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SPECIAL ISSUE ON INTRASPECIFIC VARIATION IN PLANT FUNCTIONAL TRAITS

Microanatomical traits track climate gradients for a dominant C_4 grass species across the Great Plains, USA

Seton Bachle* and Jesse B. Nippert

Division of Biology, Kansas State University, Manhattan, KS 66506, USA
**For correspondence. E-mail sbachle@ksu.edu*

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- **Background and Aims** *Andropogon gerardii* is a highly productive C_4 grass species with a large geographic range throughout the North American Great Plains, a biome characterized by a variable temperate climate. Plant traits are often invoked to explain growth rates and competitive abilities within broad climate gradients. For example, plant competition models typically predict that species with large geographic ranges benefit from variation in traits underlying high growth potential. Here, we examined the relationship between climate variability and leaf-level traits in *A. gerardii*, emphasizing how leaf-level microanatomical traits serve as a mechanism that may underlie variation in commonly measured traits, such as specific leaf area (SLA).
- **Methods** *Andropogon gerardii* leaves were collected in August 2017 from Cedar Creek Ecosystem Science Reserve (MN), Konza Prairie Biological Station (KS), Platte River Prairie (NE) and Rocky Mountain Research Station (SD). Leaves from ten individuals from each site were trimmed, stained and prepared for fluorescent confocal microscopy to analyse internal leaf anatomy. Leaf microanatomical data were compared with historical and growing season climate data extracted from PRISM spatial climate models.
- **Key Results** Microanatomical traits displayed large variation within and across sites. According to AICc (Akaike's information criterion adjusted for small sample sizes) selection scores, the interaction of mean precipitation and temperature for the 2017 growing season was the best predictor of variability for the anatomical and morphological traits measured here. Mesophyll area and bundle sheath thickness were directly correlated with mean temperature (annual and growing season). Tissues related to water-use strategies, such as bulliform cell and xylem area, were significantly correlated with one another.
- **Conclusions** The results indicate that (1) microanatomical trait variation exists within this broadly distributed grass species, (2) microanatomical trait variability appears likely to impact leaf-level carbon and water use strategies, and (3) microanatomical trait values vary across climate gradients, and may underlie variation in traits measured at larger ecological scales.

Key words: Grasslands, climate variability, intraspecific trait variability, plant functional traits, microanatomy, Great Plains.

INTRODUCTION

Grasslands occupy 30–40 % of Earth's terrestrial surface, more than any other single biome (Gibson, 2009), and are characterized by the dominance of grasses (Poaceae) and grass-like species such as sedges (Cyperaceae) and rushes (Juncaceae). Grasslands range from the tropical bushvelds of Africa and the campos and llanos of South America to the temperate regions, including the Mongolian steppes, the South African velds, the pampas of Argentina and the North American Great Plains (Blair *et al.*, 2014). For many grassland ecosystems, climate (including gradients of both temperature and precipitation) is a key driver of ecosystem function (Borchert, 1950). For example, mean annual precipitation ranges from 85 to 380 mm in the Mongolian steppe (Ma *et al.*, 2012) and from 375 to 925 mm in the South African savannah (Holdo *et al.*, 2018). The North American Great Plains is a unique region because it contains both a large precipitation gradient (mean annual precipitation 400 to + 2000 mm) and a large mean annual temperature gradient (3–27 °C). Widespread species within the Great Plains can experience a wide range of temperatures and precipitation regimes (Gibson, 2009; Eters *et al.*, 2014). Many grassland plant species possess morphological, physiological and structural traits that facilitate responses to a variable climate (Linder *et al.*, 2018; Ott *et al.*, 2019; Nunes *et al.*, 2020). These traits are commonly referred to as plant functional traits and often reflect an individual's functional response to biotic and abiotic factors (Violle *et al.*, 2007; Carmona *et al.*, 2016). Many grass species have developed and refined functional traits that promote persistence in this disturbance-rich environment, including varying growth morphology (caespitose, rhizomatous), strong narrow leaves that contain specialized cells for water storage and leaf rolling (Alvarez *et al.*, 2008), physiological alterations in carbon fixation (C_3 and C_4) congruent with altered photosynthetic cell organization, and phytoliths to discourage herbivory from intense grazing (Lisztes-Szabó, 2019).

Understanding patterns of intraspecific trait variation may facilitate a deeper understanding of how climate variability drives the expression of a range of plant traits that reflect variation in growth across both temporal and spatial gradients (Valladares *et al.*, 2007, 2014; Funk and Cornwell, 2013; Becklin *et al.*, 2016). Broad suites of plant functional traits enable differential responses to abiotic factors that can foster coexistence or competition, such as rooting depth differences between grasses and woody species (Fargione and Tilman, 2005; Nippert and Holdo, 2015; Holdo *et al.*, 2018) or water-use efficiency (O'Keefe and Nippert, 2018; Nadal and Flexas, 2019). However, trait variation within a species (intraspecific variability) may also contribute to acquisition of resources, continued growth and climate buffering during adverse periods (Funk *et al.*, 2017). Consequently, a linkage between trait variation, influenced by biotic and abiotic factors, is assumed to impact population- and community-level responses (Suding *et al.*, 2003). For this reason, intraspecific trait variability within a broadly distributed dominant species may help identify ecosystem susceptibility and sensitivity to future climate changes (Avolio *et al.*, 2019).

