Monitoring the N release from organic amendments using proximal sensing

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Abstract
The use of proximal sensed vegetation indices can reduce the uncertainty linked to the N supplied by organic amendments in a horticultural field by detecting in-season crop N status. This research assessed the applicability of the three vegetation indices (VI) of NDVI, NDRE and CCCI to evaluate the in-season long term optimized strategy of applying organic amendments to a horticultural crop (lettuce) over two seasons. A conventional urea application rate (CONV) was compared with raw (Ma) feedlot manure and Ma combined with standard (Ma+CONV) and optimized urea rate (Ma+Op). NDRE most accurately predicted crop N status at the late stage of lettuce development with an R^2 of 0.67 (RMSE 0.61), compared to 0.60 (RMSE 0.67) and 0.62 (RMSE 0.66) for NDVI and CCCI respectively. The in-season acquisition of crop reflectance proved to be a valid technique to determine the efficiency of an optimized combination between organic amendments and N-fertilizer.

Key Words
Proximal sensing, vegetation indices, N, organic amendments, horticulture

Introduction
Nitrogen (N) is the single nutrient most commonly limiting agricultural production (Basso et al. 2010) and is provided to the crop mainly through the application of synthetic fertilizer and organic amendments. Farmers traditionally use organic amendments to improve soil physical properties without accounting for the plant available N supplied from these products due to its release is affected by a high grade of spatial and temporal variability. This practice, especially with fresh animal manure, can result in excessive supply of N to horticultural soil and lead to nitrate leaching in the groundwater and nitrous oxide emissions into the atmosphere. An excess in N supply can cause undesirably high nitrate concertation in leafy vegetables (Parks et al. 2012) which can be detrimental to human health. The uncertainty in predicting N release from organic amendments can also lead to insufficient supply of N and therefore to crop N deficiency, which will decrease crop yield. Reducing the uncertainty associated with the N supplied by organic amendments is therefore crucial in order to rationalize their use in horticulture and reduce the use of synthetic N in these cropping systems. Consequently, the sustainable and economically viable use of organic amendments requires on-going assessment of crop N status which can lead to an indirect estimation of soil plant available N. Proximal sensed vegetation indices (VIs) provide valuable in-season diagnostic information to determine current plant N status. VIs are an algebraic combination of reflected energy derived from plant spectral signal obtained using optical sensors (Basso et al. 2004). For instance, the normalized difference between the reflectance of near-infrared (NIR) and red (Red) wavelength (normalized difference vegetation index, NDVI) in maize crop can provide insights on crop N deficiency (Ma et al. 1996). Likewise, an experiment conducted on cotton showed that ratios between red-edge (RE) and NIR provided the best correlation with leaf N concentrations (Tarpley et al. 2000). Barnes et al. (2000) proposed the Canopy Chlorophyll Content Index (CCCI) which combines two reflectance based indices: one estimates the percentage of canopy cover (NDVI), and the other estimates the N content (normalized difference red edge, or NDRE), which in wheat is known to be highly sensitive to N content (Cammarano et al. 2014). While the robustness of these indices has been widely tested in cereals and cotton, little information is available on the applicability of VIs in horticultural crops (Padilla et al. 2014). To enable a practical application of VIs, it is essential to assess the robustness and consistency between measured optical value and crop N status. This research aimed to compare the three main VIs currently in use (NDVI, NDRE and CCCI) to monitor the N content of a horticultural crop over two seasons. The indices were then used to assess a long term optimized strategy of applying organic amendments to horticultural crops.

Methods

Site description

The study was conducted from June to September over two seasons (2014 and 2015) at Gatton Research Station in the Lockyer Valley, Queensland, Australia (latitude 27°32'56"S, longitude 152°19'39"E; 100m a.s.l.). The alluvial soil is classified as a Black Vertosol (Isbell 2016). The complete soil characteristics are provided in De Rosa et al. (2016).

