

Seasonal patterns of spectral diversity at leaf and canopy scales in the Cedar Creek prairie biodiversity experiment



Ran Wang ^{a,*}, John A. Gamon ^{a,b,c}, Jeannine Cavender-Bares ^d

^a School of Natural Resources, University of Nebraska – Lincoln, Lincoln, NE 68583, USA

^b Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB T6G 2E3, Canada

^c Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada

^d Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, MN 55108, USA

ARTICLE INFO

Edited by Marie Weiss

Keywords:

Biodiversity
Spectral diversity
Optical diversity
Scale
Phenology
Remote sensing
Grassland

ABSTRACT

The relationship between biodiversity and spectral diversity is highly scale-dependent, and temporal variation in leaf morphological, biochemical traits and canopy structure can alter this relationship. However, the temporal dependence of the spectral diversity – biodiversity relationship is poorly understood, in part due to the difficulties of obtaining consistent measurements across space and time. Using leaf pigments and leaf and canopy reflectance throughout a growing season in the Cedar Creek prairie biodiversity experiment, we explored phenological effects on the scale dependence of the spectral biodiversity – biodiversity relationship. Leaf reflectance spectra displayed larger among-species variation than leaf pigments, indicating that leaf reflectance contained more information for distinguishing species than some leaf trait measurements. At the canopy scale, spectral variation derived using reflectance was mainly driven by among-species variation. The canopy scale spectral diversity was also influenced by changing vegetation percent cover, key phenological events (e.g., flowering), and disturbance (drought). Our results revealed that contrasting phenological patterns of spectral diversity metrics emerged at leaf and canopy scales. Because a misunderstanding of these contrasting temporal effects across spatial scales can lead to possible misinterpretations of the spectral diversity – biodiversity relationship or of their underlying causes, more research effort is needed to understand these cross-scale temporal effects.

1. Introduction

The temporal dimension of species distribution has long been considered in ecology and evolution (Preston, 1960) but has been far less explored than the spatial distribution of species (Magurran, 2007). However, including the temporal dimension in biodiversity sampling is critical, since spatial and temporal patterns of biodiversity influence each other. Both the timing and duration of sampling can influence the perception of species distribution, making the temporal dimension an essential consideration in species abundance distribution analyses (Magurran, 2007). Given that collecting biodiversity data is time-consuming and expensive (Bonaldo et al., 2008), biodiversity datasets are frequently incomplete and collected only over short periods (Magurran et al., 2010). The phenology of grassland has been largely neglected compared to that of forbs and woody plants due to the challenges of identifying species and reproductive stages in the field

(Primack and Gallatin, 2017).

Remote sensing offers the possibility to enhance biodiversity sampling through time across large spatial extents and has been used to assess multiple dimensions of biodiversity including phylogenetic, taxonomic, and functional diversity (e.g., Schweiger et al., 2018; Wang et al., 2018a; Wang et al., 2019; Wang and Gamon, 2019; Wang et al., 2020; Stasinski et al., 2021). One advantage of using remote sensing to monitor diversity is its potential to provide consistent, repeated observations that might reveal critical phenological information about diversity. However, when applied to studies of biodiversity, remote sensing also poses certain challenges, one of which has been the shortage of remotely sensed datasets at the temporal, spatial or spectral scales needed to understand changing patterns of biodiversity (Gamon et al., 2019).

The spectral diversity hypothesis (a.k.a. optical diversity) suggests that spectral variation detected by remote sensing can be related to

* Corresponding author.

E-mail address: ranwangrs@gmail.com (R. Wang).

species, functional and phylogenetic diversity, because the phylogenetic differences and resource limitations affect plant growth and leaf traits, canopy structure and phenology, all of which can affect vegetation optical properties (Gamon, 2008; Ustin and Gamon, 2010; Gamon et al., 2019; Wang and Gamon, 2019). Physiological and structural traits contribute to optical properties, yet both vary over time, and this phenological influence on spectral diversity as an analog for biodiversity has received limited attention (Gamon et al., 2020). The lack of understanding of the phenological component in the spectral diversity – biodiversity relationship severely limits our ability to detect diversity using remote sensing in part due to the limited perception of biodiversity patterns over the short term. Without attention to the temporal dimension, we are potentially missing much of the information that could be used to assess biodiversity with remote sensing.

Understanding the temporal dynamics of the spectral diversity – biodiversity relationship is expected to enhance diversity estimation using remote sensing (Ustin and Gamon, 2010; Cavender-Bares et al., 2017). Despite the recognized importance of the temporal component in remote sensing of biodiversity, only limited work has considered phenology in studies involving remote sensing of biodiversity (Rocchini et al., 2015; Wang and Gamon, 2019; Gamon et al., 2020; Gholizadeh et al., 2020). Previous studies in a Southern California Mediterranean ecosystem revealed that the ability of optical measurements to distinguish plant functional types varied with season (Zutta, 2003). In a prairie study, Gholizadeh et al. (2020) noted strong seasonal differences in the ability for airborne imaging spectrometry to detect biodiversity, and these differences were further modified by episodic disturbance (e.g. fire). Additionally, because species differ in their phenological responses (such as different seasonal patterns of bud break or flowering), it is possible that temporal trajectories of spectral data can further inform biodiversity studies. Thus, capturing spectral data across time can help identify the optimal time for distinguishing taxa with optical remote sensing, which might improve the performance of spectral diversity metrics in biodiversity estimation.

In this study, we used a time series of leaf and canopy reflectance data at Cedar Creek Ecosystem Science Reserve, Minnesota, USA in the summer growing season (late May to October) 2014 to investigate the dynamics of spectral diversity at both leaf and canopy scales for this prairie ecosystem. Besides the reflectance data, we also measured pigment concentrations of chlorophyll, carotenoids—including photo-protective xanthophyll cycle pigments—and anthocyanins, because the seasonal changes of leaf pigments due to ontogeny (Gamon and Surfus, 1999), senescence (Cavender-Bares et al., 2000), light environment (Logan et al., 1998) and stress (Verhoeven et al., 1999; Savage et al., 2009; Ramirez-Valiente et al., 2015) can affect leaf spectra over time (Gamon and Surfus, 1999), presumably influencing the spectral diversity – biodiversity relationship at both leaf and canopy scales. We hypothesized that at the leaf scale, leaf reflectance could exhibit larger among-species variation than leaf pigments; while at the canopy scale, spectral diversity could also be influenced by changing vegetation structure, cover, and key phenological events (e.g., flowering).

2. Materials and methods

2.1. Study site description

This study was conducted using the BioDIV experiment at the Cedar Creek Ecosystem Science Reserve, Minnesota, USA (45.4086° N, 93.2008° W). The BioDIV experiment has maintained 168 prairie plots ($9\text{ m} \times 9\text{ m}$) with nominal plant species richness ranging from 1 to 16 since 1994 (Tilman, 1997). The species planted in each plot were originally randomly selected from a pool of 18 species typical of Midwestern prairie, including C_3 and C_4 grasses, legumes and forbs. Weeding was done 3 to 4 times each year for all the plots to maintain the species richness (Tilman, 1997; Reich et al., 2012). Of the original 168 plots, we selected 11 monocultures and 24 plots with six replicates of every other

richness level (2, 4, 8, and 16) but with differing species combinations in this study (Wang et al., 2016a; Cavender-Bares et al., 2017; Schweiger et al., 2021).

