

ORIGINAL ARTICLE

Hyperspectral reflectance-based partial least squares regression models for predicting cotton leaf physiological traits

Ved Parkash¹  | John L. Snider¹  | Christopher M. Montes² | Elizabeth A. Ainsworth^{2,3}  | Alvaro Sanz-Saez⁴

¹Department of Crop and Soil Sciences, University of Georgia-Tifton Campus, Tifton, Georgia, USA

²Departments of Crop Sciences and Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

³Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

⁴Department of Crop Soil and Environmental Sciences, Auburn University, Auburn, Alabama, USA

Correspondence

Ved Parkash, Department of Crop and Soil Sciences, University of Georgia-Tifton Campus, Tifton, GA 31793, USA.
Email: ved.parkash@uga.edu

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Abstract

Alterations in the mechanistic drivers of photosynthesis have the potential to improve crop productivity, but their measurement is inherently time-consuming using traditional methods. High-throughput approaches to estimate photosynthesis using hyperspectral reflectance could be developed by leveraging variation in cotton (*Gossypium hirsutum* L.) leaf traits generated through nitrogen (N) management, synthetic growth regulation strategies, and leaf position within the canopy. Currently, no such models exist for cotton, and interactions among the aforementioned factors are relatively unexplored for cotton leaf traits. This study aimed to (1) evaluate the effects of N application rate, mepiquat chloride (MC) management, and leaf position within the canopy on photosynthesis and its components, and (2) develop and validate hyperspectral reflectance-based partial least squares regression (PLSR) models for predicting cotton leaf physiological traits. N rate and leaf position interacted to affect net photosynthetic rate (A_N), electron transport rate, chlorophyll a, and chlorophyll b, while MC influenced only leaf pigments and specific leaf weight (SLW). A_N reductions under N deficiency were driven by declines in the maximum rate of Rubisco carboxylation ($V_{c,max}$) and ribulose-1,5-bisphosphate regeneration (J_{max}), whereas high N had no effect on A_N . PLSR models exhibited good to high predictive accuracy, with R^2 values ranging from 0.62 to 0.87 for most traits, except for SLW. These findings enhance our understanding of the physiological responses to N rate, MC strategy, and leaf position and highlight the potential of hyperspectral reflectance-based PLSR as a high-throughput tool for predicting leaf physiological traits to improve photosynthetic efficiency in cotton.

Abbreviations: %RMSE, percentage of root mean square error; ATP, adenosine triphosphate; ETR, electron transport rate; MC, mepiquat chloride; NIR, near infrared; PLSR, partial least squares regression; RACiR, rapid A-Ci response; RMSE, root mean square error; RuBP, ribulose-1,5-bisphosphate; SLA, specific leaf area; SLW, specific leaf weight; SWIR, shortwave-infrared; VIP, variable importance in projection; VIS, visible.

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Plain Language Summary

Understanding how plant traits vary and respond to the environment is key to improving crop performance. Measuring traits like photosynthesis is important but traditionally slow and labor-intensive. In this study, we explored how leaf traits linked to photosynthesis respond to nitrogen (N) fertilizer, plant growth regulation, and leaf position. We then developed models that use hyperspectral reflectance—high-resolution spectral data collected from leaves—to predict these traits quickly and nondestructively. Nitrogen and leaf position had strong effects on leaf function, while growth regulators mainly affected pigments and leaf thickness. Our models accurately predicted several traits related to photosynthesis. These findings highlight the power of combining spectral data with trait prediction to advance field-based plant phenomics. This approach could support faster screening of cotton varieties and better crop management strategies.

1 | INTRODUCTION

Recently published reviews and original research articles have identified increased photosynthetic efficiency (at the leaf or canopy scale) as an important trait to accelerate genetic yield improvement in row crops (Constable & Bange, 2015; De Souza et al., 2022; Long et al., 2015; Zhu et al., 2010). Yield improvement through selective breeding has traditionally been associated with greater partitioning of biomass to economic yield (harvest index), with no or only limited improvements in yield associated with photosynthesis per unit leaf area (Boote & Sinclair, 2006; Conaty & Constable, 2020; Koester et al., 2016). As a result, some authors have argued that increasing photosynthetic rates alone may not lead to corresponding improvements in crop yield (T. Sinclair & Rufty, 2022; T. R. Sinclair et al., 2019). Another view is that photosynthetic trait variation has simply not been exploited in breeding programs, but will be essential to accelerate genetic yield gains in the future because harvest index must have an inherent upper limit above which negative impacts on the crop would be observed (Evans, 2013).

For example, excess partitioning to reproductive organs would eventually be expected to decrease root, stem, and leaf growth, thereby limiting the ability of the plant to capture resources and respond to abiotic stress (Evans, 2013). In upland cotton (*Gossypium hirsutum* L.), the most widely grown fiber crop globally, yield improvement has primarily been driven by increases in lint percent, which is the percentage of seed cotton accounted for by fiber (Campbell et al., 2011; Conaty & Constable, 2020). However, increases in lint percent have also been associated with reductions in individual seed mass over time (Campbell et al., 2011; Virk, Snider, Bourland, 2023), which may have negative implications for seedling vigor (J. L. Snider et al., 2016; Virk et al., 2019, 2020). As a result, other reports have emphasized the

need to explore different functional drivers of yield in cotton breeding programs (Kaur et al., 2024; Virk, Snider, Chee, et al., 2023). There is also an extensive body of literature addressing the response of photosynthesis to environmental stresses such as high temperature, nutrient deficiencies, and water deficit (Chastain et al., 2014; Kaur et al., 2023; Parkash et al., 2023; Parkash, Snider, Awori Kelvin, et al., 2024; Parkash, Snider, Virk, Dhillon, et al., 2024; Salvucci & Crafts-Brandner, 2004; Scafaro et al., 2023). Such studies typically have the goal of identifying the weak links in carbon (C) assimilation under a particular stress. The solution to reduce crop susceptibility to environmental stress, proposed by the authors, consists of breeding for increased tolerance of the key stress-sensitive physiological and biochemical processes, such as electron transport, ribulose-1,5-bisphosphate (RuBP) regeneration, and Rubisco carboxylation (Parkash et al., 2023; Parkash, Snider, Virk, Dhillon, et al., 2024).

In practice, photosynthetic traits are time-consuming to assess using traditional phenotyping methods. For example, estimates of light-saturated net photosynthetic rate (A_N) may require as little as 1 min to as much as 5 min to complete using commercially available gas exchange systems (Meeks et al., 2019; J. L. Snider et al., 2022; Stinziano et al., 2017). CO_2 response curves used to derive the maximum rates of carboxylation by Rubisco ($V_{c,max}$) or RuBP regeneration (J_{max}) may require up to half an hour to complete a single measurement using traditional approaches or 5–15 min using more recently developed methods like rapid A-Ci response (RACiR) experiments (Chastain et al., 2024; Pilon et al., 2018; Stinziano et al., 2017). If other leaf traits such as leaf pigment contents are measured, additional laboratory analysis or extraction time is required (Meacham-Hensold et al., 2020). In addition, measurements must be done within a specific developmental and diurnal timeframe to ensure valid comparisons under field conditions (Ferguson et al.,

2023). The previously mentioned constraints and the need to screen hundreds or thousands of accessions render traditional assessments of photosynthetic traits impractical (Fu et al., 2022; Montes et al., 2022). One approach to address this bottleneck uses hyperspectral reflectance data and physiological, physical, or biochemical assessments conducted on the same leaves or canopy to develop predictive models using partial least squares regression (Burnett et al., 2021; Serbin et al., 2012). Using this approach, models for predicting $J_{\max}$ and $V_{c,\max}$ have been developed for maize (*Zea mays*) (Yendrek et al., 2016), tobacco (*Nicotiana tabacum*) (Meacham-Hensold et al., 2019), soybean (*Glycine max*) (Buchaillot et al., 2022; Montes et al., 2022), peanut (*Arachis hypogaea*) (Bagherian et al., 2023; Buchaillot et al., 2022), blueberry (*Vaccinium sect. Cyanococcus*) (Rahman et al., 2025), and maple trees (*Acer* spp.) (Jin et al., 2022), among other plant species. Partial least squares regression (PLSR) models have also been developed to estimate other traits from hyperspectral reflectance data, such as leaf pigment contents and specific leaf area (SLA) (Yendrek et al., 2016; Zhi et al., 2022). As a result, photosynthetic traits can be estimated from spectral signatures that require only seconds to obtain per leaf sample. Using spectra-derived photosynthetic traits, Montes et al. (2022) was able to phenotype a large soybean nested association mapping population (containing thousands of recombinant inbred lines) and map $J_{\max}$ and $V_{c,\max}$ to specific chromosomal regions. For upland cotton, there remains a critical need to develop hyperspectral reflectance-based PLSR models for the rapid and efficient evaluation of photosynthetic traits under field conditions, which can accelerate selection and crop improvement efforts. Such models can overcome the limitations of traditional gas exchange measurements, which are labor-intensive and low-throughput, thereby facilitating large-scale phenotyping for crop improvement. According to Burnett et al. (2021), best data collection practices for the development of PLSR models to predict leaf traits include (1) collecting reflectance and physiological data from the same leaves, (2) having a minimum of 100–200 observations, and (3) filling the trait space of interest. To satisfy the third point, observed values for leaf traits should represent a wide range and not be excessively clustered or skewed.

Leaf characteristics and photosynthetic traits in field-grown cotton can be strongly influenced by management practices and leaf position on the plant. For example, nitrogen (N) deficiency decreases A_N of uppermost, fully expanded leaves from 13% to 60% for cotton grown under irrigated conditions in Georgia (Parkash et al., 2023; Parkash, Snider, Virk, 2024; J. Snider, Harris, et al., 2021). Nitrogen deficiency also negatively impacts pigment concentrations (Chl A, Chl B, and carotenoids), photosynthetic electron transport rates (ETR) through photosystem II, and stomatal conductance (g_s) (Parkash et al., 2023; Radin & Ackerson, 1981; J. Snider, Harris, et al., 2021). Importantly, $J_{\max}$ and $V_{c,\max}$ are the most

Core Ideas

- Hyperspectral reflectance enables rapid prediction of cotton photosynthetic traits.
- Hyperspectral reflectance-based partial least squares regression models exhibited good to high predictive accuracy ($R^2 = 0.62$ – 0.87) for most traits.
- Nitrogen rate and leaf position significantly affected photosynthesis and pigment composition.
- Mepiquat chloride influenced pigments and specific leaf weight but not photosynthetic capacity.

sensitive processes to N deficiency and likely co-limit photosynthesis under low N conditions (Parkash et al., 2023). Mepiquat chloride (MC) is an exogenously applied plant growth regulator that limits internode elongation, produces a more compact plant, and leads to earlier maturity in cotton by inhibiting gibberellic acid synthesis (Hake et al., 1991; Kerby, 1985; York, 1983). Leaves from plants treated with MC have been shown to exhibit lower individual leaf areas (A. R. Reddy, Reddy, Hodges, 1996), higher chlorophyll contents (A. R. Reddy, Reddy, Hodges, 1996), denser leaves with lower SLA (V. R. Reddy et al., 1990; Zhao & Oosterhuis, 2000), and in some instances, higher A_N values (Zhao & Oosterhuis, 2000). Finally, leaf position on the plant significantly affects leaf characteristics in upland cotton. Milroy and Bange (2003) demonstrated that mainstem cotton leaves near the top of the canopy had higher specific leaf nitrogen and light-saturated photosynthetic rates than leaves lower in the canopy. Similar results were obtained by Parkash et al. (2023) who documented reductions in specific leaf weight (SLW), A_N , ETR, g_s , $V_{c,\max}$, and $J_{\max}$ as leaves were sampled from successively lower positions within the cotton canopy. Exploring interactions between management strategies and leaf position may provide a broad range of variation that could be used to build PLSR models to predict leaf traits from hyperspectral reflectance.

As noted above, there are extensive studies documenting the effects of N application rate and MC management or N application rate and leaf position on photosynthesis and its underlying processes in cotton. However, studies evaluating interactive effects of N rate, MC management, and leaf position on leaf photosynthetic characteristics are nonexistent. Additionally, multiple studies have developed PLSR-based models for the prediction of photosynthetic traits from hyperspectral reflectance data in multiple crop species (Burnett et al., 2021; Dechant et al., 2017; Lamour et al., 2021; Meacham-Hensold et al., 2020; Montes et al., 2022; Silva-Perez et al., 2017; Yendrek et al., 2016). Moreover, previous studies have shown that PLSR models generally have

limited cross-species transferability due to differences in leaf structure, pigment composition, and canopy characteristics (Helsen et al., 2021; Ji et al., 2024; Z. Wang, Sun, et al., 2025). However, comparable models have not been developed for field-grown cotton, despite their potential to enable high-throughput phenotyping of photosynthetic traits, which are currently underutilized in breeding programs due to the slow and low-throughput nature of traditional gas exchange methods. By capturing the combined effects of nitrogen rate, MC management, and leaf position on leaf function, this study not only advances our physiological understanding but also establishes a foundation for high-throughput phenotyping of key photosynthetic and biochemical traits using hyperspectral reflectance in field-grown cotton. Therefore, the objectives of the current study are to (1) evaluate the effects of N application rate, MC management strategy, and leaf position on A_N and its underlying processes, and (2) exploit variation in leaf traits produced by the previously mentioned factors to develop PLSR-based models to predict functional leaf traits (A_N , ETR, $J_{\max}$, $V_{c,\max}$, chlorophyll a, chlorophyll b, carotenoid content, and SLW) on attached leaves of field-grown cotton. It is hypothesized that the three factors utilized in the current study will interact to generate sufficient variation in leaf traits to develop robust PLSR-based models to predict leaf traits from hyperspectral reflectance.

2 | MATERIALS AND METHODS

2.1 | Crop management and experimental design

The study was conducted at the Lang-Rigdon research farm of the University of Georgia in Tifton, GA, during the 2023 growing season. The field site is located in the Coastal Plain region of Georgia, which is the dominant cotton production region of the state (United States Department of Agriculture NASS, 2023). The climate of this region is considered humid and subtropical. The soil type of the study site was a Tifton loamy sand (fine-loamy, kaolinitic, thermic, Plinthic Kandicudults) (Bednarz et al., 2000). The upland cotton cultivar *Gossypium hirsutum* DP 2038 B3XF was grown and managed according to University of Georgia Cooperative Extension recommendations for cotton production in Georgia (Hand et al., 2022). All production practices were consistent across plots, except for nitrogen and MC applications.

There were two treatment factors: nitrogen rate and MC application strategy. The study was designed as a split-plot experiment, with nitrogen rate as the whole plot factor and MC application strategy as the subplot factor. There were four replications of each treatment combination. A single upland cotton cultivar (DP 2038 B3XF) was evaluated across 36 plots, representing three nitrogen rates $\times$ three MC application

strategies $\times$ four replications. Three nitrogen rates were evaluated: (1) 0 kg N ha⁻¹ (N deficient treatment), (2) 135 kg N ha⁻¹ (recommended N rate), and (3) 270 kg N ha⁻¹ (excess N treatment). The three MC treatments were (1) untreated (control, no MC application), (2) moderate (two applications of MC: the first application of 0.88 L ha⁻¹ at the first flower stage and the second application of 1.17 L ha⁻¹ at 2 weeks after the first application), and (3) aggressive (three applications of MC: the first application of 0.73 L ha⁻¹ at the squaring stage (floral bud stage), the second application of 0.88 L ha⁻¹ at the first flower stage, and the third application of 1.17 L ha⁻¹ at 2 weeks after the second application) (Hand et al., 2022). Urea (46% N) was used for nitrogen applications, with 25% of the total targeted N rate applied at planting and the remaining 75% applied at the squaring stage (Hand et al., 2022).

