

Proximal hyperspectral sensing and data analysis approaches for field-based plant phenomics

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Abstract

Field-based plant phenomics requires robust crop sensing platforms and data analysis tools to successfully identify cultivars that exhibit phenotypes with high agronomic and economic importance. Such efforts will lead to genetic improvements that maintain high crop yield with concomitant tolerance to environmental stresses. The objectives of this study were to investigate proximal hyperspectral sensing with a field spectroradiometer and to compare data analysis approaches for estimating four cotton phenotypes: leaf water content (C_w), specific leaf mass (C_m), leaf chlorophyll $a + b$ content (C_{ab}), and leaf area index (LAI). Field studies tested 25 Pima cotton cultivars grown under well-watered and water-limited conditions in central Arizona from 2010 to 2012. Several vegetation indices, including the normalized difference vegeta-

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tion index (NDVI), the normalized difference water index (NDWI), and the physiological (or photochemical) reflectance index (PRI) were compared with partial least squares regression (PLSR) approaches to estimate the four phenotypes. Additionally, inversion of the PROSAIL plant canopy reflectance model was investigated to estimate phenotypes based on 3.68 billion PROSAIL simulations on a supercomputer. Phenotypic estimates from each approach were compared with field measurements, and hierarchical linear mixed modeling was used to identify differences in the estimates among the cultivars and water levels. The PLSR approach performed best and estimated C_w , C_m , C_{ab} , and LAI with root mean squared errors (RMSEs) between measured and modeled values of 6.8%, 10.9%, 13.1%, and 18.5%, respectively. Using linear regression with the vegetation indices, no index estimated C_w , C_m , C_{ab} , and LAI with RMSEs better than 9.6%, 16.9%, 14.2%, and 28.8%, respectively. PROSAIL model inversion could estimate C_{ab} and LAI with RMSEs of about 16% and 29%, depending on the objective function. However, the RMSEs for C_w and C_m from PROSAIL model inversion were greater than 30%. Compared to PLSR, advantages to the physically-based PROSAIL model include its ability to simulate the canopy’s bidirectional reflectance distribution function (BRDF) and to estimate phenotypes from canopy spectral reflectance without a training data set. All proximal hyperspectral approaches were able to identify differences in phenotypic estimates among the cultivars and irrigation regimes tested during the field studies. Improvements to these proximal hyperspectral sensing approaches could be realized with a high-throughput

phenotyping platform able to rapidly collect canopy spectral reflectance data from multiple view angles.

16 *Keywords:* cotton, chlorophyll, drought, high performance computing,
17 inverse modeling, leaf, partial least squares regression, phenotyping,
18 PROSAIL, remote sensing, spectral reflectance, spectroradiometer, water,
19 vegetation index

20 **1. Introduction**

21 To improve food security, adapt to climate change, and reduce resource
22 requirements for crop production, scientists must better understand the con-
23 nection between a plant’s observable characteristics (phenotype) and its ge-
24 netic makeup (genotype). Unprecedented advances in DNA sequencing have
25 unlocked the genetic code for many important food crops, including rice
26 (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), and maize (*Zea mays* L.)
27 (Bolger et al., 2014). However, understanding how genes control complex
28 plant traits, such as drought tolerance, time to anthesis, and harvestable
29 yield, remains challenging. Field-based plant phenomics seeks to implement
30 information technologies, including sensing and computing tools in combi-
31 nation with genetic mapping approaches, to rapidly characterize the phys-
32 iological responses of genetically diverse plant populations in the field and
33 relate these responses to individual genes (Araus and Cairns, 2014; Furbank
34 and Tester, 2011; Houle et al., 2010; Montes et al., 2007; White et al., 2012).
35 When validated, crop improvement strategies based on targeted quantitative

36 trait loci and genomic selection can be used for efficient development of crop
37 cultivars that are both high yielding and resilient to environmental stresses.

38 A variety of electronic sensors have been deployed for field-based plant
39 phenomics, mainly on ground-based vehicles. Andrade-Sanchez et al. (2014)
40 developed a sensing platform on a high-clearance tractor that collected data
41 over four Pima cotton (*Gossypium barbadense* L.) rows simultaneously. Ul-
42 trasonic sensors, infrared radiometers, and active multispectral radiometers
43 were used to measure canopy height, temperature, and reflectance, respec-
44 tively. Scotford and Miller (2004) mounted passive two-band radiometers and
45 ultrasonic sensors on a tractor boom and used the system to estimate tiller
46 density and leaf area index (LAI) of winter wheat (*Triticum aestivum* L.).
47 Other sensing systems have incorporated passive hyperspectral radiometers
48 (spectroradiometers) for measuring crop canopy spectral reflectance contin-
49 uously over a range of wavelengths, typically within the visible and near-
50 infrared spectrum. For example, the phenotyping platform of Comar et al.
51 (2012) incorporated four spectroradiometers sensitive between 400 and 1000
52 nm at 3 nm spectral resolution and two RGB digital cameras. Also, Montes
53 et al. (2011) developed a system with light curtains for canopy profiling and
54 spectroradiometers sensitive between 320 and 1140 nm at 10 nm spectral res-
55 olution. Rundquist et al. (2004) compared machine-based versus hand-held
56 deployment of a spectroradiometer and found reduced variability and higher
57 reproducibility of sensor measurements when the instrument was positioned
58 by a machine.

59 Following sensor platforms, the next challenge for field-based plant phe-
 60 nomics is the development of methodologies to extract meaningful informa-
 61 tion from the sensor data, with the ultimate goal to quantify specific crop
 62 phenotypes. However, the fundamental measurements of many sensors have
 63 little utility for crop phenotyping without additional post-processing and
 64 analysis. For simple, empirical processing of canopy spectral reflectance data,
 65 a multitude of vegetation indices have been developed (Bannari et al., 1995)
 66 and used to estimate several crop characteristics, including canopy cover,
 67 LAI, and biomass (Wanjura and Hatfield, 1987). The popular normalized
 68 difference vegetation index (NDVI) is traditionally calculated as

$$\text{NDVI} = \frac{\rho_2 - \rho_1}{\rho_2 + \rho_1} \quad (1)$$

69 where ρ_2 is the spectral reflectance in the near-infrared waveband and ρ_1 is
 70 the spectral reflectance in the red waveband. However, with the advent of
 71 hyperspectral sensors, other narrow-band indices have been developed us-
 72 ing the NDVI equation with reflectance data in different wavebands. For
 73 example, Gamon et al. (1992) developed the physiological (or photochemi-
 74 cal) reflectance index (PRI), a narrow-band index using reflectance at 531
 75 nm to track xanthophyll cycle pigments and estimate photosynthetic effi-
 76 ciency. Likewise, Gao (1996) developed the normalized difference water in-
 77 dex (NDWI) to estimate vegetation water content. Many other studies have
 78 identified optimum wavebands for a given application by calculating narrow-
 79 band NDVI for all possible waveband combinations for a given hyperspectral

80 sensor (Fu et al., 2014; Hansen and Schjoerring, 2003; Thenkabail et al.,
 81 2000; Thorp et al., 2004). Babar et al. (2006) demonstrated several narrow-
 82 band spectral reflectance indices that explained genetic variability in wheat
 83 biomass. Mistele and Schmidhalter (2008) measured spectral reflectance of
 84 maize canopies from four view angles and found the spectral reflectance in-
 85 dices were strongly correlated ($0.57 \leq r^2 \leq 0.91$) with total nitrogen uptake
 86 and dry biomass weight. In a study by Gutierrez et al. (2012), spectral re-
 87 flectance indices explained over 87% and 93% of the variability in biomass
 88 and LAI, respectively, for three upland cotton varieties. Seelig et al. (2008)
 89 correlated shortwave infrared spectral reflectance indices with relative water
 90 content and thickness of peace lily (*Spathiphyllum lynnise*) leaves ($r^2 > 0.94$).

91 Other spectral data analysis approaches consider all the visible, near-
 92 infrared, and shortwave infrared wavebands collectively. Statistical proce-
 93 dures such as principal component regression (PCR) and partial least squares
 94 regression (PLSR) reduce dimensionality by decomposing the hyperspectral
 95 data into a set of independent factors, against which crop biophysical traits
 96 are regressed. For example, Thorp et al. (2008) used PCR to estimate maize
 97 stand density from aerial hyperspectral imagery ($r^2 = 0.79$). Also, Thorp
 98 et al. (2011) used proximal spectral reflectance data with PLSR to estimate
 99 dry biomass weight, flower counts, and silique counts of lesquerella (*Les-*
 100 *querella fendleri*) with root mean squared errors of prediction equal to 2.1
 101 Mg ha⁻¹, 251 flowers, and 1018 siliques, respectively. In another study,
 102 PLSR models developed from spectral reflectance of rice canopies explained

up to 71% of the variability in plant nitrogen (Bajwa, 2006). Hansen and Schjoerring (2003) compared estimates of wheat biophysical variables using 1) linear regression on narrow-band NDVI with optimal wavebands and 2) PLSR with all wavebands from 400 to 900 nm. The NDVI approach better estimated LAI and chlorophyll concentration, while the PLSR approach better estimated green biomass weight and nitrogen concentration.

Another potential solution for quantifying crop phenotypes involves combining measured spectral reflectance data with physical models of radiative transfer in the plant canopy. Input parameters for such models describe attributes (i.e., phenotypes) of the crop canopy, which are used to simulate canopy spectral reflectance. For example, with 14 input parameters that describe plant characteristics and illumination conditions, the PROSAIL model (Jacquemoud et al., 2009) can simulate plant canopy spectral reflectance from 400 to 2500 nm in 1 nm wavebands. Using model inversion techniques, spectral reflectance measurements from spectroradiometers can be used to estimate PROSAIL input parameters. These estimates represent additional crop phenotypes that could be useful in subsequent genetic analyses. By linking crop phenotypes to sensor data through the theoretical knowledge contained in the simulation model, the approach is less empirical than the vegetation index and PLSR approaches.

