# MONITORING RATIO OF LEAF CARBON TO NITROGEN IN WINTER WHEAT BASED ON HYPERSPECTRAL MEASUREMENTS

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## ABSTRACT

Ratio of carbon to nitrogen (C/N) in leaves, defined as the ratio of LCC (leaf carbon concentration) to LNC (leaf nitrogen concentration), is a good indicator for synthetically diagnosing the balance of carbon and nitrogen, nutrient status, and growth vigor in crop plants. So it is very significant for effective diagnosis and dynamic regulation of crop growth in field to monitor changes of leaf C/N quickly and accurately. Considering the close relationships between chlorophyll, nitrogen (N) and C/N, five typical indices aimed at N estimation were tested to estimate C/N in winter wheat as well as five indices aimed at chlorophyll evaluation in this study. The multi-temporal hyperspectral data from the four stages (flag-leaf, anthesis, filling, and milk-ripe) of winter wheat were obtained to calculate these selected spectral indices for evaluating C/N in winter wheat. The results showed that some tested indices were able to estimate leaf C/N in winter wheat, especially the spectral indices, *MCARI/OSAVI2* and *MTCI* had the better performance of estimating C/N with $R^2$ of 0.51 and 0.49, RMSE of 1.35 and 1.39, respectively. In order to improve the accuracy of C/N estimates, PLS (Partial Least Squares) was used to estimate C/N in winter wheat, and the analyses showed that a better accuracy with $R^2$ of 0.60 and RMSE of 1.23 was obtained when using PLS. It indicates that applying hyperspectral reflectance measurements for monitoring leaf C/N in winter wheat appears very potential.

**Keywords**: Winter wheat, Spectral vegetation index, Leaf C/N, PLS

## INTRODUCTION

Ratio of carbon to nitrogen (C/N) in leaves, defined as the ratio of LCC (leaf carbon concentration) to LNC (leaf nitrogen concentration), has significant influence on the ultimate formation of yield and quality in crop production (Hucklesby et al., 1971; Woodard and Bly, 1998; Guo et al., 2005), and is a good indicator for synthetically diagnosing the balance of carbon and nitrogen, nutrient status, and growth vigor in crop plants(Jin et al., 1994). Thus, it is very important for dynamic regulation of crop growth in field to monitor changes of leaf C/N quickly and accurately.

The traditional methods of determinating crop C or N status have relied mainly on plant sampling and analytical equipments, usually spend much time and energy cost (Roth and Fox, 1989). In contrast, hyperspectral remote sensing with hundreds of very narrow spectral bands can detect the subtle changes of biochemical components (such as leaf chlorophyll content, and nitrogen status) in crops, and show the advantages of non-destructive and quick detection. Currently, there are more reports on assessments of N status based on hyperspectral data, but few for C/N estimates. Shi et al. (2003) reported that there existed a high correlationship between N and C/N in vegetations, and some studies indicated that there was a close relationship between N and chlorophyll (Yoder et al., 1995; Botha et al., 2006), thus there should be related each other between N, chlorophyll and C/N. Since some spectral indices have been used to estimate chlorophyll and N, it is possible that C/N in crops may be assessed by these indices. In this study, five typical indices aimed at N estimation were tested to estimate C/N in winter wheat as well as five indices aimed at chlorophyll evaluation. In addition, PLS (Partial Least Squares) was used to evaluate leaf C/N in winter wheat for improving the accuracy.

## DATA AND METHODs

### Study area and data acquisition

The study area is situated in National Experiment Station for Precision Agriculture (40˚10.6′N, 116˚26.3′E) in northeast of Beijing, China. This experiment station has been operational since 2001 and used for precision agriculture research. In this study, the experimental data were collected at 30 plots in 6 fields (5 plots each field) from the four growth stages (flag-leaf, anthesis, filling, and milk-ripe), and the collection date was on April 28$^{th}$, May 10$^{th}$, 21$^{th}$ and 31$^{th}$, 2012, respectively. In these experimental fields, the four cultivars of winter wheat, Jingdong 8 (2 fields), Zhongmai175, Nongda 211 (2 fields), Jing9843 were sown.