Andropogon gerardii (big bluestem) is a C_4 grass species found throughout the Great Plains, accounting for >70 % of annual biomass in the tallgrass prairie (Weaver, 1968; Smith *et al.*, 2017). Previous investigations of *A. gerardii* have focused on ecotypic differences and intraspecific trait variability of key traits used in the leaf economic spectrum (LES), such as specific leaf area and leaf mass per area, over large geographic regions varying in climate (Avolio and Smith, 2013; Olsen *et al.*, 2013; Bachle *et al.*, 2018). Other investigations have focused on physiological traits such as water-use efficiency and fluctuations in species establishment and cover over geographic gradients and ranges of ecotypes (Johnson *et al.*, 2015; McAllister *et al.*, 2015; Smith *et al.*, 2017). Prior investigations concerning *A. gerardii* reported large variation in physiological traits such as photosynthetic rate, water-use efficiency and leaf nitrogen content when water availability was altered (Knapp, 1985; Nippert *et al.*, 2009). Such physiological responses are influenced by the structural components existing at the microanatomical scale (Xu and Zhou, 2008; Christin *et al.*, 2013). Microanatomical traits have also been observed to influence physiological processes that impact carbon assimilation (mesophyll area and bundle sheath area), which can underlie variation in leaf mass per area, leaf thickness and chlorophyll content (De La Riva *et al.*, 2016; Reich and Flores-Moreno, 2017; Ivanova *et al.*, 2018). Consequently, alterations in water use and acquisition traits like xylem diameter/area, interveinal distance and bulliform area have been reported to influence physiological traits in agricultural species (de Souza *et al.*, 2013; Retta *et al.*, 2016; Ouyang *et al.*, 2017; Kulya *et al.*, 2018) and, less frequently, rangeland or non-agronomic species (Ocheltree *et al.*, 2016; Moinuddin *et al.*, 2017; Bachle and Nippert, 2018). Some traits, such as interveinal distance, have been previously observed to influence both water use and quantum yield in C_4 grasses, resulting in altered carbon assimilation (Ogle, 2003; Ocheltree *et al.*, 2011). However, a determination of the variability in leaf-level microanatomical traits that may influence commonly measured functional traits at the leaf level is missing from the literature (Carmo-Silva *et al.*, 2009; Ocheltree *et al.*, 2011; Rao and Dixon, 2016).

Microanatomical traits are often overlooked due to the intensive time and effort required for data collection; however, these traits may provide key insight into our understanding of species' drought tolerance, uptake of soil resources, carbon balance and leaf hydraulic traits that scale up to influence competition, productivity and overall species resiliency to disturbance (Kattge *et al.*, 2011; Reich, 2014; Funk *et al.*, 2017; Griffin-Nolan *et al.*, 2018). Species-level anatomical traits play an important role in parameterizing ecological models that link organismic and population levels to community and macroecological scales (He *et al.*, 2019). Previous research has also shown that variation of such anatomical traits can provide an understanding of how species adjust to suboptimal growing conditions (Olsen *et al.*, 2013; Guo *et al.*, 2017). Shifting focus from species means to coefficient of variation (CV) can provide a deeper understanding of intraspecific trait variability and more effectively reveal the influence of ecosystem functioning, productivity and composition (Bolnick *et al.*, 2011). To our knowledge, intraspecific microanatomical trait variation has been seldom evaluated in grass species outside of important agronomic cultivars (Bellasio and Lundgren, 2016; Kulya *et al.*, 2018).

Our study aims to characterize the variability in microanatomical traits that underlie key leaf-level traits in a widespread grass species throughout the North American Great Plains. We hypothesize that (1) due to site-level variation in climate history and environmental conditions, mean values of microanatomical traits will vary across sampling locations, while microanatomical traits will express similar within-site variation; (2) due to the variability in water availability from location to location, but general similarity in atmospheric [CO_2] within a region, anatomical traits reflecting water use/storage will exhibit more variability among sites compared with carbon assimilation traits; and (3) because specific leaf area reflects a combination of multiple leaf-level microanatomical traits, similar patterns of variation (expressed as CV) in anatomical traits will be measured in specific leaf area across climate gradients.

MATERIALS AND METHODS

Site description

Andropogon gerardii leaf samples were collected from four sites that were chosen to span a temperature and precipitation gradient characteristic of the Great Plains (Table 1). Two Long Term Ecological Research sites were used: Cedar Creek Ecosystem Science Reserve (CDR) (45°N, 93°W) and Konza Prairie Biological Station (KPBS) (39°N, 96°W). We sampled at a tallgrass prairie site affiliated with the Platte River Prairie (PRP) Nature Conservancy site (40°N, 98°W) as well as at the Rocky Mountain Research Station (RMRS), which is a United States Forest Service site (44°N, 103°W).