Leaf physiological measurements were taken during the peak flowering stage, when flower production is at its maximum. This stage also corresponds to the period of peak demand for resources, making crop responses to different nitrogen application rates most evident (J. Snider, Bange, et al., 2021). Leaf physiological measurements were obtained from three leaves per plant, specifically from the mainstem leaves at the fourth, seventh, and 10th nodes below the terminal. Research indicates that differences in leaf traits in response to nitrogen rates are most pronounced at lower leaf positions (Parkash et al., 2023). In cotton, new mainstem leaves are produced approximately every 3 days, so the selected leaves represented a 2- to 3-week difference in leaf age (Oosterhuis, 1990). Overall, it was expected that these leaf positions would result in substantial variations in leaf traits and photosynthetic rates.

2.2 | Gas exchange and chlorophyll fluorescence

Physiological assessments were conducted over a 2-week period (between 93 and 105 days after planting) as weather permitted; however, each replication was completed within a single day to avoid the effects of day-to-day variations in weather conditions or crop age. For each of the 36 plots, measurements were collected from two plants and three leaf positions per plant, resulting in six measurements per plot and a total of 216 measurements across the experiment (36 plots $\times$ 2 plants $\times$ 3 leaves = 216). Gas exchange and chlorophyll fluorescence were measured using a portable photosynthesis system with an integrated fluorometer (LI-6800, LI-COR Environmental) between 10:00 a.m. and 3:00 p.m. Leaf chamber environmental and fluorometer configurations for survey measurements were as follows: leaf aperture area: 6 cm², reference CO₂ concentration: 400 μ mol mol⁻¹, photosynthetically active radiation: 1500 μ mol m⁻² s⁻¹, relative humidity: 60 $\pm$ 10%, and air temperature in the chamber was maintained

close to the ambient air temperature during gas exchange measurements. A multiphase flash was used for chlorophyll fluorescence assessments (Earl & Ennahli, 2004). The stomatal limitation (L_s) to photosynthesis was determined using the equation: $L_s = 1 - C_i/C_a$, where C_i is intercellular CO_2 concentration and C_a is ambient carbon concentration (R. Wang, Qin, et al., 2025).

After taking the survey measurement, the fluorometer was turned off, and then the response of net photosynthesis (A_N) to intercellular CO_2 concentration (C_i) was generated using the RACiR curve approach, as described in previous studies (Coursolle et al., 2019; Parkash et al., 2023; Parkash, Snider, Virk, 2024; Parkash, Snider, Virk, Dhillon, et al., 2024; Pilon et al., 2018). To generate RACiR curves, the automatic control program was used to gradually change the reference CO_2 concentration from 10 to $1000 \mu\text{mol mol}^{-1}$ at a rate of $100 \mu\text{mol mol}^{-1} \text{ min}^{-1}$, and an auto-log program was used to record gas exchange rates every 2 s. The generated leaf sample A- C_i response curves were then processed in RStudio (R version 4.2.2) using the *plantecophys* package to estimate the maximum RuBP regeneration rate (J_{max}) and maximum Rubisco carboxylation rate ($V_{\text{c,max}}$) (Duursma, 2015; Parkash et al., 2023). Cotton-specific kinetic parameters, such as the Michaelis–Menten constants for O_2 ($K_o = 9.59 \text{ kPa}$) and CO_2 ($K_c = 35.79 \text{ Pa}$), and the CO_2 compensation point in the absence of dark respiration ($\Gamma^* = 44 \mu\text{mol mol}^{-1}$) as reported in literature (Harley et al., 1992; Lei et al., 2021; Parkash et al., 2023; Singh et al., 2013; Walker & Cousins, 2013), were used during model fitting as described previously (Duursma, 2015; Gu et al., 2010).

2.3 | Leaf reflectance spectroscopy

After taking the gas exchange and chlorophyll fluorescence measurements, leaf spectral reflectance was recorded in situ using a spectroradiometer (Analytical Spectral Devices [ASD], FieldSpec 4 Hi-Res), which measures spectral reflectance from 350 to 2500 nm at a spectral resolution of 3 nm in the visible (VIS; 350–700 nm) and near-infrared (NIR; 700–1000 nm) regions and 8 nm for shortwave-infrared (SWIR; 1000–2500 nm). The FieldSpec4 Plant Probe, fitted with the ASD Leaf Clip (Malvern Panalytical), was used to measure reflectance spectra from the adaxial surface of the leaf used for gas exchange and chlorophyll fluorescence measurements. The plant probe contains a radiometrically calibrated internal light source, which was standardized for relative reflectance prior to each measurement, using a reflective white reference panel (Spectralon; Labsphere). Six spectral reflectance measurements were taken from each leaf, avoiding the leaf midrib and the laminar area used for the gas exchange measurements. The leaf reflectance data were processed using the FieldSpectra package in R (Serbin

et al., 2019). Spectral splice correction (jump.correction function) was used to adjust discrepancies (splices or jumps) in reflectance data between sensors in the FieldSpec. The adjustments align VIS/NIR and SWIR2 sensor data with the SWIR1 sensor, which serves as the baseline. The average.spec function was used to calculate the average of six reflectance spectra. It also removed any “technical” replicates from a leaf sample that deviate by more than two standard deviations from the spectral average across all wavelengths. Additionally, spectral reflectance bands below 500 nm and above 2400 nm, corresponding to excess sensor noise and low signal-to-noise ratio, were removed from the data set prior to model building (Burnett et al., 2021). Figures S3 and S4 illustrate how leaf reflectance across the 500–2400 nm range is associated with key physiological and biochemical traits (S3) and how it responds to nitrogen rate, growth regulator treatment, and leaf position (S4), providing context for the spectral–trait relationships underpinning our analyses.

2.4 | Leaf pigments and SLW

After taking the measurements in the field, the leaf was excised and transferred to the laboratory in a ziploc bag containing a moist paper towel (to prevent desiccation). To measure the pigment content in the leaf, four 6-mm diameter leaf discs were cut from each leaf sample using a cork borer and placed in a 10-mL amber vial containing 5 mL of ethanol (96% reagent grade). For pigment extraction, the vials were stored at 4°C in the dark for 2 weeks (Dillenburg et al., 1995). After 2 weeks, a 300 μL aliquot of the extract was transferred to a well of a 96-well plate and the absorbance at 470, 649, and 665 nm wavelengths was measured using a microplate reader (Synergy HTX Multi-Mode Microplate Reader with Gen5 software, BioTek Instruments, Inc.). These absorbance values and leaf disc areas were used to determine chlorophyll a, chlorophyll b, and total carotenoid contents per unit leaf area, using the equations reported by Lichtenthaler and Wellburn (1983). After taking leaf discs for pigment extraction, leaf area and the dried leaf weight of each leaf were determined to calculate the SLW. Leaf area was measured using a bench-top leaf area meter (LI-3100C, LI-COR Environmental).

2.5 | Partial least squares regression analysis

Predictive models for the traits of the interest were built following PLSR principles (Wold et al., 2001) and by adopting the protocol by Burnett et al. (2021). Specifically, the pls package in R (Mevik & Wehrens, 2007) was used to build the predictive models for each trait. Each observation consisted of a raw, non-averaged trait measurement paired with its corresponding hyperspectral reflectance spectrum. The full dataset,

comprising 220 observations (216 from experimental plots and four from border plots), was randomly partitioned into two disjoint subsets: 80% (176 samples) were assigned to the calibration dataset (training), and the remaining 20% (44 samples) were reserved as an independent test dataset (validation) for final model evaluation. The partitioning was stratified to ensure that both subsets spanned the full range of trait values observed in the study (Burnett et al., 2021). The test set was held out entirely from model training, cross-validation, and variable selection, providing a true assessment of model generalizability.

Within the calibration dataset, cross-validation was performed to tune the number of PLSR components and avoid overfitting. Specifically, we used the predicted residual sum of squares statistic, and determined the optimal number of components by identifying the point at which additional latent variables did not significantly reduce root mean square error (RMSE), as determined by a *t*-test (Serbin et al., 2014).

To further assess model stability and robustness, we employed jackknife resampling on the calibration dataset. This involved 1000 iterations where, in each iteration, a random 70% subset of the calibration data was used for model training (i.e., inner training set), and the remaining 30% served as an internal validation set (i.e., inner test set). Performance metrics (the coefficient of determination [R^2], root mean square error of the prediction (RMSEP), percentage RMSEP (PERC_RMSEP), and model bias) from these iterations were aggregated to evaluate consistency and detect potential outliers or unstable predictions.

Additionally, variable importance in projection (VIP) scores were calculated using the final PLSR models trained on the full calibration dataset. Although $VIP > 0.8$ is generally interpreted as a high contribution of a wavelength to model prediction (Wold et al., 2001), these scores were used solely for interpretation and not to filter wavelengths during model calibration or validation.

Finally, the fully trained model (using all 80% calibration data and the selected number of components) was evaluated on the independent 20% test dataset (validation), which had not been seen at any point during model development. Final model performance was assessed using R^2 , RMSE, and model bias, providing a realistic estimate of predictive accuracy under independent conditions (Burnett et al., 2021).

2.6 | Statistical analysis

A three-way mixed-effects analysis of variance with repeated measures was conducted to assess the effects of nitrogen rate, MC management, leaf position, and their interactions on leaf physiological parameters. Leaf position was treated as the within-subject factor (repeated measure), while nitrogen rate and MC management were considered between-subject

factors. Replication and measurement day were included as random effects to account for variability among experimental units and potential environmental fluctuations across sampling days. When significant effects were detected, post hoc comparisons were performed using Tukey's honestly significant difference test at a significance level of 0.05. All statistical analyses were conducted using JMP Pro 18 (SAS Institute Inc.).

3 | RESULTS

3.1 | Gas exchange and chlorophyll fluorescence

The effects of treatment factors on our traits of interest were highly dependent on the variable evaluated. For example, there were significant effects of N rate, leaf position, and the interaction between N rate and leaf position for net photosynthetic rate (A_N) and ETR, whereas MC strategy and interactions between MC and other model factors did not affect the previously mentioned variables (Table S1). Starting with A_N , response to the N application rate was heavily influenced by leaf position (Figure 1A). At the fourth node below the terminal, A_N was 18% lower in the N-deficient treatment (0 kg N ha⁻¹) than the high N treatment (270 kg N ha⁻¹). At the 10th mainstem leaf node, A_N was 28% lower under N deficiency compared with the high N treatment. At the seventh leaf position below the terminal, A_N was significantly lower in the low N treatment (by 25%) compared to the control (recommended N rate; 135 kg N ha⁻¹), while it was comparable between the control and the high N rate. The ETR was not significantly different among N rates at the fourth leaf position below the terminal (Figure 1B). At the seventh leaf position, ETR was 27% lower in the low N rate compared to the control and was comparable between the control and the high N rates. At the 10th leaf position below the terminal, ETR was significantly lower in the control (by 28%) and the low N rate (by 34%) than the high N rate treatment.

Only N rate and leaf position significantly affected the maximum rate of Rubisco carboxylation ($V_{c,max}$) and maximum rate of RuBP regeneration (J_{max} ; Table S1), and no interactions between the two factors were observed for these variables. When significant differences were observed in $V_{c,max}$, values were significantly lower in the low N rate treatment (by 28% at the fourth leaf position and by 39% at the seventh leaf position) and significantly higher in high N rate treatment (by 19%, 32%, and 66% at the fourth, seventh, and 10th leaf positions, respectively) compared to the control (Figure 1C). J_{max} was significantly lower by 24% in the low N rate treatment compared to the control treatment, but it was comparable between the control and high N rate treatment at the fourth leaf position (Figure 1D). Again,

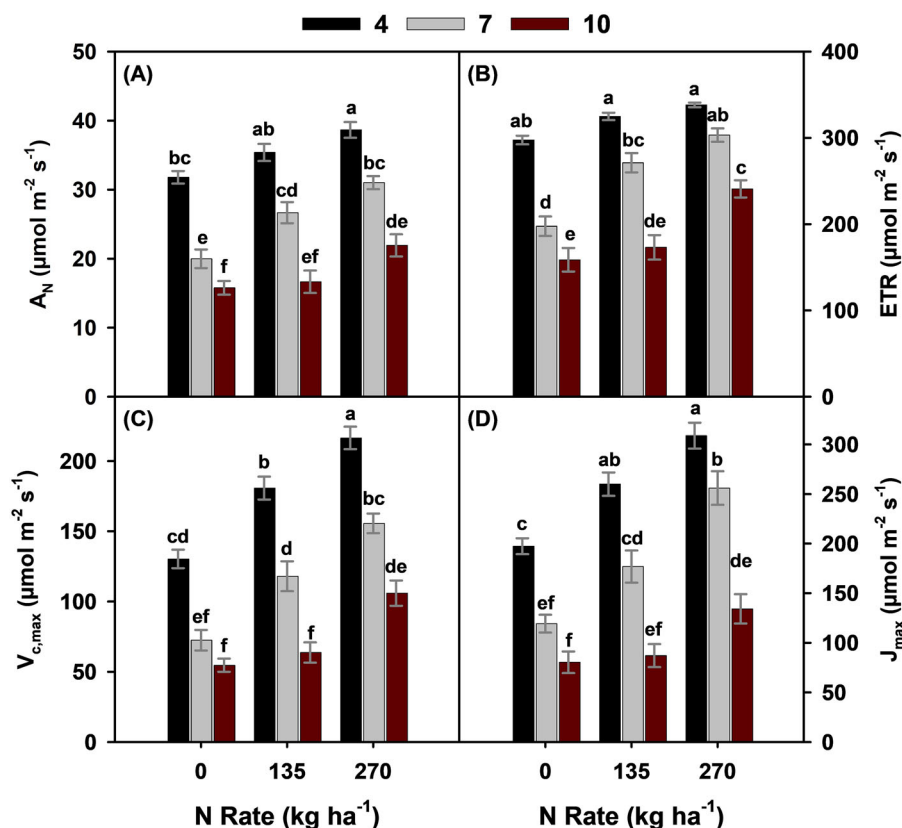


FIGURE 1 Effects of nitrogen application rate (0, 135, and 270 kg N ha⁻¹) and leaf position (fourth, seventh, and 10th leaves below the terminal) on observed values of net photosynthetic rate (A_N ; A), electron transport rate (ETR; B), maximum rate of carboxylation ($V_{c,max}$; C), and maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration (J_{max} ; D) of cotton leaves during the 2023 growing season. Bar plots represent mean $\pm$ standard error. Different lowercase letters above bars indicate statistically significant differences among treatment combinations based on Tukey's honestly significant difference (HSD) test ($p < 0.05$).

it was significantly lower by 33% in the low N treatment compared to control, but it was significantly higher by 45% in the high N treatment compared to control at the seventh leaf position. At the 10th leaf position, J_{max} was comparable between the low N rate and the control and also between the control and the high N rate. Within each N rate, most of the gas exchange and chlorophyll fluorescence parameters significantly decreased from the upper to the lower positions in the canopy (Figure 1).

Stomatal conductance (g_s) was significantly influenced only by leaf position, while intercellular CO₂ concentration (C_i) was affected solely by nitrogen (N) rate. Stomatal limitations (L_s), on the other hand, were significantly impacted by both N rate and leaf position (Table S2). The g_s values were not affected by N rate at each leaf position. However, within each N rate, g_s decreased from the uppermost measured leaf positions to the lowest leaf position in the canopy (Figure 2A). The C_i values were comparable among N rates at the fourth leaf position below the terminal. However, at the seventh and 10th leaf positions below the terminal, C_i was significantly higher under the low N rate compared to the control and high N rates (Figure 2B). Stomatal limitations were significantly

lower under the low N rate compared to the control at the seventh and 10th leaf positions below the terminal, while they were comparable between the control and high N rates at each leaf position (Figure 2C).

