Estimation of carbon and nitrogen contents in citrus canopy by low-altitude remote sensing

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Abstract: The study aimed to investigate the fast and nondestructive method for detecting carbon and nitrogen content in citrus canopy. The multispectral imagery of Tarocco blood orange (*Citrus sinensis* L. Osbeck) plant canopy was obtained by a multispectral camera array mounted at an eight-rotor unmanned aerial vehicle (UAV) flying at an altitude of 100 m above the canopy in Wanzhou District of Chongqing Municipality, China. Average spectral reflectance data of the whole canopy, mature leaf areas and young leaves areas were extracted from the imagery. Two spectral pre-processing methods, multiplicative scatter correction (MSC) and standard normal variable (SNV), and two modeling methods, the partial least squares (PLS) and the least squares support vector machine (LS-SVM), were adopted and compared for their prediction accuracy of total content of nitrogen, soluble sugar and starch in the leaves. The results showed that, based on the spectral data extracted from the mature leaves in the multispectral imagery, the PLS model based on the original spectrum obtained a R_p (correlation coefficient) of 0.6469 and *RMSEP* (root mean squares error of prediction) of 0.1296, suggested that it was the best for the prediction of total nitrogen content; the PLS model based on MSC (multiplicative scatter correction) spectrum pre-processing was the best for predicting total soluble sugar content (R_p=0.6398 and *RMSEP*=8.8891); and the LS-SVM model based on MSC was the best for the starch content prediction (R_p=0.6822 and *RMSEP*=14.9303). The prediction accuracy for carbon and nitrogen contents based on the spectral data extracted from the whole canopy and the young leaves were lower than that from the mature leaves. The results indicate that it is feasible to estimate the carbon and nitrogen contents by low-altitude airborne multispectral images.

Keywords: citrus canopy, low-altitude remote sensing, carbon and nitrogen contents, soluble sugar, starch, estimation
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1 Introduction

Carbon-nitrogen metabolism is the most basic and important nutrient metabolism of plants, and its dynamic changes in the plant directly affect the absorption, transformation of mineral nutrition and formation of

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protein and so on[1,2]. Carbon metabolism provides the protein metabolism with the carbon source and energy, and the nitrogen metabolism can provide enzymes and photosynthetic pigments, and the carbon-nitrogen metabolism also needs to share the reduction ability, adenosine triphosphate (ATP) and carbon skeleton[3]. Therefore, carbon and nitrogen metabolism and their harmony affect not only the plant growth and development, but also the fruit yield and quality[4]. Citrus is the subtropical perennial evergreen fruit crop, and it has high requirements on the carbon-nitrogen interaction balance. In the process of citrus production management, it is very important to reasonably regulate carbon-nitrogen nutrition and scientifically coordinate the carbon-nitrogen metabolism in order to obtain higher fruit yield and quality of citrus.

At present, crop nutrition diagnosis methods mainly include commonly-used morphological diagnosis and chemical diagnosis[5,6]. The morphological diagnosis method is limited by the practitioner's experiences and technical level, and the chemical diagnosis method is expensive and cannot obtain the results in time. Therefore, both methods are not conducive to timely guidance and scientific fertilization in orchard. With the citrus industry changing to intensive production and specialized management in China, the requirements for timely diagnosis and nutrition regulation to fruit trees are more and more pressing, especially for the regulation of fruit growth and flower yield. Remote sensing is being increasingly used in agriculture in recent years, it can be used to nondestructively obtain the information of ground objects in real time, and quickly and efficiently monitor and evaluate the physiological and productive conditions[7-9]. Remote sensing technology has been well studied for crop identification and classification[10], disease identification[11,12] and yield prediction[13,14]. Some studies were carried out to monitor the change of wheat coverage by extracting the thresholds of remote sensing images[15]. Furthermore, Li et al.[15] acquired hyperspectral image of the test area by the airborne hyperspectral system, and the nitrogen content in the leaves of potato was predicted by spectral data transform and partial least square regression. Based on previous studies[17-20], Ye and Sakai[21] investigated the applicability of airborne hyperspectral imagery to the estimation of fruit yield in citrus. The canopy features of individual trees were identified using pixel-based average spectral reflectance values at various wavelengths from the acquired images. Fruit yields of 48 individual trees were recorded and the yield prediction models were developed using different prediction variables. However, estimation of carbon-nitrogen nutrition level of different citrus plants based on low-altitude remote sensing is still rarely studied and reported.

