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Non-destructive estimation of foliar chlorophyll and carotenoid contents: Focus on informative spectral bands

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ABSTRACT

Leaf pigment content provides valuable insight into the productivity, physiological and phenological status of vegetation. Measurement of spectral reflectance offers a fast, nondestructive method for pigment estimation. A number of methods were used previously for estimation of leaf pigment content, however, spectral bands employed varied widely among the models and data used. Our objective was to find informative spectral bands in three types of models, vegetation indices (VI), neural network (NN) and partial least squares (PLS) regression, for estimating leaf chlorophyll (Chl) and carotenoids (Car) contents of three unrelated tree species and to assess the accuracy of the models using a minimal number of bands. The bands selected by PLS, NN and VIs were in close agreement and did not depend on the data used. The results of the uninformative variable elimination PLS approach, where the reliability parameter was used as an indicator of the information contained in the spectral bands, confirmed the bands selected by the VIs, NN, and PLS models. All three types of models were able to accurately estimate Chl content with coefficient of variation below 12% for all three species with VI showing the best performance. NN and PLS using reflectance in four spectral bands were able to estimate accurately Car content with coefficient of variation below 14%. The quantitative framework presented here offers a new way of estimating foliar pigment content not requiring model re-parameterization for different species. The approach was tested using the spectral bands of the future Sentinel-2 satellite and the results of these simulations showed that accurate pigment estimation from satellite would be possible.

Introduction

Pigments are integrally related to the physiological function of leaves. Chlorophylls (Chl) absorb light energy and transfer it into the photosynthetic apparatus. Carotenoids (Car) can also contribute energy to the photosynthetic system. When incident light energy exceeds that needed for photosynthesis, the Car that compose the xanthophyll cycle dissipate excess energy avoiding damage to the photosynthetic system (Demmig-Adams and Adams, 1996). Because of the importance of pigments for vegetation function, pigment content may provide information concerning plant productivity and physiological state.

There are several reasons why leaf pigmentation is important from an applied perspective to both land managers and ecophysiologists (Richardson et al., 2002). First, the amount of solar radiation absorbed by a leaf is largely a function of the foliar contents of photosynthetic pigments, and therefore Chl content directly relates to photosynthetic potential and hence primary production (Curran et al., 1990; Filella et al., 1995; Kergoat et al., 2008). Second, much of leaf nitrogen is incorporated in Chl so quantifying Chl content gives an indirect measure of nutrient status (Filella et al., 1995; Moran et al., 2000; Baret et al., 2007; Kergoat et al., 2008; Schlemmer et al., 2013). Third, pigmentation can be directly related to stress physiology, as contents of Car increase and contents of Chl generally decrease under stress and during senescence (Peñuelas and Filella, 1998). Fourth, the relative contents of pigments are known to change with abiotic factors such as light (*e.g.*, sun leaves have a higher Chl-a/Chl-b ratio; Larcher, 1995) and so quantifying these proportions can provide important information about relationships between plants and their environment.

Traditional methods of pigment analysis, extraction and spectrophotometric or HPLC measurement, require destruction of the measured leaves and do not permit measurement of changes in pigments over time for a single leaf. These techniques are time

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consuming and expensive, thus making assessment of the overall vegetation state of landscapes and ecosystems impractical. Alternative solutions of leaf pigment analysis are non-destructive optical methods. Monitoring plant physiological status *via* measuring leaf reflectance possesses a number of distinct advantages over traditional destructive approaches. The most important ones are simplicity, sensitivity, reliability and a high throughput. These methods are non-destructive, inexpensive, and quick and can therefore be applied across spatial scales (Gamon and Qiu, 1999). Non-destructive techniques save a great deal of manual labor and therefore have a great potential for application in studies of plant productivity, physiology and so on. Developing methods to quantify pigment content and composition using non-destructive remote reflectance measurement would clearly provide a capability that could advance understanding of photosynthetic processes (*e.g.*, light regulation, photooxidation, chlorophyll fluorescence) and insight into detection and monitoring of foliar condition (*e.g.*, environmental stressors).

A large number of reflectance-based methods have been proposed to detect plant pigments, ranging from empirical pigment content *vs*. reflectance relationships to radiative transfer models. Analytical radiative transfer models have the potential to produce accurate and consistent prediction of pigment contents because they use the full spectrum rather than individual bands (Jacquemoud and Baret, 1990; Maier et al., 1999; Feret et al., 2008, 2011). Analytical models were successfully used to optimize remotely sensed vegetation indices (VI) for estimating leaf chemical constituents (Feret et al., 2011). Synthetic reflectance spectra generated by a radiative transfer model, PROSPECT-5, were used to develop statistical relationships between leaf optical and chemical properties, which were applied to experimental data without any readjustment. Two methods used in remote sensing to estimate vegetation chemical composition, VI and Partial Least Squares (PLS) regression, were trained both on the synthetic and experimental datasets, and validated against observations. The study used synthetic data to establish several relationships to estimate leaf Chl and Car content and validated these on a large variety of leaf types. The straightforward method described brought the possibility to apply or adapt statistical relationships to any type of leaf.

