

Assessment of leaf carotenoids content with a new carotenoid index: development and validation on experimental and model data

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Abstract

Leaf carotenoids content (LCar) is an important indicator of plant physiological status. Accurate estimation of LCar provides valuable insight into early detection of stress in vegetation. With spectroscopy techniques, a semi-empirical approach based on spectral indices was extensively used for carotenoids content estimation. However, established spectral indices for carotenoids that generally rely on limited measured data, might lack predictive accuracy for carotenoids estimation in various species and at different growth stages. In this study, we propose a new carotenoid index (CARI) for LCar assessment based on a large synthetic dataset simulated from the leaf radiative transfer model PROSPECT-5, and evaluate its capability with both simulated data from PROSPECT-5 and 4SAIL and extensive experimental datasets: the ANGERS dataset and experimental data acquired in field experiments in China in 2004. Results show that CARI was the index most linearly correlated with carotenoids content at the leaf level using a synthetic dataset ($R^2 = 0.943$, $RMSE = 1.196 \mu\text{g}/\text{cm}^2$), compared with published spectral indices. Cross-validation results with CARI using ANGERS data achieved quite an accurate estimation ($R^2 = 0.545$, $RMSE = 3.413 \mu\text{g}/\text{cm}^2$), though the RBRI performed as the best index ($R^2 = 0.727$, $RMSE = 2.640 \mu\text{g}/\text{cm}^2$). CARI also showed good accuracy ($R^2 = 0.639$, $RMSE = 1.520 \mu\text{g}/\text{cm}^2$) for LCar assessment with leaf level field survey data, though PRI performed better ($R^2 = 0.710$, $RMSE = 1.369 \mu\text{g}/\text{cm}^2$). Whereas RBRI, PRI and other assessed spectral indices showed a good performance for a given dataset, overall their estimation accuracy was not consistent across all datasets used in this study. Conversely CARI was more robust showing good results in all datasets. Further assessment of LCar with simulated and measured canopy reflectance data indicated that CARI might not be very sensitive to LCar changes at low leaf area index (LAI) value, and in these conditions soil moisture influenced the LCar retrieval accuracy.

Key words

Leaf carotenoids content, Hyperspectral, spectral index, radiative transfer model

1. Introduction

Photosynthetic pigments that mainly include chlorophylls and carotenoids are of great importance in the biosphere. Their photosynthetic function is essential for plant and mammal survival (Blackburn, 2007). Within leaf chloroplasts, chlorophylls (Chl), composed of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*), represent the principal class of pigments responsible for light absorption in photosynthesis (Nobel, 1999). Carotenoids (Car), that include carotenes and xanthophylls, are the second major group of plant pigments (Blackburn, 2007). They are part of the essential structures of the photosynthetic antenna and reaction center, and help stabilize chlorophyll–protein complexes (Frank and Cogdell, 1996; Strzałka et al., 2003). Besides their function in photosynthesis, previous studies suggest that the assessment of the variation of Car and of their ratio to Chl could shed light on the understanding of photoprotection, photosynthetic acclimation, and photosynthetic efficiency in plants (Demmig-Adams and Adams, 1996; Fang et al., 1998; Gamon and Surfus, 1999; Merzlyak et al., 1999; Richardson et al., 2002; Young and Britton, 1990). Within the plant growth cycle, Chl decrease normally indicates that plants are affected by environmental stresses, while the variation of Car reflects the physiological status of vegetation (Young and Britton, 1990). For instance, it has been observed that Car content would change when plants are in sun-intense and high temperature conditions, or when nitrogen availability is low, or at the onset of leaf senescence (Demmig-Adams and Adams, 1996; Kirchgeßner et al., 2003; Munné-Bosch and Peñuelas, 2003). Therefore, quantitative estimation of Car content is extremely useful, in order to clarify the mechanisms of photoprotection and light-adaption, and for early diagnosis of stress in vegetation.

During the last decade, a series of attempts have been undertaken to use spectroscopy techniques to estimate Car content at both the leaf and canopy level, exploiting its absorption features in the visible range (Gitelson et al., 2002). Based on ratio analysis of reflectance spectra (RARS) method, Chappelle et al. (1992) suggested that the absorption band at 500 nm had the highest correlation with Car and was least affected by confounding effects from other pigments, thus they proposed a ratio index ($RARSc, R_{760}/R_{500}$) for Car estimation. Research conducted by Datt (1998) indicated that the maximum sensitivity of reflectance to variation in pigment content was in the green band region at 550 nm and at 708 nm in the red edge region, thus they put forward a reflectance band ratio index ($RBRI, R_{672}/(R_{550} \times R_{708})$), which had a good correlation with Car. In an attempt to evaluate spectral indices for estimating pigment concentrations at the leaf scale, Blackburn (1998) developed two new indices (PSSRc and PSNDc) with the optimal wavebands 470 nm and 800 nm for Car retrieval. Having found that the spectral band around 510 nm was sensitive to Car content, Gitelson et al. (2002) developed two carotenoid reflectance indices (CRI_{550} and CRI_{700}), with the reciprocal reflectance at 510, 550 and 700 nm and found that these two indices were accurate indicator of leaf carotenoids content (LCar). Furthermore, Gitelson et al. (2006) investigated the applicability of a conceptual three-band model to estimate the content of various pigments and they established two carotenoid indices ($CAR_{red\ edge}$ and

CAR_{green}) with three bands located at 510 – 520 nm, 690 – 710 nm (560 – 570 nm for CAR_{green}) and a NIR band, which showed accurate estimation of Car. Hernández-Clemente et al. (2012) found that vegetation canopy structure severely affected the performance of spectral index for Car assessment at crown level. A simple ratio index ($SR_{car}, R_{515}/R_{570}$) was then proposed and it showed good correlation with Car content at both leaf and canopy levels.

For the development of robust indices for plant biochemical content assessment with spectroscopic techniques, the quality of the training dataset, the selection of the wavelengths and the availability of an independent dataset for the validation are essential (Féret et al., 2011). The above mentioned studies have indeed made much progress in Car content estimation in different vegetation species at the leaf or canopy scales. Nevertheless, most of the research focused on establishing spectral indices or models for Car retrieval, with calibration and validation datasets that were generally limited. These limited data might not be generic enough in order to provide a robust method of assessing Car composition and distribution, at a range of phenological stages and leaf structures. Spectral indices or models based on these datasets might be site- or species-specific, their robustness and capability deserves further investigation when applied to a wide variety of plant leaves and conditions.

Radiative transfer models (RTMs) are effective tools to clarify the mechanism describing the relationships between spectral reflectance and plant parameters. They provide an analysis of the remote sensing signal based on a robust understanding of the physical, chemical, and biological processes, allowing to assemble rapidly abundant simulation datasets (Féret et al., 2008). In recent years, the RTMs have been used extensively for various applications on vegetation studies (Jacquemoud et al., 2009). Based on spectral data sets simulated from leaf scale RTMs, Blackburn and Ferwerda (2008) proposed a method to estimate leaf chlorophylls content (LChl) from reflectance using wavelet analysis. By coupling the leaf model PROSPECT (Jacquemoud and Baret, 1990) with the multi-layer canopy model Scattering by Arbitrary Inclined Leaves (SAIL) (Verhoef, 1984) into the PROSAIL model, le Maire et al. (2008) conducted a research to select optimal narrow-band vegetation indices for the retrieval of LChl and leaf mass per area (LMA). Féret et al. (2008) successfully estimated the concentrations of carotenoids and total chlorophyll by inverting the PROSPECT model from tree leaf reflectance and transmittance measurements. Di Vittorio (2009) focused on the incorporation of three pigments, including chlorophyll *a*, chlorophyll *b*, and total carotenoids, into the Leaf Incorporating Biochemistry Exhibiting Reflectance and Transmittance Yields (LIBERTY) model (Dawson et al. 1998), obtaining good estimates of the concentrations of these pigments. Vincini et al. (2016), used PROSAIL simulations to explore the sensitivity of canopy scale estimators of leaf chlorophylls, obtainable with Sentinel-2 satellite spectral resolution, to soil, canopy and leaf mesophyll factors.