Data acquisition included canopy spectral reflectance measurements in fields and the determination of LNC indoors. Data acquisition included canopy spectral reflectance measurements in fields and the determination of LNC indoors. An ASD spectrometer (FieldSpec Pro FR2500, Anaytical Spectral Devices, Inc., USA) that operates in a spectral range from 350nm to 2500nm was utilized to measure canopy spectral reflectance in winter wheat fields. When measuring canopy spectral reflectance, twenty representative wheat plants from each sample plot were collected for determination of Leaf C/N. All green leaves as separated from the plants indoors were de-enzymed at 105℃, then oven-dried at 80℃ to constant weight for chemical analysis. Leaf N and C content (g 100 g-1, %) measurements from the dried leaf samples were performed by using an elemental analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Germany).

### spectral indices

In this study, some typical spectral indices considered to be good candidates for evaluating N and chlorophyll status were selected to test their capabilities of estimating Leaf C/N in winter wheat (Table 1). Among these indices, ones aiming at N estimation such as *DCNI*, *VIopt*, RVI2, *MCARI/MTVI2*, *REP-le* are tested, and the other as the better indicators of assessing pigments, especially chlorophyll, such as *MCARI/OSAVI2*, *MCARI*, *TCARI/OSAVI*, *R-M*, and *MTCI* are also utilized to evaluate C/N in winter wheat.

**Table 1. Spectral indices used in this study**

| Spectral indices | Formulas | References |
|---|---|---|
| *MCARI/OSAVI2*# | *MCARI2*: $[(R_{750}-R_{705})-0.2(R_{750}-R_{550})](R_{750}/R_{705})$<br>*OSAVI2*: $(1+0.16)(R_{750}-R_{705})/(R_{750}+R_{705}+0.16)$ | Wu et al., 2008 |
| *MCARI* | $[(R_{700}-R_{670})-0.2(R_{700}-R_{550})](R_{700}/R_{670})$ | Daughtry et al., 2000 |
| *TCARI/OSAVI* | *TCARI*: $3[(R_{700}-R_{670})-0.2(R_{700}-R_{550})(R_{700}/R_{670})]$<br>*OSAVI:* $1.16(R_{800}-R_{670})/(R_{800}+R_{670}+0.16)$ | Haboudane et al., 2002 |
| *R-M* | $R_{750}/R_{720}$ -1 | Gitelson et al., 2005 |
| *MTCI* | $(R_{750}-R_{710})/(R_{710}-R_{680})$ | Dash et al., 2004 |
| *DCNI* | $(R_{720}-R_{700})/(R_{700}-R_{670})/(R_{720}-R_{670}+0.03)$ | Chen et al., 2010 |
| *VIopt* | $(1+0.45)(R_{800}^{2}+1)/(R_{670}+0.45)$ | Reyniers et al., 2006 |
| *RVI2*# | $R_{810}/R_{560}$ | Xue et al., 2004 |
| *MCARI/MTVI2* | *MCARI*: $[(R_{700}-R_{670})-0.2(R_{700}-R_{550})](R_{700}/R_{670})$<br>*MTVI2*: $1.5[1.2(R_{800}-R_{550})-2.5(R_{670}-R_{550})]/\mathrm{sqrt}[(2R_{800}+1)^{2}-(6R_{800}-5\mathrm{sqrt}(R_{670}))-0.5]$ | Eitel et al., 2007 |
| *REP-le* | Red edge position based on linear extrapolation method | Cho et al., 2006 |

# Denotes named by this study.

### Partial least squares regression

Partial least squares (PLS) regression is an extension of the multiple linear regression model, which integrates the functions of MLR (multiple linear regression), PCA (principal component analysis) and CCA(canonical correlation analysis) together. This method is particularly useful when one needs to predict a set of dependent variables from a large number of independent variables. In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable *y* and a set of predictor variables, the *x* variables, so that

$$y = a_0+a_1x_1 +a_2x_2 + \cdots + a_nx_n$$

In this equation, $a_0$ is the regression coefficient for the intercept, and the $a_i$ values are regression coefficients (for variables 1 to n) computed from the data.

## RESULT AND ANALYSIS

### Relationship of leaf C/N to spectral indices

After extracted from spectral reflectance measured from the four growth stages (i.e. flag-leaf, anthesis, filling, and milk-ripe) in winter wheat fields, the ten spectral indices in Table 1 were related with Leaf C/N to find the sensitive indices.

