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

Hyperspectral leaf reflectance of grasses varies with evolutionary lineage more than with site

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Abstract

To predict ecological responses at broad environmental scales, grass species are commonly grouped into two broad functional types based on photosynthetic pathway. However, closely related species may have distinctive anatomical and physiological attributes that influence ecological responses, beyond those related to photosynthetic pathway alone. Hyperspectral leaf reflectance can provide an integrated measure of covarying leaf traits that may result from phylogenetic trait conservatism and/or environmental conditions. Understanding whether spectra-trait relationships are lineage specific or reflect environmental variation across sites is necessary for using hyperspectral reflectance to predict plant responses to environmental changes across spatial scales. We measured hyperspectral leaf reflectance (400–2400 nm) and 12 structural, biochemical, and physiological leaf traits from five grass-dominated sites spanning the Great Plains of North America. We assessed if variation in leaf reflectance spectra among grass species is explained more by evolutionary lineage (as captured by tribes or subfamilies), photosynthetic pathway (C_3 or C_4), or site differences. We then determined whether leaf spectra can be used to predict leaf traits within and across lineages. Our results using redundancy analysis ordination (RDA) show that grass tribe identity explained more variation in leaf spectra (adjusted $R^2 = 0.12$) than photosynthetic pathway, which explained little variation in leaf spectra (adjusted

$R^2 = 0.00$). Furthermore, leaf reflectance from the same tribe across multiple sites was more similar than leaf reflectance from the same site across tribes (adjusted $R^2 = 0.12$ and 0.08, respectively). Across all sites and species, trait predictions based on spectra ranged considerably in predictive accuracies ($R^2 = 0.65$ to <0.01), but R^2 was >0.80 for certain lineages and sites. The relationship between Vc_{max}, a measure of photosynthetic capacity, and spectra was particularly promising. Chloridoideae, a lineage more common at drier sites, appears to have distinct spectra-trait relationships compared with other lineages. Overall, our results show that evolutionary relatedness explains more variation in grass leaf spectra than photosynthetic pathway or site, but consideration of lineage- and site-specific trait relationships is needed to interpret spectral variation across large environmental gradients.

KEYWORDS

Chloridoideae, grasslands, Great Plains, niche conservatism, phylogenetic conservatism, phylogeny, plant functional types, remote sensing, spectroscopy, Vc_{max}

INTRODUCTION

Plant functional types (PFT) link physiology to community and ecosystem processes, providing a predictive framework for modeling ecosystem responses to global change across scales (Box, 1996; Díaz et al., 1998). The grass family (Poaceae) is one of the most diverse plant families with over 11,500 species (Soreng et al., 2017) and contains a high degree of variation in evolutionary history and ecological behavior, yet grasses are often simplified into coarse functional groupings based on photosynthetic pathway, that is, C_3 versus C_4 (Hattersley, 1983; Paruelo & Lauenroth, 1996; Woodward et al., 2004). The C_4 photosynthetic pathway describes a series of biochemical and morphological modifications to the ancestral C_3 pathway that reduces photorespiration and increases plant productivity, especially in low CO_2 conditions and in warm, high-light environments (Ehleringer & Monson, 1993; Pearcy & Ehleringer, 1984). The biogeography of C_3 and C_4 grasses is thought to reflect this advantage and has revealed patterns of productivity and distinct responses to climate variations (Epstein et al., 1997; Knapp et al., 2020; Ricotta et al., 2003; Tieszen et al., 1997; Wang et al., 2013). For example, in the Great Plains region of North America, there is a well-documented northwest to southeast gradient of increasing C_4 grass dominance associated with warmer and wetter climates (Paruelo & Lauenroth, 1996; Sage & Monson, 1999; Still et al., 2003).

Despite firmly established patterns of C_3 and C_4 differences, consideration of grass evolutionary lineages has revealed a new understanding of grass biodiversity and ecological functioning (Edwards et al., 2010; Lehmann et al., 2019). The C_4 photosynthetic pathway has evolved independently at various times in roughly 26 grass lineages (Grass Phylogeny Working Group II, 2012) that differ in their ecological and climatic niches (Edwards et al., 2007; Edwards & Still, 2008). This phylogenetic diversity may confound understanding of functional differences that have previously been attributed to the photosynthetic pathway (Liu et al., 2012; Taylor et al., 2010). Grass evolutionary lineages have distinct biogeographic distributions from C_3–C_4 distributions (Griffith et al., 2020). In the Great Plains, C_4-dominated regions are represented by two evolutionary lineages with contrasting environmental preferences—the tribe Andropogoneae (within the subfamily Panicoideae) tends to occur in warmer and wetter regions that experience frequent fire, whereas the subfamily Chloridoideae tends to occur in warmer and drier regions (Figure 1; Griffith et al., 2020; Lehmann et al., 2019). C_3-dominated regions are generally comprised of grasses in the large subfamily Pooideae, which is represented by several independent tribes. Importantly, the environmental responses of grass lineages can correlate with trait differences in ways that are obscured when considering only the photosynthetic pathway. For example, in a North American tallgrass prairie, grass species with the same life history strategy in two different C_4 tribes differed in their specific leaf area (SLA), leaf dry matter content (LDMC), leaf thickness, and vegetative height (Donnelly et al., 2023). These findings for grasses align with a review and synthesis of plant-water relations that found a phylogenetic signal more often in plant structural traits than in physiological traits (Ávila-Lovera et al., 2023). Given these and other studies, there is mounting evidence suggesting that lineage-based functional types may lead to a more