In this research, the multi-spectral image information of Tarocco blood orange orchard were obtained by the multi-spectral cameral array mounted at an eight-rotor UAV flying at an altitude of 100 m above the canopy. The average spectral reflectance of the whole canopy, mature leaves and young leaves were extracted from the multi-spectral image and the better methods of the spectral pre-processing and the prediction modeling were selected to build the best prediction methods of plant carbon and nitrogen content. The objectives of this study were as follows: 1) Exploring the possibility of using low-altitude remote sensing to determine carbon and nitrogen content in citrus canopy. 2) Determining what kind of leaves of canopy is better to predict carbon and nitrogen content. 3) Screening a better method to determine carbon and nitrogen content in citrus canopy by low-altitude remote sensing.

2 Materials and methods

2.1 Experimental orchard and sample selection

The experiment was conducted in the hilly orange orchard in Ganning Town, Wanzhou District, Chongqing Municipality, China in October, 2014. The soil type of the orchard is purple and the citrus trees planted in the orchard were healthy five-year-old Tarocco blood orange tree. A total of 90 trees were randomly selected to obtain low-altitude remote sensing images on a sunny slope in the orchard.

2.2 Instruments and equipment

A multispectral camera, mini-MCA12 equipped with an incident light sensor (Tetracam, Inc., Chatsworth, CA, USA) was used to collect the remote sensing images of the tree canopy. There were twelve channels arranged in a 3×4 array in the camera. One channel was the light intensity sensor for the image correction and the other eleven channels were the spectral sensors (Table 1). Each channel had a focal length of 9.6 mm and a 1.3-megapixel (1280×1024 pixels) CMOS sensor, and the images were stored in a CF-card in raw format. The images were acquired with 10-bit radiometric resolution.

Table 1 Wavelengths and half band widths of 11-channel multispectral camera

Channel	1	2	3	4	5	6	7	8	9	10	11
Wavelength/nm	490	550	570	671	680	700	720	800	840	900	950
Half band width /nm	10	10	10	10	10	10	10	10	10	20	40

The platform for image acquisition was an eight-rotor UAV (TT Aviation Technology Development Co. Ltd. Beijing, China). The UAV is capable of lifting 4.0 kg with a flight time about 15 minutes. In order to eliminate the effects of vibration in the spectral image acquisition process, the UAV was equipped with an attitude control system which could always keep the mini-MCA12 camera perpendicular to the ground.

2.3 Multispectral image and leaf sample collection

A sunny region on the gentle slope in the experimental orchard was selected. Multispectral images of citrus plant canopy were collected by the airborne acquisition system between 12:00 and 14:00, October 9, 2014. Scene of the experiment is shown in Figure 1. The UAV flew with a speed of 3.6 m/s at an altitude of 100 m above the plant canopy.

