Postprint: Estimation of Chlorophyll Content in Flue-Cured Tobacco Leaves Based on UAV Hyperspectral Remote Sensing

Authors: Lai Jiazheng, Li Beibei, Cheng Xiang, Sun Feng, Chen Juting, Wang Jing, Zhang Qian, Ye Xiefeng

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Abstract

[Purpose/Significance] Leaf Chlorophyll Content (LCC) of flue-cured tobacco leaves is a crucial indicator characterizing photosynthesis, nutritional status, and growth vigor of flue-cured tobacco. The objective of this study is to efficiently and accurately estimate LCC of flue-cured tobacco at different growth stages. [Method] Taking Zhongyan 100 tobacco leaves as the research object, canopy reflectance data of flue-cured tobacco at six critical growth stages were collected using an unmanned aerial vehicle (UAV) equipped with a Resonon Pika L hyperspectral imager. Based on correlation analysis, 21 sensitive spectral indices for LCC were selected. By comparing the prediction accuracy of different spectral combinations and different regression analysis algorithms, an LCC regression estimation model based on multiple spectral index combinations was ultimately established. Five modeling methods were employed for LCC estimation: Unary Linear Regression (ULR), Multivariable Linear Regression (MLR), Partial Least Squares Regression (PLSR), Support Vector Regression (SVR), and Random Forest Regression (RFR). [Results and Discussion] The correlation between most spectral parameters and LCC reached extremely significant levels ($P<0.01$) at different growth stages. Compared with traditional vegetation indices, the newly combined spectral indices significantly improved the correlation with LCC. For the univariate LCC estimation model ULR, the univariate modeling accuracy was highest using the newly combined normalized spectral index and red ratio spectral index at 75 days after transplanting, with Coefficient of Determination (R2) and Root Mean Square Error (RMSE) values of 0.822 and 0.814, and 0.226 and 0.230, respectively. The prediction results of MLR, PLSR, SVR, and RFR modeling methods indicated that the RFR algorithm performed best in LCC estimation, where the R2 and RMSE for the validation set using data from 75 days after transplanting could reach 0.919 and 0.146. [Conclusion] This study constructed a reliable estimation model for LCC

of flue-cured tobacco leaves by analyzing the response patterns between multiple spectral indices and LCC of flue-cured tobacco, which can provide theoretical basis and technical support for LCC estimation of flue-cured tobacco leaves and growth monitoring of flue-cured tobacco.

Full Text

Abstract

[**Objective**] Leaf chlorophyll content (LCC) of flue-cured tobacco is a critical indicator for characterizing photosynthesis, nutritional status, and growth vigor. The objective of this study was to efficiently and accurately estimate LCC of flue-cured tobacco at different growth stages. [**Methods**] Using Zhongyan 100 tobacco leaves as the research object, canopy reflectance data of flue-cured tobacco at six key growth stages were collected using a UAV equipped with a Resonon Pika L hyperspectral imager. Sensitive spectral indices were screened based on correlation analysis, and by comparing the prediction accuracy of different spectral combinations and different regression algorithms, a regression estimation model based on multiple spectral index combinations was finally established. Five modeling methods were employed: unary linear regression (ULR), partial least squares regression (PLSR), multivariable linear regression (MLR), support vector regression (SVR), and random forest regression (RFR). [**Results and Discussions**] The results showed that for most growth stages, the correlation between spectral parameters and LCC reached extremely significant levels ($P<0.01$). Compared with traditional vegetation indices, the newly combined spectral indices significantly improved the correlation with LCC. In univariate modeling, the highest accuracy was achieved using the normalized spectral index at 75 days after transplanting, with coefficients of determination (R^2) of 0.822 and 0.814 for the modeling and validation sets, respectively. The prediction results of the five regression models showed that the RFR algorithm performed best in LCC estimation, with R^2 and root mean square error (RMSE) values of 0.891 and 0.205 for the modeling set, and 0.919 and 0.146 for the validation set at 75 days after transplanting. [**Conclusions**] This study analyzed the response patterns between multiple spectral indices and flue-cured tobacco LCC, constructing reliable LCC estimation models that provide theoretical basis and technical support for monitoring tobacco leaf development and growth.