Anatomical sampling and analyses

Healthy leaf samples were randomly collected in July of 2017 from ten individuals from each site by clipping the newest mature leaf tissue (~30-mm sections) and immediately placing into FAA (10 % formalin/5 % glacial acetic acid/50 % ethanol

TABLE 1. *Historical climate for each location (1981–2017), mean values with italicized standard deviations and underlined variability (CV). Climate data from only 2017 are located at the bottom cell for each location. Variation refers to the CV from the historical dataset*

Location (year)	MAP (mm)	Variation	MAT (°C)	Variation (%)
KPBS (1981–2017)	870.82 ± 167.13	19.19 %	12.46 ± 0.88	7.6 %
KPBS (2017)	748.64		13.71	
PRP (1981–2017)	679.04 ± 109.13	16.14 %	10.47 ± 0.87	8.31 %
PRP (2017)	792.00		11.46	
RMRS (1981–2017)	467.29 ± 114.28	24.45 %	8.39 ± 0.97	11.56 %
RMRS (2017)	354.86		9.06	
CDR (1981–2017)	803.96 ± 136.22	16.95 %	6.62 ± 1.06	25.17 %
CDR (2017)	790.37		7.21	

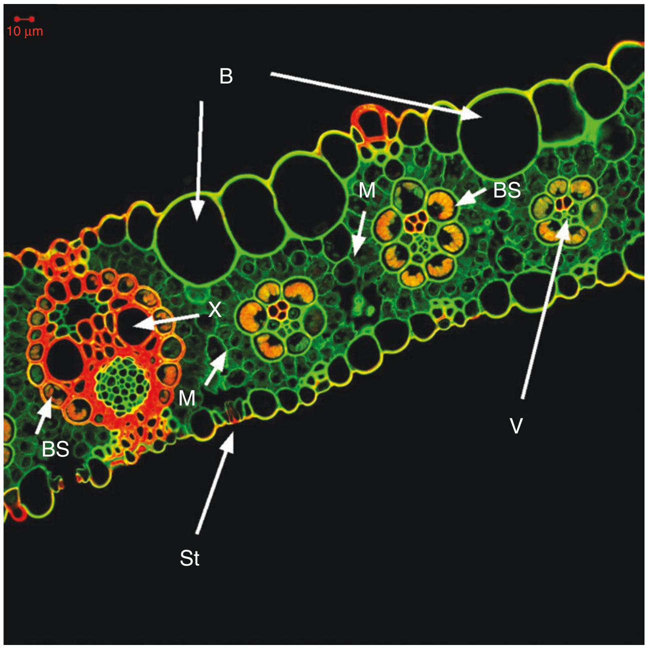

FIG. 1. Example of leaf cross-section of *A. gerardii* stained with Safranin Red and Fast Green to more clearly identify anatomical structures. B, bulliform cells; St, stoma; M, mesophyll; BS, bundle sheath; V, vein; X, xylem. Image taken with a Zeiss 880 confocal microscope.

(95 % EtOH)/35 % DI water) for vacuum infiltration. Samples were kept at room temperature until processing in August 2017.

Anatomical trait analyses

Leaf tissues were cross sectioned to a 4-µm thickness with a Leica RM2135 microtome (Leica Biosystems, Newcastle, UK) at the Kansas State University College of Veterinary Medicine Histopathology Laboratory. Samples were mounted in paraffin and then adhered to charged slides for biological staining. The tissues were stained with Safranin-O and Fast Green (Ruzin, 2000), coverslipped and imaged at ×100 and ×200 when necessary on a Zeiss 880 confocal microscope (Carl Zeiss, Walldorf, Germany) (Fig. 1). Image analysis was performed at ~850 × 850 µm with a 0.83-µm pixel size using a multi-track configuration, digital dual-bandpass filters and a GaAsP detector for enhanced sensitivity. All microanatomical data were collected using ImageJ software (Rasband, 1997–2020). Analysis consisted of measuring two tissue regions from each side of the blade midrib collected between two major vascular bundles (Bachle and Nippert, 2018). Traits measured from subsampled areas were averaged for each leaf, prior to analysis. Measurements included bundle sheath thickness (BS_t), xylem area (X_A), xylem wall thickness (t), xylem diameter (b), xylem reinforcement (t/b) (Hacke *et al.*, 2001) and interveinal distance (IVD). Xylem measurements (X_A, t/b) included all major conduits within major vascular bundles and averaged within the subsampled area. Interveinal distance was measured by averaging the distance between the centre of each vascular bundle across the whole leaf cross-section (not subsampled). Traits measured on an area basis (as a percentage of subsampled area) included mesophyll (MS_A), bundle sheath (BS_A), vein (V_A) and bulliform (B_A) (Fig. 1). While mesophyll tissue is located throughout the leaf, the majority is found within major and minor vascular bundles due to the reduced intercellular space observed in C_4 leaf anatomy. Therefore, MS_A and BS_A measurements were concentrated in both major and minor vascular bundles. We determined V_A as all tissues enclosed within the interior of the bundle sheath layer (xylem and phloem vessels). A portion of the original whole-leaf tissue collection was used to calculate specific leaf area (SLA; ratio of leaf area to dry mass) by measuring the 2D leaf area and recording oven-dried mass after 2 d of drying at 60 °C (Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2013).