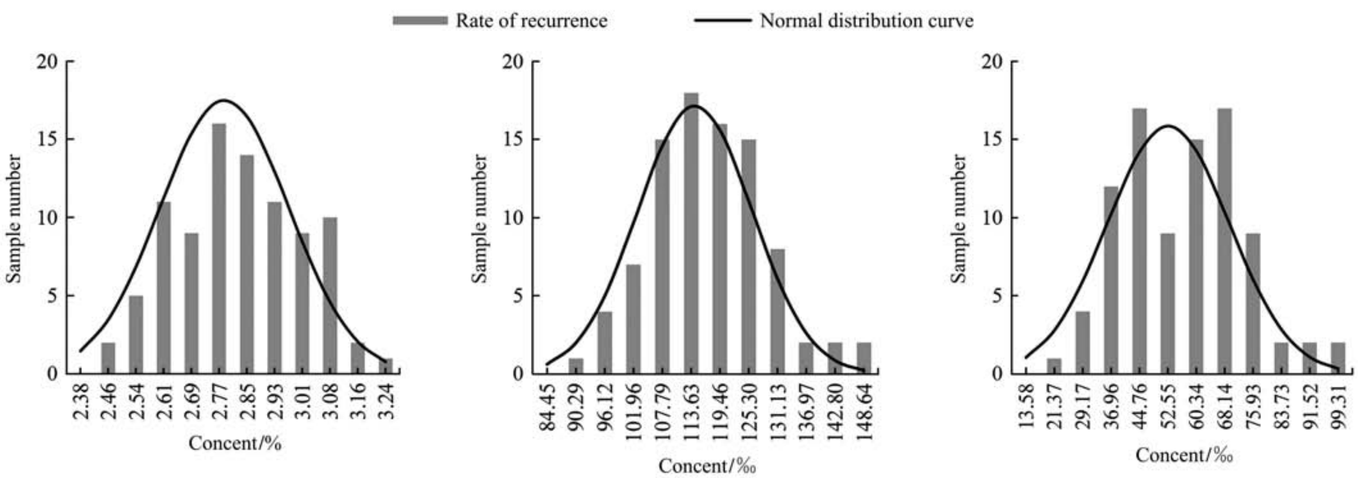
Figure 1 Scene of the experiment

After image collection, 20 mature leaves were collected from the spring shoots around the canopy of each sample plant and stored immediately in the refrigerated containers with ice packs for nutrition analysis.

2.4 Leaf carbon-nitrogen content chemical analysis

The leaf samples were cleaned and immediately placed into a 105ºC oven for 30 min to inactivate enzyme, and then were dried to constant weight at 75ºC. The total nitrogen content was obtained by the Kjeldahl determination[22], and soluble sugar and starch content were measured by anthrone colorimetric method[23].

The total nitrogen, soluble sugar and starch content of 90 test samples were shown in Figure 2, and the distributions of the three nutrients in the plant were approximately normal, indicating that the selected test sample had a better representation and wider range and was suitable for the further analysis and observation.



a. Total nitrogen b. Soluble sugar c. Starch

Figure 2 Distribution of total nitrogen, soluble sugar and starch content in citrus plants

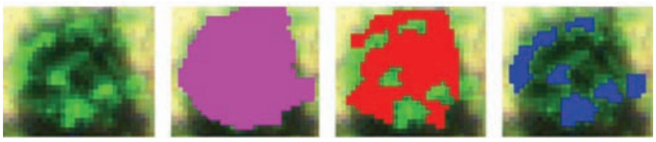
A total of 90 test samples were randomly grouped into two sets: 72 samples as the calibration set and the remaining 18 samples as the prediction set, and the statistical results of the three nutrients in the two groups are shown in Table 2. It was found that the maximum carbohydrates group in citrus plant was soluble sugar, and followed by the starch. The large standard deviation of starch content indicated that the stability of starch content was poor. The ranges of total nitrogen, soluble sugar and starch contents of the prediction set fell within the ranges of the calibration set, indicating that the model built from the calibration set could be used for prediction analysis.

Table 2 Statistical results of total nitrogen, soluble sugar and starch content in citrus plants

Nutrient	Type	Sample number	Range	Mean	Standard deviation
Total nitrogen/%	Calibration	72	2.3806-3.1642	2.7855	0.1842
	Prediction	18	2.4750-3.0035	2.7801	0.1713
Soluble sugar/‰	Calibration	72	89.4641-147.8151	114.6738	11.8539
	Prediction	18	90.6625-133.9953	113.2772	10.9284
Starch/‰	Calibration	72	21.3462-99.3869	52.2502	16.2468
	Prediction	18	27.3730-91.7628	54.8432	19.3078

2.5 Canopy spectral information extraction

First, PiexlWrench2 software (Tetracam, Inc., Chatsworth, CA, USA) was used to carry out the white board standard correction to the multispectral images and then the raw images were exported to TIF format. The citrus canopy contained young and mature leaves in the growing season. Using the ENVI 4.7 polygon tool, the areas of the whole canopy (magenta area in Figure 3b), mature leaves (red area in Figure 3c) and young leaves (blue area in Figure 3d) were selected as a region of interest from each plant canopy image (Figure 3a). Three types of canopy spectral information were extracted from the 90 experimental trees one by one and the average spectral reflectance values were used for modeling and analysis.



a b c d

Note: R: 671 nm, G: 550 nm, B: 490 nm.

