QUANTIFYING INTRA-CANOPY HYPERSPECTRAL HETEROGENEITY WITH RESPECT TO SOYBEAN ANATOMY

by

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Dedicated to my loves across the country.
Your courage inspires me, every day.
I am lucky to have you.

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ABSTRACT

To support the growing human population, plant phenotyping technologies must innovate to rapidly interpret hyperspectral (HS) data into genetic inferences for plant breeders and managers. While pigment and nutrient concentrations within canopies are known to be vertically non-uniform, these chemical distributions as sources of HS noise are not universally addressed in scaling leaf information to canopy data nor in detecting spectral plant health traits.

In this project, soybeans (*Glycine Max,* cultivar Williams 82) were imaged with a Spectra Vista Corporation (SVC) HR-1024 spectroradiometer (350-2500 nm) at the highest five node positions. The samples were subjected to nitrogen and drought stress in factorial design (n=12) that was validated via relative water content (RWC) and PLS Regression of photopigments (chlorophyll a, chlorophyll b, lutein, neoxanthin, violaxanthin, and zeaxanthin in mg/g DW) and N concentration (%) for each imaged tissue. Welch's ANOVA and Tamhane's T2 post-hoc testing quantified spectral heterogeneity with respect to treatments and node positions through spectral angle measurements (SAMs) and percent NDVI difference. Drought-stressed samples had the lowest SAM between node positions compared to other treatments, and SAM node comparisons were greatest when including the highest sampled tissues. Taking ratios of NDVI between node positions proved more statistically effective at discerning between all factorial treatments than individual leaf NDVI values. Finally, intra-canopy spectral heterogeneity was exploited by training Linear Discriminant Analysis (LDA) classifiers on relative reflectance between node positions, tuning for the F1-Score. A classifier built on Node 1 vs. Node 3 reflectance outperformed in class-specific accuracies compared to analogous models trained on point-view data. Accounting for intra-canopy spectral variability is an opportunity to develop more comprehensive phenotyping tools for plant breeders in a world with rapidly rising agricultural demand.

1. INTRODUCTION

Agricultural management techniques and plant phenotyping must innovate to accommodate the needs of a 9.7 billion human population in time for 2050 (DESA, 2019; Walter, Finger, Huber, & Buchmann, 2017). Plant phenotyping aims to measure the manifestation of genomes with respect to their environments, with particular attention to traits supporting yield and stress resistance (Pieruschka & Schurr, 2019; Poorter et al., 2012). Hyperspectral imaging (HSI) techniques have delivered the benefits of radiometers' spectral resolution in addition to spatial data, evolving into a potent tool for plant phenotyping over the past decades (Curran, Dungan, & Peterson, 2001; Fahlgren, Gehan, & Baxter, 2015; Fiorani & Schurr, 2013; L. Li, Zhang, & Huang, 2014; Mahlein, Kuska, Behmann, Polder, & Walter, 2018). One of plant phenotyping's acute challenge is linking imaging and spectral data collected from varying resolutions (leaf, canopy, etc…) to actionable genetic information (Furbank & Tester, 2011; Minervini, Scharr, & Tsaftaris, 2015; Mochida et al., 2018).

HS data can permit nondestructive inferences on targets' chemical profiles, a crucial feature to monitoring crops over seasons. Absorptions features at specific wavelengths are used to generate vegetative indices (VIs) that contain information on water content (Gao, 1996; Tilling et al., 2007), photochemical concentrations such as chlorophylls, carotenes, and xanthophylls (Blackburn, 2007; Gamon & Surfus, 1999; Haboudane, Miller, Pattey, Zarco-Tejada, & Strachan, 2004; Peñuelas, Josep; Filella & Gamon, 1995; Sims & Gamon, 2002; Yoder & Pettigrew-Crosby, 1995), and structural chemicals such as lignin (Green et al., 1998; Kokaly, Asner, Ollinger, Martin, & Wessman, 2009). The Normalized Difference Vegetation Index (NDVI) is a ubiquitous example, which is frequently used to represent general vegetative health (Fischer et al., 1998). However, many VIs exist that are correlated to specific changes in pigment concentrations indicative of environmental stresses (Altangerel et al., 2017). High dimensional spectra are also examined with spectral angle measurements (SAM) (Kruse et al., 2008), signal derivatives (Le Maire, François, & Dufrêne, 2004), and even spectral ratios (Matsuda, Tanaka, Fujita, & Iba, 2012).

To generate HS data that accurately characterizes reflectance of a target, radiometric calibration is essential to produce useful data from gantry, imaging chamber, and point-view sources (Bai et al., 2019; V. S. Ciganda & Gitelson, 2008; Condorelli et al., 2018; Martínez-Martínez, Gomez-Gil, Machado, & Pinto, 2018; Römer et al., 2012). Controlled imaging facilities aim to manage variables that influence HS data quality such as background reflectance (Elsayed, Mistele, & Schmidhalter, 2011; Kolber et al., 2005; Zarco-Tejada, Ustin, & Whiting, 2005), sensor height and angle (Crusiol et al., 2017; He et al., 2016; Herrmann et al., 2018), and shading (Corti, Marino Gallina, Cavalli, & Cabassi, 2017; Jay et al., 2017). Another approach to calibrating plant spectra is taking into account anatomy, reconstructing 3D canopies (Behmann et al., 2015; Bellasio, Olejníčková, Tesař, Šebela, & Nedbal, 2012; Biskup, Scharr, Schurr, & Rascher, 2007; Neilson et al., 2015; Paproki, Sirault, Berry, Furbank, & Fripp, 2012; Paulus, 2019; Thapa, Zhu, Walia, Yu, & Ge, 2018; Zhou et al., 2019). This focus on architecture has led to organ-level segmentation, where leveraging spatial data has effectively predicted diseases (Abdu, Mokji, & Sheikh, 2019; Nagasubramanian et al., 2019), linked geometric traits to desired heritable genes (Miao et al., 2020), and identified drought stress via leaf incident angle (Behmann et al., 2016). Spectra carry useful data that becomes more relevant when accounting for lurking environmental factors.