Nevertheless, attention on Car assessment using RTMs has been smaller than that for Chl estimation. For LCar retrieval with leaf spectra, the proper combination of various plant parameters in leaf model PROSPECT, could generate a series of simulated datasets useful for an investigation of the spectral interactions among Car and other leaf characteristics, also providing

a database for evaluating the performance of spectral indices for LCar assessment. Different from LCar retrieval with leaf level reflectance, LCar assessment with canopy spectra is much more complex, since spectra acquired at the canopy could be affected by complicating factors other than biochemical content, such as canopy structure, illumination and viewing geometry, as well as the optical properties of the soil (Lemaire, 2012). These effects can induce ambiguities in LCar assessment from canopy reflectance. Among these factors, leaf area index (LAI), one of the key parameters describing the canopy structure, and the soil background, has a large effect on canopy reflectance signals (Yu et al., 2014; Zou et al., 2015). The utilization of the PROSAIL model could generate an extensive canopy level dataset useful for better understanding the relationship between canopy geometry, background environment and canopy reflectance, thus it could shed light on the effect of LAI and soil background on LCar assessment and provide the basis for an accurate and robust LCar estimation with spectral index methods.

Therefore, the aim of the present study was to develop an accurate and robust LCar estimation index, using simulated and measured datasets based on their absorption features in the visible spectrum. The specific objectives were to: i) establish a new carotenoid index (CARI) for LCar estimation, assess and compare its performance with published carotenoid indices (Table 1) using leaf level simulated data obtained from PROSPECT-5; ii) evaluate the capability and robustness of the newly CARI and published carotenoid indices with various leaf level measured data including the widely used ANGERS dataset (Féret et al., 2008) and field survey data; iii) clarify the effect of LAI and soil background on LCar assessment with the CARI using an extensive synthetic dataset obtained from 4SAIL and measured data at the canopy scale.

2. Material and Methods

2.1 Study site

The study site was located at the National Experimental Station for Precision Agriculture (40°10.6' N, 116°26.3' E), Beijing, China. The field site has a warm temperate climate, with a mean annual rainfall of 507.7 mm, a mean annual temperate of 13.8 °C and the soil is classified as silt-clay loam. Winter wheat (*Triticum aestivum* L.), one of the major crops in China, was used in this study. In 2004, twenty-one cultivars of winter wheat were grown in plots of 30 m × 5.4 m size. Fertilization and irrigation were applied according to local standard practice in order to provide non-limiting conditions. The cultivars included rather more erectophile leaf canopy types Lumai 21, Jing 411, P7, Laizhou 3279, Nongda 3291, Xiaoyan 54 and I-93; medium leaf canopy types Zhongmai 16, Jingwang 10, CA16, 95128, 9158, Jingdong 8 and Chaoyou 66; and more open leaf canopy types 9507, Nongda 3214, 6211, 8901, 9428, Linkang 2 and 4P3. During the whole growing season, measurements were conducted on specific growth stages including booting (April 28), head emergence (May 11), pollination (May 28) and milk development (June 08). For each growth period, 11 different cultivars, including 3 erectophile, 4 medium and 4 open leaf canopy types, were used for sampling at both the canopy and leaf levels. A sample size of 44 was collected for both the leaf and canopy level measurements.

2.2 Field measurements

2.2.1 Canopy spectra measurements

On each sampling date, a 1 m × 1 m area of winter wheat was selected for canopy reflectance measurements, which were carried out using an ASD FieldSpec spectrometer (Analytical Spectral Devices, Inc., Boulder, CO, USA) under clear, blue-sky conditions between 10:00 h and 14:00 h (Beijing Local Time). The spectrometer was configured with a spectral range of 350 to 2500 nm and a field of view of 25°, and its spectral resolution was 3 nm for the region 350 to 1000 nm and 10 nm for the region 1000 to 2500 nm. Measurements were obtained from a nadir position at approximately 1.3 m above the ground and taken by averaging 10 scans. Reflectance spectra were derived through calibration (both before and after plant canopy measurements) relative to a 0.4 m × 0.4 m white reference panel.

2.2.2 Leaf spectra measurements

Crop above-ground biomass from the 1 m² quadrat where canopy spectral measurements had been made, were collected immediately following canopy spectral measurements, kept in a portable refrigerator, and then transferred to a laboratory for leaf reflectance measurement and biochemical analysis. Leaf spectra were obtained using the ASD spectrometer coupled with a Li-Cor 1800-12 integration sphere (Li-Cor, Inc., Lincoln, NE, USA). For each leaf sample, measurements were made on five different areas (avoiding leaf veins) to quantify the small but not negligible within leaf variability. The sample was illuminated by a focused beam, and the radiation that was captured by the spectrometer was the average reflected radiation within the Li-Cor 1800-12 integration sphere (Huang et al., 2014).

2.2.3 Plant measurement

Laboratory analyses were made on the 1 m² quadrat wheat samples just after leaf spectral measurement. Leaf carotenoids content, leaf dry mass and leaf area index were measured according to standard procedures. Two leaf disks of 0.25 cm² were collected from the samples. One was used for the determination of dry weight, which was measured after drying the samples in an oven at 70 °C for 48 hours, while the other was grinded in 10 ml 80% acetone after the measurement of fresh weight. Then other 15 ml of acetone were added for a total of 25 ml in each tube. Each sample for pigment determination was filtered and placed in a cuvette, and absorbance measured at 470 nm, 646 nm and 663 nm using an L6 ultraviolet-visible spectrophotometer (INESA, China) after having been stored in the dark at 25°C for 48 hours. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids (Car) concentrations were calculated using the extinction coefficient derived by Gao (2006) and absorbance measured at 470 nm, 646 nm and 663 nm with Eqs. (1) to (3):

$$\text{Chl } a \text{ (mg/L)} = 12.21 \times A_{663} - 2.81 \times A_{646} \quad (1)$$

$$\text{Chl } b \text{ (mg/L)} = 20.13 \times A_{646} - 5.03 \times A_{663} \quad (2)$$

$$\text{Car (mg/L)} = (1000 \times A_{470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b) / 229 \quad (3)$$

where A_x is the absorbance of the extract solution at wavelength x . Then, the unit of total carotenoids could be converted into content unit, i.e. mass per unit leaf dry weight (mg/g), and concentration unit, i.e. mass per unit leaf area ($\mu\text{g}/\text{cm}^2$), using data on the volume of leaf pigment extract, the leaf dry weight and the leaf disc area, with Eqs. (4) and (5):

$$\text{Car (mg/g)} = [\text{Car (mg/L)} \times V_T (\text{ml})] / [\text{DW (g)} \times 1000] \quad (4)$$

$$\text{Car } (\mu\text{g}/\text{cm}^2) = [\text{Car (mg/g)} \times \text{DW (g)} \times 1000] / \text{leaf area (cm}^2) \quad (5)$$

where V_T (ml) is the volume of leaf pigment extract solution and DW (g) is the leaf dry weight.

LAI was determined using a dry weight method (Wang et al., 2005). Leaf segments of approximate area 0.06 m^2 were cut from the central part of about 30 leaves selected from all the green leaves in the 1 m^2 quadrat as standard leaves for LAI calculation. Both the standard leaves and the remaining leaves were oven dried at 70°C to constant weight and weighed. LAI was calculated as Eq. (6):

$$\text{LAI} = (S_r \times W_t) / (S_l \times W_r) \quad (6)$$

where S_r (m^2) is the area of the standard leaves, W_t (g) is the total dry weight of the 1 m^2 quadrat sampled leaves, S_l is the sampled land area (m^2) and W_r (g) is the dry weight of the standard leaves.

2.3 ANGERS dataset

Apart from the field survey data, the ANGERS dataset (download from <http://opticleaf.ipgp.fr/>) was also used in present study. The dataset was collected in 2003 on temperate plants at the National Institute for Agricultural Research (INRA), Angers, France. It contains leaf directional-hemispherical reflectance and transmittance spectra measured at 1 nm resolution from 400 nm to 2400 nm using an ASD FieldSpec instruments equipped with integrating spheres. Chlorophyll a and b (Chl), total carotenoids (Car), water (Cw, also named equivalent water thickness) and dry matter (Cm, also named leaf mass per area) content are available for each sample. The pigments were extracted in ethanol 95% in a test tube using fresh material, and Chlorophyll a , b and total carotenoids content were determined using a multi-wavelength analysis at 470 , 648.6 and 664.2 nm . For a detailed description of the dataset, readers can be referred to Féret et al. (2008).

