

Anatomical constraints on grass physiological responses depend on water availability

by

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B.A., Hastings College, 2014
M.S., Kansas State University, 2017

AN ABSTRACT OF A DISSERTATION

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Abstract

Grasslands occupy more of Earth's terrestrial surface than any other biome and are characterized by the dominance of grasses (Poaceae), fire, grazing, and an inherently variable climate. Future climate projections forecast dramatic shifts in precipitation within the next 100 years for grassland systems, negatively impacting most native grassland species. Over the past 30 years, a substantial focus has been placed on understanding the ecological consequences of grassland responses to drought. During this period, most research has focused on identifying changes in productivity and species diversity and the larger scale consequences of drought on carbon, nutrient, and water cycling. While this ecosystem and community focus has been foundational for forecasting future scenarios, it typically lacks detailed mechanistic investigation of leaf physiological and anatomical responses to drought. For this reason, research focused on the physiological responses of dominant grassland species to changes in water availability provide a missing perspective for *WHY* and *HOW* drought impacts grassland function. Prior research on a dominant grass species, *Andropogon gerardii*, has illustrated that leaf-level physiological responses were constrained by anatomical traits that dictate carbon assimilation and water-use strategies (Bachle & Nippert, 2018 – *Acta Oecologica*). In this dissertation, I used both naturally occurring climate gradients and rainfall manipulation experiments to explore responses of a dominant grass species and provide a detailed perspective of differences in grass anatomy and physiology in response to water limitations across the climate gradients of the Great Plains.

During a greenhouse dry down experiment I imposed an extreme drought by withholding water from 12 species of grasses from 6 tribes that are known to vary in drought tolerance (Bachle *et al.* - *In Prep*). I analyzed physiological responses at three stages: prior to drought ("Initial"), when samples reached stomatal closure ("Stressed"), and two days after re-watering ("Recovery"). I paired this physiological data with measurements of above- and belowground productivity, leaf and root economic traits, and leaf – level microanatomy. Results from this experiment revealed species response to drought were similar within tribe, while recovery from drought was highly variable across species. In addition, drought responses were framed by phylogenetic relatedness, physiology, morphology and microanatomical traits while drought recovery was influenced by one primary trait: the number of stomata. These data show the

diverse physiological responses and variability of microanatomical traits across and within tribes. Furthermore, these results suggest that the biogeographic histories frame a species' ability to respond to changes in low soil moisture.

To investigate trait variability, I performed a literature search to assess intraspecific variability in commonly measured traits (such as specific leaf area - SLA) in *A. gerardii* from 13 climatically-distinct grasslands. Results indicated that SLA varied widely between locations, but with no discernable trends with climate parameters (Bachle *et al.*, 2018 *Frontiers in Ecology and Evolution*). Ecologists commonly measure SLA because differences in this trait reflect growth strategies and leaf carbon investment, and because it's a relatively easy trait to measure. However, the investment strategies inferred from SLA result from a suite of underlying anatomical tissues (bundle sheath, mesophyll, xylem) that are seldom measured. To understand how physiology/anatomy varies across latitudinal population gradients, I collected *A. gerardii* leaf tissues from locations varying in temperature and precipitation (KS, NE, SD, MN). I analyzed the relationship between SLA and internal leaf anatomical traits (Bachle & Nippert, 2020 – *Annals of Botany*). SLA was statistically similar in *A. gerardii* across locations, while anatomical traits related to carbon assimilation (mesophyll area, bundle sheath thickness) varied by mean annual temperature. Anatomical traits connected with water transport and storage (xylem area, cavitation resistance) exhibited 3 – 4 times the coefficient of variation than did carbon-related traits. Results from this chapter illustrate that variation in anatomical traits, influenced by climate, may underlie patterns of growth and productivity in this species at larger ecological scales.

For my final chapter, I collected physiological and microanatomical data from *A. gerardii* in Kansas and Nebraska prairies managed with or without cattle in 2018 and 2019 (Bachle & Nippert – *In Prep*). The precipitation differences between successive years at each location had a larger impact on physiological and anatomical differences among sites compared to the grazing treatments. In addition, photosynthetic rates increased with leaf – level nitrogen content, while cavitation resistance increased with higher C:N ratios. Microanatomical traits, such as bundle sheath tissue area, were found to correlate with photosynthetic rates; however, the direction of this relationship was dependent on the year sampled. These results indicate that leaf-level nutrient content can influence microanatomical leaf structures and physiological responses to changes in climate.

Overall, the results from my dissertation highlight the integral role of leaf anatomical traits in contextualizing our interpretation of physiological responses to drought, and across regional gradients in climate. My data clearly show that commonly used whole-leaf traits (e.g., SLA), unlike leaf microanatomy, typically don't vary predictably with leaf physiology or climate gradients. Moving forward, the research framework I have organized can be applied to other species. The increased anatomical trait representation of other native species will help elucidate leaf form and function while also adding beneficial data that may be useful for forecasting future grassland responses in this era of global change.

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Of course, without the support of my incredible wife, Kaitlin, I would not be half the person I am today – words can't express how incredible lucky and blessed I am to have her in my life. Well, words can express that....but this is an official document, so I'll keep it professional – she's cool.

Dedication

To my wife Kaitlin, who listens to me talk about grass...

Gnu

Chapter 1 - Introduction

The North American Great Plains is one of the largest grassland ecoregions in the world, second only to the Serengeti in Africa (Dixon *et al.*, 2014). The Great Plains ecoregion has many unique characteristics beyond its geographic breadth. This ecosystem was once home to millions of migratory bison, elk, and pronghorn, thousands of unique plant species, and played a fundamental role in the westward expansion of the United States as these grasslands were cultivated into commercial agriculture to support a growing nation (Blair *et al.*, 2014). Collectively, the Great Plains represents one of the most unique and important regions of North America. The rich soils of the Great Plains, along with a temperate climate conducive to growing highly productive grass species was key to the conversion of these grasslands into modern agriculture (Gibson, 2009; Thornton *et al.*, 2019).

Native grasslands in the Great Plains that have not been converted to agriculture have been maintained by three key ecosystem drivers: fire, grazing, and climate. Fire, in the proper frequency, is necessary for removing dried biomass which then allows for the growth of new grasses with high nutritional content (Knapp & Seastedt, 1986; Raynor *et al.*, 2015). In addition, fire deters the encroachment of woody species before establishment, which otherwise would have decreased grass cover and reduced productivity of the system (Ratajczak *et al.*, 2014; Collins *et al.*, 2021). The productive grasses within the Great Plains have historically provided forage for large mammals and insects, which preferentially feed on dominant grass species (Collins & Calabrese, 2012). Suppression of the dominant grasses as a result from grazing, increases floral diversity and subsequent ecosystem function (Vinton *et al.*, 1993; Allred *et al.*, 2011; Koerner & Collins, 2014). Due to the immensity of the Great Plains region, a substantial latitudinal and longitudinal climate gradient exists. This climate gradient encompasses a ~20° C

range in mean annual temperature from the northern to southern regions, while also containing a ~2,000 mm precipitation gradient from west to east (Anderson, 2006; Bachle *et al.*, 2018). The temperature (latitudinally) and precipitation (longitudinally) gradients and inherent variability in the Great Plains has also shaped productivity, species composition, and ecosystem services (Axelrod, 1985; Epstein *et al.*, 1996). Grasses have adapted unique functional traits that are adaptive under the variable conditions of the Great Plains (Carpenter, 1940; Blair *et al.*, 2014). The concept of plant functional traits (*PFTs*) originated in the late 1990's and was used to distinguish a group of traits that can be used to predict species' responses to environmental conditions (Diaz & Cabido, 1997; Chapin *et al.*, 2000; Lavorel *et al.*, 2007). Under this framework, it was postulated that predicting species and community responses from species traits instead of species identity was the 'Holy Grail' in plant ecology (Lavorel & Garnier, 2002; Funk *et al.*, 2017). It is now well recognized that *PFTs* have been developed over evolutionary time, and lend insight to physiological responses (Díaz *et al.*, 2013; Funk *et al.*, 2017; Griffith *et al.*, 2020).

Grasses have developed unique morphologies, such as large shallow fibrous rooting systems that allow for quick absorption of water and nutrients; investment into meristematic tissues that allow for rapid regrowth after a disturbance; and narrow leaves that reduce excess irradiance and subsequent water loss (Weaver & Darland, 1949; Ocheltree *et al.*, 2011; Nippert & Holdo, 2015; Ott *et al.*, 2019). In addition, grasses have developed unique microanatomical traits/structures that impact physiological responses, such as water storage cells that also serve to aid in leaf rolling (bulliform cells) (Matschi *et al.*, 2020), dumbbell guard cells flanking stomata that allow for rapid movement (Nunes *et al.*, 2020), and a large representation of the C₄ photosynthetic pathway, which serves to increase carbon assimilation and reduce water loss

(Furbank, 2016; Von Caemmerer *et al.*, 2017). While Poaceae (grasses) are not the only family to contain the C₄ pathway, it has evolved ~24 times independently, making it one of the greatest examples of convergent evolution (Sage, 2017). In addition, C₄ photosynthesis would not be possible without a complete rearrangement of the microanatomical tissues that are responsible for carboxylation and assimilation of CO₂ inside the leaf (Hatch, 1987; Lundgren *et al.*, 2014; Furbank, 2016). The importance of the influence of microanatomical traits on physiology can also be appreciated when considering other processes, including: water transport within leaves and roots, which is constrained by the size and shape of the xylem vessels (Baas *et al.*, 2004; Hacke & Sperry, 2015); photosynthate transport (phloem loading), limited by architecture of phloem and plasmodesmata at cell wall interfaces (Esau *et al.*, 1957; Botha, 1992; Danila *et al.*, 2019); and stomatal characteristics, ranging from aperture to density on leaf surfaces (Xu & Zhou, 2008; Bertolino *et al.*, 2019). While microanatomical data are obtained at an individual leaf or root level, these data are useful for inferring controls on whole plant physiology, which is often the key to understanding the population, community, and ecosystem level consequences of altered environmental conditions.

The collection of *PFT* data across multiple individuals within a species also lends insight to the intraspecific trait variability of a species (ITV), which results from genotypic variation and trait expression (Schlichting, 1986; Pigliucci, 2001). Measuring ITV, or the coefficient of variation (*CV*), can offer context as to why a single species can exist in climatically variable region or across naturally occurring climate gradients (Westerband *et al.*, 2021). For instance, these ITV data can answer how or why common grasses can exist across the Great Plains and surrounding regions (Bachle & Nippert, 2018, 2021). Knowing the mechanistic reasoning of this variability is key to predicting future responses to potential ecosystem change. In this

dissertation, I investigated how leaf - level microanatomical traits in grass influence/constrain physiological responses to changes in water availability.

- In Chapter 2, I investigate the response of a diverse assemblage of Poaceae species to a simulated drought. Distinct evolutionary histories provide a basis for altered drought responses and recoveries, mechanistically driven by physiological and anatomical traits. In this chapter I measured whole - plant, economic, and microanatomical traits paired with physiological responses of 12 grass species during a dry - down experiment. Results from this chapter indicate that drought responses are similar within grass lineages (tribes) and can be attributed to a myriad of plant functional traits (*PFTs*). In contrast the ability of species to recover when water was available was dissimilar within tribes and was most impacted by the number stomata present. Additionally, a separation between productivity and economic traits such as specific leaf area and specific root length was observed, likely reflecting a tradeoff in above and belowground growth strategies.
- In Chapter 3, I investigated how a grass species can be found across the climatically variable Great Plains region. *Andropogon gerardii* is a dominant species in the tallgrass prairie, and it is also found in over 80% of the contiguous United States. In this chapter, I performed a literature search to collect commonly measured *PFT* data for *A. gerardii* across 13 locations in the Great Plains of North America to determine how intraspecific *PFT* variability is impacted by climate parameters. Strong relationships were lacking between most functional traits and climate predictors, excluding above and belowground productivity. The major finding from this chapter was the dearth of *A. gerardii* data in the

literature, leading to a lack in statistical power. Thus, these findings underline the need to increase data collection on this species across this region.

- In Chapter 4, I investigate how internal leaf anatomical structures in *A. gerardii* are affected by differences in climate means and variability. Historically, temperature and precipitation have played key roles in driving evolution, most notably C₄ photosynthesis – which requires cellular modification of leaf tissues for altered biochemistry. In this chapter, I analyzed a myriad of internal microanatomical leaf traits in *A. gerardii* across four climatically distinct locations in the Great Plains. Traits associated with carbon assimilation displayed small variation across locations and were correlated with mean annual temperatures, but not precipitation. Interestingly, traits associated with water-use strategies displayed heightened levels of variability. In addition, specific leaf area (*SLA*) was found to be statistically similar across all locations, however, differences in variability were observed. These results indicate that changes in leaf morphology *SLA* may not be evident, while the microanatomical traits integral to whole leaf structure and physiology are impacted by climate.
- In Chapter 5, I investigated the impact of grazing and climate on leaf – level anatomy and physiology. The effects of grazing on plant physiological responses have been previously observed, however, little research has focused on grazing’s impact to leaf – level microanatomy. In this chapter, I collected physiological, anatomical, and stoichiometric data in grazed and un-grazed watersheds across three locations along a latitudinal gradient in Kansas and Nebraska. I observed very few treatment effects, but rather distinct variation across locations and years sampled - most likely due to disparate growing season conditions in 2018 and 2019. However, there were significant

relationships with physiological and microanatomical traits with nutrient availability.

These results underline the importance in understanding the interplay between anatomy and physiology in changing climate conditions.

Chapter 2 - Physiological responses and recovery to drought are associated with differences in leaf function and microanatomy among grass lineages

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Summary

Grasses are cosmopolitan, existing in many ecosystem types ranging from xeric to tropical; however, traits that relay physiological responses to varying levels of drought are based on evolutionary histories. Here, I withheld water from 12 species representing 6 tribes of grasses to examine drought responses across grass phylogenies while measuring physiological, morphological, and microanatomical traits. Phylogenetic relatedness influenced drought sensitivity but was not a key determinant of drought recovery. Increased *iWUE* decreased species' ability to remain physiologically active in drought conditions because *iWUE* coincided with greater number of stomata, resulting in greater water loss. Restriction of growth strategies to mitigate the impacts of drought were observed in all species, resulting in either high *SLA* or *SRL*, but not both. Drought tolerance in Poaceae varied by phylogenetic relatedness, physiological traits related to carbon and water usage, morphologic traits in both leaves and roots, and anatomical traits specific to stomata and xylem characteristics. These results illustrate how functional responses to drought vary among diverse grass lineages.

Introduction

Grasslands play a major role in regional and global carbon sequestration because grasses invest in extensive rooting systems and storage organs (Pendall *et al.*, 2018; Veldman *et al.*, 2019) that promote belowground C storage (Sitters *et al.*, 2020). Carbon dynamics are highly influenced by water availability in grassland systems, as evident in drought years that result in decreased productivity (Fay *et al.*, 2003; Hoover & Rogers, 2016; Carroll *et al.*, 2021).

Grasslands experiencing extreme droughts can have reduced plant physiological functioning (Cook *et al.*, 2015; Hoover *et al.*, 2015), increased invasibility from non-native species (Linder *et al.*, 2018), disruption of fire intervals (Wilcox *et al.*, 2020), and loss of ecosystem functioning (Eters *et al.*, 2014; Mainali *et al.*, 2014). While many grass species in grassland ecosystems have evolved in the context of an inherently variable climate, future climate projections emphasize large shifts in water availability, resulting in extreme drought and deluge events (Post & Knapp, 2019; Knapp *et al.*, 2020). For these reasons, it is increasingly important to understand how diverse lineages of grass species that vary in climate and evolutionary histories will respond to extreme drought conditions.

Climate change continues to modify precipitation seasonality and amount with sizable and diverse impacts on ecosystem function (Maurer *et al.*, 2020). For many ecosystems, these alterations will result in increased frequency or severity of drought events, where water availability is anomalously low (Carroll *et al.*, 2021). The magnitude and nature of, and sensitivity to drought events has variable impacts on ecosystem functioning (Carroll *et al.*, 2021). Factors determining drought sensitivity in individual plant species, include precipitation and temperature variability (Knapp *et al.*, 2015), plant productivity and species richness, (Burri *et al.*,

2018), and potentially the dominant species and their functional traits (Avolio *et al.*, 2019). Furthermore, the evolutionary history of drought exposure in plant lineages is likely to frame future drought responses. For example, lineages of plants from arid and semi-arid regions have functional traits (narrow leaves, stomatal regulation, absorptive rooting systems) that allow them to acquire and conserve water (Ocheltree *et al.*, 2020), whereas lineages from tropical regions may have wider leaves and altered stomatal traits that result in different water - use strategies (Liu *et al.*, 2018; Buckley, 2019). These evolutionary tradeoffs have shaped functional differences across lineages and directly impacted ecological dynamics (Griffith *et al.*, 2020). However, the extent of such evolutionary tradeoffs has not been utilized to identify lineage specific trait responses to extreme drought conditions. There are even fewer investigations that combine physiology, microanatomy, morphology, and structural economic data in grass species spanning several Poaceae tribes in response to drought.

Annual variation in precipitation is a feature of many grassland ecosystems and, in combination with CO₂ and temperature, has likely played a major role in the evolution and biogeographic history of major grass lineages (Osborne, 2008; Cleland *et al.*, 2013; Cotton *et al.*, 2016). The most notable evolutionary example is that of C₄ photosynthesis—a complex trait conferring rapid growth in warm conditions or during periods of low [CO₂] (Fox *et al.*, 2018). C₄ photosynthesis has evolved in ~26 lineages of grasses, each with a unique history and combination of functional traits. Importantly, a myriad of traits with varying degrees of drought tolerance have evolved across the phylogeny, providing the opportunity for comparison. These varying evolutionary histories of grasslands have resulted in different adaptations of grass species that likely account for observed differences in temporal drought responses (Ocheltree *et al.*, 2020; Knapp *et al.*, 2020). For example, some species, such as native species in the arid

American southwest, have the ability to initially tolerate the impacts of drought by maintaining physiological functioning for prolonged periods of time (Thomey *et al.*, 2014; Skelton *et al.*, 2015). The ability of some species to maintain physiological functions despite drying soils represents an ability to withstand shifts in equilibrium and may be due to increased cuticle thickness, decreased stomatal size and densities, less negative turgor loss point, and more conservative growth strategies (*SLA*, *SRL*) (Reich, 2014; Habermann *et al.*, 2019; Bertolino *et al.*, 2019; Ocheltree *et al.*, 2020). Alternatively, the production of cheaper leaves and roots and tight stomatal regulation is associated with the ability to avoid desiccation, and quickly recover once drought breaks (Poorter *et al.*, 2009; Lin *et al.*, 2015; Garbowski *et al.*, 2020). The ability to quickly resume pre-drought physiological rates via rapid recovery may or may not be associated with the ability to tolerate drought in the first place (Hoover *et al.*, 2014a; Volaire, 2018).

While many grassland systems are projected to experience drought-like conditions within the coming century (Stocker *et al.*, 2013; Cook *et al.*, 2015; Griffin-Nolan *et al.*, 2019a), the drought intensity is unknown (Carroll *et al.*, 2021). However, most future climate predictions indicate a rise in climate variability, which will likely increase the frequency of pulse and press droughts (Hoover & Rogers, 2016b). Hoover & Rogers (2016b) detail the difference between these drought types: *pulse droughts* follow short-sharp declines in rainfall, *press droughts* are less intense but longer in length, and the combination of both creates the most extreme drought conditions. These contrasting drought conditions have varying effects on water and carbon cycles and elicit major changes in the plant community due to the negative physiological effects in plant species (Dusenge *et al.*, 2019; Griffin-Nolan *et al.*, 2019a). However, due to the previously mentioned variations in phylogenetic relationships and evolutionary adaptations of grass species, there are likely to be large differences in temporal responses of plants to drought

(Ocheltree *et al.*, 2020; Knapp *et al.*, 2020). Such differences may be expressed in response to drought events (ability to withstand shifts from equilibrium) and in drought recovery (ability to regain equilibrium following release from drought) (Hoover *et al.*, 2014a; Volaire, 2018). However, drought or rainfall variability research has been mainly focused on grassland system dynamics with the use of rainfall manipulations (Fay *et al.*, 2000; Koerner & Collins, 2014) or focusing efforts on a single dominant species across precipitation gradients (Gray *et al.*, 2014; Maricle *et al.*, 2017; Bachle & Nippert, 2018). While such studies have allowed a greater understanding of productivity/community responses and the physiological responses of dominant species, less attention has been given to the responses from a wide representation of Poaceae species. Those studies that do emphasize drought responses across Poaceae have focused on many species within few families (Liu & Osborne, 2015); Liu & Osborne (2015) collected a myriad of plant functional traits (*PFTs*) ranging from leaf morphology, hydraulics, and gas exchange on two of the most dominant Poaceae subfamilies. These data are invaluable for understanding the intricacies of water relations in grasses and have been incredibly impactful for trait-based research.

During the evolutionary development of Poaceae, separate lineages have evolved different suites of traits, including traits that confer drastically different relationships with water (Osborne, 2008; Edwards *et al.*, 2010). For instance, the two most abundant monophyletic groups of C₄ grasses: Andropogoneae (water spenders) and Chlorodoideae (water savers) vary in water-use strategies because of distinct biogeographic histories (Taub, 2000; Grass Phylogeny Working II, 2012; Griffith *et al.*, 2020). Both lineages occupy warmer climates but their global distribution vary due to high (Andropogoneae) and low precipitation and water availability (Cynodonteae) (Williams *et al.*, 1998; Liu & Osborne, 2015). However, there are a myriad of

evolved characteristics impacting water-use and drought response that include morphological, physiological, and anatomical traits. Morphological traits include production of fine roots to increase water absorption (McCormack *et al.*, 2015; Roumet *et al.*, 2016; Iversen *et al.*, 2017), leaf rolling to decrease irradiance (Cardoso *et al.*, 2015), and growth form (caespitose and rhizomatous) (Blair *et al.*, 2014; Ott & Hartnett, 2015). These traits are often communicated in terms of economics, reflecting the investment of carbon and nitrogen in both leaf and root structures (Pérez-Harguindeguy *et al.*, 2013). More specifically, these traits are framed by underlying structures at the microanatomical level in leaf and root tissues (John *et al.*, 2017). One of the most notable examples of convergent evolution that resulted in spatially separated photosynthetic tissues is C₄ photosynthesis, which is heavily expressed in Poaceae. C₄ photosynthesis provides a physiological advantage that allows for increased carbon assimilation while limiting water loss through stomatal regulation (Taylor *et al.*, 2010; Zhou *et al.*, 2018). While it is recognized that C₄ species are not inherently more drought tolerant than C₃ species (Ehleringer, 2005; Nippert *et al.*, 2007; Knapp *et al.*, 2020), there is evidence that an increased *WUE* (water-use efficiency), inherent to C₄ species, can be advantageous when water is limiting (Lambert *et al.*, 2011; Kimball *et al.*, 2012; Leakey *et al.*, 2019). Higher *WUE* in C₄ grasses is made possible by the reorganization of internal leaf tissues (Kranz anatomy), which concentrates CO₂ and thereby allows decreased stomatal apertures (Lundgren *et al.*, 2014). Microanatomical leaf traits within and across families in Poaceae have also been observed to influence physiological responses most often associated with hydraulics (xylem area/diameter, resistance to cavitation) (Hacke *et al.*, 2001; Bachle & Nippert, 2018, 2021). However, the aforementioned physiological, morphological, and anatomical traits may not convey equal benefits in drought response or drought recovery across and within Poaceae lineages.

Here, I conducted a robust assessment of physiological and anatomical traits from multiple grass lineages in response to and following recovery from drought. The species under investigation were selected based on expected divergent drought responses (varying sensitivity to low water availability) within lineages. I performed a dry-down experiment to impose severe drought on 12 species of grasses across 6 tribes within the Poaceae lineage. I collected physiological, morphological, microanatomical, economical trait, and productivity (above and belowground) data while closely monitoring drought responses to capture both drought sensitivity and recovery. I hypothesized that (1) species within tribes would exhibit a similar response to drought duration, based on evolutionary histories and drought traits specific to withstand long periods of low water availability (serving as an advantage to dry-adapted species); (2) species within tribes would also exhibit similar responses in drought recovery, based on shared evolutionary histories and functional traits that serve to quickly utilize resources when available (advantaging to wet – adapted species); (3) leaf-level microanatomical traits would best describe species response to, and recovery from drought due to the constraints of structures that influence water transport and availability.

Materials and Methods

Twelve grass species belonging to six tribes were grown from seeds obtained from the USDA Germplasm Resources Information Network or locally sourced from Konza Prairie Biological Station. Species include: *Paspalum juergensii*, *Paspalum notatum*, *Festuca ovina*, *Panicum virgatum*, *Setaria viridis*, *Urochloa ruziziensis*, *Andropogon gerardii*, *Sorghastrum nutans*, *Danthonia spicata*, *Rytidosperma semiannulare*, *Bouteloua dactyloides*, *Bouteloua gracilis* (accession information in Supplemental table 1). Species were selected to represent

different major lineages of the family *Poaceae*, including C₃ (BEP and PACMAD) and C₄ species. Seeds were germinated in 868.5 cm³ size pots with a mix of potting soil and general-purpose sand with a ratio of two parts soil one-part sand and placed in a Kansas State University greenhouse under ambient conditions and raised to maturity throughout 2016 – 2018. Once reaching maturity, the samples were subjected to 100% water reduction (referred to ‘dry-down’), simulating an extreme drought, as previously described (Qiu *et al.*, 2020b,a). During the dry-down, samples were monitored daily and graded into conditions based on their physiological state: “Initial”, “Stressed”, and “Recovery”. Conditions were determined by measuring leaf-level photosynthetic rate (A_n). The condition: “Initial” was measured on Day 1 (first day of drought after being watered the previous day) in order to avoid biased measurements from saturated soils. When plants reached stomatal closure and extremely low photosynthetic rates ($A_n < 25\%$) of Day 1 “Initial” A_n , they were categorized into the new condition “Stressed”. At this point, water was re-applied until soil saturation after the pertinent data was collected. Plants were allowed two days to recover before post-drought physiological data was collected (“Recovery”).

Physiological

Physiological leaf traits were monitored daily on droughted individuals and include: photosynthesis (A_n), stomatal conductance (g_s), transpiration (E), and instantaneous water use efficiency ($iWUE$; calculated as the ratio between A_n and E). Data were collected with a LI-6400 system (LiCOR Biosciences Inc., Lincoln, NE, USA) equipped with an LED light source (light intensity maintained at 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and with CO₂ concentration at 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and relative humidity at ambient levels (35-50%).

