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ARTICLE

Special Feature: Advancing Spectral Biology to Understand Plant Diversity across Scales

All the light we cannot see: Climate manipulations leave short and long-term imprints in spectral reflectance of trees

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Abstract

Anthropogenic climate change, particularly changes in temperature and precipitation, affects plants in multiple ways. Because plants respond dynamically to stress and acclimate to changes in growing conditions, diagnosing quantitative plant-environment relationships is a major challenge. One approach to this problem is to quantify leaf responses using spectral reflectance, which provides rapid, inexpensive, and nondestructive measurements that capture a wealth of information about genotype as well as phenotypic responses to the environment. However, it is unclear how warming and drought affect spectra. To address this gap, we used an open-air field experiment that manipulates temperature and rainfall in 36 plots at two sites in the boreal-temperate ecotone of northern Minnesota, USA. We collected leaf spectral reflectance (400–2400 nm) at the peak of the growing season for three consecutive years on juveniles (two to six years old) of five tree species planted within the experiment. We hypothesized that these mid-season measurements of spectral reflectance capture a snapshot of the leaf phenotype encompassing a suite of physiological, structural, and biochemical responses to both long- and short-time scale environmental conditions. We show that the imprint of

Authors of the Artur's Round Table—detailed roles and contributions can be found in the Author Contribution section.

environmental conditions experienced by plants hours to weeks before spectral measurements is linked to regions in the spectrum associated with stress, namely the water absorption regions of the near-infrared and short-wave infrared. In contrast, the environmental conditions plants experience during leaf development leave lasting imprints on the spectral profiles of leaves, attributable to leaf structure and chemistry (e.g., pigment content and associated ratios). Our analyses show that after accounting for baseline species spectral differences, spectral responses to the environment do not differ among the species. This suggests that building a general framework for understanding forest responses to climate change through spectral metrics may be possible, likely having broader implications if the common responses among species detected here represent a widespread phenomenon. Consequently, these results demonstrate that examining the entire spectrum of leaf reflectance for environmental imprints in contrast to single features (e.g., indices and traits) improves inferences about plant-environment relationships, which is particularly important in times of unprecedented climate change.

KEYWORDS

B4WarmED, climate change, climate imprint, drought, functional ecology, functional traits, leaf-level spectral reflectance, phenotype, spectral signatures, warming

INTRODUCTION

Anthropogenic climate change, including increases in temperature and changes in the timing and magnitude of precipitation, affects plant life in a variety of ways. However, diagnosing how plants respond to climate is complex because they respond dynamically to resource availability and stress and acclimate to changes in growing conditions. For example, plants may alter the structure (e.g., leaf mass per area [LMA]) and/or pigmentation of their leaves, allowing them to cope with environmental variation in light, heat, and drought, and on different timescales (Doughty et al., 2018; Kothari et al., 2018; Poorter et al., 2009). Plant-environment relationships are often described via variation in functional trait values using environmental conditions as explanatory variables that influence plants' performance (e.g., growth and survival; Laughlin et al., 2011; Violle et al., 2007). However, while functional traits are often used to describe plant-environment relationships, there is generally a weak relationship between individual traits and environmental conditions (Anderegg, 2023; Reich et al., 2007). This is likely a result of: (1) a wide variation in the expression of traits (Albert et al., 2010; Reich et al., 2007), (2) single traits lacking a direct relationship with climate (Aubin et al., 2016; Violle et al., 2007), and (3) multiple functions of traits and/or multi-trait coordinated responses as constrained by genotype and species-specific adaptations (Falster et al., 2017; Sack & Buckley, 2020) among many others. Hence, examining the combination of multiple traits within an organism may provide more insight into plant-environment relationships, and spectral data may provide one avenue for doing so.

Spectral measurements of plants have emerged as a critical tool in plant physiology and ecology that captures an extraordinary wealth of information about the plant phenotype (Sapes et al., 2024). Leaf spectroscopy offers relatively rapid, inexpensive, and nondestructive measurements (Lamour et al., 2021) that provide individual trait detection (Chlus & Townsend, 2022), and improve understanding of plant-environment interactions (e.g., phenotypes; Cavender-Bares et al., 2017), plant diversity (Schweiger et al., 2018), and ecosystem function (Williams et al., 2021). The hyperspectral signal combines multiple physiological, structural, and chemical traits into a single measure which may enable us to make inferences about physiological processes and associated ecological consequences (Schweiger et al., 2021). While past research has often focused on single spectral features (e.g., spectral indices defining plant stress) or traits to describe plant-environment relationships (Serbin et al., 2019; Wang et al., 2022; Wu et al., 2016), the full spectrum of light reflected off the leaf surface (e.g., 400–2400 nm) may carry an integrative measure of a plant's phenotypic response to the environment and thus be more informative.

However, while we know a considerable amount about how stress caused by drought affects leaf spectral reflectance (Dao et al., 2021; Penuelas et al., 1997; Römer et al., 2012), it is currently unclear whether and how multiple ecologically important climate change factors (i.e., warming and rainfall reduction) and their interactions cause consistent spectral changes across species. Several key challenges remain unaddressed in unlocking this potential. One challenge is the inherent multicollinearity of spectral data. A second challenge arises from the complex interaction of simultaneous changes in warming and moisture conditions with multiple plant traits and physiological processes, which can influence spectral reflectance in many ways, such as (1) stress-driven changes in pigment activity and total pool sizes, (2) responses to resource availability, including changes in carbon assimilation and leaf water content in response to water availability, and (3) changes in morphology/structure driven by both stress and resource availability (e.g., LMA). A third challenge is that a spectral measurement at any given time reflects the environmental conditions to which a leaf has been exposed over both long and short time scales.

For example, environmental conditions early in the season can influence or even stimulate leaf development and alter leaf morphology (Poorter et al., 2009). These influences can accumulate over time in the leaf as it moves through developmental stages, causing changes in pigment cycles and pools in response to stress or through turgor pressure that influences cell expansion and leaf size (Ali et al., 2023; Demmig-Adams et al., 2014). Over seasonal time scales, temperature, water availability, and light availability—the latter driven by the location in the crown where a developing leaf is located—can all affect the morphological and structural properties of a leaf and its growth trajectory (Poorter et al., 2009; Thornton & Zimmermann, 2007; Williams et al., 2021). At shorter time scales, that is, hours to weeks where acute weather conditions may impact physiological processes—leaf attributes that are influenced by weather conditions may also be captured by spectral measurements (Alonso et al., 2017; Gamon et al., 1992). Thus, the spectral signals provide a snapshot of leaf phenotypes at the moment of measurement, that integrates both the long- and short-term experience of the leaf as well as how the plant responds physiologically to environmental stress.

To our knowledge, most studies to date consider leaf spectral reflectance as either a proxy for or an intermediary tool in understanding trait-environment relationships by using them to predict functional traits or taxa of interest (Fassnacht et al., 2016; Serbin et al., 2019; Wang et al., 2022; Wu et al., 2016). Moreover, because both conventional (Fajardo & Siefert, 2016; McKown et al., 2013) and spectral (Chlus & Townsend, 2022; Yang et al., 2016) trait measurements have demonstrated that trait values change over the course of the growing season and are accompanied by variation in the shape and magnitude of spectral reflectance (Richardson et al., 2021), we expect that the influence of the environment on spectral reflectance will vary over the course of leaf development. Thus, we study spectra collected at the peak of the growing season to examine the influence of environmental conditions across a range of time scales from budbreak until the moment of measurement. To examine whether leaf spectral reflectance carries a distinctive climatic signal and integrates a wide range of environmental and biological information (Figure 1), we test the following hypotheses: (1) spectral reflectance captures a snapshot of leaf phenotypic response to two major climate change drivers (i.e., warming and rainfall reduction) encompassing a suite of leaf physiological, structural, and biochemical responses that are a result of both long-and short-term environmental exposure, (2) experiencing elevated temperatures and water limitation during leaf development will leave a long-lasting imprint primarily via structural changes associated, for example, with pigment content and ratios, whereas (3) exposure to stress later in the season from similar variation in temperature and moisture availability will be primarily associated with acute stress responses, likely of a less permanent nature, for example, xanthophyll cycles, and (4) spectral responses are generalizable and will show similar directional shifts among a set of five broad-leaved tree species. To address these hypotheses, we ask the following: (1) Can we detect the influence of climate change on spectral reflectance; and if so, do all species respond similarly? (2) Is there an environmental variable that integrates the influence of rainfall and warming treatments on spectra, or do these represent divergent drivers? The former is more likely if rainfall and warming influence plants through a similar proximal pathway (such as through environmental drying; Reich et al., 2022; Stefanski et al., 2023); the latter is expected if distinct physiological processes are involved in responses to changes in moisture versus temperature. (3) Is there a time period during leaf development when the environment has the strongest influence on the spectrum observed at the peak of the growing season? (4) Which regions of the spectrum are the most sensitive to environmental influence and how do they relate to plant chemistry, structure, and physiology?