Field treatments and measurements

Lettuce (*Lactuca sativa*) was transplanted on raised beds at the beginning of June 2014 and June 2015. Irrigation was applied regularly with sprinklers weekly. Four treatments were installed in a randomized block design with four replicates each. Plot sizes was 10 m × 1.5 m with a 1.5 m wide planted buffer row. For both seasons a treatment representing standard farm practices (CONV, 100 kg N/ha as urea) was used to relate the results to common farm management practice in the region. A nil input treatment (0N, no fertilizer or organic amendment) was used to account for background soil mineralization. In both years, a single surface application of feedlot manure (Ma) was distributed nine months before lettuce transplanting. Utilizing values from the literature (Eghball et al. 2002) it was estimated that Ma would have mineralized approximately 34 and 20 kg N/ha during 2014 and 2015 seasons, respectively. In the first season Ma was combined with urea at the CONV rate (Ma+CONV 100 kg N/ha) and a reduced urea rate (Ma+Opt 66 kg N/ha). In the second season Ma was applied without fertilizer or combined with reduced rate (Ma+Opt 80 kg N/ha). Urea was surface-applied in three split applications and followed immediately by irrigation. The urea split applications consisted of a basal application of 54 kg N/ha and two side dressing of 23 kg N/ha for both seasons in CONV and only for the 2014 season in Ma+CONV. For the reduced treatment, the urea applications consisted in one basal of 20 kg N/ha and two side dressing of 23 kg N/ha for the 2014 and one basal of 34 kg N/ha and two side dressing of 23 kg N/ha for the 2015 season. Crop yield was estimated by harvesting the entire plot area of each treatment. Representative plant samples were oven-dried for 24-48 hours at 70 °C, and subsequently ground and analysed for total N and C content using a Leco Trumac CNS Analyser (LECO Corporation, USA). Soil mineral N level was monitored during the two seasons by taking soil samples and analysed by a commercial laboratory for mineral N (NO_3^- plus NH_4^+) using a 1:5 2M KCl extract.

Canopy reflectance

Four measurements per plot of canopy reflectance at 670 ,730 and 780 nm were taken before and after each urea application event and at harvest using a hand-held radiometer RapidSCAN CS-45 (Holland scientific Inc., Lincoln, NE, USA) with a sensor field of view of 45 by 25 degrees. All spectral readings were conducted under clear sky condition between 10 and 11 am. Radiometer data were used to calculate NDVI, NDRE and CCCI using the equations described by Cammarano et al. (2014).

Statistical analysis

Regression analyses were performed to evaluate the robustness of the relationship between vegetation indices and plant N content. Linear and non-linear relationship have been tested between VIs and Crop N content. We reported the model that provided the best fit. *P*-values of the regression analyses were used to determine significant relationship at $P<0.05$. Benjamini and Hochberg (BH) adjustment was performed to assess significant differences between treatments on crop yield and crop N content and soil mineral N content. Statistical and graphical presentations were performed in R environment (R Core Team 2015).

Results and discussion

Canopy reflectance and crop N content

A statistically significant ($P<0.001$) linear relationship of proximal sensed VIs with crop N content was obtained over two seasons (Table 1.). These results are consistent with previous studies from other vegetable crops e.g. broccoli (El-Shikha et al. 2007) and muskmelon (Padilla et al. 2014). While CCCI returned the best predictor of crop N content in cereals (Cammarano et al. 2014), in the present study NDRE was the index with the highest coefficient of determination for lettuce (R^2 0.67, RMSE 0.61) followed by CCCI and NDVI with R^2 of 0.61 (RMSE 0.66) and 0.60 (RMSE 0.67), respectively. The RE band returned the lowest R^2 with a value of 0.23 (RMSE 0.93). The CCCI index is based on NDRE and NDVI where the latter is used to separate the soil signal from the plant signal.

Table 1. Regression analysis, and its significance between the three vegetation indices, RE reflectance and crop N content. RMSE is the root mean square error of VIs and RE from the regression model.

	Vegetation index and reflectance vs. crop N content			
	NDVI	NDRE	CCCI	RE
R^2	0.60	0.67	0.61	0.23
significance	***	***	***	***
RMSE	0.67	0.61	0.66	0.93
Model	Linear	Linear	Linear	Linear