Literature provides examples of PROSAIL model inversion for vegetation characterization in diverse environments, but field-based plant phenomics is a novel application. Jacquemoud (1993) first investigated the practical

126 limitations of PROSAIL model inversion using synthetic spectra. A subse-
127 quent study tested field spectroradiometer data with PROSAIL model in-
128 version to retrieve sugar beet (*Beta vulgaris*) canopy characteristics, such as
129 chlorophyll $a + b$ concentration, leaf water thickness, LAI, and leaf inclina-
130 tion angle (Jacquemoud et al., 1995). At coarser spatial and spectral scales,
131 Zarco-Tejada et al. (2003) used data from the Moderate Resolution Imaging
132 Spectroradiometer (MODIS) satellite to invert PROSAIL for estimation of
133 chaparral vegetation water content in a central California shrub land. Yang
134 and Ling (2004) estimated leaf water thickness of New Guinea impatiens
135 (*Impatiens hawkeri*) in a controlled environment using PROSAIL model in-
136 version from 1300 nm to 2500 nm, but spectral artifacts between 400 and
137 1300 nm due to artificial lighting prevented the estimation of other plant
138 characteristics. PROSAIL model inversion also provided estimates of LAI
139 and chlorophyll $a + b$ concentration for potato (*Solanum tuberosum* L.) and
140 wheat managed with variable nitrogen fertilization rates (Botha et al., 2007,
141 2010). Others have linked PROSAIL with dynamic models of crop growth
142 and development for wheat (Thorp et al., 2012) and maize (Koetz et al.,
143 2005), which permitted model inversion using time-series spectral reflectance
144 measurements of the crop canopy.

145 In many previous studies, iterative optimization was used to solve the
146 PROSAIL model inversion problem (Botha et al., 2007, 2010; Jacquemoud
147 et al., 1995; Thorp et al., 2012; Yang and Ling, 2004; Zarco-Tejada et al.,
148 2003). Optimization aims to find solutions in a computationally efficient

149 manner, but convergence to local minimums is a risk. Others have used
150 lookup tables to solve the inversion problem (Combal et al., 2003; Darvishzadeh
151 et al., 2012; Koetz et al., 2005). Lookup tables are a relatively simple way to
152 characterize model responses, but the computational expense can be great
153 if many simulations are required to adequately characterize the parameter
154 space. High-performance computers increase the practicality of the lookup
155 table approach.

156 The goal of this study was to assess the utility of proximal hyperspectral
157 data and related data analysis techniques for estimating crop phenotypes
158 among Pima cotton cultivars grown in Arizona field studies. Specific objec-
159 tives were 1) to compare NDVI, NDWI, PRI, PLSR, and PROSAIL model
160 inversion methods to estimate leaf water thickness, specific leaf mass, chloro-
161 phyll $a + b$ concentration, and LAI in cotton and 2) to assess differences
162 between phenotypic estimates among irrigation and cultivar treatments im-
163 posed during the field studies.

164 **2. Materials and Methods**

165 *2.1. Field experiments*

166 As described in detail by Andrade-Sanchez et al. (2014), field experiments
167 were conducted during the summers of 2010, 2011, and 2012 at the Maricopa
168 Agricultural Center (33.068° N, 111.971° W, 360 m above mean sea level)
169 near Maricopa, Arizona. Twenty-five Pima cotton cultivars were grown under
170 well-watered (WW) and water-limited (WL) conditions using a 5×5 lattice
171 design with four replications per treatment. Experimental units were one

172 row with length of 8.8 m and row spacing of 1.02 m. A subset of four cotton
173 cultivars in 2010 (Monseratt Sea Island, Pima 32, Pima S-6, and Pima S-7)
174 and five cotton cultivars in 2011 and 2012 (89590, Monseratt Sea Island, P62,
175 PSI425, and Pima S-6) were selected for intensive field measurements and
176 proximal hyperspectral data collection. These cultivars were chosen based
177 on their different release dates to increase the range of expected responses to
178 heat and water deficit (Carmo-Silva et al., 2012). Subsurface drip irrigation
179 methods were used with irrigation schedules determined from a daily soil
180 water balance model based on FAO-56 methods (Allen et al., 1998). When
181 50% of treatment plots had one visible flower, the WL treatment received
182 one-half the irrigation rate of the WW treatment.

183 *2.2. Field data collection*

184 Intensive field data collection to characterize leaf water content and canopy
185 spectral reflectance for the selected Pima cultivars occurred on five occasions
186 during the three field experiments (Table 1). Measurements were collected
187 in August during the cotton boll filling period. Collection times in 2011
188 and 2012 were focused in the morning hours after the 2010 data analysis
189 revealed larger differences in relative leaf water content between WW and
190 WL treatments earlier in the day (Carmo-Silva et al., 2012).

191 During each data collection outing, ground-based radiometric measure-
192 ments were collected over the selected Pima cultivars using a hand-held field
193 spectroradiometer (Fieldspec 3, Analytical Spectral Devices, Inc., Boulder,
194 CO, USA). Radiometric information was reported in 2151 narrow wavebands

195 from 350 to 2500 nm in 1 nm intervals. The instrument was equipped with
196 a 25° field-of-view fiber optic. To avoid soil background effects, a wand con-
197 structed from PVC tubing was used to position the fiber optic at a nadir
198 view angle approximately 0.25 m above the canopy. Because of the proxim-
199 ity of the sensor to the target, the methods are termed “proximal sensing”
200 as opposed to “remote sensing.” Frequent radiometric observations of a cal-
201 ibrated, 0.6 m², 99% Spectralon panel (Labsphere, Inc., North Sutton, New
202 Hampshire) were used to characterize incoming solar radiation throughout
203 the data collection period. Because atmospheric absorption led to insuffi-
204 cient light in some wavebands, subsequent analyses of all spectral data used
205 1703 wavebands from 400 to 1350 nm, 1450 to 1770 nm, and 1970 to 2400
206 nm. Canopy reflectance factors in each waveband were computed as the ra-
207 tio of the canopy radiance over the corresponding time-interpolated value for
208 Spectralon panel radiance. Reflectance factors from six to twelve radiomet-
209 ric measurements over each experimental plot were averaged to estimate the
210 overall canopy spectral reflectance response. Variability in the number of
211 scans per plot was dependent on manual triggering of the spectroradiometer
212 while slowly walking through the field.

213 Simultaneously with canopy spectral reflectance measurements, two leaf
214 tissue samples were collected from two leaves in each plot with a 2 cm²
215 punch. Two leaf disks were collected per sample from one leaf at the top of
216 the canopy, sealed in a 3 × 4 cm² pre-weighed ziplock bag, and stored on
217 ice in an insulated cooler. In the laboratory, the fresh weight of leaf samples

218 (m_f) was measured on an electronic balance (AE 160, Mettler-Toledo, LLC,
 219 Columbus, OH, USA). Leaf disks were then removed from the bags and oven
 220 dried prior to dry weight (m_d) measurements. The leaf water thickness (C_w)
 221 was calculated as the depth of water per unit leaf area (cm):

$$C_w = (m_f - m_d) / (\rho_w \times A_{ls}) \quad (2)$$

222 where ρ_w is the density of water (1.0 g cm^{-3}) and A_{ls} is the total area of the
 223 leaf sample. The specific leaf mass (C_m , g cm^{-2}) was also calculated:

$$C_m = m_d / A_{ls} \quad (3)$$

224 Within two weeks of proximal hyperspectral measurements (Table 1),
 225 additional leaf samples were collected for measurements of chlorophyll $a + b$
 226 concentration (C_{ab}). Two 0.3 cm^2 leaf disks were obtained from each exper-
 227 imental plot and stored at $-80 \text{ }^\circ\text{C}$. Using the method of Porra et al. (1989),
 228 100% methanol (1 mL) was added to each sample for pigment extraction in
 229 the dark at $4 \text{ }^\circ\text{C}$ for 48 h with mixing. A $200 \text{ }\mu\text{L}$ sample of the supernatant
 230 was collected for absorbance measurements at 652 nm (A_{652}) and 665 nm
 231 (A_{665}), which were used to estimate C_{ab} ($\mu\text{g cm}^{-2}$):

$$C_{ab} = (22.12A_{652} + 2.71A_{665}) / A_{ls} \quad (4)$$

232 Within one day of proximal hyperspectral measurements (Table 1), the
 233 field-based high-throughput phenotyping system of Andrade-Sanchez et al.

234 (2014) was used to measure canopy reflectance, height, and temperature in
 235 each experimental plot. Sensors were deployed on an open rider sprayer
 236 (LeeAgro 3434 DL, LeeAgra, Lubbock, TX, USA) capable of sensing four
 237 cotton rows simultaneously. Canopy reflectance was measured in 10 nm wave-
 238 bands centered at 670, 720, and 820 nm using active multispectral radiome-
 239 ters (Crop Circle ACS-470, Holland Scientific, Lincoln, NE, USA). Equation
 240 1 was used to calculate NDVI from these data with ρ_1 and ρ_2 equal to re-
 241 flectance values at 670 and 820 nm, respectively. Although canopy height was
 242 measured by the phenotyping platform using sonar proximity sensors (Pul-
 243 sar dB3, Pulsar Process Measurement Ltd, Malvern, UK), this study used
 244 canopy height data measured manually using an electronic bar code scanner
 245 with a coded measurement stick. Using the approach of Scotford and Miller
 246 (2004), the NDVI from active radiometers and manual canopy height data
 247 were used to calculate a compound canopy index (CCI), from which LAI was
 248 estimated:

$$\text{LAI} = \beta \times \text{CCI} = \beta \left(\frac{c}{c_{max}} \right) \left(\frac{h}{h_{max}} \right) \quad (5)$$

249 where β is a constant, c and h are respectively the instantaneous canopy
 250 cover and canopy height measurements, and c_{max} and h_{max} are respectively
 251 the maximum cover and height expected during the growing season. Co-
 252 located data to parameterize this calculation were collected during other
 253 upland cotton experiments conducted at MAC from 2009 to 2013. Analysis
 254 of these data led to values of 5.5, 87.9%, and 110.5 cm for β , c_{max} , and h_{max} ,

255 respectively. The NDVI data from the active radiometers were used as a
256 direct estimate of c in Equation 5. Compared with 75 measurements from a
257 LAI meter (LAI-2200 Plant Canopy Analyzer, Li-Cor Biosciences, Lincoln,
258 NE, USA) and with LAI calculated using 75 measurements of leaf area from
259 biomass samples on an area meter (LAI-3100, Li-Cor Biosciences, Lincoln,
260 NE, USA), the index estimated LAI with a root mean squared error of 0.48
261 (15.9%).