PRISM climate data

Climate data from 1981 to 2017 from each site were extracted from the AN81d dataset made available by PRISM Climate Group sites (PRISM Climate Group, 2014). The AN81d dataset utilizes Climatologically Aided Interpolation (CAI) series values and the Advanced Hydrometeorological Prediction System (AHPS) for individual sites, and includes mean annual precipitation (MAP), mean annual temperature (MAT) and mean maximum annual temperature (MMAT). Each variable was recorded daily and averaged over a given year. Growing season means were extracted from mean climate data to include the months of May to August, as they best reflect the dominant growth period of *A. gerardii* (Knapp *et al.*, 1993; Fay *et al.*, 2003).

Statistical analyses

All analyses were conducted in the statistical program R V3.4.3 (R Core Team, 2019). Normality was checked with Levene's and Shapiro–Wilk tests. Xylem area was the only microanatomical trait that necessitated a non-parametric approach, which entailed the use of a Kruskal–Wallace test paired with a *post hoc* pairwise Wilcox test. Comparisons between locations were analysed using multiple mixed-effects model ANOVA performed with SLA as the response variable, anatomical traits and climate data as predictor variables and sites as the random effect. Tests were performed using the lmer function within the lme4 package (Bates *et al.*, 2015). To compare models using climate data extracted from PRISM, SLA and leaf trait data, we utilized Akaike's information criterion adjusted for small sample sizes (AICc). Model selection was used to determine the best model given PRISM climate parameters and all leaf traits by using the model.sel function within the MuMIn package (Grueber *et al.*, 2011; Bartoń, 2017).

RESULTS

Intraspecific trait variation

Microanatomical leaf traits in *A. gerardii* had statistically significant variability across sites but did not exhibit latitudinal trends of variation (Supplementary Data Table S1). Bundle sheath area was relatively similar across most locations, excluding *A. gerardii* at PRP, where it was significantly smaller than at KPBS and CDR ecotypes ($P < 0.05$; $P < 0.005$). Mesophyll area varied between locations (Supplementary Data Table S1; $P < 0.005$), most likely due to the reduced proportion of MS_A at CDR when compared with other locations. Mean V_A differed across locations ($P < 0.005$), corresponding to reduced V_A at PRP (Supplementary Data Table S1), where it was significantly less than at CDR ($P < 0.005$) and RMRS ($P < 0.001$). *A. gerardii* at PRP displayed the largest B_A (25.8%) of total leaf area, which was significantly higher than at both KPBS ($P < 0.003$) and RMRS ($P < 0.005$). Xylem area was observed to be the smallest at PRP ($P < 0.05$), while KPBS ecotypes were nearly double in area (Supplementary Data Table S1). *Andropogon gerardii* at PRP also displayed the highest xylem cavitation resistance (t/b; Supplementary Data Table S1; $P < 0.05$), which was twice that of KPBS ecotypes (Supplementary Data Table S1). Interveinal distance was the largest at KPBS and RMRS, and the smallest at CDR and PRP (Supplementary Data Table S1; $P < 0.05$), while the level of variability was relatively low (<15 %). The degree of variation (assessed using CV) in microanatomical traits associated with water use (X_A and t/b) was greater than in traits associated with carbon assimilation (MS_A and BS_A) (Fig. 2; Supplementary Data Table S1). Carbon assimilation traits (MS_A and BS_A) had relatively small variation across collection sites (Fig. 2A, C). In contrast, X_A and t/b (Fig. 2B, D) displayed three to four times the trait variation in comparison with carbon assimilation-related tissues (Fig. 2A, C). Specific leaf area was observed to be statistically similar across all collection sites (Fig. 3B, $P = 0.078$); however, the CV within sites ranged from 5.4% (CDR) to 20% (PRP) (Fig. 3A).