3.2 | Leaf pigment contents and SLW

Chlorophyll a was affected by N rate, leaf position, and MC individually, and there was an interaction between N rate and leaf position, but no interactions among the other factors (Table S3). At each measured leaf position, chlorophyll a was significantly lower under low N rate conditions (by 28%, 35%, and 43% at the fourth, seventh, and 10th leaf positions) and significantly higher under high N application rate (by 18%, 30%, and 30% at the fourth, seventh, and 10th leaf positions) compared to the control (Figure 3A). Chlorophyll b was significantly influenced by N rate, MC, and the interaction between N rate and leaf position (Table S3). Chlorophyll b was lower by 34%, 34%, and 48% at the fourth, seventh, and 10th leaf nodes in the low N rate compared to control and significantly higher by 34% in high N rate compared to control

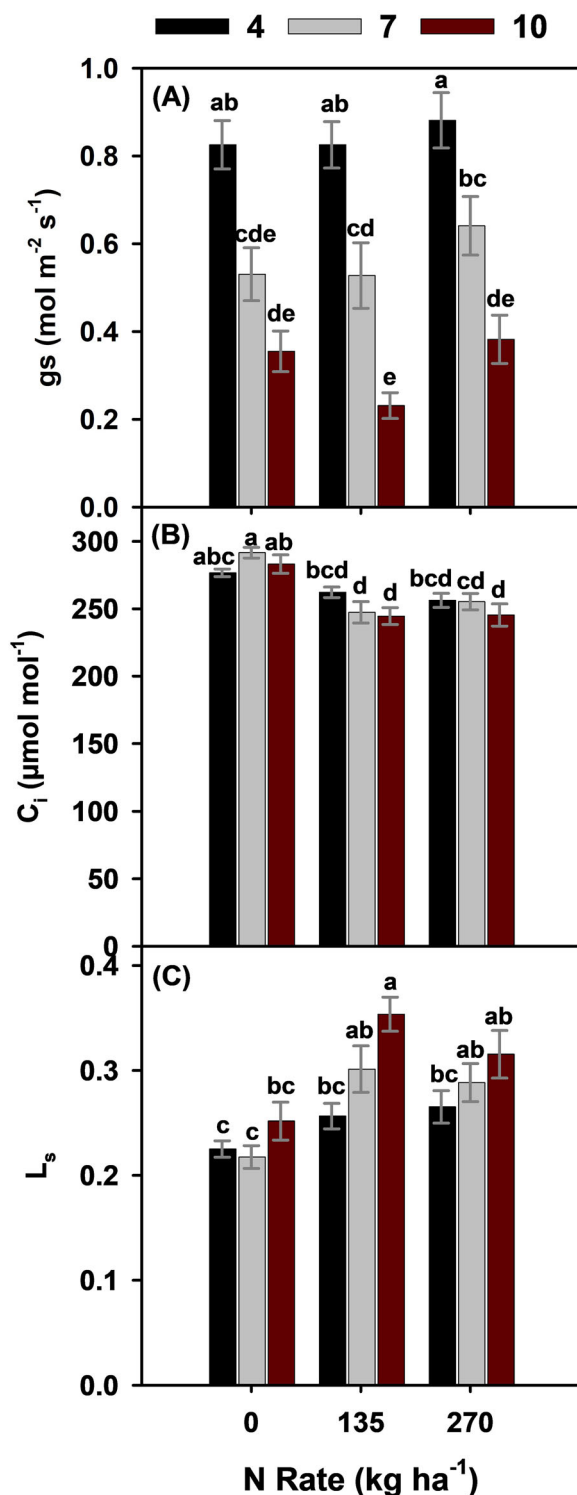


FIGURE 2 Effects of nitrogen rate (0, 135, and 270 kg N ha⁻¹) and leaf position (fourth, seventh, and 10th leaves below the terminal) on observed values of stomatal conductance (g_s ; A), intercellular CO₂ concentration (C_i ; B), and stomatal limitations (L_s ; C) of cotton leaf during the 2023 growing season. Bar plots represent mean $\pm$ standard error. Different letters above bars denote statistically significant differences among treatment combinations (Tukey's honestly significant difference [HSD], $p < 0.05$).

at the seventh leaf position only (Figure 3B). Total carotenoid content in the cotton leaf was affected solely by the main factors, and no interactions were observed (Table S3). Total carotenoid content was 24%–34% lower in the low N treatment than the control across all leaf positions and 20%–27% higher in the high N rate treatment compared to the control (Figure 3C). When averaged across N rates and MC treatments, total carotenoid content was significantly lower at the 10th leaf position compared to the fourth and seventh leaf positions. SLW was significantly impacted only by MC and leaf position (Table S3). When averaged across all N rates and MC treatments, SLW was lowest at the 10th leaf node from the terminal (Figure 3D). Chlorophyll a, chlorophyll b, total carotenoid content, and SLW were significantly higher in plants receiving some form of growth regulation (either aggressive or moderate growth control) than in the untreated control treatment (Table 1).

3.3 | Correlation between photosynthetic and leaf traits

When pairwise correlations between photosynthetic and leaf morphological or pigment traits were analyzed, A_N showed a strong correlation with g_s ($r = 0.81$), ETR ($r = 0.85$), $V_{c,max}$ ($r = 0.91$), and J_{max} ($r = 0.8$; Table 2). Although, A_N was significantly correlated with chlorophyll a, chlorophyll b, carotenoid content, and SLW, the correlations were low ($r = 0.35$ – 0.53 ; Table 2). Other significant ($p < 0.05$) and strong, positive correlations ($r > 0.7$) were observed between g_s and $V_{c,max}$ ($r = 0.73$), ETR and $V_{c,max}$ ($r = 0.81$), ETR and J_{max} ($r = 0.87$), and $V_{c,max}$ and J_{max} ($r = 0.83$). Additionally, chlorophyll a was strongly correlated with chlorophyll b ($r = 0.94$) and carotenoid content ($R^2 = 0.95$), while chlorophyll b was strongly correlated with carotenoid content ($r = 0.86$; Table 2). The significance levels for all correlations are provided in Table 2.

In addition to pooled Pearson correlations, pairwise correlations were analyzed separately within each N rate and canopy position (Table S4), revealing that the traits most strongly correlated with A_N varied across treatments. When compared among ETR, J_{max} , $V_{c,max}$, pigments, and SLW, A_N at upper leaf positions (leaf 4) was most strongly correlated with $V_{c,max}$ under 0, 120, and 240 kg ha⁻¹ N ($r = 0.52$, 0.83, and 0.79, respectively), while its correlations with ETR, pigments, and SLW were generally weak or nonsignificant. At middle leaf positions (leaf 7), A_N consistently showed strong correlations with both $V_{c,max}$ ($r = 0.88$, 0.85, and 0.70 for 0, 120, and 240 kg ha⁻¹ N, respectively) and J_{max} ($r = 0.68$ and 0.77 for 0 and 120 kg ha⁻¹ N, respectively), although the correlation with J_{max} weakened under 240 kg ha⁻¹ N (0.19 ns). At lower leaf positions (leaf 10), A_N was strongly associated with J_{max} across all N levels ($r = 0.72$, 0.80, and 0.71 for 0,

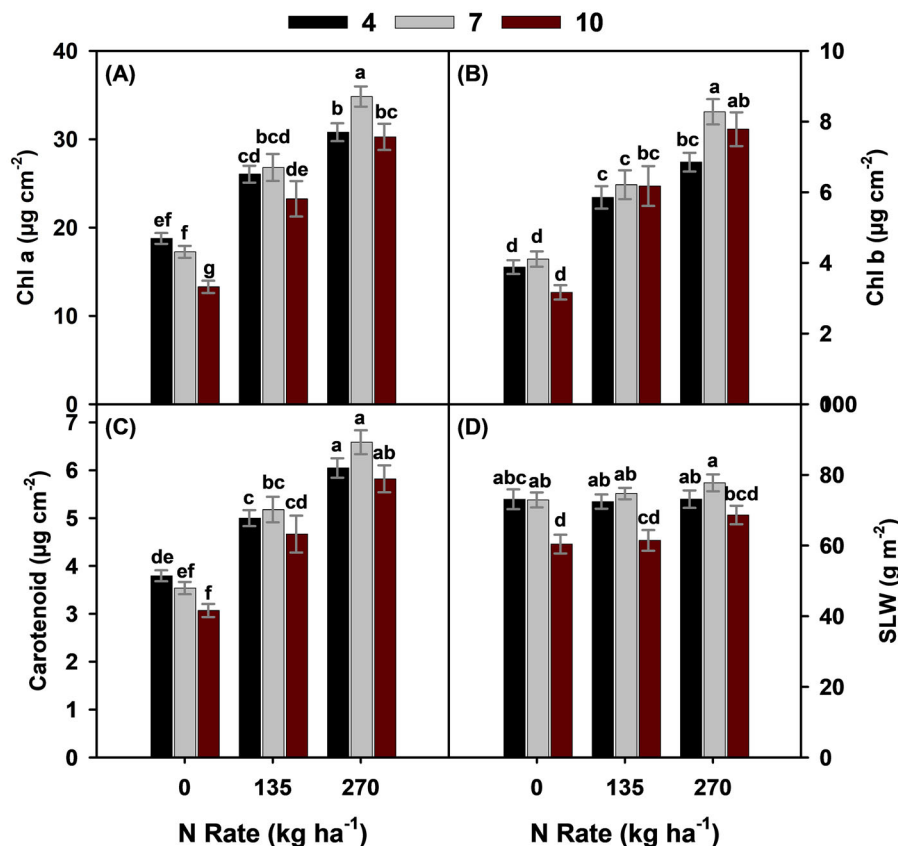


FIGURE 3 Effects of nitrogen application rate (0, 135, and 270 kg N ha⁻¹) and leaf position (fourth, seventh, and 10th leaves below the terminal) on observed values of chlorophyll a (A), chlorophyll b (B), carotenoid content (C), and specific leaf weight (SLW; D) of cotton leaves during the 2023 growing season. Bar plots represent mean $\pm$ standard error. Different lowercase letters above bars indicate statistically significant differences among treatment combinations based on Tukey's honestly significant difference [HSD] test ($p < 0.05$).

TABLE 1 Effects of mepiquat chloride strategy on observed and predicted values of chlorophyll a, chlorophyll b, total carotenoid, and specific leaf weight (SLW) of cotton leaf during the 2023 growing season.

Mepiquat chloride		Aggressive	Moderate	Untreated
Chl a (μg cm ⁻²)	Observed	25.1 $\pm$ 1.2a	25.9 $\pm$ 1.1a	22.7 $\pm$ 0.9b
	Predicted	26.2 $\pm$ 1.1a	26.4 $\pm$ 1.1a	22.9 $\pm$ 0.8b
Chl b (μg cm ⁻²)	Observed	6.1 $\pm$ 0.3a	6.1 $\pm$ 0.3a	5.2 $\pm$ 0.2b
	Predicted	6.6 $\pm$ 0.3a	6.3 $\pm$ 0.3a	5.2 $\pm$ 0.2b
Carotenoids (μg cm ⁻²)	Observed	5 $\pm$ 0.2a	5 $\pm$ 0.2ab	4.6 $\pm$ 0.2b
	Predicted	5.1 $\pm$ 0.2a	5.1 $\pm$ 0.2ab	4.6 $\pm$ 0.2b
SLW (g m ⁻²)	Observed	74 $\pm$ 1.4a	72.7 $\pm$ 1.6a	66.7 $\pm$ 1.3b
	Predicted	74.6 $\pm$ 1.4a	71.8 $\pm$ 1.7a	66 $\pm$ 1.3b

Note: Mean values within each column followed by the same letter are not significantly different, as determined by Tukey's honestly significant difference (HSD) test at a significance level of 0.05. Predicted values were derived from the full dataset, which included both training and validation observations.

120, and 240 kg ha⁻¹ N, respectively) and with ETR at 0 and 120 kg ha⁻¹ ($r = 0.75$ and 0.69 , respectively). Correlations of A_N with chlorophyll a and chlorophyll b increased in lower leaves, particularly under 0 kg ha⁻¹ N, but remained weaker or nonsignificant under high N conditions. A_N showed little association with SLW except for strong positive correlations at middle leaves under 0 kg ha⁻¹ N ($r = 0.74$).

Across all N levels and leaf positions, stomatal conductance (g_s) consistently exhibited the strongest positive correlation with A_N , often exceeding 0.7 and reaching as high as 0.95 at lower leaves (leaf 10) under 240 kg ha⁻¹ N. In contrast, C_i showed weak or negative correlations with A_N across all treatments, with most relationships being nonsignificant.

TABLE 2 Pearson product–moment correlation coefficients for pairwise association among leaf traits, including net photosynthetic rate (A_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), electron transport rate (ETR), maximum carboxylation rate of Rubisco ($V_{c,\max}$), maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration ($J_{\max}$), chlorophyll a, chlorophyll b, carotenoid, and specific leaf weight (SLW).

	C_i	g_s	ETR	$V_{c,\max}$	$J_{\max}$	Chl a	Chl b	Carotenoids	SLW
A_N	−0.08 ^{ns}	0.81***	0.85***	0.91***	0.8***	0.53***	0.38***	0.49***	0.35***
C_i		0.41***	−0.28***	−0.06 ^{ns}	−0.21**	−0.35***	−0.27***	−0.4***	−0.2*
g_s			0.57***	0.73***	0.52***	0.27***	0.19*	0.21**	0.22**
ETR				0.81***	0.87***	0.58***	0.39***	0.57***	0.5***
$V_{c,\max}$					0.83***	0.61***	0.47***	0.55***	0.33***
$J_{\max}$						0.59***	0.4***	0.6***	0.39***
Chl a							0.94***	0.95***	0.39***
Chl b								0.86***	0.27***
Carotenoids									0.39***

Note: Data were combined from all treatments (N rates, mepiquat chloride, and leaf positions; $n = 216$). Correlation coefficients in bold indicate strong associations with $r \geq 0.70$.

ns, *, **, and *** represent non-significant ($p > 0.05$), $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Principal component analysis (PCA) revealed that the traits influencing A_N varied across N rates and canopy positions (Figures S1 and S2; Tables S5 and S6), with the first two principal components (PC1 and PC2) explaining most of the variance (62% for leaf 4, 67%–72% for leaf 7, and 70%–74% for leaf 10). In upper canopy leaves (leaf 4), A_N loaded primarily on PC2 (0.72) under 0 kg N ha^{−1}, together with ETR (0.60) and $J_{\max}$ (0.61). At 120 and 240 kg N ha^{−1}, A_N loaded strongly on PC1 (0.76 and 0.74) along with $V_{c,\max}$ (0.88 and 0.80), chlorophyll a and b (0.73–0.85), and carotenoids (0.63–0.75).

In middle canopy leaves (leaf 7), A_N was aligned with PC1 under 0 and 120 kg N ha^{−1}, where it co-varied strongly with $V_{c,\max}$ (0.91–0.88), $J_{\max}$ (0.66–0.80), and ETR (0.83–0.73). At 240 kg N ha^{−1}, A_N (0.85) shifted to PC2, where $V_{c,\max}$ (0.91) also exhibited the highest loading. In lower canopy leaves (leaf 10), A_N remained aligned with PC1 across all N levels, co-varying strongly with $J_{\max}$ (0.75–0.88) and ETR (0.68–0.81). At most leaf positions and across all N levels, g_s loaded strongly on the same principal components as A_N , whereas C_i exhibited consistently weak loadings.