Figure 3 RGB image of citrus plant canopy

2.6 Spectral pre-processing methods

Two spectral pretreatment methods were selected to pre-process the multispectral images by software "The Unscrambler V 9.7" (CAMO AS, Oslo, Norway). The multiplicative scatter correction (MSC) method was employed to eliminate the interferences induced by the scattering of baseline shift and migration and improve the spectral signal to noise ratio[24], and the standards normal variate (SNV) was adopted to eliminate the surface scattering and optical path difference of spectral reflectance[25].

2.7 Modeling and model evaluation

2.7.1 Partial least squares (PLS)

Partial least squares (PLS) regression[26] is one of the classical methods of multivariate regression analysis. The absorbance and the concentration matrix were decomposed as a feature vector and the load vector, and the best latent variables were determined by cross test method, then the mathematical model of the absorbance matrix and concentration was set up. PLS analysis mainly used for regression modeling of multiple dependent variables on multiple independent variables, which can regress model with severe multiple correlation of independent variate existing in the argument. It is one of the most widely used modeling methods in quantitative analysis at present.

2.7.2 Least squares-support vector machine (LS-SVM)

Support vector machine (SVM) is a machine learning method based on statistical learning theory. According to the limited sample information in the model, it is the best way to solve the problems with small samples, over learning, nonlinearity, high dimension and local minima points. The LS-SVM regression model is an improved method of the classical SVM model which used to solve a set of linear equations to replace the classical SVM in the two complex optimization problems for reducing the computational complexity, and improving the calculation speed[27].

2.7.3 Model evaluation

The model performance was mainly evaluated by the indices: correlation coefficients of calibration R_c and prediction R_p, root mean squares error of cross-validation *RMSECV*, and root mean squares error of prediction *RMSEP*. The best model should have a maximum value of R_c and R_p and a minimum value of *RMSECV* and *RMSEP*.

3 Results and analysis

3.1 Spectral features of citrus canopy

The average reflectance spectra of the whole canopy, young leaf region and mature leaf region of the citrus tree are shown in Figure 4. In general, spectral curves of the three canopies and leaf regions were similar, but the reflection intensity of the reflectance was different. The spectral reflectance was the highest for the young leaf region, followed by the whole canopy and then the mature leaves region.

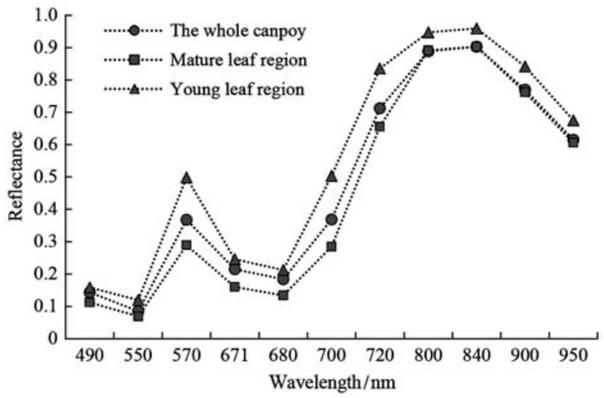
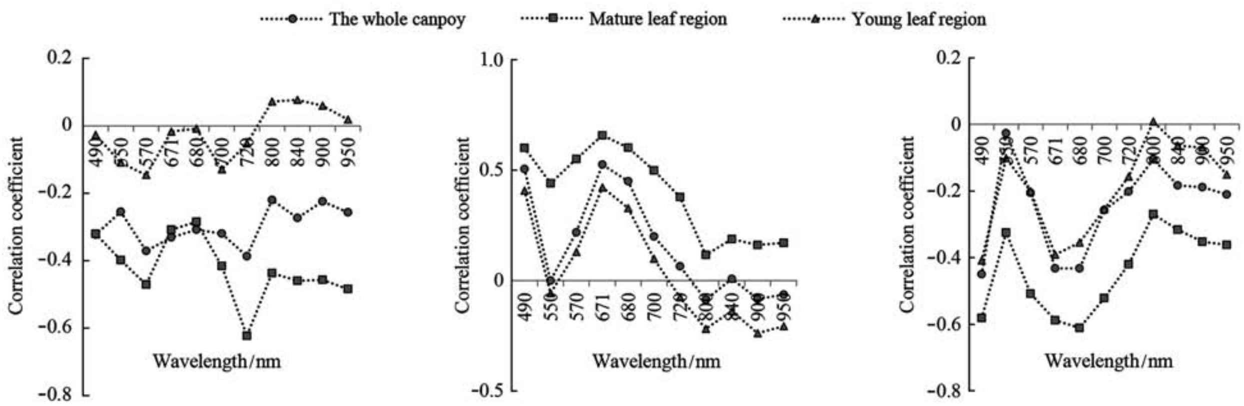