Economic

After physiological “Recovery” data were collected, above and belowground tissues were harvested to determine productivity of all species and samples that were subjected to dry-down conditions. Leaf-level economic and microanatomical data were collected from samples that included all non-droughted individuals but excluded *P. juergensii*, *P. notatum*, or *F. ovina* due to the lack of samples. The leaf tissue was collected and included: Leaf area (*LA*), specific leaf area (*SLA*; leaf area divided by dry mass), and leaf dry-matter content (*LDMC*; fresh leaf mass divided by dry mass). *SLA* and *LDMC* were analyzed with the standardized rehydration method (Garnier *et al.*, 2001a; Pérez-Harguindeguy *et al.*, 2013), while *LA* data was obtained by processing images in ImageJ (Rasband, 1997). Roots were washed and cleaned of debris for digital root imaging; analysis of root images was completed with a root imaging software (WinRhizo; Regent Instruments, Inc., Nepean, Ontario, Canada). Root imaging provided the following traits: total root length, root diameter, and specific root length (*SRL*; root length divided by dry mass). After scans were completed, above and belowground biomass were dried for 48 hours at 65°C and weighed for productivity comparisons.

Microanatomy

The newest mature leaf was used for microanatomical analysis prior to the initiation of drought from the following species: *Setaria viridis*, *Urochloa ruziziensis*, *Danthonia spicata*, *Rytidosperma semiannulare*, *Bouteloua dactyloides*, and *Bouteloua gracilis*. *Sorghastrum nutans*, *A. gerardii*, and *P. virgatum* were collected from parent populations in the field at peak physiological performance. *Festuca ovina*, *P. notatum*, and *P. juergensii* samples were not included in these analyses, due to experimental oversight. Anatomical samples, roughly 30 mm

in length, were collected (4 - 8 samples per species; $n = 33$) by clipping leaf tissue and placing them into a fixative FAA (10% formalin / 5% glacial acetic acid / 50% ethanol (use 95% EtOH) / 35% DI water) under a vacuum. Tissues were then cut (cross sectioned) to $4\mu\text{m}$ in thickness with a Leica RM2135 microtome (Leica Biosystems, Newcastle, UK), and mounted in paraffin at Kansas State's College of Veterinary Medicine Histopathology lab. Tissue was stained with Safranin-O and Fast Green (Ruzin, 2000), cover slipped, and then imaged on a Zeiss 880 confocal microscope (Carl Zeiss, Walldorf, Germany) at 10X and 20X when necessary with a multitrack configuration, digital dual-bypass filters and a GaAsP detector (Fig 2.1).

Microanatomical data were collected using IMAGEJ software (Rasband, 1997) by analyzing two tissue regions from either side of the midrib between two major vascular bundles which were then averaged together from each leaf sample (Bachle & Nippert, 2018, 2021). Here, the total subsampled area is referred to as the cross - sectional area or the area between two major vascular bundles (CSA). Microanatomical traits collected from subsamples include: xylem area (X_{area}), xylem diameter ($X_{diameter}$), t/b (xylem wall thickness/diameter), and stomatal count (S_{count}). In this study, we did not collect stomatal densities - as that would entail epithelial peels or impressions, therefore we do not equate density measurements and interpretations with the S_{count} . Instead, we utilize S_{count} to inform how many stomata are serving major and minor vascular bundles within the subsampled area (CSA).

Data Analyses

The selected traits were averaged by species, tribe, and photosynthetic pathway (C_3 and C_4); separated into three categorical 'stages' (Initial, Stressed, and Recovery) based on observable physiological responses. All data were checked for assumptions of normality before

analyses began. Comparisons among tribes and dry-down ‘stages’ were analyzed using mixed-effect model ANOVA with physiological data as the response variables and tribe and condition as predictor variables. Tests were performed with the lmer function within the lme4 package (Bates *et al.*, 2015). To assess bivariate relationships between plant functional traits, we performed simple regression analyses (using the ‘lm’ function). Non-parametric data were analyzed via Kruskal-Wallis rank sum test paired with a post hoc pairwise Wilcox test. We also performed Akaike’s information criterion, adjusted for small sample sizes and AICc model selection to determine the most impactful trait parameters for drought response using the “MuMIn” package (Grueber *et al.*, 2011; Bartoń, 2018). All data were analyzed in the statistical program R V3.4.3 (R Core Team 2017). In order to summarize the relationships and range of physiological, functional, and microanatomical diversity represented in our dry-down sample, we conducted a Principal Component Analysis (PCA) on the mean trait data across species, which cumulatively explained 92 % of the variation in traits (Fig 2.2). Not all traits were measured for every species, and so we focused on key traits coming from each of the data types I measured. I was also required to filled in mean *SLA* traits for 2 species (*F. ovina* and *P. notatum*) from the literature and also imputed $X_{diameter}$ data for those two species.

Results

Drought responses to the experimentally induced dry-down differed among species. Physiological viability following the initiation of the drought ranged from 4 – 33 days (Fig 2.3A). Drought duration (days in drought) was similar among species within tribes, but varied significantly across tribes (Fig 2.3A, $P < 0.001$). Species within the tribe Cynodonteae (*B. dactyloides* & *B. gracilis*) were the most physiologically active during the dry-down, reaching

the “stressed” stage only after 30 days in drought (Fig 2.3A, Table A.2). Whereas the most drought sensitive species were within Paspaleae and Poae. These tribes were similar ($P > 0.05$), reaching the ‘stressed’ stage more than 20 days earlier than species in Cynodonteae (Fig 2.3A, Table A.2). The recovery of grass species and tribes following re-watering displayed a more variable response (Fig 2.3B). There were no statistical differences among tribes in recovery responses ($P > 0.05$), though significant differences were observed among species ($P < 0.05$). *Sorghastrum nutans* and *B. gracilis* were the only species that displayed recoveries that exceeded pre-drought photosynthetic levels (114% and 121%, Fig 2.3B). *Festuca ovina* and *B. dactyloides* were the only species that did not regain at least 50% of ‘Initial’ physiological measurements. Species within Paspaleae, Paniceae, Danthoneae, and Andropogoneae did not fully recover (100%) to ‘Initial’ physiological levels. However, all recovered to at least 50% of Day 1 measurements (Fig 2.3B). Andropogoneae displayed the highest drought recovery, regaining on average over 92% of physiological functioning while the only species measured in Poae (*F. ovina*) was the least resilient, with only 42% recovery following re-watering.

Economic trait data were harvested at the conclusion of the dry-down when recovery data was collected for each individual sample. Production of aboveground biomass varied significantly at both tribe and species level (Table A.4; $P < 0.001$). *SLA* was statistically similar within Andropogoneae, Cynodonteae, and Danthoneae ($P > 0.05$) while *SLA* within Paniceae displayed significant variation ($P < 0.05$), ranging from $29 \text{ cm}^2 \text{ g}^{-1}$ (*P. virgatum*) – $143 \text{ cm}^2 \text{ g}^{-1}$ (*U. ruziziensis*). Similarly, *LDMC* was statistically similar across tribes, except for two species within Paniceae ($P < 0.05$) (*S. viridis* and *U. ruziziensis*) (Table A.4). The production of fine root length (diameter $< 0.5\text{mm}$) differed among tribe ($P < 0.0001$) and species ($P < 0.0001$). The largest disparity within a tribe was found in Cynodonteae: *B. dactyloides* produced over 8,000

cm more fine roots than *B. gracilis* (Table A.5). *Festuca ovina* produced the most fine roots, which was significantly higher ($P < 0.001$) and 2,330 mm more than the second highest fine root length by *P. juergensii* (Table A.5). Significant differences in *SRL* were observed across tribes and species as well ($P < 0.001$; Table A.4). All species within their respective tribes were found to have statistically similar *SRL* except for Paniceae ($P < 0.05$), Paspaleae ($P < 0.05$), and Danthoneae ($P < 0.01$) due to the 294.99 cm g^{-1} difference in *SRL*.

Most microanatomical traits displayed significant among between tribes ($P < 0.05$) but typically had reduced variability between species within the same tribe. X_{area} was statistically different between tribes ($P < 0.001$) with the exception of Danthoneae and Cynodonteae ($P = 0.485$). Andropogoneae ($589.308 \mu m^2$) had the largest X_{area} , and was 5x larger than the smallest X_{area} found in Cynodonteae ($108.957 \mu m^2$) ($P < 0.01$; Table A.3). While there were significant species differences found across all tribes ($P < 0.001$; Table A.3) there were no observable species differences within tribe ($P > 0.05$). $X_{diameter}$ reflected a similar pattern to that of X_{area} : significant differences between tribes ($P < 0.001$) and statistically similar values within tribes ($P > 0.15$; Table A.3). Xylem resistance to cavitation (t/b) differed significantly across tribes ($P < 0.001$), species ($P < 0.001$), but not among species within a tribe ($P > 0.05$; Table A.3). Stomata within the subsampled area (S_{count}) showed high variation; the significant differences among tribes ($P < 0.01$) are likely attributed to Paniceae, which had higher S_{count} (Table A.3).

There were few statistically significant relationships explaining drought responses and recovery among tribes (Figure A.1), except for X_{area} and S_{count} . Surprisingly, given the large volume of literature designating *iWUE* as a pivotal *PFT* reflecting drought tolerance, there was no statistically significant correlation of *iWUE* with drought resistance or resilience ($P > 0.05$). However, stomata number was significantly negatively correlated with drought duration ($P <$

0.01) (Fig. 4), X_{area} ($P < 0.05$), and $iWUE$ ($P < 0.05$) (Figure A.1). Results also indicate a differentiation between sample productivity and economic growth strategies (Fig 2.5). A significant relationship was observed when comparing above and belowground biomass (Fig 2.5A), yielding a tight positive relationship ($P < 0.001$; $r^2 = 0.825$). Yet, when above and belowground economic strategies (SLA and SRL , respectively) were calculated, a breakdown in the previous relationship was observed ($P > 0.05$; $r^2 = 0.008$) (Fig. 2.5B). While SLA displayed no bivariate relationships with other traits, $LDMC$ correlated with t/b ($P < 0.01$) and S_{count} ($P < 0.05$) (Figure A.1). The AICc model selection process indicated how the selected $PFTs$ influence both drought resistance and resilience. The model explaining the greatest variation in drought sensitivity included Tribe, S_{count} , $iWUE$, $LDMC$, and SLA . However, the best explanation for variation in drought resilience included a single anatomical trait: S_{count} .

Discussion

Here, we showed considerable variation in functional traits among diverse species within Poaceae in response to drought and recovery. Shared phylogenetic and biogeographic histories have resulted in unique adaptations and trait development in Poaceae that are reflected in the patterns of global distribution of grasses and their responses to drought (Christin *et al.*, 2010; Liu & Osborne, 2015; Watcharamongkol *et al.*, 2018). Our data illustrate that the traits and adaptations that confer an ability to withstand extreme drought conditions are not the same traits and adaptations that confer an ability to recover from drought, as they are driven by the coordination of different factors (Fig 2.2; Fig 2.3).

The selected Poaceae species used here displayed variable sensitivity to the dry-down; visible in both drought duration/sensitivity and recovery (Fig 2.3). The physiological responses

observed during the dry-down were statistically related to variation in anatomical features, mainly those influencing water relations at the leaf-level (Fig 2.4). We also found a separation between productivity and economic strategies in both above and belowground tissues of the selected Poaceae species (Fig. 2.5), reflecting a trade-off in leaf and root growth economic strategies. Differences in economic traits indicated different water-use strategies related to drought responses (Funk *et al.*, 2017; Reich & Flores-Moreno, 2017; Cui *et al.*, 2020). For example, plants with lower *SLA* and higher *SRL* are indicative of lower metabolic upkeep, resource – poor environments, and increased levels of resource acquisition (Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2013); allowing for sustained physiological tolerance and quicker recovery from drought conditions. While evolutionary relatedness interacts with physiological, morphological, and anatomical traits in determining drought responses, drought recovery was mainly driven by S_{count} alone. The number of stomata within the subsampled cross-sectional area (CSA) should not be interpreted as a stomatal density measurement because the subsampling method is not limited to a set area. Instead, S_{count} indicates the number of stomata that are supplying CO₂ to major and minor vascular bundles, which may be a more direct mechanistic comparison to that of stomatal densities which identify a leaf's surface as a uniform and homogeneous surface and has little to no consideration for microanatomical traits (i.e., mesophyll area, bundle sheath area, and the diffusion distance through mesophyll) that are known to impact carbon assimilation and subsequent water loss (Esau, 1953; Sack & Frole, 2006; Ocheltree *et al.*, 2011; Lundgren *et al.*, 2019).

Low soil moisture negatively impacts growth, increases xylem tension, and decreases carbon assimilation (Lemoine *et al.*, 2018; Jardine *et al.*, 2021). The ability to mitigate and recover from drought is based on anatomical and physiological traits (Taylor *et al.*, 2011; Olson

et al., 2020). While the impacts of severe drought on the physiology of grassland species have been observed in previous research, few studies combine physiological, whole-leaf, and microanatomical trait data (Fry *et al.*, 2013; Liu & Osborne, 2015). Here, we intentionally chose species (within the same tribe) that were previously reported to have varying responses to low soil moisture. However, our results indicate that closely related grasses can respond similarly during a decreasing soil moisture event (Fig 2.3A; Fig 2.4) but display variable responses when water becomes available again (Fig 2.3B), which supports previous claims that drought responses are not uniform within a functional type (Liu & Osborne, 2015; Griffith *et al.*, 2020). This diversity in physiological responses among species has been observed to protect individuals and populations while protecting ecosystem functioning from detrimental effects of drought (Mori *et al.*, 2013; Kreyling *et al.*, 2017; Roberts *et al.*, 2019).

Given the fundamental role that past evolutionary histories have played in shaping current species distributions (Fox *et al.*, 2018; Folk *et al.*, 2020), species that possess variable responses to ecosystem disturbances would benefit more than species that maintain static responses (Isbell & Wilsey, 2011; Isbell *et al.*, 2015). For instance, species that were more drought resistant (Cynodonteae) are broadly represented in the mixed and shortgrass prairies of North America, regions that are known to have less rainfall and more frequent drought (Carroll *et al.*, 2021). In contrast, the drought sensitive species (Paspalum) are from tropical locations where moisture is not the limiting resource. In addition, Cynodonteae were also observed to have less stomata and decreased gas exchange rates compared to Paniceae and Paspalum species, leading to greater water loss (Fig 2.4; Table A.2, A.3). Therefore, it stands to reason that phylogenetically dissimilar species evolving under different environmental constraints would exhibit disparate drought response to the imposed dry-down, while more closely related species

would respond more uniformly (Fig 2.3A; Fig 2.4). Our data also displays a clear indication of an evolved plasticity in physiological responses to variable climate conditions, as native grasses typically occupy regions with similar climate variability (Bachle *et al.*, 2018). Grasses sampled in this experiment were severely desiccated and recovered >50% of pre-drought physiological functioning and in several cases, physiological rates that were 20 – 30% higher (Fig 2.3B), highlighting a potentially unique characteristic of grasses across lineages. The ability to quickly acquire water and other nutrients likely facilitates grasses competing with other neighboring functional types with deeper access to water (Holdo *et al.*, 2018; Kulmatiski *et al.*, 2020).

Water availability directly impacts plant physiological responses, which are constrained by internal anatomical machinery (Fig 2.1) (Christin *et al.*, 2013; Bachle & Nippert, 2021). For example, the spatial separation of C₄ photosynthesis allows for a reduced stomatal conductance and decreased water loss (higher water-use efficiency) (Farquhar & Sharkey, 1982; Ehleringer & Monson, 1993; Berry & Patel, 2008; Way *et al.*, 2014). This characteristic is often assumed to underlie success under conditions of increased aridity. Our findings, however, do not support this claim (Figure A.1). *iWUE* was not observed to directly aid in drought resistance or resilience of grasses in this study but positively related to the number of stomata (Figure A.1), indicating that the presence of more stomata is associated with higher *iWUE*. This counterintuitive result does not indicate a more drought tolerant strategy, rather, it suggests higher gas exchange rates resulting in greater loss of water leading to desiccation (Xu & Zhou, 2008; Bertolino *et al.*, 2019; Knapp *et al.*, 2020). However, previous research has indicated that stomatal patterning, morphology, and densities can greatly influence/alter physiological responses to water stress (Nunes *et al.*, 2020; Harrison *et al.*, 2020). Figure 2.4 clearly indicates species with more stomata

have a decreased resistance to drought, which may require reevaluations of previously held claims regarding the functional significance of *iWUE* (Condon, 2004).

Xylem characteristics also impact an individual's water-use (Wahl & Ryser, 2000; He *et al.*, 2020; Olson *et al.*, 2020). Xylem area is a commonly measured trait because it corresponds with the amount of water that can be transported at any given time. Here, our results indicate two water transport strategies. Larger xylem (X_{area}) decreases drought resistance while conveying a positive relationship with recovery, when excluding *A. gerardii* and *S. nutans* (drought resilience) (Figure A.1). This strategy enables individuals with larger X_{area} to transport greater amounts of water, when available (seen in recovery). But, drought conditions can lead to increased strain on the water column inside xylem vessels, ultimately leading to an embolism during drought conditions (McCulloh *et al.*, 2019). Previous research has highlighted how increased thickness of xylem wall tissue with smaller diameter lumen (t/b) can protect from embolism events in water limiting conditions (Blackman *et al.*, 2018; Guérin *et al.*, 2020); however, our data do not corroborate such findings (Figure A.1). Anatomical traits measured were highly variable, which attribute to two main factors: (1) our sample size was relatively small, due to the time - consuming nature of anatomical studies, and (2) microanatomical traits are intricate and have large variability among individuals and within grass leaves (Fig 2.1) (Ocheltree *et al.*, 2011; Bachle & Nippert, 2021).

While microanatomical traits and leaf- level physiological rates provide key mechanistic insights into drought sensitivity and resilience, whole-plant traits are more easily observable and requires less detailed scientific instrumentation (and training) (John *et al.*, 2017; Reich & Flores-Moreno, 2017). Whole-plant traits illustrate broader growth strategies by the individual, such as resistance or avoidance of detrimental growth conditions. Our results indicate a linear

relationship between above and belowground productivity (Fig 2.5A), indicating a constant proportional investment by the selected grasses. However, when observing two widely utilized traits within the leaf and root economic spectrum (*SLA* and *SRL*), the previous relationship breaks down to reveal tradeoffs in grass growth strategies (Fig 2.5B). Individuals that invest in a root system designed for quick absorption of water and nutrients (high *SRL*) can only produce an inexpensive leaf (low *SLA*), while more ‘expensive’ leaves (high *SLA*) appear to be associated with a less economically efficient rooting strategy (Fig 2.5A). This finding highlights the inability of grasses to produce tissues at both the upper limits of the leaf and root economic spectrum. However, it's important to recognize and consider the potential for convoluted interpretations of economic traits, as they are the amalgamation of several underlying traits (Pérez-Harguindeguy *et al.*, 2013; Bachle & Nippert, 2021). One such trait is leaf dry matter content (*LDMC*), which was correlated with the thickening of xylem vessel walls or xylem reinforcement: *t/b*. This thickening of water transport tissues (or xylem reinforcement) increases the strength at which the water column can be under tension, allowing for a more negative water potential while decreasing the likelihood of cavitation, a physiological indicator of drought stress (McCulloh *et al.*, 2019).

There is currently a dearth of available functional trait data in grasses, a severely underrepresented functional type in trait databases. Results from this study indicate the need for increased collection of functional traits across a diverse assemblage of species within a functional type. Functional types are often used in ecosystem models to more easily group plants by common features; however, as our results indicate, this may lead to poor parameterization and model output. Our AICc selection indicates that anatomical traits, such as stomatal count, in addition to physiological and economic traits in addition to phylogeny were essential to

understanding species ability to withstand drought, while stomatal count was the best explanation for recovery following rewatering. Collecting plant functional trait data in grasses will contribute to current model parameterization (Still *et al.*, 2018). In addition, gathering these data with consideration to grass lineage, an under represented functional type, will be invaluable contribution to Land Surface Models (LSMs) (Griffith *et al.*, 2020).

Conclusion

The evolutionary histories of Poaceae have led to the development of unique morphology (leaves and roots) and anatomy (stomatal shape and photosynthetic tissue arrangement), with observable physiological consequences. These traits have allowed this plant functional type to flourish on every continent (excluding Antarctica). Phylogenetic divergences and subsequent trait adaptations have led to contrasting responses to, and recovery from, drought (Fig 2.3).

Anatomical traits were key in explaining physiological drought response and recovery, most specifically traits concerned with water usage. Species that exhibited increased *iWUE* were more prone to quicker desiccation, most likely due to these species possessing a higher number of stomata. Interestingly this same trait (S_{count}) was responsible for recovery from drought, which did allow for greater recovery. This study underscores the importance of collecting a myriad of in-depth trait data from several Poaceae lineages to better understand the mechanisms that communicate responses to and recovery from drought.

Acknowledgements

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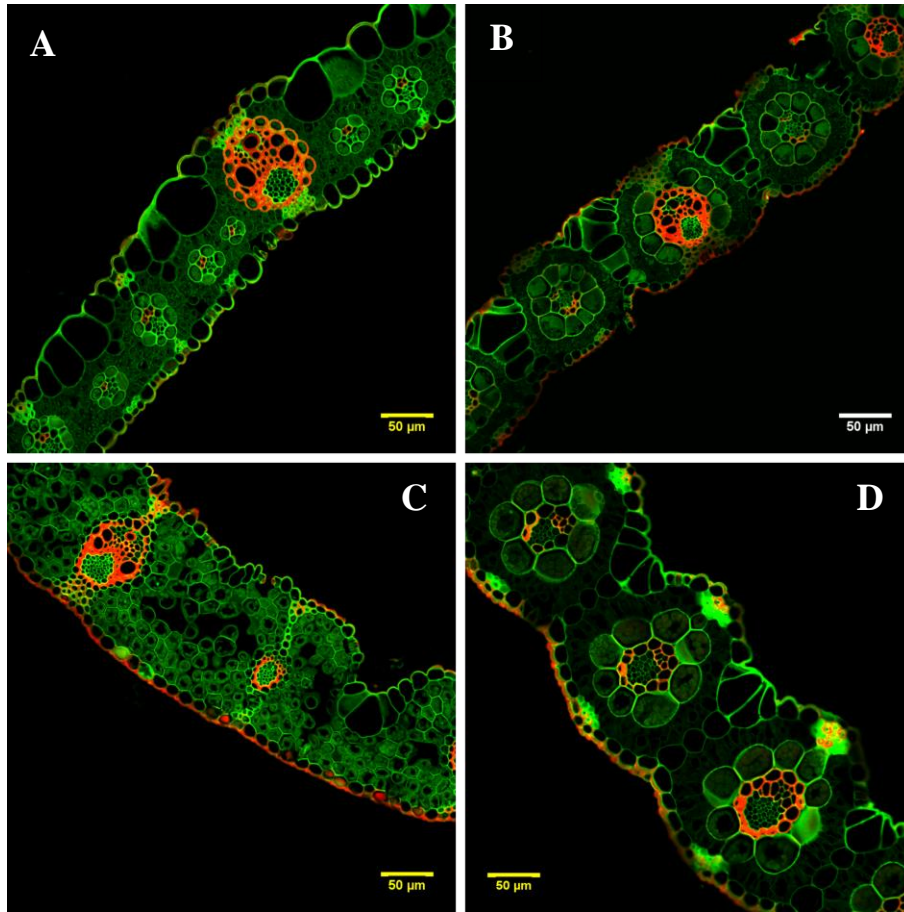


Figure 2.1 Leaf cross-sections of each major grass tribe stained with Safranin Red and Fast Green. A) *Andropogoneae* B) *Cynodonteae* C) *Danthoneae* D) *Paniceae*. Image taken with a Zeiss 880 confocal microscope.

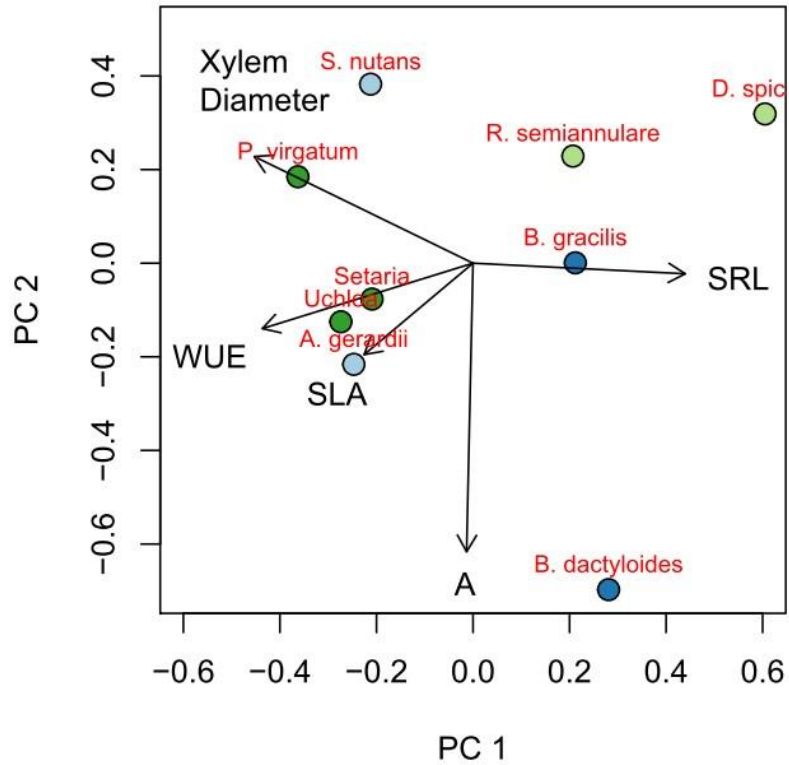


Figure 2.2 PCA Principal components analysis (PCA) of mean trait values of species in the dry down phase of the experiment. This biplot provides a summary of species in multivariate trait space using the first two PC axes, which together account for 72 % of the trait variation. PC1 was most associated with variation in water use and rooting strategies whereas PC2 was primarily associated with photosynthetic rate. Information concerning PCA axes importance and subsequent loadings are located in Table A.6 and A.7.

