12-2013

Remote estimation of nitrogen and chlorophyll contents in maize at leaf and canopy levels

Michael Schlemmer
Bayer Crop Science, michael.schlemmer@bayer.com

Anatoly A. Gitelson
University of Nebraska-Lincoln, agitelson2@unl.edu

James S. Schepers
USDA-ARS, Lincoln, NE, james.schepers@gmail.com

Richard B. Ferguson
University of Nebraska-Lincoln, rferguson1@unl.edu

Y. Peng
University of Nebraska-Lincoln

See next page for additional authors

Published in *International Journal of Applied Earth Observation and Geoinformation* 25 (December 2013), pp. 47–54;
doi: 10.1016/j.jag.2013.04.003

Submitted 5 March 2013; revised 4 April 2013; accepted 5 April 2013; published online 7 May 2013.

Remote estimation of nitrogen and chlorophyll contents in maize at leaf and canopy levels

M. Schlemmer,[1] A. Gitelson,[2] J. Schepers,[1] R. Ferguson,[1] Y. Peng,[2] J. Shanahan,[1] and D. Rundquist[2]

1 Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
2 CALMIT, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Corresponding author — A. Gitelson, School of Natural Resources, University of Nebraska-Lincoln, 303 Hardin Hall, Lincoln, NE 68583, USA; tel 402 472 8386, email agitelson2@unl.edu

M. Schlemmer is currently with Bayer CropScience, Lincoln, NE 68583, USA.
J. Shanahan is currently with Pioneer Hi-Bred International, Johnston, IA 50131, USA.

Abstract
Leaf and canopy nitrogen (N) status relates strongly to leaf and canopy chlorophyll (Chl) content. Remote sensing is a tool that has the potential to assess N content at leaf, plant, field, regional and global scales. In this study, remote sensing techniques were applied to estimate N and Chl contents of irrigated maize (*Zea mays* L.) fertilized at five N rates. Leaf N and Chl contents were determined using the red-edge chlorophyll index with R^2 of 0.74 and 0.94, respectively. Results showed that at the canopy level, Chl and N contents can be accurately retrieved using green and red-edge Chl indices using near infrared (780–800 nm) and either green (540–560 nm) or red-edge (730–750 nm) spectral bands. Spectral bands that were found optimal for Chl and N estimations coincide well with the red-edge band of the MSI sensor onboard the near future Sentinel-2 satellite. The coefficient of determination for the relationships between the red-edge chlorophyll index, simulated in Sentinel-2 bands, and Chl and N content was 0.90 and 0.87, respectively.

Abbreviations: Chl, chlorophyll, N, nitrogen, NDV, Inormalized difference vegetation index, EVI, enhanced vegetation index, MTCI, MERIS terrestrial chlorophyll index, CI, chlorophyll index

Keywords: Chlorophyll, Nitrogen, Reflectance, Remote sensing, Vegetation index

1. Introduction

Numerous leaf-level studies have demonstrated a strong link between nitrogen (N) content and photosynthetic activity (Field and Mooney, 1986; Wullschleger, 1993). Kergoat et al. (2008) analyzed the relationship between leaf N content and the eddy covariance CO_2 flux measurements obtained at a range of diverse sites located in mid to high latitudes, encompassing managed and unmanaged stands, mono- and pluri-specific canopies. They concluded that leaf N content was a strong factor influencing both optimum canopy light use efficiency and canopy photosynthesis rate. Gitelson et al. (2003a, 2006a) found a close, consistent relationship between gross primary productivity (GPP) and total plant Chl content in maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). Moreover, it was shown that despite great differences in leaf structure and canopy architecture in the C_3 and C_4 crops studied, the relationship between GPP and Chl content was not species specific. Thus, monitoring of N and Chl content provides important information about crop photosynthetic status.

Evans (1983, 1989) demonstrated the partitioning of N between protein fractions of soluble and thylakoid proteins remained unaltered with increasing N content. Therefore, changes in leaf N content will result in similar changes to the thylakoid pigment protein complex, that consists primarily of Chl, and the carbon fixing soluble protein enzyme activity of ribulose 1,5-bisphosphate (RuBP). An accurate measure of one component can provide estimates of the other two. Walters (2003) found a very close relationship between leaf Chl and N contents in maize, and also between leaf N content and crop yields. Baret et al. (2007) found that canopy Chl content was well suited for quantifying canopy-level N content. They concluded that canopy Chl content was a physically sound quantity that represents the optical path in the canopy where absorption by Chl dominates the radiometric signal. Thus, absorption by Chl provides the necessary link between remote sensing observations and canopy-state variables that are used as indicators of N status and photosynthetic capacity.

Reflectance in the green and red-edge spectral regions was shown to be optimal for non-destructive estimation of leaf Chl content in a wide range of its variation (Blackburn, 2006; Gitelson et al., 2003b; Gitelson, 2011a; Hatfield et al., 2008; le Maire et al., 2004; Richardson et al., 2002; Ustin et al., 2009). Féret et al. (2011), using a large set of leaf data collected from all over the world, showed that the

Table 1. Vegetation indices examined.

Index	Equation	Reference
Normalized difference vegetation index (NDVI)	(NIR − Red)/(NIR + Red)	Rouse et al. (1974)
Enhanced vegetation index 2 (EVI2)	2.5 × (NIR − Red)/(NIR + 2.4 × Red + 1)	Jiang et al. (2008)
Red edge inflection point (REP)	$708.75 + 45 \times [(\rho_{665} + \rho_{778.75})/2) - \rho_{708.75})/(\rho_{753.75} - \rho_{708.75})]$	Guyot and Baret (1988) and Clevers et al. (2001)
MERIS terrestrial chlorophyll index (MTCI)	(NIR − Red Edge)/(Red Edge + Red)	Dash and Curran (2004)
Green chlorophyll index (CI_{green})	(NIR/Green) − 1	Gitelson et al., 2003b and Gitelson et al., 2005
Red edge chlorophyll index ($CI_{red\ edge}$)	(NIR/Red Edge) − 1	Gitelson et al., 2003b and Gitelson et al., 2005

Prospect 5 radiative transfer model provided an accurate estimation of leaf Chl content using reflectance in the red-edge and near infrared spectral regions.

However, at canopy level the estimation of Chl and N content is much more problematic and only a few papers have demonstrated a way to estimate them (Gitelson et al., 2005; Gitelson, 2011b; Lee et al., 2008; Sripada et al., 2008; Takahashi et al., 2000; Tian et al., 2011; Xue et al., 2004; Zhu et al., 2008). Among recently published results, it is worthy to note technique for the accurate assessment of N content in rice using hyperspectral measurements (Inoue et al., 2012). They found that the combination of reflectance values at two wavebands (near infrared at 825 nm and red-edge around 735 nm) has a significant and consistent role in the assessment of rice N content. High predictive accuracy was achieved when using models involving the normalized difference or the ratio of reflectance at these wavebands. Clevers and Kooistra (2012), using PROSAIL simulations, showed that the red-edge chlorophyll index ($CI_{red\text{-}edge}$, Table 1) with a wide red-edge band (700–730 nm) was linearly related to the canopy Chl content over the full range of potential Chl values. At their study sites, the $CI_{red\text{-}edge}$ was also found to be a good and linear estimator of canopy N content in both grassland and potato cropping systems.

