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EVALUATING THE CONTRIBUTION OF C_X TO LEAF NITROGEN QUANTIFICATION USING FLUSPECT AND AIRBORNE IMAGING SPECTROSCOPY IN ALMOND ORCHARDS

*Y. Wang [1, *], L. Suarez [1, 2], D. Ryu [1], P.J. Zarco-Tejada [2, 1, 3]*
** E-mail address: wang.y@unimelb.edu.au*

[1] Department of Infrastructure Engineering, Faculty of Engineering and Information Technology (FEIT), University of Melbourne, Melbourne, VIC 3010, Australia
[2] School of Agriculture, Food and Ecosystem Sciences (SAFES), Faculty of Sciences (FoS), University of Melbourne, Melbourne, VIC 3010, Australia
[3] Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Cientificas (CSIC), Avenida Menendez Pidal s/n, 14004 Cordoba, Spain

ABSTRACT

Among all essential nutrients, nitrogen (N) is required by plants in large quantities throughout the entire developmental process. This is due to its importance for plant growth and development and as a primary source of energy for photosynthesis. Previous research has demonstrated that solar-induced chlorophyll fluorescence (SIF) coupled with chlorophyll *a+b* content (C_{ab}) improved the estimation of leaf N, outperforming standard vegetation indices. The present study investigates the contribution of leaf C_x, a measure of the de-epoxidation state of the xanthophyll cycle, for explaining leaf N variability, concluding that it ranks third after C_{ab} and SIF consistently over two growing seasons. Among the rest of the biochemical constituents estimated by model inversion, C_x contributed more than anthocyanins (Anth), the total carotenoid content (C_{car}), and crown-level structural traits.

Index Terms — C_x, airborne hyperspectral, nitrogen, xanthophyll cycle, de-epoxidation, PRI

1. INTRODUCTION

Nitrogen (N) is one of the major nutrients taken up during active plant growth and plays a significant role in preserving high fruit quality and yield [1, 2]. Consequently, a precise and sustainable agricultural management strategy in almond orchards requires an accurate leaf N status assessment in order to fine-tune fertilizer applications.

Conventional remote sensing (RS) methods to assess leaf N rely on empirical algorithms involving chlorophyll-sensitive vegetation indices (VIs) calculated from spectral bands in the visible, red-edge, and near-infrared regions, such as $CI_{red\text{-}edge}$ [3], TCARI/OSAVI [4], NDRE [5], and CCCI [6] among others. Additionally, the PRI family of indices, which involves 2-3 spectral bands in the green region, is sensitive to changes in xanthophyll pigments composition and has been proposed as a proxy for photosynthesis rate through light-use efficiency [7-9], therefore being suggested as N-induced stress indicators [10, 11].

As alternatives to VI-based methods, a number of studies have focused on the estimation of leaf N using models based on plant traits, such as chlorophyll [12] content derived through radiative transfer model (RTM) inversion [13, 14]. The C_x parameter in the Fluspect-Cx RTM [15] tracks the dynamics of the de-epoxidation state of the xanthophyll cycle, thus receiving considerable attention in recent years. The model assessment of the xanthophyll epoxidation is based on in vivo absorption coefficients for two extreme states of the carotenoid [16] pool, corresponding to the two states of xanthophyll de-epoxidation and describes the intermediate states as a lineal mixture of these two extreme states.

Recent advances have proposed models with leaf biochemistry and dynamic spectral traits linked to photosynthesis, such as solar-induced fluorescence (SIF), to explain the leaf N variability. SIF has been demonstrated as a plant stress indicator and proxy for leaf N content in various crop species. In a recent study, SIF was found to improve the leaf N estimation in almonds [17], concluding that C_{ab} and SIF were the two most important predictors for leaf N content. As a step forward, we investigate the potential contribution of several plant traits linked to photosynthesis to assess leaf N variability in almond orchards, particularly the xanthophyll pigments.

2 MATERIALS AND METHODS

2.1. Study area and field data collection

The study site consists of a 1,200-hectare commercial almond orchard (see Fig. 1a for an overview of the orchard in a false-color composite image) in Robinvale, northwest Victoria, Australia, with a Mediterranean climate. An almond tree planting program was undertaken in 2006 (northern blocks oriented N-S) and 2007 (southern blocks with mixed N-S and E-W orientations. Fig. 1b), including varieties of Nonpareil, Price, and Carmel. A drip fertigation system is used to supply nutrients, with one-hour intervals between rows of trees. Fertigation is adjusted based on previous year observations, resulting in different application rates between varieties.

