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Non-destructive estimation of foliar carotenoid content of tree species using merged vegetation indices

Fabian E. Fassnacht[a,*], Stefanie Stenzel[a], Anatoly A. Gitelson[b,c]

[a] Karlsruhe Institute of Technology, Institute of Geography and Geoecology, Kaiserstraße 12, 76131 Karlsruhe, Germany
[b] Israel Institute of Technology (Technion), Civil and Environmental Engineering, Rabin Building, 32000 Haifa, Israel
[c] School of Natural Resources, University of Nebraska, Lincoln, USA

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ABSTRACT

Leaf pigment content is an important indicator of plant status and can serve to assess the vigor and photosynthetic activity of plants. The application of spectral information gathered from laboratory, field and remote sensing-based spectrometers to non-destructively assess total chlorophyll (Chl) content of higher plants has been demonstrated in earlier studies. However, the precise estimation of carotenoid (Car) content with non-destructive spectral measurements has so far not reached accuracies comparable to the results obtained for Chl content.

Here, we examined the potential of a recently developed angular vegetation index (AVI) to estimate total foliar Car content of three tree species. Based on an iterative search of all possible band combinations, we identified a best candidate AVI_{car}. The identified index showed quite close but essentially not linear relation with Car contents of the examined species with increasing sensitivity to high Car content and a lack of sensitivity to low Car content for which earlier proposed vegetation indices (VI) performed better. To make use of the advantages of both VI types, we developed a simple merging procedure, which combined the AVI_{car} with two earlier proposed carotenoid indices. The merged indices had close linear relationship with total Car content and outperformed all other examined indices. The merged indices were able to accurately estimate total Car content with a percental root mean square error (%RMSE) of 8.12% and a coefficient of determination of 0.88. Our findings were confirmed by simulations using the radiative transfer model PROSPECT-5. For simulated data, the merged indices again showed a quasi linear relationship with Car content. This strengthens the assumption that the proposed merged indices have a general ability to accurately estimate foliar Car content.

Further examination of the proposed merged indices to estimate foliar Car content of other plant species is desirable to prove the general applicability of the index for non-destructive estimation of Car from leaf reflectance data.

Introduction

Photosynthesis is the essential process for all life, producing oxygen and organic material (Davies, 2004; Nelson and Yocum, 2006). Within this process, photosynthetically active tissue of plants captures light energy and stores it as chemical energy in form of carbohydrates (Govindjee and Krogmann, 2004; Richardson et al., 2002). The series of electron transfers that occur during photosynthesis on the thylakoid membranes in chloroplasts highly depend on several plant pigments, including chlorophyll (Chl; green pigments), carotenoid (Car; yellow pigments) and anthocyanin (Anth; red pigments) (Lichtenthaler, 1987; Ustin et al., 2009).

Chlorophylls a and b are responsible for harvesting light and transfer its energy further on (Sims and Gamon, 2002). Leaf Chl controls the amount of solar radiation that a leaf absorbs. Hence, Chl content is directly linked to the photosynthetic potential of a plant and therefore its primary production (Blackburn, 2006; Curran et al., 1990). Chlorophyll content also serves as an indirect estimation of the nutrient status as a substantial part of leaf

Abbreviations: Car, carotenoid; Chl, chlorophyll; Anth, anthocyanin; AVI, angular vegetation index; Chap, chappelle index; RMSE, root mean square error; EWT, equivalent water thickness; LMA, leaf mass per area; BP, brown pigment content.

* Corresponding author. Tel.: +49 721 60843828.
E-mail addresses: fabian.fassnacht@kit.edu (F.E. Fassnacht), stefanie.stenzel@kit.edu (S. Stenzel), agitelson2@unl.edu (A.A. Gitelson).

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nitrogen is incorporated in Chl (Filella et al., 1995; Moran et al., 2000). Furthermore, Chl content is closely related to plant stress and its senescence (Merzlyak and Gitelson, 1995; Carter and Knapp, 2001; Hatfield et al., 2008).

Anthocyanins are water-soluble pigments abundant in juvenile and senescing plants. High content of Anth in plant leaves is known to be an indicator of environmental stresses like intense sunlight, extreme temperature, drought or infections (Chalker-Scott, 1999; Gould et al., 2002). Anthocyanins were also found to regulate photosynthesis due to their photoprotective function (Steyn et al., 2002; Close and Beadle, 2003). However, their presence and functioning is not yet fully understood (Blackburn, 2006).

The third important class of pigments is represented by carotenoids (Car). Carotenoids are part of the essential structures of the photosynthetic antenna and reaction center. Furthermore, they play an important role in the xanthophyll cycle, which was found to protect the plant cells from photooxidation and photoinhibition (Bartley and Scolnik, 1995; Demmig-Adams and Adams, 1996) which can occur if light intensity exceeds the capacity for electron transfer in the photosynthetic chain. Hence, Car have both a photosynthetic and photoprotective role (Edge et al., 1997; Hatfield et al., 2008; Peterman et al., 1997).

