Chapter 11

Monitoring Crop Carotenoids Concentration by Remote Sensing

Wenjiang Huang, Xianfeng Zhou,
Weiping Kong and Huichun Ye

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Abstract

Assessment of carotenoids (Car) content provides a valuable insight into clarifying the mechanisms of plant photoprotection and light-adaption and is critical for stress diagnoses in plants. Due to their small proportion in the overall total pigment content and to the overlapping of spectral absorption features with chlorophylls (Chl) in the blue region of the spectrum, accurate estimation of Car content in plants, from remotely sensed data, is challenging. Previous studies made progress in Car content estimation at both the leaf and canopy level with remote sensing techniques. However, established spectral indices and methods for Car estimation in most studies that generally rely on specific and limited measured data might lack predictive accuracy for Car estimation and lack sensitivity to low or high Car content in various species and at different growth stages. In this chapter, a new carotenoid index (CARI) was proposed for foliar Car assessment with abundant simulated leaf data and various measured leaf reflectances. Detailed analysis on the mechanism, formation and performance of the new spectral index on Car retrieval was presented. Analysis results suggested that accurate nondestructive estimation of foliar Car content with CARI could be achieved at the leaf scale, through remote sensing techniques.

Keywords: carotenoids, retrieval, hyperspectral, remote sensing, radiative transfer model

1. Introduction

Photosynthetic pigments, mainly including chlorophylls (Chl) and carotenoids (Car), are of tremendous significance in the biosphere. Their photosynthetic function could provide necessities, such as oxygen and organic matters, for plant and mammal survival [1]. Generally, chlorophylls, composed of chlorophyll a (Chl a) and chlorophyll b (Chl b), represent the principal class of pigments responsible for light absorption in photosynthesis [2]. Carotenoids, that include carotenes and xanthophylls, are the second major group of plant pigments [1]. They are part of the essential structures of the photosynthetic antenna and reaction center and help stabilize chlorophyll-protein complexes [3, 4]. 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 [5–10]. Within the plant growth cycle, a normal decrease in Chl indicates that plants are affected by environmental stresses, while the variation of Car reflects the physiological status of vegetation [10]. 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 [5]. 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 plants.

The absorption features of Car in the visible range make it possible for Car content retrieval with remote sensing techniques. Based on its absorption features, researches on Car content estimation at both the leaf and canopy level with spectroscopic analysis have been conducted in recent years [11]. With ratio analysis of reflectance spectra (RARS) method, Chappelle et al. [12] suggested that reflectance at the 500 nm wavelength correlated best with Car content, and the reflectance was less affected by other pigments. Thus, they proposed a ratio analysis of reflectance spectra ((RARSc, R_{760}/R_{500}) for Car assessment. Research conducted by Datt [13] indicated that the maximum sensitivity of reflectance to variation in pigment content was in the green band region at 550 nm and in the red-edge region at 708 nm; a reflectance band ratio index (RBRI) ($R_{672}/(R_{550} \times R_{708})$) was then proposed for pigment content estimation, which had a good correlation with Car content. Based on the reflectance of the Car absorption band at 470 nm, Blackburn [14] put forward two spectral indices with the optimal wavebands 470 and 800 nm, that is, pigment specific simple ratio (PSSRc) and pigment specific normalized difference (PSNDc), for Car estimation at the leaf level. Gitelson et al. [11] found that the first-order derivative reflectance around 510 nm was the most sensitive to Car content. They established two spectral indices, that is, carotenoid reflectance index 550 (CRI_{550}) and carotenoid reflectance index 700 (CRI_{700}), for foliar Car content assessment. Based on a conceptual three-band model, Gitelson et al. [15] further put forward green carotenoid index (CAR_{green}) and red-edge carotenoid index ($CAR_{red\text{-}edge}$) with three bands located at 510–520 nm, 690–710 nm (560–570 nm for CAR_{green}) and a NIR band, for Car retrieval at the leaf level. Research conducted by Hernández-Clemente et al. [16] indicated that vegetation canopy structure severely affected the performance of CRI_{550} for Car content assessment at the canopy level. A simple ratio index (SRcar, R_{515}/R_{570}) was then proposed and it showed good correlation with Car content at both leaf and canopy levels.

Overall, previous studies have indeed made much progress in Car content estimation both at the leaf and canopy level; nevertheless, most of the research focused on establishing spectral indices or models for Car content retrieval, with limited measured datasets. 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 deserve further investigation when applied to a wide variety of plant leaves and conditions. Thus, to develop robust spectral indices or models for Car content retrieval with spectroscopic techniques, the quality of the training dataset, the selection of the optimal wavelengths and the availability of an independent dataset for the validation are critical [17].

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 [18]. In recent years, the RTMs have been used extensively for various applications on the vegetation studies [19]. Based on simulated data at the leaf and canopy level with leaf model PROPSECT [20] and multilayer canopy model Scattering by Arbitrary Inclined Leaves (SAIL) [21], there are researches on leaf biochemical parameters retrieval, such as leaf chlorophylls content (LChl), leaf mass per area (LMA) and leaf carotenoids content (LCar), with spectral indices methods and RTMs inversion [18, 22–24]. However, less attention was given to the application of RTMs in Car content retrieval than to Chl content assessment. For foliar Car content estimation, leaf model PROSPECT could simulate abundant leaf level data through combining plant biochemical parameters, which could be used for the investigation of optical characteristic of Car and other pigments and for quantitative evaluation of estimating results of foliar Car content with different spectral indices as well. In addition, for assessment of leaf Car content with plant canopy spectra, canopy spectra were influenced by more than biochemical parameters, canopy structure, illumination and observation geometry, and soil background properties affected canopy spectrum as well [25]. 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 [26, 27]. Utilization of PROSAIL model (coupled by leaf model PROSPECT and canopy model SAIL) 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 foliar Car content assessment and provide basis for an accurate and robust LCar estimation with spectral index methods.