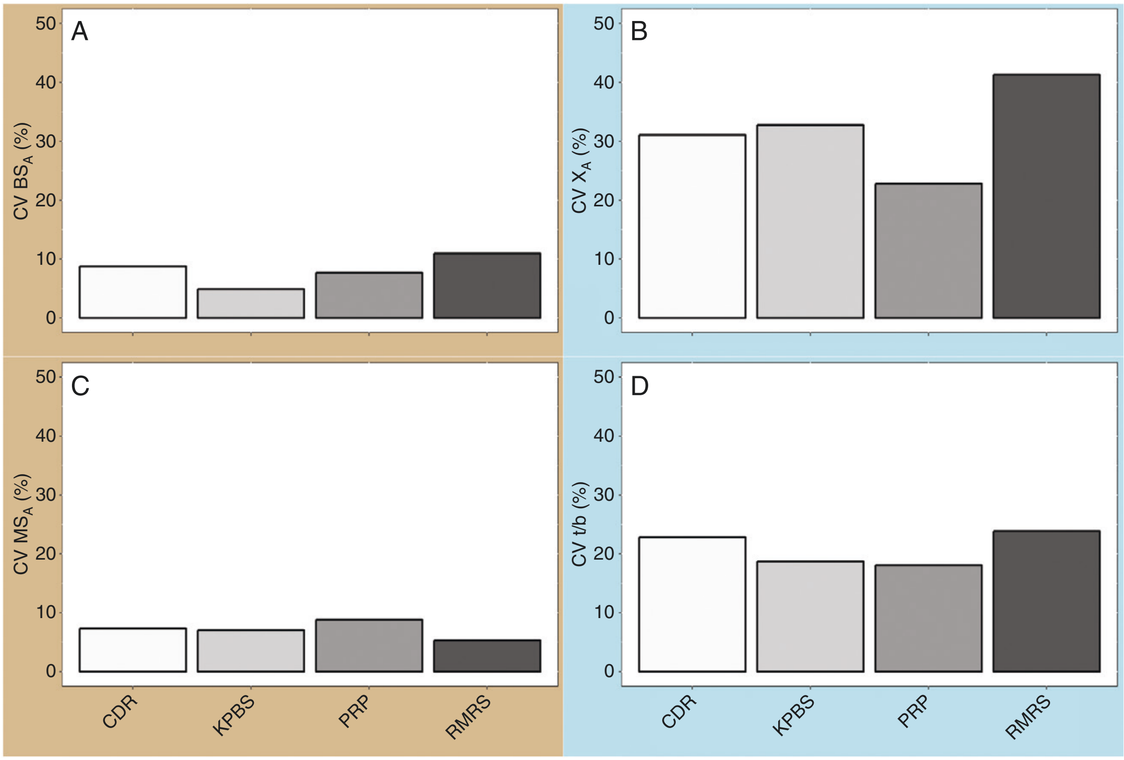

FIG. 2. Coefficient of variation of anatomical traits measured in *Andropogon gerardii* at each sampled location. (A) Bundle sheath area; (B) xylem area; (C) mesophyll area; (D) xylem reinforcement. Plot colour is related to trait function: tan, carbon assimilation; blue, water transport.

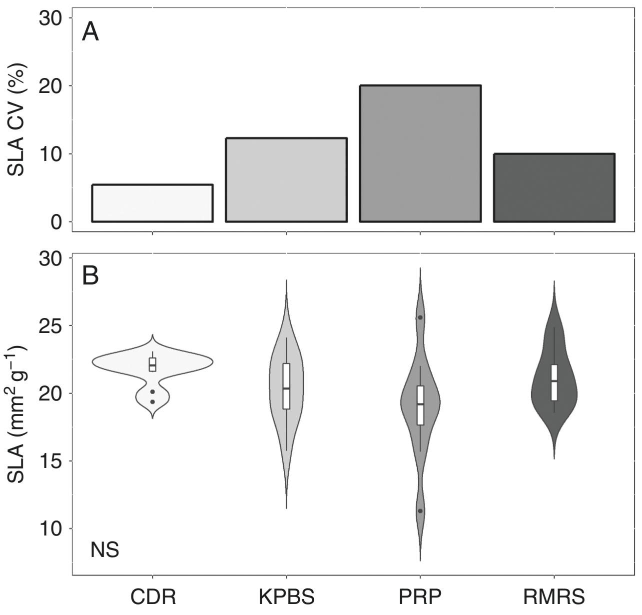

FIG. 3. Specific leaf area (SLA) measurements collected from *A. gerardii* in 2017. (A) Coefficient of variation for each location. (B) Violin plots displaying mean and distribution of SLA. NS, not significant.