Currently, spectral profiles of plants collected from either top or side view cameras are averaged to a single spectral profile (Bruning et al., 2019; Herrmann et al., 2018; Pandey, Ge, Stoerger, & Schnable, 2017) (Du et al., 2016; Haboudane et al., 2004). This approach provides limited information about plant condition because it does not take into account within canopy variation in neither plant traits (Blackburn, 1998; Lemaire & Gastal, 1997; H. Li, Zhao, Yang, & Feng, 2015) nor illumination patterns (Mercado et al., 2006). Vegetative age and development stage confound the predictive power of spectral data (Elsayed et al., 2011; Fiorani, Rascher, Jahnke, & Schurr, 2012; Zarco-Tejada et al., 2005). Standardizing sampling positions for HS point-collections (V. Ciganda, Gitelson, & Schepers, 2008; Yuan et al., 2016) and designing multi-angled metrics (He et al., 2016) are strategies to mitigate the impact of non-uniform canopy spectra. Despite its potential to obfuscate HS signals of plant health and distress, the literature does not characterize variance in spectral reflectance along vertical canopy profiles, (Gara, Darvishzadeh, Skidmore, & Wang, 2018; Gara, Skidmore, Darvishzadeh, & Wang, 2019; H. Li, Zhao, Huang, & Yang, 2013; H. Li et al., 2015; Ye et al., 2018). A knowledge gap exists in that

spectral variability has not explicitly been measured between canopy positions, nor utilized to classify crop responses to environmental stresses.

The following research aimed to directly leverage HS variability within the highest five nodes of soybean plants (*Glycine Max*) to detect drought and nitrogen stress treatments. Soybean was investigated due to the species' ubiquity in contemporary agriculture, and tissue biochemical concentrations were established via High-performance liquid chromatography (HPLC) analysis of extract separations and Partial Least Squares Regression (PLSR). While prior work has scaled leaf-level data by its cumulative LAI (Gara, Skidmore, et al., 2019), in this experiment spectra are directly compared between node positions with spectral angle measurements (SAM) and percent difference between NDVI to represent commonly utilized VIs. Single NDVI values and ratios between node positions are compared in efficacy at discerning each of the treatment groups using post-hoc testing. Finally, Linear Discriminant Analysis (LDA) classifiers built on relative reflectance between node positions are compared against analogous models built on point-view spectra.

2. MATERIALS AND METHODS

2.1 Experimental Design

In 14-1 of the Lily Hall of Life Sciences Greenhouse at Purdue University, dry-down and nitrogen deprivation treatments were executed in a factorial experimental design in replicates of 12. Williams 82 soybean plants (*Glycine Max*) were grown in randomized block without Rhizobium inoculation in Berger soilless media (coarse sphagnum peat moss (70-75%), perlite, composted sphagnum peat moss, and vermiculite). Control samples were watered to saturation with one liter of nutrient solution every four days, and on the data collection day. Fertilization was delivered with ICL 20N-1.3P-15.8K Specialty Fertilizers solution using reverse osmosis water at a concentration of 150 N, 9.8 P, 119 K, 12 Mg, 21 S, 1.5 Fe, 0.4 Mn and Zn, 0.2 Cu and B, and 0.1 Mo mg/L, with 61% nitrate and 39% ammoniacal sources. Using 0.734 gallon pots with a diameter of 16.2 cm, this fertilization is equivalent to 72.8 kg N/hectare and 36.4 kg N/hectare respectively for control and nitrogen-deprived treatments.

Dry-down lasted for 10 or 11 days, beginning when most of the plants were at V5 maturity. Visual inspection monitored for pests, which did not emerge over the experiment. Greenhouse temperature maintained within 23.9-32.2°C over the 32 days growing period extending from planting on October 6th, 2019 to the data collection days on November 7th and 8th 2019. Five equally spaced 500W halogen lamps supplemented lighting from 6:00am to 8:00pm.

2.2 HS Measurements

A Spectra Vista Corporation (SVC) HR-1024 spectroradiometer captured samples' point measurements. Using a leaf clip, two spectra were collected from the center trifoliate at the highest five nodes per plant to be averaged together, resulting in 235 point-view spectra. White references were taken every six samples. The sensor has spectral resolution from 350 nm to 2500 nm with respective intervals of 1.5 nm, 3.8 nm, and 2.5 nm for the 350-1000 nm, 1000-1890 nm, and 1890-2500 nm regions. Spectra were interpolated into 1.0 nm steps. NDVI was computed for each point-sampling position as $(R_{800} - R_{650})/(R_{800} + R_{650})$. Analysis was limited to the first trifoliate leaves from Nodes 1-4 from the top, excluding the highest due to challenging sampling logistics and

higher noise. Within each plant, SAMs were computed exhaustively between node position spectra as in Kruse et al. 1993. Spectral angle between spectrum can by calculated with the following formula, where y and r are sources with reflectance values in bands from i to n:

$$\alpha = \cos^{-1} \frac{\sum_{i=1}^{n} y_i r_i}{\sqrt{\sum_{i=1}^{n} y_i^2} \sqrt{\sum_{i=1}^{n} r_i^2}}$$

Each datum was labeled based on the source leaflet's position from the highest node of the plant, inspired by the Lindenmayer naming convention (Prusinkiewicz, 1998). This system is suited to soybean's anatomical plasticity and applicable to indeterminate, determinate, and semi-determinate varieties.