2.4 Simulated datasets

PROSPECT-5 (Féret et al., 2008), simulates leaf directional-hemispherical reflectance and transmittance from 400 to 2500 nm with six input variables: leaf chlorophylls content (LChl), leaf carotenoids content (LCar), leaf structure parameter (N), leaf mass per area (LMA), equivalent water thickness (EWT) and brown pigments (Cbrown). The specific absorption coefficient of these input variables in the $400 - 2500 \text{ nm}$ range, used in PROSPECT-5, are shown in Fig. 1. Pigments absorb light in the visible range ($400 - 760 \text{ nm}$), whereas water has a high absorbance in the near-infrared band ($1000 - 2500 \text{ nm}$). Dry matter and refractive index variations extend through the whole wave range ($400 - 2500 \text{ nm}$). Since the aim of the present study was to estimate leaf

carotenoids content mainly from visible wavebands, and the visible range was unaffected by EWT, the EWT value was kept fixed at the average EWT value of ANGERS dataset. Instead, the range of variation of LChl, LCar, N, and LMA obtained from ANGERS dataset was used in PROSPECT-5 simulations. Detailed values for the input parameters used in PROSPECT-5 simulations are shown in Table 2.

Using PROSPECT-5 Matlab version (<http://teledetection.ipgp.jussieu.fr/prosail/>), 2500 leaf reflectance simulations were obtained with Matlab 7.8 software (The Math Works, Inc., Natick, MA, USA) by random combination of parameters values extracted from those reported in Table 2. However, some of these combinations were meaningless in terms of realistic leaf properties. To avoid such unrealistic combinations, we made use of carotenoids/chlorophylls (Car/Chl) ratio to restrain the combinations. The Car/Chl ratio value in a healthy plant remains nearly constant, changing with phenology or when the plant is stressed. Statistics of Car/Chl ratio in ANGERS show that ratio values ranging from 0.1 to 0.6 account for 97 percent of the samples. The ratio range from 0.1 to 0.6 was then used to eliminate invalid combinations and finally 1700 leaf reflectance were kept. With leaf reflectance and transmittance simulated from PROSPECT-5, canopy reflectance could be simulated by 4SAIL (Verhoef et al., 2007) with a series of input parameters. To investigate the effect of LAI and soil background on LCar assessment, LAI values were set to change from 1 to 8 in a step of 1; the soil moisture parameter values were set to vary from 0 to 1 in a step of 0.5. Other input variables were fixed and defined based on Yu et al. (2014). Input values used for 4SAIL are shown in Table 3. Based on the 1700 leaf simulations in PROSPECT-5 and parameters in Table 3, 40800 canopy reflectance were then obtained using PROSAIL Matlab version (<http://teledetection.ipgp.jussieu.fr/prosail/>).

2.5 Spectral indices

Table 1 summarized the spectral indices that were used in the present study for LCar retrieval. These established spectral indices were investigated for LCar assessment with both simulated and measured datasets. In addition to the existing spectral indices, we attempted to develop a new spectral index for LCar estimation based on the spectral absorption features of Car and Chl observed with the leaf level simulated dataset. The correlation between Car and Chl with reflectance ranging from 400 to 800 nm was first investigated. Fig. 2a shows that the correlation peak region is located in the range 500 to 540 nm for Car, and band 521 nm showed the maximum correlation, suggesting that reflectance in this range is very sensitive to Car content (Gitelson et al., 2002). Additionally, correlation between Car and leaf reflectance is also apparent from 550 to 760 nm. Nevertheless, the specific absorption coefficient of $Cx+c$ in Fig. 1 suggests that it only absorbs light from 400 to 550 nm. The reasons for this could be attributable to the intercorrelation between Car and Chl, since a low correlation between them ($R^2 = 0.235$) was found in foliar simulated data. For Car, the range of its maximum sensitivity overlapped with Chl absorption features (Fig. 2a), which complicates its retrieval using absorption features in this range. For Chl, the correlation extended from 400 to 760 nm and two strong correlation peaks

were observed in green and red edge regions. Previous studies indicated that reflectance in green and red edge areas was sensitive to a wide range of Chl values (Gitelson et al., 2006).

To establish a new spectral index for LCar estimation, Band 521 nm was chosen on the account that it had the highest correlation with LCar ($R^2 = 0.607$, $RMSE = 3.144 \mu\text{g}/\text{cm}^2$, Fig. 2b), although a strong correlation with LChl also existed. Band 720 nm was selected to reduce the influence of Chl on LCar estimation since it showed the highest relationship with LChl ($R^2 = 0.906$, $RMSE = 7.981 \mu\text{g}/\text{cm}^2$, Fig. 2c). The proposed new carotenoid index (CARI, $R_{720}/R_{521} - 1$) was then established, based on the formula of chlorophyll indices (i.e. $CI_{\text{red edge}}$ and CI_{green}). Simulated and measured datasets were then used to investigate its capability and robustness for LCar assessment.

2.6 Statistics analysis

Linear regression models between leaf carotenoids content and spectral indices derived from simulated and measured datasets were obtained using the SPSS 18.0 software (SPSS Inc., Chicago, IL). A k-fold ($k = 6$) cross-validation procedure was used to evaluate the performance of spectral index methods using ANGERS and experimental data, and all the selected spectral indices were tested using the same k-fold partitions. The overall performances of these models were evaluated by statistics including a coefficient of determination (R^2), root mean square error (RMSE), relative RMSE (RRMSE) and mean absolute error (MAE). Details about these statistics can be found in Richter et al. (2012).

3. Results

3.1 Simulation results at the leaf scale

The results for LCar assessment with the newly proposed CARI using leaf level PROSPECT-5 simulations are shown in Fig. 2d. CARI exhibited a significant linear trend with LCar with a R^2 value of 0.943 and a $RMSE$ of $1.196 \mu\text{g}/\text{cm}^2$, suggesting its capability to assess LCar with leaf level simulations. In comparison, the relationships between existing carotenoid indices and LCar are presented in Fig. 3. The performance of these spectral indices varied markedly. The carotenoid indices (CRI_{550} , CRI_{700} , $CAR_{\text{red edge}}$ and CAR_{green}) proposed by Gitelson et al. (2002) and Gitelson et al. (2006) show the highest correlation with LCar ($R^2 > 0.77$, $RMSE < 2.40 \mu\text{g}/\text{cm}^2$) among these indices. However, at high values of LCar, the relationship of these indices with LCar was somehow divergent. This indicates that these indices might be less sensitive at high LCar values ($> 15 \mu\text{g}/\text{cm}^2$). Compared with CRI_{550} and CRI_{700} , the addition of a NIR band (770 nm) in CAR_{green} and $CAR_{\text{red edge}}$ did not improve the estimation accuracy of LCar. RARSc exhibited a general relation with LCar ($R^2 = 0.603$, $RMSE = 3.160 \mu\text{g}/\text{cm}^2$), however, evident nonlinear variation existed, so that RARSc was better correlated with low to medium LCar values (5 to $10 \mu\text{g}/\text{cm}^2$) (Fig. 3e). For RBRI, its relationship with LCar was rather poor ($R^2 = 0.165$, $RMSE = 4.584 \mu\text{g}/\text{cm}^2$). The scatterplot of RBRI versus LCar exhibited large dispersion. PSSRc and PSNDc showed low correlation with LCar. Compared with PSNDc, PSSRc showed slightly better results ($R^2 = 0.387$, $RMSE = 3.928 \mu\text{g}/\text{cm}^2$). Nevertheless, an obvious nonlinear response was shown by these two

indices when LCar exceeded 10 $\mu\text{g}/\text{cm}^2$ (Fig. 3g and 3h). The behavior of PRI in correlating with LCar was very poor ($R^2 = 0.120$, $\text{RMSE} = 4.705 \mu\text{g}/\text{cm}^2$) with a very large scatter of data points (Fig. 3i). As for its modified version PRI_{m1} , it showed almost no correlation with LCar, indicating that it might be unable to assess LCar. PSRI exhibited a low correlation with LCar ($R^2 = 0.191$, $\text{RMSE} = 4.511 \mu\text{g}/\text{cm}^2$) and the scatterplot of PSRI versus LCar showed an apparent nonlinear trend. Different from all these indices, SR_{car} behaved with a small negative relationship ($R^2 = 0.142$, $\text{RMSE} = 4.645 \mu\text{g}/\text{cm}^2$), its scatterplot versus LCar exhibited great dispersion.