Keywords: flue-cured tobacco; chlorophyll content estimation; unmanned aerial vehicle; spectral parameters; random forest regression; multivariable linear regression; partial least squares regression; support vector regression

1. Introduction

Chlorophyll is the primary photosynthetic pigment in crop leaves. Leaf chlorophyll content (LCC) is an important indicator reflecting crop photosynthetic capacity and canopy nutritional status. Tobacco is an important economic crop with leaves as the main harvest product, making chlorophyll content monitoring crucial. Traditional tobacco agricultural monitoring is limited by field management practices and production equipment, being time-consuming, labor-intensive, and inefficient. Compared with satellite remote sensing and ground-based hyperspectral manual collection methods, UAV remote sensing offers advantages of high resolution, fast efficiency, and wide coverage, leading to rapid development and large-scale application in crop growth monitoring.

Research has shown that remote sensing monitoring based on vegetation indices (VI) has spatiotemporal continuity, facilitating long-term monitoring of crop growth information and demonstrating high application value in chlorophyll estimation. Feng et al. analyzed the correlation between VI and wheat chlorophyll relative content (SPAD), using selected linear combination indices, plant biochemical indices, normalized difference indices, and green normalized difference indices as input variables for a partial least squares regression (PLSR) model to construct a SPAD-PLSR estimation model, achieving the best accuracy at the flowering stage with modeling and validation R^2 values of 0.78 and 0.81, respectively. Qiao et al. studied the response characteristics of LCC and vegetation indices in field maize under different coverage conditions, constructing canopy LCC estimation models using random forest (RF) and partial least squares methods. Tian et al. selected 28 vegetation indices highly correlated with crop chlorophyll and found that the PLSR model constructed from multiple spectral parameters provided the best estimation of cotton LCC.

Researchers have attempted to randomly combine two bands from hyperspectral data to form new combined spectral indices for retrieving crop growth information. Yin et al. used arbitrary two-band combination spectral indices to construct potato LCC estimation models, achieving better accuracy and stability compared to traditional vegetation indices, particularly with normalized difference spectral indices combining 586 and 498 nm and ratio spectral indices combining 586 and 462 nm. Jia et al. demonstrated that new combined spectral indices help identify sensitive bands and enhance correlation with leaf nitrogen content, showing reliable performance in regression models for nitrogen content in flue-cured tobacco leaves. In recent years, many scholars have combined machine learning algorithms to predict crop agronomic parameters, achieving satisfactory model accuracy and robustness. Chen et al. used optimal spectral indices under different spectral transformations combined with BP neural network and RF to construct winter wheat leaf area index prediction models, with RF outperforming BP neural network while effectively avoiding overfitting and underfitting. Wang et al. used random forest regression (RFR) to estimate winter wheat nitrogen nutrition index, obtaining an ideal model (R^2=0.79, RMSE=0.13). Yang et al. showed that RFR could significantly improve above-ground biomass estimation accuracy during potato tuber formation and expansion stages while reducing spectral data collinearity and redundancy.

Most published UAV remote sensing research focuses on field crops such as maize and winter wheat, with limited studies on flue-cured tobacco. Quantitative analysis of tobacco canopy LCC using hyperspectral data is of great significance and application value. To achieve rapid estimation of flue-cured tobacco LCC, this study utilized UAV-acquired hyperspectral images at six different growth stages after transplanting. Through screening spectral parameters and using unary linear regression (ULR), multivariable linear regression (MLR), and PLSR as traditional regression methods, along with SVR and RFR as machine learning methods, LCC estimation models for flue-cured tobacco were constructed to obtain optimal estimation models and provide technical support for rapid, non-destructive monitoring of field flue-cured tobacco growth information using UAV remote sensing.