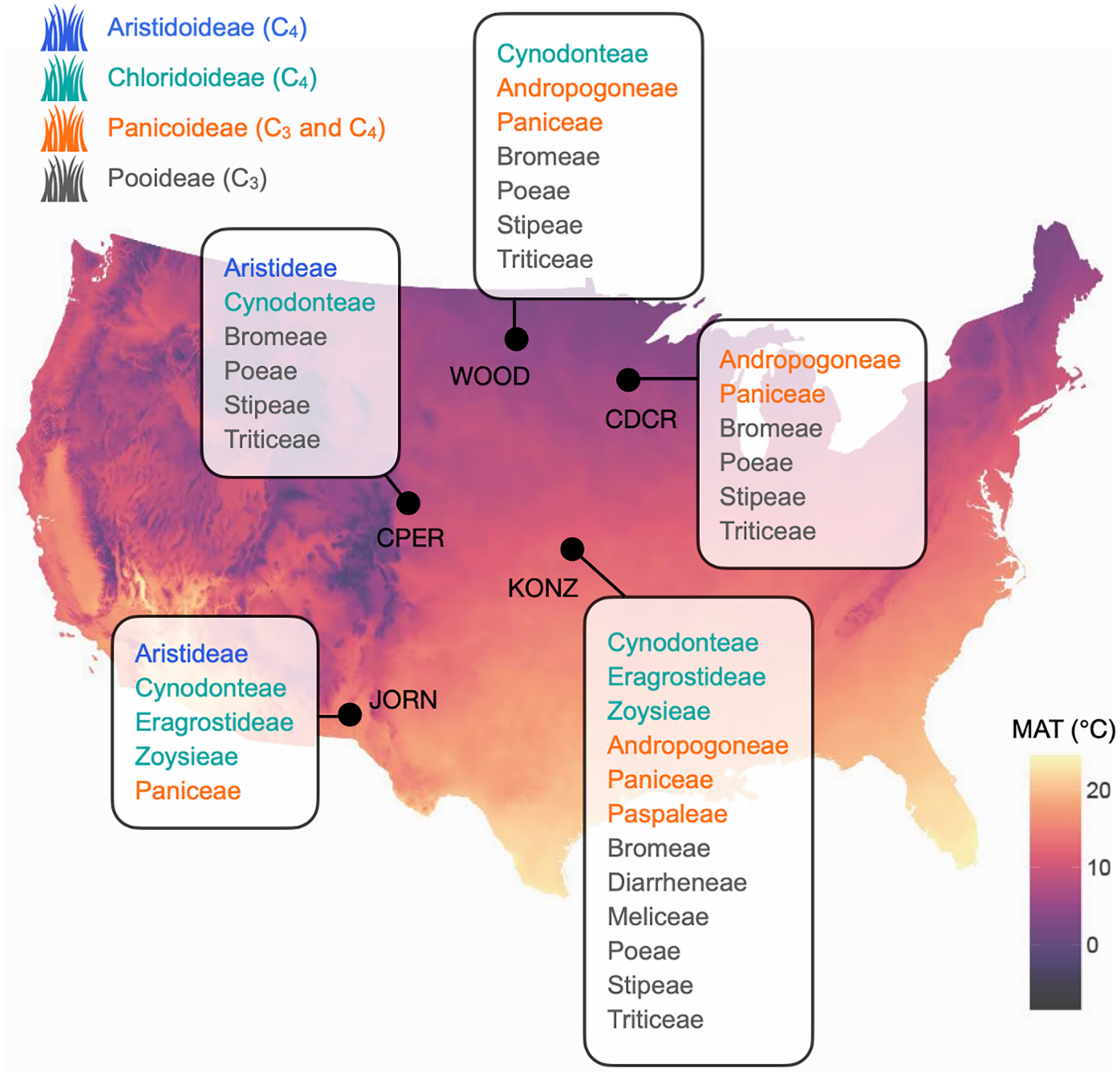

FIGURE 1 Hyperspectral leaf reflectance was collected from 66 grass species across five sites that vary in their climate and composition of grass evolutionary tribes (colored text for each tribe indicates subfamilies and associated photosynthetic pathway). MAT, mean annual temperature.

accurate representation of grass ecological behavior (Griffith et al., 2020).

Plant traits and functional group identity have been explored using leaf and canopy spectroscopy (e.g., Asner & Martin, 2009; Roberts et al., 1998; Schweiger et al., 2017; Serbin et al., 2014; Ustin et al., 1998; Wang et al., 2020). Spectroscopy provides a rapid method of assessing plant phenotypic differences across taxa and environmental gradients (Cavender-Bares et al., 2017). Leaf reflectance from the visible to the short-wave infrared regions (~350–2500 nm) can capture both structural and physiological differences in leaves, which may be linked to resource constraints and environmental differences (Ustin et al., 2004). Many plant traits, including in grasses, show coordinated trait strategies (Aspinwall et al., 2013; Taub, 2000; Wright et al., 2004), thus relationships between leaf spectra and individual traits may be confounded by trait covariation. On the other hand, relationships between spectra and plant traits that arise from trait covariance can be valuable for understanding plant growth strategies with a single integrated measure provided by spectroscopy (Cavender-Bares et al., 2016; Kothari & Schweiger, 2022; Ustin & Gamon, 2010).

Plant reflectance spectra can also reveal differences in evolutionary history if spectra are more similar among closely related species than among more distantly related species, a phenomenon known as phylogenetic conservatism (Cavender-Bares et al., 2016, 2017, 2022; Schweiger et al., 2018). Spectra may be phylogenetically conserved if trait covariance is constrained by evolutionary relationships and those traits affect plant reflectance.

Phylogenetic signal has been demonstrated in plant spectra at the leaf scale (e.g., Cavender-Bares et al., 2016; Griffith, Byrd, Taylor, et al., 2023; Meireles et al., 2020; Schweiger et al., 2018; Slapikas et al., 2024) as well as at canopy and landscape scales (e.g., Doughty et al., 2011; Griffith, Byrd, Anderegg, et al., 2023; McManus et al., 2016). Slapikas et al. (2024) showed high classification accuracy (>90%) of leaf spectra into grass evolutionary lineages. However, spectral differences due to site variation and photosynthetic pathway were not explicitly examined.