3.2 LCar assessment with spectral indices using ANGERS dataset

The ANGERS dataset, which contains samples with a wide range of LCar, collected from various plant species, was at first used to evaluate the capability of the newly proposed CARI and of published spectral indices for LCar assessment. The cross-validation results are reported in Table 4. Compared with CRI_{550} and CRI_{700} , estimation accuracy in LCar for $\text{CAR}_{\text{green}}$ and $\text{CAR}_{\text{red edge}}$ improved slightly. Nevertheless, in contrast to their good behavior with simulated leaf data, CRI_{550} , CRI_{700} , $\text{CAR}_{\text{green}}$ and $\text{CAR}_{\text{red edge}}$ all showed poor accuracy in LCar assessment with ANGERS data. RARSc had a good accuracy for LCar retrieval with a R^2 value of 0.438 and a RMSE of 3.792 $\mu\text{g}/\text{cm}^2$. Although it had a poor correlation with simulated data (Fig. 3f), RBRI showed the best estimation performance ($R^2 = 0.727$, $\text{RMSE} = 2.640 \mu\text{g}/\text{cm}^2$) of LCar with ANGERS data. Compared with PSNDc , PSSRc exhibited a slightly better performance in ANGERS data. This was in agreement with their behavior in simulated data. PRI showed low accuracy ($R^2 = 0.199$, $\text{RMSE} = 4.527 \mu\text{g}/\text{cm}^2$) for LCar retrieval with ANGERS data. Similarly, the modified PRI_{m1} exhibited rather poor results for LCar estimation. PSRI showed the worst prediction of LCar mainly on the account of its insensitivity to LCar. The performance of SR_{car} was somehow better ($R^2 = 0.213$, $\text{RMSE} = 4.489 \mu\text{g}/\text{cm}^2$) and ranked fifth among all the indices. The newly proposed CARI showed a quite good accuracy ($R^2 = 0.545$, $\text{RMSE} = 3.413 \mu\text{g}/\text{cm}^2$) in LCar assessment with ANGERS dataset, being second only to RBRI , suggesting that CARI was accurate and robust when used for LCar estimation with ANGERS data.

Scatterplots of measured LCar versus predicted LCar of the best performing four indices in Table 4, for ANGERS dataset, are shown in Fig. 4. RBRI gave the best prediction of LCar. The fitted line of its scatterplot of measured LCar versus predicted LCar was more close to the 1:1 line (slope = 0.730) than for all the other indices. Moreover, RBRI seemed more sensitive to high LCar ($> 15 \mu\text{g}/\text{cm}^2$) compared with other indices. CARI showed good estimation of LCar, and most of the data points were uniformly distributed around the 1:1 line except for samples that had higher LCar than 15 $\mu\text{g}/\text{cm}^2$. Compared with RBRI , CARI showed more sensitivity and accuracy for low LCar ($< 3 \mu\text{g}/\text{cm}^2$) estimation, nevertheless, it was insensitive to LCar changes when LCar exceeded 15 $\mu\text{g}/\text{cm}^2$. RARSc and PSSRc indices also showed satisfactory estimation. However, similar to CARI, these indices suffered from insensitivity to high LCar ($> 15 \mu\text{g}/\text{cm}^2$).

3.3 LCar retrieval with leaf level experimental data

Leaf level measured data on winter wheat were used to further assess the ability of the

different indices for LCar estimation. The cross-validation results are shown in Table 5. CRI_{550} and CRI_{700} showed the worst prediction of LCar. In contrast, CAR_{green} and $CAR_{red\ edge}$ provided slightly better results. RARSc index showed a good accuracy for LCar retrieval ($R^2 = 0.674$, $RMSE = 1.443\ \mu g/cm^2$), in agreement with its performance with ANGERS dataset. Different from their excellent behavior with ANGERS data, the performance of RBRI with wheat leaves data was rather poor ($R^2 = 0.222$, $RMSE = 2.234\ \mu g/cm^2$). PSNDc and PSSRc showed good estimation of LCar with a R^2 value larger than 0.57 and a RMSE value less than $1.65\ \mu g/cm^2$. In contrast to its poor performance with simulated and ANGERS data, PRI showed the best prediction of LCar ($R^2 = 0.710$, $RMSE = 1.369\ \mu g/cm^2$) with wheat leaves experimental data. PRI_{m1} showed a rather low accuracy of prediction, in agreement with its performance with simulated and ANGERS data. Compared to their results with ANGERS data, the behavior of PSRI and SR_{car} improved with wheat data. CARI also showed an excellent prediction of LCar for winter wheat data ($R^2 = 0.639$, $RMSE = 1.520\ \mu g/cm^2$), similar to its performance with ANGERS data. This would confirm that CARI is robust and quite accurate for LCar estimation with measured data of a single vegetation species as well.

Scatterplots of measured LCar versus predicated LCar were then inspected to assess the response of carotenoid indices to the experimental data, and results of the best four indices in Table 5 are shown in Fig. 5. PRI showed the best accuracy in the prediction of LCar. Points of predicted versus measured LCar mainly converge around the 1:1 line and the slope of the fitted line is 0.761. Also, RARSc, CARI and PSNDc also provided a good estimation of LCar. Differently from the ANGERS data (Fig. 4), the LCar of winter wheat was in the low to medium range, spanning from 3.05 to $12.59\ \mu g/cm^2$. It can be observed that most of the points clustered around a LCar value of $10\ \mu g/cm^2$. This was mainly because most of the samples that were collected at the booting, head emergence and pollination stages had little LCar variation.

3.4 Assessing CARI for LCar retrieval with canopy spectra

Simulated canopy reflectance from 4SAIL and measured canopy data, using a parameterization that corresponded to the leaf level measured data, were used to investigate the effect of LAI and soil background on LCar assessment with the newly proposed CARI. Results from simulations (Fig. 6a) revealed that the overall relationship between CARI and LCar at a wide range of LAI values was quite good ($R^2 = 0.675$, $RMSE = 2.862\ \mu g/cm^2$), but worse than that what was found using simulated leaf data ($R^2 = 0.943$, $RMSE = 1.196\ \mu g/cm^2$, Fig. 2d). Relationships between CARI and LCar for different LAI values (Fig. 6a) showed that the correlation was the lowest ($R^2 = 0.455$, $RMSE = 3.705\ \mu g/cm^2$) at the minimum LAI value (i.e. $LAI = 1$), indicating that CARI is less sensitive to LCar when the LAI is small. Indeed, when LAI is around 1, most of the information conveyed by canopy reflectance relates to the soil background. This might hinder LCar estimation. The effect of the variation of the soil moisture parameter (P_{soil}) on LCar estimation, at a LAI value of 1, was further investigated using canopy simulations. Results in Fig. 7 show that increasing P_{soil} values negatively affected the relationship between CARI and LCar: the correlation between CARI and

LCar was the lowest ($R^2 = 0.614$, $RMSE = 3.116 \mu\text{g}/\text{cm}^2$) when a dry soil background condition was simulated ($P_{\text{soil}} = 1$). In contrast, the relationship was good ($R^2 = 0.922$, $RMSE = 1.398 \mu\text{g}/\text{cm}^2$) when a wet soil condition was simulated ($P_{\text{soil}} = 0$). However, in general, R^2 between CARI and LCar increased with increasing LAI values (Fig. 6a), until LAI exceeded 4, when R^2 reached a plateau at around 0.89. Moreover, the fitted line for scatter points of CARI versus LCar hardly varied when the LAI values were larger than 4. This indicates that CARI might be insensitive to LCar changes when LAI exceeds 4.

The cross-validation results of CARI in assessing LCar with measured canopy spectra are shown in Fig. 6b. Compared with its performance with leaf level data (Fig. 5), CARI showed low accuracy ($R^2 = 0.366$, $RMSE = 2.020 \mu\text{g}/\text{cm}^2$) for LCar prediction with canopy reflectance, and samples with LCar values lower than $5 \mu\text{g}/\text{cm}^2$ were obviously overestimated (Fig. 6b). However, it should be noted that these low LCar samples were collected at the wheat kernel milk stage, when leaves were close to senescence, and LAI values were less than 1. The inaccurate estimation of these low LCar samples would confirm to use caution in the assessment of LCar from CARI, using canopy reflectance, when LAI values are low.

