

Physiological and morphological responses of grass species to drought

by

Seton Bachle

B.A., Hastings College, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Division of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Approved by:

Major Professor
Jesse Nippert

Copyright

© Seton Bachle 2017.

Abstract

The impacts of climate change over the next 100 years on North American grasslands are unknown. Climate change is projected to increase rainfall and seasonal temperature variability, leading to increased frequency of drought and decreased rainfall amounts for many grassland locations in the central Great Plains of North America. To increase our ability to predict the effects of a changing climate, I measured multiple morphological and physiological responses from a diverse suite of C₃ and C₄ grasses. Due to varying characteristics associated with the different photosynthetic pathways, these grass species respond differently to altered temperature and precipitation. I monitored grass physiology and microanatomy in conjunction with varying watered availability to replicate drought. In the second chapter, I observed leaf-level physiology and root level morphology of C₃ and C₄ grasses when exposed to 100% water reduction. Results indicated that response to water reduction are not always dependent on the photosynthetic pathway. Root-level morphological measurements were found to vary significantly between species in the same genus; *F. ovina* had the highest specific root length (SRL), which is an indicator of tolerance to environmental variability. Results also indicated that grasses of interest have thresholds that when passed result in a photosynthetically inactive plant; however it was shown that they are able to recover to near pre-drought gas exchange rates when water is re-applied. The third chapter investigated both leaf-level physiology and morphology in dominant C₄ grasses across Kansas' rainfall gradient over the growing season. I hypothesized that variation within a species' physiology would be greater than its' morphology. I also hypothesized that morphology would predict variability in a species physiological response to changes in climate. This research discovered within a location and species, leaf morphology is fixed across the growing season. Strong correlations between leaf physiology and morphology were observed,

however, the strength and relationship changed among the species compared. *A. gerardii* and *P. virgatum* exhibited opposing relationships when comparing their photosynthetic rates to the amount of bundle sheath cells. This result highlights strong species-specific relationship between physiology and morphology. My results illustrate the importance of utilizing plant physiology and morphology to understand how grasses may respond to future climate change scenarios.

Table of Contents

List of Figures	vii
List of Tables	ix
Acknowledgements	x
Dedication	xi
Chapter 1 - Introduction	1
Grassland Evolution	1
Climate Change	1
Physiology	3
Morphology	4
Current Research	4
References	6
Chapter 2 - Drought Effects on C ₃ and C ₄ Grasses	10
Introduction	10
Materials and Methods	12
Leaf-Level Physiology	13
Root Morphology	14
Statistical Analysis	15
Results	15
Leaf-Level Gas Exchange	15
Leaf-Level Chlorophyll Fluorescence	18
Root Morphology	20
Discussion	20
References	25
Tables and Figures	36
Chapter 3 - Physiological and Morphological Trait Plasticity	41
Introduction	41
Methods	44
Experimental Design	44
Trait measurements	45

Statistical Analysis	47
Results.....	47
Leaf-Level Gas Exchange.....	47
Chlorophyll Fluorescence	48
Leaf Morphology	49
Discussion.....	51
References.....	55
Tables and Figures	66
Chapter 4 - Conclusions.....	76
References.....	79
Appendix A - Additional Information	81

List of Figures

Figure 2.1 Average daily photosynthetic rates for study species. Left, <i>Paspalum</i> species with two-day recovery. Right, <i>Festuca</i> species with one-day recovery.	36
Figure 2.2 Average daily stomatal conductance rates for study species. Left, <i>Paspalum</i> species with two-day recovery. Right, <i>Festuca</i> species with one-day recovery.	37
Figure 2.3 Average daily transpiration rates for study species. Left, <i>Paspalum</i> species with two-day recovery. Right, <i>Festuca</i> species with one-day recovery.....	38
Figure 2.4 Maximum daily electron transport rate for study species. Left, <i>Paspalum</i> species with two-day recovery. Right, <i>Festuca</i> species with one-day recovery	39
Figure 2.5 Average light response curves for <i>Paspalum</i> . Left, <i>P. jeurgensii</i> ; Right, <i>P. notatum</i> species.	39
Figure 2.6 Root morphological measurements for <i>Festuca</i> and <i>Paspalum</i>	40
Figure 3.1 <i>Andropogon gerardii</i> leaf cross section from Konza Prairie, August of 2016 magnified 10X. Subsamples were taken between numbers 1 and 2; 3 and 4. Midrib was used as the reference point, X indicates a major vein that was unmeasured.....	66
Figure 3.2 Cross section of <i>Andropogon gerardii</i> from Konza Prairie, August of 2016 magnified 40X. Mesophyll cells (M), Bundle sheath cells (B), Vein area (V), Xylem area (X), yellow line (AVE D_m), blue line (AVE BS _{Thick}), black line (Stomata _{xylem}).....	67
Figure 3.3 Mean maximum photosynthetic rate A_n of each species at all sampling locations. BB: <i>A. gerardii</i> ; IG: <i>S. nutans</i> ; LB: <i>S. scoparium</i> ; SG: <i>P. virgatum</i>	68
Figure 3.4 Mean stomatal conductance rate g_s of each species at all sampling locations. BB: <i>A. gerardii</i> ; IG: <i>S. nutans</i> ; LB: <i>S. scoparium</i> ; SG: <i>P. virgatum</i>	68
Figure 3.5 Mean transpiration rate E of each species at all sampling locations. BB: <i>A. gerardii</i> ; IG: <i>S. nutans</i> ; LB: <i>S. scoparium</i> ; SG: <i>P. virgatum</i>	69
Figure 3.6 Light response curves at all sampling locations of June (A)(C) and August (B)(D). Photosynthetically available radiation (PAR). Values represent means, +/- 1 SE.	70
Figure 3.7 Light response curves at all sampling locations of June (A)(C) and August (B)(D). Photosynthetically available radiation (PAR). Values represent means, +/- 1 SE.	71
Figure 3.8 Mean xylem area for each species at all three research location in both June and August.	72

Figure 3.9 Correlation of bundle sheath area (expressed as a % of subsample) and maximum photosynthetic rate (A_n) for *A. gerardii*. 73

Figure 3.10 Correlation of bundle sheath area (expressed as a % of subsample) and maximum photosynthetic rate (A_n) for *P. virgatum*. 74

Figure 3.11 Correlation of bundle sheath thickness (μm) and maximum photosynthetic rate (A_n) for *A. gerardii*. 75

List of Tables

Table 2.1 ANOVA results for species, sample day, and interaction effects for photosynthetic rate. Numbers in bold show significance ($P < 0.05$).....	36
Table 2.3 ANOVA results for species, sample day, and interaction effects for stomatal conductance rate. Numbers in bold show significance ($P < 0.05$).....	37
Table 2.4 ANOVA results for species, sample day, and interaction effects for stomatal transpiration rate. Numbers in bold show significance ($P < 0.05$).....	38
Table A.1 Precipitation data (mm) of each research site. Percentage of annual rainfall received, and average soil moisture of location Acquired from local weather and airport stations.....	81
Table A.2 ANOVA results for species, location, month, and interaction effects for photosynthetic rate. Numbers in bold show significance ($P < 0.05$).....	81
Table A.3 ANOVA results for species, location, month, and interaction effects for stomatal conductance rate. Numbers in bold show significance ($P < 0.05$).....	82
Table A.4 ANOVA results for species, location, month, and interaction effects for transpiration rate. Numbers in bold show significance ($P < 0.05$).....	82
Table A.5 Mean values for each measured physiological and morphological trait of <i>A. gerardii</i> . Missing values indicate trait could not be measured for sample.	83
Table A.6 Mean values for each measured physiological and morphological trait of <i>S. nutans</i> ..	84
Table A.7 Mean values for each measured physiological and morphological trait of <i>S. scoparium</i> . Missing values indicate trait could not be measured for sample.	85
Table A.8 Mean values for each measured physiological and morphological trait of <i>P. virgatum</i>	86

Acknowledgements

I want to thank everyone who helped me develop into a better student and researcher. None of what I have accomplished would have been possible without the support I received from Jesse. I can't thank him enough for taking a chance on someone who had not taken a plant physiology course, yet listened to my thoughts on the field. He was always available to answer my questions and allow mistakes to grow from. I also want to thank Mark Ungerer and Carolyn Ferguson who served as committee members, they were always available to answer questions and give their insight into my research, and ask how my classes and writing were coming.

I want to thank current and past members of the Nippert lab. Rory O'Connor and Kim O'Keefe were incredibly helpful helping me settle in to graduate life. Rory was always willing to help set-up experiments in the field and answer questions with the Li6400. I could not have obtained data for the root morphology and leaf microanatomical measurements without Lindsey Swartz's hard work. My wife Kaitlin, has allowed me talk over research, and she sat through several presentations in their roughest forms. My family's support has always driven me and I'm incredibly thankful for them.

None of this would be possible without the resources from Kansas State University Division of Biology, Konza LTER, the use of prairies at University of Kansas and Fort Hays University, and the National Science Foundation for funding.

Dedication

To my wife Kaitlin,
Who loved me so much
She moved to Kansas

Chapter 1 - Introduction

Grassland Evolution

The Central Great Plains of North America is a large geographic area that extends from southern Canada to northern Mexico and contains a large precipitation range from 260 to 1200 mm (Axelrod 1985; Sala *et al.* 1988). The United States has over three million km² of grasslands representing nearly 12.5% of North America, most of which is in the Great Plains (Bailey 1998; Lauenroth, Burke & Gutmann 1999). Over the past several thousand years, this region has been molded by frequent burning, climate variability, and grazing (Borchert 1940; Anderson 2006). These drivers have led to dominance by C₄ grass species (Carpenter 1940; Smith & Knapp 1999; Sage 2004), while other species respond negatively or exhibit reduced responses compared the C₄ grasses.

Climate Change

The Central Great Plains are subject to climate change effects, which may reduce water availability in the region due to increased climate variability (Houghton *et al.* 2001; IPCC 2007). These changes are expected to cause a myriad of negative effects in agriculture and the native ecological communities that have not been observed since the 1930's dustbowl drought (Adler & Levine 2007; Romm 2011; Eters, Tarks & Hernandez 2014; Cook, Ault & Smerdon 2015). With the loss of native dominant species, altered rainfall patterns and increased air temperatures may increase the susceptibility of plant communities to plant invasion (Bellard *et al.* 2012). Although droughts are a common occurrence in the Great Plains region, an increase in the frequency and magnitude of drought has the potential to reduce productivity and limit maximal crop production in the United States (Lobell *et al.* 2014). Current model forecasts of future climate change impacts in this region include both higher frequency of droughts, as well as higher intensity

(longer days between rain events) of droughts (Cook *et al.* 2015; Hoover, Duniway & Belnap 2015). Different types of droughts can be categorized by “pulse-droughts” and “press-droughts”, which are differentiated by their magnitude (Hoover & Rogers 2016). According to Hoover & Rogers (2016), pulse-droughts are shorter in duration but characterized as more extreme in magnitude. Press-droughts, however, are higher in frequency but not as damaging to plant health (Hoover & Rogers 2016). Both types have been projected to increase in frequency due to climate change, and therefore warrant additional understanding.

Plant species have evolved various strategies attempting to overcome drought conditions (Chaves, Maroco & Pereira 2003). Plants have evolved to tolerate drought conditions through physiological and morphological adaptations. However, frequent drought conditions may cause increased plant mortality and water-stress, regardless of adaptations. As water-stress increases, hydraulic failure or cavitation can cause plant mortality and also carbon starvation due to stomatal closure for water conservation (McDowell *et al.* 2008). Therefore, the dominant prairie grasses are C₄ warm season perennials that can maintain leaf-level physiological processes during times of water stress. Without drought tolerant species in the grasslands, a major decline in productivity would follow droughts occurring in the region (Craine *et al.* 2012). These grass species utilize drought tolerant mechanisms to survive seasonal periods of low water availability, allowing some species to become increasingly dominant. Understanding the responses of these dominant grass species to drought is essential to answering how drought will alter the landscape of the Central Great Plains (Hoover, Knapp & Smith 2014a). In order to predict the responses of dominant grasses to drought, physiological and morphological plasticity over a range of precipitation inputs must be characterized.