2. Materials and Methods

2.1 Experimental Materials

The experimental field was located in a tobacco-growing area in Jia County, Pingdingshan City, Henan Province (112°14 ~113°45 E, 33°08 ~34°20 N), characterized by a temperate continental monsoon climate with moderate climate, abundant rainfall, annual sunshine rate of 53%, average temperature of 15.7°C, frost-free period of 220 days, and annual precipitation of 678.5 mm.

The flue-cured tobacco variety was Zhongyan 100, transplanted on May 8, 2022. All fertilizers were applied as base fertilizer at one time without additional topdressing. Five nitrogen fertilizer gradient levels were established: N0~N4 treatments of 0.0, 24.0, 48.0, 72.0, and 96.0 kg/hm^2, respectively, with three replications per treatment, totaling 15 plots, each approximately 144 m^2 (15.2 m × 9.5 m) as shown in Figure 1 [Figure 1: see original paper]. Other cultivation and management practices followed local high-quality tobacco cultivation methods.

2.2 Data Acquisition

2.2.1 UAV Hyperspectral Image Acquisition Images were acquired at six growth stages: 32, 48, 61, 75, 89, and 109 days after transplanting. Data collection was conducted under clear, windless, cloudless conditions at noon. A DJI M600 Pro hexacopter UAV was used with maximum payload of 5 kg, maximum takeoff weight of 15.5 kg, maximum horizontal flight speed of 18 m/s, and endurance of approximately 30 minutes. The UAV was equipped with a Resonon Pika L hyperspectral imager containing 150 spectral bands with main parameters listed in Table 1 . Ground sample collection was synchronized with UAV operations.

Measurements were conducted between 11:00–14:00 at the aforementioned growth stages. During UAV operation, target cloths were placed in the experimental field for radiometric calibration. Flight altitude was set at 100 m, flight speed at 3 m/s, minimum timed photo interval at 1.0 s, forward overlap at 80%, side overlap at 70%, lens facing vertically downward, and focal length at 17 mm.

2.2.2 Laboratory Determination of Flue-Cured Tobacco LCC Canopy leaves of flue-cured tobacco were sampled at six growth stages (32, 48, 61, 75, 89, and 109 days after transplanting). Three plants were randomly and destructively sampled from each plot, yielding 45 ground samples per data collection.

Canopy leaves were transferred to the laboratory for LCC determination. Fresh samples were collected using a 2 mm punch, mixed, and 0.1 g of fragments were weighed and immersed in 15 ml of 95% ethanol solution. After preparation, the extract was stored in a low-temperature, dark, sealed container for 24 hours of chlorophyll extraction in darkness. Following dark treatment, the leaves appeared white-green, and LCC (Ct) was measured using a Spectrumlab 722PC spectrophotometer by measuring absorbance at 665 and 649 nm. LCC was calculated using equations (1)-(3):

$$
\begin{aligned}
Ca &= 13.95 \times A_{665} - 6.88 \times A_{649} \\
Cb &= 24.96 \times A_{649} - 7.32 \times A_{665} \\
Ct &= (Ca + Cb) \times V/1000 \times W
\end{aligned}
$$

where Ca and Cb represent chlorophyll a and chlorophyll b concentrations (mg/L), V represents extract volume (mL), W represents leaf mass (g), and A_{665} and A_{649} are absorbance values at 665 and 649 nm, respectively.

2.3 Selection of Spectral Parameters

To reduce interference from environmental factors such as soil background and atmospheric moisture, most researchers utilize vegetation indices (VI) to enhance crop spectral features and achieve dimensionality reduction of hyperspectral data. This study selected spectral indices for constructing flue-cured tobacco LCC estimation models through two approaches: (1) based on 18 published vegetation indices sensitive to crop leaf LCC; (2) based on random combinations of any two bands in the 400-1000 nm wavelength range. Difference Spectral Index (DSI), Ratio Spectral Index (RSI), and Normalized Spectral Index (NDSI) were calculated, and correlation contour maps between the three spectral indices and LCC were generated. The selected spectral parameters are shown in Table 2 .