Figure 4 Spectral reflectance of three different leaf regions in a citrus plant canopy

The spectral reflectance of the citrus canopy is very similar to that of a typical plant. The chlorophyll of citrus tissues can result in a strong radiation energy absorption and low reflectance, and the higher reflectance in the near infrared region may be caused by the multiple scattering[28]. Because of the higher chlorophyll content in the mature leaf and the stronger absorptive capacity of chlorophyll on the spectra in the visible light region (490-700 nm), the visible spectral reflectance of the young leaves was higher than that of the mature leaves in the tree canopy in near infrared region (720-950 nm). The higher spectral reflectance of the young leaves than mature leaves was caused mainly by the stronger scattering capability of the spongy mesophyll tissue cells of the young leaves.

2.2 Correlation analysis

Correlation coefficients of citrus canopy spectra with total nitrogen, soluble sugar, and starch content for the whole canopy, mature leaves and young leaves are shown in Figure 5. Correlations with leaf total nitrogen, soluble sugar and starch content for the mature leaves were significantly higher than those for the whole canopy and young leaves. Therefore, the spectral information from the mature leaves was chosen for further analysis. It can be seen that the correlation coefficients between total nitrogen and the 11 bands were significantly negative and the correlation coefficient at 720 nm was the highest (0.632). The content of soluble sugar was significantly positively correlated with spectral data at 490 nm, 570 nm, 671 nm, 680 nm, 550 nm, 700 nm and 720 nm, and the correlation coefficient was the highest at 671 nm (0.658). All the 11 bands were significantly negatively correlated with starch content and the highest correlation coefficient was 0.611 at 680 nm.



a. Total nitrogen b. Soluble sugar c. Starch

Figure 5 Correlations of citrus canopy spectra with total nitrogen, soluble sugar, and starch content

2.3 Modeling and optimization

In order to better monitor the content of carbon and nitrogen in citrus trees, the spectral data of mature leaves that had the highest correlation with carbon and nitrogen content were used as the independent variables, and the total nitrogen, soluble sugar and starch content were the dependent variables, the PLS model and LS-SVM model were established respectively.

2.3.1 PLS prediction models

The PLS prediction models were developed based on the original spectrum (OS) and the MSC and SNV pretreatment spectrum (Table 2). The prediction accuracy of the PLS models for carbon and nitrogen content were compared by comparing the value of R_p and *RMSEP*. The PLS model based on the original spectrum was the best for the prediction of total nitrogen content of the tree canopy (R_p=0.6469, *RMSEP*=0.1296). For the prediction of soluble sugar content, the PLS model based on the MSC pretreatment spectrum was the best (R_P=0.6398, *RMSEP*=8.8891). The PLS model based on the SNV pretreatment spectrum obtained the highest accuracy to predict starch content (R_P=0.6434 and *RMSEP*=15.6366).