Physiology

Drought manipulations have been conducted for many years with the use of rain-out shelters and greenhouse experiments that decrease the amount of water available (Fay *et al.* 2000). Field experimental rainfall manipulations have been a proven method of measuring physiological responses of particular species (Knapp *et al.* 2002a; Nippert *et al.* 2009; Hoover, Knapp & Smith 2014b). Most precipitation manipulations entail large decreases in water (~50% in many cases) (Knapp 1984; Fay *et al.* 2000). By utilizing natural precipitation gradients, one can assess genotypic variation and phenotypic responses to varying rainfall amounts. To fully understand changes in species physiology, a multitude of traits are required for measurement. Measurements of gases exchanged through stomatal pores have been a key measurement for many years to measure species response to microclimate because of ecophysiolgists' understanding of key leaf-level processes (Nippert *et al.* 2009). Nippert *et al.* (2009) utilized experimental increases in temperature and changes in precipitation to measure important physiological traits of grassland species at the leaf-level. In addition to experimental increases in temperature, chlorophyll fluorescence and water potential measurements have been utilized in previous research (Maxwell & Johnson 2000; Nippert *et al.* 2009). Chlorophyll fluorescence is a measurement of the efficiency of photosystems II; which is an indicator of how plants respond to stress (Murchie & Lawson 2013). Leaf water potential is measured to describe the tension that exists within the xylem. This tension gradient describes the difference in potential between the soil (high potential) and the atmosphere (low potential). If the tension gradient become too great, hydraulic failure or cavitation may occur (McDowell *et al.* 2008). Climate changes that alter precipitation may promote the frequency and intensity of dry soils, increasing negative effects of drought. Quantifying physiological traits that exist among co-existing plant species may provide

insight into susceptibility to drought, and the resilience of ecosystems under novel future climate scenarios.

Morphology

C₄ grassland species become dominant during the summer heat because of their unique leaf morphology termed ‘Kranz Anatomy’ (Edwards *et al.* 2001; Christin *et al.* 2013). This unique anatomical arrangement of photosynthetic cells contain distinct layers of bundle sheath and mesophyll cells which surround the vein tissues (Berry & Patel 2008). C₄ photosynthesis allows for separation of the chemical reactions of CO₂ carboxylation and assimilation, allowing sustained photosynthetic efficiency and increased water-use efficiency during high temperatures and decreased water availability (Edwards *et al.* 2001; Von Caemmerer & Furbank 2003; Berry & Patel 2008). Without separation of photosynthetic tissues, photorespiration occurs in C₃ species due to high temperatures and may lead to mortality (Ehleringer, Cerling & Helliker 1997). The size of mesophyll cells, bundle sheath cells, and xylem diameter are significant traits due to importance for water-use in grasslands and photosynthetic pathways that evolved 24-35 million years ago (Hatch 1987; Ehleringer *et al.* 1997; Sage 2004). It is advantageous to understand how C₄ grass morphology varies within a species over a growing season and across a regional climate gradient. Linkages between leaf morphology and leaf physiology may provide insight into species differences in water-use efficiency, patterns of growth, and potential responses to climate variability.

Current Research

Climate change is projected to increase climate variability, leading to drought conditions in North America’s grasslands. Future projections depict the region to have decreased water

availability and increased temperatures which will lead to plant stress, mortality, and eventual shifts in plant communities. An assessment of intra-specific and inter-specific physiology and morphological traits can aid in understanding responses to current and future climatic conditions. Species response to environmental conditions can be influenced by the photosynthetic pathway utilized. Physiological responses of C_3 and C_4 grasses to drought conditions can aid future physiological, morphological, and agricultural research between closely related species.

This thesis investigated how precipitation variability affected physiology and morphology of grassland species. In Chapter 2, I examine how high intensity drought conditions alter both C_3 and C_4 physiology in a greenhouse setting. In Chapter 3 I investigate the plasticity of physiological and morphological traits in dominant C_4 grasses over a growing season within the precipitation gradient of Kansas.

References

- Adler, P.B. & Levine, J.M. (2007) Contrasting relationships between precipitation and species richness in space and time. *Oikos*, **116**, 221–232.
- Anderson, R.C. (2006) Evolution and origin of the Central Grassland of North America : climate, fire, and mammalian grazers. *Journal of the Torrey Botanical Society*, **133**, 626–647.
- Axelrod, D. (1985) Rise of the Grassland Biome , Central North America. *Botanical Review*, **51**, 163–201.
- Bailey, R.G. (1998) *Ecoregions*, 2nd ed. Springer Berlin Heidelberg, London.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of Climate Change on the Future of Biodiversity. *Ecol Letters.*, **12**, 365–377.
- Berry, J.O. & Patel, M. (2008) Kranz Anatomy and the C4 Pathway. *Encyclopedia of life sciences (ELS)*, 1–6.
- Borchert, J. (1940) The Climate of the Central North. *Annals of the Association of American Geographers*, **40**, 1–39.
- Von Caemmerer, S. & Furbank, R. (2003) The C4 pathway: An efficient CO2 pump. *Photosynthesis Research*, **77**, 191–207.
- Carpenter, R. (1940) The Grassland Biome. *Ecological Monographs*, **10**, 617–684.
- Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought - From genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.
- Christin, P.A., Osborne, C.P., Chatelet, D.S., Columbus, J.T., Besnard, G., Hodkinson, T.R., Garrison, L.M., Vorontsova, M.S. & Edwards, E.J. (2013) Anatomical enablers and the evolution of C4 photosynthesis in grasses. *Proceedings of the National Academy of*

- Sciences of the United States of America*, **110**, 1381–6.
- Cook, B.I., Ault, T.R. & Smerdon, J.E. (2015) Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances*, **1**, e1400082–e1400082.
- Craine, J.M., Ocheltree, T.W., Nippert, J.B., Towne, E.G., Skibbe, A.M., Kembel, S.W. & Fargione, J.E. (2012) Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*, **2**, 1–5.
- Edwards, G.E., Franceschi, V.R., Ku, M.S., Voznesenskaya, E. V, Pyankov, V.I. & Andreo, C.S. (2001) Compartmentation of Photosynthesis in Cells and Tissues of C4 Plants. *Journal of experimental botany*, **52**, 577–590.
- Ehleringer, J., Cerling, T. & Helliker, B. (1997) C4 Photosynthesis, Atmospheric CO₂, and Climate. *Oecologia*, **112**, 285–299.
- Eters, D., Tarks, P. & Hernandez, M. (2014) Functional response of U.S. grasslands to the early 21st-century drought. *Ecology*, **95**, 2121–2133.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M. & Collins, S.L. (2000) Altering rainfall timing and quantity in a mesic grassland ecosystem: Design and performance of rainfall manipulation shelters. *Ecosystems*, **3**, 308–319.
- Hatch, M. (1987) C4 Photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochimica et Biophysica Acta*, **895**, 81–106.
- Hoover, D., Duniway, M. & Belnap, J. (2015) Pulse-drought atop press-drought: unexpected plant responses and implications for dryland ecosystems. *Oecologia*, **179**, 1211–1221.
- Hoover, D., Knapp, A.K. & Smith, M.D. (2014a) Contrasting sensitivities of two dominant C4 grasses to heat waves and drought. *Plant Ecology*, **215**, 721–731.
- Hoover, D., Knapp, A. & Smith, M. (2014b) Resistance and resilience of a grassland ecosystem

- to climate extremes. *Ecology*, **95**, 2646–2656.
- Hoover, D.L. & Rogers, B.M. (2016) Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology*, **22**, 1809–1820.
- Houghton, J., Y, D., DJ, G., M, N., PJ, van der L., X, D., K, M. & C, J. (2001) Climate Change 2001: The Scientific Basis. *Climate Change 2001: The Scientific Basis*, 881.
- IPCC. (2007) *Climate Change 2007 Synthesis Report*.
- Knapp, A.K. (1984) Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia*, **65**, 35–43.
- Knapp, A., Fay, P., Blair, J., Collins, S., Smith, M., Carlisle, J., Harper, C., Danner, B., Lett, M. & McCarron, J. (2002) Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland. *Science*, **298**, 2202–2205.
- Lauenroth, W.K., Burke, I.C. & Gutmann, M.P. (1999) The Structure and Function of Ecosystems in the Central North American Grassland Region. *Great Plains Research*, **9**, 223–259.
- Lobell, D.B., Roberts, M.J., Schlenker, W., Braun, N., Little, B.B., Rejesus, R.M. & Hammer, G.L. (2014) Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science (New York, N.Y.)*, **344**, 516–9.
- Maxwell, K. & Johnson, G.N. (2000) Chlorophyll fluorescence--a practical guide. *Journal of experimental botany*, **51**, 659–668.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yezpez, E. a. (2008) Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to

- drought? *New Phytologist*, **178**, 719–739.
- Murchie, E.H. & Lawson, T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany*, **64**, 3983–98.
- Nippert, J.B., Fay, P.A., Carlisle, J.D., Knapp, A.K. & Smith, M.D. (2009) Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica*, **35**, 400–408.
- Romm, J. (2011) Desertification: The next dust bowl. *Nature*, **478**, 450–451.
- Sage, R.F. (2004) The evolution of C₄ photosynthesis. *New Phytologist*, **161**, 341–370.
- Sala, A.O.E., Parton, W.J., Joyce, L. a & Lauenroth, W.K. (1988) Primary Production of the Central Grassland Region of the United States. *Ecological Monographs*, **69**, 40–45.
- Smith, M.D. & Knapp, A.K. (1999) Exotic plant species in a C₄ dominated grassland : invasibility , disturbance , and community structure. *Oecologia*, **120**, 605–612.
- Strömberg, C. a. E. (2011) Evolution of Grasses and Grassland Ecosystems. *Annual Review of Earth and Planetary Sciences*, **39**, 517–544.

Chapter 2 - Drought Effects on C₃ and C₄ Grasses

Introduction

Grasslands evolved multiple times on several continents during the Paleogene (Edwards, Smith & Thresholds 2010). Since the late Cretaceous period, grasslands have expanded and have become a dominant biome on Earth (Strömberg 2011). Grass species constitute the primary component of grassland ecosystems, which cover roughly 25% of the world's land surface (Ramankutty & Foley 1999). Grasslands also globally influence climate and carbon cycles (Sage 2004; Kidder 2005; Strömberg 2011). Despite considerable species diversity and the contribution to ecological complexity within grassland ecosystems, a few grasses species have disproportional influence on human diet (Tilman *et al.* 2002), ecological processes (Knapp *et al.* 2002a), and grazer proliferation (Forrestel, Donoghue & Smith 2015). Expected shifts in climate over the next 50 years are likely to impact energy, nutrient, and hydrological cycling in grassland ecosystems. For this reason, further research that explores unique traits among phylogenetically conserved grass species is warranted (Grass Phylogeny Working II 2012).

Nearly 50 years have passed since the discovery of C₄ photosynthesis, and from that time scientists have described the unique biochemistry of this pathway (Kellogg 1999; Sage 2004; Edwards *et al.* 2010), the physiological and morphological variability among C₄ grass species (Grass Phylogeny Working II 2012; Christin & Osborne 2014), and the ecological significance of C₄ photosynthesis on grassland ecosystem function (Epstein *et al.* 1998).