262 2.3. Vegetation indices

263 Equation 1 was used to calculate three vegetation indices from the proxi-
264 mal hyperspectral data. The indices were selected based on their relevance to
265 monitor physiological stress in vegetation. A traditional broad-band NDVI
266 was calculated with ρ_1 and ρ_2 equal to the average spectral reflectance in
267 wavebands corresponding to the red (665 to 675 nm) and NIR (815 to 825
268 nm) filters used with the Crop Circle reflectance sensors onboard the pheno-
269 typing vehicle. The NDWI (Gao, 1996) was calculated with ρ_1 and ρ_2 equal
270 to the average spectral reflectance in wavebands corresponding to MODIS
271 Band 5 (1230 to 1250 nm) and Band 2 (841 to 876 nm), respectively. Fi-
272 nally, the PRI (Gamon et al., 1992) was calculated with ρ_1 and ρ_2 equal
273 to spectral reflectance at 531 nm and 570 nm, respectively. Linear regres-
274 sion models were developed to estimate C_w , C_m , C_{ab} , and LAI using each of
275 these spectral indices. While these three indices were specifically highlighted,
276 Equation 1 was also used to calculate NDVI for all possible combinations of
277 the 1703 proximal hyperspectral wavebands.

278 2.4. *PLSR modeling*

279 PLSR was used to assess the relationships between each of the four bio-
280 physical variables and canopy spectral reflectance in 1703 wavebands. Thorp
281 et al. (2011) provided the details on the PLSR methodology used in the
282 present study. Briefly, if $\mathbf{Y}$ is an $n \times 1$ vector of responses (measured crop
283 phenotypes) and $\mathbf{X}$ is an n -observation by p -variable matrix of predictors
284 (hyperspectral reflectance measurements in p wavebands), PLSR aims to de-
285 compose $\mathbf{X}$ into a set of A orthogonal scores such that the covariance with
286 corresponding $\mathbf{Y}$ scores is maximized. The X-weight and Y-loading vectors
287 that result from the decomposition are used to estimate the vector of regres-
288 sion coefficients, β_{PLS} , such that

$$\mathbf{Y} = \mathbf{X}\beta_{PLS} + \boldsymbol{\epsilon} \quad (6)$$

289 where $\boldsymbol{\epsilon}$ is an $n \times 1$ vector of error terms.

290 The “pls” package (Mevik and Wehrens, 2007) within the R Project for
291 Statistical Computing (<http://www.r-project.org>) was used for PLSR in this
292 study. Four models were developed: one each for estimating C_w , C_m , C_{ab} ,
293 and LAI from the canopy spectral reflectance data. To choose the appro-
294 priate number of factors for each model (A from above), leave-one-out cross
295 validation was used to test model predictions for independent data, and scree
296 plots (not shown) provided the number of factors for which the root mean
297 squared error of cross validation (RMSECV) was minimized. The PLSR
298 models for C_w , C_m , C_{ab} , and LAI were developed from the first five, eight,

299 eight, and ten factors, respectively.

300 2.5. *PROSAIL simulations*

301 The PROSAIL canopy reflectance model was developed by linking the
302 PROSPECT leaf optical properties model and the SAIL canopy bidirectional
303 reflectance model (Jacquemoud et al., 2009). PROSAIL uses 14 input param-
304 eters to define leaf pigment content, leaf water content, canopy architecture,
305 soil background reflectance, and illumination characteristics. Four of the
306 PROSAIL input parameters are the four biophysical variables measured in
307 this study: C_w , C_m , C_{ab} , and LAI. In addition to C_{ab} , other leaf pigment pa-
308 rameters include the carotenoid content ($\mu\text{g cm}^{-2}$) and the brown pigment
309 content (unitless fraction from 0.0 to 1.0). Another leaf-scale parameter is
310 the leaf structural coefficient (N ; unitless), defined as the number of leaf
311 mesophyll layers. In addition to LAI, canopy architecture is defined by the
312 average leaf inclination angle (θ_l ; degrees). The background soil reflectance
313 parameter ranges from 0.0 for wet soils to 1.0 for dry soils. Specular prop-
314 erties of the canopy surface are characterized by the hot spot size parameter
315 (s ; unitless fraction from 0.0 to 1.0). The skylight parameter (%) defines
316 the percentage of diffuse solar radiation. Illumination and viewer geometries
317 are characterized by the solar zenith angle (degrees), viewer zenith angle
318 (degrees), and relative solar and viewer azimuth angle (degrees). Based on
319 these inputs, the model calculates canopy bidirectional reflectance from 400
320 to 2500 nm in 1 nm increments.

321 PROSAIL has been developed in several programming languages. Initial

322 simulations were conducted using the Fortran version, which was compiled
323 using the “g95” Fortran compiler (<http://www.g95.org>) on a Linux operating
324 system. Later, PROSAIL for Python was deemed better for the simulation
325 analysis, because it encapsulated the Fortran code as an extension for the
326 Python programming language (<http://www.python.org>). This permitted
327 the model to be called from the Python command line and eliminated hard
328 disk access requirements for model input and output.

329 PROSAIL simulations were conducted on the “Stampede” supercomputer
330 at the Texas Advanced Computing Center (TACC), located at the University
331 of Texas in Austin. A single job submission was used to conduct 3.68 billion
332 PROSAIL simulations to test the effects of multiple parameter combinations
333 on simulated canopy spectral reflectance. Because proximal hyperspectral
334 measurements were collected in a total of 184 plots over all the field experi-
335 ments, 184 processing cores were requested such that the simulation analysis
336 could be explicitly conducted for the conditions of each experimental unit.
337 The maximum run time for a job submission on Stampede is 48 h. Thus, the
338 design objective was to conduct as many PROSAIL evaluations as possible
339 within the time limit.

340 Seven parameters were adjusted during the PROSAIL simulation exercise
341 (Table 2). A Sobol quasirandom sequence algorithm for Python was used to
342 sample the parameter space. Although “less random” than a pseudorandom
343 number sequence, the approach tends to sample the parameter space “more
344 uniformly.” Another advantage is that the sequence is repeatable, so identi-

cal parameter combinations could be tested for each experimental unit. For C_w , C_m , C_{ab} , and LAI, the lower and upper bounds were specified using the range of measured values. Ranges for N , θ_l , and s were specified using published values (Combal et al., 2003; Jacquemoud et al., 1995). Leaf carotenoid content and brown pigment content were less sensitive parameters and were fixed at $10.0 \mu\text{g cm}^{-2}$ and 0.0 (unitless), respectively. Because subsurface drip irrigation was used, the soil surface was normally dry. Thus, the soil reflectance parameter was fixed at 1.0 for all simulations. The fraction of diffuse skylight was fixed at 10% based on observations of a shaded versus sunlit Spectralon panel during the field study. By implementing the solar position algorithm of Reda and Andreas (2004), solar zenith angles were calculated from the timestamps of radiometric observations in the field. Observer zenith and relative azimuth angles were both fixed at 0° . This approach provided an evaluation of 20 million combinations of seven PROSAIL parameters for each of the 184 experimental units monitored during the field studies.

2.6. PROSAIL model inversion

Available storage allocation on Stampede became the limiting factor when PROSAIL simulation results were initially written to the hard drive (i.e., 1703 simulated reflectance values for 3.68 billion simulations would have exceeded the available storage allocation on Stampede). Thus, objective function evaluations were incorporated into the simulation exercise to reduce storage requirements. Tested parameter sets were stored in a lookup table with their corresponding objective function evaluations, including the root mean

368 squared error (RMSE) and the spectral angle (α) (Kruse et al., 1993) between
 369 measured and simulated reflectance over all spectral wavebands ($n = 1703$):

$$\text{RMSE} = \sqrt{\sum_{i=1}^n (\mathbf{S}_i - \text{PROSAIL}(\mathbf{P}, \mathbf{C})_i)^2} \quad (7)$$

370 and

$$\alpha = \cos^{-1} \left(\frac{\sum_{i=1}^n \mathbf{S}_i \times \text{PROSAIL}(\mathbf{P}, \mathbf{C})_i}{(\sum_{i=1}^n \mathbf{S}_i^2)^{0.5} (\sum_{i=1}^n \text{PROSAIL}(\mathbf{P}, \mathbf{C})_i^2)^{0.5}} \right) \quad (8)$$

371 where $\mathbf{S}$ is the vector of measured canopy spectral reflectance and $\text{PROSAIL}(\mathbf{P}, \mathbf{C})$
 372 is the vector of simulated canopy spectral reflectance as a function of adjusted
 373 parameters, $\mathbf{P}$, and constant parameters, $\mathbf{C}$. The main advantage of α is its
 374 insensitivity to illumination, because Equation 8 incorporates only vector
 375 direction and not vector length. This was considered advantageous because
 376 proximal canopy spectral reflectance measurements were largely affected by
 377 the fraction of sunlit versus shaded leaves in the instrument’s field of view.
 378 Inversion of the PROSAIL model involved the identification of $\mathbf{P}$ that mini-
 379 mized each of these objective functions for each experimental unit.

380 2.7. Statistics

381 For proximal hyperspectral sensing to be useful in field-based plant phe-
 382 nomics, metrics obtained from the data must demonstrate differences among
 383 the treatments imposed and be repeatable (i.e., heritable). Different culti-
 384 vars can then be identified and selected as parents of breeding populations
 385 for development of improved cultivars. Hierarchical linear mixed modeling

386 was used to assess differences among all data and metrics evaluated in this
 387 study: field measurements, measured spectra, vegetation indices, PLSR re-
 388 sults, and estimates from PROSAIL model inversion. Cultivar, water level,
 389 and their interaction were modeled as fixed effects. Measurement date (Ta-
 390 ble 1) and its interaction with both cultivar and water level were modeled
 391 as random effects. Replicate plot, nested within measurement date and wa-
 392 ter level, was also included as a random effect in the model. Hierarchical
 393 tests required fitting random effects with 1) cultivar fixed effects alone, 2)
 394 water level fixed effects alone, 3) both cultivar and water level fixed effects,
 395 and 4) cultivar and water level fixed effects and their interaction. Likelihood
 396 ratio tests were used to compare these hierarchical models, which showed
 397 whether a given data set was different among cultivars, water levels, or their
 398 interaction. Tukey’s multiple comparisons tests were also conducted to iden-
 399 tify specific cultivars that were different for a given measurement. Statistics
 400 were computed using the “lme4” package within the R Project for Statistical
 401 Computing software.