Table 2 PLS prediction models based on different spectral forms

Nutrient	Spectral preprocessing method	Latent Variables	R_C	*RMSECV*	R_P	*RMSEP*
Total nitrogen	OS	4	0.6780	0.1345	0.6469	0.1296
	MSC	2	0.6092	0.1451	0.5484	0.1398
	SNV	3	0.6431	0.1401	0.5118	0.1474
Soluble sugar	OS	2	0.6390	9.0548	0.6051	9.1703
	MSC	2	0.6763	8.6713	0.6398	8.8891
	SNV	2	0.6763	8.6711	0.6396	8.8908
Starch	OS	2	0.6245	12.6002	0.5811	16.1320
	MSC	2	0.6564	12.1707	0.6431	15.6435
	SNV	2	0.6565	12.1696	0.6434	15.6366

2.3.2 LS-SVM prediction model

The radial basis function (RBF) kernel was chosen for strong generalization ability[29]. Therefore, kernel function parameters were optimized after the determination of the kernel function. The LS-SVM model had two major parameters: hyper parameter γ and RBF kernel function parameter σ^2[30]. Cross validation with grid search options was used to find the optimal combination of γ and σ^2, namely in the space of γ and σ^2, seeking the optimal combination of the space point of the parameters among the various points in the space. From Table 3, the LS-SVM models based on the original spectrum for total nitrogen and soluble sugar were the best with R_P of 0.6161 and 0.6076 and *RMSEP* of 0.1313 and 8.8375, respectively. The LS-SVM model based on the MSC pretreatment spectrum was the best for prediction of starch content with R_p of 0.6822 and *RMSEP* of 14.9303.

Table 3 Results of LS-SVM models based on different spectral preprocessing methods

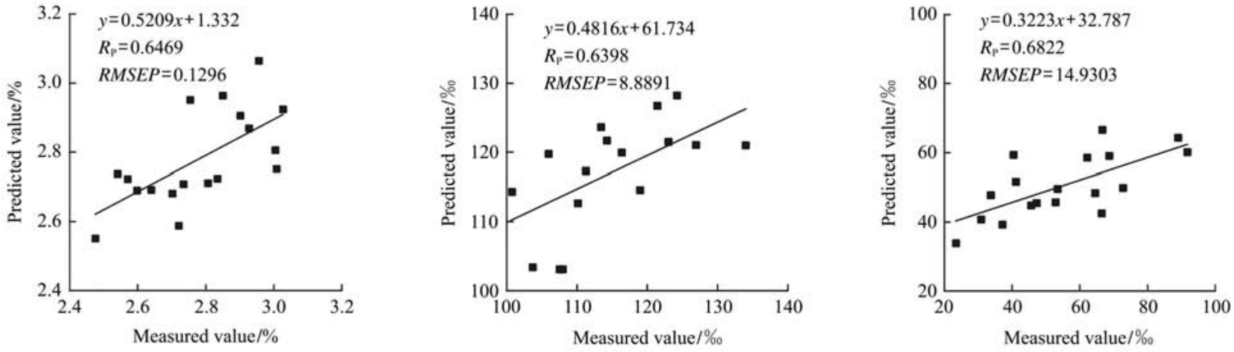
Nutrient	Spectral preprocessing method	Latent Variables	R_C	*RMSECV*	R_P	*RMSEP*
Total nitrogen	OS	4	0.6454	0.1405	0.6161	0.1313
	MSC	4	0.6480	0.1396	0.5141	0.1455
	SNV	4	0.6501	0.1393	0.5101	0.1462
Soluble sugar	OS	2	0.6585	8.9043	0.6076	8.8375
	MSC	1	0.6154	9.3121	0.6560	9.2736
	SNV	1	0.6153	9.3131	0.6559	9.2720
Starch	OS	5	0.6793	11.9012	0.6783	14.9456
	MSC	7	0.7306	11.1258	0.6822	14.9303
	SNV	3	0.6561	12.2447	0.6464	15.4035

2.3.3 Optimal prediction model selection

According to the results in Tables 2 and 3, the PLS prediction model based on the original spectrum was the best for nitrogen. The prediction accuracy of the PLS model based on the MSC pre-processing spectra was the best for total soluble sugar content and the LS-SVM model based on the MSC pre-processing spectra was the best for prediction of starch content. The optimal prediction method was selected from these different modeling methods (Table 4 and Figure 6). The correlation coefficient of the carbon-nitrogen prediction model based on the multispectral data of citrus canopy was higher than 0.6. The results suggested that it is feasible to estimate the carbon and nitrogen of citrus plants by the models of PLS and LS-SVM based on the spectrum of the plant canopy.