Grasses that utilize the C₄ photosynthetic pathway represent almost 2% of all 250,000 terrestrial plant species, rather than the more common and evolutionarily older C₃ photosynthetic mechanism (Sage 2004). Terrestrial plants have evolved the C₄ photosynthetic pathway an estimated 45 times accounting for over 7,500 grass species (Still *et al.* 2003; Sage 2004;

Edwards & Still 2008). The C₄ pathway differs from the C₃ pathway because of morphological and physiological differences that lead to the concentration of CO₂ around the enzyme Rubisco in the mesophyll cells (Brown 1975; Hatch 1987; Dengler *et al.* 1994; Edwards *et al.* 2001). The C₄ pathway allows for increased photosynthetic rates, enhanced water-use efficiency, and the elimination of photorespiration (Knapp 1993, 2013; Taylor *et al.* 2011). Eliminating photorespiration is a unique advantage, and phylogenetic data suggest that the C₄ pathway developed independently multiple times coinciding with increased warming between 20 and 30 million years ago (Kellogg 1999; Strömberg 2005; Edwards & Still 2008). Increased understanding of the phylogenetic evolution of the C₄ pathway has led to its consideration for ecosystem ecology and global change research, and its consideration for ecosystem ecology and global-change research (Edwards, Still & Donoghue 2007). Edwards *et al.* (2007) states that understanding an organism's evolutionary past will allow researchers to understand how traits have been spread across species and biomes. However, it is important to note that trait relationships vary between species, and these differences are highlighted by different evolutionary origins (Edwards *et al.* 2007).

Variation of physiological traits are also evident in congeners (species in same genera) of C₃ and C₄ grass species. For this reason, controlling for phylogeny is critical for the examination and identification of traits with functional value (Edwards *et al.* 2007; Edwards & Still 2008; Taylor *et al.* 2011). Leaf-level physiology is highly differentiated between species of grasses and typically varies according to local environmental drivers (Carmo-Silva *et al.* 2009). A similar level of understanding on the degree of variation between similar species is poorly understood. Leaf-level physiological measurements include gas exchange rates such as photosynthesis (measurement of CO₂ exchanged from leaf to atmosphere), stomatal conductance (rate of

stomatal opening to allow gas exchange), transpiration (measurement of H₂O lost from the plant to the atmosphere), and chlorophyll fluorescence (measurement of electron's loss of excitement or energy within a chlorophyll) measurements. Photosynthesis and transpiration rates differ between and within photosynthetic pathways due to inherent species characteristics and also in response to changing environmental factors such as water availability and temperature (Fay *et al.* 2002; Knapp *et al.* 2002a; Nippert *et al.* 2009). Due to the photosynthetic constraints of C₃ species to warming temperatures and C₄ species ability to maintain photosynthetic efficiency under increased temperatures and decreased water availability (Berry & Patel 2008), future climate predictions will cause unknown plant responses (Hoover *et al.* 2014a, 2015). However, a higher water-use efficiency (maximum photosynthetic rate/transpiration rate) is an inherent characteristic of C₄ species which should ultimately increase fitness during drought conditions.

The objective of this research was to determine if key leaf-level physiological traits vary in response to experimental drought simulation in a pair of C₃ and C₄ grasses, and if the results correspond to long standing notions of these pathways to decreased water availability. I hypothesize: (1) C₄ species would remain at an optimal physiological state for a longer duration during dry-down compared to the C₃ species; and (2) C₄ species would show less gas exchange variability in response to simulated drought, while C₃ species would exhibit larger fluctuations.

Materials and Methods

Two species of the genus *Festuca* were used: *F. ovina* and *F. pseudovina*. Both *Festuca* species are cool-season C₃ bunch grasses native to South Eastern Europe (Alderson & Sharp 1994), and are adapted to growing in dry locations. Ten samples of each *Festuca* species were germinated 23 September 2015 and monitored between 10 November to 21 November 2015.

When photosynthetic measurements read $0 \mu\text{mol m}^{-2} \text{s}^{-1}$, water was applied to the sample individual; and gas exchange and fluorescence was measured the following day to monitor a simulated drought recovery.

Two species of the genus *Paspalum* were used: *P. notatum* and *P. jeurgensii*. The *Paspalum* species are warm-season C_4 grasses native to Brazil (Alderson & Sharp 1994), and can be commonly found in the southern United States. Eight samples of *Paspalum jeurgensii* and seven samples of *Paspalum notatum* were germinated January 14th and monitored in complete drought conditions during 28-March to 3-April-2016. Samples were grown from seeds (purchased from the USDA Germplasm Resources Information Network). When a sample measurement indicated a plant was photosynthetically inactive, water was applied; gas exchange and fluorescence were measured for the following two days to monitor a recovery.

This dry-down experiment used four grass species. All species were subjected to 100% water reduction while physiological traits were measured daily. Grasses were watered daily and arranged randomly in blocks containing one of each species. Samples were grown in 868.5cm² 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.

Leaf-Level Physiology

All physiological measurements were collected between 11:00 and 14:00 CDT, including gas exchange, chlorophyll fluorescence, and pot weight. For each individual plant, leaves were marked for repeated sampling to ensure consistent measurements throughout the simulated drought. Four to five *Festuca* leaves were grouped laterally in order to increase surface area for gas exchange and fluorescence; single *Paspalum* leaves were used because their larger leaf

widths were of sufficient size for gas exchange and fluorescence measurements. Stomatal conductance and maximum photosynthetic rate were measured with a LI-6400 system (LiCOR, Inc., Lincoln, NE, USA) equipped with an LED light source (light intensity was maintained at $2,000 \mu\text{mol m}^{-2}\text{s}^{-1}$, CO_2 concentration at $400 \mu\text{mol mol}^{-1}$, and relative humidity at ambient levels). Measurements from the Li-6400 were logged when maximum photosynthesis was stable for 1 minute to ensure accurate measurements. Chlorophyll fluorescence was measured with the use of a miniaturized pulse-amplitude-modulated photosynthesis yield analyzer (Mini-PAM) (OS1-FL, Opti-sciences, Inc., Tyngsboro, MA). Measurements were used to create light response curves (electron transport rate against light intensity) with the actinic intensity set at two to accommodate a better range for the species and reduce the risk of overheating. To observe general water loss by samples, overall pot weight was measured using a Pioneer balance (Ohaus, Inc., Pine Brook, NJ, USA).

Root Morphology

Following the recovery day, samples were harvested and separated into above and below-ground biomass. Roots were washed thoroughly in order to be scanned while suspended in water by a digital root imaging program (WinRhizo; Regent Instruments, Inc., Nepean, Ontario, Canada). WinRhizo calculates total root length and total root diameter by length. Root diameters were binned into ten size classes that began with $< 0.5\text{mm}$, $0.5\text{-}1.0 \text{ mm}$, and increased by 0.5 mm up to 4.5 mm and greater. After the scanning process roots and shoots were dried for 48 hours at 60°C and then weighed to determine aboveground and belowground biomass. Dried roots were used to determine specific root length, which is calculated as total root length (cm) divided by total belowground biomass (g).

Statistical Analysis

Eight traits were chosen as the key traits of interest. Leaf-level physiological traits included: maximum photosynthesis, stomatal conductance to vapor, and chlorophyll fluorescence. Morphological traits included: aboveground biomass, belowground biomass, total root length, root diameter, and specific root length. Traits were analyzed by analysis of variance (ANOVA) in Sigmaplot (Systat Inc., San Jose, CA, USA) and R 2.14.2 (R Core Team).

Results

Leaf-Level Gas Exchange

The dry-down manipulation impacted leaf-level physiological processes of all four species, but the susceptibility to dry conditions and the associated response varied among the C₃ and C₄ species. The photosynthetic rates between *Paspalum* species were significantly different ($P < 0.05$); optimal A_n in *P. notatum* was 50% higher than optimal rates for *P. jeurgensii* (Figure 2.1) (Table 2.1). *P. jeurgensii* continued photosynthesis at optimal level for three days until rates dropped by 98% prior to the water application and recovery (Figure 2.1). Recovery was used to describe the observed rate returning to pre-drought levels after water was re-applied. The photosynthetic rate in *P. notatum* remained maximal for three days but fell by 63% after five days (Figure 2.1). While both species expressed an abrupt negative response to drought conditions, the response for *P. notatum* was more gradual because of the additional day observed in its decrease. Recovery of both species was abrupt after one day, and increased slightly the second day (Figure 2.1). The photosynthetic rate in *P. jeurgensii* increased by 99% in of the previous day's measurement after re-watering, but was 32% lower than its optimal pre-drought

rate. The photosynthetic rate of *P. notatum* increased by 56% after re-watering, but was still 27% below the maximal rate of photosynthesis.

Festuca had lower photosynthetic rates compared to both of the *Paspalum* species in this study. Both species responded with a gradual decrease in A_n , however, both species eventually experienced more than 100% decrease in A_n . A large difference between species can be seen in A_n of *F. ovina*, which remained positive for a longer period (day 7) compared to *F. pseudovina* (day 5) (Figure 2.1). Another major difference between both *Festuca* species is the recovery observed after re-watering. *F. pseudovina* exhibited a 72% recovery, or a 28% decrease from its optimal A_n at the start of the dry-down. *F. ovina* recovered by 27%, or decreased by 73% from its optimal A_n on day 1; *F. ovina* experienced a recovery more than half of *F. pseudovina* species (Figure 2.1).

Stomatal conductance (g_s) between *Paspalum* species was significantly different ($P < 0.05$) (Table 2.2); optimal g_s of *P. notatum* was nearly 50% higher than optimal rates for *P. jeurgensii* (Figure 2.2). *P. jeurgensii* continued its optimal conductance rate for three days before sharply decreasing by 90% before re-watering and recovery (Figure 2.2). Figure 2.2 also displays *P. notatum* exhibited an increased conductance rate for three days before declining sharply by 64% before re-watering. Both species experienced a rapid decrease in their conductance rates due to the dry-down; however, *P. notatum* decreased its average rate more sharply compared to *P. jeurgensii* (Figure 2.2). Recovery of both *Paspalum* species was immediate after one day, but only slightly increased the second day (Figure 2.2). After re-watering, g_s of *P. jeurgensii* was 66% of its original rate, but increased to 77% of the optimal rate on day 6 (Figure 2.2). g_s for *P. notatum* following re-watering was 64% of the maximum rate; but, day 6 was 93% of maximum pre-drought stomatal conductance or a 62% increase from day 5 (Figure 2.2).

Both *Festuca* species had stomatal conductance rates was to that of *Paspalum jeurgensii*, but did not reach a maximum rate close to *P. notatum* (Figure 2.2). A major difference between *Festuca* species was the varying response to the dry-down experiment. *F. ovina* experienced a small increase in g_s until day 4, from there g_s decreased gradually reaching 77% of optimal g_s before re-watering (day 7). This gradual decrease was compared to *F. pseudovina* abrupt fall in g_s which resulted in a 100% decrease before re-watering (day 5) (Figure 2.2). Little difference was seen between *Festuca* species as both species exhibited a similarly swift recovery response (Figure 2.2). *F. pseudovina* exhibited an 80% recovery, or 20% decrease from its maximal g_s . *F. ovina* increased g_s by 70% after re-watering (75% recovery), but was still 25% below its optimal g_s at the start of the dry-down (Figure 2.2). The transpiration rates between *Paspalum* species was significantly different ($P < 0.05$) (Table 2.3); pre-drought E for *P. notatum* was more than 1.5 times that of *P. jeurgensii* (Figure 2.3). *P. notatum* had a maximum transpiration rate more than two times that of *P. jeurgensii* (Figure 2.3). Transpiration rate of *P. jeurgensii* experienced subtle step-wise decrease until a sharp decrease on day 4 that was 90% of its optimal E (Figure 2.3). Whereas, the transpiration rate for *P. notatum* increased on day three where it decreased sharply to 58% of its original optimal E (Figure 2.3). Although there was an increase of transpiration rate observed in both species during recovery, *P. notatum* displayed a rapid recovery of 68% after re-watering (Figure 2.3). At the conclusion of the dry-down, *P. notatum* increased its transpiration rate by 24% from its original pre-drought level while *P. jeurgensii* recovered by 85%, and was 93% of its optimal level by day 6 (Figure 2.3).