Therefore, the goal of this chapter is to propose a nondestructive method to assess LCar with remote sensing techniques, through developing 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: (1) establish a new carotenoid index (CARI) for LCar estimation, assess and compare its performance with published carotenoid indices using leaf level simulated data obtained from PROSPECT-5; (2) evaluate the capability and robustness of the new CARI and published carotenoid indices with various leaf level measured data including the widely used ANGERS dataset and field survey data; (3) clarify the effect of LAI and soil background on LCar assessment with the CARI using an extensive synthetic dataset obtained from PROSAIL and measured data at the canopy scale.

2. Materials and methods

2.1. Field experiment

A field experiment was designed and conducted in 2004 to collect measured data at both the leaf and canopy level for foliar Car content assessment. The experiment site was located at the National Experimental Station for Precision Agriculture (40°10.6′ N, 116°26.3′ E), Beijing, China. Winter wheat (*Triticum aestivum* L.) was used in this experiment, and 21 cultivars of winter wheat were grown in plots of 30 × 5.4 m size in the experiment site. Fertilization and irrigation were applied according to local standard practice so as to provide nonlimiting conditions. During the whole growing season, field 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, different cultivars were used for sampling at both the canopy and leaf levels.

2.2. Field measurements

2.2.1. Reflectance spectrum measurements

On each sampling date, a 1 × 1 m area of winter wheat was first selected for canopy reflectance measurements, with an Analytical Spectral Devices (ASD) FieldSpec spectrometer (Analytical Spectral Devices, Inc., Boulder, CO, USA) under clear, blue-sky conditions between 10:00 and 14:00 h (Beijing Local Time). Measurements were obtained from a nadir position at approximately 1.3 m above the wheat canopy and taken by averaging 10 scans. Reflectance spectra were derived relative to a 0.4 × 0.4 m white reference panel, which was placed horizontally just above the wheat canopy.

Crop aboveground biomass from the 1 m^2 area was collected immediately after 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). The sample was illuminated by a focused beam, which was produced by a Li-Cor 787 halogen lamp light source (6 V, 10 W, 3100 K), and the radiation that was captured by the spectrometer was the average reflected radiation within the Li-Cor 1800-12 integration sphere [28].

2.2.2. Plant measurements

Laboratory analyses were made on the 1 m^2 quadrat wheat samples just after leaf spectral measurement. LCar, leaf dry mass and LAI were measured according to standard procedures. Leaf dry mass was determined drying the samples in an oven at 70°C for 48 h, leaf Car content was determined using an L6 ultraviolet-visible spectrophotometer (INESA, China). Chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids' (Car) concentrations were calculated with Eqs. (1) to (3) [29]; the unit of total carotenoids could then be converted into content unit, that is, mass per unit leaf dry weight (mg/g), and concentration unit, that is, mass per unit leaf area (μg/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):

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

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

$$\text{C}ar\ (\text{mg/L}) = (1000 \times \text{A}_{470} - 3.27 \times Chla - 104 \times Chlb)/229 \tag{3}$$

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

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

where A_X is the absorbance of the extract solution at wavelength x, 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 [30]. 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):

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

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

Besides the winter wheat measured data, the ANGERS dataset, which contains various plant species and different growth conditions, was also used. 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 to 2400 nm using ASD FieldSpec instruments equipped with integrating spheres. Chlorophyll *a* and *b* (Chl), total carotenoids (Car), water (also named equivalent water thickness (EWT)) and dry matter (also named leaf mass per area (LMA)) content are available for each sample [18].

2.4. Simulated datasets

PROSPECT-5 simulates leaf directional-hemispherical reflectance and transmittance from 400 to 2500 nm with six input variables: LChl, LCar, LMA, EWT, leaf structure parameter (N) and brown pigments (Cbrown). Generally, pigments absorb light in the visible range (400–760 nm), whereas water has a high absorbance in the near-infrared band (1000–2500 nm). Dry matter and refractive index variations extend through the whole wave range (400–2500 nm) [18]. Since the goal was to estimate leaf Car content mainly from visible wavebands, and the

Models	Parameters	Values
PROSPECT-5	Leaf chlorophyll content (LChl, μg/cm^2)	10/20/30/40/50/60/70/80/90/100
	Leaf carotenoid content (LCar, μg/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 (Cbrown)	0
4SAIL	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 1. Input parameters for PROSPECT-5 and 4SAIL models used for leaf and canopy reflectance modeling.

visible range was unaffected by EWT, the EWT value was kept fixed at the average EWT value of ANGERS dataset. The range of variation of LChl, LCar, N and LMA obtained from ANGERS dataset was used in simulations. Detailed values for the input parameters used in PROSPECT-5 simulations are shown in **Table 1**.

With PROSPECT-5 model, 2500 leaf reflectance simulations could be obtained by random combination of the parameters values. To avoid unrealistic combinations, we made use of the content ratio of Car to Chl to restrain the combinations. Statistics of the content ratio of Car to Chl in the ANGERS data show that the ratio values ranging from 0.1 to 0.6 account for 97% of the samples. This criterion was then used to eliminate invalid combinations and finally 1700 leaf reflectance were kept. To investigate the effect of LAI and soil background on LCar assessment, LAI values were set to change from 1 to 8 with a step of 1; soil moisture parameter values were set to vary from 0 to 1 with a step of 0.5. Other input variables were fixed and defined based on [26]. Input values used for 4SAIL are shown in **Table 1**. Then, 40,800 canopy reflectance were obtained using the PROSAIL model.