Clevers and Gitelson (2013) focused on the potential of Sentinel-2 (http://www.esa.int/Our_Activities/Observing_the_Earth/GMES/Sentinel-2) and Sentinel-3 (http://www.esa.int/Our_Activities/Observing_the_Earth/GMES/Sentinel-3) satellites for estimating total crop and grassland Chl and N contents. The Multi Spectral Instrument (MSI) on the Sentinel-2 satellite system has bands centered at 560 nm (green), 665 nm (red), 705 and 740 nm (red edge) and 783 nm (NIR). The Ocean and Land Color Instrument (OLCI) on the Sentinel-3 satellite system has one spectral band in red edge region centered at 709 nm. Clevers and Gitelson (2013) found that the $CI_{red\text{-}edge}$, the green chlorophyll index, CI_{green}, and the MERIS terrestrial chlorophyll index, MTCI (Table 1) were accurate and linear estimators of canopy Chl and N contents. Bands of MSI in the green and red edge are well positioned for deriving these indices. Results confirm the particular importance of the Sentinel-2 sensor for agricultural applications because it provides access to green and red-edge waveband data with high spatial resolution of 20 m.

Importantly, papers describing non-destructive N and Chl content retrieval employed spectral bands located far from the main red absorption band of Chl where absorption saturates at low-to-moderate Chl values. Use of either green or red-edge spectral regions makes it possible to avoid this saturation, retaining a high sensitivity to changes in Chl content (Gitelson, 2011a, 2011b; Hatfield et al., 2008; Ustin et al., 2009).

Collectively, the previously mentioned studies indicate that the use of remote sensing techniques may provide accurate measures of N content. This paradigm suggests that absorption by Chl provides the necessary link between remote sensing observations and canopy-state variables that are used as indicators of N status (Baret et al., 2007). However, previous studies dealt separately with N or Chl content estimation and, thus, cannot test the concept. To prove this concept, performance of remote sensing techniques to estimate *both Chl and N* contents in leaf and canopy should be investigated. This study evaluates performance of remote sensing techniques to estimate both N and Chl leaf and canopy contents in maize and, specifically, accuracy of N and Chl estimation using near future satellites Sentinel-2 and Sentinel-3. Firstly, we established relationships between N and Chl contents at the leaf and canopy levels and then tested the performance of Chl-related vegetation indices to retrieve N and Chl contents in both leaf and canopy scenarios. Secondly, we identified optimal spectral ranges in the green and the red-edge regions allowing accurate estimation of N and Chl contents in maize over a wide range of leaf area index values. Finally, we assessed accuracy of suggested technique using spectral bands of Sentinel-2, which allows monitoring of Chl and N contents in crops with high spatial and temporal resolutions.

2. Materials and methods

Field plots used to address the study objectives were grown in 2006 on a Hord silt loam, a fine-silty textured mollic soil with a 0–1% slope near Shelton, Nebraska, USA (40° 45′ 01″ N, 98° 46′ 01″ W, elevation of 620-m above sea level). These plots were part of a long term cropping system and N management study established in 1991. The maize crop was seeded on 9 May 2006 at a target density of 78,500 seeds ha^{-1} using conventional tillage methods. To satisfy P requirement at this site, liquid ammonium phosphate fertilizer was applied at the rate of 94 L ha^{-1} beneath the seed at planting, providing approximately 12 kg N ha^{-1} and 18 kg P ha^{-1}. All other plant nutrients were determined, by annual soil test results, to be adequate. The crop received irrigation throughout the growing season according to established sprinkler irrigation scheduling principles; no additional N was supplied to the study through irrigation water. Weed control was accomplished through a combination of cultivation and herbicide application.

Using monoculture maize as a subset of the original long term N management study provided conditions with the greatest range in N availability. Crop physiological data and canopy reflectance were collected from two architecturally contrasting Pioneer brand hybrids (Pioneer Brand 31N28 and 33V16) and five N treatments (0, 50, 100, 150, and 200 kg N ha^{-1}). The five N rates were applied shortly after emergence using a solution of urea ammonium nitrate (UAN) containing 28% N. It was our intention to use hybrids with the greatest architectural diversity available (erectophile vs. planophile) providing dramatically different light environments. Data were analyzed as a split-plot treatment design within a randomized complete block experiment with four replications. Maize hybrid was assigned as the main plot with N treatments as the subplots. Individual plot dimensions were 7.3 by 15.2-m, consisting of eight 0.91-m rows planted in an east-west direction.

Data collected throughout the growing season were referenced by thermal time (Growing Degree Days, GDD) and calculated from the acquisition of climatological data recorded using an automated weather station (High Plains Regional Climate Center Network, University of Nebraska) located on site. The GDD were logged and accumulated beginning from the planting date. Computations were made using Method II of McMaster and Wilhelm (1997) with a temperature base of 10 °C, and a temperature threshold of 30 °C.

Data acquisition occurred on six occasions between GDD 554 and 918. This span in thermal time covered plant development from

approximately six fully expanded leaves during the vegetative stage through the early reproductive stage when pollination begins and ear silks beginning to emerge. For each acquisition event, three separate canopy reflectance measurements were acquired within a one-square meter sampling area of each plot. The three readings were averaged to produce one spectral signature representative of each plot. Canopy spectral reflectance was measured using dual Ocean Optics USB2000 spectroradiometers (Ocean Optics, Dunedin, Florida, USA) in the range from 350 nm to 1024 nm in 0.37 nm increments. This dual sensor system allows for simultaneous measurements of target radiance and available incoming irradiance. The fiber-optic for the radiance sensor was fitted with a 20° full field of view optical restrictor and placed 1-m above the canopy in a nadir position directly over the row of plants. This orientation provided a circular field of view with a 0.35-m dia. footprint at the top of the canopy. The fiber optic for the irradiance sensor was mounted to a cosine corrected irradiance probe and placed directly above the radiance sensor. A white reference panel, utilizing the Spectralon diffuse reflectance material (Labsphere, Inc., North Sutton, New Hampshire, USA), was used for calibration. Calibration scans are necessary to adjust one sensor to the other and were acquired before and after each data collection event. The reference panel was placed beneath the radiance sensor to collect simultaneous measurements with the irradiance sensor. With the sensors matched, canopy reflectance can then be calculated as the ratio of the simultaneously measured radiance sensor value to the irradiance sensor value.

Plants from the same one-square meter of plot were harvested and the leaves removed. Leaves were grouped into upper and lower canopy zones for each plot providing a better, more homogenous, representation of the Chl and N distributions throughout the canopy. Zones were defined differently for the vegetative and reproductive growth stages. The two zones were separated into mature and elongating leaves for the vegetative growth stages while for the reproductive stages the zones were defined relative to the ear leaf position. Four specific sample locations were identified on leaves representative of each canopy zone. Leaf reflectance was measured using the Ocean Optics USB2000 spectroradiometer connected to a Li-Cor 1800IS integrating sphere (Li-Cor Inc., Lincoln, Nebraska, USA). Measurements were then averaged, resulting in a single reflectance spectrum representing the plot and leaf zone.