To test our hypotheses and address these questions, we used the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment that manipulates two climate change factors (i.e., warming and rainfall) through free-air plant and soil warming and rainfall exclusion. The experiment is located at two sites in the boreal-temperate ecotone of northern Minnesota, USA (Rich et al., 2015). In the middle of the growing season for

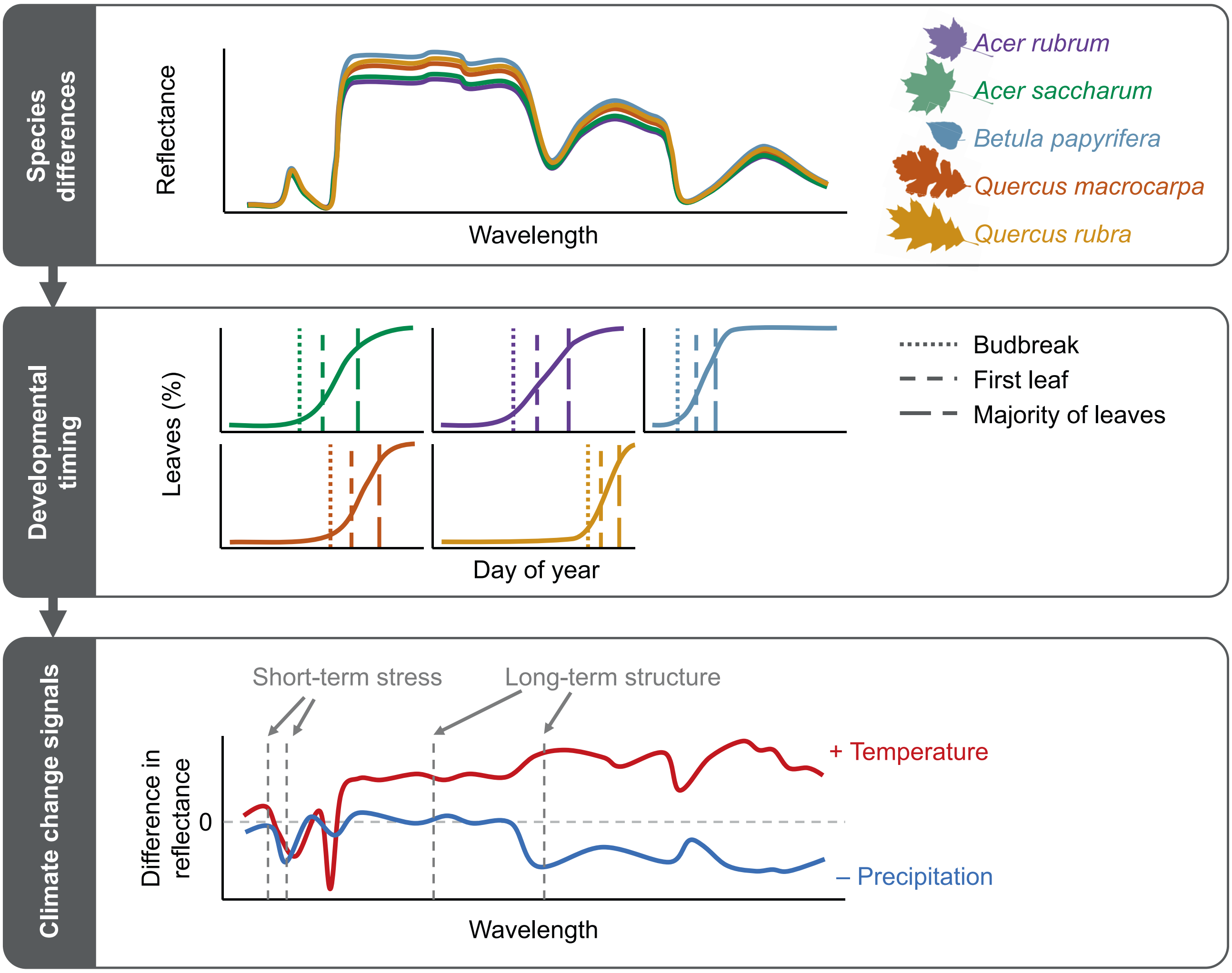

FIGURE 1 Conceptual figure outlining the sources of variation in leaf-level spectra related to species identity, developmental stage, and climate, which together guide our stepwise analysis. The effects of climate (warming treatments and rainfall reduction) on leaf spectra exist in the context of the effects of species identity (different colored lines) and canopy developmental stage (phenological phases); to better understand the former, we need to account for or standardize the latter. This conceptual figure in its entirety is the original work of the coauthor of this manuscript, Laura J. Williams.

three consecutive years, we measured full-range green foliar spectral reflectance (350–2500 nm) on juveniles (2- to 6-year-old transplanted seedlings) of five tree species from the boreal-temperate ecotone. At the time of measurements, seedlings had been growing within the treatment for at least one and up to four seasons (for details see *Methods* and Appendix S1: Table S2). In total, we measured spectra on ~1612 green leaves and analyzed them in a stepwise process using three distinctive analyses.

METHODS

Site description and experimental design

This research was conducted at a long-term climate change experiment in northern Minnesota, USA, established in 2008 in the ecotone of the boreal-temperate forest. The research sites, the experimental design, and the methodology for the warming and rainfall treatments are outlined in detail in Appendix S1 and in previous studies (Rich et al., 2015; Stefanski et al., 2020). In brief, B4WarmED is a factorial experiment consisting of two sites (i.e., Cloquet MN, and Ely MN), three warming (i.e., ambient conditions, +1.7°C and +3.3°C above ambient), and two rainfall manipulation (i.e., ambient rain and ~30% reduction in summer rainfall) treatments for a total of 36 research plots, each 3 m in diameter.

The warming treatment was implemented simultaneously for the above- and belowground parts of the plot and designed to maintain a fixed temperature differential between ambient and warmed plots. Across all 3 years, the warming system achieved +1.7°C and +3.3°C average temperature differentials between treatments

(Appendix S1: Table S1, Figures S1 and S2), we will refer to warming treatments as such throughout the paper. The summer rainfall reduction treatment used rainout shelters to reduce both total summer rainfall and the number of rain events in each year from June 1 to September 30. Across the 3 years of summer rainfall removal, we saw an average reduction of 28.1% in summer rainfall in comparison to ambient plots (Appendix S1: Table S1). Consequently, our rainfall treatments were representative of a dry summer (~10–15th percentile wettest) and severely dry summer (~0–5th percentile driest) for ambient and reduced rainfall, respectively, as compared with the broader temporal context of the 100 years of the weather record (1922–2022 available for the Cloquet site).

Over the course of this experiment, we grew seedlings of more than 10 tree species in the research plots, including the five native angiosperms used in this study: *Acer rubrum* L., *Acer saccharum* Marshall, *Betula papyrifera* Marshall, *Quercus rubra* L., and *Quercus macrocarpa* Michx. (abbreviated to Aceru, Acesa, Betpa, Queru, and Quema, respectively). The seedlings were planted into the existing matrix of native vegetation. Seedlings were sourced from local ecotypes produced by Minnesota Department of Natural Resources (DNR) nurseries. Note that *Q. macrocarpa* (bur oak) includes three distinctive ecotypes sourced from populations from across a climate gradient, representing distinctive populations from the southwestern (Oklahoma population), center (Illinois population) and northern (Minnesota population) parts of the species range. The three distinctive bur oak populations were grown from seed in the Vallonia, Indiana DNR tree seedling nursery. For the purpose of this study, we did not study differences in responses of spectral signal among populations. All seedlings were one or two years old when they were planted in the experiment and were measured in their second to sixth growing seasons in the experiment and ranged in their average maximum size across all species from ~97 cm in the first year to 156 cm in the last year (Appendix S1: Table S2).

Spectra-environment modeling approach

Our treatments manipulate two main climatic variables (i.e., temperature and rainfall) on a factorial basis, creating three distinctive warming treatments and two rainfall manipulations. However, because this is a chamberless free-air design, all plants also experience local diurnal and seasonal weather patterns; and thus experience both within-treatment and among-treatment variability in moisture and temperature (Appendix S1: Table S1, Figures S1–S3). We took a three-step approach to test whether and how environmental conditions that plants experience during their growth affect the spectral reflectance of the leaf. This approach allows us to test climate change factors that define environmental variables on factorial (i.e., in accordance with the experimental set up) and continuous scales (i.e., as the leaf experiences the environment) using physiologically meaningful timeframes and environmental variables.

Selection of climatic variables for modeling

We considered eight environmental variables that were calculated and averaged for selected timeframes (see below) based on data collected via an automated array of sensors that monitor environmental conditions on the plot, block, or site level (Rich et al., 2015). The variables that we explored are as follows: (1) atmospheric vapor pressure deficit in each plot based on the plot upper canopy surface temperature and relative humidity (VPD, the difference between actual and saturated vapor pressure of the air, in kilopascals), (2) soil volumetric water content (VWC, in cubic centimeters of H_2O per cubic centimeter of soil), (3) rainfall (in millimeters), (4) aboveground temperature (in degrees Celsius), (5) total average cumulative photosynthetic photon flux density (PPFD, a total of photosynthetically active radiation accumulated throughout the day, in micromoles per square meter per day), (6) belowground temperature (in degrees Celsius), (7) atmospheric water potential (AWP), and (8) soil water potential (SWP).

These variables were selected as candidates for characterizing the environmental conditions in which the plants grow and leaves develop because we hypothesized that they would best capture the relationship between spectra and the environment, and they offer ecological and physiological meaning. However, examining the correlations between each of the eight environmental variables and the spectral signal revealed some notable patterns (data not shown). There was often coordination in the spectral response to a single variable within the broad regions of the visible (VIS, 400–700 nm), near-infrared (NIR, 700–1100 nm), and short-wave infrared (SWIR, 1100–2500 nm). Some environmental variables revealed very similar responses to others, for example, soil temperature was almost identical to the long-timescale aboveground temperature, as one would expect from soil temperature integrating aboveground temperature.

There is considerable covariation among the environmental variables (Appendix S1: Figure S4) and so several variables (i.e., PPFD, belowground temperature, AWP, and SWP) have a reduced emphasis in further analyses. Instead, we focus on VPD, aboveground temperature, VWC, and rainfall measured or estimated on a plot level. These variables: (1) best integrate our environmental treatments, (2) have the most ecophysiological meaning in the temperature and water availability continuum, (3) have, at most, modest correlation among each other (i.e., VPD, VWC, and rainfall), and (4) performed best with the partial least square regression (PLSR) and multiple linear regression (MLR) models. However, because VPD and temperature were so highly correlated, their effects cannot be separated, so all VPD (or temperature) influences must be interpreted as the combined influence of VPD and temperature; and because VPD was better predicted from spectra using the PLSR analysis, we use three environmental variables—omitting aboveground temperature—for the final MLR analysis. See *Spectra-environment modeling approach* for further details on both the PLSR and MLR analyses.