2.2. Leaf reflectance

At the beginning of the season (late May to mid-June), leaf reflectance measurements were taken once a week for the 11 monocultures, since it was easier to identify those prairie species in the monoculture plots than in the high richness plots when plants were young. From July to early October, leaf reflectance measurements were taken every two weeks for 17 prairie species (Table S1) in all of the 35 plots. We randomly picked 3 leaves per plant on 5 plants per species to sample leaf reflectance using a field spectrometer (Unispec, PP Systems, Amesbury, MA, USA) coupled with a needle leaf clip (UNI501, PP Systems, Amesbury, MA, USA) with an internal halogen light source. The needle leaf clip allowed a narrow field of view (0.6-mm-diameter), which enabled sampling of small, narrow leaves (e.g. grass blades). For each species, leaf measurements were preceded with a dark and a white reference scan (Spectralon, Labsphere, North Sutton, NH, USA). Leaf reflectance was calculated by dividing each leaf measurement by a white reference scan after subtracting a dark spectrum from each measurement. The spectrometer has a nominal spectral range from 350 to 1100 nm with 2–3 nm band spacing and 10 nm full width at half maximum (FWHM), and a linear interpolation was used to estimate reflectance at 1-nm intervals. To track the change of leaf ‘greenness’ through the season, we used leaf-level NDVI formulated as follows:

$$NDVI = \frac{\rho_{800} - \rho_{680}}{\rho_{800} + \rho_{680}} \quad (1)$$

where ρ_{680} and ρ_{800} indicate the reflectance at 680 and 800 nm, respectively. On each sampling day, leaf reflectance were taken from 10 am to 4 pm. This leaf reflectance dataset (doi: [10.21232/i6N36jZ7](https://doi.org/10.21232/i6N36jZ7)) is available at the EcoSIS Spectral Library (ecosis.org).

2.3. Leaf pigments

To investigate the phenological properties of leaf pigments, we sampled leaf materials to determine leaf pigment concentrations over the growing season. Leaf disks (0.2 cm^2) were collected using a hole punch from the same leaves used for leaf reflectance sampling. These leaf disks were wrapped in aluminum foil, stored in liquid nitrogen immediately, and later transferred to a -80°C freezer for long term storage. High-performance liquid chromatography (HPLC; Agilent 1200, Agilent Technologies Inc., Santa Clara, California, USA) was used for pigment analysis at the University of Minnesota following methods previously described (Savage et al., 2009; Kothari et al., 2018; Schweiger et al., 2018). We calculated pigment concentrations for total chlorophyll (chlorophyll *a* and *b*), xanthophyll cycle pigment pools (V + A + Z; the sum of violaxanthin, antheraxanthin and zeaxanthin concentrations), neoxanthin, lutein, β -carotene and anthocyanins.

2.4. Canopy reflectance

In the 35 plots, canopy spectral reflectance was measured every two weeks over most of the 2014 growing season (late May to late August) and once a month during senescence (September to October) with a hand-held, dual-channel spectrometer (Unispec DC, PP Systems, Amesbury, MA, USA). On each sampling day, canopy reflectance were taken from 11 am to 3 pm under clear skies, i.e. within about 2 h of solar noon. Taking simultaneous measurements of both upwelling and downwelling radiation and cross-calibrating with a white reference calibration panel (Spectralon, Labsphere, North Sutton, NH, USA) allowed us to correct for the atmospheric variation during the sampling period. The spectrometer foreoptic was held at a distance of 2 m above the ground,

providing a ground sampling size (instantaneous field of view) of approximately 0.5 m. In each plot, reflectance measurements were taken at a half-meter interval along the northern-most plot edge on each sampling date, providing a consistent subsample of each plot ($n = 17$) over the growing season. Because of the relatively short stature of prairie vegetation, a typical sample contained multiple individual plants and anywhere from 1 to 16 species, depending upon the plot. The constraint of sampling only the northernmost plot edge of each was imposed to avoid disturbance from people entering the experimental plots and because sampling the entire plot using automated means (e.g. a tram system, Wang et al., 2016a) was prohibitive in terms of time (it takes several weeks to fully sample 30 plots due to the time required to set up automatic sampling).

To show the seasonal vegetation structural changes, canopy-level NDVI was calculated using canopy reflectance values at 680 and 800 nm following Eq. (1). We also applied a vector normalization method (Feilhauer, 2010) on the canopy reflectance data to calculate a brightness corrected reflectance product that kept the overall spectral shape while removing brightness differences. Vector normalization is often used for plant trait studies where the effects of canopy structure and illumination can cause large differences in “brightness,” possibly obscuring certain leaf traits. This allowed us to compare non-normalized reflectance spectra (influenced by brightness changes) to vector-normalized spectra (minimizing the brightness changes). This canopy reflectance dataset (doi:10.21232/C2Z070) is available at the EcoSIS Spectral Library (ecosis.org).

2.5. Partitioning of variance at leaf and canopy scales

To better understand the component contributions to the variation in the leaf pigment concentrations and the leaf and canopy reflectance data, we partitioned the total variance (total sum of squares, SS_t) into within- (within species sum of squares, SS_w) and among-species (among-species sum of squares, SS_a) components at leaf and canopy scales using a multivariate analysis of variance (MANOVA) method (Anderson, 2001). For the pigment data, we ran a principal components analysis on the pigment correlation matrix and partitioned the variance of pigment values in the principal components space to normalize differences in absolute values among pigments. For the leaf and canopy (raw and vector normalized) reflectance, MANOVA was applied for partitioning the spectral variance based on Euclidean distance between spectra (Price, 1994):

$$SS_a = \sum_{i=1}^S d_i^2 * n_i \quad (2)$$

$$SS_t = \sum_{i=1}^{M-1} \sum_{j=i+1}^M d_{ij}^2 \quad (3)$$

$$SS_w = SS_t - SS_a \quad (4)$$

where S indicates species richness and M indicates total measurements. d_i is the distance from the centroid of i^{th} species to the centroid of all the measurements multiplied by the number of individuals of the i^{th} species (n_i), and d_{ij} is the distance between two individual spectra (i and j). The Euclidean distance (d) between two spectra (ρ_1 and ρ_2), is the root mean square difference between them, averaged over the whole spectral range:

$$d = \left[\frac{1}{N} \sum_{i=1}^N [\rho_1(\lambda_i) - \rho_2(\lambda_i)]^2 \right]^{1/2} \quad (5)$$

where N is the number of wavelengths.

To compensate for the variation in sample size among different sampling dates and compare variance partitioning across the growing

season, we calculated the mean sum of squared (MSS) Euclidean distance deviations by dividing SS_a , SS_t and SS_w with their associated degrees of freedom as:

$$MSS_a = \frac{SS_a}{S - 1} \quad (6)$$

$$MSS_t = \frac{SS_t}{M - 1} \quad (7)$$

$$MSS_w = \frac{SS_w}{M - S} \quad (8)$$

2.6. Spectral diversity – biodiversity relationship at canopy scale

To investigate the spectral diversity – biodiversity relationship at the canopy scale, we took ground vegetation percent cover measurements of the selected 35 plots on June 19 and August 1 in 2014. Percent cover was determined by visual inspection within nine 0.5 m × 0.5 m quadrats, placed every meter, starting 50 cm from the north-facing edge of the plot for a total of 9 subsamples per plot. Percent cover was estimated for each species as the nearest 10% that each species occupied of the total quadrat area, and then summed. Vegetation coverage did not necessarily sum to 100% if bare ground was exposed, or if species overlapped (Wang et al., 2016a). To represent the species diversity of each plot, we calculated Shannon's index (H) for each plot on a vegetation percent cover basis as

$$H = - \sum_{i=1}^D p_i \times \log p_i \quad (9)$$

where D indicated the total number of species and p_i was the percentage cover of species i in the plot.

We calculated the coefficient of variation (CV) of the spectral reflectance in space as the spectral diversity index for each plot as

$$CV_{plot} = \frac{\sum_{\lambda=400}^{1000} \left(\frac{\sigma(\rho_\lambda)}{\mu(\rho_\lambda)} \right)}{N} \quad (10)$$

where ρ_λ denotes the reflectance at wavelength λ , and $\sigma(\rho_\lambda)$ and $\mu(\rho_\lambda)$ indicate the standard deviation and mean value of reflectance at wavelength λ across all the measurements in one plot, respectively. To minimize the soil effects on canopy scale spectral diversity, we multiplied the spectral diversity indices by the vegetation percent cover datasets to calculate soil corrected spectral diversity indices for each plot on the same two sampling dates (Gholizadeh et al., 2018). CV was then related to Shannon's index for the two sampling dates to test the canopy-scale spectral diversity – biodiversity relationship.

2.7. Time series spectral diversity at leaf and canopy scales

To investigate the seasonal dynamics of pigments and spectral diversity at leaf and canopy scales over the growing season, we calculated the convex hull volume (CHV), which has been used as an index both in trait-based community ecology (Cornwell et al., 2006) and spectral diversity (Dahlin, 2016), for each species using the first three PCs of leaf pigments, leaf reflectance and canopy reflectance, respectively.