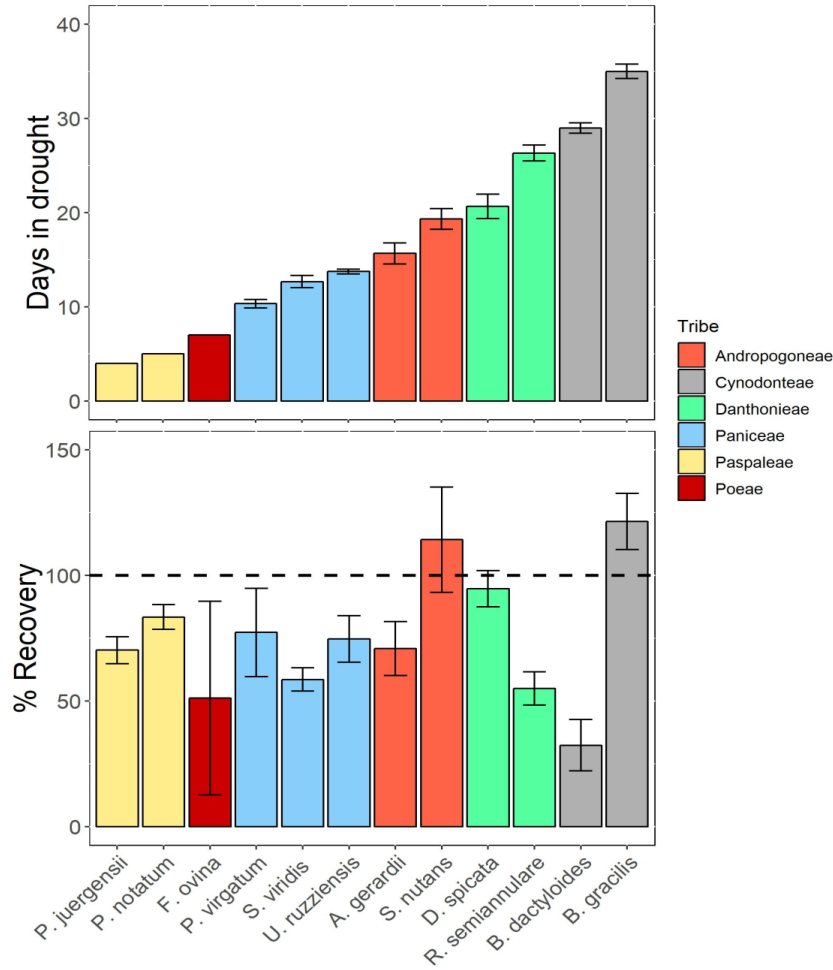


Figure 2.3 Top) Number of days that each species and tribe lasted before stomatal closure and rewatering occurred. Bottom) The physiological recovery (A_n) compared to Day 0 or Initial physiology (measured here as a percent). Dashed line signifying a complete 100% recovery of physiological function.

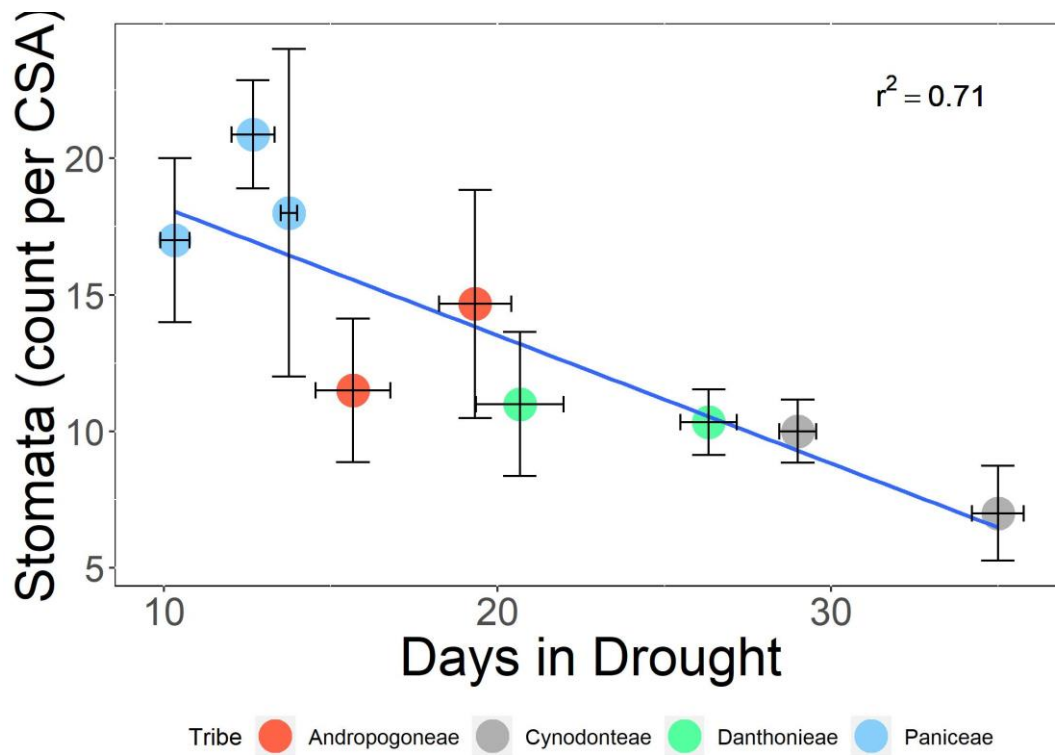


Figure 2.4 Relationship between stomatal count per cross sectional area (CSA) and days in drought before “Recovery”. Andropogoneae (red), Cynodonteae (grey), Danthonieae (green), and Paniceae (blue); each point is a species mean and \pm SE.

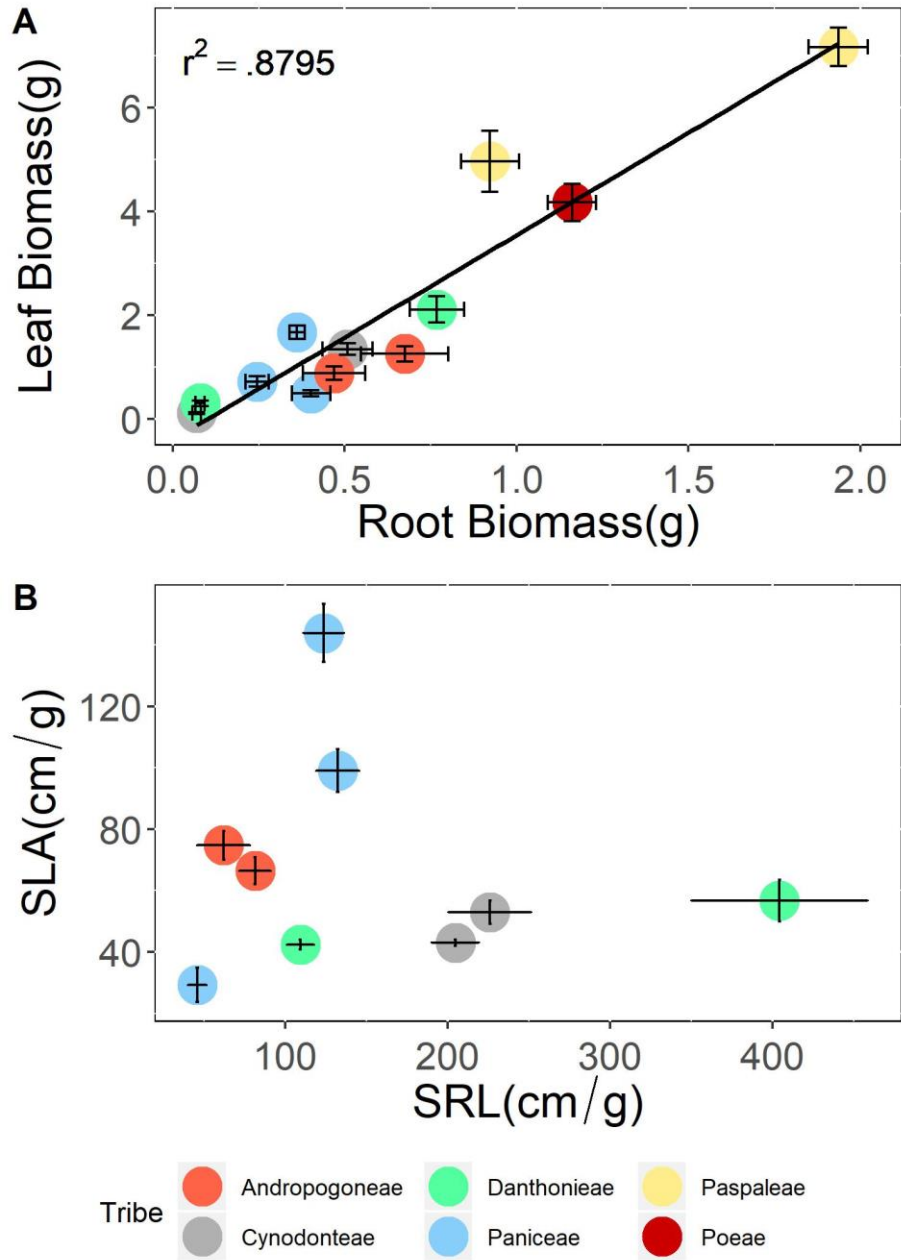


Figure 2.5 A) The relationship between leaf biomass and root biomass. B) Specific leaf area against specific root length. Andropogoneae (red), Cynodonteae (grey), Danthonieae (green), Paniceae (blue), Paspaleae (yellow), Poeae (red); each point is a species mean and \pm SE.

Chapter 3 - Intraspecific trait variability in *Andropogon gerardii*, a dominant grass species in the US Great Plains

This chapter is formatted for the journal "Frontiers in Ecology and Evolution"

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Abstract

The climatic conditions in the North American Great Plains are highly variable, characteristic of an inter-continental climate. Antecedent climate history has impacted the flora of Great Plains grasslands, resulting in high species richness as well as dominance by only a few grass species, such as *Andropogon gerardii*. While the productivity of *A. gerardii* is well described, the individual physiological and morphological characteristics that confer species dominance over wide spatial gradients are not clearly understood. We performed a literature search to assess intra-specific trait variability of *A. gerardii* from as many locations as possible. Ultimately, only 13 locations in the Great Plains have reported common plant functional traits (PFTs) for this species. To best represent site-specific climate conditions, plant functional trait data (8 PFTs) were collected from literature reporting ambient growing conditions, and excluded experimental manipulations. For most PFTs, we found insufficient data to fully quantify the range of variation across the geographical extent of *A. gerardii* dominance. This is surprising given that we focused on the most abundant grass in one of the most well-studied regions globally. Furthermore, trait data collected from our literature search showed a high degree of

variability, but no strong relationships were observed between mean trait values and climate predictors. Our review of the literature on *A. gerardii* suggests a role for trait variability as a mechanism enabling the dominance of this species across large regions such as the Great Plains of North America.

Introduction

Biomes are often spatially delineated with sharp boundaries and attributed functional properties based on the primary vegetation represented (Bailey, 1998, 2005). These vegetation types are comprised of species that likely exhibit variation in functional traits that may respond to climatic gradients or change through time. Interspecific variation has been used as a proxy for understanding climate change, because it represents how plant functional types and ecosystem function may be altered (Ryan, 1991; Adler *et al.*, 2006; Taylor *et al.*, 2014). However, the focus on mean-trait values in the literature suggests that the true magnitude of intraspecific trait variation is commonly overlooked, and is rarely incorporated into climate and vegetation process models (Lambert *et al.*, 2011; Johnson *et al.*, 2015; Funk *et al.*, 2017), although intraspecific trait variability appears promising for predicting species change (Lu *et al.*, 2017). This is partly due to a lack of empirical work focusing on patterns of ecotypic and intraspecific trait variation (Violle *et al.*, 2012). Intraspecific trait variation is the occurrence of a genotype expressing various phenotypes in a given environment; it's a combination of genetic (i.e. evolution) and environmental factors reflected in the variation within populations (forming ecotypes) (Valladares *et al.*, 2007, 2014; Turcotte & Levine, 2016; Barbour *et al.*, 2019). Measuring intraspecific trait variation allows for an in-depth understanding of a species' ability to respond and adapt to environmental changes (Molina-Montenegro *et al.*, 2018). In this review, we focus

on the average trait expression (reflected in functional traits) change in a single species spanning a large continental gradient. Our goal is to assess the degree to which intraspecific trait variation in dominant species might contribute to the functional responses of grassland ecosystems.

The Central and Great Plains region of the United States experiences a continental climate of temperature extremes and both intra- and inter-annual variability in precipitation (Borchert, 1950; Weaver, 1968). A noteworthy characteristic of the grasslands in this region is the high floristic richness (Collins & Calabrese, 2012), yet dominance by a few C₄ grass species that encompass the majority of annual production (Dietrich & Smith, 2016). A combination of site-level and regional landscape heterogeneity contributes to genotypic and phenotypic diversity within dominant species (Olsen *et al.*, 2013). This allows for a mosaic of different genotypes of dominant grass species to exist across the Great Plains region.

Previous research has shown the occurrence of broad genotypic and anatomical differences within species across regional gradients in the United States (Avolio & Smith, 2013; Olsen *et al.*, 2013; McAllister *et al.*, 2015). These differences may arise from climatic events causing changes in populations of dominant species (Hoover *et al.*, 2014b, 2015; Hoffman *et al.*, 2018). Functional trait variability may also play a large role in muting the negative impacts of large stress events, such as drought, across a landscape. Therefore, an innate advantage of increased resistance to stress events exists, and high functional trait variability may decrease the likelihood of species loss on a regional scale (Smith & Knapp, 1999).

The aim of this review is to measure within-region trait variation from a dominant species found in the Great Plains (temperate grassland; Figure 1). Focusing on several functional traits commonly used in the literature (specific leaf area, water potential, photosynthesis, stomatal conductance, above-ground productivity (individual and g/m²), and below-ground biomass

(individual and g/m^2), we have summarized the variability of functional traits within the species *Andropogon gerardii* Vitman. *A. gerardii* is a perennial C_4 grass that has significant genotypic and functional trait variability, facilitating a broad distribution throughout the Central and Great Plains. PFTs collected were analyzed to understand if the variability in a given trait varies/relates to gradients in multiple climate factors across this region, including mean annual precipitation (MAP), mean annual temperature (MAT), mean annual minimum temperature (MMinT), and mean annual maximum temperature (MMaxT) from 1980 - 2015. For example, we would expect biomass to vary across a climate gradient because productivity positively increases in this region with corresponding increases in precipitation and temperature (Nippert *et al.*, 2006; Hufkens *et al.*, 2016). Identifying trait variability within a single species may provide insight for the potential role of adaptive trait variability as a driver of population persistence across broad climatic space.

Literature search criteria for *Andropogon gerardii* PFTs

We conducted a literature search to collect specific PFT data from a widely distributed grassland species: *Andropogon gerardii*, attempting to lend insight into one of many potential reasons for why some species achieve dominance over broad climatic space. *A. gerardii* was chosen because it is a dominant species in the tallgrass prairie and encompasses roughly 70% of the total aboveground biomass in grasslands throughout the Great and Central Plains region (Rogler, 1944; Weaver, 1968; Smith *et al.*, 2017). We selected 6 important functional traits that reflect major axes of leaf economic variation and properties relevant to ecosystem function. The PFTs included in the literature search were specific leaf area (SLA), water potential (WP_{all}; pre-dawn and midday), photosynthesis (A), stomatal conductance (g_s), above-ground biomass

(AGB), and below-ground biomass (BGB). AGB and BGB data included biomass on an individual and a per square meter basis. Starting with SLA, we searched Google Scholar and Web of Science (which yielded identical results) with the string ["*Andropogon gerardii*" AND ("LMA" OR "SLA") AND "Great Plains"], which produced 60 search results. Only 12 of these studies (from 6 study sites) reported SLA. Similar searches were performed for the less common functional traits. SLA data was also collected from the TRY global database of plant traits (Kattge *et al.*, 2011), which mirrored previous search parameters with greater success. This process resulted in a PFT dataset collected from 36 separate studies, including data from 17 research locations in 7 states from 1984-2017 (Figure 1). Our goal was to include data from across the U.S. Midwestern region and to exclude redundant data that were from the same projects, and only include 'control' or 'ambient' conditions. Data collection from literature varied by state. Kansas and Illinois contributed 59 data points (each point representing a single datum), which is a substantial amount of the total dataset. In order to determine differences in geographic location, statistical analysis was conducted via ANOVA before and after the data were normalized by natural log. MAP, MAT, MMinT, MMaxT (mean from 1980 – 2015), and geographic location of each data point was collected from PRISM (PRISM Climate Group, 2014) and used as fixed variables while each PFT was used as response variables. AICc model selection was also used to determine the most impactful climate parameters in the model using the "MuMIn" package (Barton, 2018), according to Grueber *et al.* (2011). Analyses were conducted in the statistical program R V3.4.3 (R Core Team 2017). The geographic map (Figure 1) was produced using the "raster" package in program R (Hijmans, 2017).

Plant functional traits reflect Ecophysiological processes

Plant functional traits (PFTs) are commonly used to identify species' differences in growth, allocation, and competition in relation to environmental effects to reflect plant economics (Grime, 1979; Edwards *et al.*, 2007; Guo *et al.*, 2017; Volaire, 2018). PFTs represent morphological and physiological adaptations that often predict plant responses to biotic (competition, herbivory, etc), and abiotic factors (MAP, MAT, etc). PFT's typically include whole-plant traits, tissue specific traits (leaf, stem, and root), and physiological measurements (photosynthesis, stomatal conductance, transpiration, and water potential) (Pérez-Harguindeguy *et al.*, 2013; Carmona *et al.*, 2016). For instance, PFTs have been used to predict how individual *A. gerardii* and populations of other grassland species will respond to projected drought conditions (Chapin *et al.*, 2000; Nippert *et al.*, 2009; Volder *et al.*, 2010; Liancourt *et al.*, 2015; Maréchaux *et al.*, 2015; Skelton *et al.*, 2015; De La Riva *et al.*, 2016).

The biological link between ecophysiology and environmental factors aids in predicting how species will respond to climatic changes (Nippert *et al.*, 2011; Ocheltree *et al.*, 2011; Hoover *et al.*, 2014a; Griffith *et al.*, 2016); therefore, such traits reflect the ability of a species, like *A. gerardii*, to respond to changing climatic conditions that are found in the Great Plains (Grime, 2001; McGill *et al.*, 2006; Butterfield & Callaway, 2013; Losapio & Schob, 2017). *Andropogon gerardii* in the Great Plains exhibits many drought tolerant traits that allow either resistance and/or resilience in response to the abiotic stressors such as increased temperature and precipitation variability (Hoover *et al.*, 2014a; Hoover & Rogers, 2016a). Traits that are commonly correlated with drought tolerance include increased water-use efficiency (WUE), decreased leaf area (LA), higher specific root length (SRL), and lower turgor loss point (Eissenstat *et al.*, 2000; Ripley *et al.*, 2007; Hameed *et al.*, 2012; Bartlett *et al.*, 2014). Drought

tolerant traits are likely not static in species with populations that span regional gradients, as there is adaptive benefit for greater trait variability in an environment that experiences high climate variability (Chapin, 1980; Avolio & Smith, 2013; Funk *et al.*, 2017). For instance, WUE variability enables populations to maintain relatively high fitness with varying levels of water availability and mean temperatures (Briggs & Knapp, 2001; Nippert *et al.*, 2007). Nippert *et al.* 2007 showed physiological trait variability for C₄ grasses provided an advantage for fast growth under favorable conditions and the ability to withstand (resist) drought during poor (stressful) conditions (Briggs & Knapp, 2001; Nippert *et al.*, 2007). Trait variability may serve as a climate buffering mechanism (Valladares *et al.*, 2007), which may be observable on the individual physiological scale (i.e., WUE), but also in other PFTs at the regional scale.

Trait variation in *Andropogon gerardii*

Plant functional traits (PFTs) are known to differentiate between species, due to their evolutionary history (Violle *et al.*, 2012; Cornwell *et al.*, 2014; Valverde-Barrantes *et al.*, 2017). However, less work has focused on intraspecific trait variability across large geographic scales. To emphasize this point, the dominant Great Plains grass species (*Andropogon gerardii*) is arguably the most well-studied dominant grass species in the Great Plains and specific leaf area (SLA) is the most widely reported functional trait, yet we only found 12 studies from 6 research locations where PFT data were reported from natural populations without experimental manipulation. In fact, three traits accounted for nearly half the data points in the study (Figure 2A), supporting the clear need for greater reporting of PFT data within common species that span large climate gradients (Figure 2B). Because single PFT data are under-represented in the literature, we aimed to incorporate multiple PFTs for *A. gerardii* to better understand

intraspecific trait variability. More specifically, this amalgamation of PFTs may provide insight into the role of trait variability as a driver of plant species functioning over large regional scales. We assume that for a single species to extend over large geographic regions such as the Great Plains, the species would inevitably maintain a highly plastic phenotype at the population level which may buffer the whole species from variable climate conditions (Figure 2B). Figure 2C theorizes that populations from species with increased trait variability permits survival across a greater range of environmental conditions. For instance, if *A. gerardii* populations were subjected to drought conditions, varying levels of drought tolerance would be observed due to varying leaf water potential at turgor loss point (Maréchaux *et al.*, 2015). Phenotypic variability has been observed to assuage effects from harsh abiotic pressures, however this may only be realized in the short-term (Becklin *et al.*, 2016).

Long-term persistence of abiotic pressures will ultimately cause population reductions, due to climatic conditions moving out of the of historical climate parameters; this was observed in the droughts of the 1930's (Romm, 2011; Becklin *et al.*, 2016). Evidence from rain manipulation experiments provides insight into potential responses to climate extremes (Fay *et al.*, 2002; Knapp *et al.*, 2002; Nippert *et al.*, 2009). For instance, Hoover *et al.* 2015, indicated that C₄ grasses subjected to drought conditions in the Colorado Plateau (35% reduction of annual rainfall) were observed to maintain cover for the first year, but decreased cover and increased mortality with prolonged exposure. Climate buffering can also be observed in a similar experimental design (rainfall manipulation) within the tallgrass prairie. *A. gerardii* did not display the same negative responses as other similar C₄ grasses to increasing climate variability, instead a relatively static response was observed (Fay *et al.*, 2003; Avolio & Smith, 2013).

Increased trait variability within a species may provide physiological benefits in regions with high climate variability. *A. gerardii* lacks highly specific growing conditions and exists across broad geographic gradients in the U.S, including regions with hot and dry climate conditions. We hypothesize that *A. gerardii* and other generalists that dominate large geographic regions can be represented in Figure 2B as the blue line, whereas species that require more specific growing conditions are hypothetically represented as the black line. The theoretical curves in figure 1B were created by using a low standard deviation ($sd=1$; reflecting ~68% of data explained) and a higher standard deviation ($sd=3$; reflecting an increased data distribution) assuming an underlying normal distribution. Specialized species (black line) would not be capable of expanding over large heterogeneous landscapes due to the inability to withstand large fluctuations in temperature or precipitation (Linder *et al.*, 2018), which is represented by a higher/narrower trait density (Figure 2B). Trait variability is documented to lead towards a more stable system due to niche stabilization which affects community composition, the function of the ecosystem, and response to abiotic factors (Turcotte & Levine, 2016).

The PFT data from *Andropogon gerardii* varied widely between research sites, but without discernable trends due to climate parameters like increasing precipitation and temperature (locations and PFT types are found in the supplemental material). For example, *A. gerardii* from Konza Prairie (KS), exhibited photosynthetic rates that included both the maximum and minimum of observed rates from all states included in the literature search, with many data points falling along the mean. Statistical results (ANOVA) show little to no discernable trend in PFTs when considering climate conditions at the geographic locations, which supports the concept of intraspecific trait variation allowing a single species to occupy such a large geographic range containing large precipitation and temperature differences.

Photosynthetic rates did not vary by location, neither did stomatal conductance, SLA, or water potential ($P > 0.05$). Only two PFTs were observed to statistically vary by research location: above and below-ground biomass ($P < 0.05$). This result may reflect the small sample size from the literature as only 15 total data points were found for both individual and square meter collection methods. AICc model selection was performed on models containing all combinations of the PRISM climate variables to find the best model (given all variables) using the ‘*model.sel*’ function, within the MuMIn package (delta < 2, Royall’s 1/8 rule, and cumulative sum of model weights were used to identify uncertainty and differences in the models). The full model including location, MAP, MAT, MMinT, and MMaxT best explained the SLA results gathered from the literature search (AICc = 974.6; weight = 0.939); which was 23 times more likely to be the best explanation for variation compared to the next model (AICc = 980.9; weight = 0.040) that did not include location as a parameter. Production above/belowground can exhibit a positive relationship (Nippert *et al.*, 2006) with soil moisture and precipitation or relatively no change (Zhou *et al.*, 2009), providing potential for variation across precipitation gradients within the Central and Great Plains. No trends due to climate parameters were visible in the analysis based on geographic location (Figure 1), but we speculate that additional production data collected across this regional gradient may result in a positive relationship with regional precipitation gradients similar to results from precipitation manipulation experiments (Fay *et al.*, 2000, 2003).

The results from our literature review followed the ecological hypotheses presented in Figure 2B. PFTs were normalized (natural log) and combined to view large scale trends in the data. *A. gerardii* follows very closely to the ‘generalists’ species parabola, meaning that PFTs are variable or plastic across a broad range of environmental conditions (Figure 2C). *A. gerardii* also

expresses a low density of several PFTs, which indicates that traits are not static, but variable. Plastic PFTs allow *A. gerardii* the ability to respond positively in a given population, which buffers the *A. gerardii* species as a whole. This interpretation may contradict previously held claims that *A. gerardii* will experience geographic shifts or experience large population reductions due to climate change (Gray *et al.*, 2014; Smith *et al.*, 2017). Thus, more research is required to understand the role that intraspecific variation plays in the expansion and survival of *A. gerardii* in the Great Plains.

Future Directions

The literature containing plant functional traits covers many different types of ecosystems and hundreds of species (Pérez-Harguindeguy *et al.*, 2013), which have been emphasized in climate change literature encompassing major biomes across the world (Liancourt *et al.*, 2015). Here, we used PFTs to identify inherent trait variability within a single species, and identify a potential role of adaptive variability as a driver of species persistence across a regional climate gradient. Results from this literature review suggest that the PFTs observed in *A. gerardii* do not statistically differ (excluding AGB and BGB) between locations measured (Figure 1). Findings from this review underline the importance of adaptive trait variability to permit greater phenotypic plasticity, which provides population buffering for some species that exist across broad climate gradients.

Moving forward, an increased focus from interspecific to intraspecific species trait variation may provide a greater understanding of how future climate variability will impact native plant species that span large regional scales. More specifically, *A. gerardii* is the quintessential prairie species; yet an extensive examination of the literature showed a relatively

small number of sites reporting trait data. We advocate for the development of a grass trait network to examine the effect of climate change on specific dominant species in grasslands worldwide. This network could follow the framework created by Nutrient Network (NutNet; <http://www.nutnet.org>) and Drought Network (DroughtNet; <http://www.drought-net.colostate.edu>) to standardize measurements and procedures, and allow for more consistent interpretation of PFTs response to changes in abiotic factors. Increased documentation of spatial climate gradients and species distributions will increase our understanding of the role of trait variability in species resistance and resilience to future changes in the environment.