Phenology data

To place environmental data that leaves experience over the course of the growing season into a biologically meaningful framework, we used foliar phenological observations. We visited individuals of each tree species on each plot every week and scored them for three main phenological stages: budbreak, the first fully formed leaf, and the majority of fully unfolded leaves (Montgomery et al., 2020). We define five time periods that cover leaf developmental stages and a growing season that are bracketed by those phenological stages: (1) initial leaf unfolding, which spans the time between the beginning of the budbreak and the appearance of the first fully expanded leaf (BB-1L), (2) formation of the crown, which is bracketed by the appearance of the first leaf and the majority of the leaves unfolded on a given tree (1L-ML), (3) total period of leaf development, which ranges from budbreak until the majority of leaves are unfolded (BB-ML), (4) core of the growing season, which spans the majority of leaves unfolded until the measurement day (which occurred approximately at the peak of the growing season; beginning to mid-August) (ML-Meas), and (5) the whole growing season from the time of budbreak until the measurement day (BB-Meas). We defined those timeframes based on the exploratory analysis that showed that most of the variation in spectra is explained by periods that are associated with major developmental stages of the leaf life cycle.

Leaf reflectance measurements

Over the course of three growing seasons, we measured leaf reflectance spectra on ~1612 leaves of five target species (Appendix S1: Table S2). Each year, spectral measurements were conducted in mid-summer during the approximate peak of the growing season (i.e., beginning to mid-August) over the course of 6–8 days in a two-week period. During each campaign, we measured 3–24 leaves per species in each treatment. Fully mature leaves were selected from the upper part of the crown. Spectral measurements were performed using a hyperspectral (350–2500 nm), field-portable spectroradiometer (SVC-HR1024i, PA, USA) with measurement spacing (and spectral resolution) of 1.5 nm (3.5 nm) for 350–1000 nm, 3.8 nm (9.5 nm) for 1000–1890 nm, and 2.5 nm (6.5 nm) for 1890–2500 nm. Spectral measurements were taken using a leaf clip (LC-RP PRO) that holds a fiber optic cable and a tungsten halogen lamp that illuminates a leaf area of 3.14 cm^2 against a black background. Leaf relative reflectance was calculated relative to a white reference Spectralon panel on the leaf clip that approximates 100% reflectance. Spectral reflectance data were screened and examined for any erroneous values that resulted in a total of 56 out of 1612 measurements being discarded.

Spectral data preprocessing

The leaf reflectance measurements were resampled to a bandwidth of 2.0 nm over the spectral range (354–2496 nm) to ensure a uniform distribution of bands for further analysis using the “resample” function in the spectrolab package (Meireles et al., 2023) in the R software version 4.3.2 (R Core Team, 2023). The resampled leaf reflectance measurements were then transformed using two separate procedures for further analysis: vector normalization and continuous wavelet transformation (CWT). Vector normalization was performed to avoid differences among species in the magnitude of reflected light using bands between 400 and 2400 nm (from now on referred to as full spectrum). CWT was performed to enhance absorption features following (Guzmán Q. & Sanchez-Azofeifa, 2021). Our premise is that the applied treatments (i.e., warming and rainfall reduction) are likely to have a small effect on changes in leaf reflectance; thus, this transformation can guide us to explore which absorption features are more likely to be associated with these effects. For this transformation, the resampled reflectance spectra were decomposed into two wavelets (i.e., 2^3 and 2^4) using a second-order Gaussian function derivative and applying a variance of 1. The selected wavelets exclude the potential noise from the sensor while describing small variations in absorption features. The two wavelets of bands between 400 and 2400 nm were summed to create a summed-wavelet spectra.

Spectra-environment modeling approach

To examine whether and how environmental conditions that plants experience during their growth affect the spectral reflectance of the leaf, we performed a three-step analysis. First, we performed an eigendecomposition on leaf reflectance per species as a data reduction technique to subtract uncorrelated components describing the maximum variance among samples while reducing the redundancy among bands. The eigendecomposition serves as a simplified signal to directly test treatment effects (i.e., the effect of temperature and rainfall reduction) using linear mixed-models analysis. Second, we used PLSR to predict selected environmental variables from summed-wavelet reflectance spectra based on continuously measured environmental data that were averaged for each combination of species, plots, sites, and year for the five time periods defined above (BB-1L, 1L-ML, BB-ML, ML-Meas, and BB-Meas; which are unique windows of time for each species in accordance with their respective phenology) for both the main (i.e., all species combined) and species-specific models. Data were split 50% for calibration and 50% for validation. For each environmental variable, we selected the smallest number of components for which the Root Mean Squared Error (RMSE) of prediction from cross-validation dropped to one SD of the global minimum. PLSR models were run for 500 iterations, whereby for each iteration, calibration data were split using the jackknife method into 70% to train the model and 30% to test the model's predictive ability. Third, to discern both the long- and short-term effect of environmental variables on spectral reflectance and account for multiple environmental variables' effects on variation in spectral reflectance, we built a set of MLR models to predict each wavelength in the spectrum using three environmental variables: VPD, VWC, and rainfall. As noted above, due to the high correlation between VPD and aboveground temperature, all VPD influences in the MLR model must be interpreted as the combined influence of surface temperature and VPD. All three environmental variables were averaged over four timescales (similar to the PLSR analysis) unique to each site and treatment based on their phenology as follows: 6 h prior to measurement, 7 days prior to measurement, from budbreak to most leaves (BB-ML), and from most leaves to measurement (ML-Meas). Important to note is that to complete the model, each species was given an independent intercept, which resulted in a model of the following form:

$$R_\lambda = \beta_{0\lambda,\mathrm{sp}} + \beta_{1\lambda,x} W_{x,6\mathrm{h}} + \beta_{2\lambda,x} W_{x,7\mathrm{d}} + \beta_{3\lambda,x} W_{x,\mathrm{BB-ML}} + \beta_{4\lambda,x} W_{x,\mathrm{ML-M}} + \epsilon_\lambda \tag{1}$$

Equation (1) is the formal MLR model that is used to predict normalized reflectance, R. At each wavelength λ (400–2400 nm, in 2-nm increments, 1001 models in total) the reflectance is predicted by estimating a species-specific intercept $\beta_{0\lambda,\mathrm{sp}}$ and the sensitivity, $\beta_{i\lambda,x}$, to three environmental predictors W_x ($x =$ VPD, VWC, and rainfall) spread across four time periods which are indicated by the value following the x in the subscript: 6h (6 hours), 7d (7 days), BB-ML (budbreak to the majority of leaves), and ML-M (majority of leaves to measurement). For the 6 h time scale, only VPD is included but all three environmental predictors are used for the other three time scales. The ϵ_λ is the residual error.

Note that species effects were accounted for in the principal components analysis (PCA) through a Procrustes orientation, in the PLSR approaches by evaluating models for each individual species, and in the MLR by including each species as a fixed effect. More information on each modeling step is provided in supplemental materials.

Spectral indices and trait estimates—PROSPECT radiative transfer model from leaf spectral reflectance

We examined the relationships between environmental imprints on spectra and ecophysiologically relevant functional traits and processes that were derived from leaf-level spectral reflectance. To estimate functional traits, we used the radiative transfer model, PROSPECT-D (Féret et al., 2017) using optimal spectral domains (Spafford et al., 2021) to estimate LMA (leaf mass area), chlorophyll (Chl), carotenoids (Car), anthocyanins (Ant), EWT (equivalent of water thickness), and nitrogen. In addition, we calculated 22 common spectral indices (Appendix S1: Table S3) that generally are associated with the following processes: (1) the water status of the leaf, (2) carotenoids and chlorophyll content, including their ratios, and (3) physiological processes (e.g., photosynthetic activity, chlorophyll fluorescence or xanthophyll cycles). We used linear mixed models to test the effect of warming and rainfall reduction on all derived indices and functional traits, with year of measurement and site as random variables. Finally, all functional traits and spectral indices were compared with the imprints of the environmental variables (i.e., wavelengths with statistically significant model coefficients) on the spectra derived from the MLR model. These comparisons are made across leaf developmental stages and time preceding the measurements, with literature-documented regions associated with estimated functional traits and spectral indices.

All analyses were performed in R (R Core Team, 2023); on the use of specific packages for each analysis see supplemental materials.

RESULTS

Environmental conditions leave significant imprints in leaf spectral reflectance

Environmental conditions experienced by plants throughout the growing season impacted the mid-summer spectral signal; evidence for this is found in all three analyses we performed; moreover, each shows similar imprints (Appendix S1: Figure S5). Specifically, all three analyses indicate similar individual wavelengths and spectral regions are affected by environmental conditions in which the plants were growing. Species have similar directional spectral responses to warming and rainfall but vary in magnitude (Figure 2g–i; Appendix S1: Figure S6). Moreover, we show that the environmental conditions experienced during leaf development leave long-lasting distinctive imprints that differ from those that are imprinted by the conditions immediately preceding a measurement (Figure 3).

Eigendecomposition-Procrustes analysis

The three components derived from the eigendecomposition-Procrustes analysis show a significant separation of warming and rainfall removal treatments (Figure 2a–f; Appendix S1: Table S4) along all three principal component axes. The linear mixed-models analysis performed on the derived scores demonstrates the effect of experimental treatments, showing that the main effects of both warming and rainfall removal have significant separation of spectral reflectance (Appendix S1: Table S4), while species do not ($p > 0.05$), which is also demonstrated by their eigendecomposition-Procrustes ordination (Figure 2g–i). The significance level of treatment separation varies across principal components (PCs); both environmental treatment main effects are strongly significant in PC1 and PC3 ($p < 0.001$) and modestly significant for PC2 ($0.05 > p < 0.001$; Appendix S1: Table S4). Moreover, each PC shows from one to three significant interactions, and collectively across all three PCs, at least two of the four possible interactions are modestly significant ($p < 0.05$) in one of the PCs, but not a single PC has all four significant interactions.