2.5. Spectral indices

Published spectral indices for Car content assessment were summarized in **Table 2**. In addition to these existing spectral indices, a new spectral index for foliar Car content estimation was proposed 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. **Figure 1a** shows that the correlation peak region is located in the range

Spectral index	Equation	Reference
Ratio analysis of reflectance spectra (RARS*c*)	R_{760}/R_{500}	[12]
Pigment specific simple ratio (PSSR*c*)	R_{800}/R_{470}	[14]
Pigment specific normalized difference (PSND*c*)	$(R_{800} - R_{470})/(R_{800} + R_{470})$	[14]
Reflectance band ratio index (RBRI)	$R_{672}/(R_{550} \times R_{708})$	[13]
Plant senescence reflectance index (PSRI)	$(R_{678} - R_{500})/R_{750}$	[8]
Carotenoid reflectance index (CRI_{550})	$(R_{510})^{-1} - (R_{550})^{-1}$	[11]
Carotenoid reflectance index (CRI_{700})	$(R_{510})^{-1} - (R_{700})^{-1}$	[11]
Red-edge carotenoid index ($CAR_{red\ edge}$)	$[(R_{510})^{-1} - (R_{700})^{-1}] \times R_{770}$	[15]
Green carotenoid index (CAR_{green})	$[(R_{510})^{-1} - (R_{550})^{-1}] \times R_{770}$	[15]
Photochemical reflectance index (PRI)	$(R_{570} - R_{531})/(R_{570} + R_{531})$	[31]
Modified photochemical reflectance index (PRI_{m1})	$(R_{512} - R_{531})/(R_{512} + R_{531})$	[32]
Simple ratio (SR_{car})	R_{515}/R_{570}	[16]
Carotenoid index (CARI)	$R_{720}/R_{521} - 1$	[33]

R_λ is the reflectance value at wavelength λ.

Table 2. Spectral indices selected for LCar assessment.

500–540 nm for Car, and band 521 nm showed the maximum correlation, suggesting that reflectance in this range is very sensitive to Car content [11]. Besides, the range of its maximum sensitivity overlapped with Chl absorption features (**Figure 1a**). For Chl, the correlation extended from 400 to 760 nm and two strong correlation peaks were observed in green and red-edge regions.

To establish a new spectral index for LCar estimation, Band 521 nm was chosen on the consideration that it had the highest correlation with LCar (R^2 = 0.607, RMSE = 3.144 μg/cm^2, **Figure 1b**), 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 μg/cm^2, **Figure 1c**). The proposed new carotenoid index (CARI, R_{720}/R_{521}–1) was then established, based on the formula of chlorophyll indices (i.e., $CI_{red\text{-}edge}$ 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).

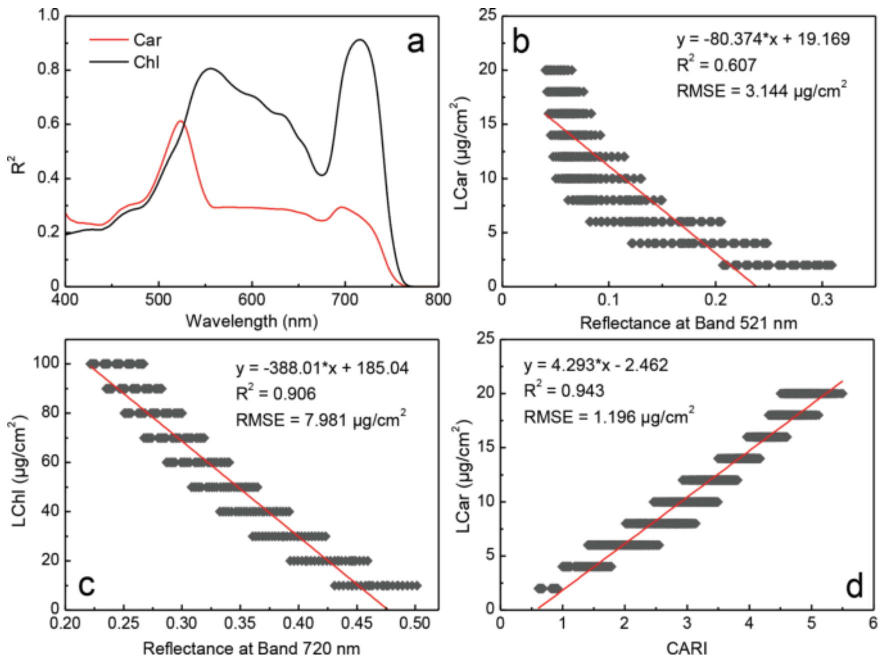

Figure 1. (a) R^2 curves for LCar (LChl) versus leaf reflectance within the wavelength range from 400 to 800 nm (b) Correlation between band 521 nm and LCar (c) Correlation between band 720 nm and LChl and (d) linear relationship between CARI and LCar.

3. Leaf car content assessment

3.1. Simulation results at the leaf level

Based on PROSPECT-5 leaf simulations, correlation between CARI and LCar is presented in **Figure 1d**. Results showed that CARI had a significant linear relationship with LCar ($R^2 = 0.943$, RMSE = 1.196 μg/cm²), indicating that CARI index was accurate in estimating LCar with leaf-simulated data. Nevertheless, relationships between established spectral indices and LCar varied in **Figure 2**. Among these established spectral indices, the carotenoid indices, that is, CRI_{550}, CRI_{700}, $CAR_{red\text{-}edge}$ and CAR_{green}, proposed by Gitelson et al. [11, 15] showed the highest correlation ($R^2 > 0.77$, the RMSE < 2.40 μg/cm²) with LCar. However, when LCar values were high, correlations between these indices and LCar presented with large dispersion, suggesting that these indices might be not sensitive to high LCar values (>15 μg/cm²). Compared with CRI_{550} and CRI_{700}, adding of a near infrared band (770 nm) in $CAR_{red\text{-}edge}$ and CAR_{green} did not improve the estimation accuracy of LCar. Correlation between RARSc and LCar was general ($R^2 = 0.603$, RMSE = 3.160 μg/cm²), and when LCar values were higher than 10 μg/cm², the correlation showed an obvious nonlinear trend. RBRI index was less correlated with LCar ($R^2 = 0.165$, RMSE = 4.584 μg/cm²), and the scatter plot of RBRI versus LCar showed