2.3 Reference Measurements

Determining total nitrogen content and the concentrations (mg/g DW) of neoxanthin, violaxanthin, lutein, zeaxanthin, chlorophyll a&b, and B-carotene in tissue samples validated the effects of the nitrogen deprivation and drought stress. For N and pigment concentrations, 118 and 116 tissues were processed for concentrations, respectively. This set of samples represented each node position and treatment combination. Referenced leaves were then used to generate a PLSR models per chemical to predict the concentrations of the rest of the spectral measurements.

Standard analytical determination of foliar carbon and nitrogen was performed using a Thermo Finnigan Flash 1112 elemental analyzer (San Jose, CA, USA). Pigments were quantified via HPLC following Cotrozzi et al (2016). Briefly, 50mg of lyophilized leaf material was homogenized with 1mL HPLC-grade methanol in a 2mL microtube. After incubating overnight at 4°C in darkness, samples were centrifuged for 15 min at 15,000 rpm. Supernatant was filtered through a 0.2 um Ministart SRT15 aseptic filter into amber HPLC vials for assessment and then run on a Shimadzu Prominence HPLC system with a PDA detector. Pigments were eluted with a solvent of 75% acetonitrile, 25% methanol for 14 minutes followed by a 1.5 linear gradient to a solvent of 68% methanol, 32% ethylacetate. The second solvent ran for another 14.5 minutes followed by a 2-minute linear gradient back to the first solvent for 5 minutes to equilibrate the

column. The flow rate was 1 mL/min with an injection volume of 25 µL, and pigments were identified based on their absorbance at 445 nm compared to pure standards.

Finally, the dry-down treatment was validated by deriving relative water content (RWC) per plant from tissue collected from the third highest node. This investigation acknowledges that these greenhouse results will not be directly transferrable to field hyperspectral data nor modern germplasm lines. Etiolation observed in samples may have affected spectral and chemical trends.

2.4 Statistical Tests

Matlab R2019a and The Field Spectroscopy Facility (FSF) Post Processing Toolbox imported, referenced, and delivered the spectroradiometer data. Python 3.6 *scipy*, *sklearn*, and *matplotlib* generated all visualizations and statistical analysis. The following methods and tests served to support the validation and evaluation of the experiment and its findings. Alpha was set to 0.05 and bootstrapping for mean values was set to 1000 resamples for 95% confidence intervals.

2.4.1 Referencing Tissue Photopigment and Nitrogen Concentrations

Photopigment concentrations were required for each imaged location in order to evaluate the significance of their relationships with treatment and node height. For chemical concentration, PLSR models built on established extracts and their spectra predicted the concentrations from the spectra of unreferenced samples (Wold, Ruhe, Wold, & Dunn, 1984; Wold, Sjostrom, & Eriksson, 2001). PLSR reduces the highly-dimensional spectra into latent variables which best predict the dependent pigment concentrations (Wegelin, 2000). Each model was refined using nested cross-validation (20 repetitions each) to tune to the optimal number of latent variables and generate train/test explained variance and root mean squared error (RMSE).

2.4.2 Quantify Significant Biochemical between Nodes and Treatments

Welch's ANOVA was used to test whether tissue concentrations were uniform across node heights or treatment groups. This test compares the means of groups, and is used on balanced, approximately normal data with heterogeneous variance between groups.

2.4.3 Evaluate Spectral Variability Pairwise Comparisons with Concentrations and VI Data with respect to Node Position and Treatment

Across treatments and node positions, a 3-way ANOVA tested the uniformity of NDVI intensities. When evaluating with respect to only treatment or node position, NDVI and NDVI ratios were evaluated with Welch's ANOVA. Following a rejected null hypothesis from a Welch's ANOVA test, post-hoc Tamhane's T2 tests identified which pairwise comparisons were significantly different between canopy positions or between treatment groups. Tamhane's T2 test conservatively evaluates data that is approximately normal with different variance between compared groups.

2.4.4 Classify Treatments based on Point Spectra or Relative Reflectance Spectra

Multiclass LDA models were trained on either spectra point-measurements or relative reflectance between various node positions, which emphasized intra-canopy spectral variability. These nonparametric classifiers performed dimensionality reduction on the spectra via singular value decomposition to maximize the distance between treatment groups in a projected linear subspace (Hastie, Tibshirani, & Friedman, 2008). Each classifier was refined using nested cross-validation (20 repetitions each) to tune based on F1-score and track precision, recall, accuracy, and kappa performance metrics. Confusion matrices summarized the classification performance across the treatments.

3. VALIDATION

3.1 Biochemical Concentrations over Node Positions

Chemical and RWC referencing characterized 47 plants (one sample died before data collection) to validate the impacts of the nitrogen-deprivation and dry-down treatments. While RWC was collected for each plant, PLSR completed the full set of photopigment and nitrogen concentrations with the unreferenced positions' spectra.

Nonparametric tests were used because homogeneity of variance was violated. Normal QQ plots deemed each concentration dataset acceptably normal, though each concentration and RWC with respect to treatment rejected homogeneous variance with $p = 1.1e4$ and $p = 3.7e4$ respectively from Levene's Test.

Table 1 summarizes the performance of the optimized chemical concentration PLSR models. Modeling ChlA/ChlB generated high RMSE (test = 1.43 +/- 1.45) with only 0.03 +/- 0.10 explained variance. To contrast, the model for nitrogen percent is comparable to the work of Bruning et. al (2019) who regressed using top-view HS data (400-2500 nm) to get validation R2 and RMSE scores of 0.60 and 0.43 respectively.