Tissue sampling was then accomplished by excising 1-cm dia. leaf disks collected at the same location, halfway from the leaf base to the tip and half way from the midrib to the leaf margin. Leaf Chl concentration was determined using photometric methods as described by Wellburn (1994) and Richardson et al. (2002). The solvent dimethyl sulfoxide (DMSO) was selected for its ability to stabilize Chl molecules for periods longer than the more commonly used acetone and eliminate material maceration and centrifuging. The four disks were held in a solution of 10 ml DMSO and placed in a 65 °C water bath for 20–30 min. The extract was then passed through a spectrophotometer to acquire the absorption (A_λ) measurements necessary to calculate Chl concentration. Equations for Chl *a* and Chl *b* are provided by Wellburn (1994):

$$\mathrm{Chl}_a, \quad \mathrm{mg\,ml^{-1}} = 12.19\,A_{665} - 3.45\,A_{649}$$

$$\mathrm{Chl}_b, \quad \mu\mathrm{g\,ml^{-1}} = 21.99\,A_{649} - 5.32\,A_{665}$$

$$\mathrm{Chl}_{total} = \mathrm{Chl}_a + \mathrm{Chl}_b$$

Leaf Chl content was derived as a function of Chl concentration, the volume of DMSO (DMSO_{vol}) used in the extraction, and the leaf disk area (LDA) sampled.

The remaining leaf tissue from each leaf zone for each plot was measured to calculate total leaf area (LA), and total dry matter (DM) on an oven dried basis. To determine N concentrations of plant materials, dried samples were first processed with a Wiley mill (20-mesh sieve). A sub-sample of approximately 0.3 g of the plant material was further ground on a roller-mill, as per Arnold and Schepers (2004). Approximately 5.5 mg of the sub-samples were used to determine N concentration using a flash combustion N analyzer following Schepers et al. (1989). The analyzer was calibrated periodically using standards with known N concentration. Chl and total N content for each leaf zone were summed for each plot and divided by the soil surface area (SA) from which they were harvested, resulting in total Chl and N contents per meter squared of surface area:

$$\mathrm{N}, \quad \mathrm{g\,m^{-2}} = \frac{\Sigma\,(\mathrm{DM} \times \%\mathrm{N})}{\mathrm{SA}}$$

$$\mathrm{Chl}, \quad \mathrm{mg\,m^{-2}} = \frac{\Sigma\,(((\mathrm{Chl}_{total} \times \mathrm{DMSO}_{vol})/\mathrm{LDA}) \times \mathrm{LA})}{\mathrm{SA}}$$

Leaf level analysis was performed on a leaf area basis:

$$\mathrm{N}, \quad \mathrm{g\,m^{-2}} = \frac{\Sigma\,(\mathrm{DM} \times \%\mathrm{N})}{\mathrm{LA}}$$

$$\mathrm{Chl}, \quad \mathrm{mg\,m^{-2}} = S\left(\frac{\mathrm{Chl}_{total} \times \mathrm{DMSO}_{vol}}{\mathrm{LDA}}\right)$$

The best-fit relationships for Chl and N contents versus vegetation indices (VI) were determined. Then, the leave-one-out technique (Stone, 1974) was used to define root mean square error (RMSE) of N and Chl estimations and coefficients of determination (R^2).
It is important to note that R^2 represents the dispersion of the points from the best-fit regression lines. It constitutes a measure of how good the regression model (best-fit function) is in capturing the relationship between N or Chl and VI. However, when the best-fit function is non-linear, the R^2 might be misleading. To determine the accuracy of N estimation, the noise equivalent (NE) of N was employed (Viña and Gitelson, 2005; Viña et al., 2011):

$$\mathrm{NE}\,\Delta\mathrm{N} = \frac{\mathrm{RMSE(VI\ vs.\ N)}}{[d(\mathrm{VI})/d(\mathrm{N})]}$$

where $d(\mathrm{VI})/d(\mathrm{N})$ is the first derivative of VI with respect to N and RMSE(VI vs. N) is the root mean square error of the VI vs. N relationship. The NEΔN provides a measure of how well the VI responds to N across its entire range of N variation. NEΔN takes into account not only RMSE of N estimation but also accounts for sensitivity of the VI to N, thus providing a metric accounting for both scattering of the points from the best fit function and the slope of the best fit function.

3. Results and discussion

3.1. Leaf Chl and N contents estimations

Linear relationships were found between leaf N content and leaf Chl content across all sampling dates in this study (Figure 1), however, these relationships varied somewhat through the growing season (Table 2). For early stages of growth (when GDD was around 550), N uptake outpaced the production of Chl. At this point the plant had approximately six fully expanded leaves producing an LAI between 0.50 and 0.75. Plant material, at this stage of development, contained higher N content with respect to Chl content and the slope of the relationship N vs. Chl was the highest. As plant growth accelerated and reached an LAI of nearly 3.0 (GDD of 660) at the 10–11 fully elongated leaf stage, a dip in the N vs. Chl relationship occurred. Available N was being used by the biochemical reactions within the plant faster

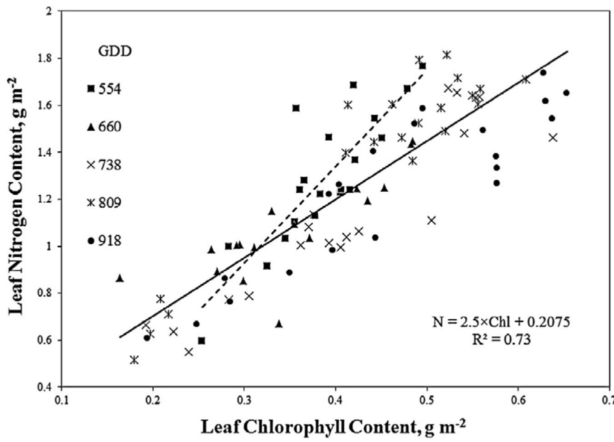

Figure 1. Relationship between leaf nitrogen and chlorophyll contents for maize at five growth stages. Solid line is best-fit function for whole growing season N vs. Chl relationship. Dashed line is best-fit function for early stage with GDD = 554. The close leaf-level relationship was the basis for using Chl-related vegetation indices to estimate N content.

Table 2. Relationships leaf nitrogen (N) content versus leaf chlorophyll (Chl) content by growing degree days (GDD) for irrigated maize. Coefficient of determination (R^2) and standard error (SE) of the relationships are indicated. N, SE and Chl contents are in g/m^2.