3. Results

The climate in this area typically features warm and wet summers. However, in the summer 2014, a period of high temperatures and drought occurred from July to early August (Fig. 1), leading to a decline in surface soil moisture and a brief period of plant water stress (Wang et al., 2016a). The drought affected plant phenology and, consequently, the vegetation reflectance, expressed as decreases in canopy-level NDVI in the mid-season (Fig. 1). It also provided clues on how a short period of

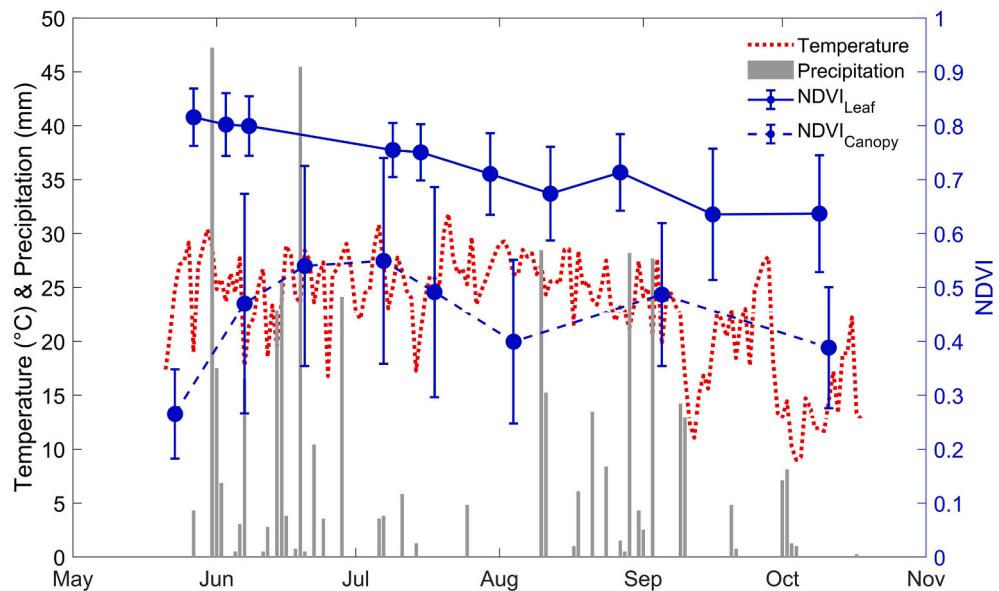


Fig. 1. Time series of maximum daily air temperature, precipitation and leaf- and canopy-level NDVI in the summer 2014. Temperature and precipitation records were collected from Cedar Creek weather station located approximately 0.76 km from the BioDIV experimental plots. The vertical bars of time series NDVI indicate the standard deviation.

disturbance can affect the spectral diversity – biodiversity relationship.

Partitioning of the total sum of squares (SS_t) between the among-species sum of squares (SS_a) and the within-species sum of squares (SS_w) allowed us to understand the among- and within- species contributions to the total variation, presuming that a larger SS_a to SS_t ratio indicated a larger difference between species than within species (Anderson, 2001). For leaf pigments, the maximum MSS_a occurred in late June, while MSS_w and MSS_t peaked in mid-July and decreased during senescence (Fig. 2). The maximum SS_a to SS_t ratio was found in mid-July with more than 60% of the total variation explained by the among species variation, while in early June, less than 35% total variation was explained by among species variation.

For the leaf reflectance data, the maximum SS_a/SS_t occurred in mid-July when about 70% of the total spectral variance was explained by the

among species variance (Fig. 3). The high temperature and drought at peak season (Fig. 1) apparently affected the total- and within-species spectral variance and coincided with an increase in the total and within-species spectral variance and a decrease in among-species variance in leaf reflectance spectra (SS_a/SS_t) was slightly higher than the same ratio calculated using pigment data (Fig. 2) collected at a similar time.

For the monoculture canopy reflectance, among-species variance explained most of the total variance across the growing season (Fig. 4). For the canopy reflectance without vector normalization, the maximum SS_a/SS_t value occurred in mid-June and was subsequently reduced during the mid-season drought. The SS_a/SS_t derived using vector normalized canopy reflectance was far less sensitive to the mid-season drought, suggesting that the mid-season drought mainly affected

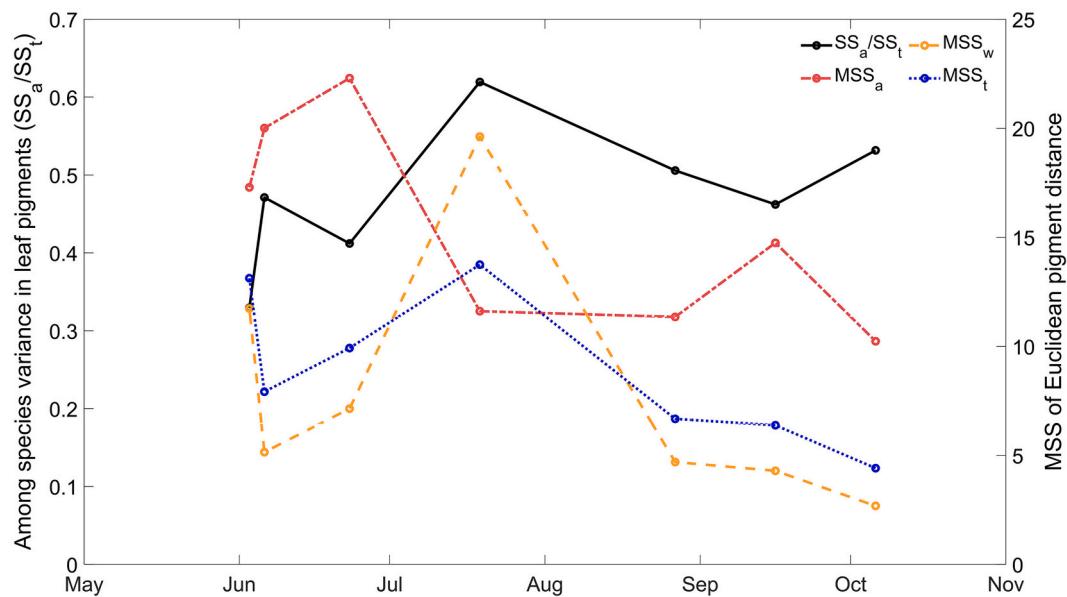


Fig. 2. Proportion of among species variance (SS_a) expressed relative to the total variance (SS_t) and mean sum of squares (MSS) of within-species variance (SS_w), among species variance (SS_a) and total variance (SS_t) over the course of the growing season derived using leaf pigment data.

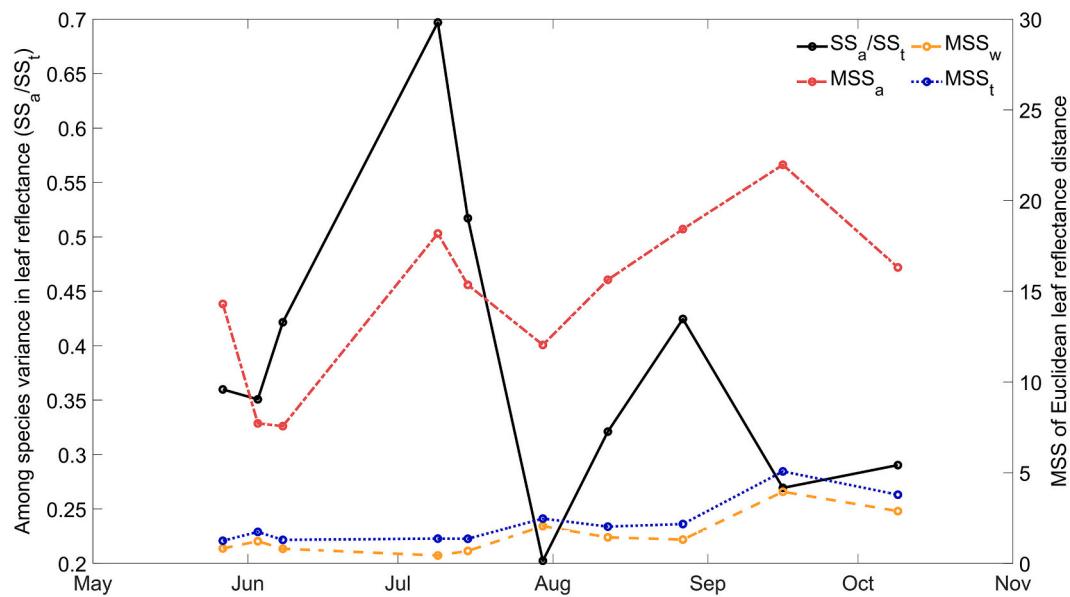


Fig. 3. Proportion of among species variance (SS_a) expressed relative to the total variance (SS_t) and mean sum of squares (MSS) over the course of the growing season derived using leaf reflectance.