Table 1: PLSR Train and Test Cross Validation Performance Metrics

Metric	Neoxanthin (mg/g DW)	Violaxanthin (mg/g DW)	Lutein (mg/g DW)	Zeaxanthin (mg/g DW)	Chlorophyll a+b (mg/g DW)	Chlorophyll a/Chlorophyll b	B-carotene (mg/g DW)	Nitrogen (%)
NLV	4	3	3	5	4	2	3	16
Train Explained Var.	0.67 (+/- 0.07)	0.42 (+/- 0.06)	0.65 (+/- 0.07)	0.75 (+/- 0.10)	0.57 (+/- 0.10)	0.10 (+/- 0.04)	0.42 (+/- 0.09)	0.96 (+/- 0.02)
Test Explained Var.	0.55 (+/- 0.37)	0.25 (+/- 0.25)	0.57 (+/- 0.25)	0.31 (+/- 1.18)	0.46 (+/- 0.28)	0.03 (+/- 0.10)	0.44 (+/- 0.26)	0.20 (+/- 2.90)
Train RMSE	0.03 (+/- 0.01)	0.02 (+/- 0.01)	0.20 (+/- 0.07)	0.01 (+/- 0.01)	2.29 (+/- 0.86)	1.45 (+/- 0.95)	0.71 (+/- 0.29)	0.17 (+/- 0.10)
Test RMSE	0.03 (+/- 0.02)	0.02 (+/- 0.01)	0.22 (+/- 0.13)	0.01 (+/- 0.01)	2.48 (+/- 1.49)	1.43 (+/- 1.45)	0.67 (+/- 0.48)	0.80 (+/- 1.59)

NLV is Number of Latent Variables

The complete chemical concentrations dataset is displayed in Figure 1. When evaluated within treatments and along nodes with Welch's ANOVA, concentrations are significantly non-uniform for except for nitrogen percent in irrigated groups, B-carotene in the drought-stress treatment, and zeaxanthin in any of the treatment groups. B-carotene concentrations may have increased in the drought conditions due to accumulation in tissues due to stunting. When evaluating with Welch's ANOVA at each node position, the only concentrations which did not vary with respect to treatment were neoxanthin at nodes 3 and 4 as well as ChlA/ChlB at node 2. The ChlA/ChlB model's weak performance precluded researchers to not rely on its predictions.
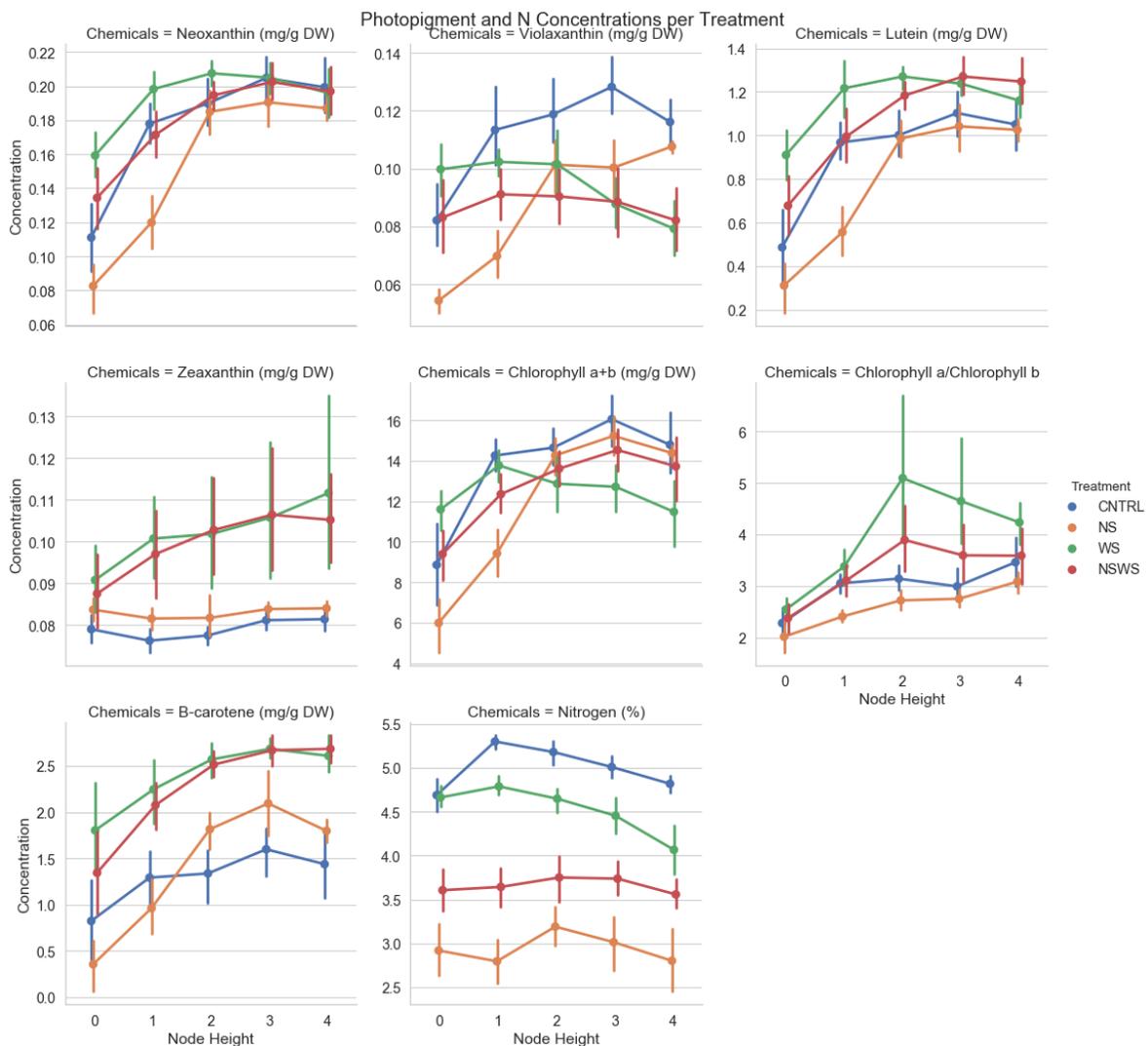
Figure 1: PLSR generated photopigment and nitrogen concentrations were generally significantly non-uniform per treatment and node positions according to Welch's ANOVA. Reduced nitrogen (%) and total Chl validate the nitrogen stress, while elevated zeaxanthin, and lutein validated the drought-stress.