3.4 | Partial least squares regression models

PLSR models were developed to predict leaf photosynthetic and pigment traits from leaf reflectance spectra, with varying predictive performance across different traits (Figures 4–8). In this study, we define low predictive accuracy as models with $R^2 < 0.50$, good predictive ability as models with R^2 between 0.60 and 0.69, strong predictive performance as models with R^2 between 0.70 and 0.80, and very high predictive accuracy for models with $R^2 > 0.80$, following conventions in plant physiology and remote sensing studies (Meacham-Hensold et al., 2019; Yendrek et al., 2016).

The PLSR model for A_N exhibited strong predictive performance during cross-validation ($R^2 = 0.70$, RMSE = 4.44 $\mu\text{mol m}^{-2} \text{s}^{-1}$, %RMSE [percentage of root mean square error] = 10.46, and model bias = 0.0302) but showed good predictive ability during external validation ($R^2 = 0.62$, RMSE = 5.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and model bias = −0.9747; Figure 4A,B). In contrast, the PLSR model for ETR demonstrated very high predictive accuracy in both cross-validation ($R^2 = 0.83$, RMSE = 23.92 $\mu\text{mol m}^{-2} \text{s}^{-1}$, %RMSE = 8.08, and model bias = −0.0865) and external validation ($R^2 = 0.80$, RMSE = 31.63 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and model bias = 2.0154; Figure 4C,D). The PLSR model for $V_{c,\max}$ achieved strong predictive performance in cross-validation ($R^2 = 0.69$, RMSE = 24.44 $\mu\text{mol m}^{-2} \text{s}^{-1}$, %RMSE = 9.61, and model bias = 0.1685) and very high predictive accuracy in external validation ($R^2 = 0.87$, RMSE = 21.06 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and model bias = 1.7453; Figure 5A,B). Similarly, the PLSR model for $J_{\max}$ exhibited strong predictive ability in cross-validation ($R^2 = 0.72$, RMSE = 40.53 $\mu\text{mol m}^{-2} \text{s}^{-1}$, %RMSE = 8.68, and model bias = 0.0982) but showed good accuracy in external validation ($R^2 = 0.62$, RMSE = 58.63 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and model bias = −8.8206; Figure 5C,D).

The PLSR model for leaf chlorophyll a content demonstrated very high predictive accuracy in both cross-validation ($R^2 = 0.86$, RMSE = 2.41 $\mu\text{g cm}^{-2}$, %RMSE = 6.63, and model bias = 0.0257) and external validation ($R^2 = 0.85$, RMSE = 2.81 $\mu\text{g cm}^{-2}$, and model bias = −0.3993; Figure 6A,B). The model for chlorophyll b content exhibited strong predictive performance during cross-validation ($R^2 = 0.75$, RMSE = 0.92 $\mu\text{g cm}^{-2}$, %RMSE = 9.34, and model bias = 0.0012) and very high accuracy during external validation ($R^2 = 0.83$, RMSE = 0.83 $\mu\text{g cm}^{-2}$, and model bias = −0.0527; Figure 6C,D). Similarly, the PLSR model for total carotenoid content demonstrated very

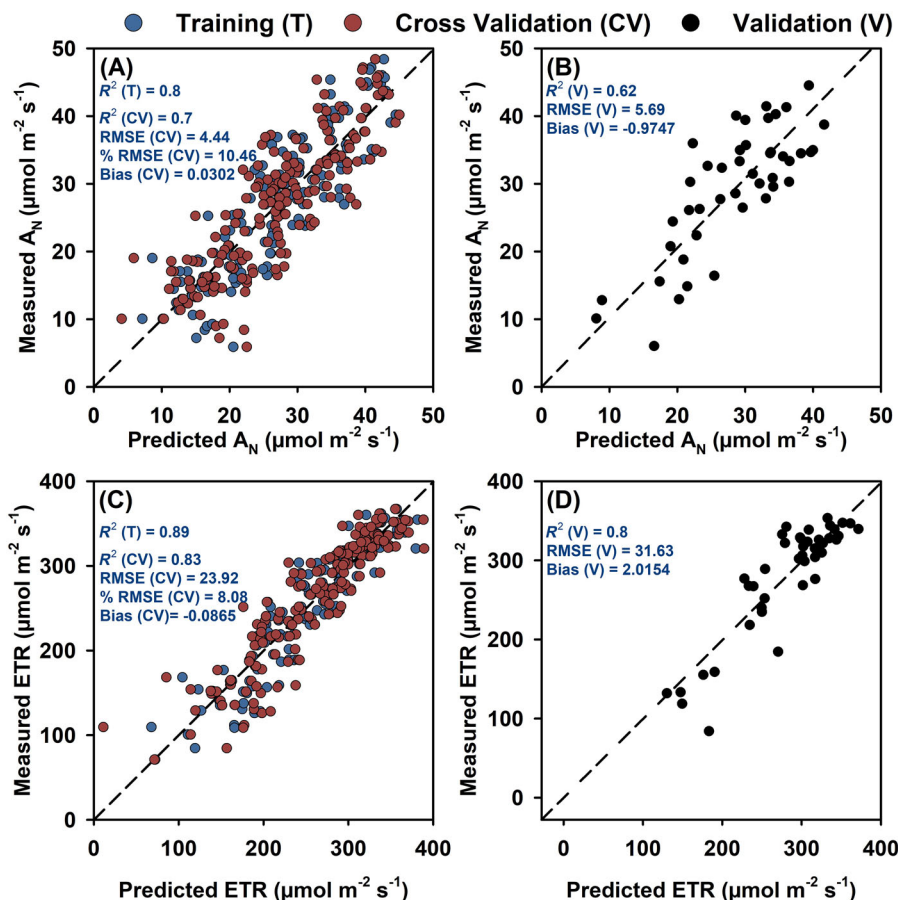


FIGURE 4 Plots of observed versus predicted values for net photosynthetic rate (A_N ; A and B) and electron transport rate (ETR; C and D) during model calibration or training (T), cross-validation (CV), and validation (V). The blue dots represent training data, red dots represent cross-validation data, and black dots represent validation data. The black dashed line represents the 1:1 line. Model performance metrics include the coefficient of determination (R^2), root mean square error (RMSE), percentage of root mean square error of prediction (%RMSEP), and model bias for both cross-validation (CV) and validation (V).

high predictive accuracy in cross-validation ($R^2 = 0.82$, $\text{RMSE} = 0.48 \mu\text{g cm}^{-2}$, $\% \text{RMSE} = 6.88$, and model bias = 0.0046) but only strong accuracy during external validation ($R^2 = 0.74$, $\text{RMSE} = 0.68 \mu\text{g cm}^{-2}$, and model bias = 0.054; Figure 7A,B). Lastly, the PLSR model for SLW showed good predictive ability in cross-validation ($R^2 = 0.59$, $\text{RMSE} = 6.83 \text{ g m}^{-2}$, $\% \text{RMSE} = 11.8$, and model bias = -0.027) but exhibited low predictive accuracy during external validation ($R^2 = 0.34$, $\text{RMSE} = 9.03 \text{ g m}^{-2}$, and model bias = -0.4765 ; Figure 7C,D).

Most wavelengths in the 500–1900 nm region contributed considerably ($\text{VIP} > 0.8$) to the prediction of A_N , with major contributions observed at 553 nm ($\text{VIP} = 1.91$), 710 nm ($\text{VIP} = 3.01$), 1059 nm ($\text{VIP} = 1.31$), 1389 nm ($\text{VIP} = 2.00$), and 1866 nm ($\text{VIP} = 1.36$; Figure 8A). For the prediction of ETR, considerable contributions were observed in the spectral regions 500–768 nm, 905–906 nm, 915 nm, 1043–1077 nm, 1320–1463 nm, and 1778–1900 nm. Major contributions were at 552 nm ($\text{VIP} = 2.39$), 716 nm ($\text{VIP} = 3.66$), 1389 nm ($\text{VIP} = 2.15$), and 1866 nm ($\text{VIP} = 1.50$;

Figure 8B). Eight spectral regions contributed significantly to the prediction of $V_{c,\text{max}}$, including 500–784 nm, 890–948 nm, 995–1132 nm, 1269–1301 nm, 1323–1427 nm, 1704–1725 nm, 1834–1883 nm, and 2242–2244 nm. The most important contributions occurred at 552 nm ($\text{VIP} = 2.07$), 714 nm ($\text{VIP} = 3.74$), 1060 nm ($\text{VIP} = 1.25$), 1389 nm ($\text{VIP} = 1.62$), and 1867 nm ($\text{VIP} = 1.13$; Figure 8C). For the prediction of J_{max} , the spectral regions with considerable contributions included 500–766 nm, 1041–1086 nm, 1318–1444 nm, 1654–1671 nm, 1677–1728 nm, 1749–1889 nm, 1912–1941 nm, and 2236–2246 nm. Major contributions were observed at 553 nm ($\text{VIP} = 2.25$), 716 nm ($\text{VIP} = 3.75$), 1062 nm ($\text{VIP} = 0.98$), 1388 nm ($\text{VIP} = 1.78$), and 1868 nm ($\text{VIP} = 1.20$; Figure 8D). Additionally, most wavelengths in the 500- to 777-nm spectral region contributed considerably to the prediction of chlorophyll a, chlorophyll b, and total carotenoid contents, with major contributions occurring around 554 and 719 nm (Figure 8E–G). For the prediction of SLW, the spectral regions with considerable contributions included 508–851 nm, 1040–1107 nm, 1238–1441 nm,

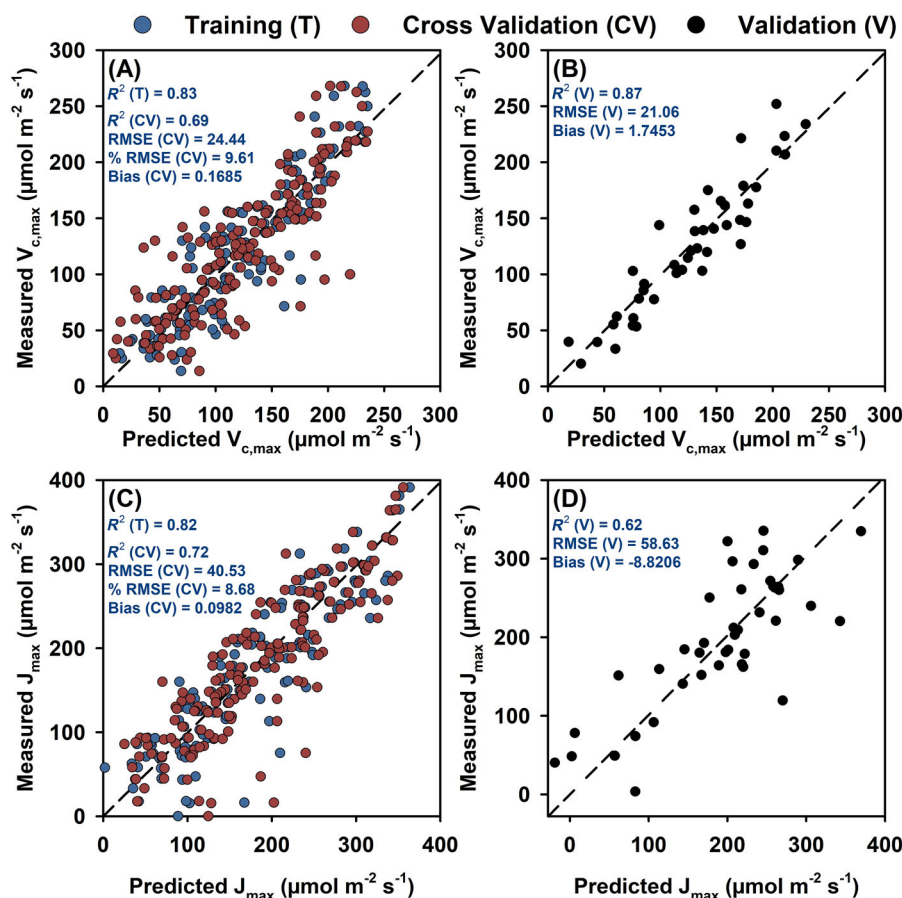


FIGURE 5 Plots of observed versus predicted values for maximum carboxylation rate of Rubisco ($V_{c,max}$; A and B) and maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration (J_{max} ; C and D) during model calibration or training (T), cross-validation (CV), and validation (V). The blue dots represent training data, red dots represent cross-validation data, and black dots represent validation data. The black dashed line represents the 1:1 line. Model performance metrics include the coefficient of determination (R^2), root mean square error (RMSE), percentage of root mean square error of prediction (%RMSEP), and model bias for both cross-validation (CV) and validation (V).

1539–1780 nm, 1845–1885 nm, 1894–2058 nm, and 2124–2175 nm. Major peaks in variable importance ($VIP > 1$) were observed at 550 nm ($VIP = 2.07$), 716 nm ($VIP = 3.75$), 1388 nm ($VIP = 1.78$), and 1661 nm ($VIP = 1.40$; Figure 8H).

3.5 | Model performance: Comparison of predicted versus observed treatment trend

The PLSR model accurately reproduced treatment-related trends and canopy gradients across all measured traits (Figures 1, 3, and 9). For A_N , ETR, $V_{c,max}$, and J_{max} , predicted values closely mirrored the observed increases with higher N rates and consistently captured the canopy gradient, with the highest values in upper leaves (leaf 4) and the lowest in lower leaves (leaf 10). Similarly, predictions for chlorophyll a, chlorophyll b, carotenoids, and SLW aligned well with observed data, reflecting both N-driven increases and leaf position differences. The model also successfully replicated the effects of MC, where untreated plots exhibited lower pig-

ment content and SLW compared with treated plots. A t -test comparing observed and predicted mean values further confirmed the strong agreement between the datasets (Table S7). For A_N , ETR, $V_{c,max}$, and J_{max} , predicted means (27.3 ± 0.6 , 265 ± 5 , 127 ± 4 , and $190 \pm 6 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) closely matched observed values (26.9 ± 0.7 , 262 ± 5 , 124 ± 4 , and $185 \pm 7 \mu\text{mol m}^{-2} \text{s}^{-1}$), with no significant differences ($p > 0.44$ for all traits). Predictions for chlorophyll a, chlorophyll b, carotenoids, and SLW were also statistically indistinguishable from observed means ($p > 0.27$).