4. Discussion

The absorption features of Car in the visible range offer valuable opportunities for non-destructive detection of Car content with spectroscopic analysis. Nevertheless, the overlap between Car and Chl absorption peaks in the visible region challenges the retrieval of Car content (Hernández-Clemente et al., 2012). The present study took advantage of extensive simulated data derived from PROSPECT-5 and 4SAIL models and measured data from the ANGERS and field experiment obtained at both the leaf and canopy level to establish a new carotenoid index (CARI), and comprehensively evaluate its capability for LCar estimation. CARI was derived by examining the chlorophyll indices (i.e. $CI_{\text{red edge}}$ and CI_{green}). These chlorophyll indices make use of a red edge (or green) band that is sensitive to Chl, and a NIR band to remove the influence of other pigments and decrease the backscattering effect (Gitelson et al., 2005). They have proved to be robust and accurate indicators of Chl content in many previous studies (Clevers and Gitelson, 2013; Clevers and Kooistra, 2012; Schlemmer et al., 2013). After investigation of the correlation between Car and reflectance at wavelengths ranging from 400 to 800 nm, using leaf level simulations, a waveband at 521 nm was selected for the CARI. This band is located in the spectral absorption region of Car and it was found to be highly correlated to LCar. But a strong correlation between band 521 nm and LChl also existed. Therefore, to reduce the influence of Chl on LCar estimation, band 720 nm was also added in CARI, since it exhibited the highest correlation with LChl. With PROSPECT-5 simulated data, the proposed CARI showed the best linear relationship (ranking 1st) with LCar among all the tested indices. Moreover, it exhibited a rather low correlation with LChl with a R^2 value of 0.315, suggesting that it was rather insensitive to LChl variations. The use of a band at 720 nm could apparently relieve the effect of LChl variation in LCar retrieval with CARI. LCar assessment with measured leaf level data showed that CARI achieved quite good LCar

estimation performances, both with the ANGERS data (ranking 2nd, Table 4) and the winter wheat leaf data (ranking 3rd in Table 5), indicating that it was consistently accurate and robust for LCar retrieval with measured data regardless of plant species and status.

The performance of published spectral indices for LCar assessment with PROSPECT-5 simulations, the ANGERS data and the winter wheat leaf data varied. The carotenoid indices (i.e. CRI₅₅₀, CRI₇₀₀, CAR_{red edge} and CAR_{green}) showed good results for leaf scale simulations. Nevertheless, these indices all exhibited rather low accuracy in LCar assessment when tested with ANGERS data and field experimental data. Their different performance in simulated and measured data suggests that the robustness of these indices in LCar estimation with measured data needs to be improved. Compared with CRI₅₅₀ and CRI₇₀₀, CAR_{green} and CAR_{red edge} showed slightly better results using ANGERS dataset and field survey data, indicating that adding a NIR band (i.e. 770 nm) in CRI₅₅₀ and CRI₇₀₀ could improve the estimation accuracy of LCar in measured datasets (Gitelson et al., 2006). RARSc also showed a consistently good estimation of LCar with ANGERS data (ranking 3rd) and wheat data (ranking 2nd) at the leaf level. Its results with these measured data agree with previous studies that used the RARSc for LCar assessment (Fassnacht et al., 2014; Yi et al., 2014), suggesting that RARSc is quite robust for LCar estimation. The performance of RBRI with simulated and measured data varied markedly: it exhibited a low relationship with LCar with leaf simulations, showed the best estimation accuracy for LCar with ANGERS data and performed poorly with wheat field data. RBRI was devised for Chl and Car content retrieval and it was developed based on Chl absorption features by Datt (1998). In his research, Chl was significantly correlated with Car. However, in our foliar simulated data, the correlation between LChl and LCar was rather low ($R^2 = 0.235$), even though LChl was remarkably related with RBRI ($R^2 = 0.847$). This could explain the poor correlation between RBRI and LCar. As for ANGERS data, a significant relationship existed between LCar and LChl with a R^2 value of 0.908, moreover, RBRI was highly correlated with LChl with a R^2 value of 0.785. This could result in its best prediction of LCar in ANGERS data. The RBRI was constructed on the equation $R_{672}/(R_{550} \times R_{708})$, which was different from the normalization and ratio form that most indices adopted. The $R_{550} \times R_{708}$ in the denominator of RBRI might enhance the numerical range of RBRI, thus making it sensitive to high LCar. However, RBRI might be lacking sensitivity to low LCar values ($< 3 \mu\text{g}/\text{cm}^2$), since it showed an overestimation when LCar was less than $3 \mu\text{g}/\text{cm}^2$ (Fig. 4). With wheat leaf experimental data, RBRI showed poor estimation of LCar despite the fact that LChl and LCar were well correlated in this dataset, with a R^2 value of 0.888. This suggests that RBRI might be unstable for LCar retrieval when used in different datasets. Blackburn (1998) pointed out that the convolution of Chl and Car absorption features might disturb the relationship between Car and PSNDc (or PSSRc). Moreover, it was shown that 470 nm, used in these indices, is not the best absorption band for Car retrieval. The results of PSNDc and PSSRc in our study support these views, since PSSRc and PSNDc showed low estimation accuracy for LCar with both simulated data and ANGERS data. Nevertheless, PSNDc and PSSRc showed good estimation of LCar with wheat leaf data. Differently from ANGERS data, the range of LCar values in the wheat experiment

dataset, was restricted, from 4 to 12 $\mu\text{g}/\text{cm}^2$. PSNDc and PSSRc could be more sensitive to LCar in this range. PRI is a versatile index and has been successfully used for various aims (Filella et al., 2009; Zarco-Tejada et al., 2013). In our present study, its behavior in correlating with LCar using both leaf level simulations and ANGERS data was very poor. The 531 nm wavelength in PRI was originally selected to optimize the detection of changes in the de-epoxidation state of the xanthophyll cycle (Gamon et al., 1992). The poor correlation between PRI and LCar might have been overly influenced by the activity of this single class of carotenoids (Garritty et al., 2011). In contrasting to the poor performance in simulated and ANGERS data, PRI showed the most accurate prediction of LCar with wheat leaf data. A previous study demonstrated that PRI exhibited good results for Car estimation in cotton (Yi et al., 2014). This might suggest that PRI could be used for LCar assessment in a single species. Compared to PRI, PRI_{m1} did not show any improvement in LCar estimation in all the datasets. The reason could be that the PRI_{m1} was modified to reduce canopy structure effects and used as a water stress indicator instead of an index for LCar (Hernández-Clemente et al., 2011). Its poor behavior in our research indicates that it is not suitable for LCar estimation. Similarly, the PSRI was designed to be used as a quantitative measure of leaf senescence and fruit ripening, and it was found to be sensitive to the Car/Chl ratio by Merzlyak et al. (1999). Its poor performance in both simulated and measured data suggests that it is not appropriate for LCar detection. Our results suggested that SR_{car} exhibited low accuracy for LCar assessment with both simulated and ANGERS data. Its disappointing performance in simulations could be due to the fact that the PROSPECT-5 simulation data used in the present work encompassed a wider range of variation of leaf parameters than those used by Hernández-Clemente et al. (2012). Similarly, the reasons for its low accuracy with ANGERS data, might be linked to the wide range of plant species used. Accurate estimation of LCar with SR_{car} was still achieved when used for winter wheat data, this might imply that SR_{car} could be effectively used for LCar retrieval for a single species.

Although the newly proposed CARI showed consistently accurate and robust estimation of LCar with varied datasets at the leaf level, investigation of LCar assessment with CARI using simulated canopy data with 4SAIL suggested that CARI was insensitive to LCar variations when the plant canopy had low LAI values (i.e. LAI = 1). Soil moisture influenced LCar estimation accuracy when LAI values were low. When LAI is low and soil background is in a dry condition, canopy reflectance is mainly dominated by soil reflection, this could weaken plant canopy information, thus reducing LCar estimation accuracy (Fig. 7c). When soil is wet, the overall soil reflectance is lower, thus its confounding effect on LCar estimation seems to be reduced (Fig. 7a). Our results with measured datasets thus supported the insensitivity of CARI to LCar detection using canopy reflectance when LAI is low. Further investigations on CARI using canopy reflectance acquired with hyper- or multi-spectral sensors (such as Sentinel-2), are still needed to achieve accurate and robust LCar calibrations, thus providing a promising new tool for assessing information on plant physiological status at the regional scale.

5. Conclusion

In this study, we examined the potential of a new developed carotenoid index (CARI) to estimate Car content at the leaf level, employing a large synthetic dataset simulated from leaf and canopy RTMs (PROSPECT-5 and 4SAIL). Its Car estimation capability was assessed and compared with published spectral indices using both simulated and measured datasets including PROSPECT-5 simulations, ANGERS dataset and field survey data obtained in 2004. The leaf level simulation analysis showed that correlation with LCar varied among different spectral indices. CARI exhibited the best linear relationship ($R^2 = 0.943$, $RMSE = 1.196 \mu\text{g}/\text{cm}^2$) with LCar among the investigated spectral indices, and it showed strong sensitivity to foliar carotenoids content variation. Assessment of spectral indices for LCar retrieval, using ANGERS dataset, demonstrated that CARI was accurate and robust for LCar retrieval in measured data that contained different species and plants status. CARI also exhibited accurate results for LCar retrieval with wheat leaf field data, which further supported the robustness and capacity of CARI for LCar assessment. Evaluation of CARI in LCar estimation with simulated and measured canopy reflectance indicated that LAI variation affected the performance of the index, when LAI value was low, soil moisture influenced LCar estimation accuracy, particularly when soil background was in a dry condition. However, on the basis of all tests carried out in this work with simulated and measured data, it can be concluded that CARI can be considered as an optimal index for LCar estimation.