In contrast to leaf spectra showing phylogenetic conservatism, spectra and associated traits can also vary across environmental gradients because of intra-and interspecific trait variation (Meerdink et al., 2019; Sánchez-Azofeifa et al., 2009; Seeley et al., 2023). Trait variation across sites may be due to inter- and intraspecific trait differences from either plastic (variation within one genotype) or ecotypic (variation due to different genotypes) responses to environmental conditions, as well as differences in ontogeny and phenology. Although previous work showed that grass leaf spectra and traits are phylogenetically conserved (Slapikas et al. 2024), it is unclear what the relative effect of site differences is on spectral variation. Determining whether spectra-trait relationships are specific to evolutionary lineages or shaped by environmental variation across sites is pivotal for mapping across scales and understanding how grasslands and grass functional types will respond to environmental change. Here, we examine variation in grass leaf hyperspectral reflectance collected from five sites across the Great Plains of North America. We ask whether variation in spectra is better explained by evolutionary lineage, photosynthetic pathway, or site differences. We then compare the ability of spectra to predict 12 structural, biochemical, and physiological leaf traits. Specifically, we ask: (1) Does lineage explain more variation in spectra than photosynthetic pathway? (2) Does lineage explain more variation in spectra than site differences? (3) How well do leaf spectra predict leaf traits, and are spectra-trait relationships lineage-specific?

METHODS

Study sites and species

We analyzed leaf-level reflectance spectra from 66 grass species from five National Ecological Observatory Network (NEON) and/or Long-term Ecological Research (LTER) sites in North America (Figure 1). Before sampling at each site, we reviewed species lists and available plot data to create rank abundance curves, and targeted sampling of the most dominant species at each site. We additionally sampled species that occurred across multiple sites even when they were not dominant, and sampled species that were representative of dominant lineages. Measurements were made during peak greenness in the summers of 2020–2022: (1) Konza Prairie (KONZ), Kansas, (2) Colorado Plains Experimental Range (CPER), Colorado, (3) Chase Lake National Wildlife Refuge (WOOD), North Dakota, (4) the Cedar Creek Ecosystem Science Reserve (CDCR), Minnesota, and (5) Jornada Experimental Range (JORN), New Mexico. These sites span a broad geographic gradient ranging from cool and dry conditions (WOOD) to cool and mesic (CDCR), warm and dry conditions (CPER) to warm and mesic (KONZ), and finally hot and dry conditions (JORN). These sites also vary in photosynthetic pathway abundance and grass tribe representation. Mean annual temperature at each site is 15.7°C at JORN, 12.4°C at KONZ, 8.6°C at CPER, 6.7°C at CDCR, and 4.9°C at WOOD. Mean annual precipitation at each site is 271.2 mm at JORN, 344.2 mm at CPER, 419 mm at WOOD, 660 mm at CDCR, and 870 mm at KONZ.

We grouped the 66 grass species into their eight respective tribes: Andropogoneae (C_4; $n = 8$ spp), Aristideae (C_4: $n = 2$), Bromeae (C_3; $n = 3$ spp), Cynodonteae (C_4; $n = 13$), Diarrheneae (C_3; $n = 1$), Eragrostideae (C_4; $n = 2$), Meliceae (C_3; $n = 1$), Paniceae (C_3 or C_4; $n = 12$ spp), Paspaleae (C_4; $n = 2$), Poeae (C_3; $n = 6$), Stipeae (C_3; $n = 4$), Triticeae (C_3; $n = 7$), and Zoysieae (C_4; $n = 5$). See Appendix S1: Table S1 for the number of species in each tribe at each site.

Fifteen species occurred at more than one site: *Bromus inermis* (four sites; Bromeae), *Andropogon gerardii* (four sites; Andropogoneae), *Elymus smithii* (three sites; Triticeae), *Panicum virgatum* (three sites; Paniceae), *Phalaris arundinacea* (three sites; Poeae), *Schizachyrium scoparium* (three sites; Andropogoneae), *Aristida purpurea* (two sites; Aristideae), *Bouteloua curtipendula* (two sites; Cynodonteae), *Bouteloua dactyloides* (two sites; Cynodonteae), *Dichanthelium oligosanthes* (two sites; Paniceae), *Dichanthelium praecocius* (two sites; Paniceae), *Hesperostipa spartea* (two sites; Stipeae), *Poa pratensis* (two sites; Poeae), *Sorghastrum nutans* (two sites; Andropogoneae).

Leaf sampling and reflectance measurements

Fresh leaves were collected from dominant species at each site and sampled during peak greenness to account for potential differences in phenology. Peak greenness was determined from a NEON model using more than 15 years of Moderate Resolution Imaging Spectroradiometer (MODIS) normalized difference vegetation index (NDVI) data. Leaves were kept in a cooler until spectra were measured within a 2-h window of field collection. Five to eight fresh individual leaves for each species at each site were measured using an ASD FieldSpec 4 Hi-Res spectroradiometer with a leaf contact probe and the adaxial surfaces oriented toward the sensor. The contact probe contains a calibrated internal light source and a leaf clip with the standard black surface behind the leaves (Malvern Panalytical, Malvern, United Kingdom). In the case of a single leaf being too small to cover the area of the black background, several leaves were carefully placed side-by-side as close as possible to ensure no visible gaps between leaves while avoiding leaf overlap (Slapikas et al., 2024). The FieldSpec 4 Hi-Res NG measures radiance in the electromagnetic spectrum from 350 to 2500 nm with 2150 bands with a spectral resolution of 3 nm in the Visible Near Infrared range and 6 nm in the Shortwave Infrared range. A standard white reference built into the leaf clip (ASD Leaf Clip 2) was scanned for calibration and conversion to reflectance before measuring each species and also every 10 min. The FieldSpec spectroradiometer was set to 25 internal repetitions to reduce measurement variability and noise and then averaged to generate one spectral reflectance for each sample. The data were corrected for spectral discontinuities between the three spectroradiometer sensors using a jump correction as in Dorigo et al. (2006). Wavelengths shorter than 400 nm or longer than 2400 nm were removed to avoid spectrally noisy regions. Median reflectance values from the five to eight individual leaves across the 400–2400-nm wavelengths were used in analyses. A spline interpolation with a moving window of 10 nm using the "hsdar" package (Lehnert et al., 2019) in the software R (R Core Team, 2023) was used to reduce spectral bands from 2000 to 201 bands.