Running the eigendecomposition-Procrustes analysis allows a rotation of coefficients, which permitted us to superimpose species coefficients by orienting them on the same axis/dimension (Figure 2g–i; Appendix S1: Figure S6) while conserving the variability of the spectra. Thus, newly derived scores from this analysis that summarize the variance of the spectra as associated with the treatments when plotted by species (Appendix S1: Figure S6) show that species organize along the same dimension with their responses affected by experimental treatments along PC1, PC2, and PC3 and primarily varying in the magnitude of their responses. In other words, this approach effectively removes the species effect from the treatment effect and allows us to use the scores to conduct linear mixed models to test the effect of experimental treatments on spectra.

Moreover, an eigendecomposition-Procrustes analysis allowed us to study the eigenvectors' importance associated with five specific bands (i.e., 532, 684, 740, 1418, and 1906 nm; Appendix S1: Figures S7 and S8) across the entire spectrum of leaf reflectance that are related to all three principal components that explain ~94% of the data variation. In visible light (VIS), the eigenvectors indicate three primary wavelengths 532, 684, and 740 nm associated with pigments and structural features of the leaf (Appendix S1: Figure S8). Also important for explaining variation in the spectral data in the PCA are two bands associated with the SWIR region 1418 and 1906 nm that are related to the structure and biochemistry of the leaf (Appendix S1: Figure S8).

PLSR modeling for environmental variables prediction

Main models

The general PLSR models, for four focal environmental variables (VPD, rainfall, aboveground temperature, and VWC) showed that environmental conditions during leaf and canopy development were stronger predictors of mid-summer spectral signatures than conditions over the entire season up to the time of measurement, or shorter periods just prior to the measurements (Appendix S1: Table S5). The VPD model had $\text{RMSE} = 17.16$ and $R^2 = 0.59$ (Figure 3a) for a period from budbreak until the appearance of the first leaves (BB-1L). The aboveground temperature for the period from budbreak to the appearance of the first leaf was best predicted from spectra with $\text{RMSE} = 19.81$ and $R^2 = 0.38$ (Figure 3b). The VWC had $\text{RMSE} = 21.88$ and $R^2 = 0.43$ (Figure 3d) for a period when leaves are developing throughout the crown (1L-ML). Rainfall showed the highest predictability of all four environmental variables ($\text{RMSE} = 16.21$ and $R^2 = 0.79$; Figure 3c) that was achieved for the period that spans the entire crown development (BB-ML; Appendix S1: Table S5). However, a continuous gradient in observed rainfall was predicted to fall into just two clusters, suggesting some concern about patterns of residuals.

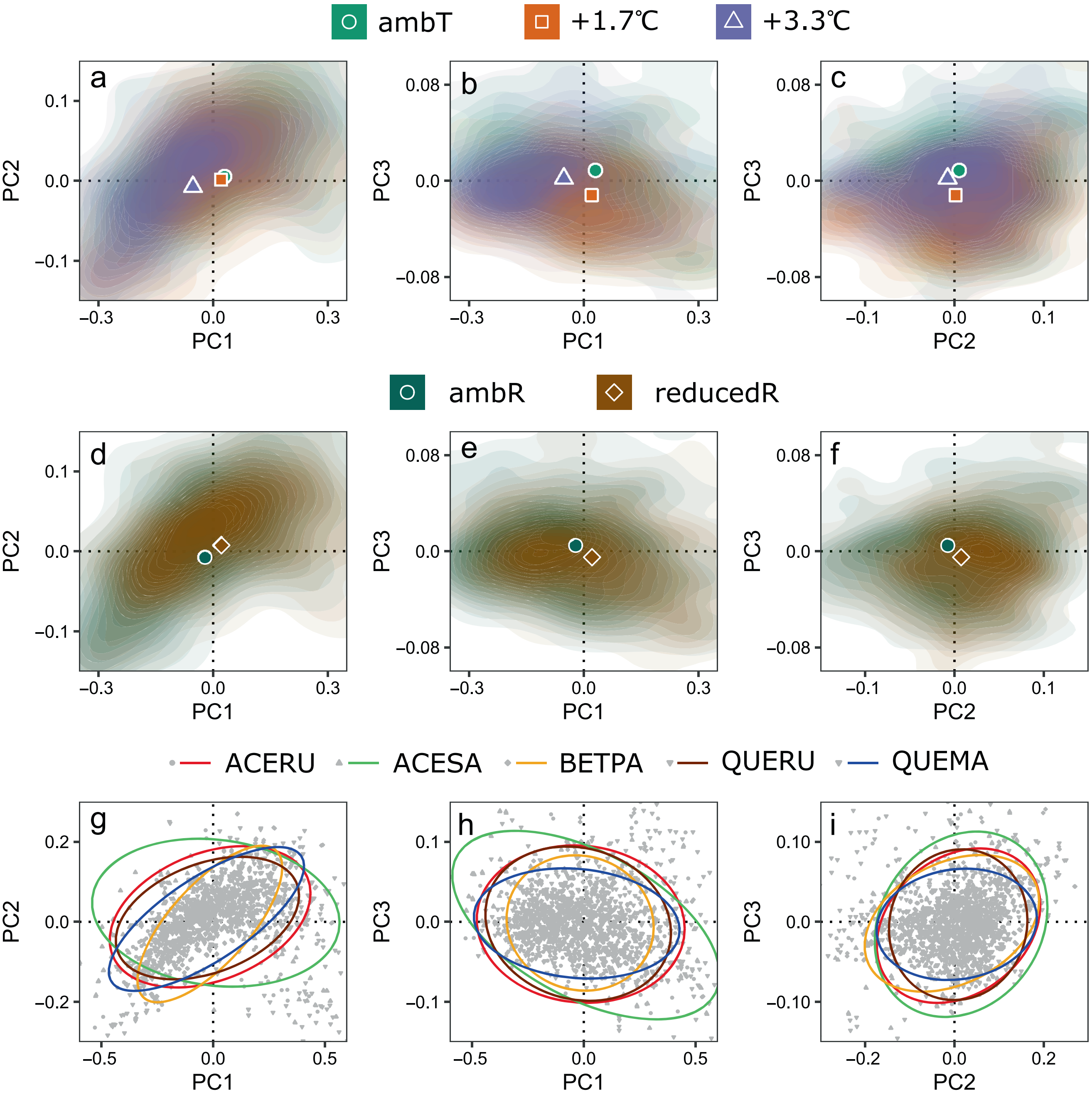

FIGURE 2 Ordination of the eigendecomposition-Procrustes analysis of the wavelet transformed spectral data. Panels (a–c) depict the distribution of warming treatments, while panels (d–f) depict rainfall reduction treatments. Panels (g–i) show the ordination of species after the superimposition of species coefficients to the same axis, while retaining the variability of the spectra as summarized by the eigendecomposition. Note that to visually emphasize the effect of the treatments in panels (a–f), we shortened both axes; as a result, ~18% of all points are not visible. PC, principal component.

Species-specific models

Species-specific PLSR models overall had similar predictive power for environmental variables as general models and were also typically the best for one or more times that spanned leaf development times at the beginning of the growing season. However, the exact time windows for which environmental variables were best predicted (that were uniquely constructed for each species based on their phenology) varied slightly among species. Rainfall and VPD were overall the best predicted from spectra (Appendix S1: Table S5).

Rainfall exhibited the most uniform and highest predictability across species. For all species, spectra best