402 **3. Results**

403 *3.1. Field measurements*

404 Measured values for C_w , C_m , C_{ab} , and LAI ranged from 0.01 to 0.02 cm,
 405 0.003 to 0.009 g cm⁻², 26.0 to 59.0 μg cm⁻², and 1.7 to 8.3, respectively,
 406 over all measurements collected (Fig. 1). Hierarchical linear mixed modeling
 407 revealed differences in all four measured plant traits among cultivars ($p <$
 408 0.01, Table 3). Differences in measured C_m and LAI were found among

409 the water levels ($p < 0.05$). The interaction of cultivar and water level
 410 was significant for C_w and C_m ($p < 0.05$). Results for measured C_w and
 411 C_m corroborate the results of Carmo-Silva et al. (2012), who conducted an
 412 independent analysis using data from the 2010 season only. Typically, the
 413 lowest and highest C_w were found for the Monseratt Sea Island and P62
 414 cultivars, respectively (Fig. 1a), and Tukey tests confirmed C_w differences
 415 between P62 and both Monseratt Sea Island and Pima S-6 for both WW
 416 and WL treatments ($p < 0.05$). For WL conditions, C_m for Monseratt Sea
 417 Island was less than four other cultivars: P62, 89590, PSI425, and Pima S-6
 418 ($p < 0.05$). For WW conditions, C_m was lower for Monseratt Sea Island as
 419 compared to P62 ($p < 0.01$, Fig. 1b). The C_{ab} for P62 was greater than
 420 both Monseratt Sea Island and 89590 ($p < 0.05$) for WW conditions, but no
 421 C_{ab} differences were found among cultivars for the WL treatment (Fig. 1c).
 422 With WW conditions, LAI for P62 was less than that for five other cultivars:
 423 Monseratt Sea Island, Pima32, PSI425, Pima S-6, and Pima S-7 ($p < 0.10$,
 424 Fig. 1d). Also, LAI for 89590 was less than that for Monseratt Sea Island,
 425 Pima32, Pima S-6, and Pima S-7 ($p < 0.05$). With WL conditions, LAI for
 426 P62 was less than that for Monseratt Sea Island, Pima 32, and Pima S-6.
 427 Based on measurements from five data sets, these results highlight the main
 428 differences for measured traits among cultivars.

429 Proximal hyperspectral measurements of the cotton canopy followed typ-
 430 ical patterns for spectral reflectance of vegetation (Fig. 2). Generally, scat-
 431 tering of near-infrared radiation led to greater variability in reflectance from

432 760 to 1350 nm as compared to the visible (400 to 700 nm) and shortwave
 433 infrared (1450 to 2400 nm) wavebands where chlorophyll and water, respec-
 434 tively, absorb radiation. Results from hierarchical linear mixed modeling
 435 demonstrated the wavebands with different reflectance values among water
 436 levels and cultivars ($p < 0.05$, Fig. 3). Among cultivars, spectral reflectance
 437 differences were found in wavebands from 400 to 725 nm, 1470 to 1800 nm,
 438 and 2000 to 2400 nm. Thus, reflectance in the entire visible portion of the
 439 spectrum was different among cultivars, likely due to effects of radiation ab-
 440 sorption by chlorophyll. Also, reflectance differences in two regions of the
 441 shortwave infrared suggest effects of C_w or total plant water status. A fewer
 442 number of wavebands demonstrated reflectance differences among water lev-
 443 els, and four main regions were identified: 528 to 569 nm, 667 to 736 nm,
 444 1681 to 1785 nm, and 2153 to 2353 nm. Wavebands around 550 nm sug-
 445 gested that water level affected greenness of the canopy, while reflectance in
 446 the far red and red edge bands were also affected. Reflectance differences
 447 in the shortwave infrared bands again suggest effects of water level on plant
 448 water status, as expected. Neither cultivar nor water level led to differences
 449 in near-infrared reflectance, suggesting that other factors contributed to the
 450 variability in those wavebands. There were also no significant cultivar by
 451 water level interaction effects on reflectance.

452 3.2. *Vegetation indices*

453 Differences in broad-band NDVI from the spectroradiometer were found
 454 for both the cultivar and water level treatments (Table 3), demonstrating

455 its robustness for proximal and remote sensing applications in agriculture.
456 Differences in broad-band NDWI were also found among cultivar and water
457 level treatments. Thus, the NDVI and NDWI could collectively provide es-
458 timates of both crop growth and water status. No differences in PRI were
459 found among cultivars or water levels. Also, unlike NDVI from the spectro-
460 radiometer, no differences in NDVI from the Crop Circle sensors were found
461 among cultivars. With a coefficient of determination (r^2) of only 0.26 (not
462 shown), the relationship between Fieldspec NDVI and Crop Circle NDVI was
463 weak. This was likely related to different fields-of-view, measurement heights,
464 and light sources among the two sensors. Effects of soil background in the
465 instrument field-of-view was likely more of an issue for the tractor-mounted
466 Crop Circle than for the hand-held spectroradiometer.

467 Many of the narrow-band NDVI calculations were different among cul-
468 tivars ($p < 0.05$, Fig. 4). When NDVI was computed using a waveband
469 from 400 to 1350 nm and any other waveband, the values often varied among
470 cultivars ($p < 0.05$). An exception was apparent when a red edge band was
471 used with any band greater than 1450 nm. Also, as shown in Table 3, the
472 wavebands used for PRI (i.e., 531 and 570 nm), which is itself a narrow-band
473 NDVI, did not lead to differences. Fewer differences among cultivars were
474 noted when NDVI was calculated using two wavebands greater than 1970 nm.
475 Fewer waveband combinations led to narrow-band NDVI differences among
476 water levels (Fig. 4). Notably, wavebands used for NDWI calculation (i.e.,
477 approximately 1240 and 858 nm) led to different narrow-band NDVI among

478 water levels ($p < 0.05$). Narrow-band NDVIs often did not demonstrate sig-
479 nificant cultivar by water level interactions, although significant interaction
480 effects were more common when two wavebands in either the near-infrared
481 (i.e., 730 to 1000 nm) or shortwave infrared (i.e., 1450 to 1770 nm) were used.

482 Linear regression models to estimate the measured crop phenotypes from
483 the vegetation indices were unfavorable compared to PLSR models, discussed
484 in the next section. None of the indices could estimate C_w , C_m , C_{ab} , and
485 LAI with root mean squared errors better than 9.6%, 16.9%, 14.2%, and
486 28.8%, respectively. Cross-validated estimates from PLSR were better than
487 the estimates from linear relationships with vegetation indices. For LAI and
488 C_{ab} , this result differed from that of Hansen and Schjoerring (2003), but
489 they compared narrow-band NDVI with PLSR and did not have spectral
490 reflectance measurements beyond 900 nm. Due to the linear nature of the
491 regression models, another concern is that the statistical results for traits
492 estimated in this way (not shown) were identical to that for the vegetation
493 index itself (Table 3). Thus, using linear regression to estimate traits from
494 vegetation indices did not provide any new information for hierarchical linear
495 mixed modeling.

496 3.3. PLSR modeling

497 The PLSR models developed from 1703 wavebands of canopy spectral
498 reflectance estimated C_w , C_m , C_{ab} , and LAI with RMSECV of 6.8%, 10.9%,
499 13.1%, and 18.5%, respectively (Fig. 5). Full spectrum data reduced root
500 mean squared errors between measured and modelled phenotypes as com-

501 pared to vegetation indices using reflectance in select wavebands. Addition-
502 ally, the PLSR results were cross-validated, so the PLSR models have been
503 properly tested with independent data.

504 Although the PLSR models provided better trait estimates than other
505 techniques, hierarchical linear mixed modeling results for PLSR estimates
506 were somewhat different than that for the field measurements (Table 3).
507 Whereas field-measured C_w , C_m , C_{ab} , and LAI were all different among cul-
508 tivars, the PLSR estimates were different only for C_w and C_m ($p < 0.01$).
509 Also, whereas field measurements were different among water levels only for
510 C_m and LAI, the PLSR estimates for all four traits were different among
511 water levels ($p < 0.05$). Thus, the PLSR technique led to different trait
512 estimates among cultivars and water levels, but the results did not always
513 corroborate results for the field-measured traits.

514 3.4. *PROSAIL simulations*

515 Most biophysical models like PROSAIL were not originally designed with
516 high-performance computing in mind. Thus, efforts to use such models on
517 supercomputers demonstrate what is possible with modern computing re-
518 sources. Using the Fortran-compiled PROSAIL model, which required hard
519 disk access for model input and output, 40 million simulations were com-
520 pleted in 40.4 h for an average of 275 simulations per second. However, when
521 using the PROSAIL model compiled as a Python extension, 3.68 billion sim-
522 ulations were completed in 37.3 h for an average of 27,395 simulations per
523 second. Simulations could be multiplied 100 times by using a model that did

524 not require hard drive access.

525 Storage requirements were also a concern for the PROSAIL simulation
526 exercises. For trials with the Fortran-based PROSAIL model, the overall job
527 size was small enough to write simulated reflectance data in 1703 wavebands
528 to the hard disk. Using binary files to write reflectance data as 4-digit in-
529 tegers, simulated data for 40 million PROSAIL runs required 136.4 GB of
530 storage. Increasing the job size to 3.68 billion would thus increase storage
531 requirements to several TB, which exceeded allocation limits on Stampede.
532 Therefore, only the RMSE (Eq. 7) and α (Eq. 8) metrics were stored for the
533 larger job, which required only 36 GB. Decisions like these are central to the
534 design of supercomputing jobs for models like PROSAIL.

535 3.5. PROSAIL model inversion

536 For the PROSAIL model inversion with the objective to minimize RMSE
537 between measured and simulated canopy spectral reflectance in 1703 wave-
538 bands (Eq. 7), C_w , C_m , C_{ab} , and LAI were estimated with RMSE of 37.6%,
539 31.1%, 16.6%, and 29.5%, respectively (Fig. 6). When the objective was to
540 minimize α between measured and simulated canopy spectral reflectance (Eq.
541 8), C_w , C_m , C_{ab} , and LAI were estimated with RMSE of 38.1%, 36.1%, 15.9%,
542 and 28.2%, respectively. Clearly, results from both objective functions were
543 inferior to that from PLSR models (Fig. 5). Discrepancies between measured
544 and simulated C_w suggested problems in how PROSAIL simulated effects of
545 leaf-level water content on canopy-level spectral reflectance (Fig. 6a). In-
546 versions with both objective functions resulted in higher C_w than measured,

547 and many optimum C_w estimates were near the imposed upper bound of 0.02
548 cm (Table 2). This effect did not occur when reflectance in 501 wavebands
549 from 400 nm to 900 nm were used for PROSAIL model inversion. In this
550 case, RMSE between measured and simulated values dropped from 38% to
551 23% (not shown). Thus, discrepancies in the near-infrared wavebands above
552 900 nm and the shortwave infrared wavebands (discussed below) likely drove
553 the high error between simulated and measured C_w . This result highlights
554 the potential for model inversion outcomes to be affected by methodological
555 choices. Estimates of C_m based on minimum RMSE were often underesti-
556 mated, while C_m based on minimum α were overestimated for all but a few
557 cases (Fig. 6b). With high RMSE and low correlation between measured and
558 simulated values, C_w and C_m were the most difficult parameters to estimate
559 using PROSAIL model inversion.