The field collection of leaf samples and ground data measurements were conducted at the pre-harvest stage for two growing seasons, 2019-2020 (March 2020) and 2020-2021 (February 2021). Fifteen monitoring plots were sampled throughout the orchard, averaging two Nonpareil trees and two Carmel trees per plot. As part of the measurement process, 20 fully exposed mature leaves per tree were measured for leaf C_{ab}, anthocyanins (Anth), flavonol content, and the nitrogen balance index (NBI) using a Dualex 4 Scientific instrument (FORCE-A, Orsay, France). We also determined leaf steady-state chlorophyll fluorescence (Ft) and leaf reflectance spectra within the visible and near-infrared (VNIR) region with FluorPen FP 110 and PolyPen RP 400 instruments (PSI, Brno, Czech Republic), respectively. Moreover, 20 additional leaves were sampled per plot for laboratory nutrient analysis using a LECO Nitrogen analyzer (LECP Corporation, MI, USA).

2.2. Acquisition of airborne hyperspectral imagery

Airborne campaigns were carried out within a week of each field campaign. The piloted aircraft, operated by the HyperSens Laboratory at The University of Melbourne, was equipped with a hyperspectral line-scanning sensor (Micro-Hyprspec VNIR model, Headwall Photonics, Fitchburg, MA, USA) with 5.8 nm FWHM covering 371 spectral bands over the VNIR region. The flights' height at 550 m above ground level yielded a spatial resolution of 40 cm, enabling the identification of each tree crown and shaded features. Image pre-processing and calibration were performed following the method in [18]. Consequently, image mosaics of reflectance and radiance were derived over the orchard.

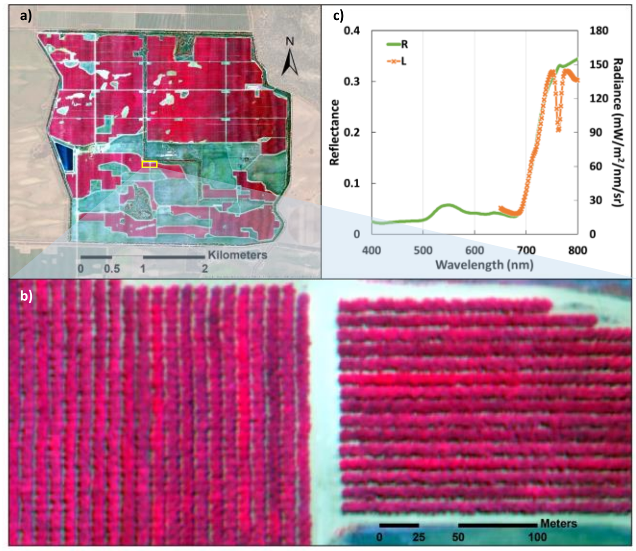

Fig. 1. (a) Colour-infrared overview of the airborne hyperspectral image acquired over a study area of 1,200 hectares at a 40-cm spatial resolution using 371 visible and near-infrared spectral bands. (b) Zoomed view of the planting blocks for almond rows that are oriented east-west and north-south. (c) Sample reflectance (R, green colour) and radiance (L for SIF calculation, orange colour) spectrum extracted from the airborne hyperspectral image.

2.3. SIF quantification and plant traits estimation

Based on pure sunlit vegetation pixels extracted from radiance image, SIF was quantified by the Fraunhofer Line Depth (FLD) method [19] from O_2-A oxygen absorption features at 762 nm. The reflectance mosaic was used to extract spectra from tree crowns used for the calculation of vegetation indices and the inversion of plant traits from RTM. C_x, along with other biochemical constituents (e.g., C_{ab}, C_{car}, and Anth), and structural traits (e.g., LAI) were retrieved simultaneously by constructing a 10-hidden layer artificial neural network (ANN) based on 500,000 simulations using the coupled Fluspect-Cx and 4SAIL model [20].

2.4. Nitrogen prediction model assessment

As part of a previous two-year validation study performed in the orchard, C_{ab} and SIF were identified as the most critical plant traits for leaf N estimation [17]. With the retrieved plant traits, Gaussian process regression models were constructed for each year incorporating single plant traits (i.e., C_{car}, C_x, Anth, LAI) in addition to C_{ab} and SIF. The training and testing steps were performed using leave-one-out cross-validation. Furthermore, the variance inflation factor (VIF) and out-of-bag predictor importance were employed to assess the input collinearity and relative contribution of the inputs, respectively.