Along analogous tissues subjected to the nitrogen-stress, overall chlorophyll concentration and percent nitrogen were reduced. This behavior validated the nitrogen deprivation, as it is typical of nutrient deprivation (Fang, Bouwkamp, & Solomos, 1998; Liu et al., 2009). Furthermore, nitrogen's apparent non-uniform descending trend is supported in the literature, where vertical nitrogen concentrations are tied to LAI (Grindlay, 1997; Hirose & Werger, 1987; Lemaire & Gastal, 1997; Pons, Schieving, Hirose, & Werger, 1990; Shiraiwa & Sinclair, 1993)

As displayed in Figure 2, RWC was significantly different between the irrigated and drought-treated groups. Water-stress was further validated in the significant increase in concentration of xanthophyll pigments zeaxanthin and lutein with respect to control as protections against oxidative stress (Altangerel et al., 2017; Havaux, 1998; Jaleel et al., 2009).

Significantly distinct by Welch's ANOVA
Significantly unique by Tamhane T2's Test
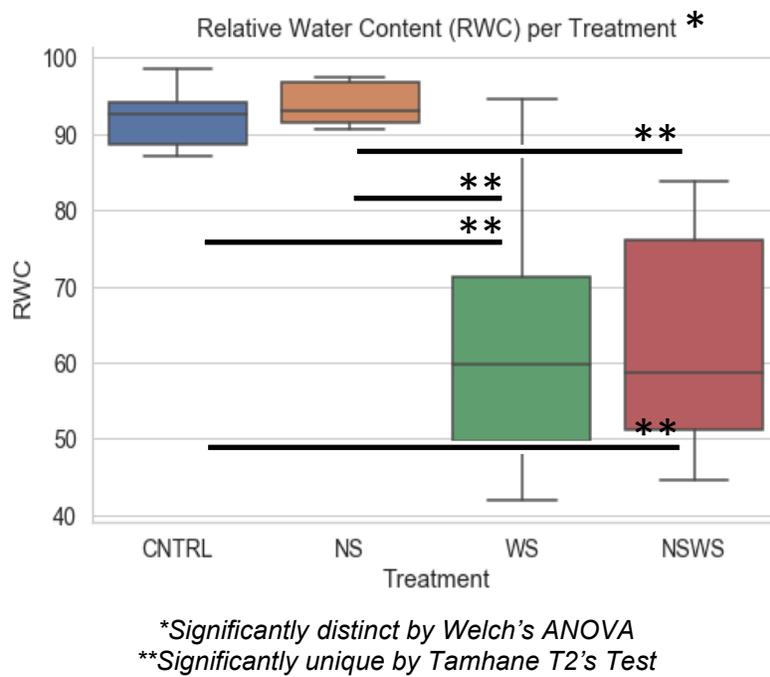
Figure 2: RWC per treatment was visibly and significantly distinct. Each treatment's RWC was respectively significantly indistinguishable and distinct from their shared and opposed irrigation regimens.

4. RESULTS AND DISCUSSION

4.1 Spectral variability across node positions

SAMs were generally greater between nodes that were further apart, but treatment-specific responses appeared in the magnitudes. Across intra-canopy comparisons shown in Figure 3, drought-stressed spectra vary less between node positions than in the other treatments, even at the most extreme pairings where the data is more variable (this is explicitly presented in Table 2).

*Significantly distinct by Welch's ANOVA
**Significantly unique by Tamhane T2's Test
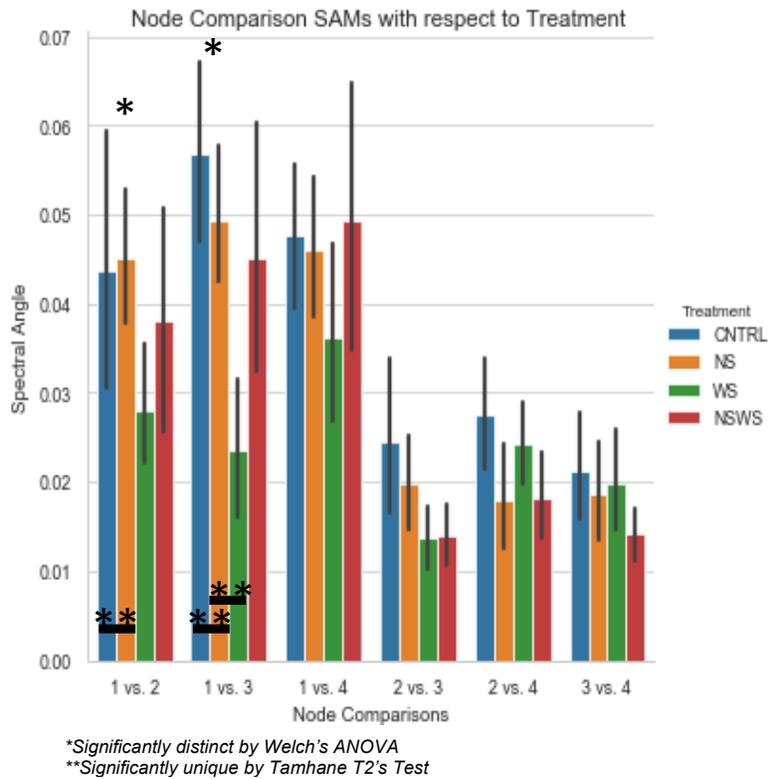
Figure 3: Spectral divergence between nodes of greater relative age difference or distance trends to be greater. These magnitudes, however, are significantly influenced by treatment when comparing nodes 1 vs. 2 and nodes 1 vs. 3.