canopy albedo related to leaf or canopy structure, i.e. the “brightness” of the spectra (Fig. S2 and Everitt and Nixon, 1986). The two peaks of SS_a/SS_t ratio in the vector normalized canopy reflectance (Fig. 4) coincided with flowering seasons of *Lupinus perennis* with purple flowers and *Amorpha canescens* having leaves with dense, white pubescence (Fig. S3 in the supplemental section) that can increase visible reflectance values (Ehleringer and Björkman, 1978; Doughty et al., 2011). In this case, flowering and leaf pubescence appeared to alter the canopy-level spectral diversity.

The soil corrected canopy-level spectral diversity indices (CV) showed linear relationships with Shannon's index on both sampling dates (Fig. 5). The weaker relationships derived from the June 19 data than from the August 4 data were largely influenced by the three points that had low species diversity level (Shannon's Index ≈ 0.5) but high spectral diversity (Figs. 5 and S3 in the supplemental section). These three big spectral diversity values occurred in two plots dominated by *Lupinus perennis* that had large number of purple flowers (Fig. S3 in the supplemental section) and the *Amorpha canescens* monoculture that had highly reflective leaves. In this case, flowering and leaf pubescence appeared to confound the canopy level spectral diversity and increased the spectral diversity values to a greater extent than the most species-diverse plots.

The time series of convex hull volume (CHV) showed the change in both the intraspecific (mean) and interspecific (standard error) diversity through the season, revealing different seasonal patterns of spectral diversity at leaf and canopy scales (Fig. 6). The largest CHV derived using pigment data peaked in early June, while the maximum CHV derived using leaf reflectance occurred in September (Fig. 6). Leaf senescence caused increased spectral diversity, shown as large convex hull volume (Fig. 6a), and small among-species variance to total variance ratio (Fig. 3). At the canopy level, vector normalization influenced the PCA loadings across the wavelength (Fig. S2) and consequently affected the phenological pattern of canopy CHV (Fig. 6). For CHV obtained using canopy reflectance without vector normalization, CHV values peaked around early July (Fig. 6b), a time when species-specific traits that influence optical properties—such as flowers and leaf pubescence—in addition to leaf pigments affected overall spectral diversity. For CHV calculated using vector normalized canopy reflectance, a large CHV value occurred in the early August, indicating that the vector normalized reflectance emphasized the variations in spectral

shape due to variation in pigments (Fig. 6a) by removing the brightness variation caused by short-term water stress (Figs. 6b and S2 in the supplemental section).

4. Discussion

Our results revealed a strong scale dependence on the seasonal spectral biodiversity – biodiversity relationship in a prairie ecosystem, resulting from contrasting phenological influences on spectral diversity indices at leaf and canopy scales. Similarly, seasonal variation of reflectance at the leaf scale was different from that of pigments, which represent common leaf traits that affect both leaf and canopy reflectance spectra. These results appeared to vary with the data analysis method used (i.e., with or without vector normalization). The results of this study indicate that different factors, both biological and statistical, affect the seasonal patterns of spectral diversity at leaf and canopy scales.

4.1. Phenology of spectral diversity at the leaf scale

Leaf reflectance spectra comprise integrative representations of plant phenotypes (Schweiger et al., 2018) that vary with time (Ustin and Gamon, 2010). Besides variations in pigment, temporal changes in leaf biochemical variation among species, such as variation in water and structural carbohydrates can affect leaf reflectance (Roberts et al., 2004). During the early to peak growing season, while pigment concentrations vary across species and functional groups (Kothari et al., 2018), the among-species variation to total-variation ratio for leaf reflectance was larger than its counterpart for leaf pigments (Figs. 2 and 3), indicating that leaf reflectance can reveal larger among-species variation than leaf pigment concentrations. This agrees with observations that leaf reflectance spectra are aggregated indicators of leaf biochemical and biophysical properties, and often contain more information than a limited set of traditional plant traits (pigments in this case), and generally outperform trait-based models in differentiating plant species (Cavender-Bares et al., 2016; Schweiger et al., 2018), detecting phylogenetic and functional diversity (Schweiger et al., 2018), and tracking leaf age (Chavana-Bryant et al., 2017).

Another study that used the same pigment data (Kothari et al., 2018) reported declining pigment concentrations in the early-to-midseason, possibly due to the ontogenetic changes in the developing leaves

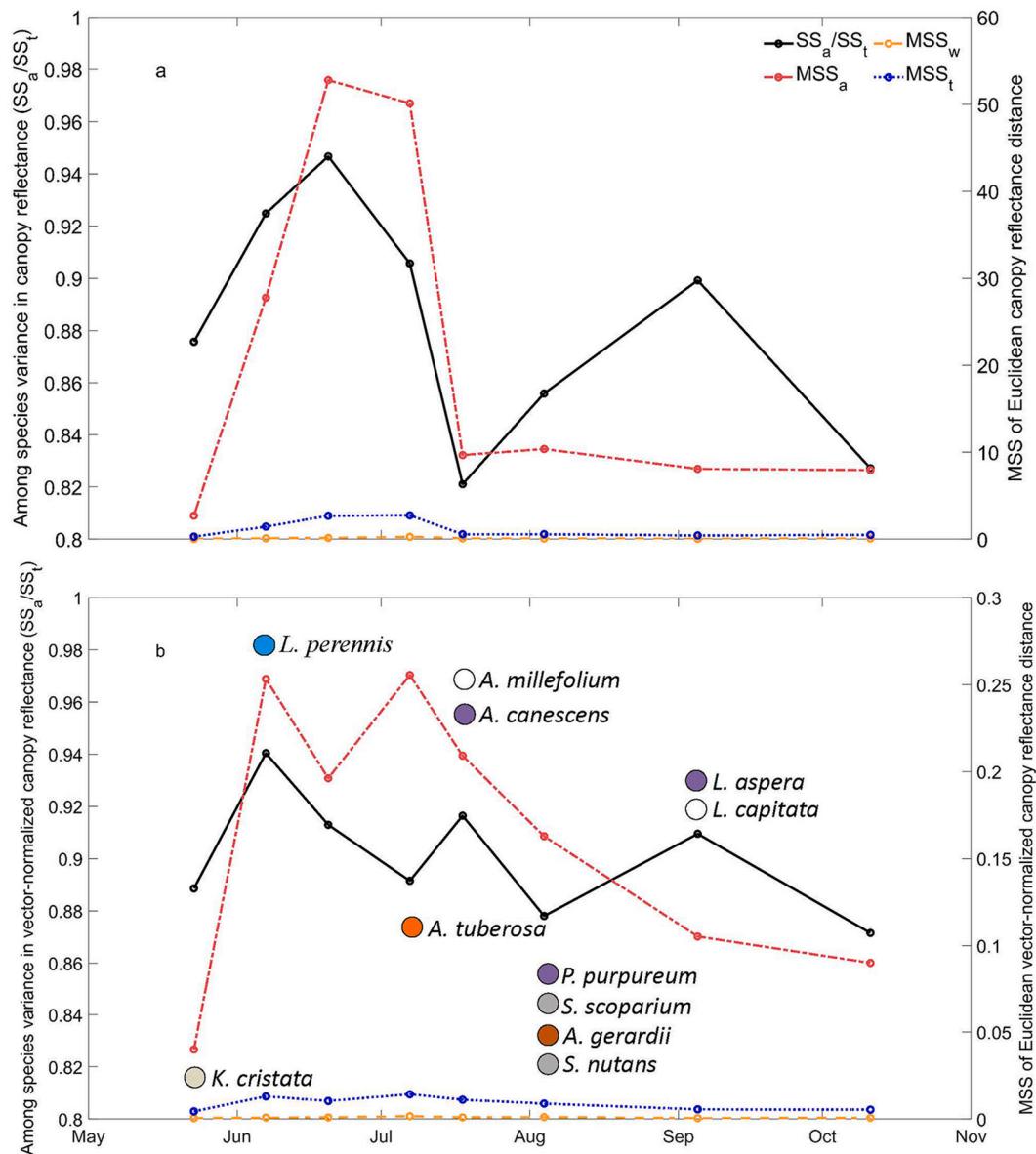


Fig. 4. Proportion of among species variance (SS_a) expressed relative to the total variance (SS_t) and mean sum of squares (MSS) over the course of the growing season derived using canopy reflectance (a) and vector normalized (b) canopy reflectance. The flowering season of each monoculture species is labeled separately in panel (b) and the approximate flower color is indicated by the colored circles.