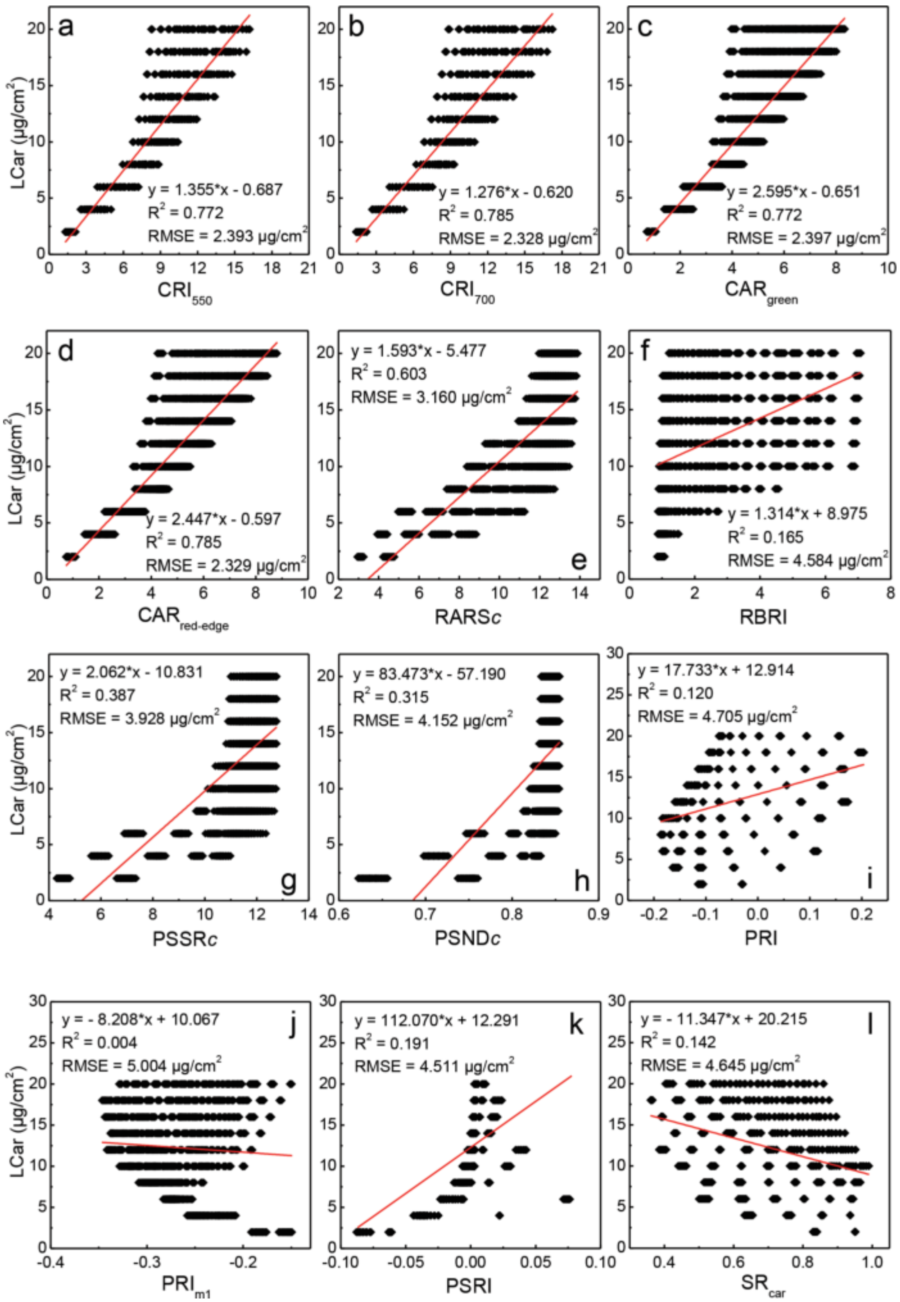

Figure 2. Relationships between published spectral indices and leaf carotenoids content from leaf level data simulated with PROSPECT-5.

large dispersity. PSSRc and PSNDc had low correlations with LCar. Compared with PSNDc, PSSRc showed a slightly better correlation with LCar (R^2 = 0.387, RMSE = 0.387 μg/cm^2). However, when LCar values exceeded 10 μg/cm^2, PSSRc and PSNDc present obvious nonlinear correlations with LCar (**Figure 2g** and **h**). Correlation between PRI and LCar was poor (R^2 = 0.120, RMSE = 4.705 μg/cm^2), and the scatter diagram showed obvious dispersion (**Figure 2i**). As for its modified version PRIm1, it showed almost no correlation with LCar, indicating that PRIm1 might not be suitable for the estimation of LCar. PSRI showed a low correlation with LCar (R^2 = 0.191, RMSE = 4.511 μg/cm^2), and the correlation was nonlinear. Different from these vegetation indices, SRcar showed a lower negative correlation with LCar (R^2 = 0.142, RMSE = 4.645 μg/cm^2), and the scatter diagram also showed strong dispersity.