Table 2: Mean SAM and Standard Deviation between Node Positions per Treatment

Node Comparison	CNTRL	NS	NSWS	WS
1 vs. 2	0.044, 0.025	0.045, 0.013	0.038, 0.024	0.028, 0.011
1 vs. 3	0.057, 0.018	0.049, 0.014	0.045, 0.026	0.024, 0.014
1 vs. 4	0.048, 0.014	0.046, 0.015	0.049, 0.028	0.036, 0.018
2 vs. 3	0.025, 0.015	0.020, 0.009	0.014, 0.006	0.014, 0.006
2 vs. 4	0.028, 0.012	0.018, 0.010	0.018, 0.009	0.024, 0.009
3 vs. 4	0.021, 0.011	0.019, 0.010	0.014, 0.005	0.020, 0.011

Node position's influence on SAM magnitude could be partially explained by tissues' relative maturities, as developing mesophilic tissue influences the path and absorption of light, fundamentally shifting vegetation's reflectance (Rapaport, Hochberg, Rachmilevitch, & Karnieli, 2014). Leaf age has been shown to influence abiotic and biotic stress responses, which inform HS data collection methods (Berens et al., 2019; Elvanidi, Katsoulas, & Kittas, 2018). Additionally, the dynamics of photosynthesis with respect to younger, more intensely illuminated tissues compared to those with more diffuse illumination could also be culpable (Gara, Darvishzadeh, Skidmore, Wang, & Heurich, 2019; Mercado et al., 2006). To model canopy compositions, sensor positioning balances the need to capture representative canopy data (Elvanidi et al., 2018; Ye et al., 2018) with maintaining signal over noise (Martínez-Martínez et al., 2018).

4.2 Efficacies of intra-canopy combination VIs at distinguishing abiotic distress treatments

SAM reports the magnitude of spectral variability, but without specifying which chemometrically relevant wavelengths are responsible. As an illustrative example of potential VI non-uniformity, NDVI was plotted with respect to treatment and node position, displayed in Figure 4A. Results from a 3-way ANOVA evaluated the relationship of watering, fertilizing, and node position as features with respect to NDVI in Table 3. Water treatment and node height were significant factors individually, though the nitrogen treatment was significant in its interaction with irrigation or with irrigation and node height. With these findings, NDVI does not appear equally sensitive to water and nitrogen stresses.

Table 3: Node height, nitrogen treatment, and water treatment relationships with NDVI intensity

Feature	Sum of Squares	DoF	F	P-value
Intercept	8.111	1	21648.68	**7.91e-183**
C(Water)	7.77e-3	1	20.731	**9.97e-6**
C(Nitrogen)	2.43e-4	1	0.649	0.422
C(Node Height)	0.010	3	9.303	**9.83e-6**
C(Water):C(Nitrogen)	3.86e-3	1	10.300	**1.59e-3**
C(Water):C(Node Height)	0.022	3	19.929	**3.91e-11**
C(Nitrogen):C(Node Height)	2.66e-4	3	0.237	0.871
C(Water):C(Nitrogen):C(Node Height)	3.58e-3	3	3.183	**0.025**

Within each treatment group, post-hoc analysis after a failed Welch's ANOVA reveal significant variability at specific node comparisons (Figure 4B). In stressed treatments, comparisons between the highest and lowest tissues were significant, and the greatest proportion of significant NDVI node comparisons came from the nitrogen-stressed samples. The directionality of NDVI changes between nodes in nitrogen-stressed samples were mirrored in the control, but the magnitude made the stress response distinct. Water-stressed treatments shared a characteristic NDVI decline in from the highest to lowest canopy positions.
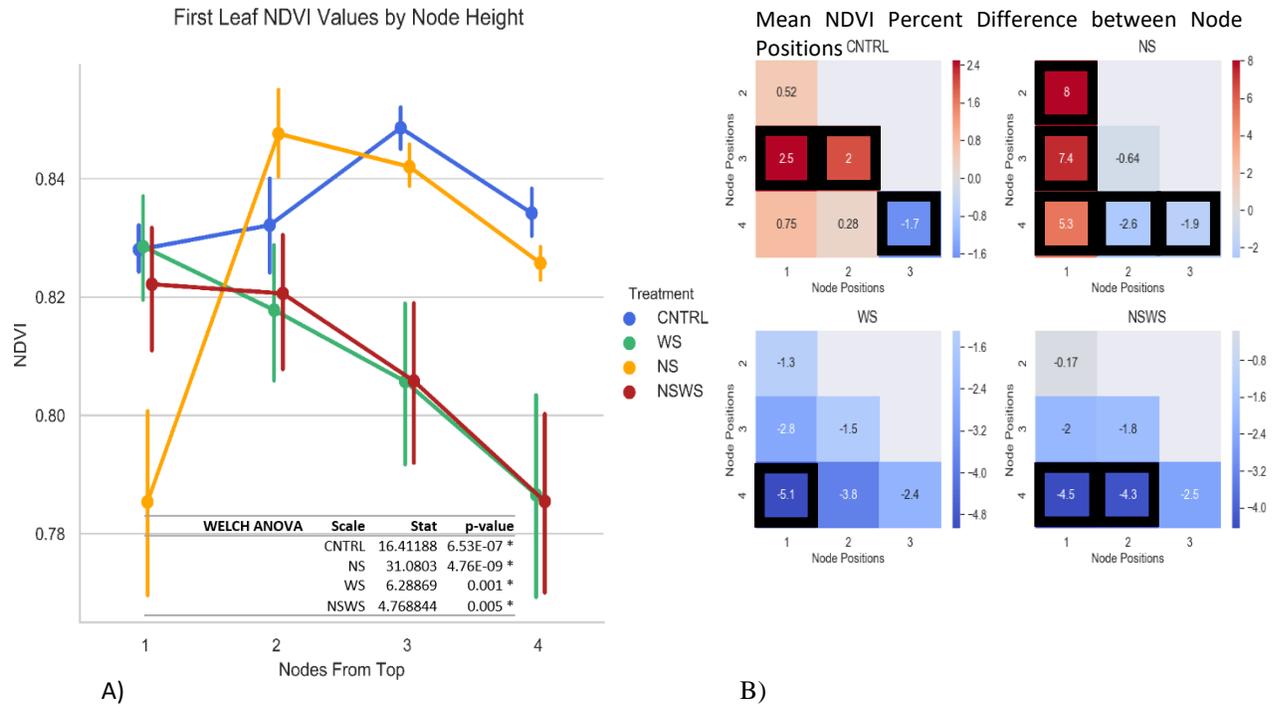
Figure 4: Nonlinear trends appear in NDVI intensity along node positions, which include changes in rank between treatments depending on node height. Error bars are 95% CI (A). Significant changes in NDVI intensity between node positions characterize sample responses to drought and nitrogen stresses via magnitude and directionality of the changes (B).