Changes of plant pigment contents, particularly contents of Chl and Car, have been linked to phenology as well as to stress symptoms. Decline in Chl content during the vegetation period often indicate stress and complementary Car content provides information on the physiological status of vegetation (Young and Britton, 1990). Variance of Car content and their proportion to Chl are therefore commonly used for the analysis of plant physiological state (Demmig-Adams and Adams, 1996; Fang et al., 1998; Richardson et al., 2002; Young and Britton, 1990; Gamon and Surfus, 1999; Merzlyak et al., 1999).

Reliable, fast and in ideal case non-destructive methods for estimating plant pigment contents can serve as a valuable tool to assess the vegetation status and therefore support decisions on agricultural and ecological issues. The non-destructive assessment of plant pigments has been examined with absorbance, reflectance and transmittance techniques (Le Maire et al., 2004; Ustin et al., 2009; Gitelson, 2011). Amongst those, the reflectance-based approaches are best known and were in some comparative studies also identified to be a better indicator of plant pigments than absorbance and transmittance based approaches (Richardson et al., 2002).

Various vegetation indices (VI) for estimating the abundance of Chl, Car and Anth contents have been presented in earlier studies (Blackburn, 2006; Ustin et al., 2009). The obtained results varied amongst the pigment types and the estimation of Car content was found to be more difficult than the estimation of Chl (Sims and Gamon, 2002; Hatfield, 2008) and Anth (Gitelson et al., 2006, 2009). One reason for this is that Car have absorption peaks overlapping with the ones of Chl (Sims and Gamon, 2002). Due to the typically higher contents of Chl, accurate subtraction of the effect of Chl absorption from reflectance where Car absorb is challenging.

Several spectral indices have been proposed to estimate leaf total Car content from reflectance data. Early studies include those of Chappelle et al. (1992), Datt (1998) and Gitelson et al. (2002) as well as indices for estimating the Car/Chl ratio (Filella et al., 1995; Merzlyak et al., 1999). However, none of these indices was able to reach similarly high accuracies as comparable approaches reached for the estimation of Chl content (e.g., Gitelson and Merzlyak, 1994a,b; Richardson et al., 2002; Sims and Gamon, 2002; Chappelle et al., 1992; Lichtenthaler et al., 1996; Le Maire et al., 2004). Furthermore, some of the proposed indices were found to be not transportable to other data sets (Sims and Gamon, 2002; Blackburn, 1998).

Since then, several new approaches have been presented and the introduction of theory-based methods, such as the application of radiative transfer models (Le Maire et al., 2004; Féret et al., 2011; Zarco-Tejada et al., 2013; Hernández-Clemente et al., 2014), or the conceptual three-band model for pigment content estimation (Gitelson et al., 2006). These approaches helped to understand better the functioning and interaction of leaf-pigments with spectral reflectance data and thereby to improve the reliability of the results of pigment estimations. However, in many cases, the proposed methods and indices still reported limited sensitivities for the estimation of foliar Car contents of trees (e.g., Gitelson et al., 2006; Lhotáková et al., 2013), especially when compared to the successes in estimating Chl contents. Therefore, the current state of knowledge still leaves space for improvements concerning the non-destructive assessment of Car content from reflectance data. In this context, Ustin (2009) stated that "new methods to identify and quantify individual pigments in the presence of overlapping absorption features would provide a major advance in understanding their biological functions, quantifying net carbon exchange, and identifying plant stresses."

One possible approach for improving the sensitivity of reflectance data is the application of VIs that built on other mathematical principles than the well-established and widely used ratio and normalized ratio indices. Examples of such VIs include integral-based indices (e.g., Oppelt and Mauser, 2003), derivation-based methods (e.g., Vogelmann et al., 1993) as well as the recently introduced angle-based indices (Palacios-Orueta et al., 2006; Khanna et al., 2007; Fassnacht et al., 2012).

In this study, we examined the potential of an angle-based vegetation index approach (AVI) for estimating foliar Car contents. We examined spectroscopic data of three tree species for which corresponding reference measurements of Car content obtained with chemical methods were available. A further focus of the study was the development of a merging procedure, to combine the advantages of two spectral indices; one of them was more sensitive to low Car content while another to high Car content. The suitability of the merged indices was validated with the available experimental data as well as with simulated reflectance data originating from the leaf radiative transfer model PROSPECT-5. Consequently, the main objective of the study was to develop a procedure that enables accurate estimation of Car content across multiple species and wide ranges of Car values.

Methods

Spectrometer data

Spectrometer data for the tree species Norway maple (*Acer platanoides* L.), horse chestnut (*Aesculus hippocastanum* L.) and European beech (*Fagus sylvatica* L.) were applied. Maple and chestnut samples were collected in a park at Moscow State University in the time period between 1992 and 2000. The samples were collected throughout the vegetation period and include juvenile, mature and senescent leaves (details are in Gitelson et al., 2002). Beech leaves were taken on the campus of the University of Karlsruhe in August 1996 and 2000. The selection of the leaves was according to their visual appearance. Only healthy leaves without Anth pigmentation and damages were used. For all collected leaves spectrophotometer measurements were conducted and the leaf pigment contents were obtained using chemical procedures. Both measurements are described with more details in Gitelson et al. (2002) and Lichtenthaler (1987). Statistical summaries of the Car measurements for the three datasets and the combined dataset are provided in Table 1. Fig. 1 shows species-specific relations between Car and Chl for the applied samples.