3. Results and Analysis

To ensure adequate model training while minimizing overfitting risk, tobacco sample data were randomly divided according to a 2:1 ratio of modeling to validation sets, with 45 samples per growth stage. First, correlation analysis was performed between selected spectral parameters and LCC to screen spectral indices sensitive to LCC. The screened spectral parameters were then used as independent variables. Referencing models selected in other UAV hyperspectral crop growth estimation studies and advantages of common machine learning models, three traditional linear models (ULR, MLR, PLSR) and two machine learning methods (SVR, RFR) were employed to estimate LCC for individual growth stages and the entire growth period.

Model accuracy and robustness were evaluated using coefficient of determination (R^2) and root mean square error (RMSE).

3.1 Changes in Flue-Cured Tobacco LCC

Table 3 describes LCC variation in flue-cured tobacco canopy at different growth stages (laboratory measurements). Results showed that canopy LCC increased rapidly during early growth stages, peaking at the rosette or rapid growth stage. After entering the maturity stage, nitrogen transferred from canopy leaves and LCC began to decrease, with further reduction at the budding stage. Overall, canopy LCC exhibited an initial increase followed by a decrease, reaching maximum at 75 days after transplanting (rapid growth stage) before gradually declining. Among growth stages, the coefficient of variation was highest at 89 days after transplanting (28.43%), with other stages ranging from 20.38% to 25.26%, all lower than that of the entire growth period.

The entire growth period sample ranged from 0.52 to 2.95 mg/g, with standard deviation and coefficient of variation values indicating large dispersion in LCC, reflecting fertility differences between treatments and ensuring model applicability within a certain range.

3.2 Correlation Analysis Between Flue-Cured Tobacco LCC and Spectral Parameters

3.2.1 Correlation Between Vegetation Indices and Flue-Cured Tobacco LCC Correlation analysis between LCC and vegetation indices across six growth stages is presented in Table 4 . Except for 109 days after transplanting, most vegetation indices showed extremely significant correlation with LCC ($p<0.01$) and were largely positively correlated. At 32 days after transplanting, all indices except TCARI and PPR showed extremely significant correlation, with NDRE showing the maximum correlation coefficient of 0.771. At 48 days after transplanting, TCARI and PPR showed no significant correlation, TCARI/OSAVI showed significant correlation ($p<0.05$), while others were extremely significantly correlated, with mND705 as the optimal index (r=0.779). From 61 to 89 days after transplanting, all vegetation indices were extremely significantly correlated, with PPR, TCARI, and TCARI/OSAVI showing extremely significant negative correlation. The highest-correlation indices differed across these three stages: mND705 at 61 days (r=0.870), NIR at 75 days (r=0.902), and TCARI/OSAVI at 89 days (r=0.877). At 109 days after transplanting, PPR, TCARI, and TCARI/OSAVI showed extremely significant negative correlation, REP showed significant correlation, while other indices showed no significant correlation. In the entire growth period dataset, all vegetation indices were extremely significantly correlated with LCC, with VOG showing the highest correlation (r=0.771).

Vegetation indices using red-edge position and near-infrared bands, such as NDRE, CIre, RNDVI, VOG, and mND705, showed correlation coefficients above 0.67 across growth stages (except 109 days), all extremely significantly correlated. These indices demonstrate good application potential for LCC estimation modeling, effectively improving model accuracy and robustness. The sensitivity of VIs to LCC is closely related to crop growth stage, and determining this sensitivity at different stages is crucial for crop parameter estimation using UAV hyperspectral imagery.