GDD	N/Chl relationship	R^2	SE N content
554	N = 4.1 × Chl − 0.30	0.73	0.156
660	N = 1.9 × Chl + 0.41	0.64	0.131
738	N = 2.7 × Chl + 0.03	0.85	0.145
809	N = 2.9 × Chl + 0.14	0.89	0.136
918	N = 2.2 × Chl + 0.21	0.83	0.148
Combined	N = 2.5 × Chl + 0.21	0.73	0.180

than its ability to uptake the additional N required to maintain the pace. This was short lived as growth rates became more linear. With linear growth, the relationship between leaf N and Chl contents stabilized and the slope varied slightly between 2.2 and 2.9 resulting in a season average of 2.5. Variations in plant growth and N uptake experienced early in the growing season accounts for a large portion of the scatter observed in Figure 1. The coefficients of determination (R^2) for N vs. Chl relations were the lowest in the beginning of the growing season (GDD 554 through 660) and then increased significantly to well above 0.8. Even with the observed scattering of points at the beginning of the growing season, the N vs. Chl relationship for the combined dataset was very close with $R^2 = 0.73$ (Table 2). Additional contributions to the scatter observed can be the result of tissue age and irradiance as a function of the leaf position within the architecture of the canopy. Evans (1989) indicates slight variations in the relationship between total leaf N and Chl can exist as influenced by N nutrition and irradiance during plant growth. The result is an increased scatter and a y-intercept deviating from zero, as observed in Figure 1.

Estimation of leaf Chl content was accomplished by calculating $CI_{red\ edge} = (\rho_{NIR}/\rho_{red\ edge}) - 1$ (Ciganda et al., 2009; Gitelson et al., 2003b, 2006b) where ρ_{NIR} is reflectance in the range 770–800 nm and $\rho_{red\ edge}$ is reflectance in the range 720–730 nm. The relationship between Chl content and $CI_{red\ edge}$ was very close with R^2 above 0.94 (Figure 2). In Figure 2, the relationship established by Ciganda et al. (2009) for maize is also presented. Importantly, best fit functions of both relationships have quite close parameters: difference in slope was less than 4%, and relationship offset for our data set was −23 mg m^{-2} while −8 mg m^{-2} for the Ciganda et al. (2009) relationship. This difference in offsets is probably due to very different geometry of measurements in these

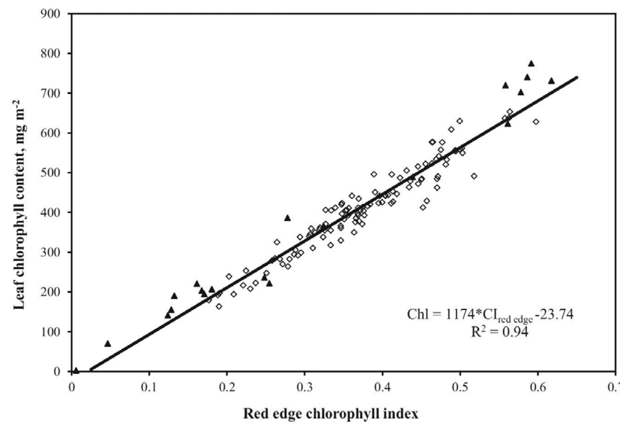

Figure 2. Maize leaf chlorophyll content versus red-edge chlorophyll index. Squares represent data taken in this study, triangles are from Ciganda et al. (2009).

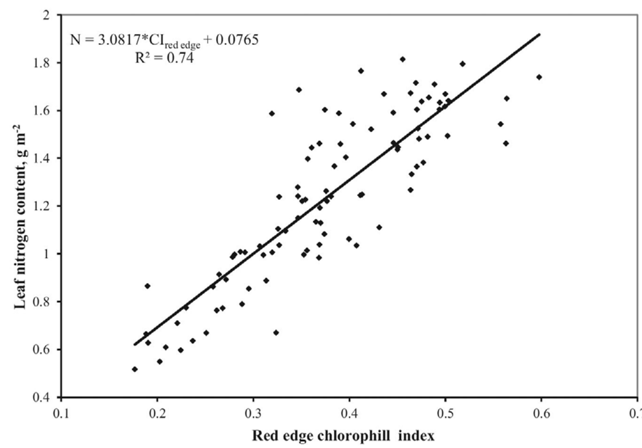

Figure 3. Maize leaf nitrogen content versus red-edge chlorophyll index.

studies: integrating sphere in our study and leaf clip in Ciganda et al. (2009). Taking into account these factors, close relationships obtained in two independent studies is remarkable.

Leaf N content estimation was accomplished using $CI_{red\ edge}$. When considering the strong relationship between leaf N and Chl contents (Figure 1), an equally strong relationship between leaf N content and $CI_{red\ edge}$ was expected; it was achieved with $R^2 = 0.74$ and RMSE of N estimation of 0.18 g m^{-2} (Figure 3). This is in accord with Baret et al. (2007) who concluded that N status could be accurately assessed through Chl estimates. The scatter observed between $CI_{red\ edge}$ and N content is expected to be similar to that observed between measured leaf N and Chl contents (Figure 1). Thus, obtained results show a strong potential for reflectance-based techniques to non-destructively estimate leaf N content.

3.2. Total canopy Chl and N contents estimation

Temporal behavior of total canopy N and Chl contents is presented in Figure 4. With increase in N content, Chl content increased nearly synchronously. Moving away from the leaf level perspective toward the integration of the full canopy within a specified surface area acted as a normalization factor, improving the N vs. Chl relationship. A very strong linear relationship was established between N and Chl contents at the canopy level (Figure 5). The strong canopy level N vs. Chl relationship was the basis for using Chl-related vegetation indices to estimate canopy N content.

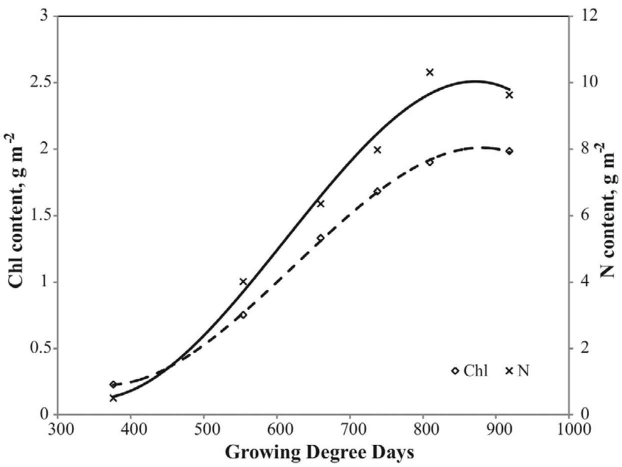

Figure 4. Average total canopy chlorophyll and nitrogen contents versus growing degree days. Lines are second order polynomial best-fit functions with coefficients of determination for both N and Chl approximations above 0.94.

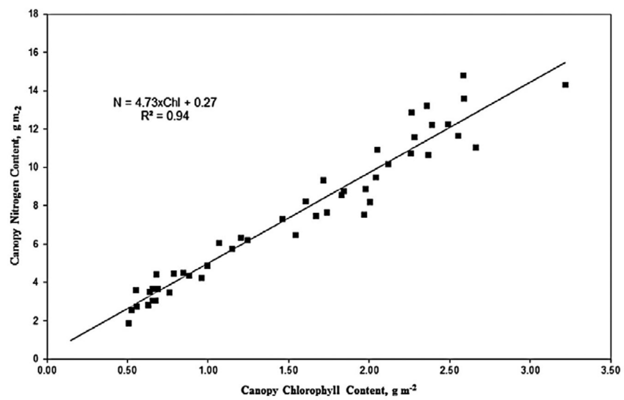

Figure 5. Relationship between canopy chlorophyll and nitrogen contents for irrigated maize.

