2004, Hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops. *Remote Sensing of Environment*, **90**, pp. 463–476.