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Lists of tables and figures

Table 1 spectral indices investigated in this study

Spectral index	Equation	Reference
Ratio analysis of reflectance spectra (RARSc)	R_{760}/R_{500}	(Chappelle et al., 1992)
Pigment specific simple ratio (PSSRc)	R_{800}/R_{470}	(Blackburn, 1998)
Pigment specific normalized difference (PSNDc)	$(R_{800} - R_{470}) / (R_{800} + R_{470})$	(Blackburn, 1998)
Reflectance band ratio index (RBRI)	$R_{672} / (R_{550} \times R_{708})$	(Datt, 1998)
Plant senescence reflectance index (PSRI)	$(R_{678} - R_{500}) / R_{750}$	(Merzlyak et al., 1999)
Carotenoid reflectance index (CRI ₅₅₀)	$(R_{510})^{-1} - (R_{550})^{-1}$	(Gitelson et al., 2002)
Carotenoid reflectance index (CRI ₇₀₀)	$(R_{510})^{-1} - (R_{700})^{-1}$	(Gitelson et al., 2002)
Red edge carotenoid index (CAR _{red edge})	$[(R_{510})^{-1} - (R_{700})^{-1}] \times R_{770}$	(Gitelson et al., 2006)
Green carotenoid index (CAR _{green})	$[(R_{510})^{-1} - (R_{550})^{-1}] \times R_{770}$	(Gitelson et al., 2006)
Photochemical reflectance index (PRI)	$(R_{570} - R_{531}) / (R_{570} + R_{531})$	(Gamon et al., 1992)
Modified photochemical reflectance index (PRI _{m1})	$(R_{512} - R_{531}) / (R_{512} + R_{531})$	(Hernández-Clemente et al., 2011)
Simple ratio (SR _{car})	R_{515}/R_{570}	(Hernández-Clemente et al., 2012)
Carotenoid index (CARI)	$R_{720}/R_{521} - 1$	This study

R_{λ} is the reflectance value at wavelength λ .

Table 2 Input parameters for PROSPECT-5 model used for leaf reflectance modeling.

Parameters	Values
Leaf chlorophyll content (LChl, $\mu\text{g}/\text{cm}^2$)	10 / 20 / 30 / 40 / 50 / 60 / 70 / 80 / 90 / 100
Leaf carotenoid content (LCar, $\mu\text{g}/\text{cm}^2$)	2 / 4 / 6 / 8 / 10 / 12 / 14 / 16 / 18 / 20

Leaf structure parameter (N)	1.6 / 1.7 / 1.8 / 1.9 / 2.0
Leaf mass per area (LMA, g/cm ²)	0.002 / 0.003 / 0.004 / 0.005 / 0.006
Equivalent water thickness (EWT, cm)	0.012
Brown pigments (<i>C_{brown}</i>)	0

Table 3 Input parameters for 4SAIL model used for canopy reflectance modeling.

Parameters	Values
Leaf area index (LAI)	1 / 2 / 3 / 4 / 5 / 6 / 7 / 8
Leaf angle distribution (LAD)	Spherical
Soil moisture parameter (P_{soil})	0 / 0.5 / 1
Solar zenith angle (SZA, °)	30
View zenith angle (VZA, °)	0
View azimuth angle (VAA, °)	0
Fraction of diffuse incident radiation	0.23
Hot spot effect	0.15

Table 4 Cross-validation results for LCar assessment using ANGERS data (n = 276)

Index	Rank	R ²	RMSE (μg/cm ²)	MAE (μg/cm ²)	RRMSE (%)
CRI ₅₅₀	10	0.139	4.693	3.363	54.179
CRI ₇₀₀	11	0.138	4.696	3.413	54.217
CAR _{green}	8	0.184	4.568	3.199	52.732
CAR _{red edge}	7	0.190	4.550	3.232	52.524
RARSc	3	0.438	3.792	2.757	43.781
RBRI	1	0.727	2.640	1.808	30.475
PSND _c	9	0.167	4.617	3.472	53.303
PSSRc	4	0.310	4.201	3.142	48.499
PRI	6	0.199	4.527	3.295	52.267
PRI _{m1}	12	0.075	4.869	3.505	56.215
PSRI	13	0.002	5.057	3.796	58.377
SR _{car}	5	0.213	4.489	3.117	51.820
CARI	2	0.545	3.413	2.345	39.400

Table 5 Cross-validation results for LCar estimation with wheat leaf level field data (n = 44)

Index	Rank	R ²	RMSE (μg/cm ²)	MAE (μg/cm ²)	RRMSE (%)
CRI ₅₅₀	12	0.124	2.395	1.998	28.531
CRI ₇₀₀	13	0.046	2.533	2.121	30.171
CAR _{green}	7	0.411	1.941	1.637	23.122
CAR _{red edge}	9	0.344	2.050	1.739	24.417
RARSc	2	0.674	1.443	1.130	17.192
RBRI	10	0.222	2.234	1.777	26.614
PSNDc	4	0.618	1.563	1.239	18.623
PSSRc	6	0.579	1.641	1.299	19.544
PRI	1	0.710	1.369	1.092	16.305
PRI _{m1}	11	0.125	2.373	1.814	28.268
PSRI	8	0.388	2.063	1.539	24.570
SR _{car}	5	0.614	1.571	1.144	18.713

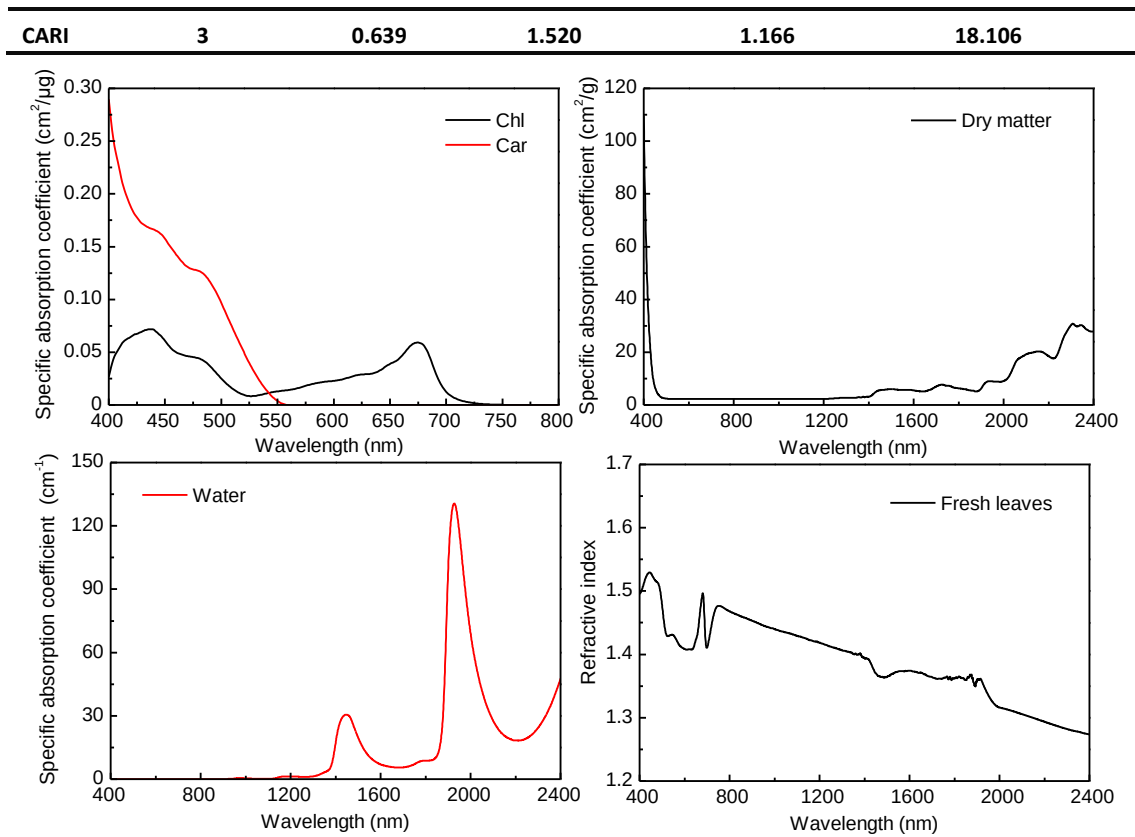


Fig. 1. Specific absorption coefficient of pigments in vivo, dry matter and water, and refractive index for PROSPECT-5.