Leaf traits

Structural, biochemical, and physiological leaf traits were measured for 52 species collected from the same populations, but on different individual leaves from the leaf spectral measurements. Twelve leaf traits were measured (all sunlit fully developed leaves): leaf area, fresh leaf thickness, dry leaf mass, SLA, LDMC, % Carbon (%C), % Nitrogen (%N), δ^{13}C, δ^{15}N, C:N, $Vc_{\max_{25}}$, and $J_{\max_{25}}$. SLA (in square centimeters per gram) is defined as the ratio of leaf area to leaf dry mass. LDMC (unitless) is defined as the ratio of leaf dry mass to leaf wet mass. Fresh leaf thickness (in centimeters) is the thickness of the leaf in field conditions and wet leaf thickness (in centimeters) is the thickness of the leaf when fully hydrated. C:N is the ratio of carbon to nitrogen in the leaf and δ^{13}C (‰) is the ratio of ^{13}C isotopes to ^{12}C isotopes compared with a lab standard. $Vc_{\max_{25}}$ is the maximum rate of carboxylation of the enzyme Rubisco (in micromoles per square meter per second) and $J_{\max_{25}}$ is the maximum rate of electron transport (in micromoles of electrons per square meter per second) during the photosynthetic light reactions. Both are corrected to a standard temperature of 25°C using the "plantecophys" package in R.

Structural traits

For SLA, LDMC, and fresh leaf thickness, one leaf was clipped from each individual plant, and all structural trait measurements were performed on that same leaf. One to nine replicates were measured for each species at each site, with most species at each site having 4–5 replicates. Fresh leaf thickness was measured immediately in the field using a micrometer, where five measurements were taken randomly at various increments along the leaf and averaged. Leaf area was measured in the field using Leafscan (Version 1.3.21), a mobile application that measures the surface area of leaves (Anderson & Rosas-Anderson, 2017). For rehydration, the leaves were submerged in water for 24–48 h. Once rehydrated, the leaves were measured for wet mass, which was used to derive LDMC. Then, the leaves were dried at 60°C for at least 48 h, until they reached a constant weight before being measured for dry mass.

Biochemical traits

For C:N, δ^{13}C, and δ^{15}N numerous leaves per replicate were collected from one or multiple individuals in the immediate area to obtain enough biomass for measurements. Leaf C, N, δ^{13}C, and δ^{15}N were measured at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. The leaves were dried at 60°C for at least 48 h before being ground and homogenized.

Gas-exchange measurements

Net CO_2 assimilation rates at different internal leaf CO_2 concentrations (i.e., A-Ci curves) were measured using portable photosynthetic devices (LI-6400 and LI-6800; Li-COR, Lincoln, Nebraska, USA) at all sites except CDCR. One to eight replicates per species per site were measured with replicates from separate individuals. Photosynthetic photon flux density (PPFD) was set at 2000 μmol m^{-2} s^{-1} for all A-Ci curves. For CPER and WOOD sites using Li6400, the reference CO_2 was changed in the sequence of 400, 300, 200, 100, 50, 35, 150, 500, 800, and 1000 ppm. For KONZ and JORN sites using LI-6800, the dynamic A-Ci program was used. Plants were secured in the closed chamber for 10 min of acclimation at a reference CO_2 concentration of 400 ppm before the program started. Reference CO_2 concentration decreased from 1600 to 400 ppm in 4 ppm decrements. Block temperature was only controlled at the JORN site (40°C). The range of VPD_L (min, max) measured at each site is as follows: CPER: (1.61, 7.84); WOOD: (1.71, 7.08); KONZ: (1.50, 5.58); JORN: (1.80, 4.80). The data from the dynamic A-Ci curve were averaged every 10 data points before curve fitting. For C_3 species, "plantecophys" R package (Duursma, 2015) was used; and for C_4 species, we followed Zhou et al. (2019) with an Excel template to fit the curves. Maximum carboxylation (Vc_{max}) and electron transport rate (J_{max}) values normalized to 25°C were estimated from the fitted curves.

Statistical analyses

Redundancy analysis ordination (RDA) followed by variance partitioning were used to examine the relative effects of tribe, photosynthetic pathway, and site on spectra. An RDA is a combination of a constrained ordination and a regression with a response matrix (Legendre & Legendre, 1998). We ran a series of separate models, first testing the effects of tribe and photosynthetic pathway on the 201 spectral reflectance bands and then, the effects of tribe and site on the same bands. We also tested the effect of subfamily (Figure 1) but subfamily resulted in lower variance explained than tribe. We used adjusted (adj) R^2 values, which account for the different number of predictors, to partition the variance explained by each predictor using the "vegan" package (Oksanen et al., 2019) and the function "varpart" in the software R. The function "rda" was used to derive p-values.

To assess the ability of leaf spectral reflectance to predict leaf traits, we performed partial least squares regressions (PLSR) using the "pls" package in R (Mevik & Wehrens, 2007). PLSR is commonly used to predict relationships among spectroscopic data and functional traits (e.g., Ollinger & Smith, 2005; Serbin et al., 2014). PLSR reduces the large predictor matrix (i.e., 201 bands of reflectance after aggregating) to fewer, uncorrelated latent components. We split the data into 80% for training and 20% for validation using stratified sampling across each tribe. To explore whether trait predictions varied across lineages, we used subfamily instead of tribe because our sample size was too small at the tribe level for model training and validation (number of species per subfamily: Chloridoideae, $n = 17$; Panicoideae, $n = 19$; Pooideae, $n = 20$). We used 30% of the data for validation (70% for training) because of the smaller sample size when running separate models by lineage. We ran a third set of models using a leave-one-out cross-validation to predict traits at each site using data from the remaining four sites. We report both validation R^2 and the root mean square error of prediction (RMSEP). For each trait model, we chose the number of components (ncomp) that resulted in the highest validation R^2. All data analyses were performed using R Statistical Software (R Core Team, 2023).