Nitrogen-stress is likely so apparent in post-hoc testing due to NDVI's constituent wavelengths NIR and red being stronger correlated to key nitrogen-incorporating pigments than to a direct water absorption feature (Peñuelas, Josep; Filella & Gamon, 1995).

The significant NDVI fluctuations within canopy were tested as a signal to separate treatment groups with additional post-hoc testing. Single and ratios of NDVI readings distinguished between the treatments to variable efficacy, summarized in Figure 5. The only NDVI point-measurement effective at differentiating all treatments was at the fifth node from the top (node 4). Alternatively, ratio NDVI metrics were also effective (Node 1 vs. Node 3, Node 1 vs. Node 4) and to greater statistical significance.
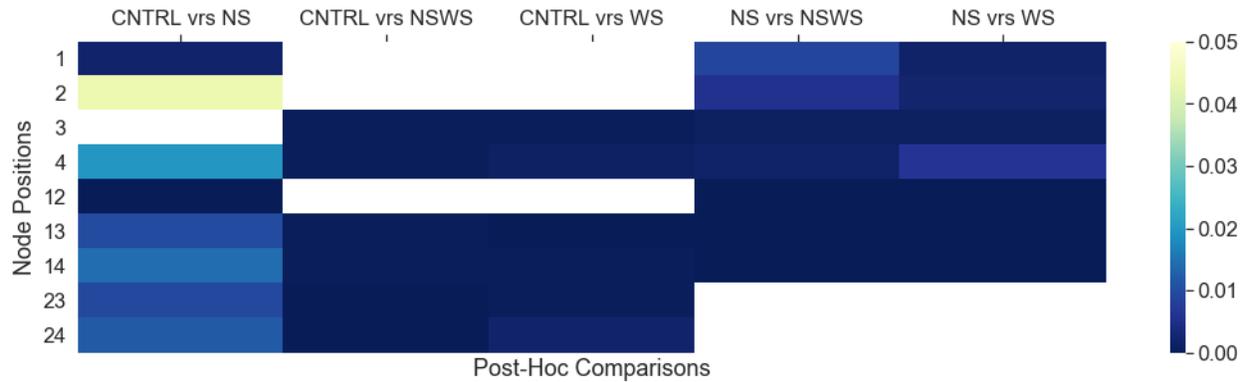
Figure 5: Post-Hoc significance of NDVI at discerning between treatments as single measurements or ratios between node positions. Point-view NDVI and combination NDVI metrics can significantly discern between all of the treatments, though the ratio metrics do so to greater statistical significance.

These results indicate that the variability in NDVI intensity between canopy positions can be more effective at discerning treatment groups than point measurements. Individual locations can be effective, but the lurking effects of canopy position are observed in the literature. Even when controlling sampling positions for collecting point view data, it is possible vegetative maturation can influence the collected spectra in addition to environmental stresses (Herrmann et al., 2018; Rapaport et al., 2014; Yuan et al., 2016)

1.3 LDA Classification using single-measurements spectra or relative reflectance between positions

Built on individual spectra or ratios between pairs of positions, multiclass LDA models were tuned using nested cross-validation (repeats=20) with an external cross-validation, selecting for F1-Score. The performance metrics from outer CV with their 95% confidence intervals are summarized in Table 4, where classifiers built on data from Node 1, Node 3, or their ratio performed superiorly.

Table 4: LDA Performance metrics with 95% CI: Overall and per Treatment

Node Data	Precision	Recall	F1-Score	Accuracy	Kappa
1 vs. 3	**0.83 (+/- 0.22)**	**0.83 (+/- 0.22)**	**0.81 (+/- 0.23)**	**0.82 (+/- 0.21)**	**0.75 (+/- 0.21)**
1	0.82 (+/- 0.15)	0.81 (+/- 0.17)	0.79 (+/- 0.19)	0.80 (+/- 0.21)	0.73 (+/- 0.21)
3	0.78 (+/- 0.30)	0.80 (+/- 0.27)	0.76 (+/- 0.29)	0.77 (+/- 0.26)	0.69 (+/- 0.26)
1 vs. 4	0.71 (+/- 0.23)	0.70 (+/- 0.22)	0.67 (+/- 0.20)	0.72 (+/- 0.19)	0.61 (+/- 0.19)
2	0.70 (+/- 0.21)	0.71 (+/- 0.13)	0.66 (+/- 0.18)	0.68 (+/- 0.17)	0.57 (+/- 0.17)
1 vs. 2	0.66 (+/- 0.19)	0.64 (+/- 0.24)	0.61 (+/- 0.20)	0.64 (+/- 0.16)	0.51 (+/- 0.16)
4	0.64 (+/- 0.25)	0.66 (+/- 0.26)	0.61 (+/- 0.23)	0.63 (+/- 0.24)	0.51 (+/- 0.24)
2 vs. 3	0.62 (+/- 0.25)	0.59 (+/- 0.25)	0.56 (+/- 0.25)	0.61 (+/- 0.24)	0.47 (+/- 0.24)
2 vs. 4	0.54 (+/- 0.18)	0.54 (+/- 0.22)	0.50 (+/- 0.20)	0.54 (+/- 0.20)	0.38 (+/- 0.20)
3 vs. 4	0.33 (+/- 0.26)	0.32 (+/- 0.24)	0.28 (+/- 0.20)	0.30 (+/- 0.21)	0.09 (+/- 0.21)