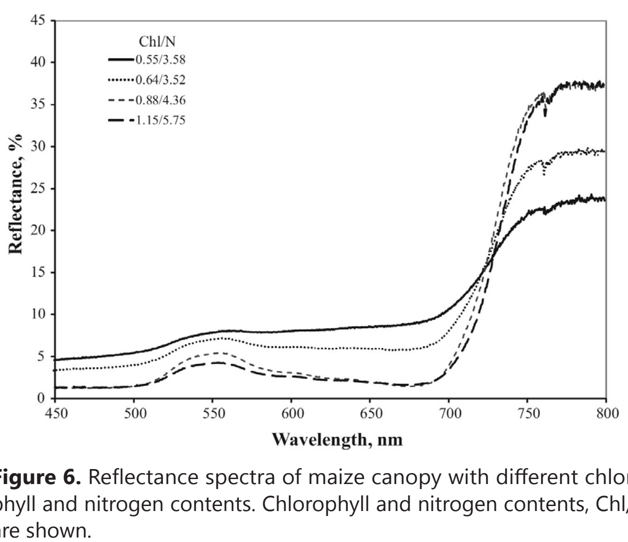

Figure 6. Reflectance spectra of maize canopy with different chlorophyll and nitrogen contents. Chlorophyll and nitrogen contents, Chl/N, are shown.

Reflectance spectra of maize canopy varied significantly during the growing season as total canopy N and Chl contents increased. The NIR reflectance increased with increasing green LAI and biomass, while reflectance in the red region (maximum Chl absorption around 670 nm) decreased (Figure 6). When Chl content approached 0.8 g m^{-2} with an N content of 4 g m^{-2}, this occurring at LAI values as low as 2.0, red reflectance stopped changing due to saturation of absorption in the red range at these moderate LAI values. In contrast to red reflectance, green reflectance (around 550 nm) and red edge reflectance (from 710 to 750 nm) continued to decrease following increases in Chl and N contents (Figure 6).

Several Chl-related vegetation indices were selected to evaluate the estimation of N content at the canopy level (Table 1): NDVI (Rouse et al., 1974) and EVI2 (Huete et al., 1997; Jiang et al., 2008) that employ NIR and red spectral bands only, as well as indices that use green and red edge bands: MERIS terrestrial chlorophyll index (MTCI; Dash and Curran, 2004), red edge position (REP; Guyot and Baret, 1988), and green and red edge chlorophyll indices (CI_{green}, $CI_{red\ edge}$; Gitelson et al., 2003b, 2005, 2006b). The NDVI and EVI2 were simulated with reflectance in spectral bands of Landsat and MODIS; CI_{green} was simulated for green spectral bands of MERIS/OLCI and Landsat; and MTCI, REP and $CI_{red\ edge}$ were simulated using spectral bands of MERIS/OLCI centered at 754 nm (NIR), 709 nm (red edge) and 665 nm (red).

The NDVI was sensitive to N below 4 g m^{-2}, but did not change thereafter (Figure 7A). The EVI2 and REP showed almost the same pattern as NDVI but reached saturation at a higher N content, above 6 g m^{-2} (Figure 7B and C). Yet, MTCI and $CI_{red\ edge}$ showed much higher sensitivity to N content over the entire range of its variation (Figure 7D and E). However, when N exceeded 10 g m^{-2}, the sensitivity of MTCI decreased more than twofold (Figure 7D), while the sensitivity of $CI_{red\ edge}$ only decreased about 60% (Figure 7E). The best fit for the CI_{green} vs. N relationship was the power function $N = 1.23 \times CI_{green}^{0.97}$ ($R^2 = 0.79$) and was quite close to linear with $R^2 = 0.73$ (Figure 7F).

Noise equivalent of N estimation (NEΔN) using VIs, is presented in Figure 8 versus canopy N content. This parameter facilitated understanding how well the VIs responded to N across the entire range of N variation. Both NDVI and EVI had quite small NEΔN and, thus, high sensitivity to N content below 2–3 g m^{-2}. This sensitivity decreased drastically with increasing N content beyond 3 g m^{-2} (Figure 7A and B) resulting in NEΔN increasing exponentially (insert to Figure 8). For REP, $CI_{red\ edge}$, and MTCI, NEΔN was below 2 g m^{-2} for N < 9 g m^{-2}, and then increased reaching values above 4 g m^{-2} for N content around 14 g m^{-2}. The behavior of NEΔN for CI_{green} was quite different. It was nearly invariant over the entire range of N variation. To estimate N content below 6 mg m^{-2}, the use of either REP, $CI_{red\text{-}edge}$, or MTCI is recommended, while for higher N content CI_{green} is more accurate.

The next step was to explore the potential of the MultiSpectral imager (MSI) sensor onboard Sentinel-2 to estimate total maize N and Chl contents. This sensor has narrow spectral bands (15-nm width) centered at 705 nm and 740 nm, providing outstanding potential for retrieving canopy Chl and N contents. In combination with the high spatial resolution (20 m) and short revisit time (near weekly with a constellation of two identical satellites), it offers improved applications in fields like precision agriculture.

To find an optimal band in the red-edge spectral region for estimating Chl content, we calculated RMSE of the total Chl estimation using the red-edge chlorophyll index, $CI_{red\ edge} = (\rho_{NIR}/\rho_{red\ edge}) - 1$ with ρ_{NIR} at 780–800 nm and variable red-edge spectral bands from 680 to 760 nm. The minimal RMSE and the highest accuracy of Chl estimation by means of $CI_{red\ edge}$ was found in the red-edge range 735–745 nm (Figure 9). This optimal spectral range is positioned more than 60 nm away from the position of in situ red absorption band of Chl. In this spectral region, the absorption coefficient of Chl is very low (Lichtenthaler, 1987), however, light penetration inside the leaf and canopy is much deeper than in the red and shortwave red-edge region (Ciganda et al., 2012; Merzlyak and Gitelson, 1995). Thus, absorption by the canopy around 740 nm (i.e., product of light pathway