RESULTS

Variance partitioning

Across all 66 species and five sites, almost all explained variation in grass leaf spectra was attributed to evolutionary lineage (i.e., tribes) rather than photosynthetic pathway (adj R^2 for the conditional effect of tribe = 0.12; adj R^2 for the conditional effect of photosynthetic pathway = 0.00; residual variance adj R^2 = 0.87; Table 1a). The effect of subfamily was also tested but resulted in lower variance explained than tribe, although subfamily still explained more variance than site. Tribe also accounted for more explained variation in spectra than site (adj R^2 = 0.12 for the conditional effect

TABLE 1 Variance explained in redundancy analysis ordination (a) by tribe and photosynthetic pathway and (b) tribe and site in leaf reflectance spectra from 66 species occurring across five sites.

Factor	Individual effect (adjusted R^2)	Conditional effect (adjusted R^2)
(a) Tribe + Photosynthetic pathway		
Tribe	0.137***	0.117**
Photosynthetic pathway	0.016**	−0.004
Both factors		0.133***
Joint		0.019
Residual		0.867
(b) Tribe + Site		
Tribe	0.137***	0.121**
Site	0.096**	0.081*
Both factors		0.217***
Joint		0.016
Residual		0.783

Note: "Both factors" refers to having both factors in the model combined, whereas "joint" refers to the amount of shared variance that is jointly explained by both factors.

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

of tribe holding the effect of site constant; adj $R^2 = 0.08$ for the conditional effect of site holding the effect of tribe constant; residual variance adj $R^2 = 0.78$). Importantly, shared variance between tribe and photosynthetic pathway, and tribe and site (i.e., the amount of variance that both factors jointly explain) was low (<2%). Overall, the combination of tribe and site better predicted leaf spectra variation than tribe and photosynthetic pathway given the lower residual variance (Table 1a,b).

Because a few common species represent each tribe across sites, we examined a subset of 15 species that occur across multiple sites. In this case, the effect of tribe relative to photosynthetic pathway was slightly larger, but adj R^2 values were not significant (Appendix S1: Table S2a). Site explained more variation in leaf spectra than tribe (adj R^2 for the conditional effect of tribe = 0.10 n.s.; adj R^2 for the conditional effect of site = 0.46; residual variance adj $R^2 = 0.52$; Appendix S1: Table S2b). Again, shared variance between lineage and site was low (<1%).

Leaf trait predictions

Using stratified sampling across each tribe, the highest validation R^2 for PLSR-based trait predictions using leaf spectra was for $Vc_{max_{25}}$ ($R^2 = 0.65$; RMSEP = 12.6%; ncomp = 3; Figure 2). Spectra also predicted $\delta^{13}C$ ($R^2 = 0.62$; RMSEP = 24.7%; ncomp = 7) reasonably well, with clear sorting by photosynthetic pathway. Remaining traits were predicted with variable accuracy by leaf spectra: LDMC ($R^2 = 0.55$; RMSEP = 15.1%; ncomp = 7), $\delta^{15}N$ ($R^2 = 0.47$; RMSEP = 9.1%; ncomp = 7), fresh leaf thickness ($R^2 = 0.44$; RMSEP = 12.0%; ncomp = 6), C:N ($R^2 = 0.38$; RMSEP = 19.0%; ncomp = 9), %C ($R^2 = 0.37$; RMSEP = 8.8%; ncomp = 5), %N ($R^2 = 0.36$; RMSEP = 19.7%; ncomp = 10), SLA ($R^2 = 0.24$; RMSEP = 12.5%; ncomp = 10), leaf dry mass ($R^2 = 0.11$; RMSEP = 10.5%; ncomp = 1), leaf area ($R^2 = 0.11$; RMSEP = 12.6%; ncomp = 3), and $J_{max_{25}}$ ($R^2 < 0.01$; RMSEP = 56.0%; ncomp = 1; Figure 2).

We also explored trait predictions for individual lineages, though we grouped by subfamily and not tribe for larger sample sizes to allow for model validation (Figure 3). Differences among lineages show that in general, Panicoideae and Pooideae spectra performed better at predicting traits (i.e., lower RMSEP). Chloridoideae had the highest RMSEP for 6 of the 12 leaf traits (LDMC, SLA, %C, C:N, $\delta^{15}N$, and $Vc_{max_{25}}$).

Trait predictions using leaf spectra were generally better at the wetter, resource-rich sites (i.e., WOOD, KONZ, and CDCR), whereas predictions for drier, resource-poor sites (i.e., CPER and JORN) were worse (Figure 3; Appendix S1: Figure S1). The trait $\delta^{13}C$ was well predicted by spectra at WOOD ($R^2 = 0.81$; RMSEP = 18.1%) and CDCR ($R^2 = 0.81$; RMSEP = 17.3%). A few leaf structural traits at select sites had an $R^2 > 0.50$ using PLSR: SLA at CDCR ($R^2 = 0.53$; RMSEP = 15.5%), LDMC at KONZ ($R^2 = 0.71$; RMSEP = 22.5%), fresh leaf thickness at WOOD ($R^2 = 0.61$; RMSEP = 17.6%).

DISCUSSION

Our results show that variation in hyperspectral leaf reflectance of grass species collected across a gradient of sites is better explained by evolutionary history than by photosynthetic pathway or site. The Great Plains are among the most endangered ecosystems in North America (Samson et al., 2004; White et al., 2000; Gibson 2009), spanning large climatic gradients that are associated with turnover in dominant grass species and ecosystem functions. Species compositional change and differences in ecosystem function across the Great Plains have typically been understood through differences in photosynthetic pathway (Knapp et al., 2020). Photosynthetic pathway was an evolutionary innovation to past periods of low atmospheric CO_2 and precipitation seasonality (Edwards & Smith, 2010; Ehleringer et al., 1991) resulting in fundamental differences in how plants fix atmospheric CO_2. However, these pathways are not necessarily connected to distinct grass traits and growth strategies (e.g., Donnelly et al., 2023; Griffith et al., 2020; Liu et al., 2012; Taylor et al., 2010).

The amount of spectral variance explained by both tribe and photosynthetic pathway was higher than for photosynthetic pathway alone (i.e., "both factors" in Table 1) indicating that both affect differences in spectra. However, the amount of shared variance explained was still relatively low, suggesting tribe and photosynthetic pathway are not confounded in their effects on leaf spectra and that they affect spectra differently. Each predictor appears to be associated with unique leaf traits, which may affect different regions of the spectra. In all our models, residual variance in spectra was high, which is not surprising given the high dimensionality of hyperspectral reflectance data (Cawse-Nicholson et al., 2022). Future work could evaluate various techniques to reduce noise beyond the spectral averaging and component selection that we performed (e.g., principal components analysis); though our approach here was to focus on the relative effects of each factor.