4 | DISCUSSION

4.1 | Photosynthetic response to nitrogen rate and plant growth regulator

The first objective of this study was to examine the photosynthetic response of main stem leaves, positioned at various levels within the canopy, to different nitrogen (N) rates and

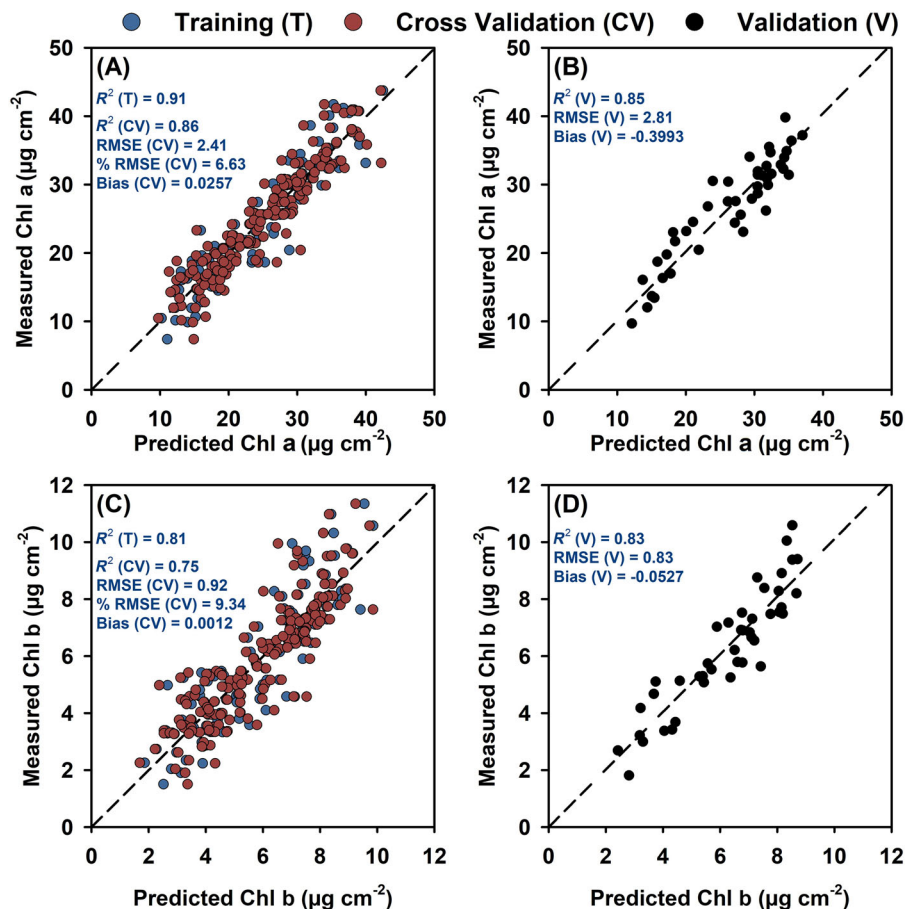


FIGURE 6 Plots of observed versus predicted values for chlorophyll a (Chl a; A and B) and chlorophyll b (Chl b; C and D) during model calibration or training (T), cross-validation (CV), and validation (V). The blue dots represent training data, red dots represent cross-validation data, and black dots represent validation data. The black dashed line represents the 1:1 line. Model performance metrics include the coefficient of determination (R^2), root mean square error (RMSE), percentage of root mean square error of prediction (%RMSEP), and model bias for both cross-validation (CV) and validation (V).

MC treatments. The net photosynthetic rate (A_N) was not significantly affected by MC, but it was significantly influenced by N rate; however, the extent of this impact varied depending on the leaf position within the canopy. For instance, A_N differed significantly between low and high N rates at the fourth and 10th leaf positions below the terminal, but it was comparable between low and control rates, as well as between control and high rates. At the seventh leaf position below the terminal, however, A_N increased significantly with each increase in N rate. Regardless of the N rate, A_N decreased with greater leaf position depth within the canopy. In our previous study (Parkash et al., 2023), a similar photosynthetic response to high and low N rates within the canopy was observed. However, that study only compared photosynthetic responses between low and high N rates. In contrast, our current study offers a more comprehensive understanding of the photosynthetic response to both N deficiency and excess in field-grown cotton. Overall, A_N was negatively affected by N deficiency but unaffected by N excess in field-grown cotton.

Stomatal conductance (g_s) consistently emerged as the dominant predictor of A_N across all canopy layers and N treatments, with correlation coefficients often exceeding 0.7 and reaching 0.95 in lower leaves under high N (Table 2 and Table S4). In contrast, intercellular CO_2 concentration (C_i) showed weak or negative correlations with A_N , with most relationships being nonsignificant. Notably, stomatal limitations were significantly lower under low N conditions compared to the control (Figure 2C). Therefore, it can be said that stomatal limitations were not major drivers of reductions in A_N under N deficiency. In our previous study (Parkash et al., 2023), we reported that stomatal limitations could not fully explain reductions in A_N under N deficiency. Similarly, in a controlled environment study, A. R. Reddy, Reddy, Padjung et al. (1996) found that g_s increased while intercellular CO_2 concentration (C_i) decreased with an increase in leaf N content in Pima cotton (*Gossypium barbadense*).

Regarding potential biochemical limitations, ETR, maximum carboxylation rate of Rubisco ($V_{c,\text{max}}$), and maximum rate of RuBP regeneration (J_{max}) were substantially lower

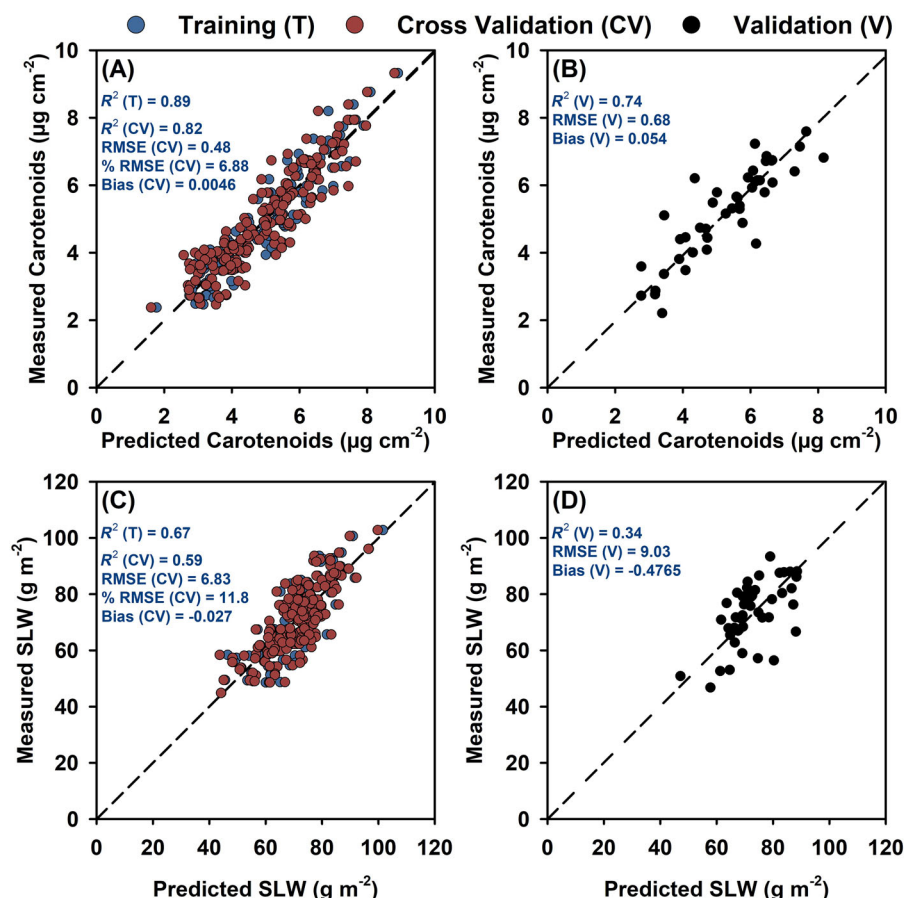


FIGURE 7 Plots of observed versus predicted values for total carotenoid content (A and B), and specific leaf weight (SLW; C and D) during model calibration or training (T), cross-validation (CV), and validation (V). The blue dots represent training data, red dots represent cross-validation data, and black dots represent validation data. The black dashed line represents the 1:1 line. Model performance metrics include the coefficient of determination (R^2), root mean square error (RMSE), percentage of root mean square error of prediction (%RMSEP), and model bias for both cross-validation (CV) and validation (V).

in the N deficiency treatment compared to the control (Figure 1B–D). These reductions were expected because N deficiency reduces the content and/or activity of key photosynthetic enzymes, leading to impaired biochemical processes (Mu & Chen, 2021; Mu et al., 2016; J. Snider, Harris, et al., 2021; Yao et al., 2015; Zhong et al., 2017). For example, Yamori et al. (2011) observed substantial reductions in cytochrome f content (an enzyme involved in electron transport) and ETR under N deficiency in rice (*Oryza sativa*). Therefore, it is possible that N deficiency might have reduced the content and/or activity of one of the enzymes (such as cytochrome f) and produced lower ETR under N deficiency in cotton. Furthermore, chlorophyll content was significantly lower in N-deficient plants, and chlorophyll plays a central role in capturing light energy and initiating the electron transport processes (G. Wang et al., 2022). Therefore, declines in chlorophyll content and accessory pigments such as carotenoids might have also contributed to observed reductions in ETR in N-deficient plants. In shaded lower leaves, light-harvesting capacity and pigment content (chlorophylls

and carotenoids) became increasingly important, as indicated by stronger correlations of A_N with pigment traits under low N conditions.

Additionally, RuBP regeneration relies on the ETR, adenosine triphosphate (ATP) synthesis, and content and/or activity of some of the Calvin cycle enzymes (Hikosaka et al., 2005; Mächler et al., 1988; Yamori et al., 2011). In our previous study (Parkash, Snider, Virk, 2024), significant reductions in ATP content were observed under N deficiency in cotton. Therefore, declines in ETR and the resultant rate of ATP synthesis could have contributed to observed declines in $J_{\max}$ under N deficiency. In the current study, considerable declines in $V_{c,\max}$ were observed in N-deficient plants. It is evident that N deficiency negatively impacts Rubisco content and also total Rubisco activity (Li et al., 2009; A. R. Reddy, Reddy, Padjung, et al., 1996). Therefore, observed declines in $V_{c,\max}$ were likely due to declines in Rubisco content and/or activity in N-deficient plants. In the current study, $V_{c,\max}$ and $J_{\max}$ were equally sensitive to N deficiency and both of these parameters decreased by an average of 32% in N-deficient

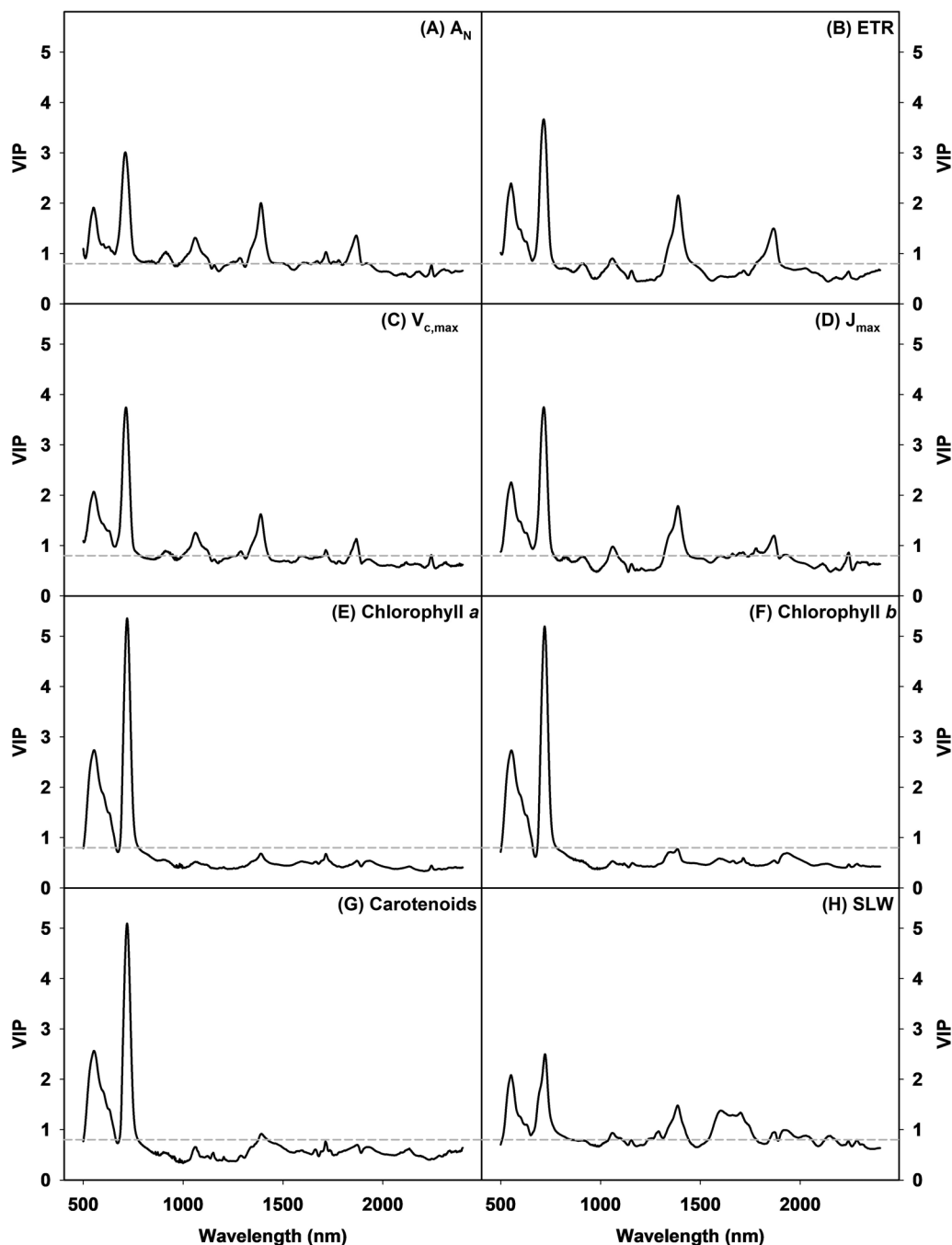


FIGURE 8 Plots showing the variable importance in projection (VIP) scores for each wavelength across the leaf spectra in partial least squares regression models for various leaf traits, including net photosynthetic rate (A_N ; A), electron transport rate (ETR; B), maximum carboxylation rate of Rubisco ($V_{c,max}$; C), maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration (J_{max} ; D), chlorophyll a (E), chlorophyll b (F), total carotenoids (G), and specific leaf weight (SLW; H). The gray dashed line represents $VIP = 0.8$.

plants compared to control plants. ETR was less negatively impacted compared to $V_{c,max}$ and J_{max} by N deficiency. These results indicate that $V_{c,max}$ and J_{max} co-limit A_N under N deficiency in field-grown cotton. Similar results were observed in our previous studies on N deficiency in field-grown cotton (Parkash et al., 2023; Parkash, Snider, Virk, 2024) and also by von Caemmerer and Farquhar (1981) in common bean (*Phaseolus vulgaris* L.) and Evans and Terashima (1988) in

spinach (*Spinacia oleracea*). Overall, Rubisco carboxylation and RuBP regeneration co-limit A_N under N deficiency in field-grown cotton.

