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### **Research Paper**

## Hyperspectral imaging of spinach canopy under combined water and nitrogen stress to estimate biomass, water, and nitrogen content



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Keywords: Crop monitoring Remote sensing Hyperspectrograms Multivariate data analysis VIS-NIR canopy reflectance This work had the goal to assess the capability of hyperspectral line scan imaging (400 -1000 nm) to estimate crop variables in the greenhouse under combined water and nitrogen stress using multivariate data analysis and two data compression methods: canopy average spectra and hyperspectrogram extraction. Hyperspectral images contain far more information than do multispectral ones, which permits discrimination among minute pattern differences in canopy spectral reflectance.

A pot greenhouse experiment of eight treatments, from the combination of four nitrogen supply levels and two water supply levels, was designed to test widely varied spinach canopies. Using partial least square regression models, the fresh and dry matter of aboveground biomasses and water and nitrogen contents were estimated from a 76-sample dataset. Both the canopy reflectance-based and hyperspectrogram-based models performed well in estimating variables strictly related to canopy leaf area index (LAI) and geometry, i.e., water content and fresh and dry matters, such that  $R^2$  in independent validation reached values of 0.87, 0.65, 0.65, and 0.86, 0.74, 0.72, respectively. Estimation of nitrogen concentration from single leaf spectra hyperspectral images produced a high cross-validation  $R^2$  (0.83), as opposed to the poor predictive results produced from canopy scans. This latter result arose from orientation effects due to canopy architecture. Finally, for estimation purposes, image hyperspectrogram compression without spatial information loss produced more encouraging results while considering canopy structure in crop variables than did average canopy spectra.

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#### 1. Introduction

Ineffective water and nitrogen (N) management is costly to both farmers and the environment, as it represents the main cause of agricultural pollution. Such effects are avoidable by accurately estimating crop needs and properly scheduling the strongly linked managements of water (irrigation) and nitrogen (fertilisation). Draining excess water from the root horizon carries away not only soluble and plant available mineral nutrients, but also nitrate, which reduces both their availability to plants and use efficiencies. Potential solution to this problem will match nitrogen and water supplies to variations in crop demand, both spatially and temporally, and that maintain yields while improving crop system environmental performance (Olfs et al., 2005). Remote sensing using optical scanners is one technique to investigate crop status quickly and without destruction because it captures spatial crop information from crop radiative behaviour. Moreover, when calibrated to describe crop response to water and nitrogen, precision crop management can be derived from sensor spectral information.

Multispectral sensors, whether handheld, ground vehiclemounted, or airborne (aircraft or unmanned aerial vehicle), are the most common sensor types applied in agricultural settings. Multispectral sensors record reflectance in a few bands within the visible and near infrared (VIS-NIR) spectral region. From these reflectance signatures, vegetation indices are calculated and correlated to crop variables that reveal plant status, such as aboveground biomass, N uptake, and N concentration. These sensors are used most to detect N stress when N is the only limiting factor. However, studies conducted to discriminate the N stress of crops grown under different water availabilities have had difficulty distinguishing the effect of a single stress (Eitel, Long, Gessler, & Hunt, 2008; Schepers, Blackmer, Wilhelm, & Resende, 1996; Strachan, Pattey, & Boisvert, 2002; Wang, Ma, Xiong, Saleem, & Li, 2011; Zillmann, Graeff, Link, Batchelor, & Claupein, 2006). Among studies undertaken to separate nitrogen from water effects on various crops, only some authors have identified vegetation indices that related more strictly to one stress over another. For instance, Peñuelas, Filella, Biel, Serrano, and Save (1993) found the ratio of reflectance at 970 and 900 nm to be indicative of plant water status; others found plant water status affected normalised difference vegetation indices (NDVI) of both the red and red-edge bands. A third vegetation index, described by the equation (R850 - R710)/ (R850 – R680) and Meris terrestrial chlorophyll index (MTCI = (R760 - R720)/(R720 - R670)), were shown to be less affected by water stress (Shiratsuchi et al., 2011). Finally, the proposed modified chlorophyll absorption ratio index and second modified triangular vegetation index in ratio (MTCAR/ MTVI2) index, based on green, red, and red edge bands, was found to be highly correlated to chlorophyll content (and thus, to N status), but not affected by water (Eitel et al., 2008).