3.2. Car assessment using ANGERS dataset

First, the ANGERS dataset was used to analyze the capability of different spectral indices in estimating LCar. Performance of different spectral indices in LCar assessment is shown in **Table 3**. The estimation accuracy of $CAR_{red\text{-}edge}$ and CAR_{green} in LCar assessment was slightly better than that of CRI_{550} and CRI_{700}. However, compared with the simulated results, these indices showed rather poor performance in LCar retrieval with the ANGERS data. RARSc exhibited good performance in LCar retrieval with a R^2 value of 0.438 and a RMSE value of 3.792 μg/cm^2. Although RBRI showed poor correlation with LCar in the leaf-simulated data, its estimation accuracy in LCar retrieval was the highest in the ANGERS data (R^2 = 0.727, RMSE = 2.640 μg/cm^2). Compared with PSNDc estimation results, estimation accuracy of PSSRc is relatively high, which is consistent with the foliar simulated results. PRI showed low accuracy in LCar estimation (R^2 = 0.199, RMSE = 4.527 μg/cm^2), while PRIm1 also showed poor estimation results.

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
$CAR_{red\text{-}edge}$	7	0.190	4.550	3.232	52.524
RARS*c*	**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
PSSR*c*	**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 3. Cross-validation results for LCar assessment using ANGERS data.

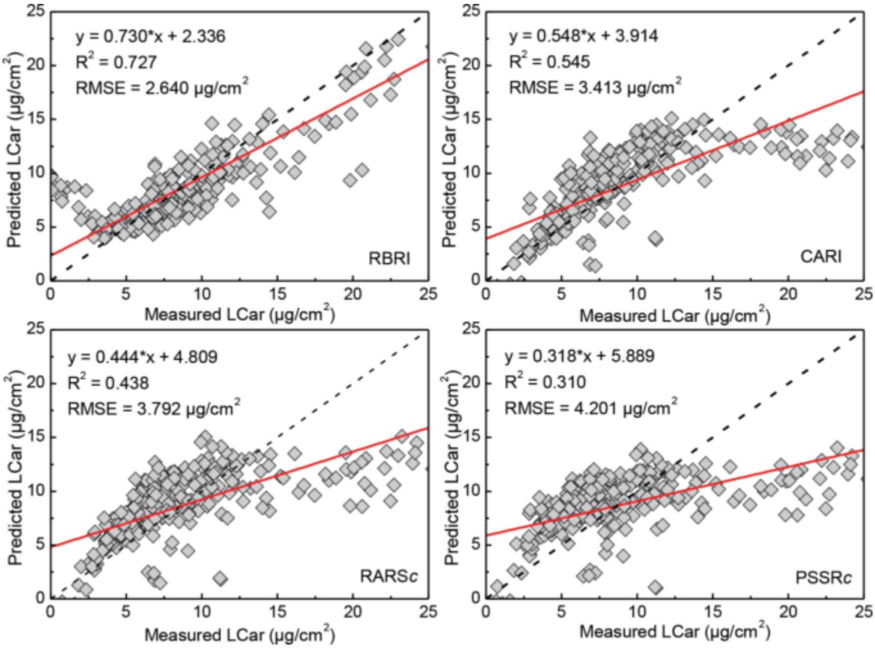

Figure 3. Scatterplots of measured LCar versus predicted LCar for spectral indices with ANGERS dataset. Dashed lines indicate 1:1 lines.

Index	Rank	R^2	RMSE (μg/cm²)	MAE (μg/cm²)	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
RARS*c*	**2**	**0.674**	**1.443**	**1.130**	**17.192**
RBRI	10	0.222	2.234	1.777	26.614
PSND*c*	**4**	**0.618**	**1.563**	**1.239**	**18.623**
PSSR*c*	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
CARI	**3**	**0.639**	**1.520**	**1.166**	**18.106**

Table 4. Cross-validation results for LCar estimation with wheat leaf level field data.

Among all the indices, PSRI had the lowest estimation accuracy, possibly due to its insensitive to LCar. The estimation accuracy of SRcar generally (R^2 = 0.213, RMSE = 4.489 μg/cm^2) ranks fifth in all estimation results. Compared with these existing spectral indices, the estimation accuracy of CARI was accurate (R^2 = 0.545, RMSE = 0.545 μg/cm^2), second to RBRI, showing that CARI data can be used to accurately estimate LCar in the ANGERS data.

Based on the estimation results of these spectral indices in LCar retrieval with the ANGERS dataset, the scatter diagrams of the best four ranking spectral indices were presented in **Figure 3**. The results showed that compared with other indices, the fitting line of the scatterplot of RBRI is closer to the 1:1 straight line (the slope of the fitting line is 0.730).In addition, RBRI index was more sensitive to higher leaf carotenoid content (>15 μg/cm^2). The CARI index also showed good estimation results, except for the samples that had LCar values greater than 15 μg/cm^2, and the estimated values of most sample points were evenly distributed around the 1:1 straight line with the measured values. Compared with RBRI, CARI was more sensitive to lower LCar values (<3 μg/cm^2), but it showed a slight "saturation effect" on high LCar values (>15 μg/cm^2). RARS and PSSRc indices also showed satisfactory estimation results. Similar to CARI, these indices were not sensitive to higher LCar values (>15 μg/cm^2).