When considering differences by site, tribe was again the dominant effect on leaf reflectance. In other words, leaf reflectance from the same tribe across sites was more similar than leaf reflectance from the same site across lineages. This result demonstrates evolutionary constraints on grass leaf spectra and associated traits despite large

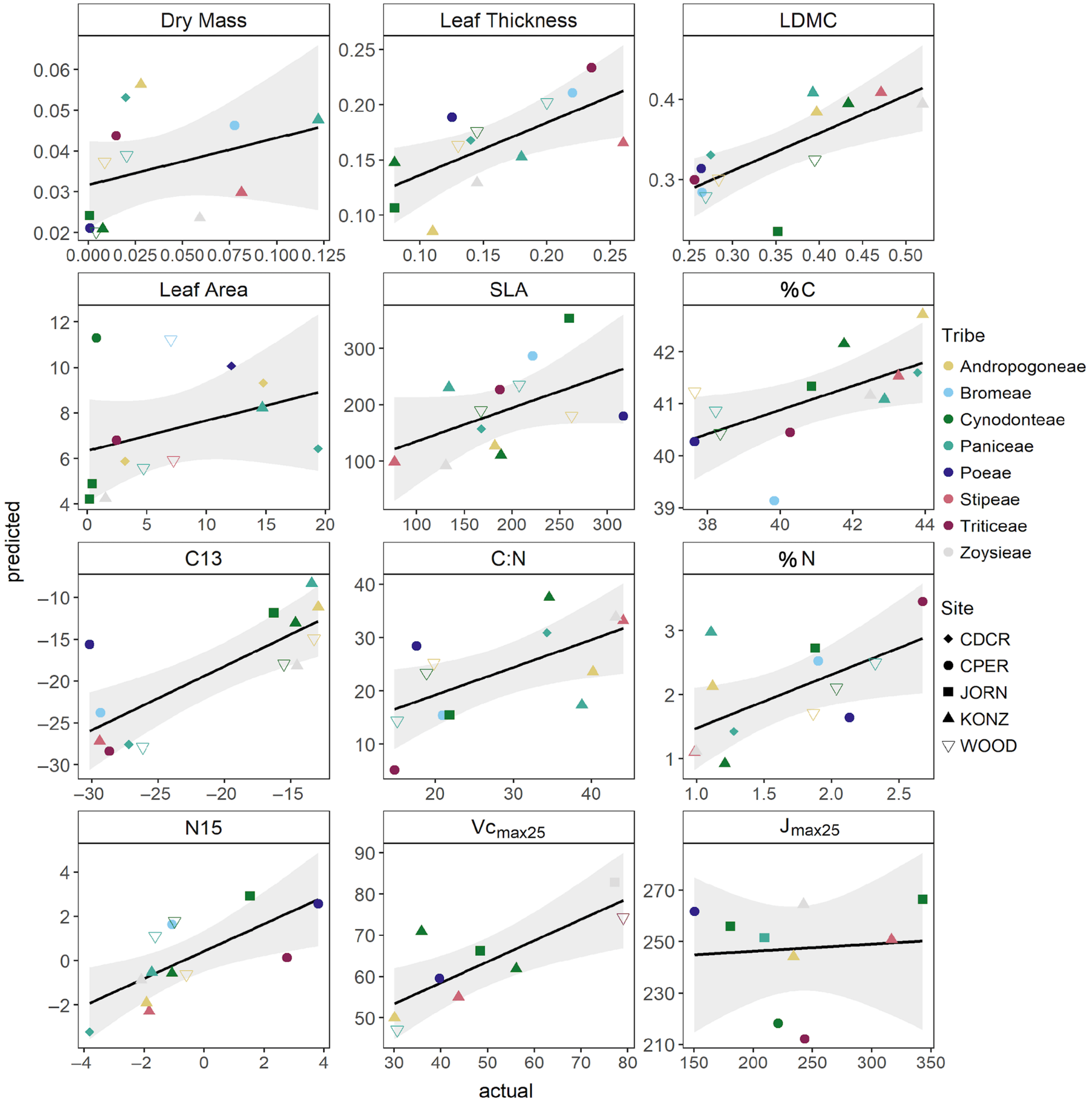

FIGURE 2 Validation plot from partial least squares regression (PLSR) predictions of 12 leaf traits using leaf spectra (400–2400 nm) from five sites across the Great Plains, USA. Points show median trait values for each species at a site against PLSR predicted values. Validation data are stratified by tribe so that 20% of species from each tribe was used for validation (80% of species from each tribe was used for training the PLSR model). LDMC, leaf dry matter content; SLA, specific leaf area. Grey shading = 95% confidence intervals around the predicted line.

environmental differences across sites. The larger effect of tribe over site furthermore suggests that there can be higher within-site spectral diversity, associated with phylogenetic diversity and possibly other factors, than across sites. Few studies have compared spectral diversity within versus across sites. However, remote sensing studies have recently shown that spectral dissimilarity can detect fine-scale species richness in grassland communities (Gholizadeh et al., 2018, 2020; Schweiger et al., 2018; Wang et al., 2018). On the other hand, Van Cleemput et al. (2023) found mixed support for the ability of spectral diversity to predict taxonomic diversity,