(Miyazawa et al., 2003; Fernandes et al., 2020). Unlike the decline in all pigments for most phylogenetic linkages (Kothari et al., 2018), leaf reflectance data exhibited larger temporal variations in the among-species variation to total-variation ratio than pigment data (Figs. 2, 3 and 6). This might be because leaf pigments (particularly chlorophyll and photoprotective carotenoid pigments), while varying with ontogeny (Gamon and Surfus, 1999; Fernandes et al., 2020), season (e.g., Cavender-Bares et al., 2000) and stress (e.g., Verhoeven et al., 1999; Savage et al., 2009), are relatively conserved traits (Ustin et al., 2009; Kothari et al., 2018), constrained by evolution within a narrow range of variation (Meireles et al., 2020).

4.2. Phenology of spectral diversity at the canopy scale

At the canopy scale, contributions of plant materials, including leaves, branches and flowers, as well as shadow, soil, and other “background” (non-vegetative) materials, to the overall canopy optical properties vary by season and by species, and can affect the spectral

diversity – biodiversity relationship in complex ways (Figs. 5 and S3 in the supplemental materials). In this study, the canopy scale spectral variation among the monocultures was dominated by among-species variance (Fig. 4) indicating that canopy information appears to improve species discrimination more than leaf spectra alone, in agreement with previous studies at this site (Wang et al., 2018a). In this case, monocultures used in this study served as a unique example of analyzing the spectral variation at canopy scale, which could be helpful to understand the mechanics behind the diversity estimation using canopy reflectance. However, the partitioning derived from the monocultures may not fully represent the situation in high diversity plots, where frequent species overlap occurs and the canopy reflectance measurements are often mixed spectra of plant materials of different species and competition may also lead to different intraspecific variation.

Unlike the large between-species variation achieved in the monoculture plots, no relationship between spectral diversity (Coefficient of Variation) and species diversity (Shannon's index) was found when reflectance from all the plots was used but a clear relationship emerged

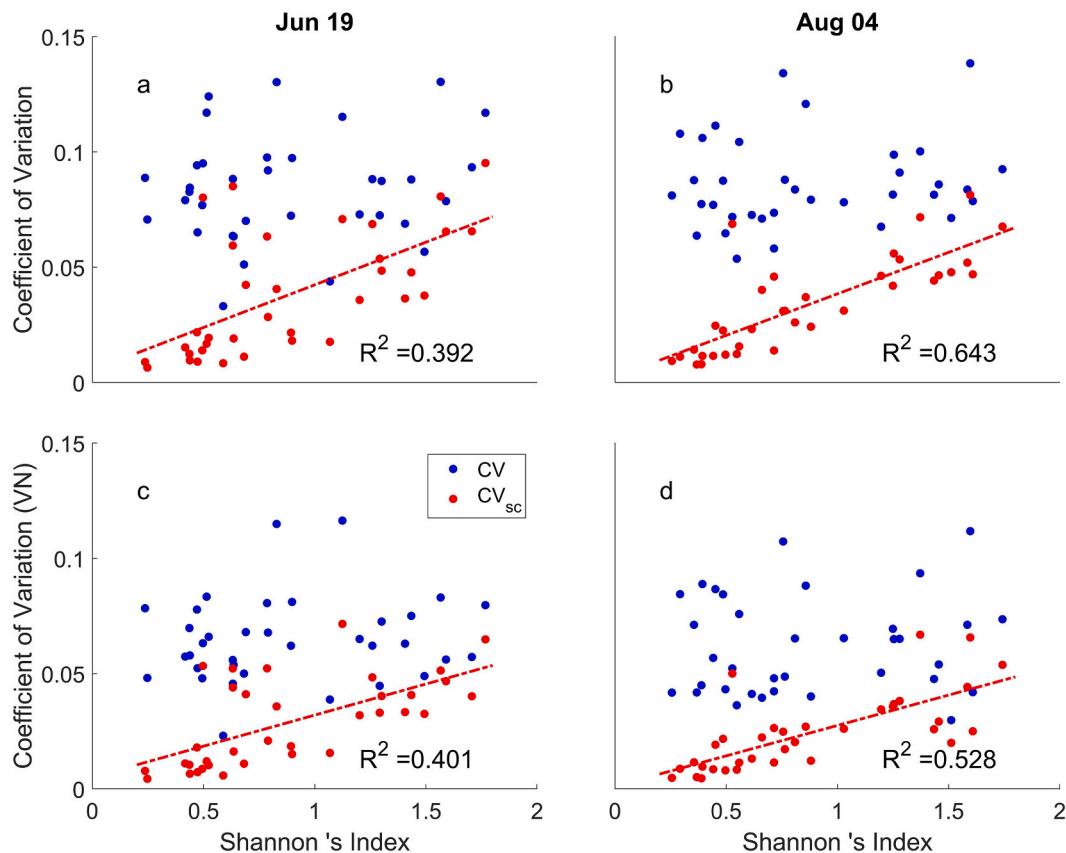


Fig. 5. Soil correction (“SC”) improved the relationship between canopy scale spectral diversity (Coefficient of Variation) and Shannon’s index on June 19 (a & c) and August 4 (b & d). Canopy reflectance (a & b) and vector normalized canopy reflectance (c & d) of all the plots were used.

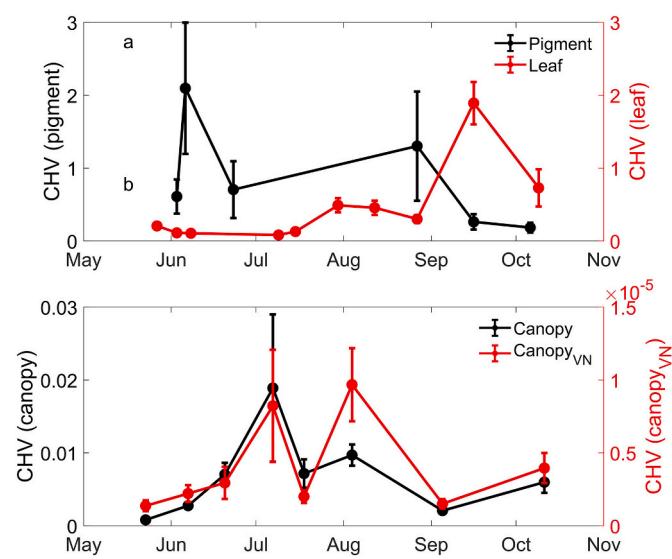


Fig. 6. Different phenological diversity patterns of pigment and leaf reflectance (a) and canopy reflectance and vector normalized canopy reflectance (b) expressed as convex hull volume of each species calculated using the first three PCs, respectively. The vertical bars indicate the standard error.

upon soil correction (Fig. 5). This might be due to two reasons: first, species discrimination is harder for high diversity areas than for low diversity areas (Lopatin et al., 2017) and second, the spatial scale dependence of the spectral diversity – biodiversity relationship can cause weak relationships at spatial scales (pixel sizes) coarser than

typical crown sizes. Previous prairie spectral diversity studies at the canopy scale showed that the ability to estimate taxonomic diversity (e.g., species richness and evenness) as a function of sampling scale (pixel size) declined rapidly above about 1–10 cm—corresponding to the size of individual plants at this site (Wang et al., 2018a). The pixel size of canopy reflectance collected in this study was approximately 0.5 m, which is the sampling scale where spectral diversity – biodiversity relationship became weak. Together with previous work, these findings emphasize the need for high-resolution images in prairie diversity estimation at fine scales (i.e., alpha diversity) (Lopatin et al., 2017; Wang et al., 2018a).