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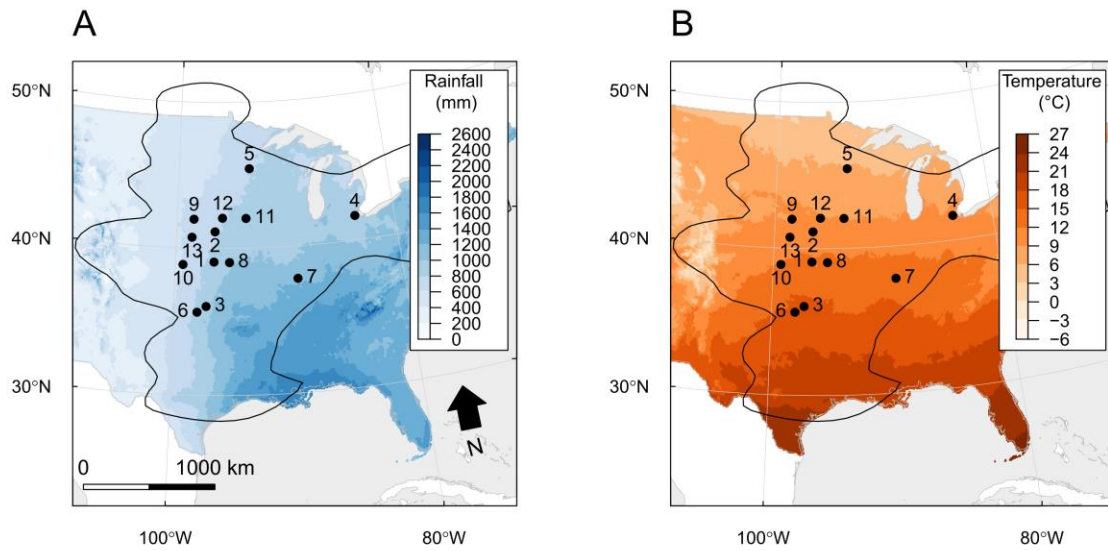


Figure 3.1 Site locations of gathered *A. gerardii* functional traits used in this study. The black contour line indicates the 90% percentile of the density of GBIF occurrences. Source ID codes, locations, precipitation (A), and temperature (B) are located in Table B.1, B2, B3.

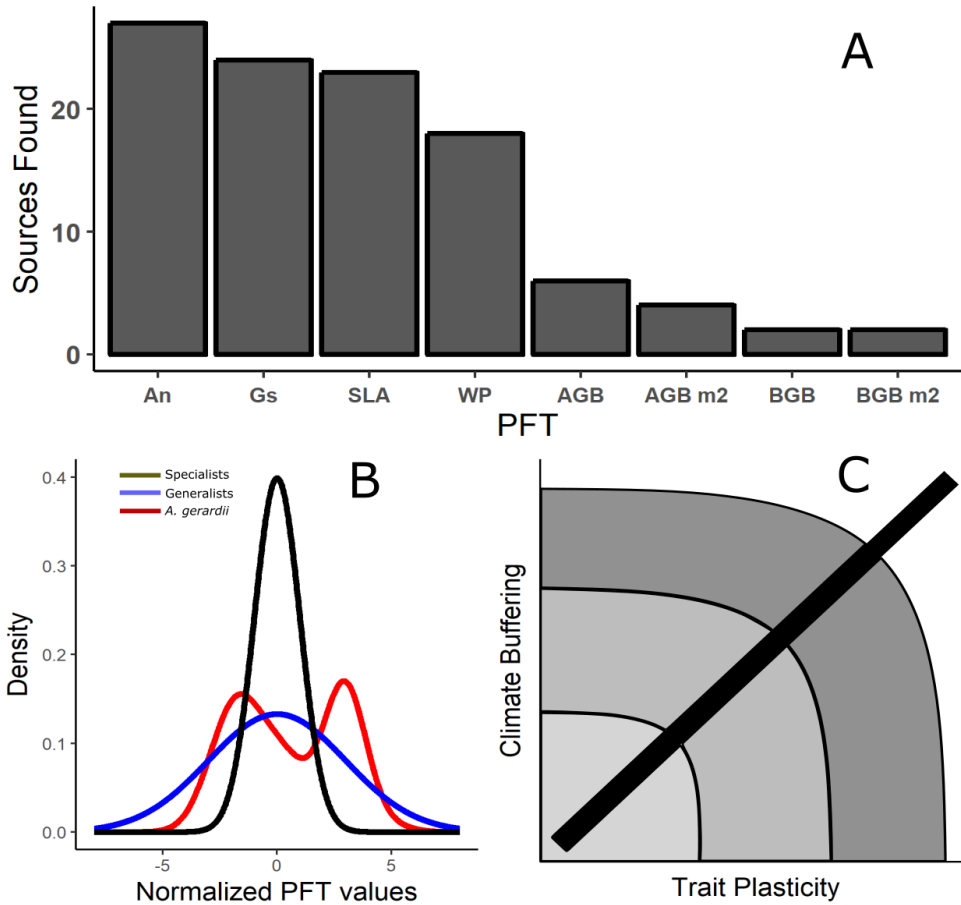


Figure 3.2 (A) Displays the number of sources found in each PFT, the data were then used to create the density plot in (B). (B) Portrays predictions of normalized PFT data variability within a specialist species exhibiting low trait variability (Black; standard deviation = 1), generalists/high trait variability (Blue, standard deviation = 3), and the normalized data reported from the literature search is represented in red. (C) Illustrates how increasing PFT variability may result in increased buffering (resilience) from environmental variability. Arcs and color describe the theoretical placement of a species on a continuum such that a low trait variability results in low climate buffering (lighter color), and high trait variability facilitates higher climate buffering (darker color). Figures produced using the “ggplot2” package in program R V3.4.3 (R Core Team, 2017).

Chapter 4 - Microanatomical traits track climate gradients for a dominant C₄ grass species across the Great Plains, USA

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Abstract

Background and Aims

Andropogon gerardii is a highly productive C₄ 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 high 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 *SLA*.

Methods

A. 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 prepped for fluorescent confocal microscopy to analyze internal leaf anatomy. Leaf microanatomical data was compared with historical and growing season climate data which was extracted from PRISM spatial climate models.

Key Results

Microanatomical traits displayed large variation within and across sites. According to AICc 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 (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) microanatomy trait values vary according to climate gradients, and may underlie variation in traits measured at larger ecological scales.

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, campos and llanos of South America to the temperate regions including the

Mongolian steppes, South African velds, 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 – 380mm in the Mongolian steppe (Ma *et al.*, 2012) and from 375 – 925 mm in the South African savanna (Holdo *et al.*, 2018). The North American Great Plains is a unique region because it contains both a large precipitation gradient (400 to +2000 MAP mm) and a large mean annual temperature gradient (3–27 °C). These dramatic climate gradients expose vegetation in the region to high variability in growing season temperatures, along with droughts and floods (Gibson, 2009; Eters *et al.*, 2014). Many grassland plant species possess morphological, physiological, and structural traits that facilitate responses to a variable climate. These traits are commonly referred to as plant functional traits (*PFTs*), 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 *PFTs* 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 allow for differential responses to abiotic factors that can foster coexistence or competition, such as

rooting depth differences between grasses and woody species (Fargione & Tilman, 2005; Nippert & Holdo, 2015; Holdo *et al.*, 2018) or water-use efficiency (O’Keefe & Nippert, 2018; Nadal & 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 (Vitman) (big bluestem) is a C₄ grass species found throughout the Great Plains, accounting for over 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 (*SLA*) and leaf mass per area (*LMA*) over large geographic regions varying in climate and heterogeneity (Avolio & Smith, 2013; Olsen *et al.*, 2013; Bachle *et al.*, 2018). Other investigations have focused on physiological traits such as water-use efficiency (*WUE*) 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 & 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 (*LMA*), leaf thickness, and chlorophyll content (De La Riva *et al.*, 2016; Reich & 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 & 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 common *PFTs* is currently missing from the literature (Carmo-Silva *et al.*, 2009; Ocheltree *et al.*, 2011; Rao & 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 (Cavender-Bares *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 & 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: (1) Due to site-level variation in climate history and environmental conditions, mean anatomical traits will vary across sampling locations, while microanatomical traits within a site will express similar variability. (2) Due to the variability in water availability from location to location, but general similarity in atmospheric [CO₂] within a region, anatomical traits reflecting water-use/storage will exhibit more variability compared to carbon assimilation traits. (3) Because *SLA* reflects a combination of multiple leaf-level microanatomical traits, similar patterns of variation (expressed as CV) in anatomical traits will be measured in *SLA* 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 4.1). 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 10 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 (95% EtOH)/ 35% DI water) for vacuum infiltration. Samples were kept at room temperature until processing in August of 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 lab. 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), cover slipped, and imaged at 100X and 200X when necessary on a Zeiss 880 confocal microscope (Carl Zeiss, Walldorf, Germany). Image analysis was performed at approximately 850 x 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). Analysis consisted of measuring two tissue regions from each side of the blade mid-rib collected between two major vascular bundles (Bachle & Nippert, 2018). Traits measured from sub-sampled 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 center of each vascular bundle across the whole leaf cross section (not subsampled). Traits measured on an area basis (as a percent of subsampled area) included: mesophyll (MS_A), bundle sheath (BS_A), vein (V_A), and bulliform (B_A). 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. V_A was determined 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 , the ratio of leaf area to dry mass) by measuring the two-dimensional leaf area and recording oven-dried mass after two days drying at 60 °C (Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2013).

PRISM Climate data

Climate data from 1981 thru 2017 from each site was 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 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 – 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, 2020). Normality was checked with Levene's and Shapiro-Wilk tests. X_A 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 analyzed 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 samples 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, 2018).

Results

Intraspecific trait variation

Microanatomical leaf traits in *A. gerardii* had statistically significant variability across sites but did not exhibit latitudinal trends of variation (Table C.1). BS_A was relatively similar across most locations, excluding *A. gerardii* at PRP, which was significantly smaller than KPBS and CDR ecotypes ($P < 0.05$; $P < 0.005$). MS_A varied between locations (Table C.1; $P < 0.005$), most likely due to the reduced proportion of mesophyll area at CDR when compared to other locations. Mean V_A differed across locations ($P < 0.005$) corresponding with reduced V_A at PRP (Table C.1), which was significantly less than 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 both KPBS ($P < 0.003$) and RMRS ($P < 0.005$). X_A was observed to be the smallest at PRP ($P < 0.05$), while KPBS ecotypes were nearly double in area (Table C.1). *A. gerardii* at PRP also displayed the highest xylem cavitation resistance (t/b ; Supplementary Table 1; $P < 0.05$) which was twice that of KPBS ecotypes (Table C.1). *IVD* was the largest at KPBS and RMRS, and the smallest at CDR and PRP (Table C.1; $P < 0.05$), while the level of variability was relatively low ($< 15\%$). The degree of variation (assessed using CV) in microanatomical traits associated with water use (xylem area, xylem cavitation resistance) was greater than traits associated with carbon assimilation (mesophyll and bundle sheath area) (Fig 4.2; Table C.1). Carbon assimilation traits (MS_A and BS_A) had relatively small variation across collection sites (Fig 4.2A, C). In contrast, X_A and t/b (Fig. 4.2B, D) displayed three to four times the trait variation in comparison to carbon assimilation related tissues (Fig 4.2A, C). *SLA* 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 4.3A).

Trait Interdependency

While *A. gerardii* leaves expressed considerable variation in their anatomical traits across sites, significant trait relationships were present (Fig 4.4; Figure C.1). Specifically, a greater area of water storage (B_A) was correlated with smaller diameter xylem vessels (Fig 4.4A, $P < 0.001$). Samples with greater CV for interveinal distance (*IVD*) were also correlated with increased CV in xylem cavitation resistance (Fig 4.4B, $P < 0.038$). Water-related traits had a higher CV at each site (Fig 4.2B, D) than carbon assimilation traits (Fig 4.2A, 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) (Figure C.1).

Few statistically significant relationships were found between *SLA* and the microanatomical traits measured here (Figure C.1). 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 ($P > 0.10$). 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 ($P > 0.05$; Figure C.1).

Using the AICc model selection process, the model that included MAP, TMAX, and the interaction MAP:TMAX 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 4.5B, 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 4.5C) and to a lesser extent BS_t (Fig 4.5A). 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* (Figure C.1). CV comparisons also displayed similar patterns; such as the negative trend between B_A and MAT.

Discussion

These data illustrate that leaf-level microanatomical traits exhibited a relationship within climate gradients of the Great Plains while also influencing broad physiological strategies in *A. gerardii* (Fig 4.5A, B). For traits related to carbon-use (mesophyll, bundle sheath area: Fig 4.2A, C) CV was low across sites, varying between 5-10%. In contrast, traits associated with water-

use (t/b , xylem area: Fig 4.2B, D) had approximately 2-5 times higher CV. Variation in anatomical traits associated with water-use were greater than that of carbon-use traits, suggesting a potentially higher coupling to water availability in local environments (Fig 4.2). 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). Some leaf-level anatomical traits can be associated with both carbon-use and water-use. 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). 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 4.3A, 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 for greater overall carbon assimilation which reduces the need for large structural leaf variation (CV) within species (Lundgren *et al.*, 2014).

Micro-anatomical 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 4.4). 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 4.4A), enabling a larger potential for water and mineral transport while decreasing the potential for water storage (Carmo-Silva *et al.*, 2009; Gibson, 2009). However, individuals with increased variation (CV) in interveinal distance (IVD) were observed to have xylem vessels with larger variation (CV) in resistance (t/b) (Fig 4.4B); 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, individuals from some populations can be highly responsive and acquire water and nutrients quickly (increased xylem area) while others exhibit a more conservative approach (increased bulliform area). The higher regional variability in microanatomical traits associated with water-use (Fig 4.2, Fig 4.4C), may facilitate populations of *A. gerardii* to persist through dry periods that would otherwise require decreased carbon assimilation caused by the stomatal closure (Dusenge *et al.*, 2019; Buckley, 2019), degradation of photosynthetic machinery (Maricle & 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 (Tsy-pin & Macpherson, 2012; Griffin-Nolan *et al.*, 2019b), which also influences plant growth and system productivity (Hoover *et al.*, 2014a; Felton & 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 & Nippert, 2018). The results in this study indicate a positive relationship between carbon assimilation tissues (mesophyll area and bundle sheath thickness) and mean annual temperature (Fig 4.5A, B), while mean annual precipitation yielded no such correlations (Fig 4.5C, D). Relationships

with temperature and carbon assimilation traits may be indirectly linked to differences in stomatal regulation across sites (Berry & Patel, 2008; Sage *et al.*, 2014). Previously, individuals growing in locations with higher temperatures had increased stomatal regulation, likely reflecting the role of increased VPD on 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 specific leaf area 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 in collection and observable plasticity within populations and species (Garnier *et al.*, 2001b; 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.*, 2001b; 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 & Flores-Moreno, 2017). As such, variation in microanatomical traits should at least theoretically coincide with variation in *SLA*. While we observed significant differences in microanatomical traits across sites, *SLA* was statistically similar across sites (Fig 4.3B), and unrelated to the microanatomical traits measured here (Figure C.1). 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 intra-specific *SLA* differences than is required for microanatomical trait differences. 3) The functional interpretation of microanatomical tissues (e.g., tissues associated with water transport, structure, C assimilation, etc.) is more straight-forward than the functional interpretation of a composite whole leaf-trait like *SLA*. 4) 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. Regardless of these caveats, 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 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.

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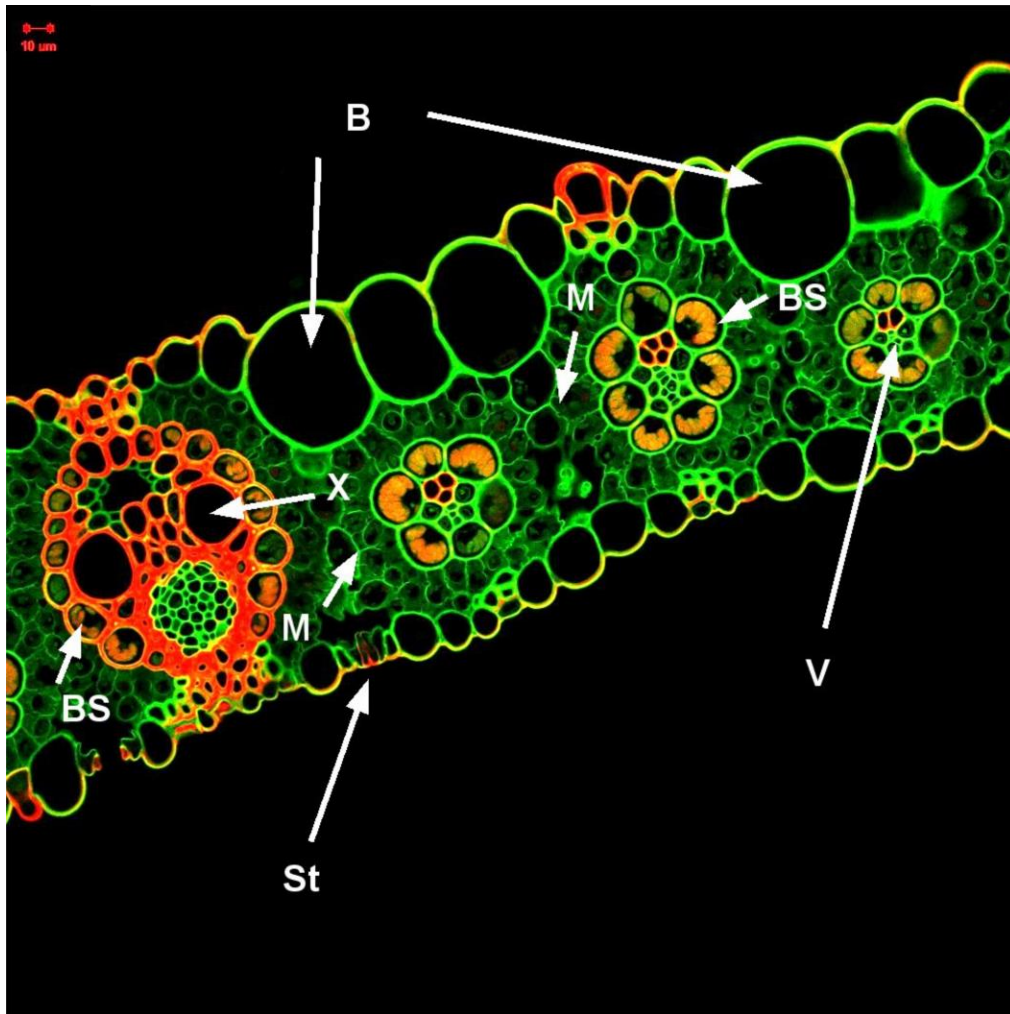


Figure 4.1 Example of leaf cross-section of *A. gerardii* stained with Safranin Red and Fast Green to more clearly identify anatomical structures. B, bulliform cells; St, stoma; M, mesophyll; BS, bundle sheath; V, vein; X, xylem. Image taken with a Zeiss 880 confocal microscope.

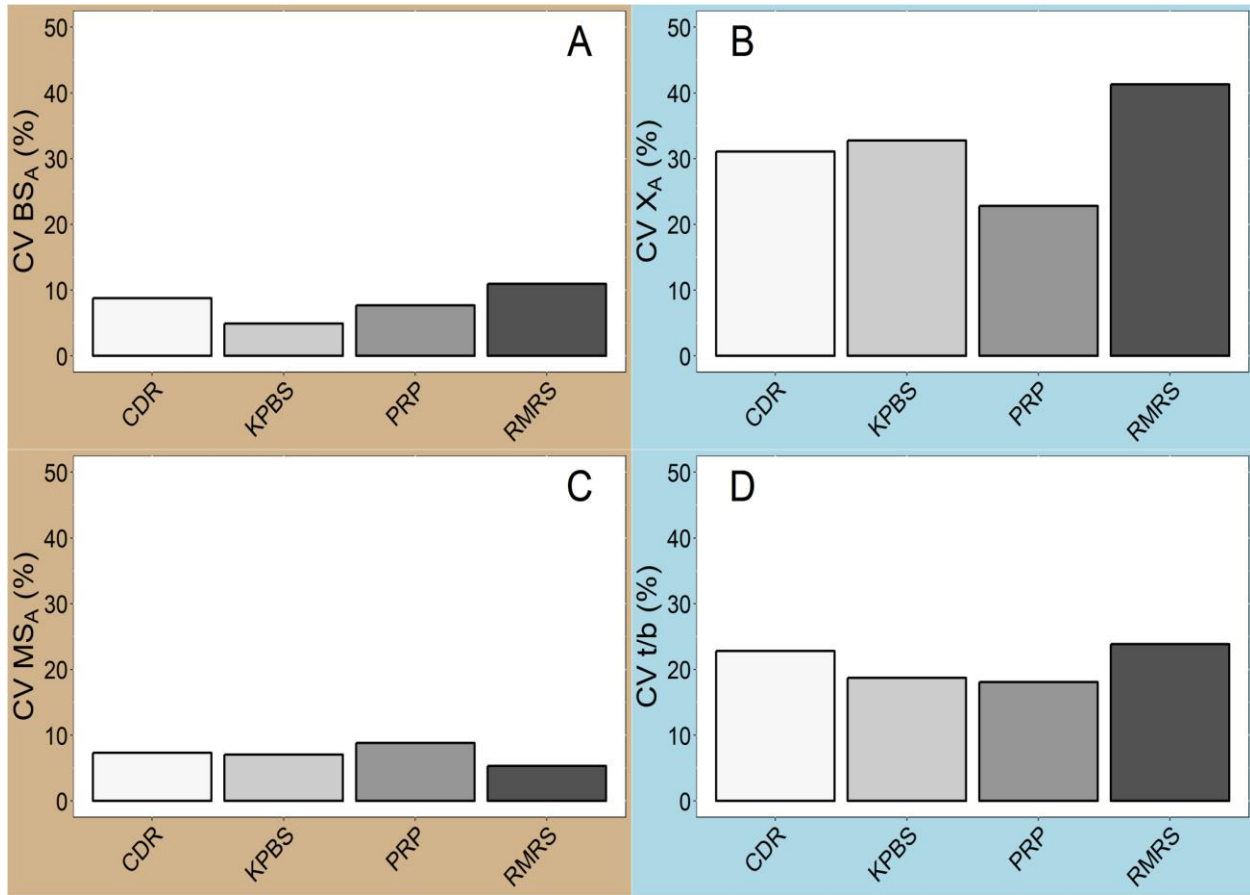


Figure 4.2 Coefficient of variation of anatomical traits measured in *Andropogon gerardii* at each sampled location. (A) Bundle sheath area; (B) xylem area; (C) mesophyll area; (D) xylem reinforcement. Plot colour is related to trait function: tan, carbon assimilation; blue, water transport.

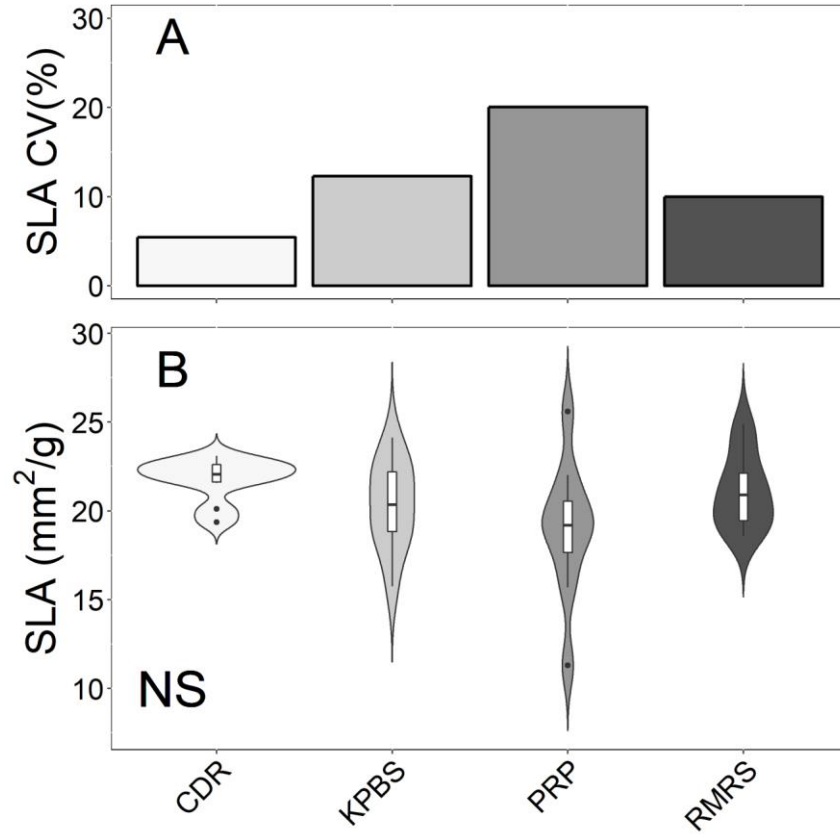


Figure 4.3 Specific leaf area (SLA) measurements collected from *A. gerardii* in 2017. (A) Coefficient of variation for each location. (B) Violin plots displaying mean and distribution of SLA. NS, not significant.

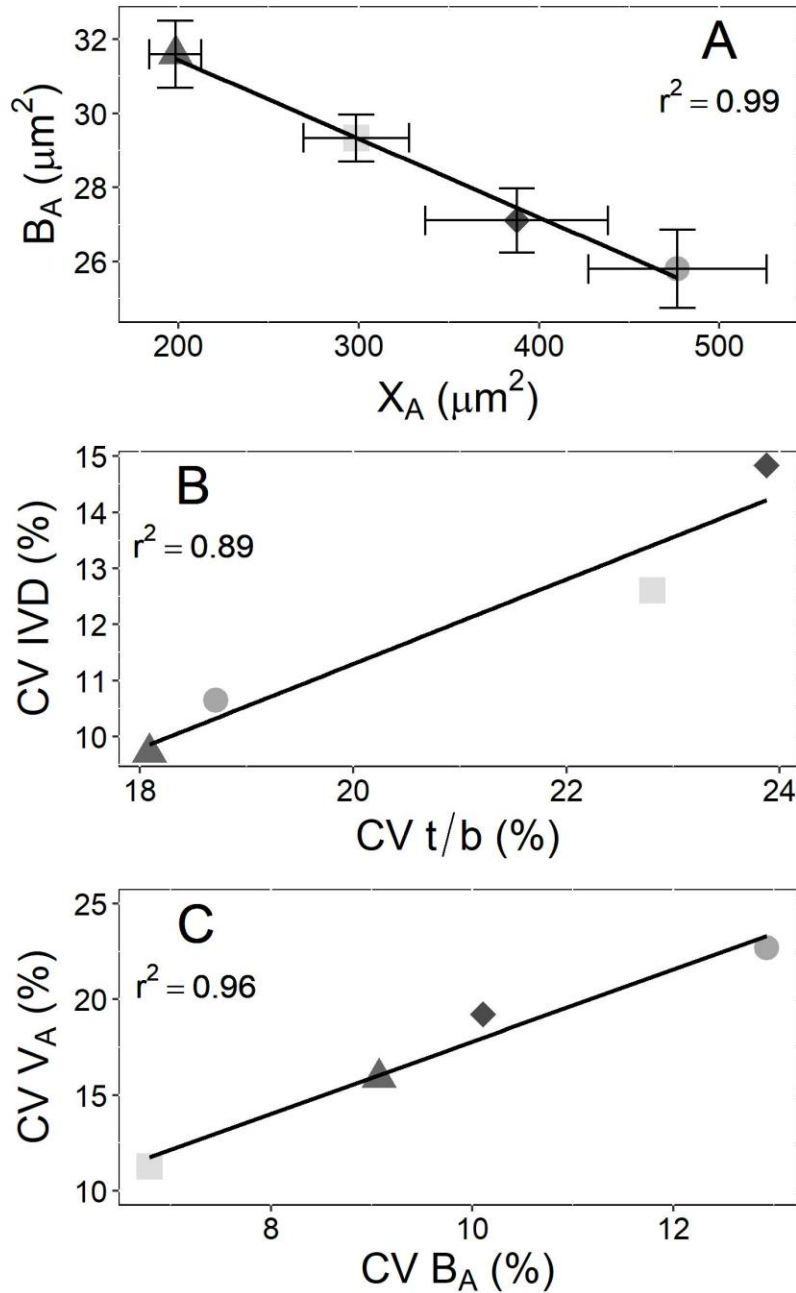


Figure 4.4 Anatomical trait relationships in *A. gerardii* across all sampling locations. (A) Xylem area (X_A) and bulliform area (B_A). (B) Xylem reinforcement (t/b) and interveinal distance (IVD) variation. (C) B_A and vein area (V_A). Sites are indicated by shading (matching previous figures) and shapes: diamonds, RMRS; squares, CDR; triangles, PRP; circles, KPBS.