The Node 1/Node 3 classifier performed the best overall, followed by the Node 1 classifier. In Table 5, confusion matrices for both classifiers illustrate how the Node 1 classifier was more confused in detecting the water stressed samples. While the Node 1/Node 3 classifier was less effective at identifying the nitrogen stressed samples, it had lower or comparable variability for the rest of the treatments.

Table 5: Mean Confusion Matrices and confidence intervals for classifiers built on Node 1 and Node 1/Node 3 spectra

Node 1/Node 3

	CNTRL	WS	NS	NSWS
CNTRL	**0.912 (+/- 0.234)**	0.0 (+/- 0.0)	0.065 (+/- 0.22)	0.022 (+/- 0.136)
WS	0.0 (+/- 0.0)	**0.837 (+/- 0.368)**	0.0 (+/- 0.0)	0.163 (+/- 0.368)
NS	0.056 (+/- 0.23)	0.0 (+/- 0.0)	0.909 (+/- 0.3)	0.035 (+/- 0.23)
NSWS	0.077 (+/- 0.27)	0.262 (+/- 0.472)	0.012 (+/- 0.108)	0.599 (+/- 0.578)

Node 1

	CNTRL	WS	NS	NSWS
CNTRL	0.912 (+/- 0.326)	0.012 (+/- 0.108)	0.025 (+/- 0.218)	0.05 (+/- 0.254)
WS	0.02 (+/- 0.174)	0.736 (+/- 0.502)	0.03 (+/- 0.146)	0.214 (+/- 0.472)
NS	0.0 (+/- 0.0)	0.0 (+/- 0.0)	**0.978 (+/- 0.136)**	0.022 (+/- 0.136)
NSWS	0.153 (+/- 0.494)	0.22 (+/- 0.436)	0.01 (+/- 0.088)	**0.617 (+/- 0.548)**

Figure 7 presents the ratio of spectra between Nodes 1 and 3, in which relative reflectance per treatment reveals distinctive features. By emphasizing spectral variability resulting from treatment, sampling groups were separated as in Matsuda et al. 2012. It is possible that the relative reflectance model's advantage was by capturing non-uniform hyperspectral signals in canopies (Römer et al., 2012) that could be tied to age-mediated responses to stresses (Berens et al., 2019). In the literature, the usefulness of HS data collected from canopies compared to leaf measures depends how much of the plants are in view (Herrmann et al., 2018; Martínez-Martínez et al., 2018; Mishra et al., 2017). SAMs between Nodes 1 and 3 were among the largest overall as they represent distinct portions of the canopy, though the water/nitrogen stressed samples had greater variability at this comparison. It is possible this unequal noise contributed to the relative reflectance model's confusion in classifying the water/nitrogen stressed samples compared to the Node 1 model.
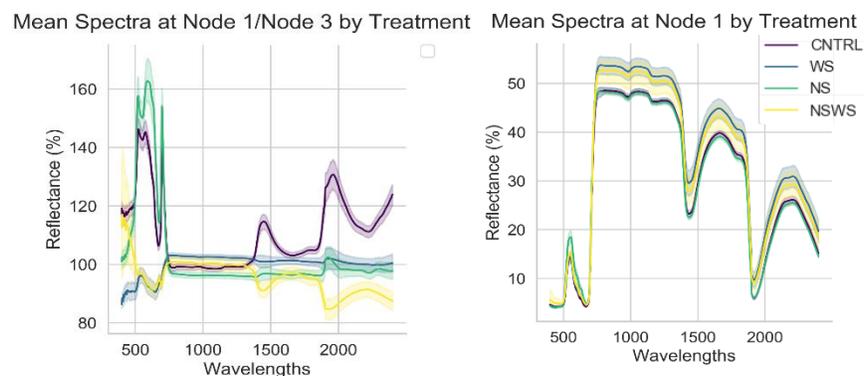

Figure 6: The transformed Node 1 vs. Node 3 spectra had distinctive shape, but overall greater variance over the range and within treatments. Node 1 data, on the other hand, had observably greater variability in the drought treatments compared to the irrigated.

5. CONCLUSIONS

As phenotyping technologies become ubiquitous in plant breeding and farming operations, it is our obligation to account for significant and known signal noise in our methods. In this project, soybeans were subjected to nitrogen and drought stress were distinguished apart by leveraging HS heterogeneity collected from the highest nodes. SAMs and percent NDVI differences between node positions quantified spectral heterogeneity with respect to treatments, and spectral stress responses displayed characteristic non-uniform, directional, and significant NDVI intensity trends. Leveraging spectral variability revealed that NDVI ratios proved more statistically effective at discerning treatments than individual leaf NDVI values and a LDA classifier built on relative reflectance delivered more uniform classification performance. This work will inform and justify the design of organ-level HS segmentation and more effective sensing methods to detect plant and canopy health statuses. Understanding the signal in canopy spectral variability is an opportunity to develop more comprehensive phenotyping tools to swiftly glean actionable information on expressed crop stresses and resiliencies.

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