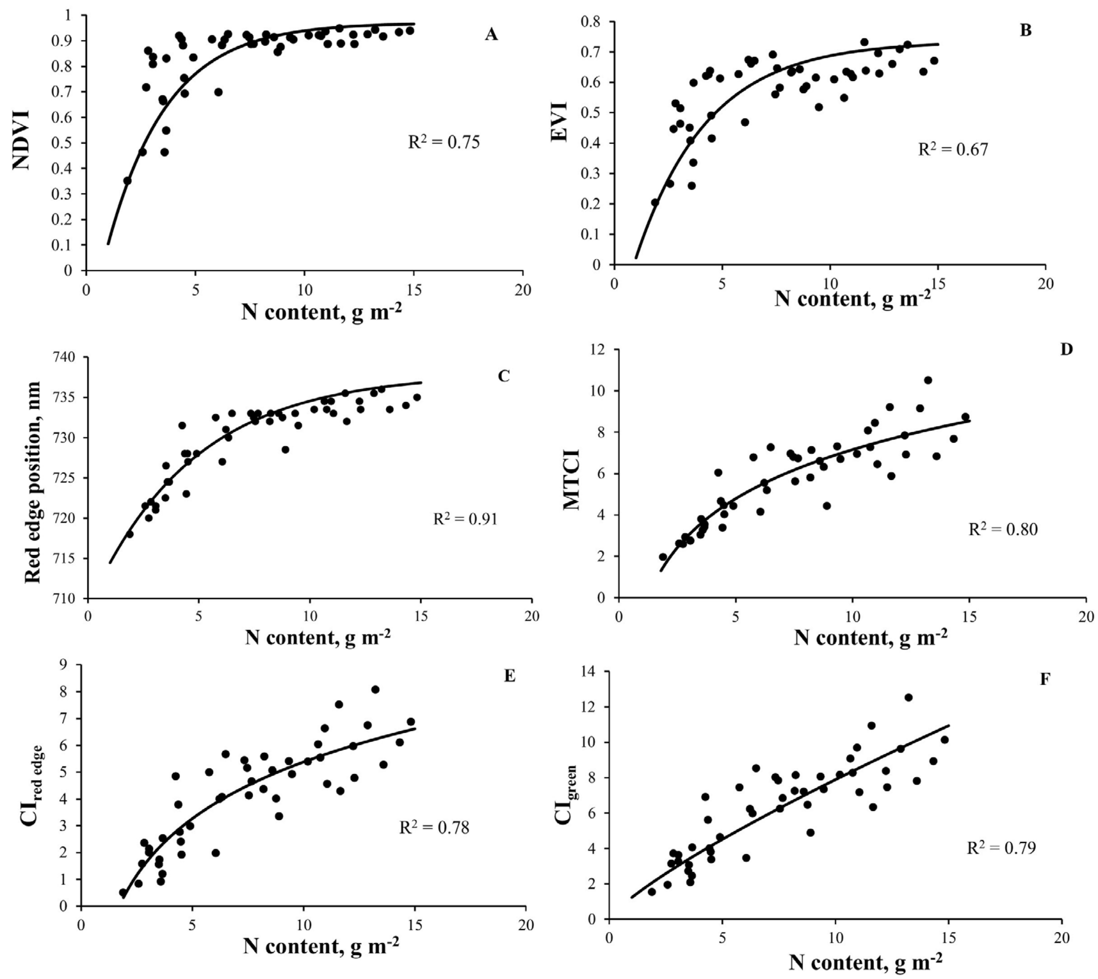

Figure 7. Canopy nitrogen content versus (a) NDVI; (b) EVI2; (c) MTCI; (d) red-edge position; (e) green chlorophyll index, CI_{green}; and (f) red-edge chlorophyll index, $CI_{red\ edge}$. NDVI, EVI and CI_{green} were calculated using spectral bands of MODIS. MTCI, red-edge position and $CI_{red\ edge}$ were calculated with spectral bands of OLCI sensor onboard Sentinel-3.

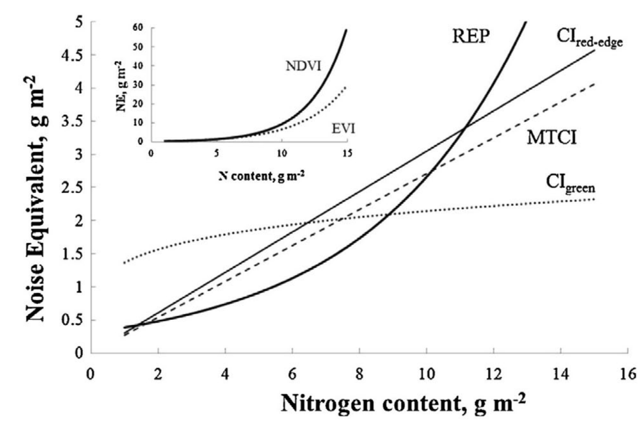

Figure 8. Noise equivalent of total nitrogen estimation by vegetation indices CIs, MTCI and red-edge position. Insert shows noise equivalents of NDVI and EVI. NDVI, EVI and CI_{green} were calculated using spectral bands of MODIS/OLCI. MTCI, red-edge position and $CI_{red\text{-}edge}$ were calculated with spectral bands of OLCI sensor onboard Sentinel-3.

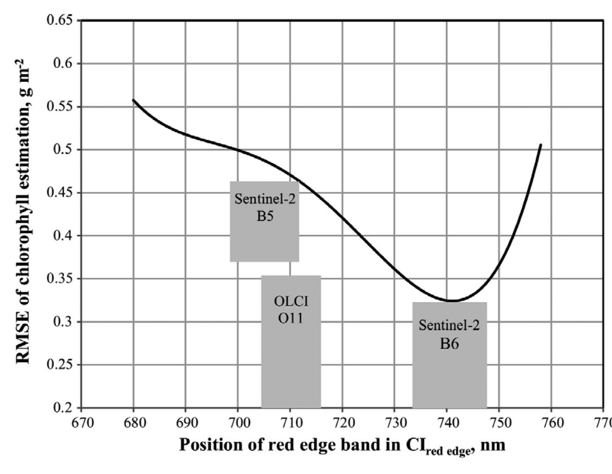

Figure 9. RMSE of total chlorophyll estimation by red-edge chlorophyll index, $CI_{red\ edge} = (\rho_{NIR}/\rho_{red\ edge}) - 1$ with ρ_{NIR} = 780–800 nm and variable red-edge spectral band from 680 to 760 nm. In the range 735–745 nm, the $CI_{red\ edge}$ had minimal RMSE and the highest accuracy of chlorophyll estimation.

and absorption coefficient) is enough to provide high sensitivity of reflectance to Chl content.

The $CI_{red\ edge}$ shown in Figure 10 was simulated using the red-edge band of the MSI sensor onboard Sentinel-2 centered at 740 nm (width 15 nm). Both N vs. $CI_{red\ edge}$ and Chl vs. $CI_{red\ edge}$ relationships were very close and nearly linear (linear approximations result in $R^2 = 0.84$ for Chl and 0.82 for N). Noise equivalent of $CI_{red\ edge}$ with 740 nm band was even lower than that of CI_{green} allowing very accurate N estimation (Figure 11).

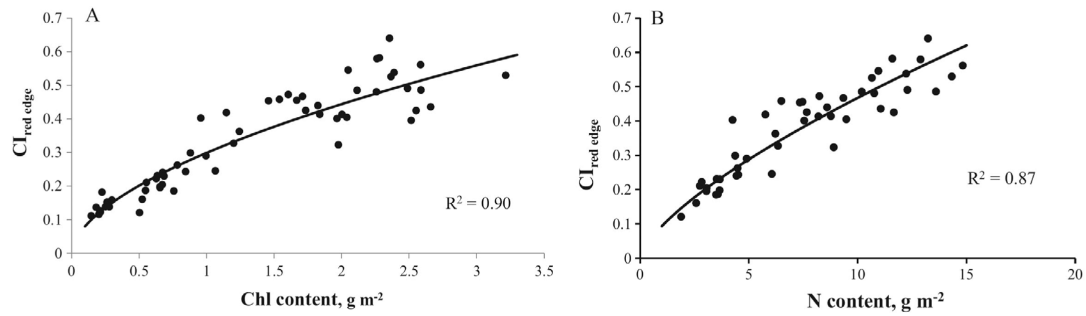

Figure 10. Relationships between canopy chlorophyll content (a) and canopy nitrogen content (b) and red-edge chlorophyll index with simulated B6 spectral band at 740 nm (width 15 nm) of MSI sensor onboard Sentinel-2.