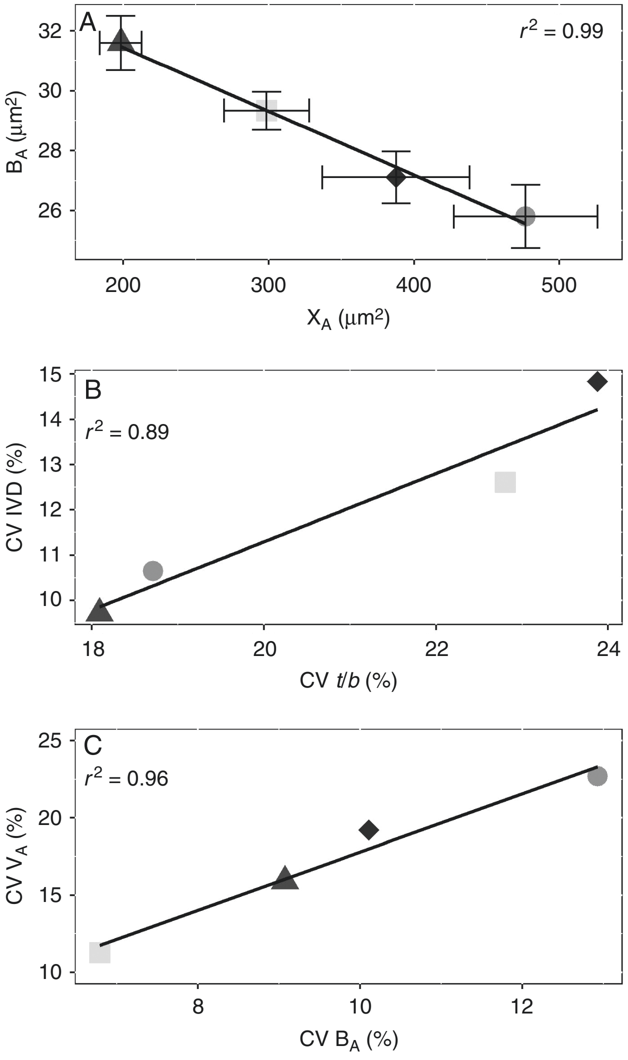

FIG. 4. Anatomical trait relationships in *A. gerardii* across all sampling locations. (A) Xylem area (X_A) and bulliform area (B_A). (B) Xylem reinforcement (*t/b*) and interveinal distance (IVD) variation. (C) B_A and vein area (V_A). Sites are indicated by shading (matching previous figures) and shapes: diamonds, RMRS; squares, CDR; triangles, PRP; circles, KPBS.

Trait interdependency

While *A. gerardii* leaves expressed considerable variation in their anatomical traits across sites, significant trait relationships were present (Fig. 4; Supplementary Data Fig. S1). Specifically, a greater area of water storage (B_A) was correlated with smaller diameter of xylem vessels (Fig. 4A; $P < 0.001$). Samples with greater CV for IVD were also correlated with increased CV in *t/b* ($P < 0.038$; Fig. 4B). Water-related traits had a higher CV at each site (Fig. 2B, D) than carbon assimilation traits (Fig. 2A, C). Mean IVD was positively correlated with several anatomical traits (BS_A, MS_A and X_A) and negatively correlated with others (BS_A and *t/b*) (Supplementary Data Fig. S1).

Few statistically significant relationships were found between SLA and the microanatomical traits measured here (Supplementary Data Fig. S1). Surprisingly, given the large proportion that M_A and BS_A (carbon assimilation tissues) encompass in a leaf, there was no statistically significant correlation with mean SLA ($P > 0.05$). Our results also indicated that water-related anatomical traits in *A. gerardii* (X_A, B_A and *t/b*) were not correlated with SLA across sites ($P > 0.10$). The CV in SLA did show a negative trend with IVD and X_A, while displaying a positive trend with MS_A and B_A CV, but these relationships were non-significant ($P > 0.05$; Supplementary Data Fig. S1).

Using the AICc model selection process, the model that included MAP, MMAT, and the interaction MAP × MMAT explained the greatest variation of economic and microanatomical traits in *A. gerardii*. Annual precipitation for 2017 was lower than the historical average for each site (excluding PRP) and did not display any correlation with MS_A or BS_t (Fig. 5B, D). Compared with long-term averages, all sites experienced higher mean temperatures in 2017. The mean air temperatures in 2017 had a positive relationship with the proportion of both MS_A (Fig. 5A) and to a lesser extent BS_t (Fig. 5B). Several microanatomical traits showed non-significant ($P > 0.10$) trends with climate data (e.g. the correlation between mean B_A and mean X_A with MAP, or the relationship between MAT and X_A, *t/b*, BS_A and SLA Supplementary Data Fig. S1). Comparisons of CVs also displayed similar patterns, such as the negative trend between B_A and MAT.

DISCUSSION

A persistent research quest in physiological ecology involves the identification of mechanistic plant traits that help explain ecological species occurrence, abundance and persistence (Reich, 2014; Funk *et al.*, 2017; Linder *et al.*, 2018; He *et al.*, 2019). Here, the data presented illustrate that leaf-level microanatomical traits varied systematically along climate gradients of the Great Plains while also influencing broad physiological strategies in *A. gerardii* (Fig. 5A, B). For traits related