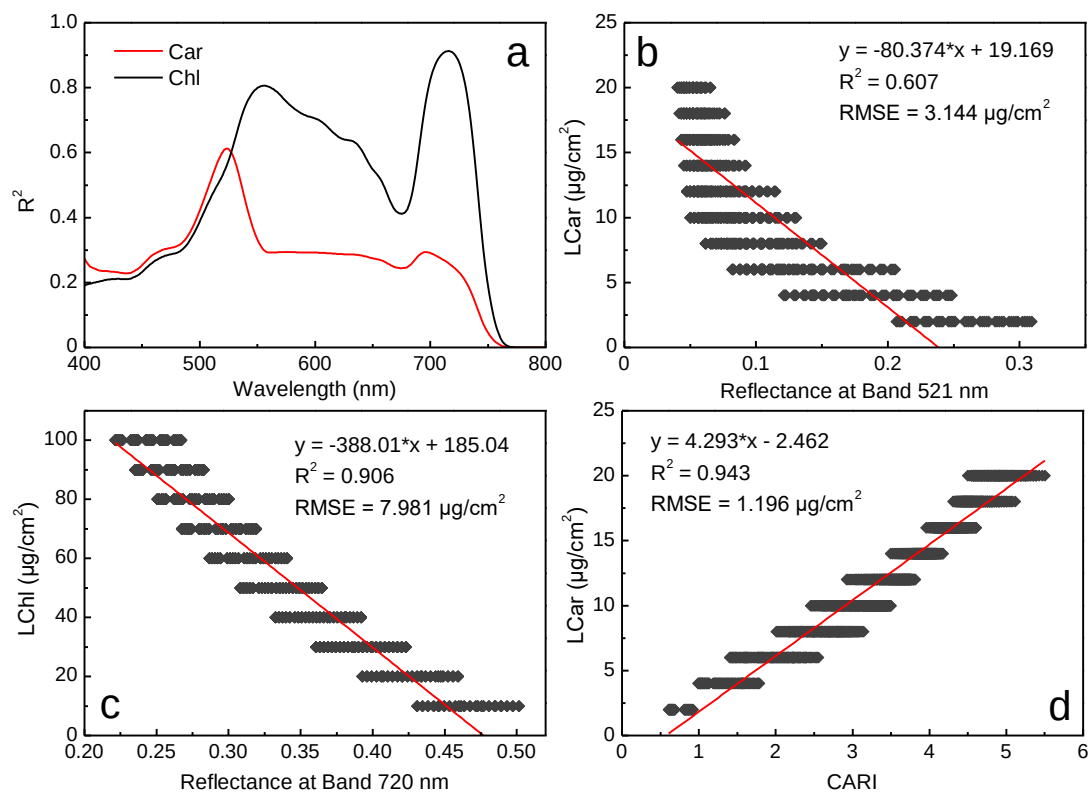


Fig. 2. (a) R^2 curves for LCar (LChl) versus leaf reflectance within the wavelength range from 400 to 800 nm. (b) Correlation between band 521 nm and LCar. (c) Correlation between band 720 nm

and LChl, and (d) linear relationship between carotenoid index (CARI) and LCar.

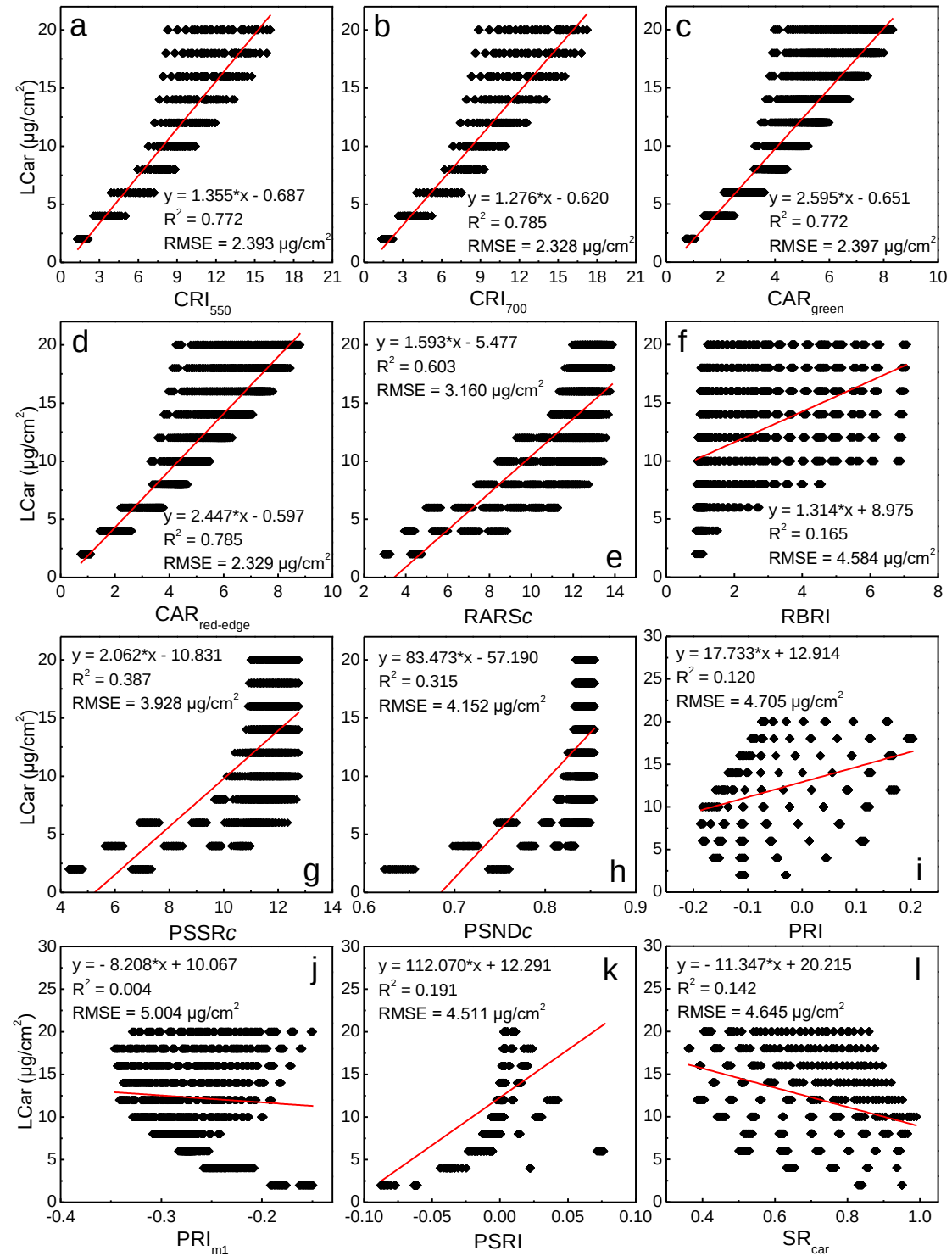


Fig. 3. Relationships between published spectral indices and leaf carotenoids content from leaf level data simulated with PROSPECT-5 (n = 1700).