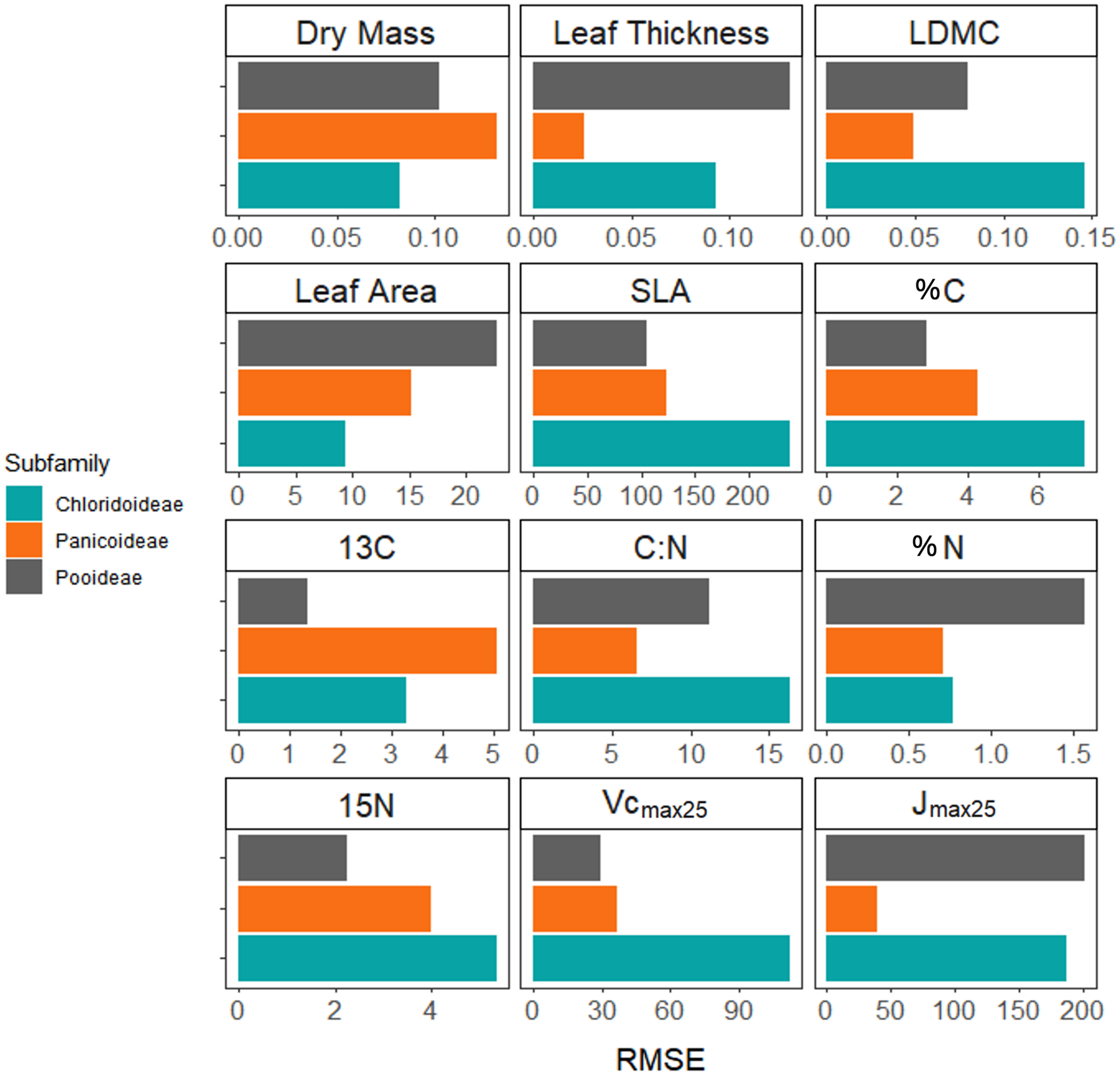

FIGURE 3 Comparison of root mean square error of prediction (RMSEP) from partial least squares regressions of 12 leaf traits using leaf spectra (400–2400 nm) for grass subfamilies. LDMC, leaf dry matter content; SLA, specific leaf area.

particularly at the sites that overlapped with our study (CPER, KONZ, and WOOD).

For a subset of species that occur across two or more sites, the most influential effect on spectra was site not tribe (Appendix S1: Figure S1). Thus, although variation in grass spectra was clearly constrained by evolutionary history, this result suggests that species with wider distributions respond to unique site conditions, whether through leaf plasticity or ecotypic variation (population local adaptation). *A. gerardii*, for example, is a perennial C_4 grass that is widespread across the Great Plains. This species displays large intraspecific trait variation, which may allow it to be dominant across a range of climatic conditions (Bachle et al., 2018). *P. virgatum* is another widespread C_4 grass species that shows local adaptation in morphological and physiological traits (Aspinwall et al., 2013; Donnelly et al., 2025). Thus, certain species and tribes may have a greater capacity to adapt or acclimate to future environmental change given their intraspecific trait variability (Valladares et al., 2014).