The goodness-of-fit of radiative transfer models to predict optical properties of leaves or needles depends on how well understood all processes affecting reflectance are, and how they are accounted for in the models (le Maire et al., 2004). However, these analytical models are difficult to invert and require information about leaf structure that may not be available or, if not accurate, result in poor model performance. Consequently, most relationships between leaf reflectance and pigment contents have been derived empirically.

Among techniques using reflectance to quantify leaf pigment content are vegetation indices (VI) employing a few spectral bands or multiple bands. These indices are based on knowledge of the reflectance properties of leaf biochemical components. le Maire et al. (2004) provided a comprehensive listing of the Chl spectral indices published until 2002. More complete reviews of the practical and theoretical considerations of reflectance spectroscopy are given by Curran et al. (1990), Gamon and Surfus, (1999), le Maire et al. (2004), Richardson et al. (2002), Blackburn, (2007a), Hatfield et al. (2008), and Ustin et al. (2009).

Various processing approaches have been investigated, such as principal components analysis (Yao and Tian, 2003), factor analysis (Coops et al., 2002), stepwise multiple regression (O'Neill et al., 2002; Osborne et al., 2002), artificial neural networks (NN) (Tumbo et al., 2002; Chen et al., 2007), or wavelet-based techniques (Blackburn, 2007b), among others. Despite 30 years of leaf reflectance spectroscopy research, it remains an area of active research. In most cases the techniques for pigment estimation have been tested for a single species or at most a few related species and thus it is not clear whether they can be applied across species with varying leaf structural characteristics. In other cases one technique (*e.g.*, VI) was applied for different species (*e.g.*, Sims and Gamon, 2002) with no comparison with other techniques. A key problem is the selection of optimal spectral bands and an appropriate processing approach for pigment estimation among the vast array of those available.

Our objective in this study was to test the performance of vegetation indices, neural network (NN) and partial least squares (PLS) regression for estimating foliar Chl and Car contents of three unrelated tree species. The attempt was made to find techniques that are insensitive to species and leaf structure variation and thus could be applied in large scale remote sensing studies without extensive calibration. In particular, we focused on finding the most informative spectral bands, *i.e.*, developing models that allow accurate estimation of pigment content with a minimum number of spectral bands. We found that all three techniques are very accurate for estimating Chl content. Rededge chlorophyll index used two quite wide spectral bands in rededge and NIR regions, while both NN and PLS used three 20 nm wide bands. NN and PLS were more accurate than carotenoid reflectance index estimating Car content in all three species taken together, thus were not species-specific. Four 20 nm wide spectral bands centered at 510, 550, 720 and 770 nm allowed accurate Car estimation in three species by single algorithm. The most informative spectral bands found using uninformative variable elimination PLS (UVE PLS) coincided with spectral bands used in three tested techniques, VI, NN and PLS. All three techniques were tested with reflectance simulated in spectral bands of near future Sentinel-2 satellite and were found to be estimating accurately pigment content.

Methods

Pigment content and reflectance measurement

For estimating the Chl and Car contents, anthocyanin free juvenile, mature and senescent leaves collected from 1992 to 2005 were used. Norway maple and horse chestnut leaves were from a park at Moscow State University (Russia), beech leaves were from the University of Karlsruhe campus (Germany). The leaf total Chl and Car content was determined analytically from the same leaf samples used for reflectance measurement (details are in Gitelson et al., 2001, 2002, 2003). Pigment content was expressed on a leaf area basis. Three data sets containing 90 leaves (beech, chestnut, and maple) were used (Table 1). Pigment content varied widely: total Chl from 4 to 675 mg m^{-2} and total carotenoids from 16 to 137.2 mg m^{-2}.

Table 1
Pigment content (in mg m^{-2}) of maple, chestnut and beech leaves examined in this study.

		Chl-a	Chl-b	Total Chl	Total Car
Maple *n* = 38	Median	181.7	62.0	242.5	45.9
	Average	192.6	69.5	262.1	48.4
	Minimum	2.8	1.0	4.0	16.0
	Maximum	419.9	150.1	570.0	82.6
Chestnut *n* = 18	Median	51.3	15.5	66.7	49.6
	Average	105.0	34.7	139.7	48.9
	Minimum	7.0	3.0	10.0	26.0
	Maximum	335.9	134.5	470.4	83.2
Beech *n* = 34	Median	256.9	95.7	352.6	90.2
	Average	271.6	101.2	371.0	87.8
	Minimum	63.5	21.9	85.5	30.1
	Maximum	541.4	192.0	675.0	137.2