Both *Festuca* species had transpiration rates that decreased over the span of the dry-down (Figure 2.3). One of the major differences between the two species was the nature of the E decline. An increased transpiration rate was observed in *F. ovina* before decreasing gradually to

18% of its optimal E (82% decrease) over seven days (Figure 2.3). The transpiration rate of *F. pseudovina* responded differently; after an increased E there was a sharp decrease on the fifth day that was a 100% decline from its maximal pre-drought rate. Recovery of both *Festuca* species was observable, however, *F. pseudovina* responded to re-watering by regaining 84% of its original E . Recovery in *F. ovina* was not as abrupt compared to *F. pseudovina*, but it did regain 62% of its original transpiration rate (Figure 2.3).

Leaf-Level Chlorophyll Fluorescence

Dry-down conditions negatively impacted chlorophyll fluorescence of both *Paspalum* and *Festuca* in the study, however, the vulnerability to dry conditions differed between and among the C_3 and C_4 species (Figure 2.4).

The maximum electron transport rate between *Paspalum* species was significantly different ($P < 0.05$). Though *P. notatum* was consistently higher compared to *P. jeurgensii*, both species had a similar abrupt decrease in ETR_{max} (Figure 2.4). *P. notatum* also displayed an increase of ETR_{max} before decreasing by 42% of its optimal electron transport rate (Figure 2.4). Figure 4A also portrays that *P. jeurgensii* experienced a gradual decrease before falling by 79% from the highest ETR_{max} . A large difference between the *Paspalum* species can be noted in the recovery of ETR_{max} . *P. jeurgensii* electron transport rate recovered by 72% one day after re-watering, and regained further to be 16% short of its pre-drought ETR_{max} . *P. notatum* increased its maximum electron transport rate increased by 33% after one day of re-watering, then, ETR_{max} increased 7% beyond its original optimal rate (Figure 2.4).

The maximum electron transport rate did not reach the same level in *Festuca* as it did in *P. notatum*, but did experience contrasting responses within species to the dry-down (Figure 2.4).

F. pseudovina decreased gradually for the first four days, and then decreased sharply to 38% of the pre-drought ETR_{max} (Figure 2.4). The maximum electron transport rate for *F. ovina* increased one day, and then decreased by 22% gradually before water was re-applied on day seven. Another difference between the two species can be observed in their recovery. *F. pseudovina* recovered by 57% after re-watering, and was measured to be 73% of its optimal ETR_{max} (Figure 2.4). Though *F. ovina* recovered 15%, which was not a large percentage, the ETR_{max} at this level is 90% of the pre-drought maximum electron transport rate (Figure 2.4).

Chlorophyll light-curve results portray differences between the *Paspalum* species during multiple days in the dry-down (Figure 2.5). Days of the dry-down are partitioned in different panels of Figure 5. Both *Paspalum* species experienced a similar drop in electron transport during the ongoing drought, they also had similar recoveries based on pre-drought ETR_{LC} (Figure 2.5). There are observable differences between measurement days. Figure 2.5 depicts *P. jeurgensii* raising its electron transport rate as light intensity is increasing, totaling a 9% increase from its initial point. *P. jeurgensii* decreased its electron transport rate as the dry-down continues; the largest ETR decrease occurred on the fourth day of the experiment (Figure 2.5). ETR of *P. jeurgensii* dropped by 83% from its initial pre-drought rate; recovery is depicted to bring ETR levels near its original transport rate of electrons (78% of original rate) (Figure 2.5). Figure 2.5 portrays Electron transport rates were comparable between *P. notatum* to *P. jeurgensii*, ETR level fell by 75% from initial measurements of *P. notatum* (day 5), but showed a steep recovery of 83% of optimal electron transport rate (Figure 2.5). Recoveries by both *Paspalum* species are seen to closely mirror the original light-response curves (Figure 2.5).

Root Morphology

Specific root length (SRL) differed between the two *Paspalum* species, but not significantly ($P < 0.05$) (Figure 2.6F). *P. jeurgensii* had a slightly higher SRL compared to *P. notatum*, even though the total length for *P. jeurgensii* was much higher than *P. notatum* (Figure 2.6F). *Festuca* specific root length varied drastically between species, and was statistically significant ($P < 0.05$) (Figure 2.6F). Of all species that experienced dry-down conditions, *F. ovina* had the highest SRL and *P. notatum* had the lowest. All species had similar proportions within the smallest root diameter class (<.5mm), yet the species with the lowest proportion had a lower SRL value compared to those with a high proportion of roots in this diameter size class (Figure 2.6C). There were observable species differences in total root length (2.6A), root production (2.6B), fine root length (2.6D), and resource allocation (2.6E); indicating various rooting strategies.

Discussion

Numerous research projects have measured physiological traits of C₃ and C₄ grasses in response to changing environmental conditions (Nippert, Knapp & Briggs 2006; Ripley *et al.* 2007; Knapp 2013; Taylor *et al.* 2014). However, few examples exist that compare physiological drought tolerant traits among congeneric species with contrasting C₃ and C₄ photosynthetic pathways. The objective of this study focused on addressing the differences between photosynthetic pathways of four grass species to drought conditions by measuring leaf and root physiological traits. In general, C₃ species exhibited a prolonged g_s while C₄ species displayed a sharper decline in g_s ; both C₃ and C₄ species recovered at higher rates than expected. These results support previous research indicating the photosynthetic capabilities of C₄ grasses (Smith

& Knapp 1999; Nippert *et al.* 2009; Volder, Tjoelker & Briske 2010) and importance of root characteristics (Monk 1966; Eissenstat 2000; Ostonen *et al.* 2007).

Previously, leaf-level comparisons of physiological traits have been focused on identifying the differences between C₃ and C₄ grasses, particularly with regard to drought. Comparisons of drought responses by photosynthetic pathways suggest that C₄ grasses retain greater physiological functioning during drought conditions compared to C₃ grasses because of the inherently higher carboxylation rates at lower rates of stomatal conductance compared to C₃ grass species, which allows for an enhanced water use efficiency (WUE) (Knapp 1993). Higher WUE may facilitate C₄ species to maintain gas exchange longer during drought conditions, by using less water per unit time. With the data collected, I have reinforced that there is significant physiological variability between photosynthetic pathways, and provided evidence for significant variability between congeneric species for physiological traits. C₄ grasses did not consistently outperform C₃ species during the induced drought. Gas exchange data (Figures 2.1-2.3) shows both *Festuca* (C₃) species being photosynthetically viable for a longer or equal period than both *Paspalum* (C₄). This was contradictory to our hypothesis that C₄ grasses would remain physiologically optimal for a longer duration during the dry-down compared to C₃ grasses. The impact of drought on *Paspalum* could be due to its leaf area which is larger compared to that of *Festuca*; leaf area has been shown to increase transpiration rates in many grass species (Xu & Zhou 2008). *Paspalum* was operating at a higher photosynthetic rate while remaining at slightly higher transpiration rates compared to *Festuca*. The water use behavior of *Paspalum* may reflect conditions experienced in its native habitat (where water is more readily available), which may point to the higher A_n and E and quicker desiccation.

Maximum electron transport rate exhibited a similar trend as shown for gas exchange traits for all species (Fig. 2.4). The *ETR* reflects the health of photosystem II (Maxwell & Johnson 2000), while *ETR_{max}* provides insight into the maximum capability of electron transport. The high *ETR_{max}* by *P. notatum* suggests that this species did not experience physiological stress from the drought treatments imposed. Again, *ETR_{max}* for *P. jeurgensii* was similar to both *Festuca* species (Figure 4), and was reflected in gas exchange data (Figures 2.1-2.3). Large differences in *ETR_{LC}* observed between congeners of *C₄ Paspalum* species portray the damage done to the photosynthetic capability of the species (Fig. 2.5) (Maxwell & Johnson 2000; Murchie & Lawson 2013). *P. jeurgensii* had a very low photosynthetic rate, corresponding with the very low *ETR* values observed during day four, which implies severely damaged photosynthetic machinery.

Congener variability was common for a variety of traits measured during this experiment. Differences between both *Paspalum* species' photosynthesis, stomatal conductance, and transpiration rates were statistically significant ($P < 0.05$) (Tables 2.1-2.3) for every sampling period (excluding day five) (Figures. 2.1-2.3). These figures indicate differences between *Festuca* species; *F. ovina* did not always have the highest rates of gas exchange, but did maintain initial rates for a longer period compared to *F. pseudovina*. Maximum electron transport rate (*ETR_{max}*) also varied between congeneric species in this study (Figure. 2.4). Significant differences ($P < 0.05$) between *Paspalum* and *Festuca* *ETR_{max}* species similarly to that of gas exchange rates (Figure 2.4). *Paspalum*'s *ETR_{LC}* had large differences throughout the length of the study, portraying photosystem II efficiency differences (Figure 2.5).

Root traits of grass species can give insight into resource acquisition, providing mechanistic insight into how grasses allocate root growth belowground. When comparing root

traits (Fig. 2.6), variability among species is evident despite evolutionary similarity among these species. Figure 2.6A depicts *F. pseudovina* placing more investment into producing a longer and heavier rooting system which could demand greater carbon for maintenance (Reekie & Bazzaz 1987a; b). *F. ovina* was seen to have a consistently larger proportion of fine roots (Fig 2.6C), while larger variation was observed in *F. pseudovina* and *P. jeurgensii*. Fine roots are typically responsible for acquisition of water and nutrients, with the tradeoff of reduced overall water transport during periods of high availability and low soil penetration power (Pérez-Harguindeguy *et al.* 2013). Root diameter is negatively correlated with nutrient uptake rate, but positively correlated with water uptake and penetrative force in soils (Roumet *et al.* 2011). This result could explain why all species have a relatively similar proportion of fine roots but alternative responses to simulated drought. Specific root length (SRL) is most frequently measured because of its ability to characterize economic features (maintenance of structural components, production of new material, etc.) of rooting systems with environmental change; similar to measuring specific leaf area (SLA) for leaf economic aspects (Ostonen *et al.* 2007; Pérez-Harguindeguy *et al.* 2013). Both *Festuca* species exhibited larger SRL values compared to the *Paspalum* species. This result may be related to the prolonged drought tolerance for *Festuca* species. More specifically, *F. ovina* had the highest SRL compared to all species indicating that its leaf economic features are ideal to survive in drought conditions (Figure 2.6F).

The goal of this research project was to determine if key leaf-level physiological traits vary in response to experimental drought simulation in C₃ and C₄ congeners, and if the results correspond to long standing notions of these pathways to decreased water availability. This project addressed two main hypotheses that revealed unique findings. Both *Paspalum* (C₄) species did not sustain an optimal photosynthetic rate during prolonged drying, as predicted.

Festuca (C₃) was found to remain photosynthetically active for more days of simulated drought. During dry-down conditions, the C₄ species exhibited larger fluctuations in gas exchange rates compared to the C₃ species. This suggests that a species photosynthetic pathway is not the sole determining factor of grass response to changing climate conditions; for instance, this study provided evidence that root-level morphological traits may explain large components of drought response that may be missed if not addressed in such studies. Research exploring root morphology's importance in conjunction with leaf-level or whole-plant response to climate variability will require further investigation.