560 Estimates of C_{ab} from PROSAIL model inversion were more reasonable
561 (Fig. 6c), although the RMSEs between measured and simulated C_{ab} were
562 still approximately 3% higher than that for the PLSR model. Estimates of
563 LAI were most problematic for values greater than 6.0 (Fig. 6d). Measure-
564 ment error is likely partially responsible for this result, because LAI mea-
565 surements were based on Crop Circle NDVI and canopy height according to
566 Equation 5. Some cultivars reached over 1.5 m in height, but Equation 5 was
567 parameterized using data from cotton with height less than 1.1 m. Thus,
568 the higher LAI “measurements” suffered from extrapolation issues. When
569 removing the LAI values above 6.0 from the calculation, the RMSE between

570 measured and simulated LAI was still above 22% which was 4% higher than
571 that for the PLSR model with all data included.

572 When the objective was to minimize RMSE between measured and simu-
573 lated canopy spectral reflectance, the resulting deviation between PROSAIL-
574 simulated and measured spectral reflectance was not greater than 0.05 at any
575 wavelength (Fig. 7a). In fact, simulated reflectance could often be optimized
576 to within 0.02 of measured reflectance for most wavelengths. This showed
577 that the inversion approach worked appropriately to find parameter values
578 that achieved the best fit between PROSAIL-simulated and measured canopy
579 spectral reflectance. When measured values for C_w , C_m , C_{ab} , and LAI were
580 then substituted for the values obtained through PROSAIL model inversion,
581 the resulting deviations between PROSAIL-simulated and measured canopy
582 spectral reflectance (Fig. 7b) explain why PROSAIL model inversion had
583 problems producing accurate values for these parameters. Foremost, there
584 were greater positive deviations in reflectance from 1100 to 2400 nm. Thus,
585 the model overestimated reflectance in these wavebands when measured pa-
586 rameters were used. Also, there were greater deviations, up to 0.13, in the
587 near-infrared wavebands from 750 to 1350 nm. These results could indicate
588 errors in both measurement and modeling, and improvements could focus in
589 the mentioned waveband intervals.

590 Plotting the ranked RMSE and α statistics for the top 1% (200,000) of
591 PROSAIL evaluations provided insights on equifinality effects (Fig. 8). Re-
592 sults showed rapid departure from the minimum function evaluation within

the top 0.1% (20,000) of total model evaluations. Deviations from the minimum function evaluation were less variable for evaluations ranked greater than 20,000, indicating greater equifinality effects with increasing evaluation rank. The results suggest that model inversion identified a relatively small fraction of parameter combinations with low RMSE and α statistics and that equifinality was more problematic for parameter combinations other than these. Parameter estimates for C_w , C_m , C_{ab} , and LAI that better agree with measured values might be found within the top 20,000 evaluations. However, equifinality renders the model inversion less useful above 20,000 evaluations. Results also showed that the α statistic offered better separation from the minimum function evaluation as compared to the RMSE statistic. Thus, equifinality was less problematic for α than RMSE, but both statistics were able to identify 0.1% of evaluated parameter combinations as top candidates. Remaining issues include 1) understanding equifinality issues among these top candidates and 2) addressing measurement and modeling errors to insure estimated parameters are more accurate (Fig. 6).

Although PROSAIL model inversion estimated phenotypes with less accuracy than other methods, many of the estimates differed among the water level and cultivar treatments imposed during the field studies (Table 3). Results were often inconsistent between the objective functions used for model inversion, which further highlighted the sensitivity of the inversion approach to methodological choices. Generally, more traits were different when the objective was to minimize α rather than RMSE ($p < 0.05$). Overall results

616 from PROSAIL model inversion were less accurate than that for PLSR mod-
617 els, but differences were nonetheless noted in parameter values estimated by
618 PROSAIL.

619 4. Discussion

620 While the differences among the C_w , C_m , C_{ab} , and LAI measurements
621 were apparent and biologically meaningful (Table 3), the manual procedures
622 used to quantify these crop phenotypes were labor intensive and time con-
623 suming. Though practical here for 4 replications of 5 or even 25 cultivars,
624 obtaining these measurements for 1000 or 10000 cultivars would amplify la-
625 bor requirements greatly. Major bottlenecks include labor requirements for
626 collecting and processing leaf samples as well as time required for chemical
627 extraction of C_{ab} and oven drying to obtain C_w and C_m . Thus, proximal or
628 remote sensing metrics that are able to discriminate these crop phenotypes
629 are essential for practical scaling of field-based plant phenomics experiments.

630 High-throughput approaches are needed for collection of field-based prox-
631 imal hyperspectral data. Time was the main limiting factor for the manual
632 approaches used in the present study. Six to twelve scans were collected in
633 each of 40 experimental plots in roughly 1.75 h. This provided data for only
634 one-fifth of the cotton cultivars grown in this relatively small study of 25
635 Pima lines. For larger studies with thousands of lines, high-throughput ca-
636 pability is a necessity. The averaged spectra for each experimental plot were
637 also highly variable in the near-infrared wavebands (Fig. 2), indicating per-

638 haps that more scans per plot were needed to ensure that spectral reflectance
639 of both sunlit and shaded portions of the canopy were adequately character-
640 ized. This is important because of the bidirectional reflectance distribution
641 function (BRDF) of the crop canopy, which defines how canopy reflectance
642 properties change with solar and viewer geometry. Because passive spec-
643 troradiometers use solar irradiance as the light source, a high-throughput
644 platform for such sensors must also collect data rapidly. This ensures that
645 BRDF effects on canopy spectral reflectance among experimental units are
646 minimal for a given data set. Use of an active field spectroradiometer with
647 its own light source could be another strategy for minimizing BRDF effects,
648 but the authors know of no such instrument for field-based proximal sensing
649 at this time. Finally, a high-throughput platform should enable canopy spec-
650 tral reflectance measurements from multiple view angles. This would permit
651 better characterization of BRDF effects and would provide more data to
652 constrain PROSAIL model inversion. A high-throughput sensing platform
653 capable of collecting much more than 12 spectral scans from a 8.8 m cotton
654 row at multiple view angles in a few seconds would be ideal for field-based
655 plant phenomics applications. To multiply efforts, sensing units with these
656 characteristics could be distributed along a tractor boom or gantry system
657 or perhaps mounted on a fleet of unmanned aerial systems.

658 To minimize BRDF impacts on canopy reflectance measurements, passive
659 reflectance sensing is often restricted to times near solar noon. In central Ari-
660 zona in August, this strategy provides two hours from 11:30 to 13:30 when

661 the solar zenith angle does not change by more than 5° . Another strategy
 662 is to maintain constant BRDF effects for spectral data collected over an en-
 663 tire growing season. For cotton in Arizona, spectral measurements around
 664 the time of a 45° solar zenith angle permits data collection with similar
 665 BRDF characteristics from April to September. In the present study, the
 666 goal was to collect spectral measurements concurrently with measurements
 667 of C_w . Because prior studies demonstrated the dynamic diurnal response of
 668 C_w and greater C_w variability among experimental treatments in the morn-
 669 ing (Carmo-Silva et al., 2012), canopy spectral reflectance measurements
 670 were primarily collected in the hours before and after solar noon (Table 1).
 671 Concurrent spectral measurements with dynamic C_w was deemed more im-
 672 portant than strict adherence to data collection at solar noon, although the
 673 average solar zenith during spectral measurements was 42° , similar to the 45°
 674 angle required for constant BRDF effects over a cotton season. Crop pheno-
 675 types that undergo dynamic diurnal changes could require a departure from
 676 traditional passive reflectance sensing techniques that restrict data collection
 677 to solar noon. If the optimum time for monitoring a given phenotype occurs
 678 while canopy spectral reflectance changes more rapidly due to BRDF effects,
 679 efforts must focus on understanding these BRDF effects and on designing
 680 sensors and sensing protocols that either characterize or minimize them. For
 681 example, multiple view angles assist with BRDF characterization while rapid
 682 spectral data collection minimizes illumination changes among experimental
 683 units.

684 The PROSAIL model offers several advantages for field-based plant phe-
685 nomics, including its ability 1) to simulate BRDF effects on canopy spectral
686 reflectance and 2) to estimate phenotypes from canopy spectral reflectance
687 data alone. This study was limited to spectral reflectance measurements from
688 a nadir view angle, which likely limited efforts to estimate phenotypes using
689 PROSAIL model inversion. Data from multiple view angles should provide
690 more information to constrain PROSAIL, leading to better estimates. There
691 were also many methodological choices that impacted the PROSAIL model
692 inversion results, including the selected wavebands and the objective func-
693 tion. Future efforts should explore these issues in greater detail. For example,
694 with high-performance computing capabilities, a large database of PROSAIL
695 simulations could be generated and permanently stored. Multiple measure-
696 ment sets of a large mapping population over multiple years and locations
697 could then be inverted using the same database. Also, the data could be
698 used to develop confidence regions within the parameter space, which would
699 assist with parameter identification and equifinality issues.

700 As compared to PROSAIL model inversion, methods involving linear re-
701 gression on vegetation indices and PLSR on canopy spectral reflectance were
702 able to better quantify crop phenotypes. At this time, these methods remain
703 the most practical approach for crop phenotyping based on canopy spec-
704 tral reflectance. A main drawback of the regression approaches is that field
705 measurements of each phenotype are required for model fitting. A practical
706 approach for field phenomics may be to directly measure phenotypes for se-

707 lected experimental plots and to measure canopy spectral reflectance over all
708 plots using a high-throughput sensing platform. Data from plots with both
709 types of measurements could be used for building regression models, which
710 would subsequently be applied to estimate phenotypes for all experimental
711 units.

712 5. Conclusions

713 Proximal hyperspectral sensing offers a wealth of information for char-
714 acterizing reflectance from crop canopies and should be a fundamental com-
715 ponent of field-based plant phenomics programs. This study showed that
716 PLSR modeling was the most robust method for estimating C_w , C_m , C_{ab} ,
717 and LAI from canopy spectral reflectance data. Vegetation indices computed
718 from selected wavebands, including NDVI, NDWI, and PRI, were informative
719 but could not estimate phenotypes as well as PLSR. With improvements to
720 the PROSAIL model and ability to rapidly collect spectral reflectance data
721 from multiple view angles, model inversion for crop phenotyping may be-
722 come more practical. In the meantime, further investigations are needed to
723 improve PROSAIL model inversion strategies and to address related equifi-
724 nality issues. High-performance computing offers much potential for these
725 efforts and for overall advancements in the use of biophysical models for
726 agricultural applications.