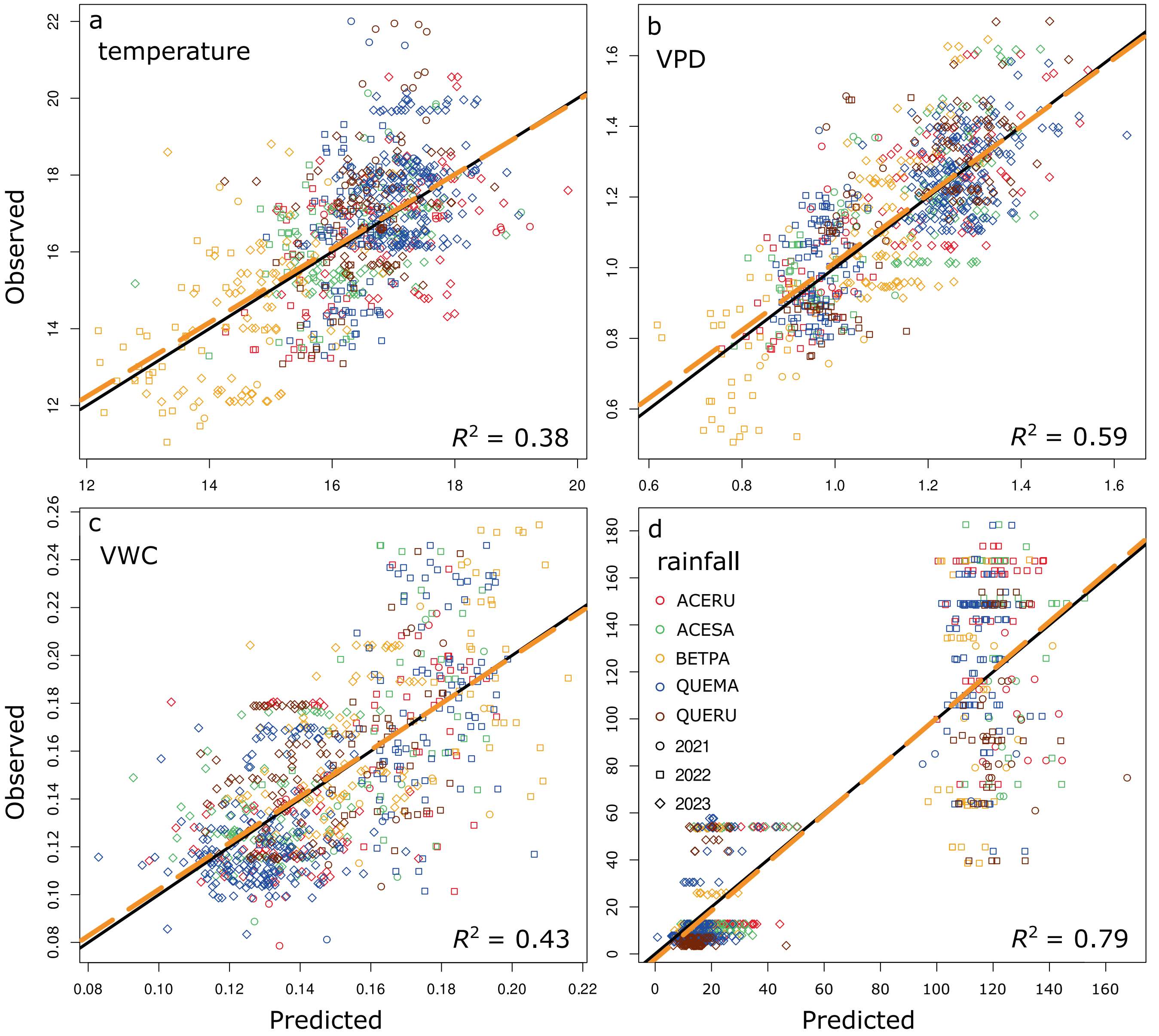

FIGURE 3 Relationship between environmental conditions during leaf development and mid-summer spectra. Panels show predicted versus observed environmental variables from analysis of leaf spectra using partial least square regression. (a) Aboveground temperature for a period of BB-1L, (b) vapor pressure deficit (VPD) for a period of BB-1L, (c) volumetric water content (VWC) for a period of 1L-ML, and (d) rainfall for a period of BB-ML. Black diagonal lines show 1:1, orange dashed lines are model fits. For acronyms, see the main text.

predicted rainfall occurring during leaf development (BB-ML), which on average had the lowest RMSE and highest R^2 (RMSE < 19.86 and $R^2 > 0.65$; Appendix S1: Figure S9). VPD was well predicted for the BB-1L timeframe for all species and ranged from RMSE $= 19.96$ and $R^2 = 0.48$ for *Q. rubra* to RMSE $= 17.14$ and $R^2 = 0.58$ for *Q. macrocarpa*. Aboveground temperature was also significantly predicted for each species (Appendix S1: Figure S9); however, in contrast to the other environmental variables, species differed in the best predicted time frames, with the best models for *A. rubrum* (RMSE $= 30.80$ and $R^2 = 0.13$), *A. saccharum* (RMSE $= 29.68$ and $R^2 = 0.39$), and *Q. rubra* (RMSE $= 29.68$ and $R^2 = 0.39$) being for a period of ML-Meas, while for *B. papyrifera* (RMSE $= 26.72$ and $R^2 = 0.36$) and *Q. macrocarpa* (RMSE $= 23.46$ and $R^2 = 0.33$) models were best for a period of 1L-ML. VWC was less reliable for any of the time frames considered for the PLSR models in general across all species except *Q. macrocarpa* (RMSE $= 20.43$ and $R^2 = 0.50$) and *A. saccharum* (RMSE $= 29.55$ and $R^2 = 0.38$) (Appendix S1: Figure S9).

Variable importance in projections

The general PLSR models with species grouped together across sites and years indicate ranges and individual wavelengths important in the predictability of the environmental variables. For each environmental variable from the leaf development timeframe, PLSR models indicated from 6 to 9 important regions with dominant wavelengths as defined by the 0.8 heuristic threshold for the selection of important wavelengths as suggested by Burnett et al. (2021) (Figure 4). While important regions and wavelengths differ across environmental variables, there are some commonalities. In particular, measurements at 1902 and 1388 nm are important for each environmental variable. These wavelengths correspond to water absorption features in the SWIR region of the leaf spectral reflectance (Jacquemoud & Ustin, 2019). Moreover, if wavelength regions are considered more broadly (i.e., characterized by the spectral breadth of a variable importance in projections [VIP] peak) there is considerable overlap among environmental variables. Across all four environmental variables, four regions covering roughly 490–586 nm, 628–760 nm, 1368–1428 nm, and 1852–1928 nm are common as indicated by PLSR general models.

Species-specific models generally agreed with the main models and indicated similar spectral regions with important wavelengths used by the PLSR models to predict environmental variables (Appendix S1:

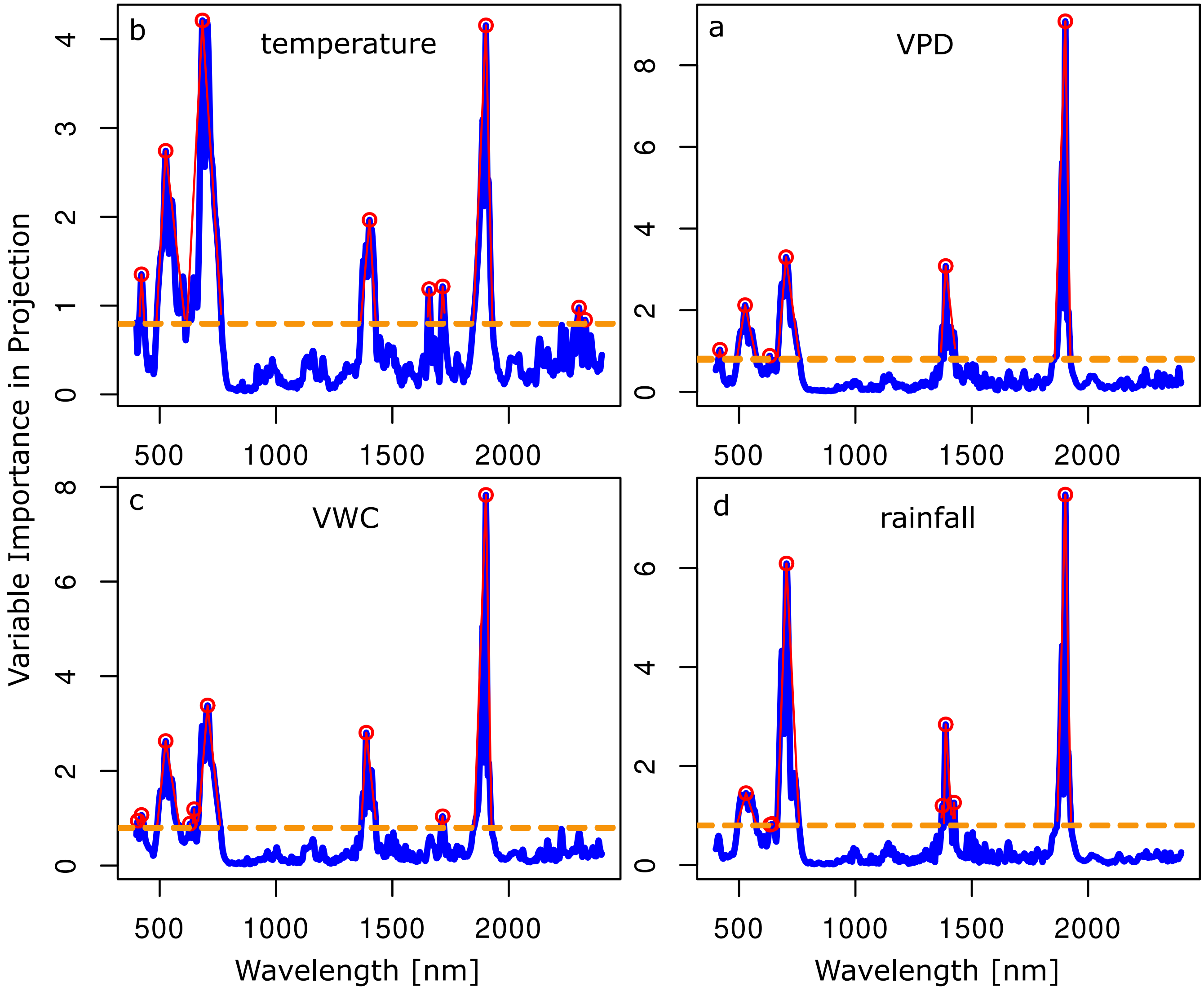

FIGURE 4 Variable importance in projection (VIP) for four environmental variables based on the Partial Least Squares Regression. (a) aboveground temperature for a period of BB-1L, (b) vapor pressure deficit (VPD) for a period of BB-1L, (c) volumetric water content (VWC) for a period of 1L-ML, and (d) rainfall for a period of BB-ML. The horizontal dashed line fixed at 0.8 identifies the heuristic threshold for importance suggested by (Burnett et al., 2021) for wavelengths in the partial least square regression model. For acronyms, see the main text.

Figure S9, Table S3). As well, common wavelengths emerge as important across species. However, for example, VWC—as described above—shows good predictions for only two of five species, but these do match spectral regions and wavelengths of the main models. Generally, the key spectral regions and wavelengths in the PLSR models overlap across species regardless of the time frame (Appendix S1: Figure S10).