4.3. Vector normalization effects on canopy spectral diversity

Vector normalization on canopy reflectance was applied to keep the overall spectral shape while removing brightness differences caused by illumination conditions (Feilhauer, 2010). By only focusing on the variations in spectral shape, the vector normalization method can better detect subtle absorption features associated with certain plant traits and has been used to improve the performance of plant traits estimation using canopy reflectance data via PLSR (Feilhauer, 2010, Serbin et al., 2014). On the other hand, vector normalization reduces some of the variation due to structure at both the canopy and leaf scales (i.e., anatomy and morphology). Thus, vector normalization can weaken the ability to distinguish species or detect conditions that effect structure. For example, drought stressed plants have higher reflectance at visible wavelengths than non-stressed plants at both leaf and canopy levels, due to lower pigment and water contents but more exposed soil background (Everitt and Nixon, 1986). As a result, applying the vector normalization can reduce the power to distinguish optical types by reducing the power to detect the structural contribution to contrasting canopy-scale optical

properties (Fig. S2 in the supplemental section and [Ustin and Gamon, 2010](#)).

4.4. Idiosyncratic effects on canopy spectral diversity

At the canopy scale, the optical properties of flowers or leaves of particular species can have substantial effects on canopy reflectance (Figs. 5, 6 & S3 in the supplemental section), complicating interpretation of the spectral diversity at particular seasons. Botanists rely largely on flowers to distinguish plant species and flowering time to investigate plant phenological responses to climate change ([Munson and Long, 2017](#)), but reproductive traits have generally not been considered in remote sensing, except for a few studies ([Carvalho et al., 2013](#); [Landmann et al., 2015](#); [Müllerová et al., 2017a, 2017b](#); [Shen et al., 2010](#)). Flower display can increase the intraspecific spectral variability at canopy scale ([Clark et al., 2005](#)), thus potentially increasing the within-species variation and confounding the spectral diversity – biodiversity relationship (Figs. 5 & 6) and weakening species discrimination using spectral data ([Roberts et al., 2004](#)). On the other hand, flowers and leaf pubescence enhances among-species variance at the canopy scale (Figs. 4 and S2 in the supplemental section) and can be used to distinguish particular species at certain season. The few studies that do examine the spectral effects of flowers have revealed that including flowering information can improve the accuracy of species mapping ([Carvalho et al., 2013](#); [Landmann et al., 2015](#); [Müllerová et al., 2017a, 2017b](#)). Therefore, flowering information, if properly used, can provide a way to increase the accuracy of biodiversity estimation. The surrogacy hypothesis ([Magurran, 2004](#); [Wang and Gamon, 2019](#)) predicts that diversity information provided at one taxonomic or sampling level can provide information at another level. Similarly, spectral effects of flowers can perhaps provide information on other trophic levels, e.g., pollinators, so can conceivably provide a link to larger community diversity levels beyond that of vegetation alone.

5. Conclusions and recommendations

The challenges of obtaining continuous or repeated remote sensing and ground biodiversity measurements often constrains our ability to investigate the spectral diversity – biodiversity relationship through the growing season. By showing contrasting seasonal patterns at different spatial scales for experimental prairie plots, this study demonstrated an interaction of temporal and spatial scale dependence of the spectral diversity – biodiversity relationship for prairie vegetation. Our results revealed clear effects of phenology on the spectral diversity for prairie plant species, and these effects varied between leaf and canopy levels, as well as with the selected spectral diversity indices ([Wang et al., 2018a, 2018b](#)) and analysis method, e.g., whether or not vector normalization is applied to canopy reflectance.

To fully implement the power of remote sensing in biodiversity studies, it is critical to include phenological information in the future remote sensing of biodiversity work, which can guide our biodiversity sampling campaign in terms of identifying the best season for overall distinguishing species or monitoring particular species and understanding the disturbance induced successional changes in species diversity. For example, the post-fire diversity of Mediterranean ecosystems is influenced by fire severity, life form, resource availability and landscape features ([Keeley et al., 2005](#); [Capitanio and Carcaillet, 2008](#)) and is further confounded by the impacts of climate change ([Slingsby et al., 2017](#)). In this study, we focused data from one single field season due to limited resources but acknowledge that a careful experimental design with data from multiple years could provide a better understanding of the spectral diversity – biodiversity relationship in the future. Emerging (e.g. EnMAP) and planned (e.g. Surface Biology and Geology, SBG) satellites with imaging spectrometers ([Schimel et al., 2020](#)) may help address phenological effects on biodiversity detection, but these sensors are designed to sample at scales (30–45 m pixels) too

coarse for detecting alpha diversity, at least for most short-statured grassland communities ([Barnett et al., 2019](#); [Gamon et al., 2020](#); [Schimel et al., 2020](#)). The relatively lower cost of flying drones compared to airborne campaigns may soon make it more affordable to get a time series dataset to investigate the phenology in remote sensing of biodiversity ([Holman et al., 2019](#)), particularly at spatial scales between those of field and satellite measurements. However, most current drone technology lacks the spectral detail and accurate georeferencing capabilities needed for detecting biodiversity at fine spatial scales over time ([Gamon et al., 2020](#)). Assuming phenological sampling methods can be improved (e.g. via drones), further attention should also be given to different analytical methods often applied to different scales, as these may lead to somewhat different results.

Author contribution

RW: Conceptualization, Methodology, Software, Data curation, Formal Analysis, Writing - original draft. JAG: Conceptualization, Methodology, Resources, Funding acquisition, Writing - review & editing. JCB: Conceptualization, Methodology, Resources, Funding acquisition, Writing - review & editing.

Data availability

These reflectance datasets are available at the EcoSIS Spectral Library ([ecosis.org](#)). The two datasets are leaf reflectance (doi: [10.21232/i6N36jZ7](#)) and canopy reflectance (doi: [10.21232/C2Z070](#)).

The ground biodiversity data is available at the University of Minnesota Cedar Creek research project data catalog ([https://www.cedarcreek.umn.edu/research/data](#)).

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors acknowledge the help from the staff at the Cedar Creek Ecosystem Science Reserve, particularly Troy Mielke and Kally Worm, and research assistant Jonathan Anderson. We appreciate Keren Bitan, Austin Pieper and Cathleen Lapadat for extracting pigment contents and analyzing them using HPLC. This study was supported by a NASA and NSF grant (DEB-1342872), NSF BII Implementation grant (DBI-2021898), and NSF-LTER grants (DEB-1234162 and NSF DEB-1831944) to J. Cavender-Bares, and by iCORE/AITF (G224150012 & 200700172), NSERC (RGPIN-2015-05129), and CFI (26793) grants to J. Gamon. The authors acknowledge constructive comments from two anonymous reviewers that greatly improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at [https://doi.org/10.1016/j.rse.2022.113169](#).

References

- Anderson, Marti, 2001. A new method for non parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.
- Barnett, D.T., Adler, P.B., Chemel, B.R., Duffy, P.A., Enquist, B.J., Grace, J.B., Harrison, S., Peet, R.K., Schimel, D.S., Stohlgren, T.J., Vellend, M., 2019. The plant diversity sampling design for the National Ecological Observatory Network. *Ecosphere* 10 (2), e02603. [https://doi.org/10.1002/ecs2.2603](#).
- Bonaldo, A.B., Joana, E., Esposito, M.C., Ferreira, L.V., Hernandez, M.I.M., Lo-manhung, N.F., Maria, M.N., 2008. The cost-effectiveness of biodiversity surveys in tropical forests. *Ecol. Lett.* 11, 139–150. [https://doi.org/10.1111/j.1461-0248.2007.01133.x](#).
- Capitanio, R., Carcaillet, C., 2008. Post-fire Mediterranean vegetation dynamics and diversity: a discussion of succession models. *For. Ecol. Manag.* 255 (3–4), 431–439. [https://doi.org/10.1016/j.foreco.2007.09.010](#).