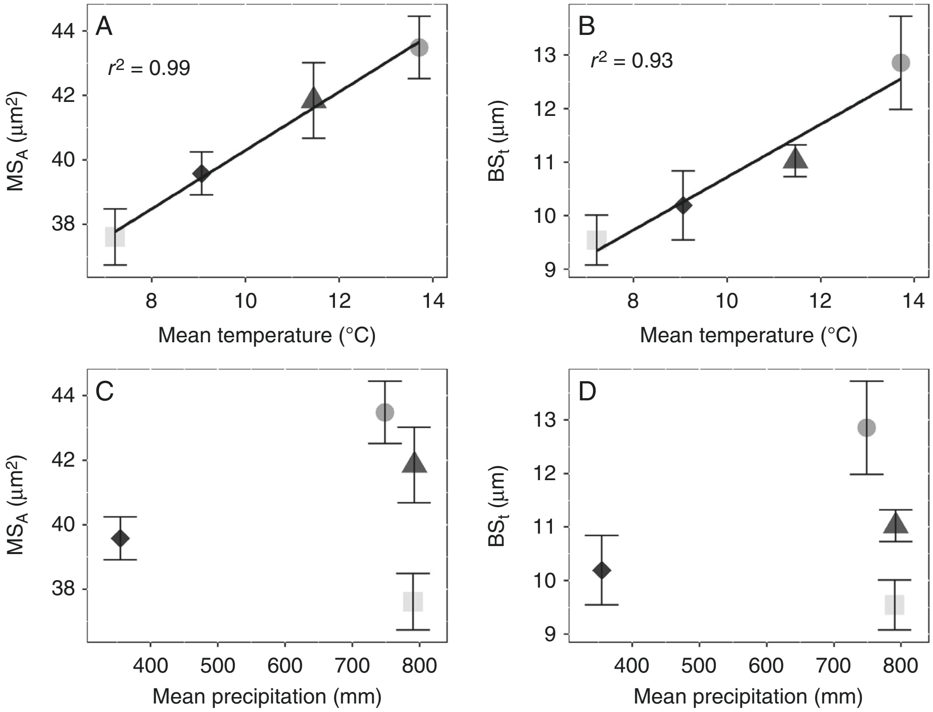

FIG. 5. Linear regressions of carbon assimilation tissues with continuous mean temperature (A, B) and mean precipitation (C, D) from each site in 2017, provided by PRISM. (A, C) Mesophyll area; (B, D) Bundle sheath thickness. Sites are indicated by shading (matching previous figures) and shapes: diamonds, RMRS; squares, CDR; triangles, PRP; circles, KPBS.

to carbon use (MS_A, BS_A; Fig. 2A, C), CV was low across sites, varying between 5 and 10 %. In contrast, traits associated with water use (*t*/*b*, X_A; Fig. 2B, D) had ~2–5 times higher CV (20–40 %). Variation in microanatomical traits associated with water use was greater than that in carbon-use traits, suggesting a potentially higher coupling to water availability within and across sites (Fig. 2). Given the fundamental role of water availability as a driver of grassland ecosystem processes and grass species physiology, the expression of a broader range of trait values associated with water availability is expected (Weaver *et al.*, 1935; Nippert *et al.*, 2011; Reichstein *et al.*, 2013; (O'Keefe *et al.*, 2019). Stomatal aperture regulation is one such physiological trait, which controls carbon gain (CO_2 uptake) and water loss (transpiration) (Brodribb *et al.*, 2007; Buckley, 2019; Nunes *et al.*, 2020). C_4 grass species typically exhibit conservative regulation in stomatal aperture, because the biochemical adaptation for concentrating CO_2 inside bundle sheath cells maximizes carboxylation per unit water loss (Hatch, 1987; Edwards *et al.*, 2001; Zhou *et al.*, 2018). Relatively low variation (CV) in carbon-use tissues (Fig. 3A, C) reflects the innate biochemical adaptations of C_4 species, resulting in a lower quantum efficiency and a higher photosynthetic capacity than C_3 species (Taylor *et al.*, 2011). While the C_4 biochemical strategy has an additional carboxylation step requiring additional ATP, the modified leaf anatomy (Kranz) allows greater overall carbon assimilation, which reduces the need for large structural leaf variation (CV) within species (Lundgren *et al.*, 2014).

Microanatomical trait data revealed novel tissue-specific water-use strategies that may facilitate the existence of *A. gerardii* across a regional gradient that experiences shifting water availability (Fig. 4). When individuals of *A. gerardii* were measured across the region, functional responses of water-use strategies illustrate a clear pattern of maximizing water transport or water storage. Individuals that produced larger xylem vessels (X_A) had decreased bulliform area (B_A) (Fig. 4A), enabling a larger potential for water and mineral transport while decreasing the potential for water storage (Carmo-Silva *et al.*, 2009; Gibson, 2009). However, variation (CV) in interveinal distance (IVD) across sites was strongly associated with variation in resistance (*t*/*b*) (Fig. 4B), equating to greater flexibility in the amount of transport vessels (IVD) and the capability of those vessels to withstand decreased water availability (*t*/*b*) (Jordan *et al.*, 2013). These strategies may allow populations of *A. gerardii* to respond to climate fluctuations (temperature and rainfall) within a growing season. For example, within populations at a given site, some individuals can acquire water and nutrients quickly (increased X_A) while others exhibit a more conservative approach (increased B_A); this variation may buffer the impact of climate fluctuations on population-level performance. The higher regional variability in microanatomical traits associated with water use (Figs 2 and 4C), may facilitate the persistence of populations of *A. gerardii* through dry periods that would otherwise require decreased carbon assimilation caused by stomatal closure (Buckley, 2019; Dusenge *et al.*, 2019), degradation of photosynthetic machinery (Maricle and Adler, 2011) and increased water stress leading to the loss of vessel integrity or cavitation (Blackman *et al.*, 2010; Ocheltree *et al.*, 2014).