Pairwise correlations and PCA analyses further highlight the central role of biochemical processes in regulating A_N under varying N and canopy conditions. In upper leaves (leaf 4), A_N was most strongly correlated with $V_{c,max}$ across all N levels ($r = 0.52$ – 0.83), while correlations with ETR and

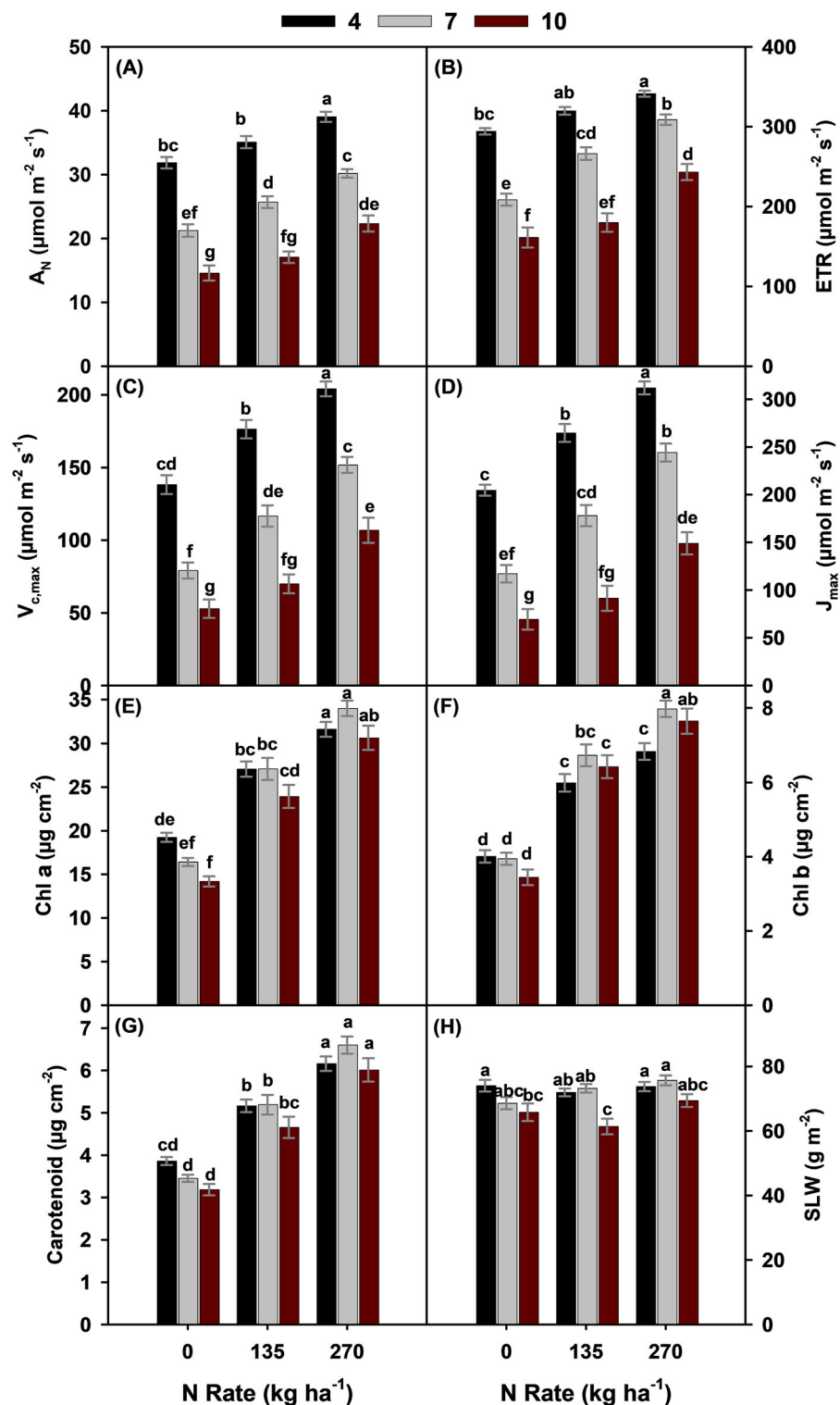


FIGURE 9 Effects of nitrogen application rate (0, 135, and 270 kg N ha⁻¹) and leaf position (fourth, seventh, and 10th leaves below the terminal) on predicted values of net photosynthetic rate (A_N ; A), electron transport rate (ETR; B), maximum rate of carboxylation ($V_{c,max}$; C), maximum rate of ribulose 1,5-bisphosphate (RuBP) regeneration (J_{max} ; D), chlorophyll a (E), chlorophyll b (F), carotenoid content (G), and specific leaf weight (SLW; H) of cotton leaves during the 2023 growing season. Bar plots represent mean $\pm$ standard error. Different lowercase letters above bars indicate statistically significant differences among treatment combinations based on Tukey's honestly significant difference (HSD) test ($p < 0.05$). Predicted values were derived from the full dataset, which included both training and validation observations.

pigments were generally weak or nonsignificant. In middle canopy leaves (leaf 7), both $V_{c,max}$ ($r = 0.70\text{--}0.88$) and J_{max} ($r = 0.66\text{--}0.80$) were strongly associated with A_N , whereas in lower leaves (leaf 10), J_{max} ($r = 0.71\text{--}0.88$) and ETR ($r = 0.69\text{--}0.75$) were dominant. PCA supported these results, showing that A_N co-varied with $V_{c,max}$ and J_{max} in upper and middle leaves and with J_{max} , ETR, and pigments in lower canopy leaves, particularly under N deficiency (Table S5). This pattern suggests a shift from biochemical control (carboxylation and RuBP regeneration) in upper leaves to combined biochemical and light-harvesting constraints in shaded lower leaves.

Although, MC-treated plants had darker green (higher chlorophyll content) and thicker leaves (higher SLW) than untreated control plants, there was no impact on the A_N . A number of other studies have also documented the increase in chlorophyll content and SLW in response to MC application in cotton (A. R. Reddy, Reddy, Hodges, 1996; Tung et al., 2018; Xu & Taylor, 1992; Zhao & Oosterhuis, 2000). However, the response of A_N to MC application varies from study to study. Some reports documented reductions in A_N (A. R. Reddy, Reddy, Hodges, 1996; Tung et al., 2018) with MC application, while others observed increases in A_N (Zhao & Oosterhuis, 2000) in response to MC application. This variability may arise from differences in cultivar, growth stage at the time of application, or environmental conditions. Another important factor may be the interaction between canopy architecture and light distribution. As shown by Gonias et al. (2012), MC can modify canopy structure by reducing plant height and leaf area index, which can alter the canopy light extinction coefficient and enhance light penetration into the mid-canopy layers. Such changes improve within-canopy light distribution and may enhance radiation use efficiency, even without altering total light interception. Therefore, the lack of change in A_N observed in our study, despite increases in chlorophyll and SLW, might be due to suboptimal canopy architecture or limited improvements in light distribution under the given conditions. This emphasizes that increased chlorophyll content alone does not guarantee higher photosynthesis unless paired with effective canopy-level light utilization (Milroy & Bange, 2003).

4.2 | Partial least squares regression

The second objective of this study was to develop PLSR-based models to predict leaf functional traits of cotton. The combination of nitrogen application rates, leaf positions, and MC strategy successfully generated substantial variation in leaf traits. This was evident from the wide ranges observed for photosynthetic parameters (A_N : $5.9\text{--}48.4\ \mu\text{mol m}^{-2}\text{ s}^{-1}$; ETR: $70\text{--}360\ \mu\text{mol m}^{-2}\text{ s}^{-1}$; $V_{c,max}$: $13\text{--}402\ \mu\text{mol m}^{-2}\text{ s}^{-1}$; J_{max} : $3\text{--}467\ \mu\text{mol m}^{-2}\text{ s}^{-1}$) and pigment concentrations (chloro-

phyll a: $7\text{--}44\ \mu\text{g cm}^{-2}$; chlorophyll b: $2\text{--}16\ \mu\text{g cm}^{-2}$; total carotenoids: $1.0\text{--}9.3\ \mu\text{g cm}^{-2}$), whereas SLW exhibited relatively low variability ($26\text{--}112\ \text{g m}^{-2}$). PLSR models were developed for a range of leaf traits, including A_N , ETR, $V_{c,max}$, J_{max} , chlorophyll a, chlorophyll b, total carotenoids, and SLW. The predictive performance of PLSR models varied across traits, with strong results for gas exchange and chlorophyll fluorescence parameters, but relatively weaker performance for SLW. However, Serbin et al. (2019) reported higher predictive accuracy ($R^2 = 0.89$; RMSE = $15.45\ \text{g m}^{-2}$) for SLW using leaf reflectance for leaves from a wide range of plant species, leaf age, and canopy positions from diverse biomes. In their study, SLW values ranged from 17 to $393\ \text{g m}^{-2}$, whereas in our study, SLW values were more constrained ($45\text{--}103\ \text{g m}^{-2}$). This limited variation in SLW for model training could be one of the key factors contributing to the lower predictive accuracy during independent validation.

Among the gas exchange and chlorophyll fluorescence traits, PLSR models for A_N and J_{max} exhibited good predictive ability during external validation. Similarly, Buchaillet et al. (2022) achieved an R^2 of 0.62 for A_N using leaf spectral reflectance-based PLSR model in peanut. In contrast, Silva-Perez et al. (2017) reported lower accuracy ($R^2 = 0.49$) for leaf reflectance-based prediction of A_N in wheat. However, Rahman et al. (2025) achieved significantly higher accuracy ($R^2 = 0.945$) for A_N using a graph convolutional network (GCN)-based model (Plant-GCN). It is well known that g_s plays a crucial role in regulating A_N , and g_s can fluctuate rapidly in response to changing environmental conditions, such as light intensity, temperature, and vapor pressure deficit. Since leaf spectra primarily predict A_N in the absence of stomatal limitations, these models do not fully account for real-time physiological constraints that significantly impact ambient photosynthesis (Doughty et al., 2011). Therefore, very high accuracy in predicting A_N solely from hyperspectral reflectance is generally not expected (Silva-Perez et al., 2017). However, previous studies have reported strong to very high predictive accuracy for J_{max} (Dechant et al., 2017; Fu et al., 2020; Serbin et al., 2012; Silva-Perez et al., 2017).

In contrast, $V_{c,max}$ and ETR exhibited very high predictive accuracy. Although direct comparisons with other studies should be made cautiously due to differences in species, experimental conditions, and datasets, the predictive performance for $V_{c,max}$ in our study is within or exceeds the range of previously reported PLSR models (Ainsworth et al., 2014; Dechant et al., 2017; Lamour et al., 2021; Meacham-Hensold et al., 2019; Yendrek et al., 2016). These results further support the idea that gas exchange traits with strong biochemical underpinnings tend to yield more accurate predictions from hyperspectral reflectance data.

For all gas exchange and chlorophyll fluorescence traits, five wavelengths made the most significant contributions (VIP > 0.8) to trait prediction, and these wavelengths were

consistent across all traits. These included 553 ± 1 nm in the visible range (450–700 nm), 714 ± 4 nm, 1060 ± 2 nm, and 1389 ± 1 nm in the NIR range, and 1866 ± 2 nm in the short-wave infrared range. Similarly, Kothari et al. (2023) reported that distinct peaks in VIP scores at the green hump (550–560 nm), the inflection point of the red edge (710–720 nm), and bands at 1390 and 1880 nm across multiple leaf traits in different plant functional groups and ecosystems. In the current study, VIP scores were highest for 714 ± 4 nm, followed by 553 ± 1 nm, 1389 ± 1 nm, 1866 ± 2 nm, and 1060 ± 2 nm among the major contributing wavelengths in trait prediction.

Additionally, Meacham-Hensold et al. (2020) compared PLSR models trained using different spectral ranges (500–900 nm, 500–1700 nm, and 500–2400 nm) and found that expanding the spectral range did not necessarily improve the predictability of $V_{c,max}$ and J_{max} . In the present study, we found that certain spectral bands contributed more significantly to model accuracy suggesting that the full spectrum (500–2400 nm) may not be required to obtain high-quality PLSR reflectance models. The wavelengths with the highest VIP scores (e.g., 714 ± 4 nm, 553 ± 1 nm, 1389 ± 1 nm, 1866 ± 2 nm, and 1060 ± 2 nm) were key contributors across multiple traits. This highlights two points, many of the measured leaf traits are likely influenced by similar underpinning physiology and the importance of specific regions in the spectrum rather than the overall range.

In the current study, PLSR models also demonstrated very high predictive accuracy for leaf pigment content traits, including chlorophyll a, chlorophyll b, and total carotenoid content. The predictive accuracy of these models is comparable to or higher than those previously reported for other plant species (Kothari et al., 2023; Meacham-Hensold et al., 2020; Montes et al., 2022; Yendrek et al., 2016). For chlorophyll a, chlorophyll b, and total carotenoid content, multiple wavelengths contributed to trait prediction, with two wavelengths exhibiting particularly strong contributions (VIP > 0.8): 553 ± 1 nm in the visible range (450–700 nm) and 714 ± 4 nm at the red edge transition between the visible and NIR regions. While spectral boundaries between VIS and NIR are not always distinct, 714 nm aligns with the red-edge inflection point, a critical region where reflectance sharply increases due to reduced chlorophyll absorption and increased leaf scattering.

The strong VIP loadings at 553 nm coincide with the “green hump”—a region of minimal chlorophyll absorption but increased reflectance, likely contributing to the spectral signal for pigment estimation. Similarly, the 714 nm peak aligns with the red-edge transition, where spectral derivatives are highly sensitive to changes in chlorophyll content and leaf structure. This is consistent with findings from Kothari et al. (2023), who reported VIP peaks at 550–560 nm (green hump) and 710–720 nm (red-edge inflection) as key wavelengths for predicting leaf pigment content. The spectral

region near 1385–1400 nm is associated with absorption features arising from hydroxyl (OH) stretching vibrations, while 1860–1900 nm corresponds to the long-wave shoulder of the strong water absorption feature centered near 1900 nm. The feature around 1400 nm, caused by the first overtone of the OH stretch, and the feature near 1900 nm, resulting from a combination of the H–O–H bend and OH stretch, are indicative of molecular water within plant tissues. The simultaneous presence of both 1400 and 1900 nm features reflects free and bound water content, whereas the presence of the 1400 nm feature alone suggests the presence of hydroxyl groups only (Murphy, 2015). These water-related features are physiologically important, as leaf water status strongly influences photosynthetic capacity and other functional traits, making these wavelengths particularly valuable for trait prediction models. In addition, the region near 1060–1100 nm is influenced by C–H stretching and deformation, which is characteristic of biochemical constituents such as oils, proteins, and lignin due to their hydrocarbon content (Curran, 1989). These spectral signatures provide biochemical insight into key physiological traits and help explain the predictive strength of these wavelengths in modeling photosynthetic function.

Independent validation of PLSR models confirms that hyperspectral reflectance-based approaches provide a powerful, nondestructive tool for assessing leaf photosynthetic and pigment traits (Burnett et al., 2021; Silva-Perez et al., 2017). The strong alignment between predicted and observed values across all measured traits underscores the robustness and reliability of the PLSR model in capturing treatment-related physiological and biochemical responses. Accurate reproduction of nitrogen-induced trends and vertical canopy gradients in A_N , ETR, $V_{c,max}$, and J_{max} demonstrates the model's sensitivity to leaf-level variations in photosynthetic capacity. Similarly, precise predictions of chlorophyll pigments, carotenoids, and SLW confirm that spectral data effectively captured both biochemical and structural traits. The model also differentiated management effects, such as the lower pigment content and SLW in untreated MC plots, with predicted means statistically indistinguishable from observations ($p > 0.27$). Using a single sensor, multiple traits can be estimated simultaneously, enabling rapid, high-throughput screening of large crop populations (Lamour et al., 2021; Silva-Perez et al., 2017). Additionally, this method allows for the measurement of a larger number of leaves within the canopy, providing a more accurate estimate of whole-canopy photosynthesis (Earl & Davis, 2003). This capability enhances phenotyping efficiency, allowing breeders to identify genotypes with superior photosynthetic performance, a key trait for improving crop productivity (Bailey-Serres et al., 2019).

Furthermore, the noninvasive nature of this technology enables continuous monitoring throughout the growing season, facilitating data-driven selection and precision

agriculture practices. Ultimately, integrating hyperspectral reflectance-based PLSR models into breeding programs can accelerate genetic gains, improve sustainability, and drive the development of high-yielding, climate-resilient cotton varieties.

PLSR has proven to be an effective approach for reflectance-based modeling of leaf traits, and previous studies (e.g., Bagherian et al. [2023]; Buchailot et al. [2022]; Fu et al. [2019]; Rahman et al. [2025]) have explored machine learning (ML) techniques such as random forests and deep learning for further refinement. While standard ML techniques may not always offer substantial improvements over PLSR, future work could focus on optimizing existing models, exploring transfer learning, or contributing to the development of universal reflectance models (e.g., GSTI; GitHub—plantphys/gsti) to enhance cross-study applicability. Additionally, incorporating diverse genotypes, along with multi-season and multi-location validation, could further improve model robustness for large-scale phenotyping applications, ensuring broader applicability across different environmental conditions and genetic backgrounds.