References

- Adler, P.B. & Levine, J.M. (2007) Contrasting relationships between precipitation and species richness in space and time. *Oikos*, **116**, 221–232.
- Alderson, J. & Sharp, W.. (1994) *Grass Varieties in the United States*.
- Anderson, R.C. (2006) Evolution and origin of the Central Grassland of North America : climate , fire , and mammalian grazers. *Journal of the Torrey Botanical Society*, **133**, 626–647.
- Axelrod, D. (1985) Rise of the Grassland Biome , Central North America. *Botanical Review*, **51**, 163–201.
- Baer, S.G., Blair, J.M., Collins, S.L. & Knapp, A.K. (2003) Soil resources regulate productivity and diversity in newly established tallgrass prairie. *Ecology*, **84**, 724–735.
- Bailey, R.G. (1998) *Ecoregions*, 2nd ed. Springer Berlin Heidelberg, London.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of Climate Change on the Future of Biodiversity. *Ecol Lett.*, **12**, 365–377.
- Berry, J.O. & Patel, M. (2008) Kranz Anatomy and the C4 Pathway. *Encyclopedia of life sciences (ELS)*, 1–6.
- Borchert, J. (1940) The Climate of the Central North. *Annals of the Association of American Geographers*, **40**, 1–39.
- Borchert, J.R. (1950) The Climate of the Central North American Grassland. *Annals of the Association of American Geographers*, **40**, 1–39.
- Bräutigam, A. & Gowik, U. (2016) Photorespiration connects C3 and C4 photosynthesis. *Journal of Experimental Botany*, **67**, 2953–2962.
- Brown, W. (1975) Variations in Anatomy , Associations , and Origins of Kranz Tissue. *American Journal of Botany*, **62**, 395–402.

- Von Caemmerer, S. & Furbank, R. (2003) The C4 pathway: An efficient CO2 pump. *Photosynthesis Research*, **77**, 191–207.
- Carmo-Silva, A.E., Francisco, A., Powers, S.J., Keys, A.J., Ascensão, L., Parry, M. a J. & Arrabaça, M.C. (2009) Grasses of different C4 subtypes reveal leaf traits related to drought tolerance in their natural habitats: Changes in structure, water potential, and amino acid content. *American Journal of Botany*, **96**, 1222–1235.
- Carpenter, R. (1940) The Grassland Biome. *Ecological Monographs*, **10**, 617–684.
- Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought - From genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.
- Christin, P.-A. & Osborne, C.P. (2014) The evolutionary ecology of C4 plants. *New Phytologist*, **204**, 765–781.
- Christin, P.-A., Osborne, C.P., Chatelet, D.S., Columbus, J.T., Besnard, G., Hodkinson, T.R., Garrison, L.M., Vorontsova, M.S. & Edwards, E.J. (2013) Anatomical enablers and the evolution of C4 photosynthesis in grasses. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 1381–6.
- Cook, B.I., Ault, T.R. & Smerdon, J.E. (2015) Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances*, **1**, e1400082–e1400082.
- Cook, B.I., Smerdon, J.E., Seager, R. & Cook, E.R. (2014) Pan-Continental Droughts in North America over the Last Millennium. *Journal of Climate*, **27**, 383–397.
- Craine, J.M., Ocheltree, T.W., Nippert, J.B., Towne, E.G., Skibbe, A.M., Kembel, S.W. & Fargione, J.E. (2012) Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*, **2**, 1–5.
- Dengler, N.G., Dengler, R.E., Donnelly, P.M. & Hattersley, P.W. (1994) Quantitative leaf

- anatomy of C3 and C4 Grass (poaceae) Bundle sheath and Mesophyll surface area relationships. *Annals of Botany*, **73**, 241–255.
- Edwards, G.E., Franceschi, V.R., Ku, M.S., Voznesenskaya, E. V, Pyankov, V.I. & Andreo, C.S. (2001) Compartmentation of Photosynthesis in Cells and Tissues of C4 Plants. *Journal of experimental botany*, **52**, 577–590.
- Edwards, E.J., Osborne, C., Stromberg, C., Smith, S. & C4 Grass Consortium. (2010) The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, **328**, 587–590.
- Edwards, E. & Still, C. (2008) Climate, phylogeny and the ecological distribution of C4 grasses. *Ecology Letters*, **11**, 266–276.
- Edwards, E.J., Still, C.J. & Donoghue, M.J. (2007) The relevance of phylogeny to studies of global change. *Trends in Ecology & Evolution*, **22**.
- Ehleringer, J., Cerling, T. & Helliker, B. (1997) C4 Photosynthesis, Atmospheric CO₂ , and Climate. *Oecologia*, **112**, 285–299.
- Eissenstat, D.M. (2000) Root Structure and function in an ecological context. *New Phytologist*, **148**, 353–354.
- Eissenstat, D., Wells, C. & Yanai, R. (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, **147**, 33–42.
- Epstein, H.E., Lauenroth, W.K., Burke, I.C., Coffin, D.P., Ecology, S.P., Coffin, P. & Lauenroth, K. (1998) Regional Productivities of Plant Species in the Great Plains of the. *Plant Ecology*, **134**, 173–195.
- Eters, D., Tarks, P. & Hernandez, M. (2014) Functional response of U.S. grasslands to the early 21st-century drought. *Ecology*, **95**, 2121–2133.

- Fay, P., Carlisle, J.D., Danner, B.T., Lett, M.S., McCarron, J.K., Stewart, C., Knapp, A.K., Blair, J.M. & Collins, S.L. (2002) Altered rainfall patterns, gas exchange, and growth in grasses and forbs. *International Journal of Plant Sciences*, **163**, 549–557.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M. & Collins, S.L. (2000) Altering rainfall timing and quantity in a mesic grassland ecosystem: Design and performance of rainfall manipulation shelters. *Ecosystems*, **3**, 308–319.
- Forrestel, E.J., Donoghue, M.J. & Smith, M.D. (2015) Functional differences between dominant grasses drive divergent responses to large herbivore loss in mesic savanna grasslands of North America and South Africa. *Journal of Ecology*, **103**, 714–724.
- Gilliam, F.S., Seastedt, T. & Knapp, A. (1987) Southwestern Association of Naturalists Canopy Rainfall Interception and Throughfall in Burned and Unburned Tallgrass Prairie. *The Southwestern Naturalist*, **32**, 267–271.
- Grass Phylogeny Working II, G. (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. *New Phytologist*, **193**, 304–312.
- Hatch, M. (1987) C4 Photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochimica et Biophysica Acta*, **895**, 81–106.
- Hoover, D., Duniway, M. & Belnap, J. (2015) Pulse-drought atop press-drought: unexpected plant responses and implications for dryland ecosystems. *Oecologia*, **179**, 1211–1221.
- Hoover, D., Knapp, A.K. & Smith, M.D. (2014a) Contrasting sensitivities of two dominant C4 grasses to heat waves and drought. *Plant Ecology*, **215**, 721–731.
- Hoover, D., Knapp, A. & Smith, M. (2014b) Resistance and resilience of a grassland ecosystem to climate extremes. *Ecology*, **95**, 2646–2656.
- Hoover, D.L. & Rogers, B.M. (2016) Not all droughts are created equal: the impacts of

- interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology*, **22**, 1809–1820.
- Houghton, J., Y, D., DJ, G., M, N., PJ, van der L., X, D., K, M. & C, J. (2001) Climate Change 2001: The Scientific Basis. *Climate Change 2001: The Scientific Basis*, 881.
- IPCC. (2007) *Climate Change 2007 Synthesis Report*.
- Kakani, V.G., Surabhi, G.K. & Reddy, K.R. (2008) Photosynthesis and fluorescence responses of C4 plant *Andropogon gerardii* acclimated to temperature and carbon dioxide. *Photosynthetica*, **46**, 420–430.
- Kellogg, E.A. (1999) Phylogenetic aspects of the evolution of C4 photosynthesis. *C4 Plant Biology* (eds R.F. Sage & R. Monson), pp. 411–444. Academic Press, San Diego.
- Kidder, D.L. (2005) Impact of Grassland Radiation on the Nonmarine Silica Cycle and Miocene Diatomite. *Palaios*, **20**, 198–206.
- Knapp, A.K. (1984) Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia*, **65**, 35–43.
- Knapp, A.K. (1993) Gas Exchange Dynamics in C3 and C4 Grasses: Consequence of Differences in Stomatal Conductance. *Plant physiology*, **74**, 113–123.
- Knapp, A.K. (2013) Effect of Fire and Drought on the Ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a Tallgrass Prairie Author (s): Alan K . Knapp Published by : Ecological Society of America Stable URL : <http://www.jstor.org/stable/1939184> . EFFECT OF FIRE. *Ecological society of America ESA*, **66**, 1309–1320.
- Knapp, A., Fay, P., Blair, J., Collins, S., Smith, M., Carlisle, J., Harper, C., Danner, B., Lett, M. & McCarron, J. (2002a) Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland. *Science*, **298**, 2202–2205.

- Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S. & McCarron, J.K. (2002b) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, **298**, 2202–2205.
- Kromdijk, J., Ubierna, N., Cousins, A.B. & Griffiths, H. (2014) Bundle-sheath leakiness in C₄ photosynthesis : a careful balancing act between CO₂ concentration and assimilation. *Journal of Experimental Botany*, **65**, 3443–3457.
- Lauenroth, W.K., Burke, I.C. & Gutmann, M.P. (1999) The Structure and Function of Ecosystems in the Central North American Grassland Region. *Great Plains Research*, **9**, 223–259.
- Lobell, D.B., Roberts, M.J., Schlenker, W., Braun, N., Little, B.B., Rejesus, R.M. & Hammer, G.L. (2014) Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science (New York, N.Y.)*, **344**, 516–9.
- Lundgren, M.R., Osborne, C.P. & Christin, P. (2014) Deconstructing Kranz anatomy to understand C₄ evolution. *Journal of Experimental Botany*, **65**, 3357–3369.
- Maai, E., Miyake, H. & Taniguchi, M. (2011) Differential positioning of chloroplasts in C₄ mesophyll and bundle sheath cells. *Plant Signaling & Behavior*, 1111–1113.
- Maricle, B.R. & Adler, P.B. (2011) Effects of precipitation on photosynthesis and water potential in *Andropogon gerardii* and *Schizachyrium scoparium* in a southern mixed grass prairie. *Environmental and Experimental Botany*, **72**, 223–231.
- Maxwell, K. & Johnson, G.N. (2000) Chlorophyll fluorescence--a practical guide. *Journal of experimental botany*, **51**, 659–668.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yezzer, E. a. (2008) Mechanisms of plant survival

- and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist*, **178**, 719–739.
- Meister, M., Agostino, A. & Hatch, M. (1996) The roles of malate and aspartate in C4 photosynthetic metabolism of *Flaveria bidentis* (L.). *Planta*, **199**, 262–269.
- Monk, C. (1966) Ecological Importance of Root / Shoot Ratios. *Bulletin of the Torrey Botanical Club*, **93**, 402–406.
- Mueller, K., Blumenthal, M., Pendall, E., Carillo, Y., Dijkstra, F., Williams, D., Follet, R. & Morgan, J. (2016) Impacts of warming and elevated CO₂ on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters*, **19**, 956–966.
- Murchie, E.H. & Lawson, T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany*, **64**, 3983–98.
- Nelson, J.A., Morgan, J.A., LeCain, D.R., Mosier, A.R., Milchunas, D.G. & Parton, B.A. (2004) Elevated CO₂ increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado. *Plant and Soil*, **259**, 169–179.
- Nippert, J.B., Fay, P.A., Carlisle, J.D., Knapp, A.K. & Smith, M.D. (2009) Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica*, **35**, 400–408.
- Nippert, J.B., Knapp, A.K. & Briggs, J.M. (2006) Intra-annual rainfall variability and grassland productivity: Can the past predict the future? *Plant Ecology*, **184**, 65–74.
- Ocheltree, T.W., Nippert, J.B. & Prasad, P.V. V. (2012) Changes in stomatal conductance along grass blades reflect changes in leaf structure. *Plant, cell & environment*, **35**, 1040–9.
- Olsen, J.T., Caudle, K.L., Johnson, L.C., Baer, S.G. & Maricle, B.R. (2013) Environmental and genetic variation in leaf anatomy among populations of *Andropogon gerardii* (Poaceae)