3. RESULTS

The xanthophyll pigment-related indices extracted from tree crowns were highly correlated with leaf N, in particular, PRI (r^2 = 0.48, p-value < 0.005 in 2020, and r^2 = 0.27, p-value < 0.05 in 2021), and PRI_{m4} (r^2 = 0.34, p-value < 0.05 in 2020, and r^2 = 0.50, p-value < 0.005 in 2021). The RTM-derived parameter C_x, however, exhibited a superior and consistently significant relationship with leaf N for both years (r^2 = 0.61 in 2020 and r^2 = 0.62 in 2021; p-values < 0.005). Relationships were obtained between C_x vs. leaf-measured PRI_{m4} (r^2 = 0.48 in 2020 and r^2 = 0.46 in 2021; p-values < 0.005) and airborne-derived PRI_{m4} (r^2 = 0.50 in 2020 and r^2 = 0.42 in 2021; p-values < 0.01, Fig. 2).

Based on the relative contribution of each input to leaf N estimation, C_x was demonstrated as the best non-collinear (VIF<10) predictor after C_{ab} and SIF. Moreover, the model incorporating C_x along with C_{ab} and SIF (e.g., RMSE = 0.079% in 2020+2021) outperformed the model built with C_{ab} and SIF alone (e.g., RMSE = 0.092% in 2020+2021). With a model consisting of C_{ab}, C_x, and SIF (N = $f(C_{ab}, C_x, \text{SIF})$: r^2 = 0.86 in 2020, r^2 = 0.65 in 2021, and r^2 = 0.87 in 2020+2021, Fig. 3), leaf N variability was better explained than any other model combinations for each individual year and when combining the two years together. These results suggest that the RTM-derived C_x estimated from airborne hyperspectral imagery is an important predictor for leaf N assessment in almond orchards, improving the model performance when coupled to C_{ab} and SIF.

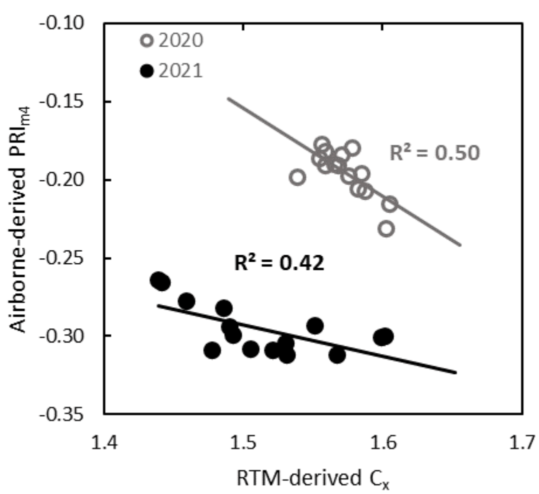

Fig. 2. Relationships between RTM-derived C_x and airborne-derived PRI_{m4} in 2020 (hollow grey circle) and 2021 (solid black circle). All p-values < 0.01.

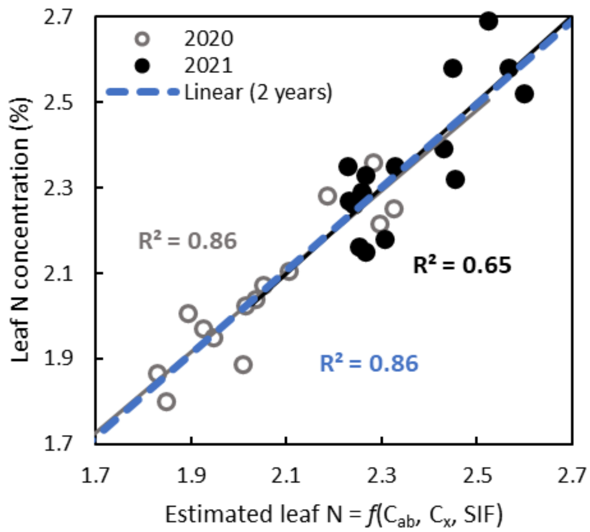

Fig. 3. Relationships between leaf N concentration (%) and predicted leaf N using models based on chlorophyll content, C_x, and SIF. The blue dashed line represents correlation when combining data from 2 years. All p-values < 0.005.

4. CONCLUSIONS

This study demonstrates that the RTM-derived C_x parameter, an indicator of the xanthophyll pigments cycle, ranked third behind C_{ab} and SIF when explaining the observed variability of leaf N in almond orchards. The leaf N prediction model that incorporated C_x in addition to C_{ab} and SIF was found to outperform any other combinations of plant traits over the course of two years. Other leaf biochemical constituents such as anthocyanins (Anth), the total carotenoid content (C_{car}), dry matter (C_{dm}), and structural traits yielded lower contributions when explaining the leaf N variability in almond orchards.

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