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Table 1: Field measurement schedule for five cotton phenomics data sets: 2010A, 2010B, 2011, 2012A, and 2012B.¹

Measurement	2010A	2010B	2011	2012A	2012B
Leaf punches for C_w and C_m	04 Aug 2010 09:00-10:30	04 Aug 2010 13:30-16:30	18 Aug 2011 09:00-10:30	03 Aug 2012 08:45-10:30	31 Aug 2012 09:00-11:00
Leaf punches for C_{ab}	30 Jul 2010 morning	30 Jul 2010 morning	10 Aug 2011 morning	09 Aug 2012 08:30-10:45	16 Aug 2012 7:45-11:00
Fieldspec canopy spectral reflectance	04 Aug 2010 08:00-09:45	04 Aug 2010 14:00-15:30	18 Aug 2011 09:00-10:30	03 Aug 2012 08:45-10:30	31 Aug 2012 09:00-11:00
Crop Circle canopy reflectance	05 Aug 2010 14:00-15:15	05 Aug 2010 14:00-15:15	18 Aug 2011 15:00-15:45	02 Aug 2012 07:00-08:30	31 Aug 2012 10:00-11:30
Manual canopy height	08 Aug 2010 morning	08 Aug 2010 morning	19 Aug 2011 morning	02 Aug 2012 morning	30 Aug 2012 morning

¹ leaf chlorophyll $a + b$ content, C_{ab} ; leaf water thickness, C_w ; specific leaf mass, C_m

Table 2: Parameterization of the PROSAIL model.

Parameter	Unit	State	Lower Bound	Upper Bound
Leaf water thickness (C_w)	cm	free	0.01	0.02
Specific leaf mass (C_m)	g cm^{-2}	free	0.003	0.008
Chlorophyll $a + b$ (C_{ab})	$\mu\text{g cm}^{-2}$	free	25.0	60.0
Leaf area index (LAI)	unitless	free	1.25	8.75
Leaf structure parameter (N)	unitless	free	1.4	2.4
Average leaf angle (θ_l)	degrees	free	10.0	70.0
Hot spot size (s)	unitless	free	0.001	1.0
Leaf carotenoid content	$\mu\text{g cm}^{-2}$	fixed	10.0	10.0
Brown pigment content	unitless	fixed	0.0	0.0
Soil reflectance parameter	unitless	fixed	1.0	1.0
Diffuse radiation fraction	%	fixed	10.0	10.0
Solar zenith angle	degrees	fixed	27.3	60.3
Viewer zenith angle	degrees	fixed	0.0	0.0
Relative azimuth angle	degrees	fixed	0.0	0.0

Table 3: Results of hierarchical linear mixed modeling for measured plant traits, vegetation indices, and plant trait estimates from PLSR models and PROSAIL model inversion.¹ Significance codes are “***” ($p < 0.001$), “**” ($p < 0.01$), and “*” ($p < 0.05$).

Trait	Cultivar			Water Level			Interaction		
	χ^2	P		χ^2	P		χ^2	P	
Measured C_w	21.5	0.0015	**	1.5	0.2176		15.2	0.0185	*
Measured C_m	27.2	0.0001	***	4.7	0.0298	*	20.0	0.0028	**
Measured C_{ab}	17.2	0.0085	**	2.3	0.1269		12.0	0.0625	
Measured LAI	22.2	0.0011	**	6.7	0.0097	**	7.1	0.3131	
Fieldspec NDVI	21.0	0.0019	**	6.3	0.0118	*	4.4	0.6287	
Fieldspec NDWI	22.5	0.0010	***	4.2	0.0410	*	8.5	0.2011	
Fieldspec PRI	10.9	0.0930		0.6	0.4343		3.9	0.6959	
Crop Circle NDVI	12.0	0.0613		4.4	0.0350	*	5.5	0.4782	
PLSR C_w	33.9	0.0000	***	5.5	0.0190	*	6.5	0.3729	
PLSR C_m	27.3	0.0001	***	7.1	0.0078	**	6.3	0.3871	
PLSR C_{ab}	12.2	0.0575		13.2	0.0003	***	2.0	0.9167	
PLSR LAI	11.4	0.0779		6.6	0.0103	*	11.8	0.0661	
PS RMSE C_w	3.8	0.6978		1.1	0.2996		6.1	0.4154	
PS RMSE C_m	24.7	0.0004	***	0.8	0.3664		11.1	0.0846	
PS RMSE C_{ab}	10.9	0.0902		7.3	0.0067	**	7.9	0.2487	
PS RMSE LAI	10.3	0.1118		2.2	0.1385		7.5	0.2739	
PS RMSE N	33.9	0.0000	***	3.6	0.0576		2.4	0.8786	
PS RMSE θ_l	10.3	0.1145		0.3	0.5744		6.3	0.3869	
PS RMSE s	8.0	0.2410		3.4	0.0666		1.7	0.9457	
PS α C_w	11.5	0.0746		0.6	0.4240		5.3	0.5013	
PS α C_m	5.4	0.4925		6.9	0.0086	**	6.8	0.3439	
PS α C_{ab}	14.7	0.0226	*	4.0	0.0460	*	11.8	0.0669	
PS α LAI	15.1	0.0191	*	4.0	0.0451	*	6.8	0.3415	
PS α N	16.6	0.0111	*	0.0	0.9072		12.9	0.0444	*
PS α θ_l	22.5	0.0010	***	0.3	0.6145		5.0	0.5386	
PS α s	22.3	0.0011	**	7.3	0.0070	**	4.6	0.5909	

¹ Chi square statistic, χ^2 ; hot spot size, s ; leaf area index, LAI; leaf chlorophyll $a + b$ content, C_{ab} ; leaf inclination angle, θ_l ; leaf structural coefficient, N; leaf water thickness, C_w ; normalized difference vegetation index, NDVI; normalized difference water index, NDWI; partial least squares regression, PLSR; physiological (or photochemical) reflectance index, PRI; probability value, P ; PROSAIL canopy reflectance model, PS; root mean squared error, RMSE; specific leaf mass, C_m ; spectral angle, α

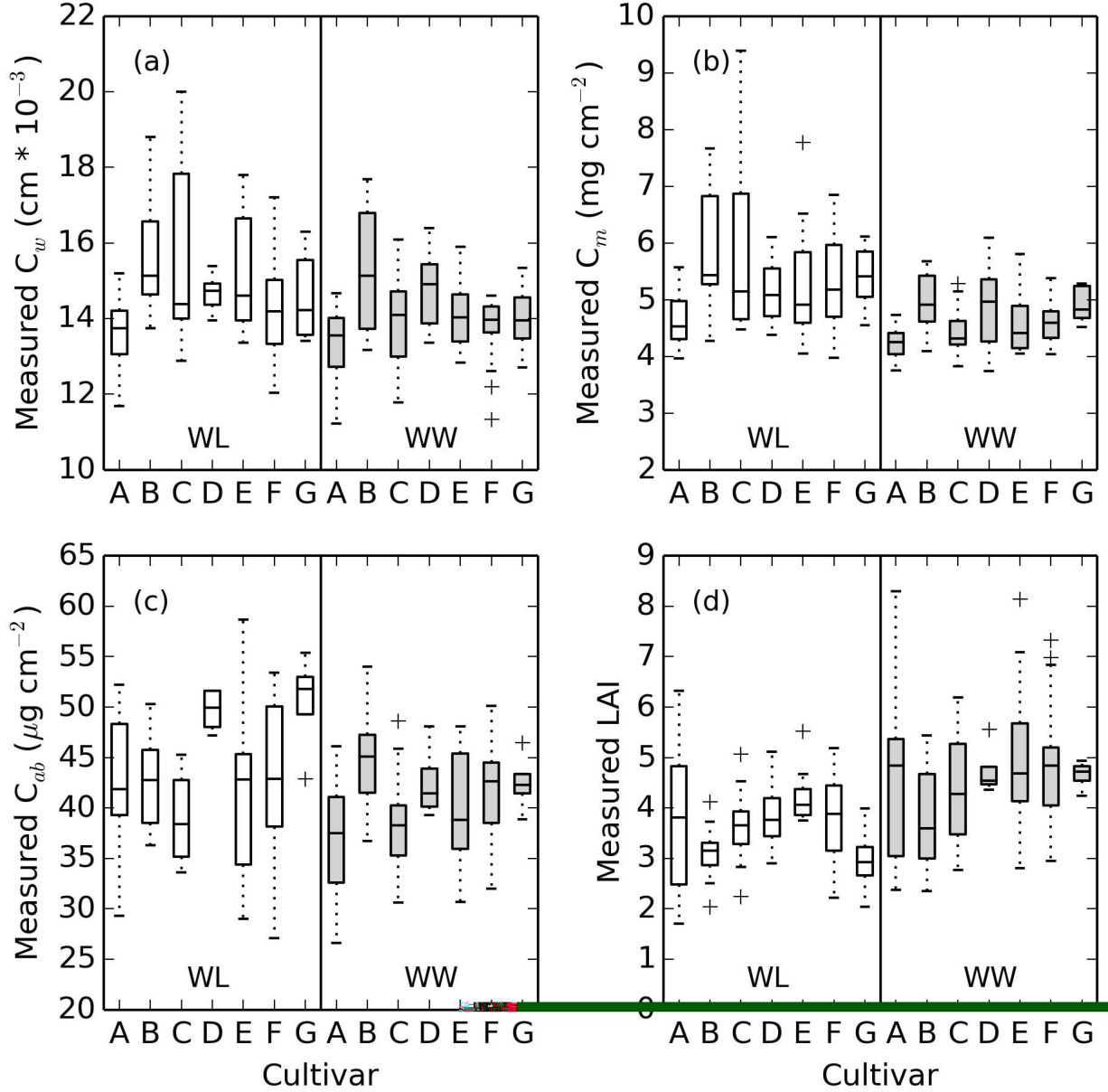


Figure 1: Box plots for a) leaf water content (C_w), b) specific leaf mass (C_m), c) leaf chlorophyll $a + b$ content (C_{ab}), and d) leaf area index (LAI) for all measurements collected for the 2010A, 2010B, 2011, 2012A, and 2012B data sets. Measurements were collected under well-watered (WW) and water-limited (WL) conditions for seven Pima cotton cultivars: A) Monseratt Sea Island, B) P62, C) 89590, D) Pima32, E) PSI425, F) Pima S-6, and G) Pima S-7.