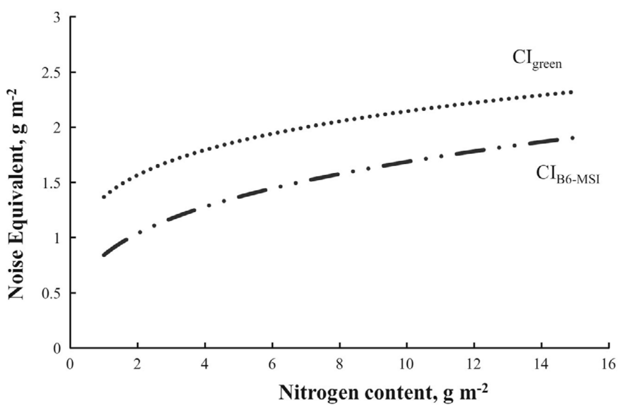

Figure 11. Noise equivalent of total nitrogen content estimation by chlorophyll indices (CI), calculated using green spectral band of MODIS/OLCI and red-edge band B6 (732–747 nm) of MSI sensor onboard Sentinel-2.

Previous studies showed that CI_{green} and $CI_{red\text{-}edge}$ are robust for estimating canopy N content (Clevers and Kooistra, 2012; Clevers and Gitelson, 2013). However, it was found that the precise position of the spectral bands in the $CI_{red\text{-}edge}$ is not very critical. This study with maize illustrated that for crops with very high Chl and N contents the precise position of the spectral bands in the $CI_{red\text{-}edge}$ is quite critical. The RMSE of Chl and N estimations using $CI_{red\text{-}edge}$ with the long-wave 740 nm band of Sentinel-2 was 50% smaller when using the short-wave 705 nm band. Different climates and irrigation regimes (and perhaps varieties) will result in variable quantities of N and Chl, and thus require both Sentinel-2 bands, centered at 705 nm for lower Chl and 740 nm for higher Chl. Importantly, the optimal spectral bands, as well as techniques to estimate Chl and N contents demonstrated in this maize study, are in accord with findings for rice (Inoue et al., 2012; Lee et al., 2008; Takahashi et al., 2000), wheat (Wu et al., 2009; Zhu et al., 2008), and maize (Gitelson et al., 2005; Wu et al., 2010).

4. Conclusions

The paper showed that chlorophyll and nitrogen content in maize can be estimated by the same remote sensing techniques and confirmed a paradigm, suggesting that absorption by Chl provides the necessary link between remote sensing observations and canopy-state variables that are used as indicators of N status. This study presents the significance of the green and long wave red-edge bands of the MSI sensor on Sentinel-2 for estimating Chl and N contents in maize. This study confirms that green chlorophyll index CI_{green} accurately estimates N content, which is consistent with findings for both crops and grassland systems (Clevers and Gitelson, 2013). Also notably, $CI_{red\text{-}edge}$ with quite a wide spectral band around 740 nm was optimal for N and Chl estimation. $CI_{red\text{-}edge}$ with red edge band 720–730 nm allowed accurate non-species specific estimation of gross primary production in maize and soybean (Peng and Gitelson, 2011) and the same range was found to be optimal for N estimation in rice (Inoue et al., 2012). Thus, it is likely that presented techniques for N and Chl estimation in maize could accurately estimate the same characteristics in other crops. This study offers possible options for users to collect the appropriate data from proximal sensors to space–borne platforms that will provide measures of such important physiological variables as Chl and N from the plant to the global scale.

References

Arnold, S.L., Schepers, J.S., 2004. A simple roller–miller grinding procedure for plant and soil samples. Communications in Soil Science and Plant Analysis 35, 537–545.

Baret, F., Houlès, V., Guérif, M., 2007. Quantification of plant stress using remote sensing observations and crop models: the case of nitrogen management. Journal of Experimental Botany 58, 869–880.

Blackburn, G.A., 2006. Hyperspectral remote sensing of plant pigments. Journal of Experimental Botany 58, 855–867.

Ciganda, V., Gitelson, A., Schepers, J., 2009. Non-destructive determination of maize leaf and canopy chlorophyll content. Journal Plant Physiology 166, 157–167.

Ciganda, V.S., Gitelson, A.A., Schepers, J., 2012. How deep does a remote sensor sense? Expression of chlorophyll content in a maize canopy. Remote Sensing of Environment 126, 240–247.

Clevers, J.G.P.W., de Jong, S.M., Epema, G.F., van der Meer, F., Bakker, W.H., Skidmore, A.K., Addink, E.A., 2001. MERIS and the red-edge position. International Journal of Applied Earth Observation and Geoinformation 3, 313–320.

Clevers, J.G.P.W., Gitelson, A.A., 2013. Remote estimation of crop and grass chlorophyll and nitrogen content using red-edge bands on Sentinel-2 and - 3. International Journal of Applied Earth Observation and Geoinformation 23, 344–351.

Clevers, J.G.P.W., Kooistra, L., 2012. Using hyperspectral remote sensing data for retrieving canopy chlorophyll and nitrogen content. IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing 5, 574–583.

Dash, J., Curran, P.J., 2004. The MERIS terrestrial chlorophyll index. International Journal of Remote Sensing 25, 5403–5413.

Evans, J.R., 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiology 72, 297–302.

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78, 9–19.

Féret, J-B., François, C., Gitelson, A.A., Barry, K.M., Panigada, C., Richardson, A.D.,

Jacquemoud, S., 2011. Optimizing spectral indices and chemometric analysis of leaf chemical properties using radiative transfer modeling. Remote Sensing of Environment 115, 2742–2750.

Field, C.B., Mooney, H.A., 1986. The photosynthesisp–nitrogen relationship in wild plants. In: Givnish, T.J. (Ed.), On the eEconomy of Plant Form and Function. Cambridge University Press, Cambridge, pp. 25–55.

Gitelson, A.A., Verma, S.B., Vina, A., Rundquist, D.C., Keydan, G., Leavitt, B., Arkebauer, T.J., Burba, G.G., Suyker, A.E., 2003a. Novel technique for remote estimation of CO2 flux in maize. Geophysical Research Letters 30 (9), 1486, doi: 10.1029/2002GL016543.

Gitelson, A.A., Gritz, Y., Merzlyak, M.N., 2003b. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. Journal Plant Physiology 160, 271–282.

Gitelson, A.A., Viña, A., Ciganda, V., Rundquist, D.C., Arkebauer, T.J., 2005. Remote estimation of canopy chlorophyll content in crops. Geophysical Research Letters 32, L08403, doi: 10.1029/2005GL022688

Gitelson, A.A., Viña, A., Verma, S.B., Rundquist, D.C., Arkebauer, T.J., Keydan, G., Leavitt, B., Ciganda, V., Burba, G.G., Suyker, A.E., 2006a. Relationship between gross primary production and chlorophyll content in crops: implications for the synoptic monitoring of vegetation productivity. Journal of Geophysical Research 111, D08S11, doi: 10.1029/2005JD006017.