5 | CONCLUSIONS

This study investigated the effects of nitrogen (N) rate, MC strategy, and leaf position on physiological traits. The results showed that net photosynthetic rate and other physiological traits were not affected by the three-way interaction of N rate, MC strategy, and leaf position. However, N rate interacted with leaf position for some traits, while MC application influenced only leaf pigments and SLW. Reductions in A_N under N deficiency were primarily driven by declines in the maximum rate of Rubisco carboxylation and RuBP regeneration, whereas high N had no significant effect. These findings provide insights into the physiological responses of cotton to nutrient availability and growth regulators, which could aid in optimizing nutrient management and growth regulation strategies for improving crop productivity.

Additionally, this study demonstrates the potential of PLSR models for predicting key photosynthetic and pigment-related leaf traits in cotton using hyperspectral reflectance data. The models encompassed a broad range of trait variation and achieved good to very high predictive accuracy for most parameters, with the exception of SLW, which showed lower accuracy likely due to limited variability in the training dataset. This study further provides evidence that key physiological and biochemical traits associated with stress responses can be accurately estimated using PLSR models developed from hyperspectral leaf reflectance data. This approach offers a high-throughput, nondestructive method for phenotyping, enabling efficient screening of stress-resilient genotypes.

AUTHOR CONTRIBUTIONS

Ved Parkash: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing—original draft; writing—review and editing. **John L. Snider:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing—review and editing. **Christopher M. Montes:** Conceptualization; data curation; formal analysis; methodology; visualization; writing—review and editing. **Elizabeth A. Ainsworth:** Conceptualization; methodology; supervision; writing—review and editing. **Alvaro Sanz-Saez:** Conceptualization; methodology; resources; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

PLSR model coefficient can be download from Dryad (<https://doi.org/10.5061/dryad.g4f4qrg32>). The other data supporting the findings of this study are available from the corresponding author upon request.

ORCID

Ved Parkash  <https://orcid.org/0000-0002-0509-0346>

John L. Snider  <https://orcid.org/0000-0001-6535-7711>

Elizabeth A. Ainsworth  <https://orcid.org/0000-0002-3199-8999>

REFERENCES

- Ainsworth, E. A., Serbin, S. P., Skoneczka, J. A., & Townsend, P. A. (2014). Using leaf optical properties to detect ozone effects on foliar biochemistry. *Photosynthesis Research*, 119(1), 65–76. <https://doi.org/10.1007/s11120-013-9837-y>
- Bagherian, K., Bidese-Puhl, R., Bao, Y., Zhang, Q., Sanz-Saez, A., Dang, P. M., Lamb, M. C., & Chen, C. (2023). Phenotyping agronomic and physiological traits in peanut under mid-season drought stress using UAV-based hyperspectral imaging and machine learning. *The Plant Phenome Journal*, 6(1), e20081. <https://doi.org/10.1002/ppj2.20081>
- Bailey-Serres, J., Parker, J. E., Ainsworth, E. A., Oldroyd, G. E. D., & Schroeder, J. I. (2019). Genetic strategies for improving crop yields. *Nature*, 575(7781), 109–118. <https://doi.org/10.1038/s41586-019-1679-0>
- Bednarz, C. W., Harris, G. H., & Shurley, W. D. (2000). Agronomic and economic analyses of cotton starter fertilizers. *Agronomy Journal*, 92(4), 766–771. <https://doi.org/10.2134/agronj2000.924766x>

- Boote, K. J., & Sinclair, T. R. (2006). Crop physiology: Significant discoveries and our changing perspective on research. *Crop Science*, 46(5), 2270–2277. <https://doi.org/10.2135/cropsci2006.01.0039gas>
- Buchailot, M. L., Soba, D., Shu, T., Liu, J., Aranjuelo, I., Araus, J. L., Runion, G. B., Prior, S. A., Kefauver, S. C., & Sanz-Saez, A. (2022). Estimating peanut and soybean photosynthetic traits using leaf spectral reflectance and advance regression models. *Planta*, 255(4), Article 93. <https://doi.org/10.1007/s00425-022-03867-6>
- Burnett, A. C., Anderson, J., Davidson, K. J., Ely, K. S., Lamour, J., Li, Q., Morrison, B. D., Yang, D., Rogers, A., & Serbin, S. P. (2021). A best-practice guide to predicting plant traits from leaf-level hyperspectral data using partial least squares regression. *Journal of Experimental Botany*, 72(18), 6175–6189. <https://doi.org/10.1093/jxb/erab295>
- Campbell, B. T., Chee, P. W., Lubbers, E., Bowman, D. T., Meredith, W. R., Johnson, J., & Fraser, D. E. (2011). Genetic improvement of the Pee Dee Cotton Germplasm Collection following seventy years of plant breeding. *Crop Science*, 51(3), 955–968. <https://doi.org/10.2135/cropsci2010.09.0545>
- Chastain, D. R., Snider, J. L., Collins, G. D., Perry, C. D., Whitaker, J., & Byrd, S. A. (2014). Water deficit in field-grown *Gossypium hirsutum* primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. *Journal of Plant Physiology*, 171(17), 1576–1585. <https://doi.org/10.1016/j.jplph.2014.07.014>
- Chastain, D. R., Snider, J. L., Singh, B., & Virk, G. (2024). Drought response modelling of leaf photosynthetic parameters in two *Gossypium* species. *Journal of Agronomy and Crop Science*, 210(3), e12709. <https://doi.org/10.1111/jac.12709>
- Conaty, W. C., & Constable, G. A. (2020). Factors responsible for yield improvement in new *Gossypium hirsutum* L. cotton cultivars. *Field Crops Research*, 250, 107780. <https://doi.org/10.1016/j.fcr.2020.107780>
- Constable, G. A., & Bange, M. P. (2015). The yield potential of cotton (*Gossypium hirsutum* L.). *Field Crops Research*, 182, 98–106. <https://doi.org/10.1016/j.fcr.2015.07.017>
- Coursolle, C., Otis Prud'homme, G., Lamothe, M., & Isabel, N. (2019). Measuring rapid A–Ci curves in boreal conifers: Black spruce and balsam fir. *Frontiers in Plant Science*, 10, 1276. <https://doi.org/10.3389/fpls.2019.01276>
- Curran, P. J. (1989). Remote sensing of foliar chemistry. *Remote Sensing of Environment*, 30(3), 271–278. [https://doi.org/10.1016/0034-4257\(89\)90069-2](https://doi.org/10.1016/0034-4257(89)90069-2)
- Dechant, B., Cuntz, M., Vohland, M., Schulz, E., & Doktor, D. (2017). Estimation of photosynthesis traits from leaf reflectance spectra: Correlation to nitrogen content as the dominant mechanism. *Remote Sensing of Environment*, 196, 279–292. <https://doi.org/10.1016/j.rse.2017.05.019>
- De Souza, A. P., Burgess, S. J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K. K., & Long, S. P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science*, 377(6608), 851–854. <https://doi.org/10.1126/science.adc9831>
- Dillenburger, L. R., Sullivan, J. H., & Teramura, A. H. (1995). Leaf expansion and development of photosynthetic capacity and pigments in *Liquidambar styraciflua* (hamamelidaceae)—Effects of UV-B radiation. *American Journal of Botany*, 82(7), 878–885.
- Doughty, C. E., Asner, G. P., & Martin, R. E. (2011). Predicting tropical plant physiology from leaf and canopy spectroscopy. *Oecologia*, 165(2), 289–299. <https://doi.org/10.1007/s00442-010-1800-4>
- Duursma, R. A. (2015). Plantecophys—An R package for analysing and modelling leaf gas exchange data. *PLoS One*, 10(11), e0143346. <https://doi.org/10.1371/journal.pone.0143346>
- Earl, H. J., & Davis, R. F. (2003). Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. *Agronomy Journal*, 95(3), 688–696. <https://doi.org/10.2134/agronj2003.6880>
- Earl, H. J., & Ennahli, S. (2004). Estimating photosynthetic electron transport via chlorophyll fluorometry without photosystem II light saturation. *Photosynthesis Research*, 82(2), 177–186. <https://doi.org/10.1007/s11120-004-1454-3>
- Evans, J. R. (2013). Improving photosynthesis. *Plant Physiology*, 162(4), 1780–1793. <https://doi.org/10.1104/pp.113.219006>
- Evans, J. R., & Terashima, I. (1988). Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant and Cell Physiology*, 29(1), 157–165.
- Ferguson, J. N., Jithesh, T., Lawson, T., & Kromdijk, J. (2023). Excised leaves show limited and species-specific effects on photosynthetic parameters across crop functional types. *Journal of Experimental Botany*, 74(21), 6662–6676. <https://doi.org/10.1093/jxb/erad319>
- Fu, P., Meacham-Hensold, K., Guan, K., & Bernacchi, C. J. (2019). Hyperspectral leaf reflectance as proxy for photosynthetic capacities: An ensemble approach based on multiple machine learning algorithms. *Frontiers in Plant Science*, 10, 730. <https://doi.org/10.3389/fpls.2019.00730>
- Fu, P., Meacham-Hensold, K., Guan, K., Wu, J., & Bernacchi, C. (2020). Estimating photosynthetic traits from reflectance spectra: A synthesis of spectral indices, numerical inversion, and partial least square regression. *Plant, Cell & Environment*, 43(5), 1241–1258.
- Fu, P., Montes, C. M., Siebers, M. H., Gomez-Casanovas, N., McGrath, J. M., Ainsworth, E. A., & Bernacchi, C. J. (2022). Advances in field-based high-throughput photosynthetic phenotyping. *Journal of Experimental Botany*, 73(10), 3157–3172. <https://doi.org/10.1093/jxb/erac077>
- Gonias, E. D., Oosterhuis, D. M., & Bibi, A. C. (2012). Cotton radiation use efficiency response to plant growth regulators. *The Journal of Agricultural Science*, 150(5), 595–602. <https://doi.org/10.1017/S0021859611000803>
- Gu, L., Pallardy, S. G., Tu, K., Law, B. E., & Wullschlegel, S. D. (2010). Reliable estimation of biochemical parameters from C3 leaf photosynthesis–intercellular carbon dioxide response curves. *Plant, Cell & Environment*, 33(11), 1852–1874.
- Hake, K., Kerby, T., McCarty, W., O'Neal, D., & Supak, J. (1991). Physiology of PIX. *Cotton Physiology Today*, 2(6), 4.
- Hand, C., Culpepper, S., Harris, G., Kemeraite, B., Liu, Y., Perry, C., Porter, W., Roberts, P., Smith, A., Virk, S., & Bag, S. (2022). *University of Georgia cotton production guide*. University of Georgia.
- Harley, P. C., Thomas, R. B., Reynolds, J. F., & Strain, B. R. (1992). Modelling photosynthesis of cotton grown in elevated CO₂. *Plant, Cell & Environment*, 15(3), 271–282.
- Helsen, K., Bassi, L., Feilhauer, H., Kattenborn, T., Matsushima, H., Van Cleemput, E., Somers, B., & Honnay, O. (2021). Evaluating different methods for retrieving intraspecific leaf trait variation from hyperspectral leaf reflectance. *Ecological Indicators*, 130, 108111. <https://doi.org/10.1016/j.ecolind.2021.108111>
- Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., & Onoda, Y. (2005). Temperature acclimation of photosynthesis: Mechanisms

- involved in the changes in temperature dependence of photosynthetic rate. *Journal of Experimental Botany*, 57(2), 291–302. <https://doi.org/10.1093/jxb/erj049>
- Ji, F., Li, F., Hao, D., Shiklomanov, A. N., Yang, X., Townsend, P. A., Dashti, H., Nakaji, T., Kovach, K. R., Liu, H., Luo, M., & Chen, M. (2024). Unveiling the transferability of PLSR models for leaf trait estimation: Lessons from a comprehensive analysis with a novel global dataset. *New Phytologist*, 243(1), 111–131. <https://doi.org/10.1111/nph.19807>
- Jin, J., Wang, Q., & Song, G. (2022). Selecting informative bands for partial least squares regressions improves their goodness-of-fits to estimate leaf photosynthetic parameters from hyperspectral data. *Photosynthesis Research*, 151(1), 71–82. <https://doi.org/10.1007/s1120-021-00873-9>
- Kaur, N., Snider, J. L., Parkash, V., Paterson, A. H., & Tishchenko, V. (2023). Genotypic variation in growth, single leaf physiology, and acclimation potential of thylakoid processes in cotton exposed to high temperature extremes. *Environmental and Experimental Botany*, 215, 105512. <https://doi.org/10.1016/j.envexpbot.2023.105512>
- Kaur, N., Snider, J. L., Paterson, A. H., Virk, G., Parkash, V., Roberts, P., & Li, C. (2024). Genotypic variation in functional contributors to yield for a diverse collection of field-grown cotton. *Crop Science*, 64(3), 1846–1861. <https://doi.org/10.1002/csc2.21232>
- Kerby, T. A. (1985). Cotton response to mepiquat chloride. *Agronomy Journal*, 77(4), 515–518. <https://doi.org/10.2134/agronj1985.00021962007700040003x>
- Koester, R. P., Nohl, B. M., Diers, B. W., & Ainsworth, E. A. (2016). Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. *Plant, Cell & Environment*, 39(5), 1058–1067.
- Kothari, S., Beauchamp-Rioux, R., Blanchard, F., Crofts, A. L., Girard, A., Guilbeault-Mayers, X., Hacker, P. W., Pardo, J., Schweiger, A. K., Demers-Thibeault, S., Bruneau, A., Coops, N. C., Kalacska, M., Vellend, M., & Laliberté, E. (2023). Predicting leaf traits across functional groups using reflectance spectroscopy. *New Phytologist*, 238(2), 549–566. <https://doi.org/10.1111/nph.18713>
- Lamour, J., Davidson, K. J., Ely, K. S., Anderson, J. A., Rogers, A., Wu, J., & Serbin, S. P. (2021). Rapid estimation of photosynthetic leaf traits of tropical plants in diverse environmental conditions using reflectance spectroscopy. *PLoS One*, 16(10), e0258791. <https://doi.org/10.1371/journal.pone.0258791>
- Lei, Z., Liu, F., Wright, I. J., Carriqui, M., Niinemets, Ü., Han, J., Jia, M., Atwell, B. J., Cai, X., Zhang, W., Zhou, Z., & Zhang, Y. (2021). Comparisons of photosynthetic and anatomical traits between wild and domesticated cotton. *Journal of Experimental Botany*, 73(3), 873–885. <https://doi.org/10.1093/jxb/erab293>
- Li, Y., Gao, Y., Xu, X., Shen, Q., & Guo, S. (2009). Light-saturated photosynthetic rate in high-nitrogen rice (*Oryza sativa* L.) leaves is related to chloroplastic CO₂ concentration. *Journal of Experimental Botany*, 60(8), 2351–2360. <https://doi.org/10.1093/jxb/erp127>
- Lichtenthaler, H. K., & Wellburn, A. R. (1983). *Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents*. Portland Press Ltd. <https://doi.org/10.1042/bst0110591>
- Long, S. P., Marshall-Colon, A., & Zhu, X.-G. (2015). Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell*, 161(1), 56–66. <https://doi.org/10.1016/j.cell.2015.03.019>
- Mächler, F., Oberson, A., Grub, A., & Nösberger, J. (1988). Regulation of photosynthesis in nitrogen deficient wheat seedlings. *Plant Physiology*, 87(1), 46–49. <https://doi.org/10.1104/pp.87.1.46>
- Meacham-Hensold, K., Fu, P., Wu, J., Serbin, S., Montes, C. M., Ainsworth, E., Guan, K., Dracup, E., Pederson, T., Driever, S., & Bernacchi, C. (2020). Plot-level rapid screening for photosynthetic parameters using proximal hyperspectral imaging. *Journal of Experimental Botany*, 71(7), 2312–2328. <https://doi.org/10.1093/jxb/eraa068>
- Meacham-Hensold, K., Montes, C. M., Wu, J., Guan, K., Fu, P., Ainsworth, E. A., Pederson, T., Moore, C. E., Brown, K. L., Raines, C., & Bernacchi, C. J. (2019). High-throughput field phenotyping using hyperspectral reflectance and partial least squares regression (PLSR) reveals genetic modifications to photosynthetic capacity. *Remote Sensing of Environment*, 231, 111176. <https://doi.org/10.1016/j.rse.2019.04.029>
- Meeks, C. D., Snider, J. L., Babb-Hartman, M. E., & Barnes, T. L. (2019). Evaluating the mechanisms of photosynthetic inhibition under growth-limiting, early-season water deficit stress in cotton. *Crop Science*, 59(3), 1144–1154. <https://doi.org/10.2135/cropsci2018.07.0432>
- Mevik, B.-H., & Wehrens, R. (2007). The pls package: Principal component and partial least squares regression in R. *Journal of Statistical Software*, 18(2), 1–23. <https://doi.org/10.18637/jss.v018.i02>
- Milroy, S. P., & Bange, M. P. (2003). Nitrogen and light responses of cotton photosynthesis and implications for crop growth. *Crop Science*, 43(3), 904–913. <https://doi.org/10.2135/cropsci2003.9040>
- Montes, C. M., Fox, C., Sanz-Sáez, Á., Serbin, S. P., Kumagai, E., Krause, M. D., Xavier, A., Specht, J. E., Beavis, W. D., Bernacchi, C. J., Diers, B. W., & Ainsworth, E. A. (2022). High-throughput characterization, correlation, and mapping of leaf photosynthetic and functional traits in the soybean (*Glycine max*) nested association mapping population. *Genetics*, 221(2), iyac065. <https://doi.org/10.1093/genetics/iyac065>
- Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G. (2016). Within-leaf nitrogen allocation in adaptation to low nitrogen supply in maize during grain-filling stage. *Frontiers in Plant Science*, 7, 699.
- Mu, X., & Chen, Y. (2021). The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry*, 158, 76–82. <https://doi.org/10.1016/j.plaphy.2020.11.019>
- Murphy, R. J. (2015). Evaluating simple proxy measures for estimating depth of the ~1900 nm water absorption feature from hyperspectral data acquired under natural illumination. *Remote Sensing of Environment*, 166, 22–33. <https://doi.org/10.1016/j.rse.2015.05.029>
- Oosterhuis, D. M. (1990). Growth and development of a cotton plant. In W. N. Miley & D. M. Oosterhuis (Eds.) *Nitrogen nutrition of cotton: Practical issues* (pp. 1–24) American Society of Agronomy.
- Parkash, V., Snider, J., Awori Kelvin, J., Katta Jhansy, R., Pilon, C., & Tishchenko, V. (2024). Distinguishing high daytime from nighttime temperature effects during early vegetative growth in cotton. *Journal of Agronomy and Crop Science*, 210(5), e12757. <https://doi.org/10.1111/jac.12757>
- Parkash, V., Snider, J. L., Sintim, H. Y., Hand, L. C., Virk, G., & Pokhrel, A. (2023). Differential sensitivities of photosynthetic processes and carbon loss mechanisms govern N-induced variation in net carbon assimilation rate for field-grown cotton. *Journal of Experimental Botany*, 74(8), 2638–2652. <https://doi.org/10.1093/jxb/erad038>
- Parkash, V., Snider, J. L., & Virk, G. (2024). Interactive effects of water deficit and nitrogen deficiency on photosynthesis, its underlying