MLR—Spectra and environment

Based on the PLSR, the MLR analyses used only the mean spectrum of each species, that is, all species were assumed to respond identically to the environment. Regardless, the model estimates of the mean vector normalized reflectance for each species show substantial differences across many regions of the spectrum (Figure 5b)

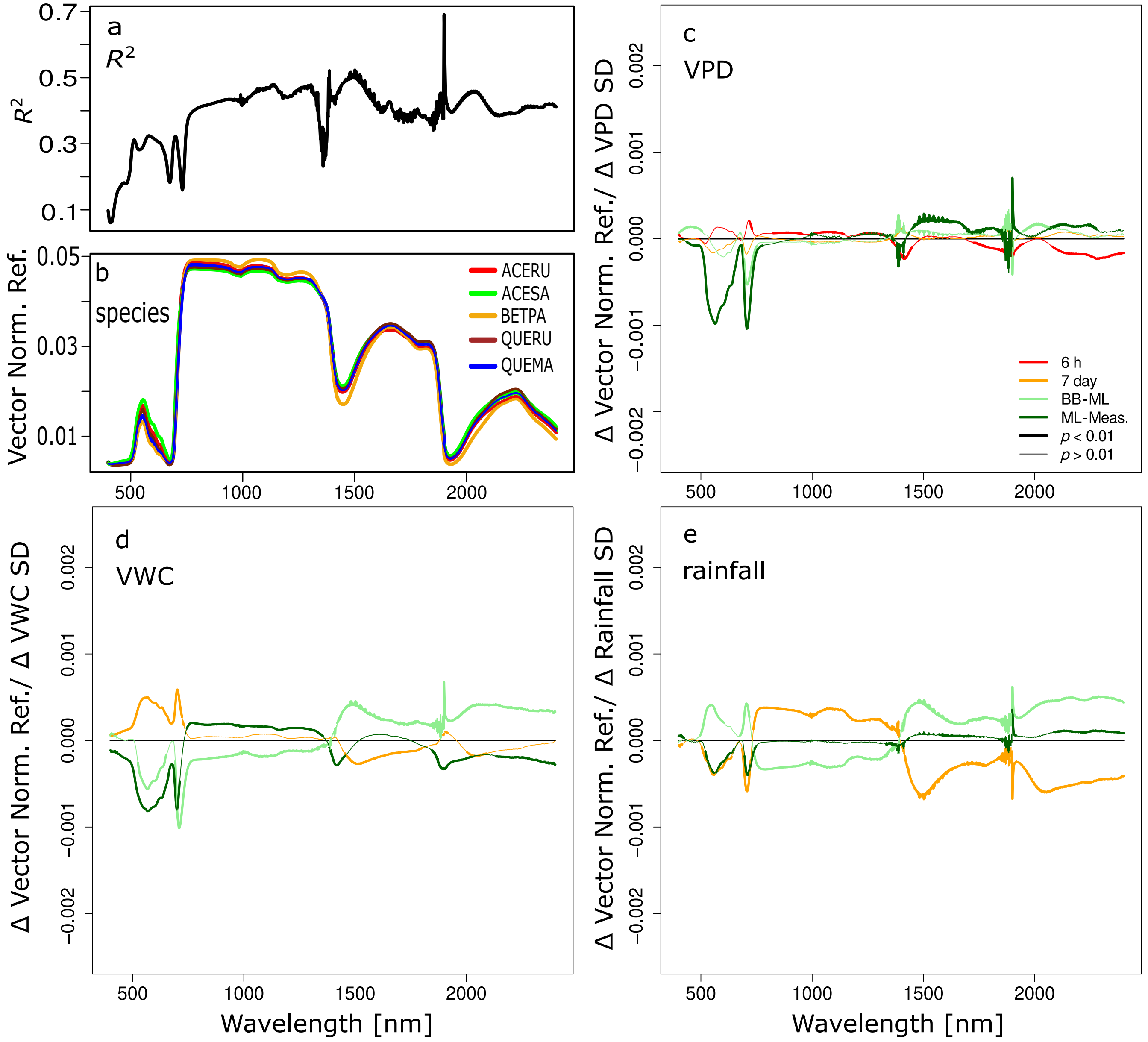

FIGURE 5 Goodness of fit and coefficients indicating the strength of the environmental covariates with spectra based on multiple linear regression (MLR). Panels (a) the coefficient of determination, R^2 (b) Species specific base spectra (the intercept of the MLR model, Equation 1). Each species is given a unique color. Panels (c–e) show coefficient strength expressed as the change in vector normalized reflectance per unit SD of the environmental metric. (c) Coefficient values for vapor pressure deficit (VPD), but note that due to the high correlation of VPD with aboveground temperature, this coefficient is indicative of the combined influence of VPD and surface temperature. The legend here applies to panels (d) and (e) as well. Statistical significance ($p < 0.01$) is indicated by the portions of the spectrum with a thicker line. (d) Coefficient values for volumetric water content (VWC). (e) Coefficient values for precipitation. All environmental data were transformed to mean zero and unit SD (z-scores) to allow for a direct comparison across environmental predictors.

that are most distinct in the NIR (e.g., *B. papyrifera* and *A. saccharum*) and within the SWIR (e.g., *Q. rubra*, *B. papyrifera*, and *A. rubrum*). These are the species mean spectra after accounting for the influence of the environmental variables that comprise the remainder of the model (Equation 1). It is unsurprising that species have distinct spectral signatures (Cavender-Bares et al., 2017; Meireles et al., 2020), but it is a useful check that this model is able to extract those differences from the data.

The MLR model has variable goodness of fit as shown by the estimate of the coefficient of determination, R^2 (Figure 5a), with generally poor goodness of fit in the visible that rises to an average of between 0.4 and 0.5 across much of the NIR and SWIR; there is also a notable variation between the main water absorption wavelengths in the SWIR region. Also, the model appears to show modest responsiveness to species identity and quite a bit to the environmental variables (Figure 5b–e). However, due to the estimated coefficients and modest correlation, the actual environmental influence on this region is much smaller because VPD, VWC, and rainfall have opposite signs (or negative correlation) for the major peaks occurring in the same time frame and thus cancel each other out (Figure 5c–e). Low sensitivity in the visible is due to the influence of light absorption by pigments. On the other hand, the MLR predictability in the NIR and SWIR is significantly higher and addresses a larger portion of the spectral variability.

The sensitivity to all three environmental variables changes with the timeframe under consideration and shows that reflectance is generally more sensitive (larger model estimated coefficients) to environmental conditions during leaf development (BB-ML) and the core of the growing season (ML-Meas) than to conditions immediately preceding measurement (days to hours prior to the measurement). For example, the MLR reveals that across the entire spectral signal, the highest sensitivity of leaf reflectance is to water (i.e., rainfall and VWC) during the leaf development time and main portion of the growing season. Rainfall, in particular, has a stronger influence in the 7 days preceding measurement than its longer-term average.

A closer examination of the MLR model sensitivity across different time frames shows three interesting features. First, the scale of sensitivity of the leaf reflectance across the entire spectrum changes with the time frame being considered. For instance, in the case of the VPD, during leaf development, the highest sensitivity is located in relatively small regions of the blue and red VIS and only in regions associated with water absorption in the SWIR. As noted above, the sensitivity of the spectrum to VPD must be interpreted as the joint sensitivity to VPD and surface temperature due to the high correlation between these environmental variables. However, in the middle of the season, the significance of the coefficients expands through the entire VIS, most of the SWIR, and parts of the NIR. In the days preceding the measurement, most of the areas previously significant in large swaths across the entire spectrum become comparatively insignificant but flip again in the hours immediately preceding the measurements to show the importance of broader areas in the NIR and SWIR that were not associated with any other time frames (Figures 5c and 6). Second, there is a notable change in sign from early leaf development (BB-ML) to a full canopy (ML-Meas) and days preceding the measurement across most of the spectrum in the case of rainfall (Figure 5e). There is a similar flip in VWC between BB-ML and ML-Meas times and days preceding the measurement (7d; Figure 5d); however, some caution is called for due to the temporal correlation within these variables (Appendix S1: Figure S4). Finally, there is more variability in model coefficients in the case of VPD during leaf development and the main core of the season specifically around water absorption bands (Figure 4a,c).

Environmental imprints across all environmental variables and time scales reveal striking patterns indicating the importance and role of water in spectral signals. For example, the positive coefficients of the VWC and Rainfall in the NIR region and negative in SWIR during ML-Meas and 7d periods suggest that at times when conditions are typically warmer and drier, the reflectance in NIR is higher while in SWIR it is lower, suggesting that leaves are going through not only water content changes but also some structural and biochemical changes. Moreover, the modest but different influence of the VWC and rainfall on the shortest time scale (7d) signal in the VIS and the tail of the SWIR likely reflects higher frequency shifts in leaf biochemistry in response to recent weather conditions and water availability (e.g., lack of rain and warmer conditions causing increased depletion of VWC). Lastly, precipitation (Figure 5e) often shows a coordinated response between the medium term (7 days) and longer term (ML-Meas) response across the NIR and SWIR. The 7-day signal is statistically significant across almost the entire spectrum and reinforces the observation (Miller et al., 1991) that rainfall within a week of a measurement campaign will have an outsized influence on the spectrum. Naturally, the influence of water on the spectrum in the NIR and SWIR is apparent in the strength of the coefficients across water absorption bands. Consequently, examination of the significant portions of the MLR coefficients and their respective locations on the leaf reflectance spectrum across variables and times suggests that the environment more strongly influences structural properties during the development of the leaf while more recent time periods are more reflective of changing chemistry and/or the physical presence of water.