Plant physiological responses are directly impacted by temperature and water availability (Tsypin and Macpherson, 2012; Griffin-Nolan *et al.*, 2019), which also influences plant growth and system productivity (Hoover *et al.*, 2014; Felton and Smith, 2017). Leaf microanatomical traits may also display similar climate–trait relationships, but have been infrequently investigated across species or systems (Carmo-Silva *et al.*, 2009; Ouyang *et al.*, 2017; Bachle and Nippert, 2018). The results in this study indicate a positive relationship between the investment in carbon assimilation tissues (MS and BS_t) and mean annual temperature (Fig. 5A, B), while mean annual precipitation yielded no such correlations (Fig. 5C, D). Relationships with temperature and carbon assimilation traits may be indirectly linked to differences in stomatal regulation across sites (Berry and Patel, 2008; Sage *et al.*, 2014). Previously, individuals growing in locations with higher temperatures had increased stomatal regulation, likely reflecting the role of increased vapour pressure deficit in leaf function (Lin *et al.*, 2015). Therefore, these results may illuminate how ranges of intraspecific values of selected microanatomical traits may explain underlying variation in whole-leaf traits (such as SLA and leaf dry matter content) commonly reported across temperature gradients (Jung *et al.*, 2014).

Specific leaf area is a frequently measured trait due to the ease of collection and observable plasticity within populations and species (Garnier *et al.*, 2001; Tjoelker *et al.*, 2005; Wellstein *et al.*, 2017). Differences in SLA are determined by biotic and abiotic factors and interpreted as varying economic strategies: conservative (high SLA: wide and thin leaves) or structurally expensive (low SLA: thick and narrow leaves) (Wohlfahrt *et al.*, 1999; Garnier *et al.*, 2001; Reich, 2014). However, economic strategies inferred from changes in SLA are mechanistically derived from underlying microanatomical tissues (mesophyll, bundle sheath, bulliform, xylem) that aggregate to form whole-leaf properties (i.e. thickness, area, mass) (Carmo-Silva *et al.*, 2009; Reich and Flores-Moreno, 2017). Thus, variation in microanatomical traits should at least theoretically coincide with variation in SLA (John *et al.*, 2017). While we observed significant differences in microanatomical traits across sites, SLA was statistically similar across sites (Fig. 3B), and unrelated to the microanatomical traits measured here (Supplementary Data Fig. S1). Thus, it is surprising that we see predictable variability in anatomical traits, but not for one of the most common leaf-level traits studied. We attribute the similarity in SLA values across this grassland region to a variety of possible factors. (1) Our microanatomical subsampling method, which utilized only a portion of the leaf may not scale to the whole leaf. (2) Insights shown here arise from four different grassland locations. Perhaps more data/sites are required to detect intraspecific SLA differences than are required for microanatomical trait differences. (3) The inclusion of additional sites could also be used to resolve any potential confounding between MAT and MAP among the sites used here. With more sites, links between climate and SLA trait variability may improve. (4) The functional interpretation of microanatomical tissues (e.g. tissues associated with water transport, structure, C assimilation, etc.) is more straightforward than the functional interpretation of a composite whole-leaf trait like SLA. (5) Here, we focused on responses of a single, common grass species. Future studies investigating the plasticity of different species representing different plant functional types may yield different results. While statistically significant differences in SLA between locations or climate variables were absent, CV did vary, suggesting that variability in SLA is not equivalent among the populations compared here. (6) Finally, the components of SLA (leaf area and leaf mass) may vary systematically across sites such that changes in SLA mask or cancel each other out. Regardless of these caveats for SLA, we do show clear mechanistic linkages among microanatomical traits, relationships with regional climate gradients and the utility of microanatomical traits for drawing physiological inference within a common grass species.

Results from this study contribute to a growing volume of research that suggests trait variability (here measured as CV) can provide insight into functional plant responses on a par with investigations of mean trait values (Funk *et al.*, 2007; Poorter *et al.*, 2009; Li *et al.*, 2016). Our data also highlight and support how increased utilization of intraspecific microanatomical trait variability, highly sensitive to lower-level parameters (Verheijen *et al.*, 2013), in a dominant grass species may reveal investment options for both carbon- and water-use tissues, which collectively vary to elucidate leaf form and function.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: mean anatomical and morphological trait data with standard error and CV for each collection. Figure S1: correlation matrix of leaf traits, mean annual precipitation and mean annual temperature at each site.

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