3.3. Car retrieval with leaf level experimental data

Leaf level experimental data of winter wheat were used to further investigate the capability of the above spectral indices in LCar estimation. Results in **Table 4** showed that the estimation accuracy of CRI_{550} and CRI_{700} is the worst. $CAR_{red\text{-}edge}$ and CAR_{green} have slightly improved LCar estimating accuracy. RARSc presented an excellent estimation result (R^2 = 0.674, RMSE = 1.443 μg/cm^2), which is consistent with its estimation results with the ANGERS data. Different from its good estimation accuracy in ANGERS data, RBRI showed poor estimation accuracy in the experimental data (R^2 = 0.222, RMSE = 2.234 μg/cm^2). PSNDc and PSSRc have a performance with good results in LCar estimation with the experimental data (R^2 > 0.57, RMSE < 1.65 μg/cm^2). Compared with its poor estimation results in the ANGERS data, PRI showed the highest estimation accuracy of LCar in the experimental data (R^2 = 0.710, RMSE = 1.369 μg/cm^2). PRIm1 showed poor estimation results in the experimental data, which was consistent with the ANGERS data. Compared with the ANGERS data, the estimation accuracy of PSRI and SRcar in LCar with the experimental data had slightly improved. Similar to its performance with the ANGERS data, CARI had high estimation accuracy in LCar retrieval with the experimental data (R^2 = 0.639, RMSE = 0.639 μg/cm^2), showing that CARI was accurate and robust for LCar estimation with different leaf level datasets.

Similar to the results with the ANGERS dataset, scatter diagrams of the best four ranking spectral indices were presented in **Figure 4**. The results showed that the estimation results obtained by PRI were the best. LCar values estimated by PRI and the measured values were concentrated near the 1:1 line, and the slope of the scatter plot was 0.76. In addition, RARSc, CARI and PSNDc also showed good estimation results. Unlike the ANGERS data (**Figure 3**), The LCar values of the leaf level experimental data were in the range from 3.05 to 12.59 μg/cm^2, and LCar was in low to moderate numerical range. According to **Figure 4**, the majority of LCar values of the samples were around 10 μg/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.

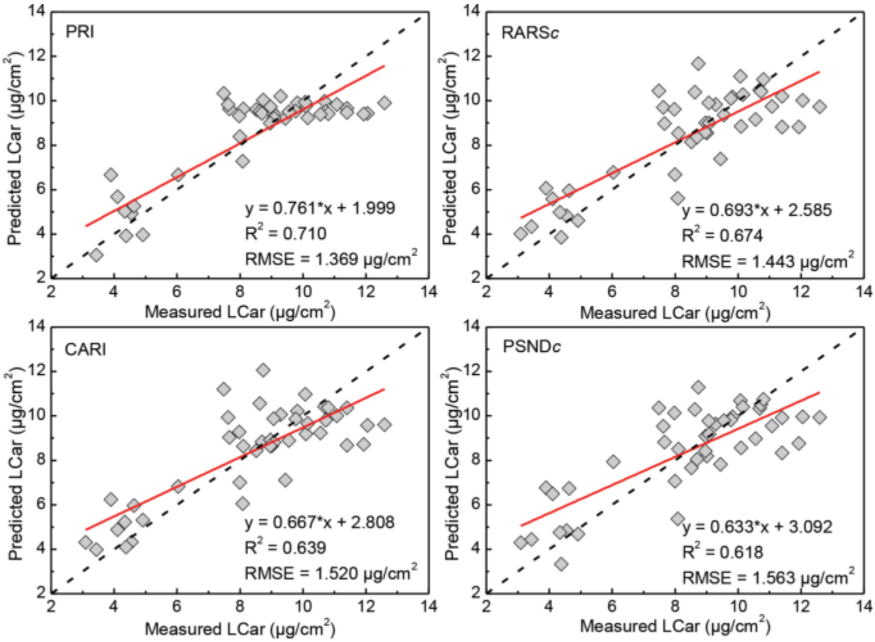

Figure 4. Scatterplots of measured LCar versus predicted LCar for spectral indices with leaf level experimental data. Dashed lines indicate 1:1 lines.

3.4. Assessing CARI for LCar retrieval with canopy spectra

The above results with leaf level measured data showed that CARI was accurate and robust in LCar estimation. Canopy level simulations and measured data were then used to further explore the effect of LAI and soil moisture on CARI for LCar retrieval. Canopy simulation results in **Figure 5a** showed that the overall correlation between CARI and LCar was high ($R^2 = 0.675$, RMSE = 0.675 μg/cm^2); however, the correlation differed when LAI values varied. The relationship between CARI and LCar was the worst ($R^2 = 0.455$, RMSE = 0.455 μg/cm^2) when LAI values were around 1, which suggests that when LAI values were small, CARI was not sensitive to LCar variations. Indeed, when LAI values were around 1, the information obtained by the canopy spectrum was mostly related to soil background, thus it affected the estimation of LCar. The influence of soil moisture parameter on LCar retrieval with CARI was then investigated when LAI values were 1. Results in **Figure 6** suggested that variations of soil moisture parameter did affect the correlation between CARI and LCar. When the value of soil moisture parameter was 1 (i.e., simulated dry soil), CARI correlated worst with LCar ($R^2 = 0.614$, RMSE = 0.614 μg/cm^2). When its value was 0 (i.e., simulated wet soil), CARI showed the best correlation with LCar ($R^2 = 0.922$, RMSE = 1.398 μg/cm^2). In general, with the increase of LAI values, the correlation between CARI and LCar increased, and when LAI exceeded 4, the correlation reached 0.89 and remained unchanged. When the LAI values exceeded 4, the fitting equations between CARI and LCar hardly changed, suggesting that when LAI values were larger than 4, CARI might be less sensitive to LCar variations based on canopy spectral data.