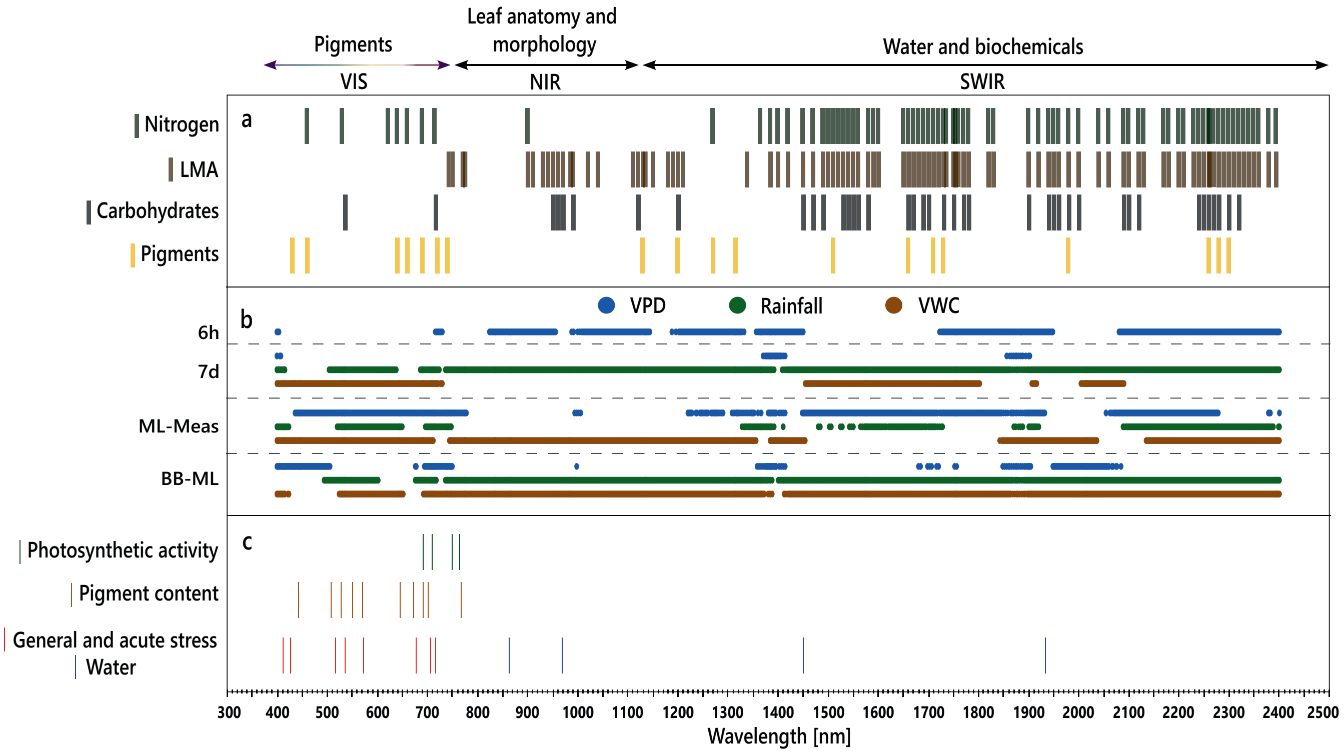

FIGURE 6 Climate imprints on spectra matched with known functional traits and spectral indices. Panel (a) indicates locations of wavelengths that are associated with selected traits' (Curran, 1989; Fourty et al., 1996; Kokaly et al., 2009; Kokaly & Skidmore, 2015; Wang et al., 2020). Panel (b) shows all important individual wavelengths as indicated by the multiple linear regression model for all three environmental variables at different times from leaf development (BB-ML), middle of the season (ML-Md), 7 days prior to measurement (7d), and 6 h before measurement (6h). Panel (c) indicates locations of wavelengths that are associated with specific spectral indices taken from the literature (Appendix S1: Table S3). LMA, leaf mass per area; NIR, near-infrared; SWIR, short-wave infrared; VIS, visible; VPD, vapor pressure deficit; VWC, volumetric water content.

Important wavelengths in relation to traits and spectral indices

All three analyses identified similar and statistically significant regions representing key chemical, structural, and physiological properties of the leaf (Figures 4–6; Appendix S1: Figures S5, S8, and S10), yet rarely did precisely the same wavelengths match across all three tests. In at least a few specific instances (e.g., 1906 nm for PCA, 1902 nm for PLSR, and 1900–1906 nm for MLR) the exact peaks were within a few nanometers of each other. So while the exact peaks were not aligned, the analyses often had similar broader regions of significance that occur near these peaks (compare and contrast Figures 4–6; Appendix S1: Figures S5, S8, and S10). Some of those common regions are associated with important features of leaf spectral reflectance related to pigments and processes in the region of VIS, structural traits, and water absorption in NIR, and biochemistry and water in SWIR.

Across the leaf developmental stages during the growing season, the important spectral regions shift and either broaden or shrink in spectral breadth. Four distinctive regions can be identified (450–580 nm, 650–750 nm, 1350–1450 nm, and 1850–1950 nm with some small differences) that are significant across all three analyses and all time periods, while the remaining spectral regions are dynamic in response to variation in the environmental variables across time periods (Figure 6; Appendix S1: Figure S5). For example, the primary importance of the VPD influence on spectra during leaf development (BB-ML) manifests on the edges of the VIS region and water absorption regions in the SWIR. However, this changes in the period spanning the middle of the season (ML-Meas) where the importance in the VIS region expands primarily in its central part (i.e., 500–580 nm), and also expands and shifts in the SWIR. The significant regions in the days preceding the measurement (7 day) disappear from the middle of the VIS region, with new significant wavelengths appearing in areas representing general stress (shorter VIS wavelengths associated with pigments), disappearing in the NIR, and shrinking considerably to narrowly reflect water absorption features in the SWIR. Finally, at the time immediately preceding measurements (6 h) significance shrinks in the visible to a very narrow portion of the shortest wavelengths, reappears at the red edge, and expands significantly in the NIR and around four distinctive features associated with water absorption in the NIR and SWIR. On the other hand, in the case of VWC, the VIS region changes only modestly through time, while the SWIR changes significantly. Effectively, the range of importance expands and contracts as time progresses throughout the season across all three environmental variables (Figures 4–6; Appendix S1: Figure S5).

Finally, a linear mixed-effects analysis of functional traits and spectral indices showed that for all six functional traits (i.e., nitrogen, Chl, Car, Ant, EWT, and LMA) and 19 out of 22 indices (e.g., Photochemical Reflectance Index, Water Band Index, Normalized Difference Water Index, Normalized Phaeophytinization Index, and CI; Appendix S1: Table S3) warming had a significant effect ($p < 0.0433$; Appendix S1: Table S6). In the case of rainfall, half of the estimated traits (i.e., EWR, LMA, and Chl) and 14 out of 22 indices were significantly affected ($p < 0.0438$; Appendix S1: Table S6). Generally, interactions were additive, and only in some individual instances did warming and rainfall reduction have interactive effects on functional traits and indices, which is not surprising because often warming has a dual effect: direct thermal and indirect nonthermal (i.e., warming also has a drying effect; see Reich et al., 2018; Stefanski et al., 2023). The most notable changes in functional trait responses estimated from the Prospect model we observed were: (1) a modestly significant increase in Nitrogen content (1%) warming, (2) a sizeable increase in chlorophyll and anthocyanins content with highest warming (8.3% and 11.3% respectively), and a significant decrease in EWT and LMA due to both warming and rainfall reduction (4.5% and 3.5% respectively).

Our stepwise approach to decomposing and analyzing the full range of green leaf spectral reflectance demonstrates that there are regions of the spectra in the VIS, NIR, and SWIR that have lasting fingerprints from the environment, likely setting up the overall trajectory of leaf development as well as more nuanced responses throughout the season across multiple functional traits and physiological processes which point toward a "fine-tuning" of leaf phenotype expression to environmental conditions.

DISCUSSION

In an open-air warming field experiment, we show that environmental variables leave distinct spectral imprints corresponding with regions of the spectra that are associated with important chemical, structural, and physiological properties of the leaf. Numerous studies have shown that spectra reflect how leaf functional traits and physiological processes are shaped by the environment (Gamon et al., 2005; Townsend et al., 2003) and that plant responses to the environment, including drought stress (Dao et al., 2021; Penuelas et al., 1997; Römer et al., 2012) impact leaf spectral properties. Historically, however, research incorporating foliar spectral information has focused on how either the whole spectrum can be used to predict traits or how single features can be used to describe plant characteristics and plant-environment relationships. Here we have demonstrated how examining the full spectrum of the leaf reflectance allows us to identify imprints of a leaf's sensitivity to the environment at short and long time scales across the growing season that capture a holistic phenotype response.

In general, we show that the spectral sensitivity to the environment varies in magnitude from modest changes across the full spectrum at short time scales to systemic changes that are fixed during sensitive periods of leaf development. Thus, we demonstrate that spectra capture complex plant-environment relationships, allowing us to make four ecologically meaningful inferences: (1) the spectra-environment relationships appear to be consistent across species (Figure 2; Appendix S1: Table S4), (2) we can predict environmental conditions that plants experienced from spectra and vice versa (Figures 3 and 5) and thus infer environmental conditions that impact spectra (which is the true goal), (3) by linking them to responses on a biochemical, structural, and process level (e.g., linking them to specific functional traits; Figure 6), and (4) these changes in the spectra-environment relationships through time reflect the trajectory of trait expression over the course of the growing season (i.e., structure during development, general stress throughout the season, and "fine-tuned" responses to recently experienced conditions; Figures 5 and 6).

The spectra-environment relationships that we have shown here appear to be consistent across species and are primarily driven by water content and water stress. Moreover, we have demonstrated that multiple environmental factors (e.g., temperature, rainfall, VPD) across a range of time scales (i.e., from the time of leaf development to hours before the measurement) alter spectral imprint in concert with the dynamic nature of leaf physiological processes affecting the direction and magnitude of influence they exert on the differences in spectral signals we observed among species (Figure 5a). This is in line with previous work that has shown that species with similar adaptations typically have similar physiological responses to warming and rainfall but vary in the magnitude of their responses (Reich et al., 2018; Stefanski et al., 2023). Some of this variation in the environmental imprints on spectral reflectance can likely be attributed to the combination of direct and indirect effects. For instance, changes in VPD at time scales of hours and days preceding the measurement influence components of the visible spectrum and the SWIR that primarily can be linked to stress activity of pigments, biochemical changes, and water content as leaves adjust to high-frequency changes in the weather (Drake et al., 2018; Xu et al., 2020). On the other hand, VPD experienced by plants during leaf development and over the bulk of the growing season likely relates to structural characteristics located in the VIS and SWIR regions of the spectral signal (Jacquemoud & Ustin, 2019; Poorter et al., 2009). Note that because VPD depends both on the amount of water in the atmosphere and on temperature the effects cannot be separated and generally VPD influence must be interpreted as the combined effect of VPD and temperature. Thus, the effects of VPD on spectra may result from changes in the gradient of water deficit on the soil–plant-atmosphere continuum across the season driven by shifts in either the water vapor or the temperature, or both. Consequently, it is possible that at the beginning of the season, any effects statistically associated with VPD may functionally be related to temperature—since water in the soil is not a limiting factor (VPD is quite low) during this part of the growing season at these study sites. Thus, VPD effect is likely associated with increased rather than decreased stomatal conductance at that time period (because conductance increases with temperature and VPD also increases with temperature) (Reich et al., 2018). However, in the middle of the growing season, when often water deficit increases on the soil–plant-atmosphere continuum because the water in soil may become limited along with a significant increase in temperatures, spectral responses to VPD are likely to be principally related to a drying creating a greater water deficit gradient on the soil–plant-atmosphere continuum affecting water status of plants.