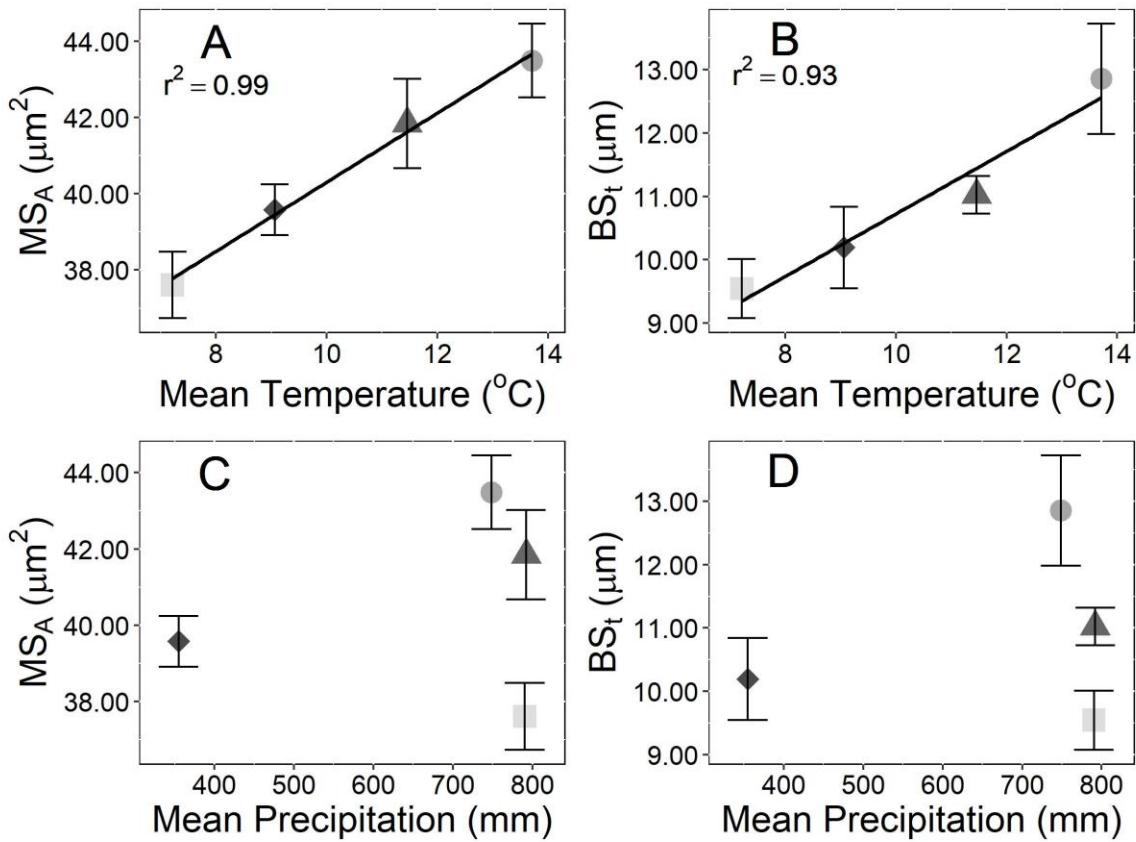


Figure 4.5 Linear regressions of carbon assimilation tissues with continuous mean temperature (A, B) and mean precipitation (C, D) from each site in 2017, provided by PRISM. (A, C) Mesophyll area; (B, D) Bundle sheath thickness. Sites are indicated by shading (matching previous figures) and shapes: diamonds, RMRS; squares, CDR; triangles, PRP; circles, KPBS.

Table 4.1 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	748.64
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	

Chapter 5 - Inter-annual climate differences supersede grazing effects on *A. gerardii* anatomy and physiology in the Great Plains

This chapter is formatted for the journal "Oecologia"

Abstract

Ecological processes in grassland systems are historically shaped by climate, fire, and grazing as essential environmental drivers. These grassland drivers influence species morphology and productivity via physiological processes, resulting in unique water and carbon use strategies among species and populations. Leaf-level physiological responses in plants are framed by microanatomical structures, which have previously been shown to alter carbon assimilation and water-use in leaf tissues. Yet, there is currently little information describing the impacts of key grassland drivers, such as climate of origin and grazing by large ungulates on leaf-level microanatomical traits. To address this knowledge gap, we sampled from three locations along a latitudinal gradient in the mesic grassland region of the central Great Plains, USA during the 2018 and 2019 growing seasons. We measured annual biomass and forage quality at the plot level, while collecting physiological and microanatomical traits at the leaf-level in un-grazed and cattle grazed locations at each site. Leaf-level measurements were focused on the dominant grass species *Andropogon gerardii* (big bluestem) because of its high abundance and forage value for native and non-native grazers. These two sampling seasons received markedly different levels of precipitation: drought conditions in 2018 and excessive early season precipitation in 2019. Ambient drought conditions negatively impacted *A. gerardii* physiology and drastically reduced productivity regardless of treatment. This is in direct contrast

with 2019 in which productivity was observed to increase to ~2x that of 2018. Leaf-level microanatomical traits, particularly traits associated with water-use, varied within and across locations and between years. My results highlight how trait plasticity can serve as an important tool for predicting future grassland responses to climate change and variable disturbances.

Introduction

The Great Plains is the largest expanse of grasslands in North America, reaching from the northern Dakotas through Texas (Robinson *et al.*, 2019; Jones *et al.*, 2020). The community composition and productivity of native grasses in the Great Plains varies as a result of the precipitation (longitudinally) and temperature (latitudinally) gradient (Teeri & Stowe, 1976; Sala *et al.*, 1988; Lura *et al.*, 2019). The impacts of these gradients are reflected in the grassland ecotones of the Great Plains (arid to mesic) that separate regions of shortgrass, mixed-grass, and tallgrass prairies (DeLuca & Zabinski, 2011; Dixon *et al.*, 2014). Each of these prairie systems are dominated by a few grass species that account for a majority of annual production. For example, *Bouteloua gracilis*, commonly found throughout the shortgrass steppe, accounts for upwards of 90% of total biomass in that system (Sasaki & Lauenroth, 2011; Hoffman *et al.*, 2020). Similarly, the northern mixed-grass prairie is dominated by *B. gracilis*, *Heterostipa comata*, and *Pascopyrum smithii* which accounts for the majority of the annual production there (Lura *et al.*, 2019). *Andropogon gerardii* can account for over 70% of annual biomass in the tallgrass prairie when ample rainfall is received (Weaver, 1968; Smith & Knapp, 2003). These dominant grasses thrive in their native habitats because each has evolved specialized plant functional traits (PFTs) as mechanisms of persistence within each region's disturbance regimes (fire, grazing, and climate variability) (Anderson, 2006; Bachle *et al.*, 2018; Jardine *et al.*, 2021).

These adaptations include but are not limited to: 1) large shallow rooting systems comprised of fine roots that quickly absorb water (Nippert & Knapp, 2007; Nippert *et al.*, 2012); 2) belowground meristematic tissues (“bud banks”) which provide new growth after senescence, fire, and grazing (Ott & Hartnett, 2015; Ott *et al.*, 2019); and 3) specialized leaf morphology and anatomy to maximize light capture and minimize water loss to combat drought (Hameed *et al.*, 2012; Nunes *et al.*, 2020; Bachle & Nippert, 2021). While these PFTs explain the continued success of grass species in their respective region, less is understood about how these traits vary, and if each morphological and physiological adaptation offers the same advantages across sites with varying local climates, yet all within the same broad geographic region. For instance, what allows *A. gerardii* to dominant locally (tallgrass prairie) and spread regionally (Great Plains)?

Research investigating local adaptations has been primarily focused on assessing productivity, whole-leaf economics, or genomics/phenotypes (Avolio & Smith, 2013; McAllister *et al.*, 2015). Much of this is built around a common garden experimental design, and has yielded many novel insights such as the genotypic changes in local populations (ecotypes) across regions (Mendola *et al.*, 2015; Maricle *et al.*, 2017; Galliard *et al.*, 2019). Ecotypes can display significant differences in growth rates, root biomass, and aboveground productivity (Mendola *et al.*, 2015). Similarly, the work of Maricle *et al.* (2017) and others have combined leaf-level physiology with known genotypic variation to determine responses within common gardens (Johnson *et al.*, 2015; Maricle *et al.*, 2017). Results indicate that genetic differences exist among *A. gerardii* ecotypes across a wide precipitation gradient (East–West). However, the negative physiological responses to reduced soil moisture availability explained most of this variation, rather than the ecotypic variation. This implies that large intraspecific variation in *PFTs* regulating physiology must exist in *A. gerardii*, enabling a single species to occupy a wide

geographic and environmental breadth (Bachle *et al.*, 2018; Westerband *et al.*, 2021). To date, investigation of genotypic and physiological variability in *A. gerardii* has provided key perspectives on population-level plasticity in *A. gerardii* across naturally occurring precipitation gradients. However, a mechanistic understanding of how this population variability interacts with naturally-occurring ecosystem drivers in native populations is less understood. Many of these genotypic studies have also been framed around common garden experiments that artificially transplant populations among sites across precipitation gradients. Here, I propose an alternate approach by investigating naturally occurring populations in their home environments but under a range of environmental conditions. This allows for an assessment of responses to climate variability within a site and then comparisons of variability across sites. Because *A. gerardii* has evolved within the Great Plains, it's important to consider the responses to climate variability in conjunction with other ecosystem drivers (i.e., fire and grazing). For example, the effects of grazing on native grass species can be exacerbated by periodic droughts, resulting in reduced ecosystem services and productivity (Koerner & Collins, 2014; Souther *et al.*, 2020). While intraspecific responses to grazing and climate may reflect genetic differences, the mechanisms that underlie physiological responses are found at the microanatomical level (Christin *et al.*, 2013; Bellasio & Lundgren, 2016; Guha *et al.*, 2018).

While microanatomical traits are not often assessed because of the tedious and labor-intensive preparation and data collection, information gleaned from these methods allows for deeper understanding of physiological mechanisms (Wahl & Ryser, 2000; McElrone *et al.*, 2004; Carmo-Silva *et al.*, 2009). Plant physiology has typically been contextualized by variation in microanatomical traits because the structural framework of tissue architecture sets limits for physiological output (Esau, 1939; Furbank, 2016; Bellasio & Lundgren, 2016). For instance, the

innovation and diversification of xylem affects survival in drought conditions across many functional types (Scoffoni *et al.*, 2014; Hammond *et al.*, 2019; Ocheltree *et al.*, 2020).

Alterations to stomatal anatomy and densities regulate water-usage, because stomata serve as the gateway for the flux of CO₂, O₂, and H₂O to and from the leaf. The regulation of CO₂ and H₂O fluxes directly impacts carbon and water balance at the leaf level, and subsequent whole organism performance. The innovation of C₄ photosynthesis is one of the best-known examples of microanatomical changes influencing physiology and plant performance (Hatch, 1987; Lundgren *et al.*, 2014; Von Caemmerer *et al.*, 2017). The biochemical changes notwithstanding, without the anatomical change to ‘Kranz’ anatomy or spatial separation of carbon assimilation and carbon reduction tissues – this pathway would not be possible (Brown, 1975; Berry & Patel, 2008; Sage *et al.*, 2014). Spatial separation of photosynthetic tissues, in conjunction with biochemical modifications, allows reduced stomatal aperture, the ability to concentrate CO₂ within bundle sheath tissues, and reduction in water loss during photosynthesis (Leegood & Walker, 1999). While the previous example illustrates the unique ability of structure to dictate function, there are several underlying factors that should be considered.

Carbon, water, and nitrogen are necessary for proper physiological functioning, and the investment of such materials is also required for cellular upkeep and development of microanatomical tissues (Chaves *et al.*, 2003; Lundgren & Fleming, 2019). Investigations focused on the anatomical changes associated with different levels of carbon, nitrogen, and water availability are typically done in greenhouses or in agricultural studies that focus on few traits – usually for developmental biology and/or few species (Henry *et al.*, 2012; Retta *et al.*, 2016; Ermakova *et al.*, 2019). While the importance of this research should not be overlooked due to its significance in feeding a growing global population, these data are collected from controlled

environments with tightly controlled environments and abundant resources. Under real-world conditions, resources for native species are not always in steady supply – typically they are variable and often limiting. In addition, data for annual agricultural species does not always translate to regions like the Great Plains, which are comprised of native perennial grasses that must maintain structural integrity and investment beyond an single annual reproductive cycle (Benson *et al.*, 2004; Benson & Hartnett, 2006). In addition, native species are not granted protection from fire and grazing, rather grasses in the Great Plains are adapted to these added stresses (Axelrod, 1985; Strömberg, 2011).

Genetic differentiation and physiological plasticity in a dominant and ubiquitous species have been used to explain responses to changes in water availability. The evolved responses within populations are due to environmental differences that occur at a single location, not across regional scales (Valladares *et al.*, 2014; Hoffman *et al.*, 2020). Therefore, understanding traits within populations exposed to different intra-annual climate within a single site will provide a better understanding for how respond to climate variability at various locations. To my knowledge, a multi-location and multi-year investigation across a climate gradient in conjunction with grazing effects on leaf-level anatomy and physiology has not been done for a native grass species. This study aims to provide a mechanistic understanding of how varying climate and grazing, impacts a dominant species' physiological and microanatomical traits within a latitudinal gradient in the Great Plains. We hypothesized that: 1) due to site-level differences in climate histories across the latitudinal gradient, and contrasting growing season conditions in 2018 and 2019, there would be significant differences in mean and variability (measured here as the coefficient of variation) of leaf-level nutrient content, microanatomical traits, and instantaneous physiological responses across sites; 2) because microanatomical traits

constrain/frame physiological responses to water availability, the existing trait relationships will show significant differences between years sampled due to the disparity in precipitation received; and 3) due to the stress of compensatory growth and reallocation of resources, grazing will accentuate leaf-level microanatomical and nutrient content differences between treatments and across locations, negatively impacting physiology.

Materials and Methods

Site Descriptions

This experiment was conducted at three separate locations dominated by *A. gerardii* within the tallgrass prairie region of the Great Plains during the 2018 and 2019 growing seasons. These locations include: 1) a Long-term ecological research site (LTER), Konza Prairie Biological Station (KPBS) located in the northern Flint Hills region of north-eastern Kansas USA (39.1° N, 96.9° W), 2) the Flint Hills Prairie Preserve (FHPP) located at the mid-point of the Flint Hills region of Kansas USA (38.2° N, 96.3° W), and 3) the Platte River Prairies (PRP) located within the Big Bend region of south – central Nebraska USA (40.4° N, 98.3° W). The FHPP and PRP are both owned by The Nature Conservancy (TNC). Data was collected from five 1-m² plots equally distributed in grazed areas and ungrazed enclosures across similar topographic positions (plots = 10) at each location. Land managers from each location were also contacted to determine cattle grazing pressures (animal units per acre: AU acre⁻¹) at each location. The FHPP was burned in the summer of 2017, but not in 2018 or 2019, and grazed at 3 AU acre⁻¹. This site is predominantly silty-clay soils that receives ~950 mm yr⁻¹ precipitation. Two separate experimental watersheds were utilized at KPBS, including the ungrazed ‘2D’ and the grazed ‘3CB’ 8 AU acre⁻¹), both of which were burned in 2019. KPBS receives ~890 mm of annual

precipitation and is characterized by silty clay soils. Experimental plots at the PRP were located in ungrazed and grazed pastures (8 Au acra⁻¹); both locations were burned in the spring of 2019. The PRP receives ~670 mm per year with soils that are predominantly sandy soils. In 2018, the FHPP and KPBS experienced a drought that drastically reduced rainfall in the early and mid-growing season.

Leaf physiology and anatomy

Gas exchange rates were measured using a Li-COR 6400XT (Li-COR Biosciences, Lincoln, NE, USA) equipped with an LED light source (maintained at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), CO₂ concentration at 400 ppm, and maintained relative humidity in the chamber between 40 – 60%. This instrument was used to collect photosynthetic rates (A_n), stomatal conductance (g_s), and transpiration rates (E) twice (June and August) between 10:00 and 14:00 CDT during the 2018 and 2019 growing seasons on three individual *A. gerardii* leaves in each plot. Measurements were recorded when gas exchange levels remained stable for ~2 minutes. These same individual leaves were also used to determine nutrient content and microanatomical traits.

Following physiological gas exchange measurements, the previously measured leaf tissues were then clipped (~30 mm) and immediately placed into a FAA (10 % formalin/5 % glacial acetic acid/50 % ethanol (95 % EtOH)/35 % DI water) for vacuum infiltration to analyze microanatomical traits. Leaf tissues were then cross sectioned to a 4 μm thickness with a Leica RM2135 microtome (Leica Biosystems, Newcastle, UK), stained with Safranin-O and Fast Green (Ruzin, 2000), and imaged at 100X and 200X on an Olympus BH-2 compound microscope (Olympus America Inc, Melville, NY) (Fig. 1). We then quantified microanatomical traits by using IMAGEJ software (Rasband, 1997) and the established procedure detailed by

Bachle and Nippert (2018). The selected microanatomical traits included: the total cross-sectional area measured (TMA), bundle sheath cell area (BS_A), mesophyll area (MS_A), bundle sheath: mesophyll area ($BS:MS$), bulliform area (B_A), xylem area (X_A), and xylem reinforcement (t/b): the ratio of xylem wall thickness (t) with xylem diameter (b). Due to the small size of minor veins in the sampled leaf tissue, xylem characteristics were limited to major vascular bundles.

Leaf stoichiometry and biomass

Carbon and nitrogen content were determined on each leaf that was used to determine photosynthetic rates. These leaves were dried and ground for elemental composition of carbon and nitrogen per plot (protocol outlined in Connell et al. 2020). Biomass was determined by clipping herbaceous biomass in one 0.1 x 0.1 m frame per plot at the conclusion of each growing season. This biomass was sorted to exclude dead biomass (when necessary) and then dried at 60°C for 48 hours and weighed to determine dry mass.

Statistical analyses

All analyses were completed in the statistical program R V3.5.3 (R Core Team, 2020). For all analyses, we evaluated homogeneity of variances by examining residuals vs fitted and also examined normality using qq-plots and when necessary, a Shapiro-wilk's test. TMA and X_T were the only traits that required non-parametric analyses via Kruskal-Wallis test accompanied with a *post hoc* pairwise Wilcoxon test. To assess the effects of grazing and climate differences between locations, we utilized repeated measures mixed-effects model ANOVAs with separate models for each physiological, microanatomical, and nutrient trait as the response variables, and

location, treatment, and year sampled as predictor variables, and plot as the random effects. Tests were performed using the “lmer” function within the “lmerTest” package (Kuznetsova *et al.*, 2017).

Results

Leaf – level physiological traits

Leaf -level physiological traits in *A. gerardii* were variable across locations ($P < .005$). However, grazing had no effect on gas exchange rates ($P > 0.40$) (Fig 5.2; Table 5.1). E was statistically similar across the years sampled ($P > 0.05$; Fig 5.2C; Table 5.1). Grasses at PRP had the highest gas exchange rates in 2018, while FHPP displayed the highest rates in 2019 (Fig 5.2). A_n and g_s increased between 2018 and 2019 ($P < 0.001$), most notably at FHPP (74% and 156% respectively) and KPBS (119% and 150% respectively) (Fig 5.2A, B). In addition, there was an interaction between location and year sampled for both A_n and g_s ($P < 0.001$; Table 5.1). No latitudinal trend was discernible in 2018 gas exchange, however, g_s and E generally decreased with increasing latitude (Fig 5.2B, C).

Internal microanatomical leaf traits

The total measurable area of internal anatomical traits (TMA) varied significantly among locations ($P < 0.05$) but remained statistically similar across treatments and year ($P = 0.29$; 0.57 respectively; Table 5.1). Specifically, TMA at FHPP was significantly smaller compared to other locations in 2019 ($P < 0.05$; Table 5.1). On average, BS_A varied significantly by location and increased significantly from 2018 to 2019 ($P < 0.001$; Table 5.1). A significant interaction between year sampled and location also existed ($P < 0.001$; Table 5.1). In 2018, BS_A in KPBS

samples was significantly higher at either FHPP or PRP; however, FHPP samples contained the highest BS_A in 2019 ($P < 0.05$; Table 5.1). In addition, MS_A and annual productivity were the only traits that were affected by the grazing treatment, but only within KPBS in 2019 ($P < 0.05$; Table 5.1). Overall, MS_A did not change between years nor among locations ($P > 0.05$), maintaining ~40% of TMA . The ratio of bundle sheath area and mesophyll area ($BS_A:MS_A$) displayed significant effects from location, year, and their interaction ($P < 0.03$, $P < 0.0001$, $P < 0.0001$; Table 5.1). V_A varied significantly between years ($P < 0.05$) but was not affected by treatment or location sampled ($P > 0.05$, $P = 0.056$; Table 5.1). V_A at FHPP and PRP increased from 2018 to 2019; in contrast, V_A at KPBS decreased (Table D.2). Tissues within V_A were consistently between 12 – 18% of TMA (Table D.2). B_A did not vary across locations ($P = 0.96$) or treatment ($P = 0.59$; excluding KPBS in 2019), but significantly decreased from 2018 to 2019 in all locations except KPBS ($P = 0.25$; Fig 5.3B; Table 1). In addition, TMA consisted of ~ 20 – 30% B_A across each location, year, and treatment (Table D.2). X_A also increased across years sampled (Fig 5.3; $P < 0.005$) but remained statistically similar across locations and treatment ($P = 0.36$, $P = 0.69$ respectively; Table 1). FHPP was the only location that exhibited a large difference in X_A between control and grazing treatment in both 2018 and 2019 ($P < 0.02$, $P < 0.005$ respectively), while grazing only impacted X_A at KPBS in 2018 ($P < 0.05$; Fig 5.3A; Table 5.1). Lastly, xylem reinforcement (t/b) followed a similar pattern to X_A , resulted in significant decreases across years sampled (Table 5.1; $P < 0.005$), but were similar across locations and unaffected by grazing ($P > 0.05$; Table 1).

Stoichiometry and Productivity

Carbon and nitrogen content in *A. gerardii* leaves varied according to year and location, but C:N was the only stoichiometric measurement affected by the grazing treatment ($P > 0.05$; Table 5.1). Nitrogen content was consistently higher in 2019 than in 2018 ($P < 0.0001$; Table 5.1); Grass leaves at PRP had the highest nitrogen content, regardless of year (Table D.1). In addition, C:N ratios were higher in 2018 compared to 2019 and also varied by location sampled and treatment ($P < 0.05$; Table 1). The C:N ratio was higher at both FHPP and KPBS relative to PRP in both years sampled, regardless of treatment (Table D.1). Aboveground biomass varied by location, year, and not surprisingly-treatment ($P < 0.05$; Table 5.1). PRP was the most productive location in both 2018 and 2019, in both grazed and control plots (Table D.1).

Trait relationships and variation

While traits did show relationships with average climate parameters (MAP and MAT) for the three sites, the disparity between 2018 and 2019 rainfall data made it difficult to discern logical trends (Figure D.2, 3, 4). However, higher temperatures were associated with lower N content and higher C:N ratios; while gas exchange data patterns were similar to N patterns (Figure D.4). Some microanatomical traits such as BS_A , V_A , and X_A decreased with rising MAT while t/b and B_A were positively correlated with increasing MAT (Figure D.3). Trait data collected across locations, years, and treatment displayed considerable, yet statistically significant variation (Fig D.2 - 4). Specifically, increased photosynthetic rates (A_n) were correlated with increased levels of N ($P < 0.001$), while increasing C:N ratios was shown to decrease A_n (Fig D.1; $P < 0.05$). In addition, BS_A and MS_A were shown to decrease with rising N while t/b was observed to decrease, regardless of treatment (Fig D.1; $P < 0.05$). Samples with

increased amounts of BS_A were also observed to have a positive relationship with A_n , (Fig D.1; $P < 0.001$).

The mean coefficient of variation (CV) in physiological traits (A_n , g_s , and E) was significantly higher than the mean CV in microanatomical traits (Fig 5.5A, B). However, water usage/storage traits (X_A , t/b , and B_A), were responsible for the majority of microanatomical variation (Fig 5.5C). In addition, slight changes in microanatomical CV were observed between years and locations, while physiology displayed significantly higher CV in 2018 than 2019 (Fig 5.5). Weak relationships between functional trait CV were visible in several traits, however many of these relationships were dependent on the year of collection (Fig D.1 - 3). The variation of several anatomical traits also displayed relationships across climate parameters (rainfall & temperature) (Fig 5.6; Fig D.1 - 3). These data also indicate yearly differentiation in relationship direction and correlation due to climate, irrespective of grazing (Fig 5.6).

Discussion

For the grasslands in the Great Plains, the east-west precipitation gradient results in three distinct grassland types: tallgrass prairie from Illinois to Kansas, associated with a region where rainfall amounts exceed evaporation losses, shortgrass steppe in the west limited by rainfall and growing season temperatures, and the mixed-grass prairie in the central portion as a transition between the wetter and drier prairies (Maricle & Adler, 2011; Olsen *et al.*, 2013; Maricle *et al.*, 2017). Across all three grassland types, long-term precipitation and temperature is more variable compared to other systems, often creating yearly and seasonally contrasting growing conditions (Zhang *et al.*, 2010; Knapp *et al.*, 2015; Flanagan *et al.*, 2017). For example, Flanagan *et al.* (2017) analyzed long-term precipitation and temperature records and found a rise in

asynchronicity in climate maxima in the Great Plains, which results in a widening disparity between abiotic patterns and plant phenology. Here, my results emphasize the large differences in physiological and anatomical responses that can exist within a widespread C₄ grass species (*A. gerardii*) across multiple years and locations with distinct climate histories (contrasting precipitation and temperature) (Fig 5.1). My data illustrates that patterns of response in this widespread species vary across locations, but perhaps most importantly, that this pattern of variation to wet/dry years is not uniform within a single latitude.