- along a precipitation gradient. *American Journal of Botany*, **100**, 1957–1968.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, a. F.M., Pronk, a., Vanguelova, E., Weih, M. & Brunner, I. (2007) Specific root length as an indicator of environmental change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, **141**, 426–442.
- Pérez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S.S., Cornwell, W.K.K., Craine, J.M.M., Gurvich, D.E.E., Urcelay, C., Veneklaas, E.J.J., Reich, P.B.B., Poorter, L., Wright, I.J.J., Etc., Ray, P., Etc., Díaz, S., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S.S., Cornwell, W.K.K., Craine, J.M.M., Gurvich, D.E.E., Urcelay, C., Veneklaas, E.J.J., Reich, P.B.B., Poorter, L., Wright, I.J.J., Ray, P., Enrico, L., Pausas, J.G., Vos, A.C. de, Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., Steege, H. ter, Heijden, M.G.A. van der, Sack, L., Blonder, B., Poschlod, P., Vaieretti, M. V., Conti, G., Staver, A.C., Aquino, S. & Cornelissen, J.H.C. (2013) New Handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.
- Ramankutty, N. & Foley, J.A. (1999) Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles*, **13**, 997–1027.
- Rao, X. & Dixon, R.A. (2016) The Differences between NAD-ME and NADP-ME Subtypes of C4 Photosynthesis: More than Decarboxylating Enzymes. *Frontiers in plant science*, **7**, 1525.
- Rasband, W. (1997) ImageJ. *U.S National Institutes of Health*.
- Reekie, E. & Bazzaz, F.. (1987a) Reproductive Effort in Plants. 1. Carbon Allocation to Reproduction. *The American Naturalist*, **129**, 876–896.

- Reekie, E.. & Bazzaz, F.. (1987b) Reproductive Effort in Plants. 2. Does Carbon Reflect the Allocation of Other Resources? *The American Naturalist*, **129**, 897–906.
- Ripley, B.S., Gilbert, M.E., Ibrahim, D.G. & Osborne, C.P. (2007) Drought constraints on C4 photosynthesis: Stomatal and metabolic limitations in C3 and C4 subspecies of *Alloteropsis semialata*. *Journal of Experimental Botany*, **58**, 1351–1363.
- Romm, J. (2011) Desertification: The next dust bowl. *Nature*, **478**, 450–451.
- Roumet, C., Urcelay, C., Díaz, S. & Diaz, S. (2011) Suites of root traits in species growing differ between the and perennial. *New Phytologist*, **170**, 357–367.
- Ruzin, S. (1999) *Plant Microtechnique and Microscopy*. Oxford University Press, New York.
- Sage, R.F. (2004) The evolution of C4 photosynthesis. *New Phytologist*, **161**, 341–370.
- Sala, A.O.E., Parton, W.J., Joyce, L. a & Lauenroth, W.K. (1988) Primary Production of the Central Grassland Region of the United States. *Ecological Monographs*, **69**, 40–45.
- Salinger, J., Sivakumar, M.V.K. & Motha, R.P. (2005) Increasing climate variability and change: Reducing the vulnerability of agriculture and forestry. *Climate Change*, **70**, 9–29.
- Schroeder-Georgi, T., Wirth, C., Nadrowski, K., Meyer, S.T., Mommer, L. & Weigelt, A. (2016) From pots to plots: Hierarchical trait-based prediction of plant performance in a mesic grassland. *Journal of Ecology*, **104**, 206–218.
- Smith, M.D. & Knapp, A.K. (1999) Exotic plant species in a C4 dominated grassland : invasibility , disturbance , and community structure. *Oecologia*, **120**, 605–612.
- Smith, M.D., Knapp, A.K. & Collins, S.L. (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology*, **90**, 3279–3289.
- Still, C.J., Berry, J.A., Collatz, G.J. & DeFries, R.S. (2003) Global distribution of C 3 and C 4

- vegetation: Carbon cycle implications. *Global Biogeochemical Cycles*, **17**, 6-1-6–14.
- Strömberg, C.A.E. (2005) Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11980–11984.
- Strömberg, C. a. E. (2011) Evolution of Grasses and Grassland Ecosystems. *Annual Review of Earth and Planetary Sciences*, **39**, 517–544.
- Taylor, S.H., Ripley, B.S., Martin, T., De-Wet, L.A., Woodward, F.I. & Osborne, C.P. (2014) Physiological advantages of C4 grasses in the field: A comparative experiment demonstrating the importance of drought. *Global Change Biology*, **20**, 1992–2003.
- Taylor, S.H., Ripley, B.S., Woodward, F.I. & Osborne, C.P. (2011) Drought limitation of photosynthesis differs between C3 and C4 grass species in a comparative experiment. *Plant, Cell and Environment*, **34**, 65–75.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature*, **418**, 671–7.
- Tucker, S.S., Craine, J.M. & Nippert, J.B. (2011) Physiological drought tolerance and the structuring of tallgrass prairie assemblages. *Ecosphere*, **2**, art48.
- Vico, G. & Porporato, A. (2008) Modelling C3 and C4 photosynthesis under water-stressed conditions. *Plant and Soil*, **313**, 187–203.
- Volder, A., Tjoelker, M.G. & Briske, D.D. (2010) Contrasting physiological responsiveness of establishing trees and a C4 grass to rainfall events, intensified summer drought, and warming in oak savanna. *Global Change Biology*, **16**, 3349–3362.
- Weaver, J.E. (1968) Prairie plants and their environment. *Agronomy and Horticulture Department*, 276.

Wilson, J. (1988) The Effect of Initial Advantage on the Course of Plant Competition. *Oikos*, **51**, 19–24.

Xu, Z. & Zhou, G. (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, **59**, 3317–3325.

Tables and Figures

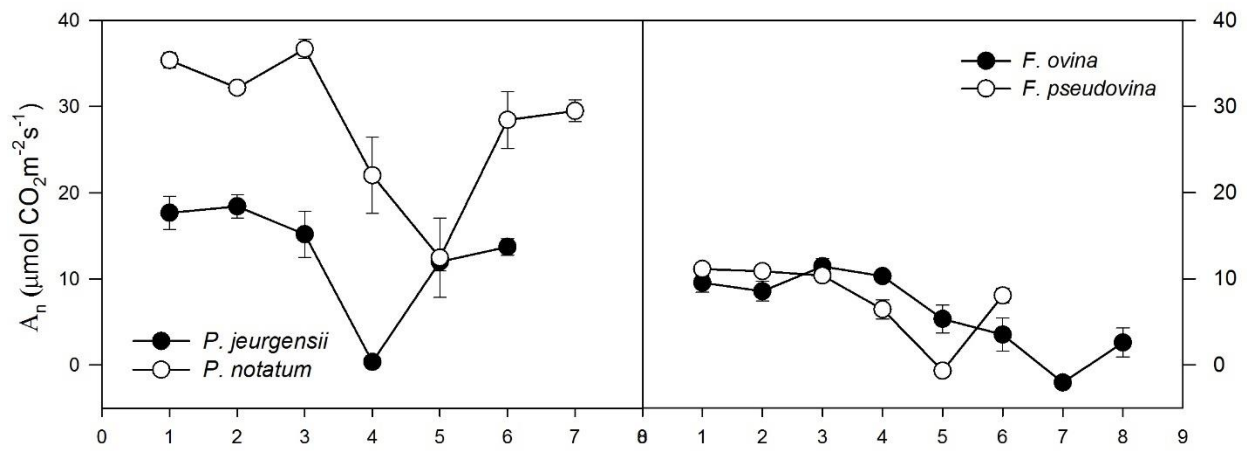


Figure 2.1 Average daily photosynthetic rates for study species. Left, *Paspalum* species with two-day recovery. Right, *Festuca* species with one-day recovery.

Table 2.1 ANOVA results for species, sample day, and interaction effects for photosynthetic rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean Square</i>	<i>F value</i>	<i>P value</i>
Species (S)	3	5973	157.656	$< 2e^{-16}$
Sample Day (D)	1	3743	98.798	$< 2e^{-16}$
S X D	3	41	1.081	.358

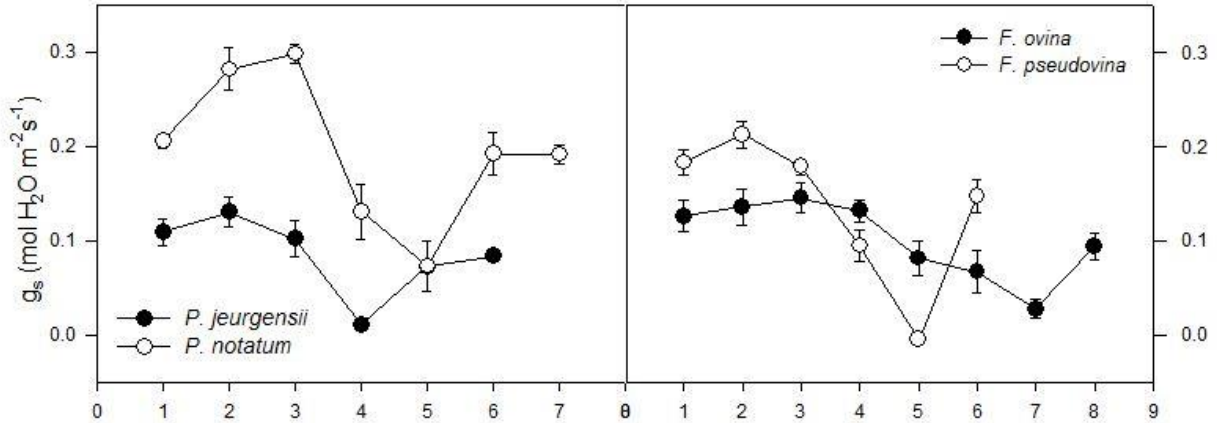


Figure 2.2 Average daily stomatal conductance rates for study species. Left, *Paspalum* species with two-day recovery. Right, *Festuca* species with one-day recovery.