The imprints on spectral signatures established by the leaf-environment relationship during early developmental stages related to structural characteristics are maintained over the season while other traits are more dynamic and change over time due to stress and resource availability (Ramirez-Valiente et al., 2015; Yang et al., 2016), or changes in biochemistry resulting from dynamic physiological adjustments (López-Hidalgo et al., 2023). The association of the environmental factors imprints with different traits and processes at different times suggests that, for example, environmental conditions experienced during a critical development period lock in trajectories for trait variation, leaving a lasting imprint in leaf optical properties at the peak of the growing season. Thus, it is not surprising that at shorter time scales and times immediately preceding the measurements, the spectra-environment relationship is generally weak. We consider that this result is likely due to increasing variability in more plastic functional trait responses to short-term changes (e.g., up to 7 days preceding the measurement) in environmental conditions, such as diurnal xanthophyll cycles and daily pigment pool sizes or changes in leaf water status. This conclusion is reinforced by the MLR multi-time scale analysis that shows the changes in the importance of different regions of spectra throughout the growing season, which supports the PLSR model's ability to detect environmental conditions from the time of leaf development but not from times immediately preceding the measurements. For example, the environmental conditions during early leaf development show a strong connection with the spectral indices that define leaf pigments and water content, nutrients, and structural compounds (e.g., cellulose, lignin, and carbohydrates; Figure 6). This is supported by numerous studies that show the seasonal change in pigment content, which typically increases dramatically at the beginning of the season, plateaus after full canopy formation, while at the same time, carbon content and some nutrients (e.g., calcium) increase gradually throughout the entire growing season, leading to an increase in LMA as well as phenological variation in other traits (Chlus & Townsend, 2022; Fajardo & Siefert, 2016; Kothari et al., 2018; McKown et al., 2013; Yang et al., 2016). Overall, our findings align with short- and long-term responses of tree carbon balance to environmental changes (Gessler & Zweifel, 2024): environmental conditions that act on short timescales can alter long-term trajectories of structural and functional traits, leading to the detected environmental imprints in spectra we show here.

Likely, the dynamic changes of imprints throughout the season along the spectrum are reflective of holistic leaf phenotype adjustments via the dynamic interplay of trait expression to the experienced environment. For example, the shift of the leaf sensitivity to VPD in the VIS region across time is likely related to a shift in importance from indices that are associated with leaf content (e.g., pigment, structure; Yang et al., 2016) to those that indicate acute and general stress as well as photosynthetic process adjustments (Figure 6; Gamon et al., 1992; Rabinowitch & Govindjee, 1965). These shifts are likely associated with environment-induced expression of specific traits and a reallocation of investments as, for example, we observed that warming treatments showed an increase in the content of chlorophyll and other pigments that play important roles in photosynthesis and stress protection (Rabinowitch & Govindjee, 1965; Xu et al., 2020). This emphasizes the increasing importance of regions of spectra that are associated with traits that can be linked to instantaneous physiological responses (e.g., photoprotection and photosynthetic activity; Drake et al., 2018; Xu et al., 2020) in the VIS and water status in the infrared. In addition, dynamic changes in the infrared region of the spectrum in response to VPD in times immediately preceding measurements are likely a response to both a decrease in water content as well as changes in solute concentration. This is supported by our observation of a decrease in the EWT and LMA in response to warming, which is likely related to a decrease in water availability in the hottest and driest treatments and matches previous work (Poorter et al., 2009), which showed a decrease in LMA with decreasing water availability and an increase in growth temperature. This apparent sensitivity in large swaths of the entire spectrum across environmental variables and time frames specifically in times immediately preceding measurements is likely related to the "fine-tuning" of leaf responses in real time to experienced weather conditions by adjusting its chemistry (pigments content), physiological processes (e.g., xanthophyll cycle), water content, and solute changes.

Effectively, because environmental conditions leave imprints throughout the entire reflectance spectrum, we have shown the importance of examining the entire spectrum rather than focusing on single spectral features and/or functional traits. Thus, this work demonstrates that whole spectrum signals can supplement work that focuses on single features of the spectra and/or functional traits, whether measured spectrally or in a conventional way, and provide new insight into the nuanced relationship between plants and the environment. Note that, given plants of differing life stages may employ similar responses to a range of environmental stressors (e.g., pigments for thermal protection and photoprotection), we anticipate these kinds of temporal imprints to be generalizable across life stages (i.e., from juveniles to mature trees). Naturally, there is great benefit in examining individual spectral features and functional traits that can be directly linked to properties and processes that are influenced by the environment, but individual features and traits may fail to capture the holistic plant-environment relationship (Anderegg, 2023; Reich et al., 2007). This is likely a result of a range of factors, including single traits lacking a direct relationship with climate (Aubin et al., 2016; Violle et al., 2007), a wide variation in the expression of traits (Albert et al., 2010; Reich et al., 2007), or multiple functions of traits and/or multi-trait coordinated responses as constrained by plants' genotype and species-specific adaptations (Falster et al., 2017; Sack & Buckley, 2020).

In sum, we showed that by examining the full spectral signal we can detect and quantify the direction and magnitude of the climate imprints in leaf spectral reflectance that are reflective of the leaf holistic phenotype expression resulting from the long-and short-term experienced environmental conditions. This holistic measure of the leaf phenotype can be associated with specific functional traits and demonstrates that spectra capture permanent and dynamic responses where the former relate to building a leaf that will be best suited to given climatic conditions while the latter suggests "fine-tuning" to environmental conditions experienced in real time. The generalizability of our findings to broader sets of species, including those with longer leaf lifespans or varying more strongly hydraulic and carbon-acquisition strategies, remains to be demonstrated and ought to be a focus of future research. However, our work has taken an initial step in quantifying the relationship between leaf spectral reflectance and global environmental change by: (1) leveraging key features of spectral data, (2) demonstrating the importance of capturing the temporal dynamics of leaf trait-environment relationships, (3) illustrating how examining the full spectral signal of plants grown in experimentally manipulated conditions offers a holistic examination of leaf phenotype response to the environment, and (4) adding to the growing body of research that demonstrates that the spectral signal can be considered a trait in its own right and moving the field toward a more universal application across species, and ultimately toward broad-scale upscaling.

AUTHOR CONTRIBUTIONS

This was highly collaborative work of the members of the ASCEND project integrating unique sets of expertise in the spirit of the "Authors of Artur's Round Table" through exciting and mind-tickling discussions, and passionate group effort through and through. Authors of the Artur's Round Table: co-first authors, Artur Stefanski and Ethan E. Butler; senior authors, Peter B. Reich and Jeannine Cavender-Bares; senior researchers, Rebecca A. Montgomery and Philip A. Townsend; junior researchers, Laura J. Williams, Raimundo Bermudez, J. Antonio Guzman Q., and Andrew Larson. However, we do recognize individual contributions as follows: Peter B. Reich and Rebecca Montgomery contributed to experiment conceptualization. Laura J. Williams, Artur Stefanski, Ethan E. Butler, Jeannine Cavender-Bares, Peter B. Reich, Rebecca Montgomery, Philip A. Townsend, Raimundo Bermudez, and J. Antonio Guzmán Q. contributed to study conceptualization. Peter B. Reich, Rebecca Montgomery, and Artur Stefanski contributed to design and methodology for the experiment. Artur Stefanski, Raimundo Bermudez, Peter B. Reich, and Rebecca Montgomery contributed to experimental implementation. Artur Stefanski, Jeannine Cavender-Bares, Raimundo Bermudez, Peter B. Reich, Rebecca Montgomery Laura J. Williams, and Ethan E. Butler contributed to study design and methodology.

Artur Stefanski, Raimundo Bermudez, Andrew Larson, and Laura J. Williams contributed to data collection and curation. Ethan E. Butler, J. Antonio Guzmán Q., Raimundo Bermudez, Laura J. Williams, and Artur Stefanski contributed to analysis. Artur Stefanski, Ethan E. Butler, Jeannine Cavender-Bares, Laura J. Williams, Peter B. Reich, Rebecca Montgomery, Philip A. Townsend, and J. Antonio Guzmán Q. contributed to interpretation. Artur Stefanski, Ethan E. Butler contributed to first draft. Jeannine Cavender-Bares, Laura J. Williams, Peter B. Reich, Rebecca Montgomery, Philip A. Townsend, Raimundo Bermudez, Andrew Larson, and J. Antonio Guzmán Q. contributed to review and editing. Artur Stefanski, Raimundo Bermudez, Peter B. Reich, Rebecca Montgomery, Philip A. Townsend, and Jeannine Cavender-Bares contributed to project administration and supervision. Peter B. Reich, Jeannine Cavender-Bares, Philip A. Townsend, Rebecca Montgomery, Laura J. Williams, Ethan E. Butler, and Artur Stefanski contributed to funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Stefanski et al., 2024) are available in the Data Repository for the University of Minnesota (DRUM) at https://doi.org/10.13020/5jj1-8040.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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