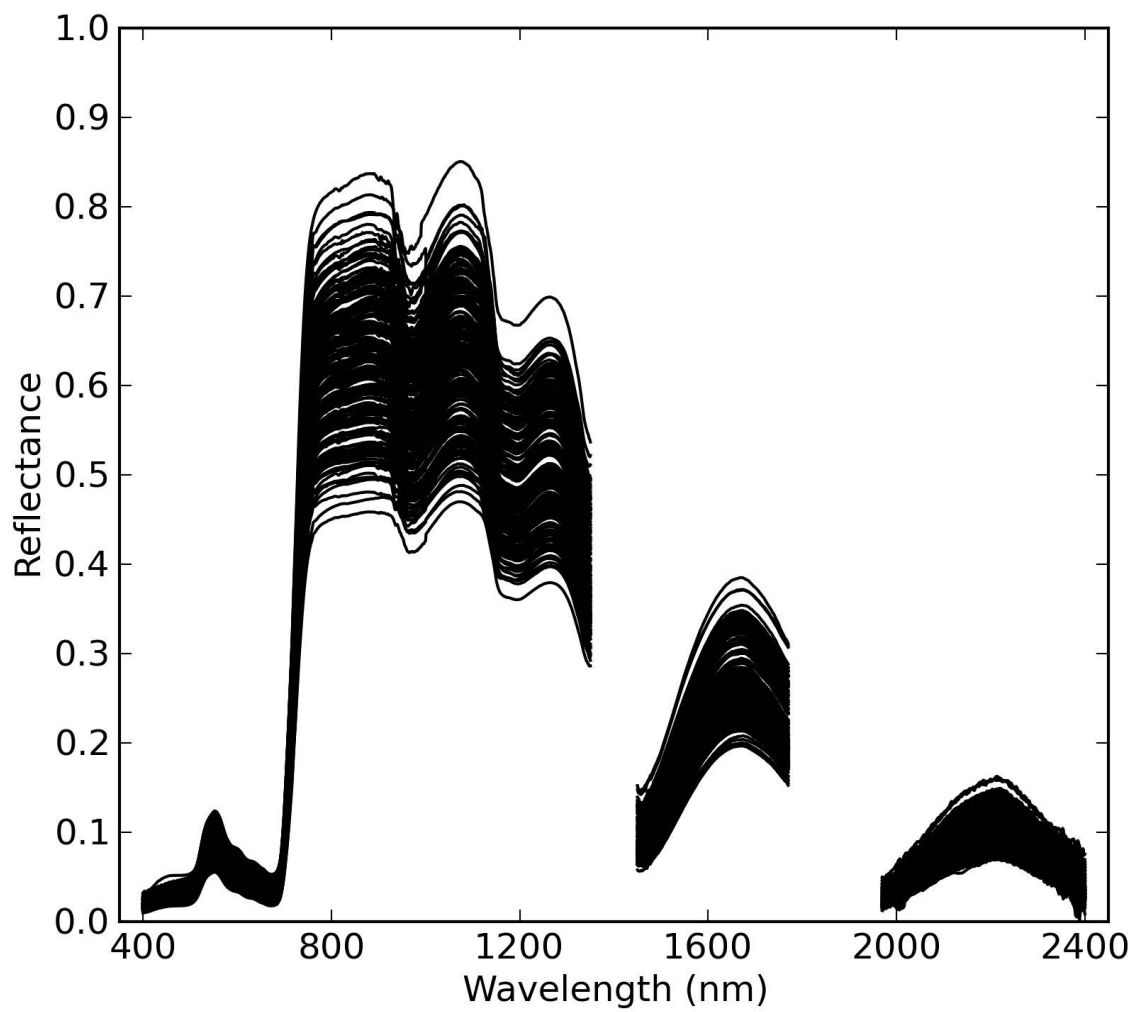


Figure 2: Cotton canopy spectral reflectance measurements for the 2010A, 2010B, 2011, 2012A, and 2012B data sets.

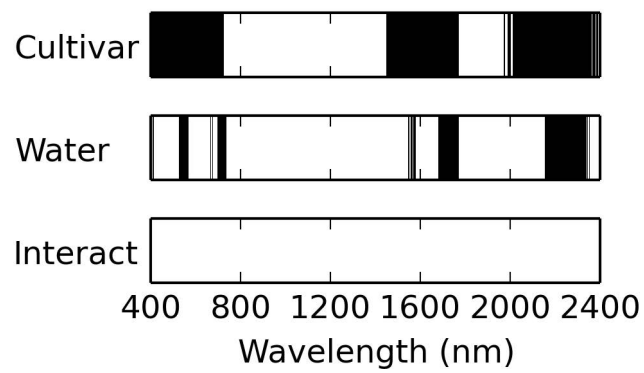


Figure 3: Results of hierarchical linear mixed modeling for canopy spectral reflectance from 400 to 2400 nm in 1 nm wavebands. Dark bands indicate different reflectance values among cultivars or water levels ($p < 0.05$). There were no significant interaction effects.

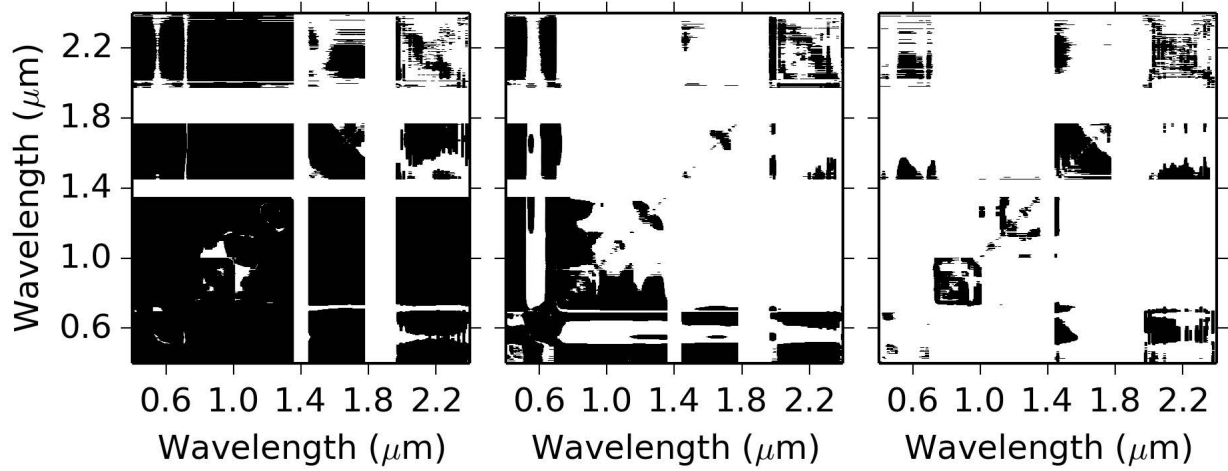


Figure 4: Results of hierarchical linear mixed modeling for narrow-band NDVI calculated using all possible combinations of canopy spectral reflectance in 1 nm wavebands from 400 to 1350 nm, 1450 to 1770 nm, and 1970 to 2400 nm. Dark areas indicate different NDVI values ($p < 0.05$) for the specified wavelengths among cultivars (left), water levels (middle), and their interaction (right).

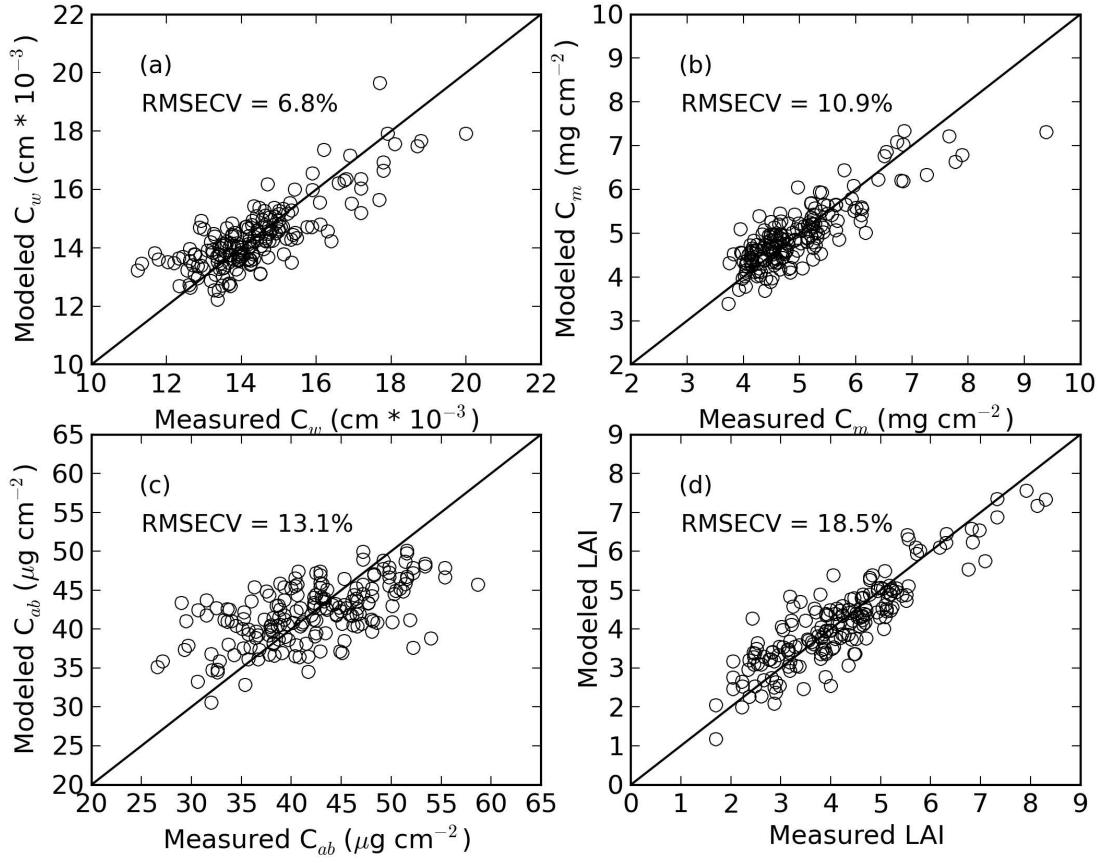


Figure 5: Modeled versus measured a) leaf water content (C_w), b) specific leaf mass (C_m), c) leaf chlorophyll $a + b$ content (C_{ab}), and d) leaf area index (LAI). Modeled estimates are from partial least squares regression (PLSR) models developed from measured canopy spectral reflectance data collected for the 2010A, 2010B, 2011, 2012A, and 2012B data sets. The root mean squared errors of cross validation (RMSECV) between measured and modeled values are provided.