Table 2.2 ANOVA results for species, sample day, and interaction effects for stomatal conductance rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean Square</i>	<i>F value</i>	<i>P value</i>
Species (S)	3	.1484	38.353	< 2e⁻¹⁶
Sample Day (D)	1	.4680	120.957	< 2e⁻¹⁶
S X D	3	.0253	6.549	.000278

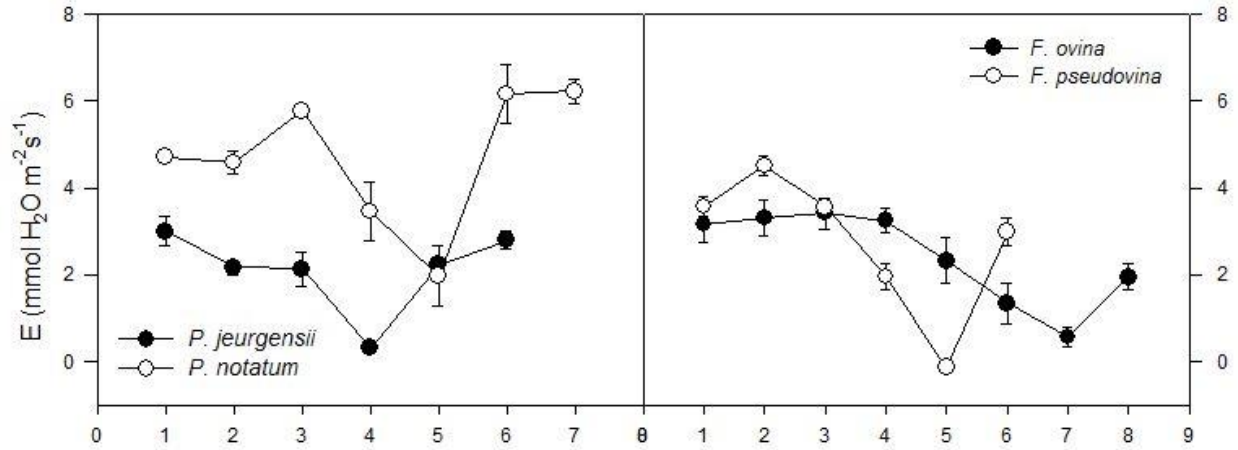


Figure 2.3 Average daily transpiration rates for study species. Left, *Paspalum* species with two-day recovery. Right, *Festuca* species with one-day recovery

Table 2.3 ANOVA results for species, sample day, and interaction effects for stomatal transpiration rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean Square</i>	<i>F value</i>	<i>P value</i>
Species (S)	3	91.81	48.87	$< 2e^{-16}$
Sample Day (D)	1	144.22	76.76	$2.79e^{-16}$
S X D	3	25.61	13.63	$2.84e^{-8}$

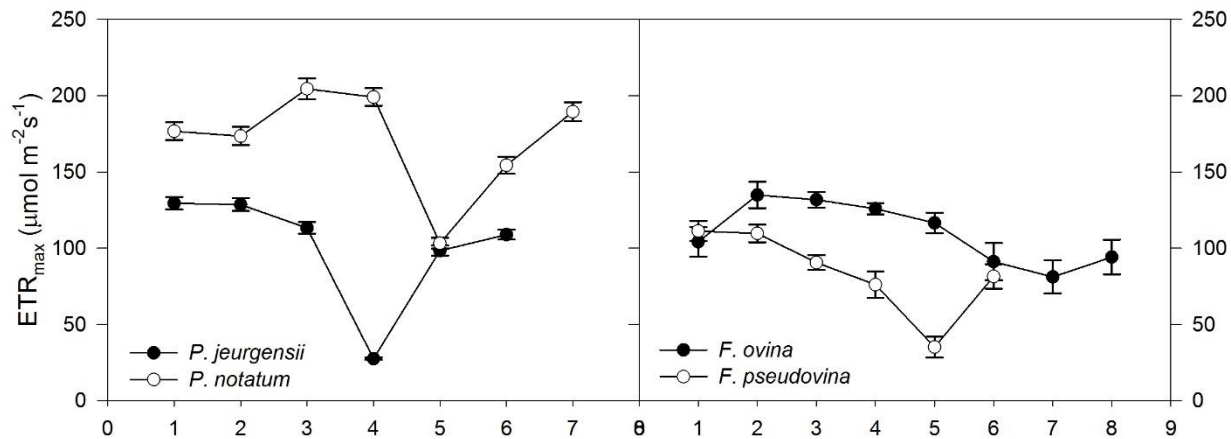


Figure 2.4 Maximum daily electron transport rate for study species. Left, *Paspalum* species with two-day recovery. Right, *Festuca* species with one-day recovery

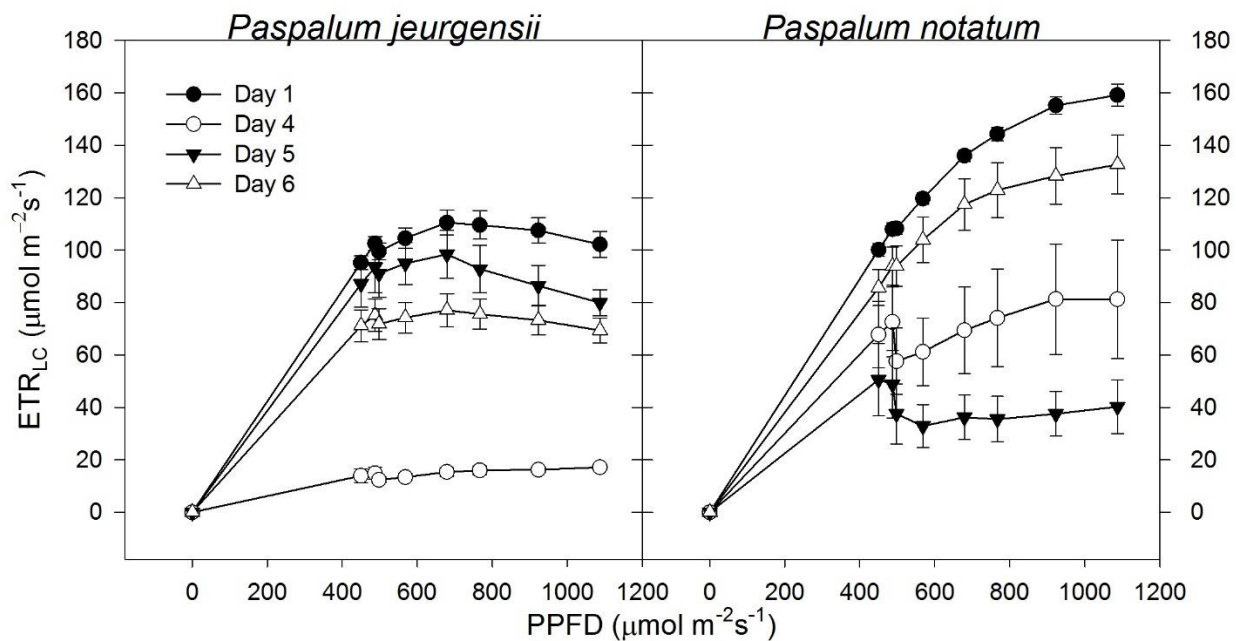


Figure 2.5 Average light response curves for *Paspalum*. Left, *P. jeurgensii*; Right, *P. notatum* species.

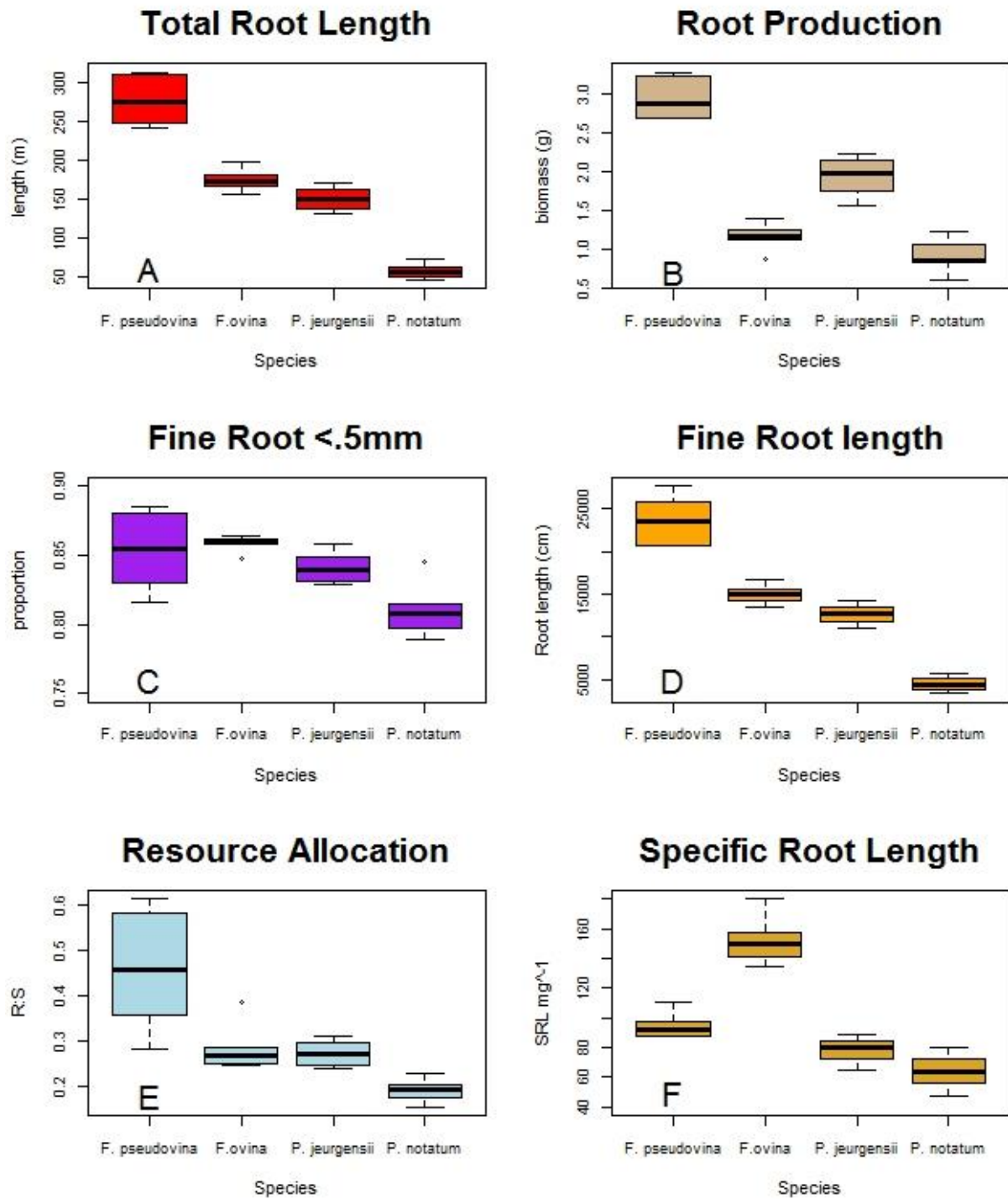


Figure 2.6 Root morphological measurements for *Festuca* and *Paspalum*.

Chapter 3 - Physiological and Morphological Trait Plasticity

Introduction

Since the appearance of grasses in the Paleogene period, grasslands have spread across Earth (Strömberg 2011) with an eventual shift to the Neogene C₄ dominated grass habitats. Grassland expansion led to cover 25% of the terrestrial surface, resulting in the establishment of a major biome type (Ramankutty & Foley 1999). Grassland expansion in North America started in the Great Plains, which were formed in the Middle-Late Miocene period (Strömberg 2011).

Grasslands comprise 15% of North America, and have been shaped by fire, grazing, and precipitation variability (Anderson 2006). North American grasslands are characterized by climatic variability and periodic drought, creating an environment with fluctuating resource availability and extremes in temperature and precipitation (Weaver 1968). Precipitation variability is a fundamental condition for the grassland biome of North America, with variable rainfall and increased temperatures occurring in the summer growing season (Borchert 1950). Despite the natural occurrence of climate variability in this region, increased frequencies and magnitude of climate variability due to climate change may have negative consequences on grassland structure and function within the Central Plains of North America (Houghton *et al.* 2001; Nippert *et al.* 2009).

Climate change is increasing the likelihood of future drought conditions in the Central Plains in North America (Cook *et al.* 2014, 2015; Hoover & Rogers 2016). Drought conditions are likely to have a negative effect on plant species due to increased temperatures and decreased water availability (Craine *et al.* 2012). However, many grasses in the Great Plains have traits that confer tolerance to drought and fluctuations in water availability (Tucker, Craine & Nippert

2011). Traits such as small vessel diameter, increased water-use-efficiency, and decreased transpiration are generally associated with species that utilize the C₄ photosynthetic pathway that has come to dominate grassland systems.