Other attempts to build a combined vegetative index univocally related to one of two stressors, *e.g.*, the Canopy Chlorophyll Content Index (CCCI) to estimate crop N status, were created from a combination of vegetation indices (Barnes et al., 2000; El-Shikha, Waller, Hunsaker, Clarke, & Barnes, 2007; Fitzgerald, Rodriguez, & O'Leary, 2010; Fitzgerald et al., 2006; Rodriguez, Fitzgerald, Belford, & Christensen, 2006; Tilling et al., 2007). In some of these experiments that attempted to differentiate the effect of two stressors using vegetation indices (Rodriguez et al., 2006; Wang et al., 2011), it was suggested that confounding effects arose from changes in canopy architecture, leaf surface properties, and/or bare soil reflectance. Such effects would be particularly true for canopy cover-related indices in limited water, a time when soil moisture, not nitrogen availability, drives the variation in leaf optical properties (Eitel et al., 2008).

Combining optical and thermal indices (Barnes et al., 2000; Cohen et al., 2013) can be used to differentiate nitrogen and water stress effects, as Cohen et al. (2013) showed when they mixed thermal and hyperspectral imaging on potato. First, they categorised (using ANOVA) thermal images into water stress classes based on estimated leaf water potential, after which they used hyperspectral data to group water classspecific images according to crop nitrogen concentration level. The two-step classification resulted in confusion matrices with accuracies of 83% and 65% on two potato varieties.

Hyperspectral sensors record hundreds of narrow bands in the VIS–NIR spectral region, and can be used to study combined two-stressor effects. With "across track" (whisk broom) or "along track" (push broom) scanning, the devices detect subtle differences in the patterns of canopy reflectance (Jones & Vaughan, 2010) from the entire vegetation spectrum. Hyperspectral sensing can be of particular benefit to precision agriculture when it employs imaging spectroscopy to extract spatial information on canopy reflectance by combining the potential of digital images with hyperspectral measurements. To handle the large giga- or even tera-byte datasets produced from hyperspectral images, multivariate statistical analysis has proved a useful and affordable technique to reduce the data to a limited number of significant components (Stellacci et al., 2012).

Multivariate approaches can also help to overcome some of the limitations associated with vegetation indices. Principal Components Analysis (PCA; Schut & Ketelaars, 2003), Discriminant Analysis (Goel et al., 2003), and Partial Least Squares regression (PLS; Alchanatis, Schmilovitch, & Meron, 2005; Hansen & Schjoerring, 2003; Li, Mistele, Hu, Chen, & Schmidhalter, 2014; Vigneau, Ecarnot, Rabatel, & Roumet, 2011) are promising multivariate techniques to assess vegetation nitrogen status separately from water status (Ullah et al., 2014; Zhang, Li, & Zhang, 2012). However, their use in the presence of different stressors has yet to be deeply explored (Karimi et al., 2005; Ray, Singh, & Panigrahy, 2010; Strachan et al., 2002). Generation of a hyperspectrogram (HSG) is a data extraction and compression technique based on PCA that manage large numbers of hyperspectral images and retain spatial information (Ferrari, Foca, & Ulrici, 2013). The HSGs are matrix signals that summarise the most relevant information carried by the original hyperspectral images (Ferrari, Foca, Calvini, & Ulrici, 2015). HSGs can then be used not only for calibration purposes—as opposed to the original image-but also for variable prediction by projecting them back into the image space or spectral domain to detect spatial and spectral regions carrying the most useful information.

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