- component processes, and carbon loss processes in cotton. *Crop Science*, 64(6), 3480–3501. <https://doi.org/10.1002/csc2.21328>
- Parkash, V., Snider, J. L., Virk, G., Dhillon, K. K., & Lee, J. M. (2024). Diffusional and biochemical limitations to photosynthesis under water deficit for field-grown cotton. *Physiologia Plantarum*, 176(2), e14281. <https://doi.org/10.1111/pp1.14281>
- Pilon, C., Snider, J. L., Sobolev, V., Chastain, D. R., Sorensen, R. B., Meeks, C. D., Massa, A. N., Walk, T., Singh, B., & Earl, H. J. (2018). Assessing stomatal and non-stomatal limitations to carbon assimilation under progressive drought in peanut (*Arachis hypogaea* L.). *Journal of Plant Physiology*, 231, 124–134. <https://doi.org/10.1016/j.jplph.2018.09.007>
- Radin, J. W., & Ackerson, R. C. (1981). Water relations of cotton plants under nitrogen deficiency: III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. *Plant Physiology*, 67(1), 115–119. <https://doi.org/10.1104/pp.67.1.115>
- Rahman, M. H., Busby, S., Hanif, S., Maruf, M. M., Ahmad, F., Ru, S., Sanz-Saez, A., Zheng, J., & Rehman, T. U. (2025). A graph convolutional network approach for hyperspectral image analysis of blueberries physiological traits under drought stress. *Smart Agricultural Technology*, 10, 100743. <https://doi.org/10.1016/j.atech.2024.100743>
- Reddy, A. R., Reddy, K. R., & Hodges, H. F. (1996). Mepiquat chloride (PIX)-induced changes in photosynthesis and growth of cotton. *Plant Growth Regulation*, 20(3), 179–183. <https://doi.org/10.1007/BF00043305>
- Reddy, A. R., Reddy, K. R., Padjung, R., & Hodges, H. F. (1996). Nitrogen nutrition and photosynthesis in leaves of Pima cotton. *Journal of Plant Nutrition*, 19(5), 755–770. <https://doi.org/10.1080/01904169609365158>
- Reddy, V. R., Baker, D. N., & Hodges, H. F. (1990). Temperature and mepiquat chloride effects on cotton canopy architecture. *Agronomy Journal*, 82(2), 190–195. <https://doi.org/10.2134/agronj1990.00021962008200020004x>
- Salvucci, M. E., & Crafts-Brandner, S. J. (2004). Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. *Plant Physiology*, 134(4), 1460–1470. <https://doi.org/10.1104/pp.103.038323>
- Scafaro, A. P., Posch, B. C., Evans, J. R., Farquhar, G. D., & Atkin, O. K. (2023). Rubisco deactivation and chloroplast electron transport rates co-limit photosynthesis above optimal leaf temperature in terrestrial plants. *Nature Communications*, 14(1), Article 2820. <https://doi.org/10.1038/s41467-023-38496-4>
- Serbin, S. P., Dillaway, D. N., Kruger, E. L., & Townsend, P. A. (2012). Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of Experimental Botany*, 63(1), 489–502. <https://doi.org/10.1093/jxb/err294>
- Serbin, S. P., Singh, A., McNeil, B. E., Kingdon, C. C., & Townsend, P. A. (2014). Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications*, 24(7), 1651–1669. <https://doi.org/10.1890/13-2110.1>
- Serbin, S. P., Wu, J., Ely, K. S., Kruger, E. L., Townsend, P. A., Meng, R., Wolfe, B. T., Chlus, A., Wang, Z., & Rogers, A. (2019). From the arctic to the tropics: Multi biome prediction of leaf mass per area using leaf reflectance. *New Phytologist*, 224(4), 1557–1568. <https://doi.org/10.1111/nph.16123>
- Silva-Perez, V., Molero, G., Serbin, S. P., Condon, A. G., Reynolds, M. P., Furbank, R. T., & Evans, J. R. (2017). Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *Journal of Experimental Botany*, 69(3), 483–496. <https://doi.org/10.1093/jxb/erx421>
- Sinclair, T., & Rufty, T. W. (2022). Photosynthesis and yield. In *Bringing skepticism to crop science* (pp. 11–18). Springer International Publishing.
- Sinclair, T. R., Rufty, T. W., & Lewis, R. S. (2019). Increasing photosynthesis: Unlikely solution for world food problem. *Trends in Plant Science*, 24(11), 1032–1039. <https://doi.org/10.1016/j.tplants.2019.07.008>
- Singh, S. K., Badgujar, G., Reddy, V. R., Fleisher, D. H., & Bunce, J. A. (2013). Carbon dioxide diffusion across stomata and mesophyll and photo-biochemical processes as affected by growth CO₂ and phosphorus nutrition in cotton. *Journal of Plant Physiology*, 170(9), 801–813. <https://doi.org/10.1016/j.jplph.2013.01.001>
- Snider, J., Bange, M., & Heitholt, J. (2021). Cotton. In V. O. Sadras & D. F. Calderini (Eds.) *Crop physiology case histories for major crops* (pp. 714–746). Academic Press.
- Snider, J., Harris, G., Roberts, P., Meeks, C., Chastain, D., Bange, M., & Virk, G. (2021). Cotton physiological and agronomic response to nitrogen application rate. *Field Crops Research*, 270, 108194. <https://doi.org/10.1016/j.fcr.2021.108194>
- Snider, J. L., Collins, G. D., Whitaker, J., Chapman, K. D., & Horn, P. (2016). The impact of seed size and chemical composition on seedling vigor, yield, and fiber quality of cotton in five production environments. *Field Crops Research*, 193, 186–195. <https://doi.org/10.1016/j.fcr.2016.05.002>
- Snider, J. L., Pilon, C., Hu, W., Wang, H. M., Tishchenko, V., Slaton, W., Chastain, D., & Parkash, V. (2022). Net photosynthesis acclimates to low growth temperature in cotton seedlings by shifting temperature thresholds for photosynthetic component processes and respiration. *Environmental and Experimental Botany*, 196. <https://doi.org/10.1016/j.envexpbot.2022.104816>
- Stinziano, J. R., Morgan, P. B., Lynch, D. J., Saathoff, A. J., McDermitt, D. K., & Hanson, D. T. (2017). The rapid A–Ci response: Photosynthesis in the phenomic era. *Plant, Cell & Environment*, 40(8), 1256–1262.
- Tung, S. A., Huang, Y., Ali, S., Hafeez, A., Shah, A. N., Song, X., Ma, X., Luo, D., & Yang, G. (2018). Mepiquat chloride application does not favor leaf photosynthesis and carbohydrate metabolism as well as lint yield in late-planted cotton at high plant density. *Field Crops Research*, 221, 108–118. <https://doi.org/10.1016/j.fcr.2018.02.027>
- United States Department of Agriculture NASS. (2023). Georgia county estimates: Cotton 2022–2023. https://www.nass.usda.gov/Statistics_by_State/Georgia/Publications/County_Estimates/2023/GACotton2023.pdf
- Virk, G., Snider, J. L., & Bourland, F. (2023). Genotypic and environmental contributions to lint yield, yield components, and fiber quality in upland cotton from Arkansas variety trials over a 19-year period. *Crop Science*, 63(3), 1284–1299. <https://doi.org/10.1002/csc2.20948>
- Virk, G., Snider, J. L., Chee, P., Virk, S., Parkash, V., Kaur, N., & Jones, D. (2023). Physiological contributors to yield in advanced cotton breeding lines. *Crop Science*, 63(6), 3264–3276. <https://doi.org/10.1002/csc2.21082>
- Virk, G., Snider, J. L., & Pilon, C. (2019). Physiological contributors to early season whole-crop vigor in cotton. *Crop Science*, 59(6), 2774–2783. <https://doi.org/10.2135/cropsci2019.03.0176>

- Virk, G., Snider, J. L., & Pilon, C. (2020). Associations between first true leaf physiology and seedling vigor in cotton under different field conditions. *Crop Science*, 60(1), 404–418. <https://doi.org/10.1002/csc2.20097>
- von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 153(4), 376–387. <https://doi.org/10.1007/BF00384257>
- Walker, B. J., & Cousins, A. B. (2013). Influence of temperature on measurements of the CO₂ compensation point: Differences between the Laik and O₂-exchange methods. *Journal of Experimental Botany*, 64(7), 1893–1905. <https://doi.org/10.1093/jxb/ert058>
- Wang, G., Zeng, F., Song, P., Sun, B., Wang, Q., & Wang, J. (2022). Effects of reduced chlorophyll content on photosystem functions and photosynthetic electron transport rate in rice leaves. *Journal of Plant Physiology*, 272, 153669. <https://doi.org/10.1016/j.jplph.2022.153669>
- Wang, R., Qin, X., Pan, H., Li, D., Xiao, X., Jin, Y., Wang, Y., & Liang, H. (2025). Assessing the effects of drought stress on photosynthetic performance and physiological resistance in camphor seedling leaves. *PLoS One*, 20(1), e0313316. <https://doi.org/10.1371/journal.pone.0313316>
- Wang, Z., Sun, Z., Liu, N., Geng, S., Wen, M., Zhang, H., & Yang, L. (2025). Characterizing key foliar functional traits of subtropical evergreen forests in South China using leaf and UAV-based spectroscopy. *Computers and Electronics in Agriculture*, 233, 110178. <https://doi.org/10.1016/j.compag.2025.110178>
- Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: A basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, 58(2), 109–130. [https://doi.org/10.1016/S0169-7439\(01\)00155-1](https://doi.org/10.1016/S0169-7439(01)00155-1)
- Xu, X., & Taylor, H. M. (1992). Increase in drought resistance of cotton seedlings treated with mepiquat chloride. *Agronomy Journal*, 84(4), 569–574. <https://doi.org/10.2134/agronj1992.00021962008400040005x>
- Yamori, W., Nagai, T., & Makino, A. (2011). The rate-limiting step for CO₂ assimilation at different temperatures is influenced by the leaf nitrogen content in several C3 crop species. *Plant, Cell & Environment*, 34(5), 764–777.
- Yao, H., Zhang, Y., Yi, X., Hu, Y., Luo, H., Gou, L., & Zhang, W. (2015). Plant density alters nitrogen partitioning among photosynthetic components, leaf photosynthetic capacity and photosynthetic nitrogen use efficiency in field-grown cotton. *Field Crops Research*, 184, 39–49. <https://doi.org/10.1016/j.fcr.2015.09.005>
- Yendrek, C. R., Tomaz, T., Montes, C. M., Cao, Y., Morse, A. M., Brown, P. J., McIntyre, L. M., Leakey, A. D. B., & Ainsworth, E. A. (2016). High-throughput phenotyping of maize leaf physiological and biochemical traits using hyperspectral reflectance. *Plant Physiology*, 173(1), 614–626. <https://doi.org/10.1104/pp.16.01447>
- York, A. C. (1983). Cotton cultivar response to mepiquat chloride. *Agronomy Journal*, 75(4), 663–667. <https://doi.org/10.2134/agronj1983.00021962007500040020x>
- Zhao, D., & Oosterhuis, D. M. (2000). Pix plus and mepiquat chloride effects on physiology, growth, and yield of field-grown cotton. *Journal of Plant Growth Regulation*, 19(4), 415–422. <https://doi.org/10.1007/s003440000018>
- Zhi, X., Massey-Reed, S. R., Wu, A., Potgieter, A., Borrell, A., Hunt, C., Jordan, D., Zhao, Y., Chapman, S., Hammer, G., & George-Jaeggli, B. (2022). Estimating photosynthetic attributes from high-throughput canopy hyperspectral sensing in sorghum. *Plant Phenomics*, 2022, 9768502. <https://doi.org/10.34133/2022/9768502>
- Zhong, C., Cao, X., Hu, J., Zhu, L., Zhang, J., Huang, J., & Jin, Q. (2017). Nitrogen metabolism in adaptation of photosynthesis to water stress in rice grown under different nitrogen levels. *Frontiers in Plant Science*, 8, 1079. <https://doi.org/10.3389/fpls.2017.01079>
- Zhu, X.-G., Long, S. P., & Ort, D. R. (2010). Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*, 61(1), 235–261. <https://doi.org/10.1146/annurev-arplant-042809-112206>

SUPPORTING INFORMATION

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