Carvalho, S., Schlerf, M., van der Putten, W.H., Skidmore, A.K., 2013. Hyperspectral reflectance of leaves and flowers of an outbreak species discriminates season and successional stage of vegetation. *Int. J. Appl. Earth Obs. Geoinf.* 24 (1), 32–41. <https://doi.org/10.1016/j.jag.2013.01.005>.

Cavender-Bares, J., Potts, M., Zacharias, E., Bazzaz, F.A., 2000. Consequences of CO₂ and light interactions for leaf phenology, growth, and senescence in *Quercus rubra*. *Glob. Chang. Biol.* 6 (8), 877–887.

Cavender-Bares, J., Meireles, J.E., Couture, J.J., Kaproth, M.A., Kingdon, C.C., Singh, A., Townsend, P.A., 2016. Associations of leaf spectra with genetic and phylogenetic variation in oaks: prospects for remote detection of biodiversity. *Remote Sens.* 8 (3) <https://doi.org/10.3390/rs8030221>.

Cavender-Bares, J., Gamon, J.A., Hobbie, S.E., Madritch, M.D., Meireles, J.E., Schweiger, A.K., Townsend, P.A., 2017. Harnessing plant spectra to integrate the biodiversity sciences across biological and spatial scales. *Am. J. Bot.* 104 (7), 966–969. <https://doi.org/10.3732/ajb.1700061>.

Chavana-Bryant, C., Malhi, Y., Wu, J., Asner, G.P., Anastasiou, A., Enquist, B.J., Gerard, F.F., 2017. Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements. *New Phytol.* 214 (3), 1049–1063. <https://doi.org/10.1111/nph.13853>.

Clark, M.L., Roberts, D.A., Clark, D.B., 2005. Hyperspectral discrimination of tropical rain forest tree species at leaf to crown scales. *Remote Sens. Environ.* 96, 375–398. <https://doi.org/10.1016/j.rse.2005.03.009>.

Cornwell, W.K., Schwilke, D.W., Ackerly, D.D., 2006. A trait-based test for habitat filtering: convex hull volume. *Ecology* 87 (6), 1465–1471.

Dahlén, K.M., 2016. Spectral diversity area relationships for assessing biodiversity in a wildland-agriculture matrix. *Ecol. Appl.* 26 (8), 2756–2766.

Doughty, C.E., Field, C.B., McMillan, A.M.S., 2011. Can crop albedo be increased through the modification of leaf trichomes, and could this cool regional climate? *Clim. Chang.* 104 (2), 379–387. <https://doi.org/10.1007/s10584-010-9936-0>.

EHleringer, J.R., Björkman, O., 1978. A comparison of photosynthetic characteristics of *Encelia* species possessing glabrous and pubescent leaves. *Plant Physiol.* 62 (2), 185–190. <https://doi.org/10.1104/pp.62.2.185>.

Everitt, J.H., Nixon, P.R., 1986. Canopy reflectance of two drought-stressed shrubs. *Photogramm. Eng. Remote. Sens.* 52 (8), 1189–1192.

Feilhauer, Hannes, et al., 2010. Brightness-normalized Partial Least Squares Regression for hyperspectral data. *J. Quant. Spectrosc. Radiat. Transf.* 111, 1947–1957.

Fernandes, A.M., Fortini, E.A., de Müller, L.A.C., Batista, D.S., Vieira, L.M., Silva, P.O., do Amaral, C.H., Poethig, R.S., Otoni, W.C., 2020. Leaf development stages and ontogenetic changes in passionfruit (*Passiflora edulis* Sims.) are detected by narrowband spectral signal. *J. Photochem. Photobiol. B Biol.* 209 (July 2019), 111931. <https://doi.org/10.1016/j.jphotobiol.2020.111931>.

Gamon, J.A., 2008. Tropical sensing — Opportunities and challenges. In: Kalacska, G., Sanchez-Azofeifa, A. (Eds.), *Hyperspectral Remote Sensing of Tropical and Subtropical Forests*. CRC Press Taylor&Francis Group, Boca Raton, FL, USA, pp. 297–304.

Gamon, J.A., Surfus, S., 1999. Assessing leaf pigment content and activity with a reflectometer. *New Phytol.* 143, 105–117.

Gamon, J.A., Somers, B., Malenovský, Z., Middleton, E.M., Rascher, U., Schaeppman, M. E., 2019. Assessing vegetation function with imaging spectroscopy. *Surv. Geophys.* 40, 489–513.

Gamon, J.A., Wang, R., Gholizadeh, H., Zutta, B., Townsend, P., Cavender-Bares, J., 2020. Consideration of scale in remote sensing of biodiversity. In: Cavender-Bares, J., Gamon, J.A., Townsend, P.A. (Eds.), *Remote Sensing of Plant Biodiversity*. Springer, New York.

Gholizadeh, H., Gamon, J.A., Zygilbaum, A.J., Wang, R., Schweiger, A.K., Cavender-Bares, J., 2018. Remote sensing of biodiversity: soil correction and data dimension reduction methods improve assessment of α -diversity (species richness) in prairie ecosystems. *Remote Sens. Environ.* 206, 240–253. <https://doi.org/10.1016/j.rse.2017.12.014>.

Gholizadeh, H., Gamon, J.A., Helzer, C.J., Cavender-Bares, J., 2020. Multi-temporal assessment of grassland α - and β -diversity using hyperspectral imaging. *Ecol. Appl.* <https://doi.org/10.1002/eaap.2145>.

Holman, F.H., Riche, A.B., Castle, M., Wooster, M.J., Hawkesford, M.J., 2019. Radiometric calibration of 'commercial off the shelf' cameras for UAV-based high-resolution temporal crop phenotyping of reflectance and NDVI. *Remote Sens.* 11 (14), 1657. <https://doi.org/10.3390/rs11141657>.

Keely, J.E., Fotheringham, C.J., Baer-keely, M., 2005. Factors affecting plant diversity during post-fire recovery and succession of mediterranean-climate shrublands in California, USA. *Divers. Distrib.* 11 (6), 525–537. <https://doi.org/10.1111/j.1365-1276.2005.00513.x>.

Kothari, S., Cavender-Bares, J., Bitan, K., Verhoeven, A.S., Wang, R., Montgomery, R.A., Gamon, J.A., 2018. Community-wide consequences of variation in photoprotective physiology among prairie plants. *Photosynthetica* 56 (1), 1–13. <https://doi.org/10.1007/s11099-018-0777-9>.

Landmann, T., Piiroinen, R., Makori, D.M., Abdel-Rahman, E.M., Makau, S., Pellikka, P., Raina, S.K., 2015. Application of hyperspectral remote sensing for flower mapping in African savannas. *Remote Sens. Environ.* 166, 50–60. <https://doi.org/10.1016/j.rse.2015.06.006>.

Logan, B.A., Grace, S.C., Adams, W.W., Demmig-Adams, B., 1998. Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* 116 (1–2), 9–17.

Lopatin, J., Fassnacht, F.E., Kattenborn, T., Schmidlein, S., 2017. Mapping plant species in mixed grassland communities using close range imaging spectroscopy. *Remote Sens. Environ.* 201, 12–23.

Magurran, A.E., 2004. *Measuring Biological Diversity*. Blackwell Publishing, Malden, Massachusetts.

Magurran, A.E., 2007. Species abundance distributions over time. *Ecol. Lett.* 10 (5), 347–354. <https://doi.org/10.1111/j.1461-0248.2007.01024.x>.

Magurran, A.E., Baillie, S.R., Buckland, S.T., Dick, J.M., Elston, D.A., Scott, E.M., Watt, A. D., 2010. Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends Ecol. Evol.* 25 (10), 574–582. <https://doi.org/10.1016/j.tree.2010.06.016>.

Meireles, J.E., Cavender-Bares, J., Townsend, P.A., Ustin, S., Gamon, J.A., Schweiger, A. K., Schaeppman, M.E., Asner, G.P., Martin, R.E., Singh, A., Schrodt, F., Chlus, A., O'Meara, B., 2020. Leaf reflectance spectra capture the evolutionary history of seed plants. *New Phytol.* 228 (2), 485–493.