A large number of studies have investigated how a dominant species (*A. gerardii*) responds to changes in precipitation (Knapp, 1985; Dietrich & Smith, 2016; Hoffman *et al.*, 2018). Such studies have elucidated how local ecotypes are genetically distinct across a precipitation gradient that exists from Kansas and Illinois, using common garden experiments (Mendola *et al.*, 2015; Kramer *et al.*, 2018) or within local landscapes (Avolio & Smith, 2013; McAllister *et al.*, 2015). These studies provide an understanding of ecotypic responses under novel climate conditions; but do not present a mechanistic understanding for physiological responses of local populations within and across sites. Common garden experiments often do not include key grassland ecosystem drivers (fire and grazing), which have been repeatedly shown to impact physiological responses, biomass, and local ecosystem function (O’Keefe & Nippert, 2017; O’Connor *et al.*, 2020; Connell *et al.*, 2020). To add a mechanistic understanding to the existing literature, we investigated how local populations of *A. gerardii* within their site of origin respond to water availability and grazing in comparison to populations across multiple sites.

Significant differences in leaf level physiology, microanatomy, stoichiometry, and biomass were observed across sites and between years in this study. The long-term climate histories of each location have been responsible for shaping PFTs of local populations (Fig 5.1),

allowing for site-specific leaf-level anatomy and physiology (Fig 5.2, 3; Table 5.1; Table D.1, 2) (Hoffman & Smith, 2020; Bachle & Nippert, 2021). However, when local populations experience water limitations (drought), negative responses ensue (Maricle *et al.*, 2017; Galliard *et al.*, 2020). Decreased soil moisture availability reduces carbon assimilation, decreases nutrient uptake, and leads to reduced productivity (Lemoine *et al.*, 2018; Jardine *et al.*, 2021). Our data illustrate similar patterns, at the FHPP and KPBS sites, which received significantly less rainfall in the 2018 growing season than the subsequent year (Fig 5.1). The drought conditions at both locations resulted in significantly reduced photosynthetic rates, stomatal conductance, and leaf nitrogen content (Fig 5.2; Table 5.1; Table D.1). Increasing water stress causes decreased stomatal aperture, allowing for decreased water loss; however, long durations of water stress can lead to carbon starvation (Lawson & Matthews, 2020; Nunes *et al.*, 2020). Similarly, reductions in X_A and increased B_A were also observed in 2018 (Fig 5.3), reflecting changes in water – use strategies. Previous research showed that increased X_A allows for greater water transport, but it also increases the likelihood of cavitation during droughts or when the water column is under high tension (Olson *et al.*, 2020).

Intraspecific trait variability (CV) was distinct between years, but relatively similar across locations (Fig 5.5). The greatest variation was within gas exchange measurements (A_n , g_s , E) in 2018, which were ~2 times higher than the following year (at both FHPP and KPBS) (Fig 5.5A). While high variability may be inherent to the instantaneous nature of gas exchange measurements, the CV of physiological responses in 2019 was similar to all microanatomical traits regardless of function (Fig 5.5B, C). This decrease in physiological CV may indicate a baseline physiology an associated physiological plasticity of *A. gerardii*, when water is less limiting. While mean microanatomical traits varied significantly between 2018 and 2019, there

was little change in variability (CV) across years (Fig 5.5B, C). In fact, most microanatomical variation resulted from water-specific traits (X_A , t/b , B_A) (Fig 5.5C). The diversity in PFT responses has been reported to protect individuals and populations from detrimental effects of drought (Mori *et al.*, 2013; Kreyling *et al.*, 2017; Roberts *et al.*, 2019).

While previous research has indicated that microanatomical traits can influence/constrain physiological responses to changes in water availability (Christin *et al.*, 2013; Guha *et al.*, 2018; Edson-Chaves & Graciano-Ribeiro, 2018; Wargowsky *et al.*, 2021), few studies have analyzed physiology, stoichiometry, and microanatomy on the same leaf. The importance of this sampling technique allowed us to analyze direct bivariate relationships of both PFTs mean and variability (CV) (Fig 5.4; Figure D.1-4). Past research focusing on anatomical and physiological relationships has been mainly constrained to greenhouses or single-year studies (Henry *et al.*, 2012; Bachle & Nippert, 2018; Sonawane *et al.*, 2021). My results emphasize how disparate climates across years can result in dissimilar relationships among traits and between traits and climate variables (Fig 5.4; Fig 5.6; Figure D.1-3). *A. gerardii* photosynthetic rates correlated positively with increasing nitrogen content (Fig 5.4A) when analyzed between years. However, this seemingly tight relationship broke down when analyzing each year separately (Fig 5.4B). Several mean trait relationships in physiological and microanatomical traits displayed opposing trends between 2018 and 2019 (Figure D.1-3), including BS_A against gas exchange traits (A_n , g_s , and E). In addition, the timing of precipitation has also been known to impact grassland productivity (Nippert *et al.*, 2006; Craine *et al.*, 2012), which is a result of altered microanatomy and physiology (Fay *et al.*, 2002; Wang *et al.*, 2016; Lemoine *et al.*, 2018). For example, early season rainfall (coinciding with tissue development) allows for the production of larger vessels

areas for greater transport potentials, while early season droughts constrain development, which results in smaller vessel areas (Mauseth, 1988).

Historically, the Great Plains have provided forage for native mammalian grazers such as *Bison bison* (bison), and grazing resulted in increased plant diversity and landscape heterogeneity (Knapp *et al.*, 1999; Elson & Hartnett, 2017). More recently however, the majority of grazing is accomplished by non-native grazers like cattle. Similar to climate variability and fire, responses to grazing are typically examined at the community or ecosystem levels, while less is understood about the physiological and microanatomical mechanisms responsible for those responses (O’Keefe & Nippert, 2017). However, grazing and other forms of herbivory can increase gas exchange rates in order to compensate for the loss of tissue (Pinkard *et al.*, 2011; O’Connor *et al.*, 2020). While this allows for greater carbon assimilation, it requires increased stomatal conductance which inherently leads to greater water loss (Bertolino *et al.*, 2019). During drought conditions, this compensatory response of recently grazed tissues would negatively impact grass physiology, thereby decreasing carbon assimilation and future productivity (Feller, 2016; Souther *et al.*, 2020). However, gas exchange rates within grazed locations in this study were nearly identical to the control (Table 5.1; Fig 5.2), even during the dry 2018 growing season. In addition, only three PFTs were impacted by the grazing treatment: MS_A , C:N ratios, and biomass production (Table 5.1). The grazing treatment at KPBS was responsible for most MS_A variation, in both 2018 and 2019 (Table D.2). In 2018, grazing increased C:N ratios in leaf tissues from FHPP and PRP (Table 5.1; Table D.1). While grazing did impact PFT trait variability, it was only observed during the 2018 growing season and only in physiological and water-use microanatomical trait CV (Fig. 5.5). The lack of treatment response may be due to several factors including: 1) stocking rates at each location may not be

conducive to infer grazing pressure; 2) the experimental design may not have adequately covered/represented each site and subsequent treatment, ultimately leading to lack of replication; 3) due to the evolutionary history of *A. gerardii* in the Great Plains, a heightened grazing intensity may be necessary to induce alternative physiological responses.

Conclusion

These results highlight how trait plasticity can serve as an important tool for understanding the anatomical and physiological mechanisms that facilitate wide distributions of a dominant grass species. This research was completed during the 2018 and 2019 growing seasons which had significantly different water availability among years. Drought conditions in 2018 resulted in decreased gas exchange rates and subsequent biomass production, irrespective of grazing. However, increased water availability in 2019 facilitated high gas exchange rates and the doubling of aboveground biomass. In addition, there was significant variation in microanatomical traits across locations and between sampling years. Together, these results indicate that there are specific leaf construction strategies based on intra-annual climate conditions across the Great Plains. Such leaf construction strategies frame instantaneous physiological responses to climate variability, and also other grassland drivers (i.e., grazing and fire). Results from this study underlie the importance of collecting multiple years of data from native species in native environments. Our data also emphasizes the need for increased microanatomical research, as we clearly demonstrate site and climate-specific leaf construction strategies are important for understanding and contextualizing physiological responses in *A. gerardii*.

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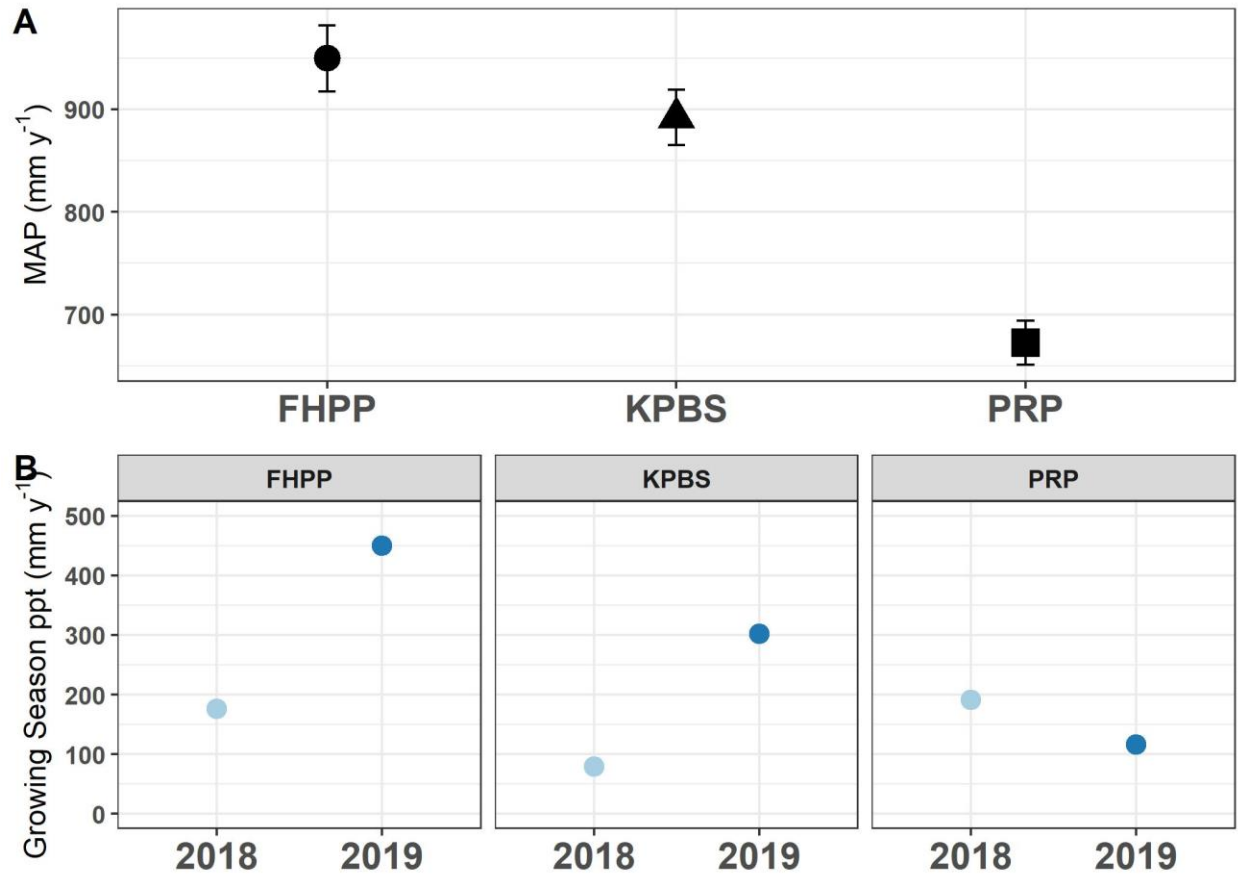


Figure 5.1 A) Long - term mean annual precipitation for each location (1981 – 2019), error bars represent standard error. **B)** Growing season precipitation from May 1st – August 10th during 2018 and 2019. Light blue represents 2018 and dark blue represents 2019.

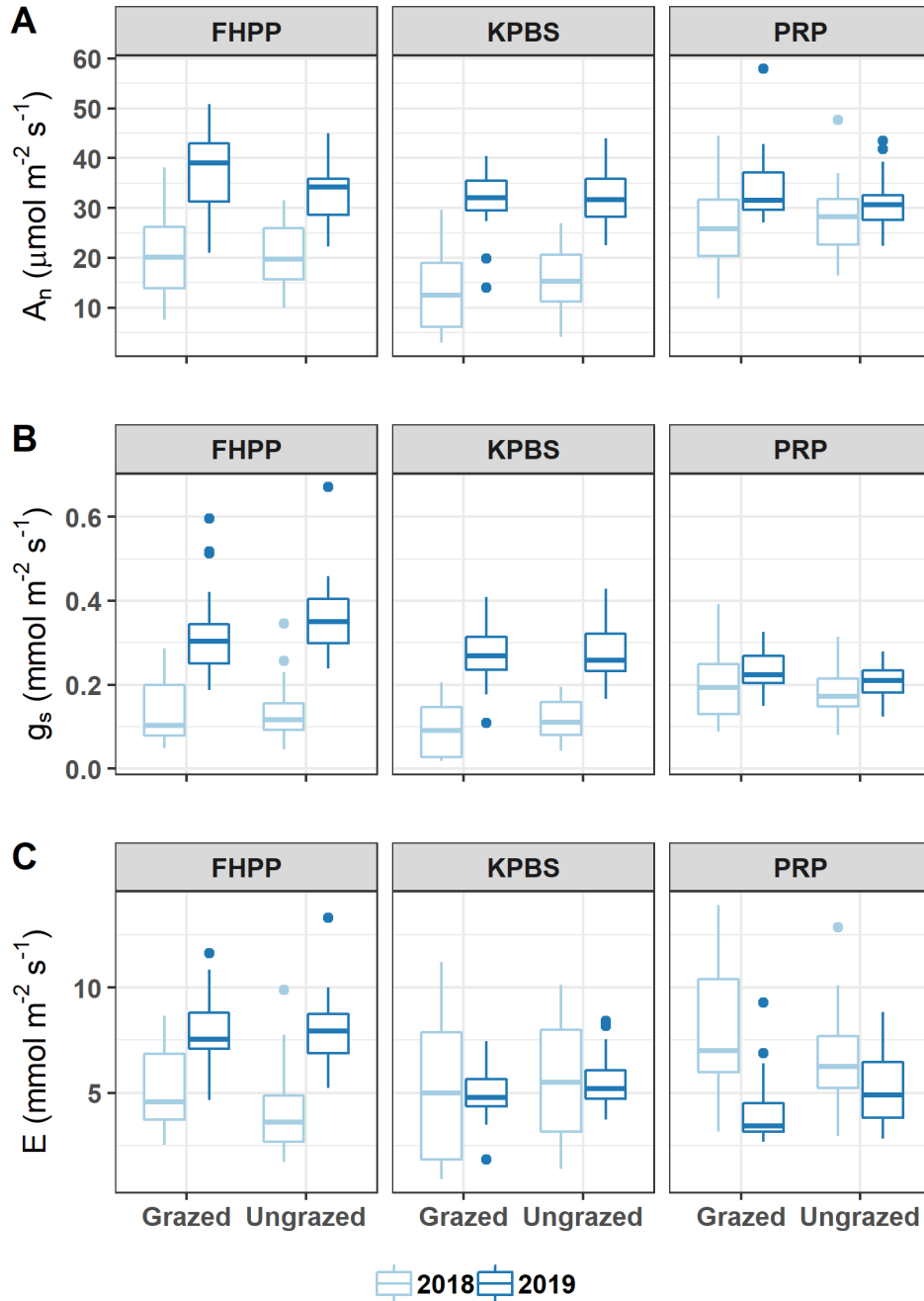


Figure 5.2 Gas exchange collected at each site and treatment during the 2018 (light blue) and 2019 (dark blue) growing. **A)** photosynthetic rate; **B)** stomatal conductance; **C)** transpiration rate. Thickened lines represent the median value, upper and lower edges of box represent the interquartile values (25th and 75th percentiles).

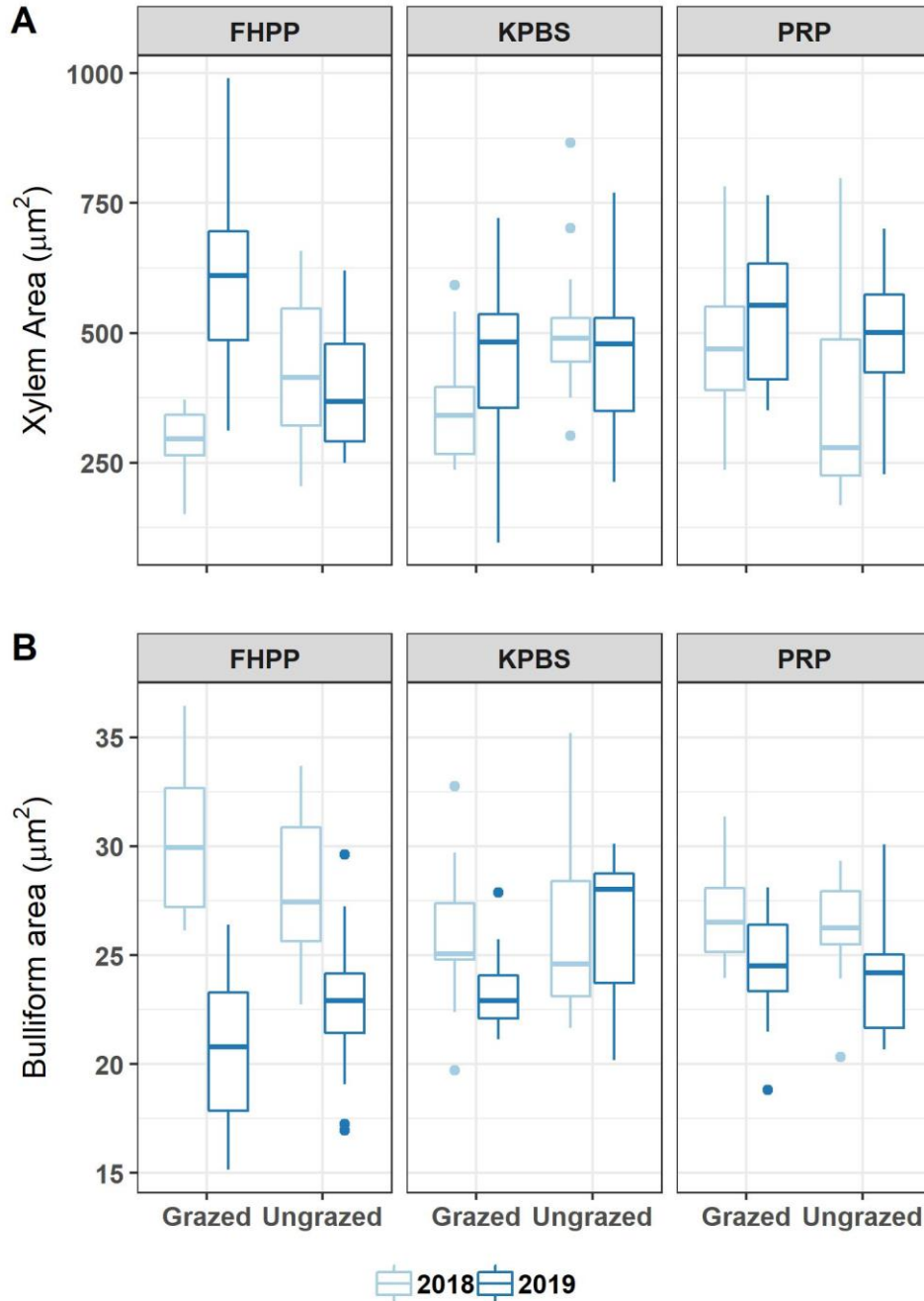


Figure 5.3 Boxplots of microanatomical data collected at each site and treatment during the 2018 (light blue) and 2019 (dark blue) growing season. A) mean xylem area; B) mean bulliform cell area. Thickened lines represent the median value, upper and lower edges of box represent the interquartile values (25th and 75th percentiles).

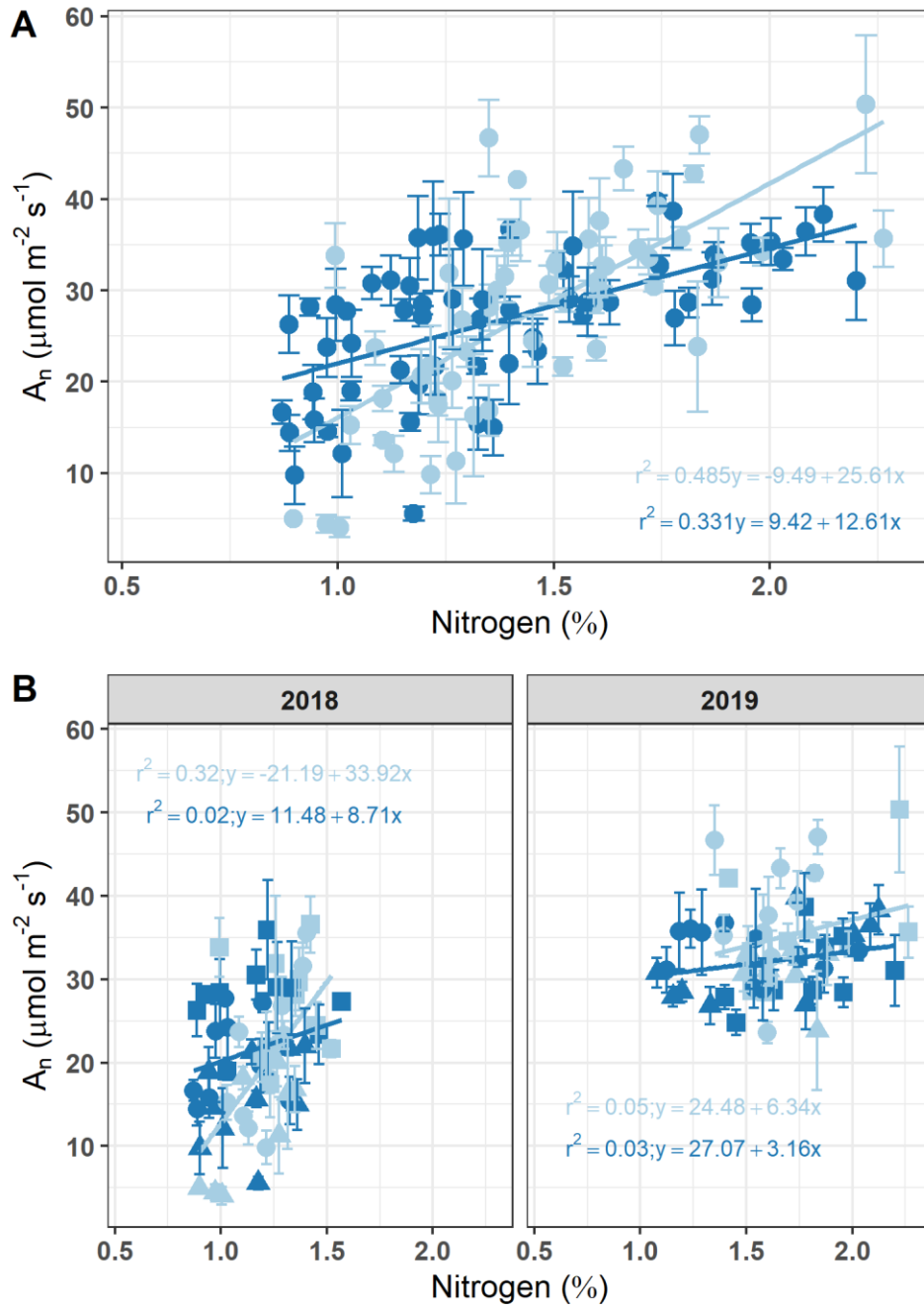


Figure 5.4 Linear regression relating leaf-level nitrogen content and mean photosynthetic rate at each location and year (mean \pm SE). **A)** Relationship across years; **B)** relationship separated by year sampled. Shapes denote location (circle, FHPP; triangle, KPBS square, PRP;) while color denotes treatment (light blue, grazed; dark blue, ungrazed).

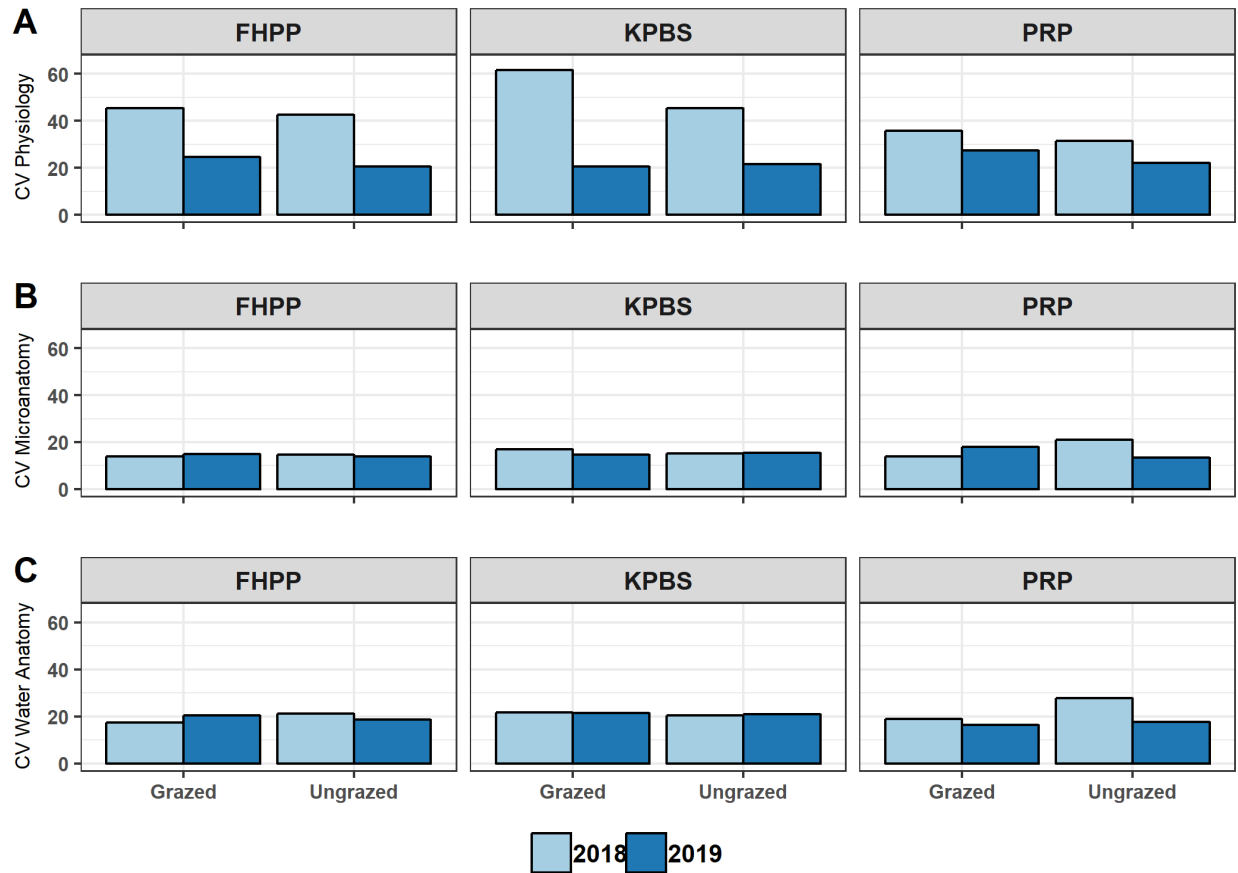


Figure 5.5 Coefficient of variation (*CV*) measured as a percent at each location and year. **A)** Combined mean *CV* for A_n , g_s , and E . **B)** Combined mean *CV* for all microanatomical traits (excluding redundancies). **C)** Combined mean *CV* for microanatomical traits that influence water storage or transport (X_A , t/b , B_A). Colors denote year of sampling (light blue, 2018; dark blue, 2019).