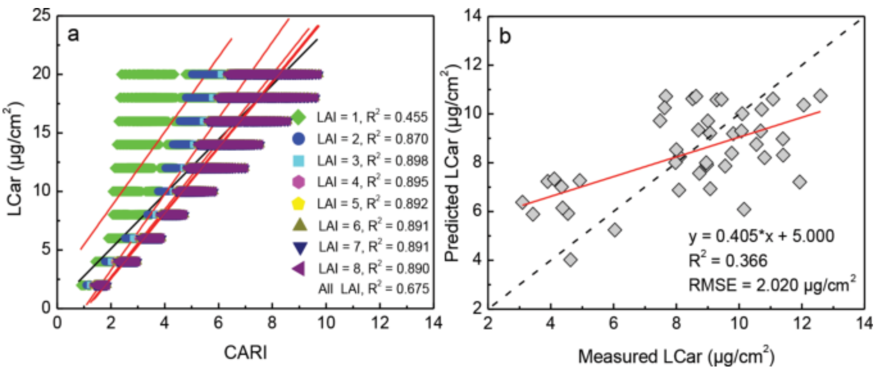

Figure 5. (a) Correlation between CARI and LCar at different LAI values, from all canopy simulations with 4SAIL model (n = 40,800). (b) Scatterplots of measured LCar versus predicted LCar for CARI with canopy reflectance obtained from field data (n = 44). Dashed lines indicate 1:1 lines.

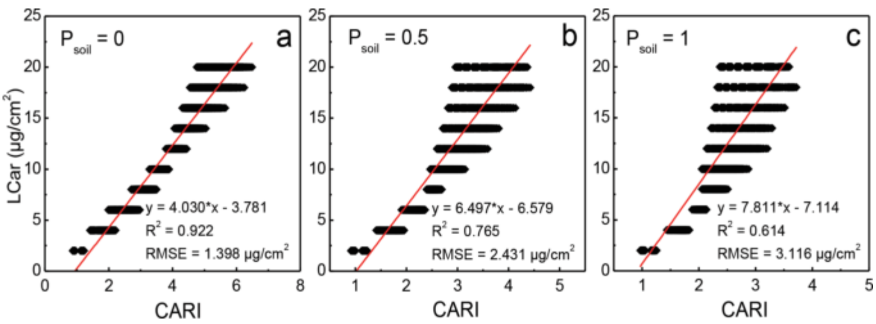

Figure 6. Relationships between CARI and LCar using canopy reflectance simulations with LAI value fixed to 1 at different soil moisture levels. P_{soil} value set as (a) 0, (b) 0.5 and (c) 1. All other parameters for 4SAIL were fixed based on **Table 3** (n = 1700).

Based on the canopy level measured data of winter wheat, LCar estimation results with CARI is shown in **Figure 5b**. Compared to the results that used leaf level data, the estimation accuracy was rather low for LCar retrieval with canopy level spectrum ($R^2 = 0.366$, RMSE = 0.366 μg/cm^2), and LCar values lower than 5 μg/cm^2 were obviously overestimated (**Figure 5b**). 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.

3.5. Discussion

The spectral absorption features of carotenoids in the visible range make it possible for analysis of nondestructive estimation of leaf carotenoids content. However, the overlaps of spectral absorption characteristics of carotenoids and chlorophylls in the visible band making it challenging to assess LCar with its own absorption features [16]. Based on the reviewed studies on LCar estimation with remote sensing techniques, this chapter established a new carotenoid index (CARI) based on the spectral absorption features of carotenoids. Abundant synthetic data simulated from leaf and canopy models, and measured dataset, including the ANGERS and winter wheat data, were then used to comprehensively investigate its capability in LCar assessment. CARI was established in the form of chlorophyll indices, that is, $CI_{red\text{-}edge}$ and CI_{green}. These chlorophyll indices proposed by Gitelson et al. [15] utilized red-edge (or green) band that was sensitive to chlorophyll variation. Meanwhile, a near infrared waveband was also considered to eliminate the effect of other pigments and backward scattering effect. Many studies had shown that $CI_{red\text{-}edge}$ and CI_{green} can be used to estimate leaf chlorophylls content accurately [34, 35]. Through analyzing the correlation between LCar and reflectance in the visible range from 400 to 800 nm wavelength, we utilized the reflectance of 521 nm band to establish the CARI index. The 521 nm waveband was located in the spectral absorption band of carotenoids and was significantly related to LCar. However, strong correlation between reflectance of 521 nm waveband and LChl existed. In order to eliminate the effect of chlorophylls on carotenoids retrieval, 720 nm waveband was also used in CARI; owing to that, the reflectance of 720 nm band was the most correlated with LChl. With PROSPECT-5 simulations, the new CARI showed a significantly strong linear relationship with LCar. Moreover, CARI showed low correlation with LChl ($R^2 = 0.315$), showing that it was less sensitive to LChl variations and the use of 720 nm band obviously decreased the effect of LChl on LCar estimation to some extent. In addition, CARI showed good estimation of LCar with both the ANGERS data and leaf level experimental data of winter wheat, indicating that it was accurate and robust for LCar assessment with CARI.

With different foliar datasets (leaf simulations, the ANGERS data and winter wheat experimental data), performance of published spectral indices in LCar estimation varied. Carotenoids index, including CRI_{550}, CRI_{700}, $CAR_{red\text{-}edge}$ and CAR_{green}, showed significant linear relationship with LCar with foliar simulated data. However, those indices exhibited poor results for LCar estimation with the ANGERS data and experimental data of winter wheat, suggesting that the accuracy and robustness of these indices in LCar estimation needed to be improved. Compared with CRI_{550} and CRI_{700}, the estimation accuracy in LCar retrieval with $CAR_{red\text{-}edge}$ and CAR_{green} slightly improved. This suggested that adding of a near infrared band (770 nm) in CRI_{550} and CRI_{700} could improve the estimation accuracy [15]. Based on foliar measured data, RARSc showed accurate estimation of LCar (ranking third with the ANGERS data, while ranking second with winter wheat data). These results were consistent with previous studies using RARSc to estimate LCar with measured data [36, 37], suggesting that RARSc was robust in LCar estimation. Performance of RBRI in LCar estimation with simulated and measured data significantly varied: it showed low correlation with LCar in foliar simulations, best results in LCar retrieval with the ANGERS data and poor results with winter wheat experimental data. Based on the spectral absorption features of chlorophylls, Datt [13] proposed the RBRI spectral index, which was used for chlorophylls and carotenoids retrieval. In his study, chlorophylls and carotenoids were significantly correlated. However, in the foliar simulations, their correlation was low ($R^2 = 0.235$), although LChl was significantly related with RBRI ($R^2 = 0.847$). This could explain the correlation between RBRI and