Miyazawa, S.I., Makino, A., Terashima, I., 2003. Changes in mesophyll anatomy and sink-source relationships during leaf development in *Quercus glauca*, an evergreen tree showing delayed leaf greening. *Plant Cell Environ.* 26 (5), 745–755. <https://doi.org/10.1046/j.1365-3040.2003.01011.x>.

Müllerová, J., Bartaloš, T., Brůna, J., Dvořák, P., Vítková, M., 2017a. Unmanned aircraft in nature conservation: an example from plant invasions. *Int. J. Remote Sens.* 38 (8–10), 2177–2198. <https://doi.org/10.1080/01431161.2016.1275059>.

Müllerová, J., Brůna, J., Bartaloš, T., Dvořák, P., Vítková, M., Pyšek, P., 2017b. Timing is important: unmanned aircraft vs. satellite imagery in plant invasion monitoring. *Front. Plant Sci.* 8 (May), 887. <https://doi.org/10.3389/fpls.2017.00887>.

Munson, S.M., Long, A.L., 2017. Climate drives shifts in grass reproductive phenology across the western USA. *New Phytol.* 213, 1945–1955. <https://doi.org/10.1111/nph.14327>.

Preston, F.W., 1960. Time and space and the variation of species. *Ecology* 41 (4), 612–627.

Price, John, 1994. How unique are spectral signatures? *Remote Sens. Environ.* 49, 181–186.

Primack, R.B., Gallatin, A.S., 2017. Insights into grass phenology from herbarium specimens. *New Phytol.* 213, 1567–1568. <https://doi.org/10.1111/nph.14439>.

Ramirez-Valiente, J.A., Koehler, K., Cavender-Bares, J., 2015. Climatic origins predict variation in photoprotective leaf pigments in response to drought and low temperatures in live oaks (*Quercus* series *Virentes*). *Tree Physiol.* 35 (5), 521–534.

Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B., Eisenhauer, N., 2012. Impacts of biodiversity loss escalate through time as redundancy fades. *Science* 336 (6081), 589–592. <https://doi.org/10.1126/science.1217909>.

Roberts, D.A., Ustin, S.L., Ogunjemiyo, S., Greenberg, J., Dobrowski, S.Z., Chen, J., Hinckley, T.M., 2004. Spectral and structural measures of Northwest forest vegetation at leaf to landscape scales. *Ecosystems* 7 (5), 545–562. <https://doi.org/10.1007/s10021-004-0144-5>.

Rocchini, D., Hernández-Stefanoni, J.L., He, K.S., 2015. Advancing species diversity estimate by remotely sensed proxies: a conceptual review. *Ecol. Informa.* 25, 22–28. <https://doi.org/10.1016/j.ecoinf.2014.10.006>.

Savage, J., Cavender-Bares, J., Verhoeven, A., 2009. Habitat generalists and wetland specialists in the genus *Salix* vary in their photoprotective responses to drought. *Funct. Plant Biol.* 36, 300–309.

Schimel, D., Townsend, P.A., Pavlick, R., 2020. Prospects and pitfalls for spectroscopic remote sensing of biodiversity at the global scale. In: Cavender-Bares, J., Gamon, J. A., Townsend, P.A. (Eds.), *Remote Sensing of Plant Biodiversity*, pp. 503–518. <https://doi.org/10.1007/978-3-03-33157-3>.

Schweiger, A.K., Cavender-Bares, J., Townsend, P.A., Hobbie, S.E., Madritch, M.D., Wang, R., Gamon, J.A., 2018. Plant spectral diversity integrates functional and phylogenetic components of biodiversity and predicts ecosystem function. *Nat. Ecol. Evol.* 2 (6), 976–982. <https://doi.org/10.1038/s41559-018-0551-1>.

Schweiger, A.K., Cavender-Bares, J., Kothari, S., Townsend, P.A., Madritch, M.D., Grossman, J.J., Gholizadeh, H., Wang, R., Gamon, J.A., 2021. Coupling spectral and resource-use complementarity in experimental grassland and forest communities. *Proc. R. Soc. B Biol. Sci.* 288, 20211290. <https://doi.org/10.1098/rspb.2021.1290>.

Serbin, S.P., Kingdon, C.C., Townsend, P.A., 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecol. Appl.* 24, 1651–1669. <https://doi.org/10.1890/13-2110.1>.

Shen, M., Chen, J., Zhu, X., Tang, Y., Chen, X., 2010. Do flowers affect biomass estimate accuracy from NDVI and EVI? *Int. J. Remote Sens.* 31 (8), 2139–2149. <https://doi.org/10.1080/0143160903578812>.

Slingsby, J.A., Merow, C., Aiello-Lammens, M., Allsopp, N., Hall, S., Mollmann, H.K., Turner, R., Wilson, A.M., Silander, J.A., 2017. Intensifying postfire weather and biological invasion drive species loss in a Mediterranean-type biodiversity hotspot. *Proc. Natl. Acad. Sci. U. S. A.* 114 (18), 4697–4702. <https://doi.org/10.1073/pnas.1619014114>.

Stasinski, L., White, D.M., Nelson, P.R., Ree, R.H., Meireles, J.E., 2021. Reading light: leaf spectra capture fine-scale diversity of closely related, hybridizing arctic shrubs. *New Phytol.* 232, 2283–2294.

Tilman, D., 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277, 1300–1302. <https://doi.org/10.1126/science.277.5330.1300>.

Ustin, S.L., Gamon, J.A., 2010. Remote sensing of plant functional types. *New Phytol.* 186, 795–816.

Ustin, S.L., Gitelson, A., Jacquemoud, S., Schaeppman, M., Asner, G.P., Gamon, J.A., Zarco-Tejada, P.J., 2009. Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. *Remote Sens. Environ.* 113, S67–S77. <https://doi.org/10.1016/j.rse.2008.10.019>.

Verhoeven, A.S., Adams, W.W., Demmig-Adams, B., Croce, R., Bassi, R., 1999. Xanthophyll cycle pigment localization and dynamics during exposure to low temperatures and light stress in *Vinca major*. *Plant Physiol.* 120 (3), 727–737.

Wang, R., Gamon, J.A., 2019. Remote sensing of terrestrial plant biodiversity. *Remote Sens. Environ.* 231, 111218 <https://doi.org/10.1016/j.rse.2019.111218>.

Wang, R., Gamon, J.A., Montgomery, R.A., Townsend, P.A., Zygielbaum, A.I., Bitan, K., Cavender-Bares, J., 2016a. Seasonal variation in the NDVI-species richness relationship in a prairie grassland experiment (Cedar Creek). *Remote Sens.* 8 (2), 128. <https://doi.org/10.3390/rs8020128>.

Wang, R., Gamon, J.A., Schweiger, A.K., Cavender-Bares, J., Townsend, P.A., Zygielbaum, A.I., Kothari, S., 2018a. Influence of species richness, evenness, and composition on optical diversity: A simulation study. *Remote Sens. Environ.* 211, 218–228. <https://doi.org/10.1016/j.rse.2018.04.010>.

Wang, R., Gamon, J.A., Cavender-Bares, J., Townsend, P.A., Zygielbaum, A.I., 2018b. The spatial sensitivity of the spectral diversity-biodiversity relationship: an experimental test in a prairie grassland. *Ecol. Appl.* 28 (2), 541–556. <https://doi.org/10.1002/eaap.1669>.

Wang, Z., Townsend, P.A., Schweiger, A.K., Couture, J.J., Singh, A., Hobbie, S.E., Cavender-Bares, J., 2019. Mapping foliar functional traits and their uncertainties across three years in a grassland experiment. *Remote Sens. Environ.* 221, 405–416.

Wang, Z., Chlus, A., Geygan, R., Ye, Z., Zheng, T., Singh, A., Townsend, P.A., 2020. Foliar functional traits from imaging spectroscopy across biomes in eastern North America. *New Phytol.* 228, 494–511. <https://doi.org/10.1111/nph.16711>.

Zutta, B., 2003. Assessing Vegetation Functional Type and Biodiversity in Southern California Using Spectral Reflectance. MS thesis. California State University, Los Angeles, CA, USA.