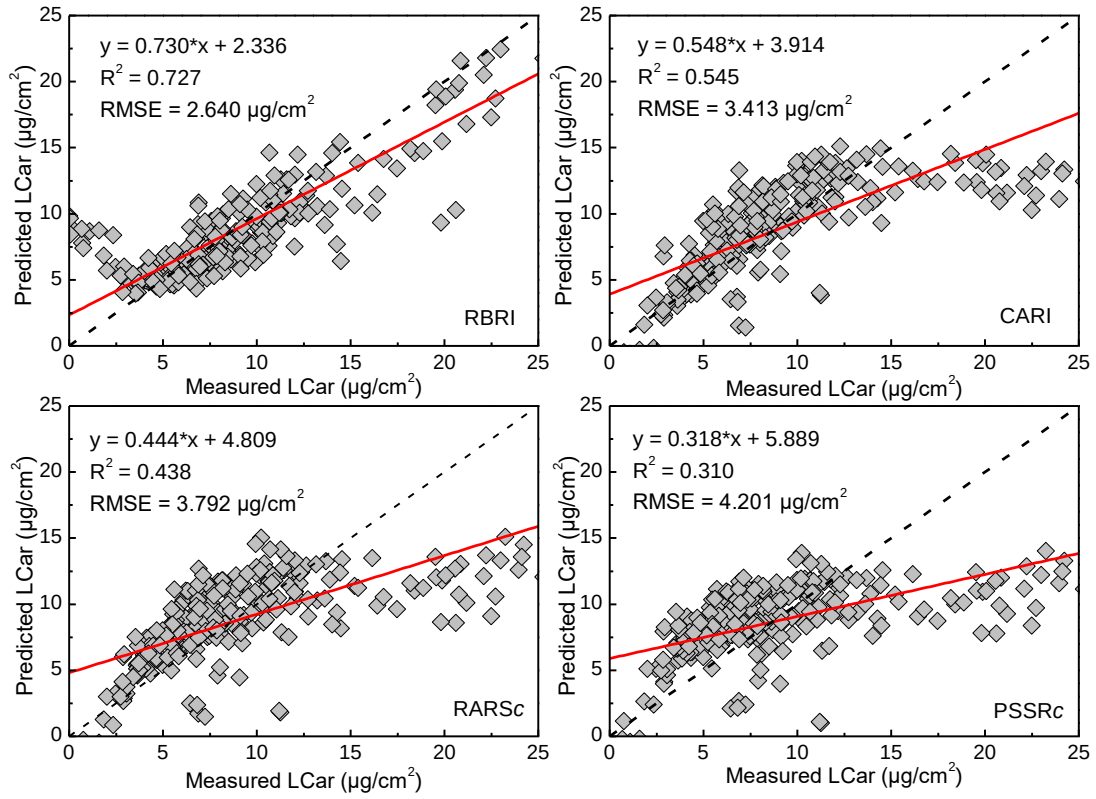


Fig. 4. Scatterplots of measured LCar versus predicted LCar for spectral indices with ANGERS dataset (n = 276). Dashed lines indicate 1:1 lines.

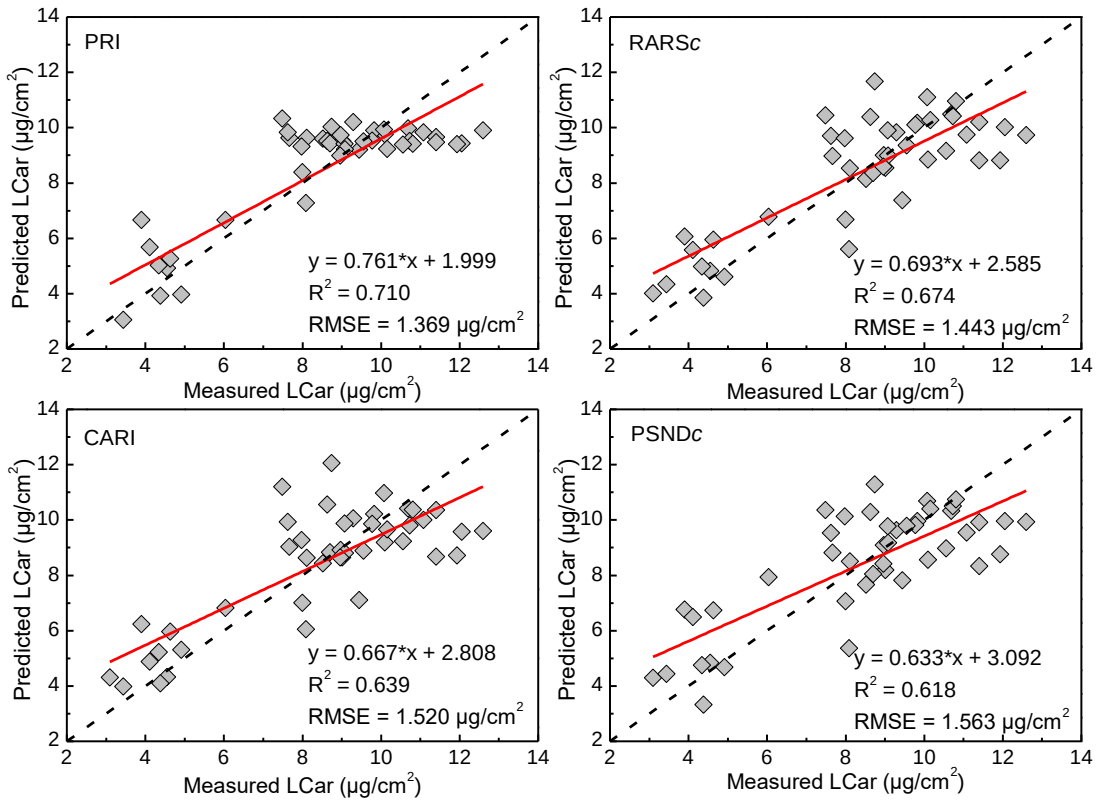


Fig. 5. Scatterplots of measured LCar versus predicted LCar for spectral indices with leaf level experimental data (n = 44). Dashed lines indicate 1:1 lines.

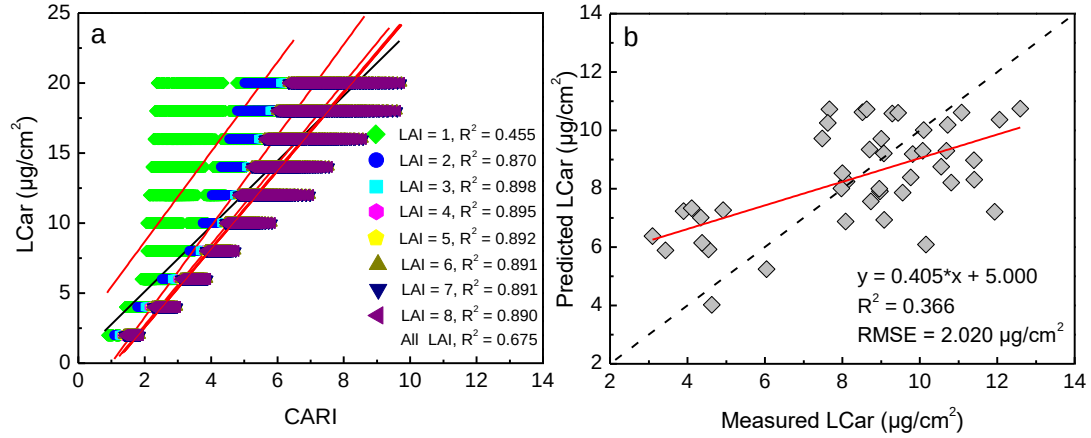


Fig. 6. (a) Correlation between CARI and LCar at different LAI values, from all canopy simulations with 4SAIL model ($n = 40800$). (b) Scatterplots of measured LCar versus predicted LCar for CARI with canopy reflectance obtained from field data ($n = 44$). Dashed lines indicate 1:1 lines.

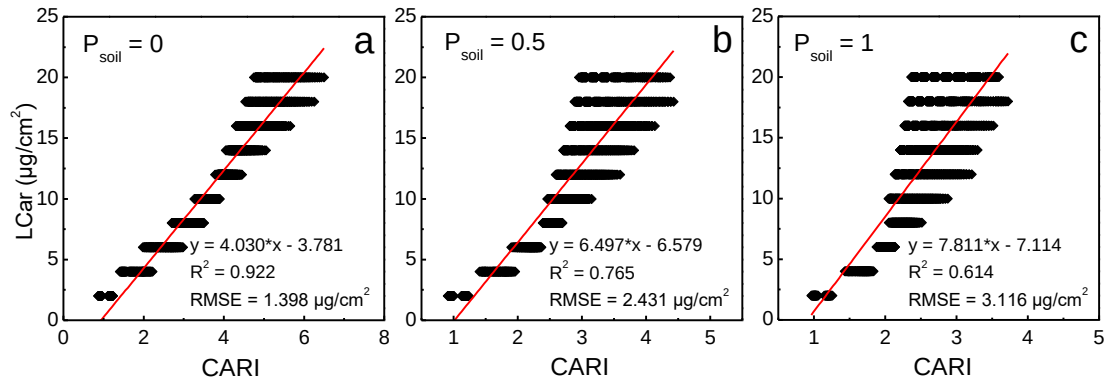


Fig. 7. Relationships between CARI and LCar using canopy reflectance simulations with LAI value fixed to 1 at different soil moisture levels. P_{soil} value set as (a) 0, (b) 0.5 and (c) 1. ALL other parameters for 4SAIL were fixed based on Table. 3 ($n = 1700$).