Table 4 Optimal modeling for prediction of carbon and nitrogen

Nutrient	Modeling method	Spectral preprocessing method	Latent Variables	R_C	*RMSECV*	R_P	*RMSEP*
Total nitrogen	PLS	OS	4	0.6780	0.1345	0.6469	0.1296
Soluble sugar	PLS	MSC	2	0.6763	8.6713	0.6398	8.8891
Starch	LS-SVM	MSC	7	0.7306	11.1258	0.6822	14.9303



a. Total nitrogen b. Soluble sugar c. Starch

Figure 6 Predicted and measured values of carbon and nitrogen content in citrus

4 Discussion

Low-altitude remote sensing based on UAV has the advantages of high efficiency, high image resolution, low cost, low risk and reusable, and it can obtain the multi-scale and temporal ground remote sensing data[9]. It provides us with a more efficient technique for the real-time monitoring in large areas. Citrus is an evergreen fruit tree and the branches in the canopy are more complex. The citrus branches can be divided into growing branches and bearing branches according to the nature; spring shoot, summer shoot, and autumn shoot according to the season[31]. Because the citrus canopy shoots are composed of mature leaves and young leaves in the growing season, it is difficult to simply extract the spectral information of citrus canopy for plant nutrient diagnosis by unmanned airborne remote sensing technology. In this experiment, the spectral information was respectively extracted from the mature leaves, young leaves and the whole canopy according to the characteristics of various types of leaves existing in the citrus canopy at the same time. The results showed that the spectral information of mature leaves from spring shoots is feasible for the diagnosis of carbon and nitrogen nutrients, it is probably related to a variety of physiological mechanisms that mature leaves may have fully developed and stable leaf internal structure. The spectral information of plants with different nutritional conditions is significantly different. The spectral information of young leaves only had a lower prediction accuracy of carbon and nitrogen content, because the growth and development of young leaves have not yet completed and the nutrient accumulation and the construction are still in progress. This study provided a reference for the research on the low-altitude remote sensing monitoring of citrus plant nutrition in the future.

In this experiment, the manual selection of the interest region from the remotely sensed image was used. It had a negative effect on obtaining better prediction accuracy and the rapid extraction of spectral information. This is also the key problem that needs to be addressed in future research.

Compared to the apple chlorophyll content monitoring model built by Fang et al.[32], the prediction accuracy of the remote sensing monitoring model built for citrus carbon and nitrogen content in this experiment is slightly higher, but it is slightly lower compared with those under the condition of leaf scale in the laboratory[33,34]. These differences may be caused by the influence of variable outdoor environmental conditions (illumination, temperature, humidity, etc.) on the spectral data acquisition. In addition, the surface sediments (dust, moss, etc.) of the leaves in the canopy could also affect the spectral reflectance. In this experiment, the spectral reflectance of plant canopy was used to estimate the nutrient status of citrus trees, and the canopy gap and the different illuminations among canopy leaves could also affect the prediction accuracy. Further research is needed to minimize the effects of these unfavorable factors and improve the accuracy of low-altitude remote sensing.

5 Conclusions

The feasibility of citrus plant nutrition diagnosis based on multispectral remote sensing was explored in this experiment. Multispectral information of citrus canopy was obtained and average spectral reflectance of the whole canopy, mature leaves and young leaves were extracted respectively. Spectral pre-processing methods and modeling methods were compared and the best methods were chosen to build the optimal prediction models for canopy leaf nitrogen and carbon content. The prediction accuracy of the PLS model based on the original spectrum was the best for total nitrogen content, the PLS prediction model based on the MSC pretreatment spectrum was the best for the content of soluble sugar and the LS-SVM model based on the MSC pretreatment spectrum was optimal for the prediction of starch content. The results of this experiment showed that the estimation of total nitrogen, soluble sugar and starch content of citrus plants was feasible by low-altitude remote sensing image of citrus canopy with appropriate spectral pretreatment and multivariate statistical analysis methods.

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