LCar in simulations. In ANGERS data, LCar and LChl were significantly linear correlated (R^2 = 0.908) and RBRI showed strong relationship with LChl (R^2 = 0.785); therefore, RBRI showed high estimation accuracy in LCar retrieval. The RBRI was established on the equation R_{672}/ ($R_{550} \times R_{708}$), which was different from the normalization and ratio form that most indices adopted. The form of the denominator ($R_{550} \times R_{708}$) might help to increase the numerical range of RBRI, making it more sensitive to large values of LCar. However, RBRI might not be sensitive to low values of LCar (<3 μg/cm^2), as samples with LCar values lower than <3 μg/cm^2 were obviously overestimated. In the experimental data of winter wheat, although LCar and LChl were significantly linear related (R^2 = 0.888), RBRI showed poor estimation results for LCar retrieval. This suggested that RBRI might not be stable when used in various datasets for LCar estimation.

Blackburn [14] pointed out that the overlaps between spectral absorption features of carotenoids and chlorophylls might affect the relationship between LCar and PSNDc (or PSSRc). Moreover, 470 nm waveband was used in these spectral indices, which was not the best absorption band for carotenoids. Their performance with the leaf-simulated data and the ANGERS data supported this viewpoint. However, PSNDc and PSSRc showed rather good estimation accuracy in LCar retrieval with winter wheat data. This may be due to the fact that LCar values of the measured foliar data of winter wheat were in the range of 4–12 μg/cm^2. Unlike the ANGERS data numerical range, these indices may be more sensitive to LCar changes in this range. PRI was successfully applied to a variety of studies [38, 39]. In this chapter, PRI showed poor performance in LCar estimation with leaf-simulated data and the ANGERS data. The 531 nm waveband of PRI was used to detect variations of xanthophyll cycle components [31], PRI's relationship with LCar may be overly influenced by a single carotenoid component [40].Compared with the estimated results in simulated data and the ANGERS data, PRI showed the best estimation accuracy in LCar retrieval with winter wheat data. Previous study had also shown that PRI was accurate in LCar estimation in cotton plants [37]. These results indicated that PRI may be suitable for LCar estimation in single-species vegetation. Unlike PRI, PRIm1 showed poor estimation results in all the used datasets. This may be because PRIm1 was devised to reduce the effect of the canopy structure effect and indicated water stress [32]. These results indicated that PRIm1 was not suitable for LCar estimation. Similarly, PSRI was devised to indicate leaf senescence and fruit ripening, it was sensitive to changes of the content ratio of carotenoids to chlorophylls [8]. The assessment results also indicated that PSRI was not suitable for LCar estimation. SRcar showed poor correlation with LCar in simulated data, this was mainly because that the parameters of the leaf-simulated data in this chapter were more complicated than that of Hernandez-Clemente et al. [16]. Similarly, the poor estimation results of LCar with SRcar in the ANGERS data may be related to the diversity of vegetation types. However, SRcar showed good estimation accuracy in LCar assessment with winter wheat data, which indicated that it might be suitable for LCar retrieval with single-species vegetation.

Although the new index CARI showed good estimation results for LCar retrieval with different foliar datasets, the simulation results with canopy level data suggested that CARI was not sensitive to LCar variations when LAI was low (e.g., LAI = 1). Moreover, soil moisture parameters affected the estimation accuracy of LCar with CARI. When LAI values are low, and soil is in a dry condition, canopy spectral reflectance of plants is mainly controlled by soil reflection; this could weaken plant canopy information, thus reducing LCar estimation accuracy (**Figure 6c**). When the soil is in a wet condition, the overall soil reflectance is lower, thus its confounding effect on LCar estimation seems to be reduced (**Figure 6a**). 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 multispectral 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.

4. Conclusions

This chapter mainly focused on leaf and canopy level radiative transfer model PROSPECT-5 and 4SAIL to simulate abundant leaf and canopy synthetic data and to establish a new carotenoid index (CARI) for LCar assessment based on the spectral absorption features of carotenoids. Abundant measured data, including the ANGERS data and experimental data of winter wheat, were then used to comprehensively evaluate the capability and robustness of CARI in LCar retrieval. Results showed that CARI correlated best with LCar among all the selected spectral indices with leaf-simulated data. Moreover, CARI showed accurate and robust estimation results of LCar with the ANGERS data and experimental data of winter wheat. Further investigation of CARI in LCar retrieval with simulated and measured canopy level data showed that CARI was insensitive to LCar variations when LAI values were low. In these conditions, soil moisture parameters affected the estimation accuracy of LCar with CARI. Overall, we suggest that CARI is suitable for LCar assessment, which could provide basis for LCar nondestructive estimation with remote sensing techniques.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Wenjiang Huang[1], Xianfeng Zhou[2]*, Weiping Kong[1] and Huichun Ye[1]

*Address all correspondence to: zhouxianfeng@hdu.edu.cn

1 Key Laboratory of Digital Earth Science, Institute of Remote Sensing and Digital Earth, Chinese Academy of Science, Beijing, China

2 College of Life Information Science and Instrument Engineering, Hangzhou Dianzi University, Hangzhou, China

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