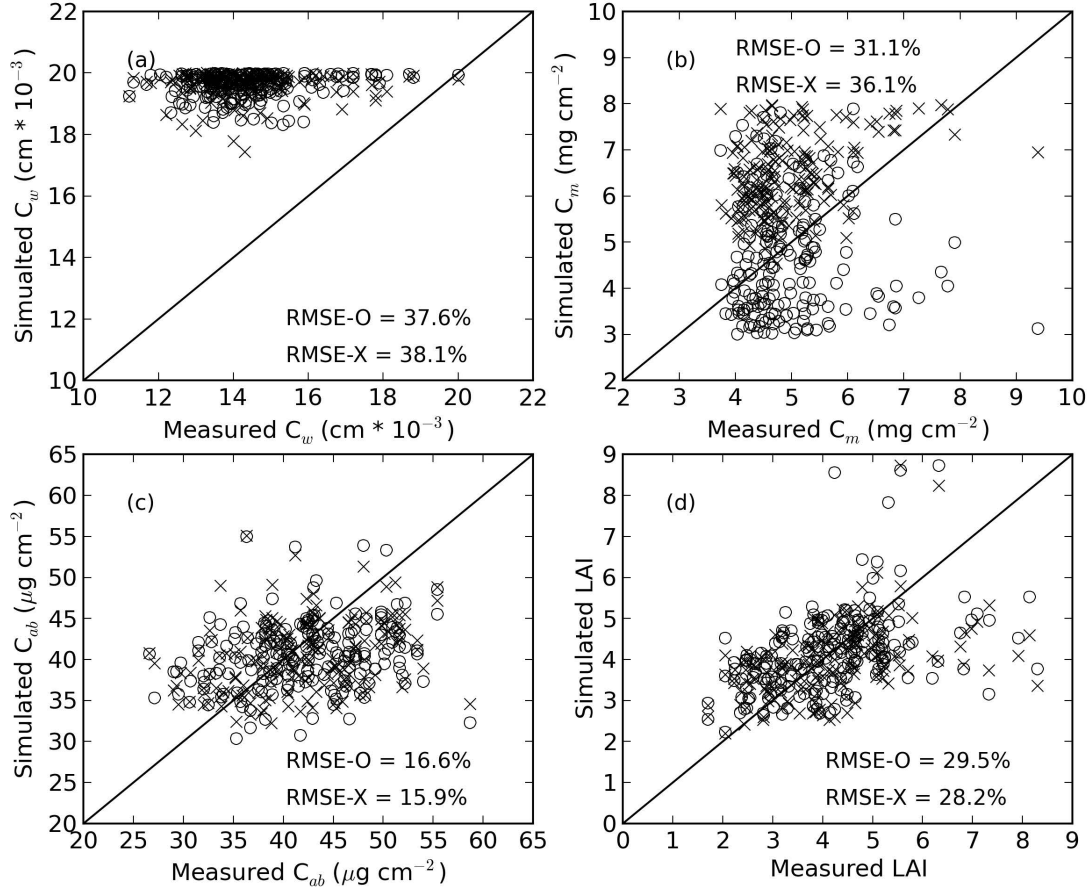


Figure 6: PROSAIL-simulated versus measured a) leaf water content (C_w), b) specific leaf mass (C_m), c) leaf chlorophyll $a + b$ content (C_{ab}), and d) leaf area index (LAI). Simulated estimates minimized the root mean squared error (O) or the spectral angle (X) between measured and PROSAIL-simulated canopy spectral reflectance for the 2010A, 2010B, 2011, 2012A, and 2012B data sets. Root mean squared errors between simulated and measured values are provided for both objective functions (RMSE-O and RMSE-X).

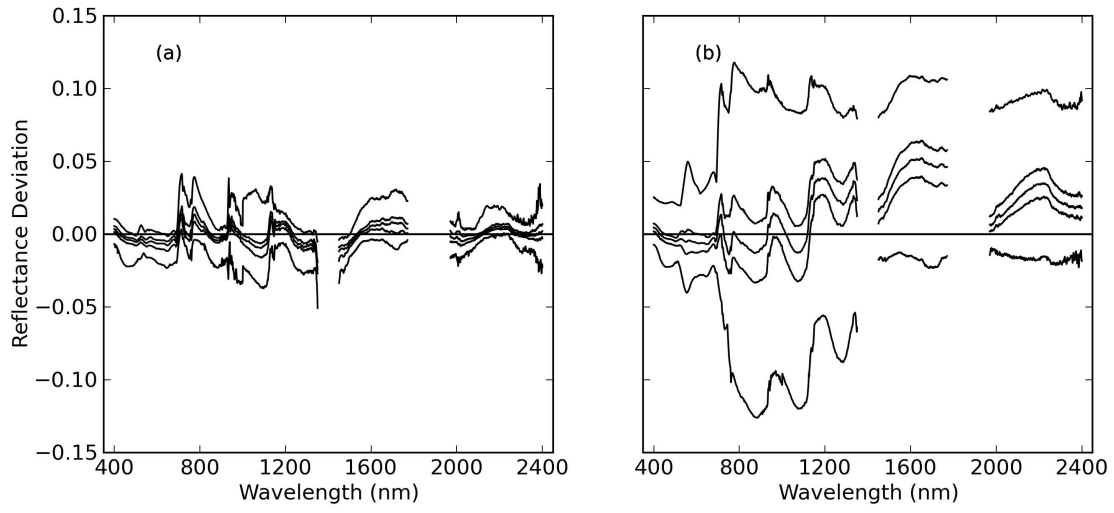


Figure 7: Minimum, lower quartile, median, upper quartile, and maximum deviations between PROSAIL-simulated and measured canopy spectral reflectance for a) the PROSAIL model inversion that minimized RMSE and b) subsequently replacing the optimum values for leaf water content (C_w), specific leaf mass (C_m), leaf chlorophyll $a + b$ content (C_{ab}), and leaf area index (LAI) with measured values.

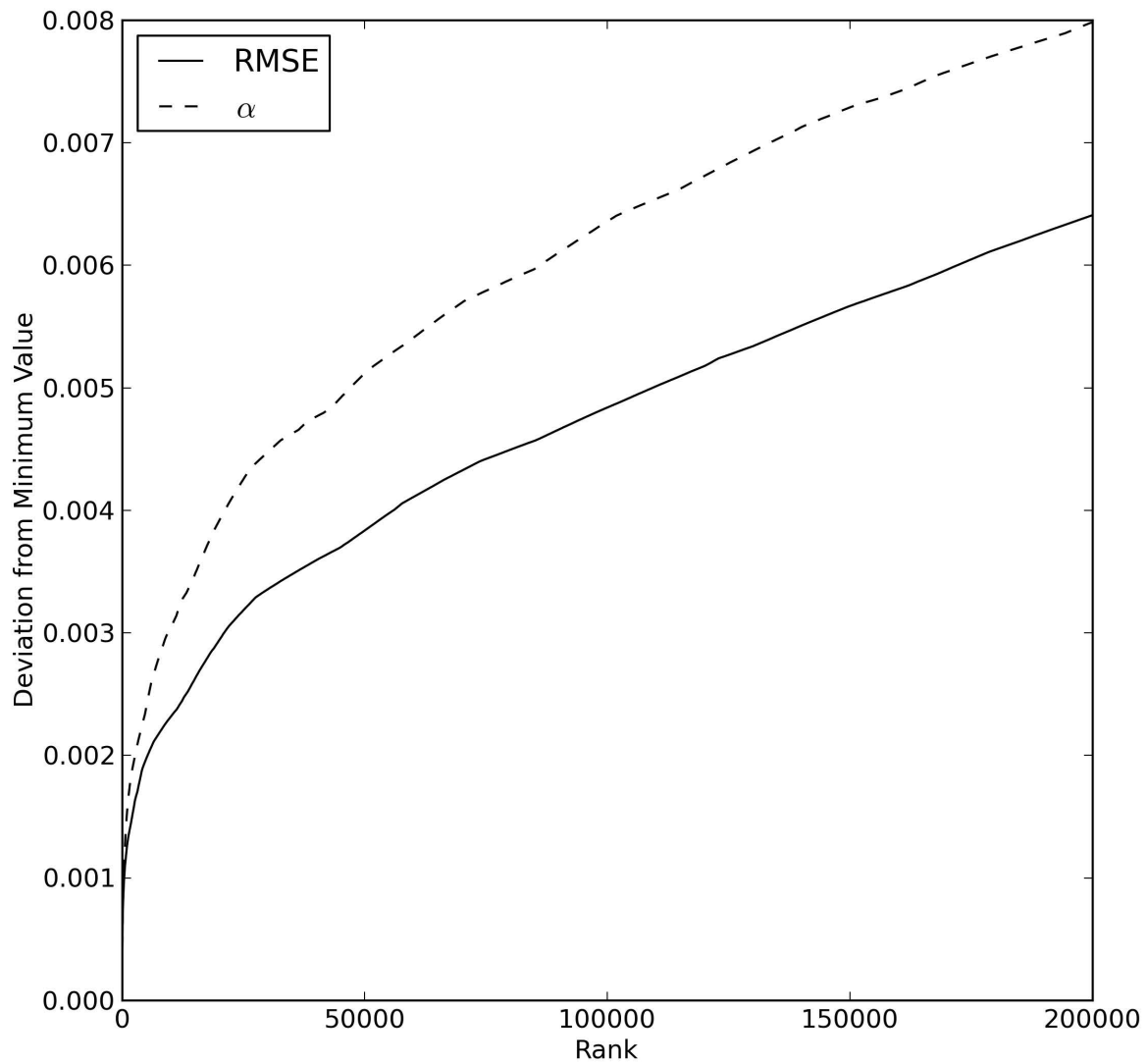


Figure 8: Deviation from the minimum value for ranked objective function evaluations of root mean squared error (RMSE) and spectral angle (α) between measured and PROSAIL-simulated canopy spectral reflectance. Results are shown for the median value among model inversion exercises for 184 experimental units (all plots for all five data sets).