3.2.2 Correlation Between Combined Spectral Indices and Flue-Cured Tobacco LCC Using arbitrary two-band combinations in the 400-1000 nm range, DSI, RSI, and NDSI were calculated and correlated with leaf chlorophyll content. Correlation contour maps were generated for individual growth stages and the entire growth period (Figures 2 [Figure 2: see original paper] and 3 [Figure 3: see original paper]). Results showed that except for 109 days after transplanting, sensitive bands at each stage were relatively concentrated, with highly correlated spectral index combinations mainly distributed between 780-940 nm and 520-710 nm. At 109 days, performance was poorer than other stages. Among the three combined spectral indices, normalized difference spectral indices showed the highest correlation with LCC at 32, 48, 61, 75, and 109 days after transplanting, with optimal band combinations of (797, 719 nm), (727, 697 nm), (736, 706 nm), (775, 745 nm), and (455, 541 nm), respectively, with correlation coefficients of 0.795, 0.805, 0.878, 0.912, and 0.669. Difference spectral indices showed highest correlation at 89 days, with DSI(R587,R579) achieving r=0.892. Ratio spectral indices showed maximum correlation in the entire growth period dataset, with RSI(R736,R723) achieving r=0.807.

Compared with traditional vegetation indices, combined spectral indices showed substantially improved correlation with LCC. Sensitive bands for the entire growth period were relatively dispersed, with minimal difference in sensitive band positions between different spectral indices. Based on the principle of maximizing correlation with LCC, 21 optimal combined spectral indices were selected for different growth stages and the entire growth period (Table 5). All selected spectral indices passed significance tests at the 0.01 level.

3.3 Construction of Flue-Cured Tobacco LCC Estimation Models

3.3.1 Single-Variable LCC Estimation Models Based on correlation analysis between leaf chlorophyll and spectral parameters, the highest-correlation vegetation indices (NDRE, mND705, NIR, TCARI/OSAVI, PPR, VOG) and spectral indices (NDSI(R797,R719), NDSI(R727,R697), NDSI(R736,R706), NDSI(R775,R745), DSI(R587,R579), RSI(R455,R541), RSI(R736,R723)) were selected to establish ULR models with tobacco leaf chlorophyll. Estimation results are shown in Table 6 .

Combined spectral indices generally outperformed traditional vegetation indices in estimation accuracy across growth stages and the entire growth period, though performance varied between stages. From 32 to 75 days after transplanting, accuracy of both modeling and validation sets gradually increased. NIR at 75 days showed the best vegetation index performance, with modeling and validation R^2 of 0.814 and 0.829, and RMSE of 0.230 and 0.253, respectively. NDSI(R775,R745) at 75 days showed the best combined spectral index performance, with modeling and validation R^2 of 0.822 and 0.862, and RMSE of 0.226 and 0.227, respectively. Accuracy began declining from 75 to 109 days, with both vegetation indices and combined spectral indices performing worst at 109 days. The entire growth period dataset (270 samples from different stages) showed relatively poor performance, with VOG achieving modeling and validation R^2 of 0.602 and 0.558, and RMSE of 0.348 and 0.349, respectively, while RSI(R736,R723) achieved modeling and validation R^2 of 0.636 and 0.686, and RMSE of 0.333 and 0.304, respectively.

3.3.2 Multi-Variable LCC Estimation Models Studies on crop physiological and biochemical parameter estimation based on hyperspectral remote sensing show that using multiple spectral parameters as independent variables significantly improves model accuracy and robustness compared to single-parameter models. However, due to collinearity between independent variables, appropriate variable selection is necessary to prevent overfitting. Therefore, the seven highest-correlation vegetation indices and three combined spectral indices from each growth stage and the entire growth period dataset were selected as independent variables for MLR, PLSR, SVR, and RFR modeling. Model performance is shown in Table 7 .

Compared with single-parameter models, multi-parameter models showed substantial accuracy improvements across all growth stages and the entire growth period. The LCC-RFR model performed best at 32, 48, 61, and 109 days (modeling R^2: 0.733, 0.744, 0.866, 0.678; RMSE: 0.186, 0.165, 0.176, 0.120), while LCC-MLR performed best at 75 and 89 days (modeling R^2: 0.893, 0.850; RMSE: 0.214, 0.217). For validation sets, LCC-MLR performed best at 32 days (R^2=0.679, RMSE=0.215), while LCC-RFR performed best at the other five stages (validation R^2: 0.757, 0.832, 0.919, 0.842, 0.580; RMSE: 0.173, 0.185, 0.146, 0.246, 0.131). In the entire growth period dataset, LCC-RFR outperformed LCC-MLR, LCC-PLSR, and LCC-SVR, with modeling R^2 increasing by 19.06%, 18.62%, and 29.51%, and RMSE decreasing by 31.93%, 29.51%, and 28.24%, respectively, compared to these models. Validation R^2 increased by 8.21%, 12.62%, and 8.17%, while RMSE decreased by 3.76%, 9.33%, and 4.55%, respectively.