Gitelson, A.A., Keydan, G.P., Merzlyak, M.N., 2006b. Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophysical Research Letters 33, L11402, doi: 10.1029/2006GL026457.

Gitelson, A.A., 2011a. Non-destructive estimation of foliar pigment (chlorophylls, carotenoids and anthocyanins) contents: espousing a semi-analytical three-band model. In: Thenkabail, P.S., Lyon, J.G., Huete, A. (eds.), Chapter 6 in Hyperspectral Remote Sensing of Vegetation. Taylor and Francis, CRC Press- Taylor and Francis group, Boca Raton, London, New York, pp. 141–165.

Gitelson, A.A., 2011b. Remote Sensing estimation of crop biophysical characteristics at various scales. In: Thenkabail, P.S., Lyon, J.G., Huete, A. (eds.), Chapter 15 in Hyperspectral Remote Sensing of Vegetation. Taylor and Francis, CRC Press- Taylor and Francis group, Boca Raton, London, New York, pp. 329–358.

Guyot, G., Baret, F., 1988. Utilisation de la haute resolution spectrale pour suivre l'etat des couverts vegetaux. In: Proceedings 4th International Colloquium 'Spectral Signatures of Objects in Remote Sensing', Aussois, France, pp. 279–286 (ESA, Paris).

Hatfield, J.L., Gitelson, A.A., Schepers, J.S., Walthall, C.L., 2008. Application of spectral remote sensing for agronomic decisions. Agronomy Journal 100, S-131-S-117.

Huete, A.R., Liu, H.Q., Batchily, K., van Leeuwen, W., 1997. A comparison of vegetation indices global set of TM images for EOS-MODIS. Remote Sensing of Environment 59, 440–451.

Inoue, Y., Sakaiy, E., Zhu, Y., Takahashi, W., 2012. Diagnostic mapping of canopy nitrogen content in rice based on hyperspectral measurements. Remote Sensing of Environment 126, 210–221.

Jiang, Z., Huete, A.R., Didan, K., Miura, T., 2008. Development of a two-band enhanced vegetation index without a blue band. Remote Sensing of Environment 112, 3833–3845.

Kergoat, L., Lafont, S., Arneth, A., Le Dantec, V., Saugier, B., 2008. Nitrogen controls plant canopy light-use-efficiency in temperate and boreal ecosystems. Journal of Geophysical Research 113, doi: 10.1029/2007JG000676, G04017, 1–19.

le Maire, G., François, C., Dufréne, E., 2004. Towards universal broad leaf chlorophyll indices using PROSPECT simulated database and hyperspectral reflectance measurements. Remote Sensing of Environment 89, 1–28.

Lichtenthaler, H.K., 1987. Chlorophyll and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology 148, 331–382.

Lee, Y., Yang, C., Chang, K., Shen, Y., 2008. A simple spectral index using reflectance of 735 nm to assess nitrogen status of rice canopy. Agronomy Journal 100, 205–212.

McMaster, G.S., Wilhelm, W.W., 1997. Growing degree-days: One equation, two 6 interpretations. Agricultural and Forest Meteorology 87, 291–300.

Merzlyak, M., Gitelson, A., 1995. Why and what for the leaves are yellow in autumn? On the interpretation of optical spectra of senescing leaves (*Acer platanoides* L.). Journal Plant Physiology 145 (3), 315–320.

Peng, Y., Gitelson, A.A., 2011. Remote estimation of gross primary productivity in soybean and maize based on total crop chlorophyll content. Remote Sensing of Environment 117, 440–448.

Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153, 185–194.

Rouse, J.W., Haas Jr., R.H., Schell, J.A., Deering, D.W., 1974. Monitoring vegetation systems in the Great Plains with ERTS. In: Third ERTS-1 Symposium, NASA, Washington, DC, pp. 309–317.

Schepers, J.S., Francis, D.D., Thompson, M.T., 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and plant material. Communications in Soil Science and Plant Analysis 20, 949–959.

Sripada, R.P., Schmidt, J.P., Dellinger, A.E., Beegle, D.B., 2008. Evaluating multiple indices from a canopy reflectance sensor to estimate corn N requirements. Agronomy Journal 100, 1553–1561.

Stone, M., 1974. Cross-validatory choice and assessment of statistical predictions. Journal of the Royal Statistical Society: Series B 36 (2), 111–147.

Takahashi, W., Nguyen-Cong, V., Kawaguchi, S., Minamiyama, M., 2000. Statistical models for prediction of dry weight and nitrogen accumulation based on visible and near-infrared hyper-spectral reflectance of rice canopies. Plant Production Science 3, 377–386.

Tian, Y.C., Yao, X., Yang, J., Cao, W.X., Hannaway, D.B., Zhu, Y., 2011. Assessing newly developed and published vegetation indices for estimating rice leaf nitrogen concentration with ground- and space-based hyperspectral reflectance. Field Crops Research 120, 299–310.

Ustin, S.L., Gitelson, A.A., Jacquemoud, S., Schaepman, M., Asner, G.P., Gamon, J.A., Zarco-Tejada, P., 2009. Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. Remote Sensing of Environment 113, S67–S77.

Viña, A., Gitelson, A.A., 2005. New developments in the remote estimation of the fraction of absorbed photosynthetically active radiation in crops. Geophysical Research Letters 32, L17403, doi: 10.1029/2005GL023647.

Viña, A., Gitelson, A.A., Nguy-Robertson, A.L., Peng, Y., 2011. Comparison of different vegetation indices for the remote assessment of green leaf area index of crops. Remote Sensing of Environment 115, 3468–3478, doi: 10.1016/j.rse.2011.08.010.

Walters, D.T., 2003. Diagnosis of nitrogen deficiency in maize and the influence of hybrid and plant density. In: North central Extension-Industry Soil Fertility Conference, 2003. Vol. 19, Des Moines, IA.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal Plant Physiology 144, 307–313.

Wullschleger, S.D., 1993. Biochemical limitations to carbon assimilation in C3 plants—a retrospective analysis of the A/Ci curves from 109 species. Journal of Experimental Botany 44, 907–920.

Wu, C., Niu, Z., Gao, S., 2010. Gross primary production estimation from MODIS data with vegetation index and photosynthetically active radiation in maize. Journal of Geophysical Research 115, D12127 doi: 10.1029/2009JD013023

Wu, C., Niu, Z., Tang, Q., Huang, W., Rivard, B., Feng, J., 2009. Remote estimation of gross primary production in wheat using chlorophyll-related vegetation indices. Agricultural and Forest Meteorology 149, 1015–1021.

Xue, L., Cao, W., Luo, W., Dai, T., Zhu, Y., 2004. Monitoring leaf nitrogen status in rice with canopy spectral reflectance. Agronomy Journal 96, 135–142.

Zhu, Y., Yao, X., Tian, Y., Liu, X., Cao, W., 2008. Analysis of common canopy vegetation indices for indicating leaf nitrogen accumulations in wheat and rice. International Journal of Applied Earth Observation and Geoinformation 10, 1–10.