Linear, logarithm, and exponential model were used to make fit, and the best fitting with highest $R^2$ and lowest RMSE were expressed, Table 2 showed the results of regression analysis between spectral indices and leaf C/N in winter wheat. It could be seen that some tested indices could be used to evaluate leaf C/N in winter wheat. Especially, the two indices, *MCARI/OSAVI2* and *MTCI*, had the better performance of estimating C/N in winter wheat, with $R^2$ of 0.51 and 0.49, RMSE of 1.35 and 1.39, respectively. Fig. 1 exhibited the plotted relationships of C/N to the two spectral indices.

**Table 2. Results of regression analysis between spectral indices and Leaf C/N in winter wheat (n=120).**

| Spectral indices | $R^2$ | RMSE | Spectral indices | $R^2$ | RMSE |
|---|---|---|---|---|---|
| *MCARI/OSAVI2* | 0.51 | 1.35 | *DCNI* | 0.11 | 1.83 |
| *MCARI* | 0.00 | 1.94 | *VIopt* | 0.01 | 1.93 |
| *TCARI/OSAVI* | 0.08 | 1.86 | *RVI2* | 0.29 | 1.63 |
| *R-M* | 0.39 | 1.52 | *MCARI/MTVI2* | 0.28 | 1.65 |
| *MTCI* | 0.49 | 1.39 | *REP-le* | 0.47 | 1.42 |

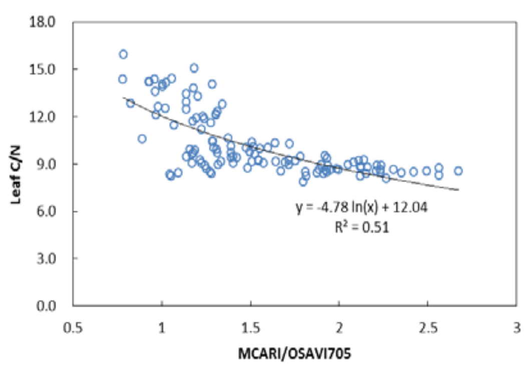


**Fig. 1. Regression relationships of C/N to the two spectral indices *MCARI/OSAVI2* and *MTCI* (n=120).**

## Leaf C/N estimates based on PLS

In order to improve the accuracy of estimating leaf C/N in winter wheat, PLS was utilized to evaluate C/N with using the above tested indices as independent variables. The analyses showed that in comparison with any tested spectral index for leaf C/N estimates, PLS had the best estimation with $R^2$ and RMSE value, 0.60 and 1.23, respectively. Fig. 2 exhibited the plotted relationships of the observed C/N to the estimated value based on PLS method.

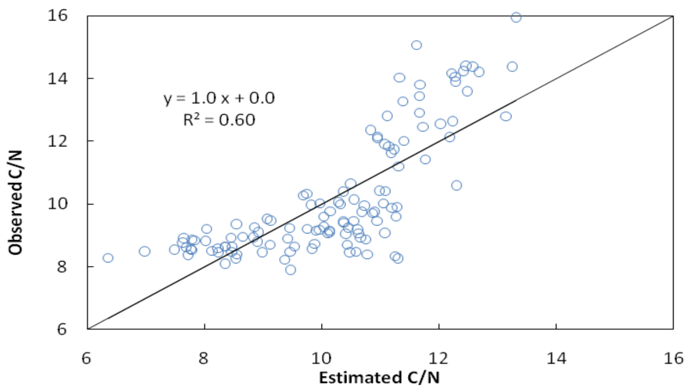


**Fig. 2. Correlationship between the observed C/N to the estimated values base on PLS (n=120).**

## CONCLUSION

In this study, the ten typical spectral indices aimed at N or chlorophyll estimation were tested to evaluate leaf C/N in winter wheat by the measurements from the four stages of four wheat cultivars. The results showed that some tested spectral indices could be used to assess leaf C/N in winter wheat, especially the two indices, *MCARI/OSAVI2* and *MTCI* had better performance of estimating leaf C/N. Based on these spectral indices, PLS (Partial Least Squares) could effectively improve the estimating accuracy of leaf C/N in winter wheat with $R^2$ and RMSE, 0.60 and 1.23, respectively. It indicates that hyperspectral technology is a valuable tool to monitor leaf C/N in winter wheat.

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