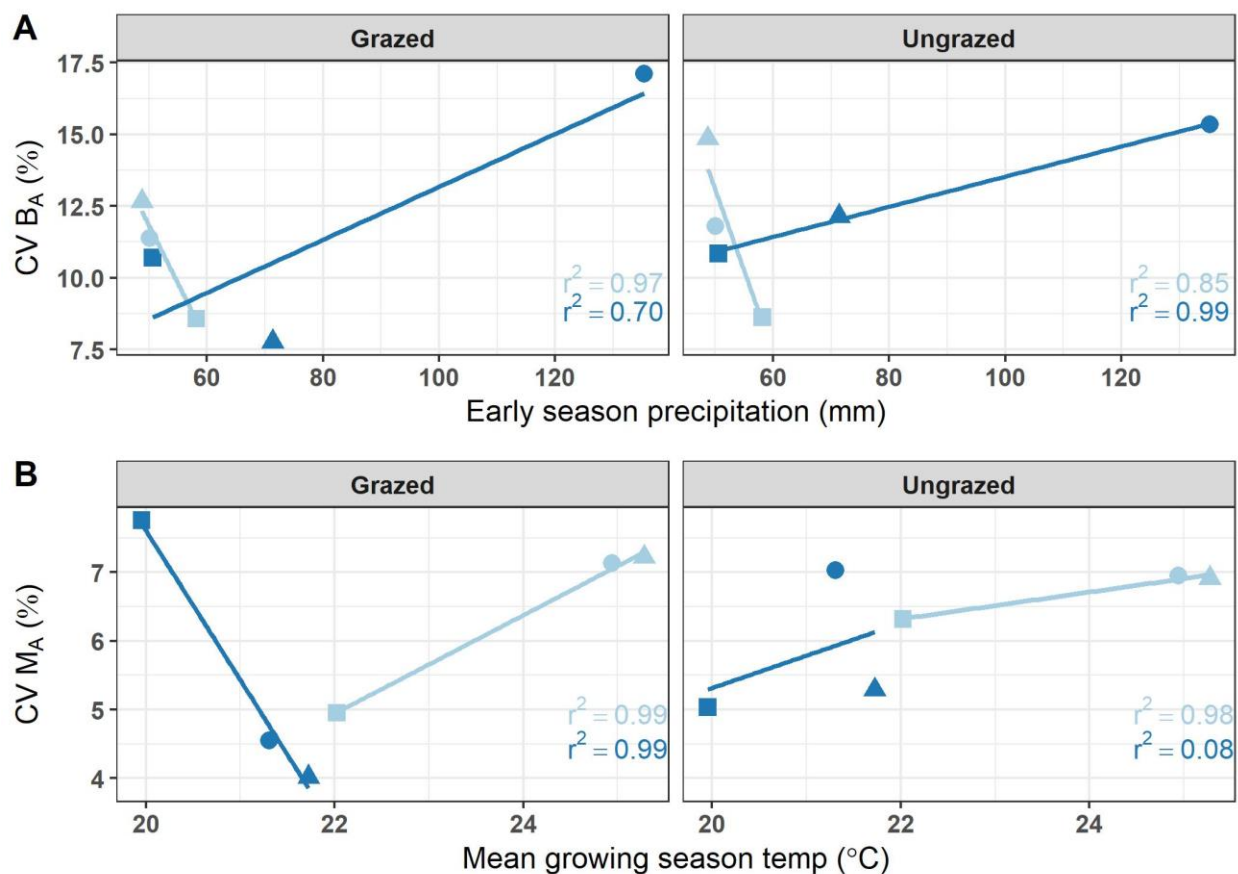


Figure 5.6 Linear regression between microanatomical traits and climate parameters delineated by treatment. **A**) Linear regression between bulliform area $CV(B_A)$ and early season precipitation (January 1st – May 31st). **B**) Linear regression between mesophyll area $CV(M_A)$ and mean growing season temperature. Shapes denote location (circle, FHPP; triangle, KPBS square, PRP;) while color denotes treatment (light blue, 2018; dark blue, 2019).

Table 5.1 ANOVA results, reported as F – values for leaf – level physiological, microanatomical, stoichiometric traits, and biomass. Subscript text in parentheses refers to data transformation necessary to meet assumptions of normality. ^ P < 0.10, *P < 0.05, **P < 0.01, *P < 0.001.**

Trait	Location	Treatment	Year	L x T	L x Y	T x Y	L x T x Y
A_N	26.91***	0.79	280.30***	2.74^	23.79***	5.14*	0.23
G_s (SQRT)	11.25***	0.37	356.52***	3.19	49.03***	0.00	1.52
E	5.73**	0.08	1.05	1.48	51.92***	7.61**	1.92
TMA_{LOG}	5.25**	1.27	0.33	0.57	0.80	0.99	0.91
BS_A	10.45***	1.21	191.00***	2.84^	15.19***	4.67*	0.39
MS_A	1.05	7.46*	1.70	7.30***	4.21*	3.09^	0.40
V_A	1.52	4.99^	9.46**	4.59*	19.73***	8.15**	4.53*
BS:MS	6.07**	0.33	61.63***	2.00	8.24***	6.25*	0.37
B_A	0.04	0.32	62.08***	1.94	19.88***	6.00*	1.70
X_A	1.03	0.17	14.00***	4.96**	3.00^	9.97**	8.19***
t/b (LOG)	1.68	2.10	31.08***	7.05**	4.94**	1.03	6.08**
N	6.66***	2.91	137.86***	1.05	1.14	0.04	0.58
C:N	11.36***	4.75*	73.38***	2.35^	0.58^	0.06	3.57*
Biomass_(LOG)	57.15***	67.19***	5.52*	3.09^	2.49^	7.50**	0.58

Chapter 6 - Conclusion

Water availability is a key driver of plant microanatomy and physiological responses, yet there is limited knowledge on how such traits vary in native grass species across the Great Plains of North America. The Great Plains region is currently projected to experience increased climate variability, leading to droughts and deluges (Joyce *et al.*, 2001; Craine *et al.*, 2011; Perkins *et al.*, 2019); therefore, identifying and analyzing the mechanistic influence of microanatomical traits in dominant species may increase our ability to predict future physiological responses of individuals and ecosystems and (Sperry & Love, 2015; Venturas *et al.*, 2017). To address this knowledge gap, I evaluated leaf – level microanatomical traits and physiological responses of grass species under varying levels of soil moisture in both greenhouse studies and in native prairies across the North American Great Plains.

First, I investigated how a diverse assemblage of grasses, 12 species representing 6 tribes, respond to decreasing soil moisture availability (Chapter 2). Specifically, I evaluated the physiological responses of all species utilizing a dry-down method, analyzing whole-plant physiological traits, economic traits, and microanatomical traits. Here, I showed that species within tribes display similar drought sensitivities, but differ considerably in drought recovery. My data also indicate that traits utilized to aid in drought duration are not necessarily associated with drought recovery.

Next, I evaluated how a single species (*Andropogon gerardii*) can spread across the climatically variable Great Plains region (Chapter 3). I performed a literature search to assess intraspecific trait variability in *A. gerardii* from 13 locations across the Great Plains. There were no discernable trends between PFTs and climate conditions across locations. The results from

Chapter 3 suggest that intraspecific trait variability (ITV) may serve as a significant mechanism that enables the presence and dominance of *A. gerardii* across the Great Plains.

Then, I evaluated the relationship between climate and ITV of leaf anatomy in *A. gerardii* (Chapter 4). Specifically, I analyzed leaf-level microanatomical traits and specific leaf area (SLA) in *A. gerardii* across four climatically distinct locations in the Great Plains. Here, I showed mean precipitation and mean growing season temperature were the best predictors of microanatomical and morphological trait variation. My data also indicate that microanatomical traits related to water-use and storage were significantly correlated with one another and these traits contained significantly higher variability (measured as coefficient of variation) than traits related to CO₂ carboxylation and assimilation.

Finally, I investigated the impact of climate and grazing on a dominant grass species across multiple locations in the Great Plains (Chapter 5). Here, I measured PFTs including, leaf – level physiology, microanatomy, and stoichiometry of *A. gerardii* in ungrazed and cattle grazed areas at three locations along a latitudinal gradient in the Great Plains during the 2018 and 2019 growing season. Most variation in PFTs were due to the severe drought in 2018 and ample precipitation of 2019, which was reflected in aboveground productivity. My data also indicate fluctuations in microanatomical trait *CV* with climate parameters such as early season precipitation and growing season temperatures, in both 2018 and 2019. In addition, results from Chapter 5 emphasize the importance of collecting data from multiple years, especially in regions that have inherently variable climates.

Taken as a whole, the results of this dissertation show that water availability plays a major role in determining leaf microanatomy, as such internal morphological structures constrain physiological responses. Additionally, there are significant differences in microanatomical

arrangements across Poaceae, likely reflecting water-use strategies in evolutionary histories (Brown, 1958; Griffith *et al.*, 2020). This in turn suggests that a single species, spread across precipitation gradients, will also have varying microanatomy and physiological responses to water availability (Bachle & Nippert, 2018, 2021; Bachle *et al.*, 2018). In addition, I showed that increasing intraspecific trait variability may buffer populations from the detrimental effects of the variable intercontinental climate that exists within the Great Plains. For example, climate variability in the Great Plains directly impacts water availability for plant species. As such, microanatomical traits related to water-use and storage (xylem characteristics and bulliform cells) displayed a larger *CV*, which allows individuals within the population to survive. These data also emphasize the need for increased microanatomical trait research for more plant species. Adding microanatomical trait information of dominant species in conjunction with previously collected genetic data to databases, will provide an increased mechanistic understanding of grass species form and function, and will ultimately increase our ability to predict future grassland responses at larger scales.

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Appendix A - Appendix for Chapter 2

Table A.1 Source and accession numbers for all species with each associated subfamily and tribe.

Species	Subfamily	Tribe	Source	Accessions Number
<i>Bouteloua dactyloides</i> (Nutt.)	Chloridoideae	Cynodonteae	USDA National Plant germplasm	W639025
<i>Bouteloua gracilis</i> (Nutt.)	Chloridoideae	Cynodonteae	USDA National Plant germplasm	PI664239
<i>Danthonia spicata</i> (P. Beauv)	Danthonioideae	Danthonieae	USDA National Plant germplasm	DAN02G
<i>Rytidosperma semiannulare</i> (Labill.)	Danthonioideae	Danthonieae	USDA National Plant germplasm	PI210172
<i>Festuca ovina</i> (L.)	Pooideae	Poeae	USDA National Plant germplasm	PI595178
<i>Paspalum notatum</i> (Fluegge)	Panicoideae	Paniceae	USDA National Plant germplasm	PI241878
<i>Paspalum juergensii</i> (Hack.)	Panicoideae	Paniceae	USDA National Plant germplasm	PI508779
<i>Setaria viridis</i> (Russian) (P. Beauv)	Panicoideae	Paniceae	USDA National Plant germplasm	PI677118
<i>Setaria viridis</i> (Iran) (P. Beauv)	Panicoideae	Paniceae	USDA National Plant germplasm	PI230135
<i>Urochloa ruziziensis</i> (Hochst. ex A. Rich)	Panicoideae	Paniceae	USDA National Plant germplasm	PI379628
<i>Andropogon gerardii</i> (Vitman)	Panicoideae	Andropogoneae	Konza Prairie Biological Station	NA
<i>Sorghastrum nutans</i> (Nash.)	Panicoideae	Andropogoneae	Konza Prairie Biological Station	NA
<i>Panicum virgatum</i> (L.)	Panicoideae	Paniceae	Konza Prairie Biological Station	NA

Table A.2 Mean physiological data and standard error for all species at each dry down stage.

Species	Condition	An	An_SE	Gs	Gs_SE	E	E_SE
A. gerardii	Initial	21.4876	2.9709	0.1442	0.0192	3.8823	0.5064
A. gerardii	Stress	1.1544	0.2991	0.0205	0.0064	0.6087	0.1677
A. gerardii	Recovery	14.1505	0.8987	0.1017	0.0108	2.7254	0.2932
S. nutans	Initial	12.7453	1.3992	0.0762	0.0078	2.3199	0.2234
S. nutans	Stress	1.8571	0.3630	0.0393	0.0079	1.0991	0.1898
S. nutans	Recovery	16.6318	3.1104	0.1213	0.0164	3.5126	0.5662
B. dactyloides	Initial	27.7674	2.5511	0.2065	0.0102	5.8000	0.2769
B. dactyloides	Stress	1.8887	0.6369	0.0320	0.0120	0.7512	0.3342
B. dactyloides	Recovery	10.7790	0.9724	0.1528	0.0293	3.3203	0.4608
B. gracilis	Initial	15.0928	1.5219	0.1114	0.0133	2.9918	0.3292
B. gracilis	Stress	4.5627	1.3154	0.0579	0.0076	1.3766	0.2143
B. gracilis	Recovery	16.7721	1.7055	0.2062	0.0469	4.5658	1.0242
D. spicata	Initial	9.0953	1.0991	0.1328	0.0175	3.7644	0.4818
D. spicata	Stress	-0.7963	0.1906	0.0505	0.0142	1.5285	0.4189
D. spicata	Recovery	10.9774	1.7805	0.1958	0.0137	4.9372	0.1638
R. semiannulare	Initial	13.7312	1.0176	0.2354	0.0273	5.2575	0.5528
R. semiannulare	Stress	0.7390	0.2769	0.0521	0.0041	1.4346	0.1470
R. semiannulare	Recovery	7.3743	0.8873	0.2400	0.0316	4.1835	0.5419
P. virgatum	Initial	12.9008	1.2572	0.0702	0.0068	1.6905	0.1549
P. virgatum	Stress	4.8485	1.0223	0.0358	0.0036	0.7718	0.0884
P. virgatum	Recovery	8.8600	1.7807	0.0507	0.0090	1.1285	0.1907
S. viridis	Initial	14.8821	0.9879	0.1065	0.0083	2.1900	0.1863
S. viridis	Stress	0.7542	0.2111	0.0096	0.0011	0.2234	0.0230
S. viridis	Recovery	8.4149	0.5410	0.0734	0.0060	1.4904	0.0958
U. ruzizensis	Initial	14.3511	1.7448	0.0958	0.0145	2.2602	0.3054
U. ruzizensis	Stress	2.7812	0.9463	0.0260	0.0089	0.5883	0.1774
U. ruzizensis	Recovery	10.4644	1.2718	0.0797	0.0107	1.8018	0.1600
P. juergensii	Initial	17.6451	1.9418	0.1089	0.0142	2.9993	0.3369
P. juergensii	Stress	0.3473	0.1867	0.0105	0.0013	0.3160	0.0391
P. juergensii	Recovery	13.7033	0.9570	0.0835	0.0058	2.7946	0.2025
P. Notatum	Initial	35.2663	1.0492	0.2059	0.0085	4.6979	0.1386
P. Notatum	Stress	9.2430	3.9080	0.0535	0.0211	1.5207	0.5926
P. Notatum	Recovery	29.5254	1.4845	0.1948	0.0110	6.2618	0.3313
F. ovina	Initial	9.5266	1.1006	0.1259	0.0169	3.1629	0.4077
F. ovina	Stress	-2.0556	0.2982	0.0277	0.0102	0.5730	0.2123
F. ovina	Recovery	2.5885	1.6819	0.0940	0.0146	1.9500	0.2960

Table A.3 Mean microanatomical data and standard error for all species. NA refers to species that have no data.

Species	X _{Area} (μm^2)	SE	X _{thick} (μm)	SE	X _{Diameter} (μm)	SE	t/b	SE	S _{count}	SE
<i>Andropogon gerardii</i>	536.19	121.51	4.06	0.14	27.71	3.86	0.16	0.027	11.5	2.63
<i>Sorghastrum nutans</i>	642.43	88.84	3.45	0.24	30.32	2.16	0.12	0.014	14.67	4.18
<i>Bouteloua dactyloides</i>	83.04	25.52	3.04	0.08	10.27	1.65	0.31	0.040	10.00	1.15
<i>Bouteloua gracilis</i>	134.87	30.16	3.02	0.27	13.46	1.30	0.23	0.015	7.00	1.73
<i>Danthonia spicata</i>	85.95	11.60	3.14	0.12	11.41	0.43	0.28	0.08	11.00	2.65
<i>Rytidosperma semiannulare</i>	268.95	86.71	4.29	0.66	17.91	2.85	0.24	0.018	10.33	1.20
<i>Panicum virgatum</i>	582.46	23.26	4.21	0.35	29.23	0.92	0.19	0.017	17.00	1.98
<i>Setaria viridis</i>	326.14	43.56	3.87	0.20	21.91	1.18	0.13	0.002	20.88	3.00
<i>Urochloa ruziziensis</i>	412.37	79.98	5.56	0.72	23.09	3.56	0.25	0.020	18.00	6.00
<i>Paspalum juergensii</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Paspalum notatum</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Festuca ovina</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table A.4 Mean leaf – based morphological data for each species with standard error (SE). NA refers to species that have no data.

Species	SLA (cm ² /g)	SE	LDMC	SE	TLA (cm ²)	SE	Leaf Biomass (g)	SE
<i>Andropogon gerardii</i>	74.76	4.63	3.16	0.18	110.68	17.39	1.25	0.15
<i>Sorghastrum nutans</i>	66.41	4.43	2.84	0.23	75.27	12.41	0.88	0.13
<i>Bouteloua dactyloides</i>	52.93	3.84	2.94	0.37	8.36	1.49	0.11	0.02
<i>Boueleuoa gracilis</i>	43.03	1.00	2.65	0.11	82.25	5.83	1.34	0.12
<i>Danthonia spicata</i>	56.71	6.79	2.88	0.13	28.92	6.21	0.30	0.05
<i>Rytidosperma semiannulare</i>	42.39	1.57	2.91	0.09	101.36	9.60	2.11	0.25
<i>Panicum virgatum</i>	29.27	5.52	3.05	0.11	16.46	2.43	0.49	0.06
<i>Setaria viridis</i>	99.01	7.00	4.16	0.12	157.59	12.85	1.67	0.13
<i>Urochloa ruziziensis</i>	143.84	9.36	4.51	0.10	98.41	8.62	0.72	0.10
<i>Paspalum juergensii</i>	NA	NA	NA	NA	NA	NA	7.17	0.37
<i>Paspalum notatum</i>	NA	NA	NA	NA	NA	NA	4.96	0.59
<i>Festuca ovina</i>	NA	NA	NA	NA	NA	NA	4.17	0.36

Table A.5 Mean root – based morphological data for each species with standard error (SE). NA refers to species that have no data.

Species	Root Biomass (g)	SE	Total length (cm)	SE	Fine length (cm)	SE	SRL (cm/g)	SE
<i>Andropogon gerardii</i>	0.68	0.13	3816.56	1144.82	3452.83	1112.49	61.92	16.08
<i>Sorghastrum nutans</i>	0.47	0.09	3586.17	499.49	3248.85	464.20	81.22	9.61
<i>Bouteloua dactyloides</i>	0.07	0.01	1552.05	328.03	1523.57	319.12	225.86	25.54
<i>Bouteloua gracilis</i>	0.51	0.07	10146.19	1468.50	9858.80	1445.45	204.57	14.62
<i>Danthonia spicata</i>	0.08	0.01	3210.78	689.14	3165.54	672.41	404.14	54.38
<i>Rytidosperma semiannulare</i>	0.77	0.08	8095.55	487.71	7831.55	462.76	109.15	8.30
<i>Panicum virgatum</i>	0.40	0.06	1630.28	106.14	1435.56	91.81	45.70	5.59
<i>Setaria viridis</i>	0.36	0.02	4555.73	291.66	4325.36	293.07	132.20	13.22
<i>Urochloa ruziziensis</i>	0.25	0.03	3046.49	498.24	2816.61	471.17	123.47	12.27
<i>Paspalum juergensii</i>	1.94	0.09	15080.03	510.20	12671.63	415.71	78.50	2.86
<i>Paspalum notatum</i>	0.92	0.08	5753.35	364.03	4479.36	329.44	64.16	4.50
<i>Festuca ovina</i>	1.16	0.07	17485.90	581.78	15001.59	474.95	94.78	3.58

Table A.6 Summary statistics and variance measurements from PCA axes for Figure 2.2.

	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.5808	1.0474	1.0067	0.54633	0.30358
Proportion of Variance	0.4998	0.2194	0.2027	0.54633	0.30358
Cumulative Proportion	0.4998	0.7192	0.9219	0.98157	1.000

Table A.7 PCA Loadings of the traits for the top three PCA axes from Figure 2.2.

	PC1	PC2	PC3
Xylem diameter ($X_{diameter}$)	-0.56643800	0.32543142	0.1666604
Photosynthetic rate (A_n)	-0.01661524	- 0.88058130	0.3695310
<i>iWUE</i>	- 0.54540790	- 0.19956644	- 0.1677293
<i>SLA</i>	- 0.28180947	- 0.27892932	- 0.8062078
<i>SRL</i>	0.54953214	- 0.03228998	- 0.3969472

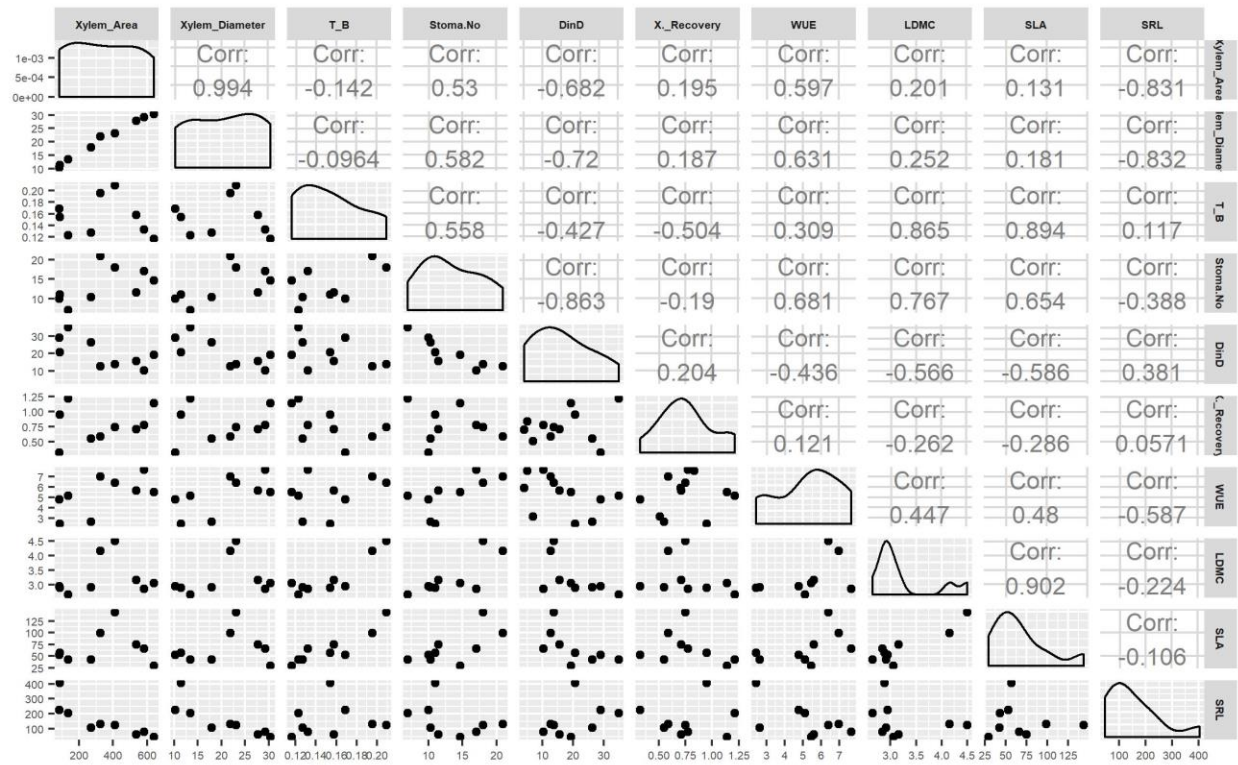


Figure A.1 Bivariate relationships between microanatomical, physiological, and economic traits alongside drought sensitivity (Days In Drought) and Recovery.