Overall, LCC-RFR demonstrated the best estimation performance, followed by LCC-MLR and LCC-SVR, with LCC-PLSR performing worst. Model prediction accuracy and stability across growth stages ranked from highest to lowest as: 75, 61, 89, 48, 32, and 109 days after transplanting.

3.3.3 Spatial Distribution Maps of Flue-Cured Tobacco LCC Based on the LCC-RFR model (which showed optimal accuracy and stability), UAV hyperspectral images from three growth stages (48, 61, and 75 days after transplanting) were used to generate LCC spatial distribution maps after removing soil background and weed interference (Figure 4 [Figure 4: see original paper]). Overall, color depth increased from 48 to 75 days, indicating increasing LCC. Differences were observed between replications, with growth vigor ranking as: replication 3 > replication 2 > replication 1. At 45 days, LCC ranged from 1.4–1.9 mg/g. From 61 days, differences between replications became more pronounced, with replication 1 mostly in the 1.8–2.3 mg/g range, while replications 2 and 3 were mostly in the 2.1–2.5 mg/g range. At 75 days, LCC distribution was dense overall, ranging from 2.2–3.1 mg/g, consistent with tobacco growth characteristics where LCC increases from early growth to rapid growth stage. The spatial distribution of LCC retrieved from UAV hyperspectral imagery matched measured results, demonstrating that UAV-borne hyperspectral imagers can provide high-resolution, high-precision spatial distribution information of LCC at small scales, effectively distinguishing growth status at different stages.

4. Conclusions

This study explored the response patterns between flue-cured tobacco LCC and UAV hyperspectral imagery in central Henan tobacco-growing areas. Through correlation analysis between multiple spectral indices and LCC, ULR, MLR, PLSR, SVR, and RFR models were constructed for estimating tobacco leaf chlorophyll content, yielding the following conclusions:

1. Most vegetation indices showed extremely significant correlation with LCC. The highest-correlation vegetation indices at 32–109 days after transplanting were NDRE, mND705, mND705, NIR, TCARI/OSAVI, and REP, respectively. In the entire growth period dataset, all vegetation indices were extremely significantly correlated with LCC, with VOG showing the highest correlation (r=0.771).

2. Based on the principle of maximizing correlation between randomly combined two-band spectral indices and LCC, 21 optimal spectral indices were selected for different growth stages and the entire growth period. Sensitive bands for both traditional vegetation indices and combined spectral indices were mostly concentrated in the red-edge region, but combined spectral indices showed superior overall correlation. NDSI(R775,R745) and RSI(R775,R745) at 75 days after transplanting showed the highest correlation (r=0.912).

3. Based on correlation analysis between leaf chlorophyll and spectral parameters, the highest-correlation spectral parameters for six growth stages and the entire growth period were selected to establish ULR models with tobacco leaf chlorophyll. Combined spectral indices outperformed traditional vegetation indices in estimation accuracy and stability across all

growth stages and the entire growth period.

4. Compared with single-parameter models, multi-parameter models showed substantial accuracy improvements across all growth stages and the entire growth period. At 75 days after transplanting, the LCC-RFR model based on multiple spectral parameters performed best, with modeling and validation R^2 of 0.891 and 0.919, and RMSE of 0.205 and 0.146, respectively. Overall, the RFR model demonstrated the most ideal LCC estimation performance, followed by MLR and SVR, with PLSR performing worst. Model prediction accuracy and stability across growth stages ranked from highest to lowest as: 75, 61, 89, 48, 32, and 109 days after transplanting.

Conflict of Interest Statement: The authors declare no conflicts of interest.

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