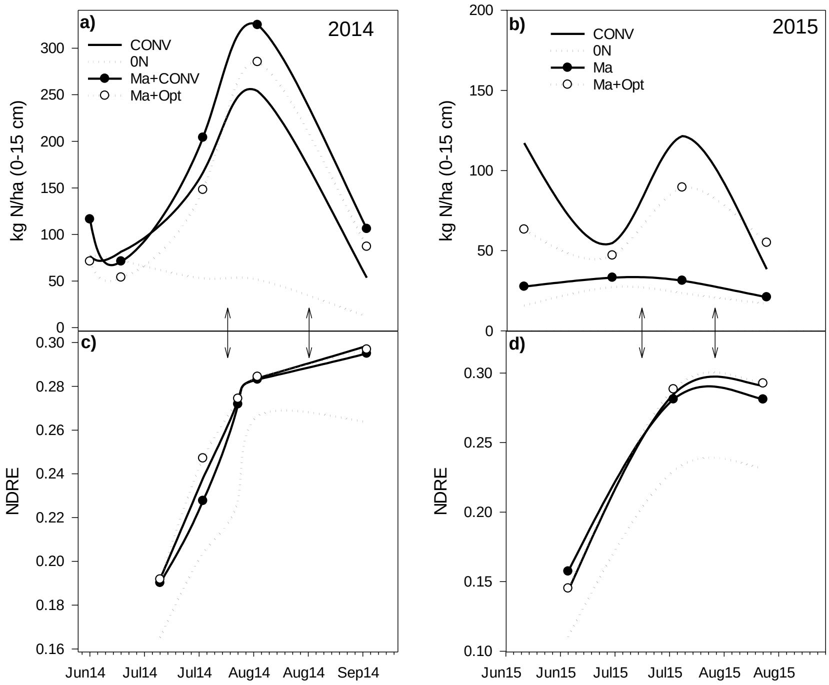
*** P<0.001

The NDVI, despite being one of the most widely used VIs in agriculture has several limitations. For example, its use in a late stage of development of a the canopy structure of lettuce, characterized by overlapping leaves, where an increase in biomass does not imply a significant variation in the NDVI due to the scattering of the NIR reflectance (Serrano et al. 2000). It is hypothesized in the current study that the NDVI, and consequently the CCCI, was influenced by the specific structure of lettuce canopy and its size, that at 35 days after planting could reach value of LAI > 4 and 95% of ground cover (TEI et al. 1996). The NDRE use the RE band which has been shown to be highly sensitive to the cop N content. However, in this study the RE band showed the lowest coefficient of determination with the highest RMSE. These results indicate that RE-derived vegetation index –such as NDRE, CCCI- are more suitable to determine the crop N status than single RE reflectance or non-RE reflectance derived index such as NDVI in lettuce.

Use of VI for N management in vegetable production

While the application of different N fertilizer rates increased the soil mineral N levels (Figure 1. a-b), the in-season NDRE temporal trend showed no differences between CONV and treatments that received organic amendments (Ma+Opt, Ma+CONV) (Figure 1. c-d). Differences in the NDRE were observed mainly between the N fertilized treatments and 0N.

Figure 1. Soil mineral N levels (0-15 cm depth) for the 2014 (a) and 2015 (b) seasons, and NDRE temporal trend during the 2014 (c) and 2015(d) seasons. Arrows indicate the timing of N fertilizer application



The absence of differences in the NDRE temporal trend during the 2014 season, highlighted that the supplementary N application of 34 kg N/ha in Ma+CONV was not necessary. Accordingly, no significant differences in lettuce crop yield were observed among treatments. The excessive N supplied in the Ma+CONV is further confirmed by the highest soil mineral N level recorded at harvests and during the growing seasons while no differences were observed between CONV and Ma+Opt (Figure 1a-b). For the 2015 season, the NDRE detected the N deficiency in the Ma treatment which was confirmed by the lower crop N content among the fertilizer treatment (Table 2). This study proved that it is possible to adopt proximal sensed VIs to assess whether the crop is receiving sufficient N under an optimized N strategy (Ma+Opt). The differences observed for the same treatments in the crop N response and soil N availability between two seasons highlight the temporal uncertainty that affects proximal sensed data. The inclusion of a non-limiting N fertilizer plot will help to determine the crop growth/N content potential under the influence of the in-season weather factors.

Table 2. Crop yield and crop N content (mean (SE)) and soil mineral N at post-harvest. Means denoted with different letter indicate significant differences between treatments (*P<0.05*)

Measurement	*Year*	*Treatments*			
	2014	*CONV*	*Ma+CONV*	*Ma+Opt*	*0N*
Yield [t/ha]	*Post-harves*	49 (1.7)	48 (7)	47 (4)	40 (7)
Crop N [%]		4 (0.2)a	4.1 (0.2)a	3.7 (0.12)a	2.6 (0.23)b
Soil min N [kg N/ha]		53.6 (21)b	106 (12)a	87 (13)ab	12 (5)c
	2015	*CONV*	*Ma*	*Ma+Opt*	*0N*
Yield [t/ha]	*Post-harvest*	63 (9)	62(10)	68 (8)	66 (10)
Crop N [%]		2.8 (0.3)a	1.6 (0.09)c	2.2 (0.4)ab	1.4 (0.1)c
Soil min N [kg N/ha]		38.5 (31)ab	21 (9)b	55 (26)a	15 (7)b

Conclusions

This study indicates that RE-derived vegetation index –such as NDRE during the mid/late stage of development of lettuce, with the inclusions of non-limiting N plot, has the capability to assess whether the crop is receiving sufficient N under an optimized organic amendments strategy. Further work is required to examine the performance of proximal sensed VIs across different crops at different growing stages.

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