Despite measuring spectra and traits on different leaves (but from the same population within each site, that is, sampled within a few meters of each other), the explained variance in many of our trait predictions from leaf spectra (e.g., $Vc_{max_{25}}$, LDMC, $\delta^{15}N$, fresh leaf thickness, C:N, %C, %N) was within the range of other studies (e.g., Doughty et al., 2011; Kothari et al., 2023; Schweiger et al., 2017; Serbin et al., 2014; Van Cleemput et al., 2018; Wang et al., 2020). For trait predictions that were low compared with previous studies (e.g., SLA, leaf dry mass, leaf area, and $J_{max_{25}}$), either a larger sample size is needed, traits should be measured on the same leaves as spectra, and/or there are weak relationships with spectra for different taxa. Notably, our results showed a promising relationship between $\mathrm{Vc}_{\mathrm{max}_{25}}$, a key measure of photosynthetic capacity and a critical parameter in land surface models (Lu et al., 2020). $\mathrm{Vc}_{\mathrm{max}}$ is the maximum carboxylation rate of the enzyme Rubisco. Although $\mathrm{Vc}_{\mathrm{max}}$ is generally lower in C_4 compared with C_3 grasses (Collatz et al., 1998), $\mathrm{Vc}_{\mathrm{max}}$ can also vary with phylogeny (Donnelly et al., 2023; Griffith et al., 2020) and environmental factors (Croft et al., 2017; Groenendijk et al., 2010; Kattge et al., 2009; Smith et al., 2019). The recognized positive relationship between $\mathrm{Vc}_{\mathrm{max}}$ and leaf chlorophyll content may underlie the correlation between leaf spectra and $\mathrm{Vc}_{\mathrm{max}}$ in our results. Chlorophyll content and $\mathrm{Vc}_{\mathrm{max}}$ can be strongly linked because the absorption of light energy by chlorophyll is related to the amount of CO_2 catalyzed by Rubisco via coordination of the light and dark reactions of photosynthesis (Croft et al., 2017; Lu et al., 2020). Chlorophyll content is one of the most well-studied leaf traits using remote sensing (Ustin et al., 2009; Van Cleemput et al., 2018) and is well correlated with optical remote sensing measurements using both physically based radiative transfer models (Croft et al., 2020; Zarco-Tejada et al., 2004) and empirically based statistical models (Sims & Gamon, 2002). While $\mathrm{Vc}_{\mathrm{max}}$ can also be correlated with other leaf traits such as leaf %N and SLA, these relationships are often inconsistent across sites and species (Croft et al., 2017; Kattge et al., 2009; Walker et al., 2014). In our data, R^2 values for SLA and %N were not high, although site-specific relationships were in some cases stronger (Appendix S1: Figure S1). Predictions of $J_{\mathrm{max}_{25}}$ were surprisingly poor given the typically tight coupling of $J_{\mathrm{max}_{25}}$ with leaf chlorophyll content and with $\mathrm{Vc}_{\mathrm{max}_{25}}$ (Walker et al., 2014), perhaps due to differences in environmental stress at the time of data collection. Barnes et al. (2017) showed that variation in pre-dawn water potential affected predictions of $\mathrm{Vc}_{\mathrm{max}}$ using hyperspectral leaf reflectance only moderately, whereas predictive accuracy for J_{max} was considerably reduced. Other studies have found higher correlations between leaf reflectance and $\mathrm{Vc}_{\mathrm{max}}$, with a principal difference being that $\mathrm{Vc}_{\mathrm{max}}$ and leaf reflectance were measured under varying temperatures rather than across species (Serbin et al., 2012, 2015).

For Chloridoideae, traits that were poorly predicted in comparison to other lineages (LDMC, SLA, %C, C:N, and $\mathrm{Vc}_{\mathrm{max}_{25}}$; Figure 3) may covary in ways unique to that lineage. In other words, single traits do not underlie plant reflectance; rather it is the combination of traits, some of which may show stronger relationships to spectra than others, that affect predictive ability. Additionally, variation is fundamental to statistical prediction. Stronger trait predictions are leveraged when there is more variance to explain, that is, a greater range of trait values. Some of these traits were well predicted when using data across all lineages (e.g., $\mathrm{Vc}_{\mathrm{max}_{25}}$), indicating that the trait itself may have a spectral signature but is not well captured in Chloridoideae leaves, which all have low $\mathrm{Vc}_{\mathrm{max}_{25}}$ values. The strength of spectrally predicted traits when using data across all sites and lineages (Figure 2) were in some cases due to variation across lineages and in other cases to variation across sites. Leaf structural traits appear to cluster by lineage in trait predictions; for example, Cynodonteae (in the Chloridoideae subfamily) show clustering in dry mass, leaf thickness, and leaf area values (Figure 2). This result aligns with previous work showing more frequent phylogenetic signal in leaf structural traits, which are more fixed, as opposed to physiological traits which are typically more plastic in response to environmental variation (Ávila-Lovera et al., 2023; Donnelly et al., 2023).

Trait predictions limited to individual sites, using spectra from remaining sites to train the models, show improved accuracy for some traits and sites, and poor transferability for others. In general, trait predictions at the wetter sites (i.e., CDCR, KONZ, and WOOD) were better, suggesting environmental filtering of traits and spectra at the drier, more marginal sites (i.e., JORN and CPER; Appendix S1: Figure S1). JORN, an arid desert grassland, and CPER, a semiarid short-grass steppe, are the two sites characterized by water limitation, short growing seasons, and pulsed precipitation events. These habitats may filter for lineages or species that can tolerate and persist under resource-poor conditions (Cornwell & Ackerly, 2009; Díaz et al., 1998; Keddy, 1992); for example, Chloridoideae is a dominant lineage in these drier habitats. Traits may also vary uniquely within a species or lineage (plasticity or ecotypic intraspecific variation) as specializations to marginal resource-poor environments (e.g., Power et al., 2019) or in response to the competitive environment (e.g., Burns & Strauss, 2012; Turcotte & Levine, 2016). Investigations of how trait-spectra relationships vary with environmental conditions have shown that both biotic and abiotic factors can alter trait-spectra relationships (Asner & Vitousek, 2005; Sánchez-Azofeifa et al., 2009; Seeley et al., 2023). Our sampling design spanned five sites across a large biogeographic and environmental gradient, which came at a cost to more intensive sampling at specific sites. For some traits, greater sampling at individual sites may improve prediction accuracy, as we found generally positive relationships with observed spectra for many traits.

CONCLUSIONS

Our results from 66 grass species occurring across five sites in North America show that leaf hyperspectral reflectance is more strongly influenced by evolutionary relationships than by photosynthetic pathway or differences across sites. Phylogenetic conservatism of grass leaf spectra indicates distinct evolutionary constraints on responses to environmental change. Although relationships with individual traits were variable in their accuracy, the predictable phylogenetic variation in leaf spectra suggests that spectra can indicate unique trait coordination across lineages. Relatedly, our work shows that the transferability of PLSR models across taxa and general predictive models across biomes may be challenging (Helsen et al., 2011). Our results suggest that future research should focus on understanding relationships between spectral reflectance and trait networks that integrate form and function, not necessarily individual traits. In modeling efforts, especially for grasslands, explicitly including the diversity of grass lineages should lead to improved predictions of responses to global change (Anderegg et al., 2022; Griffith et al., 2020); plant reflectance spectra provide a novel avenue for understanding ecological similarity at multiple spatial scales. With the development of airborne and satellite hyperspectral sensors (e.g., NEON AOP, DESIS, and the planned NASA SBG mission) our work demonstrates the potential to examine underappreciated grass diversity and their ecological responses to future global change.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Pau et al., 2025) are available from Dryad: https://doi.org/10.5061/dryad.gf1vhhn0n.

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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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