Appendix B - Appendix for Chapter 3

Table B.1 Averaged Values from literature search (after ln normalization)

SourceID	AGB	AGBgm2	BGB	BGBgm2	Cond	Photo	SLA_m2kg	WPALL
1	1.93297				-1.66073	2.944439	12	-0.69315
2							11.00625	
3					-2.20727	3.113515	5	
4								1.280934
5								-0.91629
6					-1.62985	2.943052		-0.23102
7						3.401197		
8	1.252763		1.386294					
9								0.81093
10		6.464996						
11								0.405465
12							18.58	
13					-3.34387	3.38439		
14					-1.83848	3.107304		
15					-2.57082	2.720899		
16	1.446919		1.252763		-2.81341	2.995732		0.405465
17					-2.24181	2.505318		0.405465
18						1.791759		
19								0.182322
20				5.298317				
21								-1.60944
22					-1.89712			
23		5.56452		6.35437				
24	0.91901				-1.12956	2.927738		-0.43644
25						2.99072		
26						3.218876		0.223144
27						3.391147	10.2	
28								2.014903
29					-1.89712	3.218876	22.5	0.530628
30					-1.89712	3.401197	60	
31		5.991465						
32							15.28326	
33							14.65018	
34							13.75	
35							15.3	
36							12.87	

Table B.2 Site ID and Source ID Tables for Figure 3.1

Site ID	OBS Site ID	State	Location	Site name	Latitude	Longitude	MAP (mm)	Min mean Temp (C)	MAT (C)	Max mean Temp (C)
1	1	KS	Konza	Konza	39.08333	-96.5833	863.5819	5.961111	12.40278	18.85833
2	2	NE	Mead	Mead	41.13333	-96.5	757.2575	3.852778	10.33333	16.79444
3	3	OK	OKSU	OKSU	36.06461	-97.2333	900.5664	9.338889	15.73889	22.13333
4	4	OH	Toledo	Toledo	41.65672	-83.615	885.345	5.186111	10.20556	15.23611
5	5	MN	Cedar Creek	Cedar Creek	45.41	-93.21	798.6817	0.816667	6.638889	12.44722
6	6	OK	El Reno	El Reno	35.66667	-98	820.6942	9.197222	15.68889	22.17222
7	7	IL	Jackson County	Burning Star	37.84167	-89.1667	1163.675	7.430556	13.33333	19.24167
7	8	IL	Jackson County	Freeman United Coal Mining	37.97222	-89.2928	1152.068	7.702778	13.44722	19.20556
7	9	IL	Jackson County	De Soto	37.85556	-89.2317	1152.276	7.538889	13.38889	19.24722
8	10	KS	Rockefeller Native Prairie	Rockefeller Native Prairie	39.04525	-95.2015	981.9706	6.716667	12.775	18.83056
9	11	NE	Derner Ranch	Derner Ranch	41.97308	-98.4495	651.0186	2.411111	9.230556	16.05556
7	12	IL	Jackson County	SIU	37.70906	-89.2249	1186.836	7.261111	13.275	19.275
10	13	KS	Hays	Hays	38.87918	-99.3268	581.7278	4.977778	12.28889	19.6
11	14	IA	ISU	ISU	42.02662	-93.6465	885.6306	3.288889	9.133333	14.97222
13	15	IA	BioGEN	BioGEN	42.06	-95.82	804.8603	3.511111	9.447222	15.36944
7	16	IL	Jackson County	Jackson County	37.61667	-89.2833	1193.52	7.7	13.575	19.43056
12	17	NE	Platte River Prairies	Platte River Prairies	40.74389	-98.59	679.2067	3.852778	10.39444	16.92778

Table B.3 Source ID description from literature search

<i>SourceID</i>	<i>Citation</i>
1	Allred, B., Fuhlendorf, S., Monaco, T., and Will, R. (2010). Morphological and physiological traits in the success of the invasive plant <i>Lespedeza cuneata</i> . <i>Biol. Invasions</i> 12, 739–749. doi:10.1007/s10530-009-9476-6.
2	Avolio, M. L., and Smith, M. D. (2013). Intra-specific responses of a dominant C 4 grass to altered precipitation patterns. <i>Plant Ecol.</i> 214, 1377–1389. doi:10.1007/sl.
3	Awada, T., Perry, M. E. L., and Schacht, W. H. (2003). Photosynthetic and growth responses of the C 3 <i>Bromus inermis</i> and the C 4 <i>Andropogon gerardii</i> to tree canopy cover. <i>Can. J. Plant Sci.</i> 83, 533–540.
4	Axmann, B. D., and Knapp, A. K. (1993). Water Relations of <i>Juniperus virginiana</i> and <i>Andropogon Gerardii</i> in an Unburned Tallgrass Prairie Watershed. <i>Southwest. Nat.</i> 38, 325–330.
5	Barnes, P. (1985). Adaptation to Water Stress in the Big Bluestem-Sand Bluestem Complex. <i>Ecology</i> 66, 1908–1920.
6	DeLucia, E., Heckathorn, S., and Day, T. (1992). Effects of soil temperature on growth , biomass allocation and resource acquisition of <i>Andropogon gerardii</i> Vitman. <i>New Phytol.</i> 120, 543–549.
7	Dietrich, J. D., and Smith, M. (2016). The effect of timing of growing season drought on flowering of <i>Andropogon gerardii</i> . <i>Oecologia</i> 181, 391–399. doi:10.1017/CBO9781107415324.004.
8	Gustafson, D. J., Gibson, D. J., and Nickrent, D. L. (2004). Competitive relationships of <i>Andropogon gerardii</i> (Big Bluestem) from remnant and restored native populations and select cultivated varieties. <i>Funct. Ecol.</i> 18, 451–457.
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Appendix C - Appendix for Chapter 4

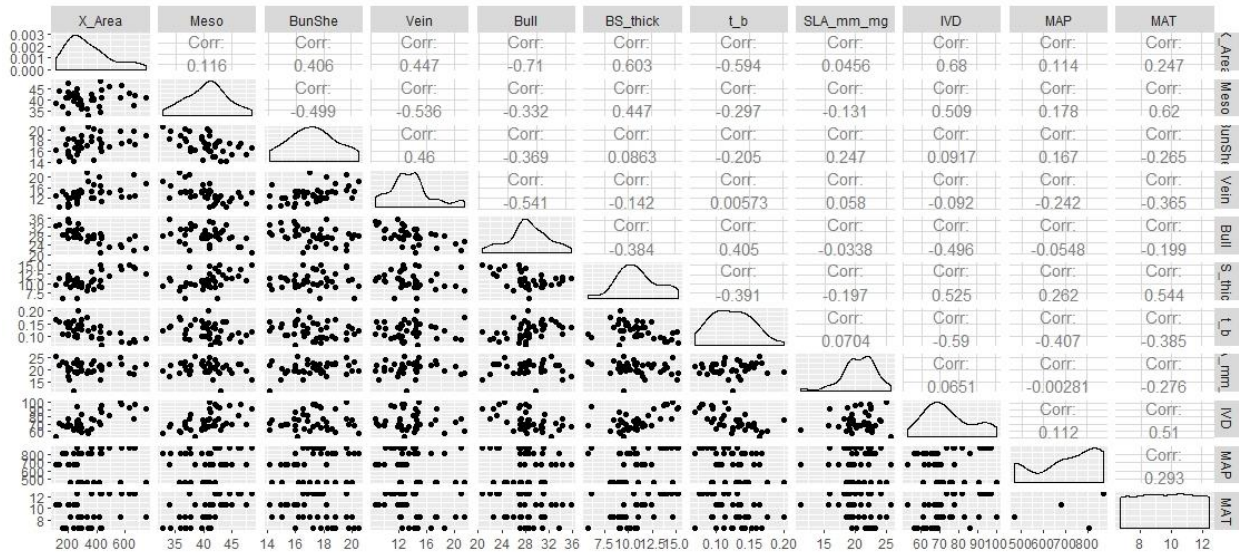


Figure C.1 Bivariate correlation matrix of leaf traits, mean annual precipitation and mean annual temperature at each site.

Table C.1 Mean anatomical and morphological trait data with standard error

TRAIT	KPBS	PRP	RMRS	CDR
<i>Mesophyll area</i> (μm^2) (%)	43.48 \pm 0.97	41.84 \pm 1.17	39.57 \pm 0.67	37.61 \pm 0.87
<i>Mesophyll area</i> (CV)	7.03	8.85	5.32	7.33
<i>Bundle sheath area</i> (μm^2) (%)	17.52 \pm 0.27	15.67 \pm 0.38	17.32 \pm 0.60	18.28 \pm 0.51
<i>Bundle sheath</i> (CV)	4.78	7.68	10.97	8.78
<i>Bundle sheath thickness</i> (μm)	12.86 \pm 0.87	11.02 \pm 0.30	10.19 \pm 0.65	9.54 \pm 0.47
<i>Bundle sheath thickness</i> (CV)	21.41	8.51	20.04	15.47
<i>Vein area</i> (μm^2) (%)	13.19 \pm 0.95	10.90 \pm 0.55	15.99 \pm 0.97	14.78 \pm 0.53
<i>Vein area</i> (CV)	22.69	15.91	19.22	11.27
<i>Bulliform area</i> (%)	25.80 \pm 1.05	31.59 \pm 0.91	27.11 \pm 0.87	29.33 \pm 0.63
<i>Bulliform area</i> (CV)	12.93	9.07	10.12	6.79
<i>Xylem area</i> (μm^2)	479.70 \pm 49.36	198.38 \pm 14.30	387.69 \pm 50.60	298.65 \pm 29.35
<i>Xylem area</i> (CV)	32.74	22.80	41.28	31.08
<i>Xylem wall thickness</i> (μm)	2.27 \pm 0.14	2.07 \pm 0.07	3.06 \pm 0.14	2.26 \pm 0.21
<i>Xylem wall thickness</i> (CV)	19.65	10.19	14.91	26.34
<i>Xylem lumen diameter</i> (μm)	23.75 \pm 1.73	16.31 \pm 0.68	23.00 \pm 1.64	20.10 \pm 1.31
<i>Xylem lumen diameter</i> (CV)	23.01	13.13	22.55	20.57
<i>Xylem reinforcement</i>	0.098 \pm 0.009	0.129 \pm 0.006	0.138 \pm 0.007	0.129 \pm 0.010
<i>Xylem reinforcement</i> (CV)	18.71	18.09	23.88	22.81
<i>Interveinal Distance</i> (μm)	87.87 \pm 2.960	66.99 \pm 2.058	77.36 \pm 3.628	65.51 \pm 2.611

<i>Interveinal Distance (CV)</i>	10.65	9.71	14.84	12.60
<i>SLA (cm²/g)</i>	20.33 ± 0.79	18.95 ± 1.20	21.11 ± .067	21.79 ± 0.37
<i>SLA (CV)</i>	12.28	20.04	9.98	5.43

Appendix D - Appendix for Chapter 5

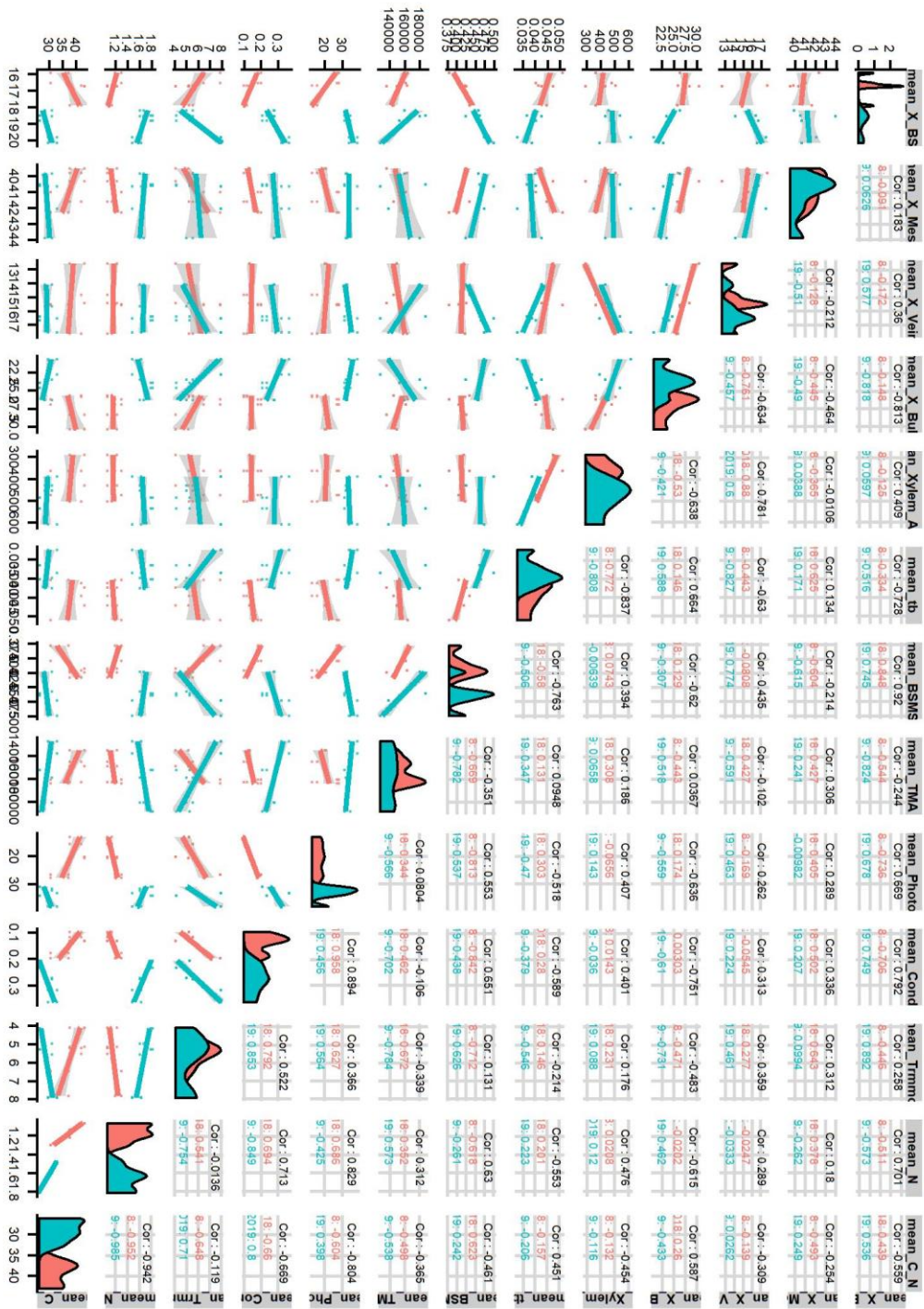


Figure D.1 Bivariate trait relationships between microanatomical, physiological, and stoichiometric traits delineated by year (red, 2018; blue 2019).

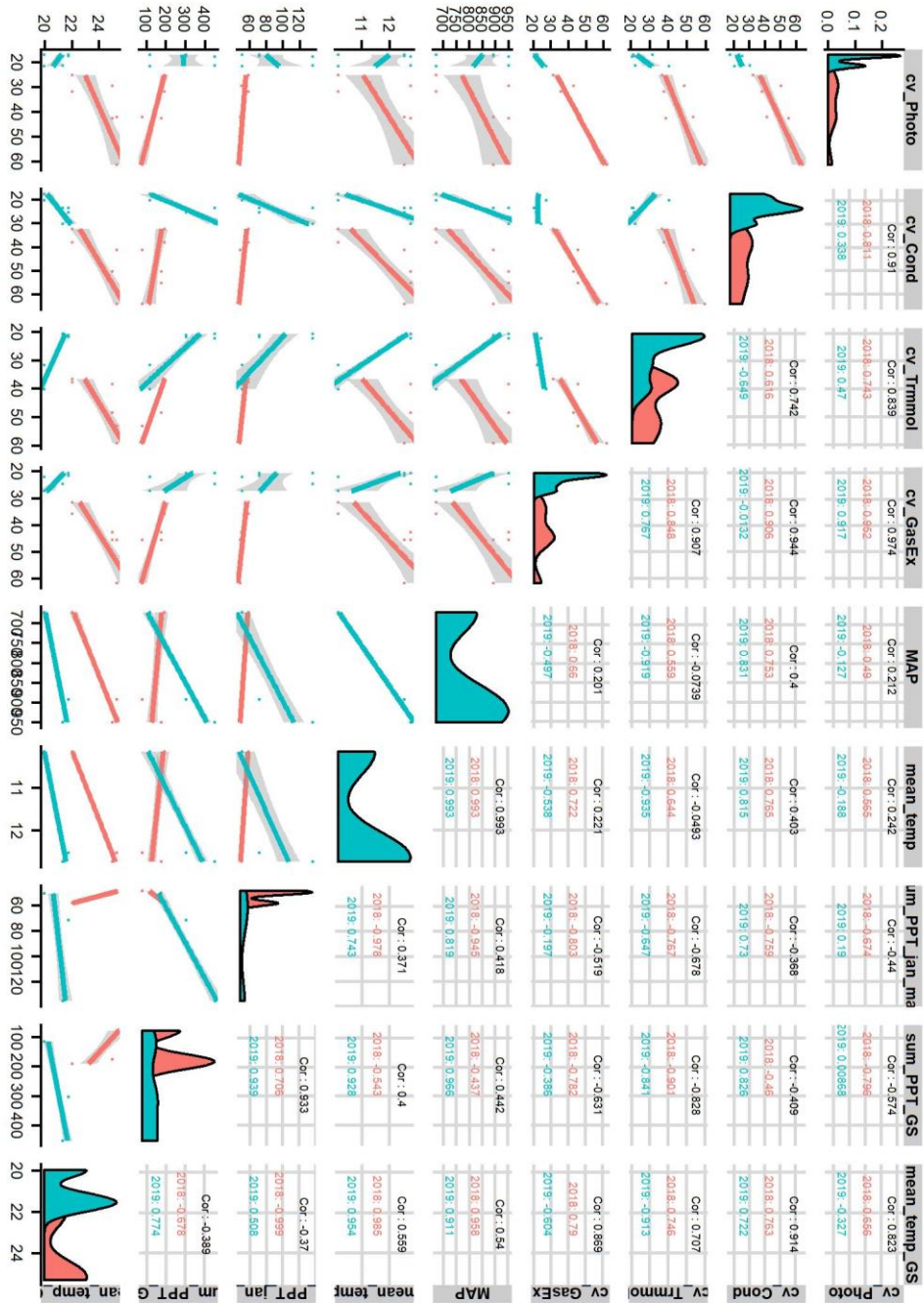


Figure D.2 Bivariate trait relationships between gas exchange and climate parameter data delineated by year (red, 2018; blue 2019).



Figure D.3 Bivariate trait relationships between microanatomical and climate parameter data delineated by year (red, 2018; blue 2019).

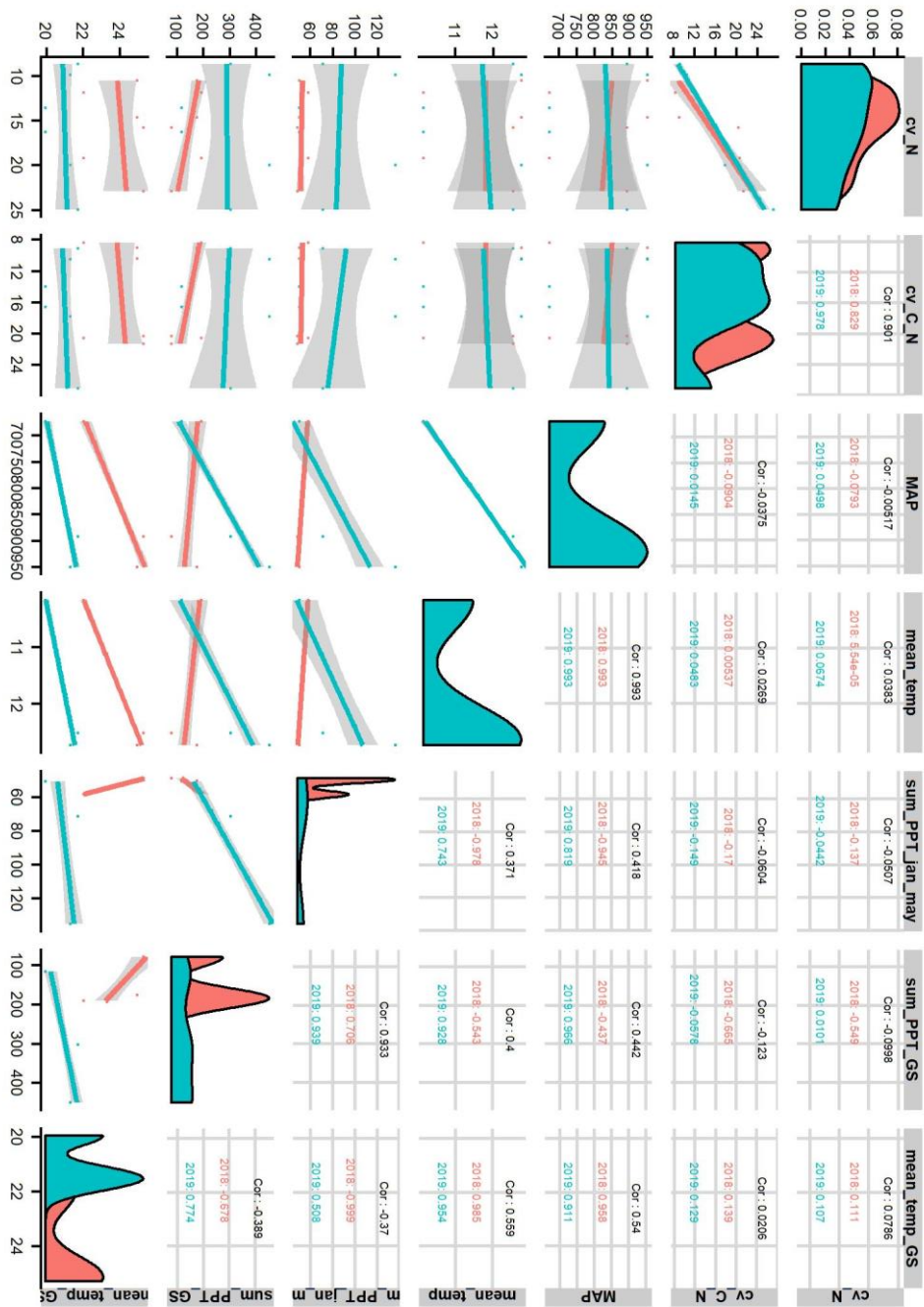


Figure D.4 Bivariate trait relationships between leaf – level stoichiometric and climate parameter data delineated by year (red, 2018; blue 2019).

Table D.1 Mean values and standard deviations for physiological, stoichiometric traits of *A. gerardii* and plot biomass for each location, year, and treatment.

Trait	Location	Grazed		Ungrazed	
		2018	2019	2018	2019
<i>A_n</i>	FHPP	20.77 ± 8.84	38.08 ± 8.11	20.15 ± 5.93	33.26 ± 5.87
	KPBS	13.06 ± 8.01	31.70 ± 5.39	15.65 ± 6.59	32.31 ± 5.62
	PRP	26.64 ± 8.45	34.08 ± 7.33	27.69 ± 6.98	30.98 ± 5.28
<i>g_s</i>	FHPP	0.13 ± 0.07	0.32 ± 0.10	0.13 ± 0.07	0.36 ± 0.09
	KPBS	0.10 ± 0.06	0.27 ± 0.06	0.12 ± 0.05	0.28 ± 0.07
	PRP	0.19 ± 0.08	0.23 ± 0.05	0.18 ± 0.06	0.21 ± 0.04
<i>E</i>	FHPP	5.15 ± 1.98	7.88 ± 1.75	4.09 ± 1.98	7.90 ± 1.63
	KPBS	5.21 ± 3.09	4.97 ± 1.05	5.53 ± 2.94	5.55 ± 1.21
	PRP	7.83 ± 2.95	4.14 ± 1.67	6.47 ± 2.37	5.27 ± 1.66
<i>N</i> (%)	FHPP	1.22 ± 0.13	1.62 ± 0.16	1.07 ± 0.16	1.48 ± 0.30
	KPBS	1.07 ± 0.24	1.67 ± 0.14	1.14 ± 0.18	1.60 ± 0.40
	PRP	1.30 ± 0.15	1.81 ± 0.30	1.19 ± 0.23	1.78 ± 0.24
<i>C:N</i>	FHPP	37.63 ± 3.91	29.61 ± 3.11	42.99 ± 3.88	32.81 ± 5.85
	KPBS	41.84 ± 8.92	28.29 ± 2.59	37.50 ± 7.61	30.89 ± 8.32
	PRP	30.51 ± 2.55	26.26 ± 4.35	37.00 ± 7.61	26.47 ± 3.69
<i>Biomass</i> (g m ⁻²)	FHPP	174.04 ± 31.47	218.50 ± 44.18	492.66 ± 50.59	452.92 ± 182.80
	KPBS	137.42 ± 43.39	255.34 ± 106.28	397.66 ± 135.71	599.92 ± 378.24
	PRP	487.86 ± 110.49	781.44 ± 226.54	1096.86 ± 317.67	878.94 ± 390.82

Table D.2 Mean values and standard deviations for microanatomical traits of *A. gerardii* and for each location, year, and treatment.

Trait	Location	Grazed		Ungrazed	
		2018	2019	2018	2019
TMA (μm^2)	FHPP	148781 \pm 32565	137626 \pm 37691	135235 \pm 37655	127206 \pm 24573
	KPBS	144733 \pm 42711	180123 \pm 51377	162726 \pm 38373	159063 \pm 49562
	PRP	160728 \pm 32409	187825 \pm 97734	163832 \pm 83120	152195 \pm 36615
BS_A (%)	FHPP	16.733 \pm 1.233	19.827 \pm 1.127	16.728 \pm 0.889	20.147 \pm 1.134
	KPBS	17.888 \pm 1.180	18.554 \pm 0.722	16.696 \pm 1.209	18.195 \pm 1.185
	PRP	16.538 \pm 0.927	18.494 \pm 1.417	15.908 \pm 0.964	18.816 \pm 0.054
MS_A (%)	FHPP	40.326 \pm 2.876	42.038 \pm 1.912	39.52 \pm 2.748	40.628 \pm 2.2856
	KPBS	41.589 \pm 3.003	43.883 \pm 1.763	39.827 \pm 2.754	39.867 \pm 2.108
	PRP	41.004 \pm 2.030	40.519 \pm 3.143	42.29 \pm 2.672	40.619 \pm 2.045
BS:MS	FHPP	0.41767 \pm 0.050	0.47226 \pm 0.030	0.42484 \pm 0.033	0.49897 \pm 0.054
	KPBS	0.43297 \pm 0.050	0.42369 \pm 0.028	0.42103 \pm 0.041	0.45728 \pm 0.034
	PRP	0.40477 \pm 0.037	0.46093 \pm 0.067	0.3783 \pm 0.041	0.46434 \pm 0.036
V_A (%)	FHPP	12.599 \pm 1.415	17.505 \pm 2.668	15.438 \pm 1.075	16.512 \pm 1.958
	KPBS	14.824 \pm 2.174	14.105 \pm 1.489	17.622 \pm 2.070	15.649 \pm 1.798
	PRP	15.529 \pm 2.370	16.383 \pm 1.945	15.561 \pm 1.681	16.644 \pm 2.030
B_A (%)	FHPP	30.342 \pm 3.453	20.631 \pm 3.530	28.315 \pm 3.339	22.712 \pm 3.488
	KPBS	25.700 \pm 3.256	23.458 \pm 1.822	25.855 \pm 3.841	26.289 \pm 3.192
	PRP	26.928 \pm 2.308	24.604 \pm 2.632	26.241 \pm 2.270	23.922 \pm 2.594
X_A (μm^2)	FHPP	289.40 \pm 65.291	609.88 \pm 190.12	430.21 \pm 136.891	390.17 \pm 111.797
	KPBS	362.10 \pm 113.592	431.25 \pm 167.453	503.36 \pm 149.089	453.59 \pm 153.114
	PRP	474.56 \pm 141.986	534.18 \pm 138.488	362.97 \pm 197.666	486.46 \pm 136.203
t/b	FHPP	0.047136 \pm 0.008	0.03254 \pm 0.004	0.042413 \pm 0.008	0.038567 \pm 0.005
	KPBS	0.04455 \pm .0010	0.04258 \pm 0.008	0.041157 \pm 0.007	0.039907 \pm 0.007
	PRP	0.040307 \pm 0.007	0.03656 \pm 0.005	0.050986 \pm 0.011	0.040236 \pm 0.006