C₄ photosynthesis provides multiple adaptive benefits to plants in dry environments, including reduced photorespiration, increased drought tolerance, and greater growth efficiency (Brown 1975; Lundgren, Osborne & Christin 2014). Species that utilize C₄ photosynthesis possess a modified leaf morphological structure referred to as 'Kranz' anatomy. Kranz anatomy is characterized by two spatially separate cell types that partition the biochemical reactions required for CO₂ carboxylation and assimilation (Hatch 1987; Edwards *et al.* 2001; Berry & Patel 2008). Concentric rings of mesophyll cells are wrapped around a ring of bundle sheath cells that surround the vascular tissue (xylem and phloem). C₄ mesophyll cells are responsible for carboxylation of atmospheric CO₂, and these cells contain large numbers of chloroplasts for initial carboxylation (Berry & Patel 2008). Bundle sheath cells are responsible for the decarboxylation and assimilation of the C₄ acid assembled in the mesophyll cells (Sage 2004; Berry & Patel 2008). The separation of the biochemical reactions facilitates maximal carboxylation rates by the enzyme Rubisco via exposure to saturating concentrations of CO₂ (Christin *et al.* 2013; Kromdijk *et al.* 2014). During times of water-stress, plants strategically close stomata to reduce water loss, thus inhibiting the ability to take in atmospheric CO₂. Therefore, species that concentrate CO₂ in the bundle sheath cells (C₄ species) are more likely to survive in water limiting conditions because the spatial separation in microanatomical tissue in C₄ species gives them a higher water use efficiency (WUE) (Hatch 1987; Vico & Porporato 2008; Taylor *et al.* 2014). WUE is recognized as a useful trait that explains how C₄ species have increased carbon assimilation while maintaining or reducing rates of water loss (Nelson *et al.*

2004). Monitoring C₄ grass gas exchange (H₂O vapor and CO₂ gas) through stomatal pores has been used in order to assess plant physiological response to drought and other altered climatic conditions (Fay *et al.* 2000; Knapp *et al.* 2002b; Nippert *et al.* 2009). Gas exchange measurements allow instantaneous measurements of photosynthesis and water lost through transpiration, which aid in understanding water limitation during drought. Chlorophyll fluorescence (measurement of electron's loss of excitement or energy within chloroplasts) is also an indicator of the photosynthetic machinery involved in the light-dependent reactions of photosynthesis and has been observed to respond to water availability (Maxwell & Johnson 2000; Kakani, Surabhi & Reddy 2008; Murchie & Lawson 2013). These physiological traits should be directly influenced by the micro-anatomical features of the species; more specifically, proportions and sizes of cell types in Kranz anatomy of C₄ plants should effect the rates of gas exchange and water use in leaves.

Plant physiological characteristics have been used to explain species specific responses to varying conditions, but little work has described species specific morphological traits. Key physiological traits may be influenced by the microanatomical structure that is characteristic of the species. C₄ grass micro-anatomical features have been previously described (Christin & Osborne 2014), but understanding how this morphology changes over time and space within and across species has been seldom described. Measuring physiological and microanatomical differences between and within species over the growing season, and across natural environmental gradients may give insight to future responses from climate change.

The objective of this research was to measure the plasticity of leaf-level physiological and anatomical traits among four common C₄ grasses across time and space. Plasticity among selected leaf-level traits across a geographical rainfall gradient may provide insight on drought

sensitivity for these dominant C₄ grass species. Four C₄ grasses were examined due to their dominance of Kansas' prairies systems: *A. gerardii*, *S. scoparium*, *S. nutans*, *P. virgatum*. Leaf-level physiological traits measured included: A_{max} , g_s , E , and chlorophyll fluorescence; leaf morphological traits included: interveinal distance (IVD), average Xylem area, % vein area, % bundle sheath area, % mesophyll area, bundle sheath: mesophyll area ($BS:MS$), distance through mesophyll (D_m), Average bundle sheath thickness (BS_{thick}), and distance from stomata to xylem ($stomata_{xylem}$). Here, I propose 3 hypotheses: (1) Species will exhibit significant variability in gas exchange characteristics across time and location (2) Leaf anatomical traits will not vary significantly within species over spatial and temporal scales, but will vary between species (3) Morphological traits will significantly predict variability in leaf physiology, with differences more pronounced by species rather than time or location.

Methods

Experimental Design

This research was conducted at three sampling locations in Kansas. Albertson Prairie is located in Hays, Kansas (38°88' N, 99°35' W). Albertson Prairie has a mean annual precipitation of 596mm, with June maximum temperatures averaging 30.78°C and 32.03°C in August. Three watersheds at Konza Prairie Biological Station (4B: 39°08' N, 96°60' W; 1D: 39°08' N, 96°56' W; K20A: 39°10' N, 96°57' W) were used in this study. I averaged responses from these site in order to increase heterogeneity. Konza Prairie receives an average of 900mm of rainfall annually, and June temperatures average 30.39°C and 32.16 °C in August. Rockefeller Prairie was the most eastern site (39°05' N, 95°20' W) and receives 1014mm annual rainfall and mean June temperatures of 30.16°C and 32.22 °C in August. Measurements at each of the three

locations were made during two separate sampling periods: June 1st, 3rd, 4th and August 9th, 10th, 11th in the summer of 2016. Precipitation and temperature data were collected from 2000-2016 from weather stations nearest to each research site and averaged.

Four C₄ grassland species were measured at each site: *Andropogon gerardii* (big bluestem), *Sorghastrum nutans* (Indian grass), *Schizachyrium scoparium* (little bluestem), and *Panicum virgatum* (switchgrass). Ten individuals from each species were labelled with metal identification tags, and marked via GPS at each sampling location. Species physiological and morphological samples were measured and collected on the same leaf and spread to encompass the majority of grassland area in order to maximize heterogeneity. Soil moisture was measured at 0-5cm depth adjacent to each individual sampled to account for differences in surface soil water availability. Measurements were conducted with a HydraProbe II (Stevens, Inc., Portland, OR, USA).

Trait measurements

All trait data were collected between 10:00 and 18:00 CDT at each sampling site. For leaf-physiology measurements, the youngest, developmentally mature leaves were used in order to reflect the most recent environmental conditions. Gas exchange rates were measured using a LI-6400 system (LiCOR, Inc., Lincoln, NE, USA) equipped with an LED light source (light intensity was maintained at 2,000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, CO₂ concentration at 400 ppm, and relative humidity between 40-60%) and included rates of photosynthesis (A_n), stomatal conductance (g_s), and transpiration (E). Measurements from the LI-6400 were logged when maximum photosynthetic rate remained stable for at least one minute. Leaf chlorophyll fluorescence was measured using a miniaturized pulse-amplitude-modulated photosynthesis yield analyzer (Mini-

PAM) (Walz, Hamburg, Germany). Measurements were utilized for the creation of light response curves (electron transport rate against light intensity) with actinic intensity set at values that ranged from 0-1087 $\mu\text{mol m}^{-2}\text{s}^{-1}$; this range of light intensities was appropriate to minimize the risk of photoinhibition in the field.

Leaf anatomy samples included leaf cross sectional tissue from newest mature leaf tissue from each species (four samples of each species) and were collected at all sites for both sampling periods. After leaf-level physiological measurements were taken, leaf anatomical samples were collected via clipping the same leaf previously measured for physiological characteristics. Leaf tissue samples were fixed in FAA (10% formalin / 5% glacial acetic acid / 50% ethanol (use 95% EtOH) / 35% DI water) and sent to Kansas State's College of Veterinary Medicine Histopathology lab for paraffin mounting and cross-sectional slide staining with Safranin-O and Fast Green (Ruzin 1999). Leaf cross sectional samples were scanned with a Panoramic MIDI (3DHitech Inc., Budapest, Hungary) and measured using IMAGEJ software (Rasband 1997).

Two major vascular bundles were selected on either side of the mid-rib, and were at least one major vein distant from the mid-rib area (Figure 3.1). Interveinal distance (*IVD*) was determined by measuring the distance between the center of two adjacent vascular bundles and averaged for the entire leaf sample, while major leaf tissue types were measured in subsamples (Figure 3.1, area from 1-2 and 3-4) mesophyll:bundle sheath area ratio (*MS:BS*), and percent area of mesophyll, bundle sheath, and vein. Four large vessels were selected for measurements (Figure 3.1. 1-4): average xylem area, distance through mesophyll (D_m), bundle sheath thickness (*BST*), and distance from stomata to nearest xylem (*Stomata_{xylem}*) (Figure 3.2).

Statistical Analysis

My primary goal was to elucidate differences in physiology and morphology among these C₄ grass species across sites and across a growing season. Therefore, I used a mixed-effects model analysis of variance (ANOVA). For each model, the physiological gas exchange data, fluorescence data, and morphological traits measured were used as the response variables. Fixed effects include species, site, sample date and their interactions. Random effects include the model intercept, and sampling locations within site. Multiple comparison tests to identify differences among species, locations, and time were conducted using post-hoc Tukey's HSD. Regression analysis was utilized to correlate physiological and morphological traits. All analyses were conducted in R 2.14.2 (R Core Team).

Results

Leaf-Level Gas Exchange

During June, mean photosynthetic rate (A_n) for all species at Konza Prairie was higher than Rockefeller (Figure 3.3, $P < 0.05$) (Table A.2). For all species at Konza, A_n consistently decreased from June to August, whereas Albertson prairie and Rockefeller prairie increased between both sampling periods (excluding *P. virgatum* at Albertson) (Figure 3.3). Although A_n varied within species across sites and the growing season, *S. scoparium* was the only species to exhibit significant differences in A_n ($P < 0.05$) (Figure A.3) between sites within sampling period (June), and within a sample site (Hays) across the growing season ($P < 0.05$) (Figure 3.3).

Stomatal conductance (g_s) rates were also higher at Konza Prairie during June, but varied in August (Figure 3.4). Within the month of June, stomatal conductance (g_s) for both *S. scoparium* and *P. virgatum* was higher on Konza compared to other sampling locations (Figure 3.4, $P < 0.001$; $P < 0.05$) (Table A.3). *S. scoparium* decreased its g_s significantly on Konza

between sampling periods and increased in Albertson and Rockefeller ($P < 0.05$, $P < 0.001$, $P < 0.05$) (Table A.3). g_s rates for *S. scoparium* in August varied between Albertson prairie and Konza prairie, due to Albertson's significantly increased g_s ($P < 0.05$) (Figure 3.4) (Table A.3).

August transpiration (E) varied more for each species at all sites (excluding *P. virgatum* which exhibited small variation), and transpiration rate for *S. scoparium* was higher than all species at either site or sampling time (Figure 3.5). *S. nutan* and *S. scoparium* exhibited significantly variable E between sites during August ($P < 0.05$), while *P. virgatum* varied significantly in June ($P < 0.05$) (Figure 3.5) (Table A.4). E rates for two species (*S. scoparium* and *S. nutans*) increased significantly over the growing season at Albertson prairie ($P < 0.01$) (Table A.4). At Konza, *S. nutans* had increased E significantly over the growing season as well ($P < 0.01$) (Table A.4).

During June, soil moisture exhibited the typical precipitation gradient of Kansas: soil moisture increased moving eastward. However; during the month of August, precipitation amounts were received in the following descending order: Konza, Rockefeller, and Albertson.

Chlorophyll Fluorescence

Measurements of electron transport rate (ETR) differed within species across different sampling locations and time (Figure 3.6-3.7). *S. nutans* and *A. gerardii* had significantly higher ETR on Konza prairie during June ($P < 0.01$), while *S. scoparium* and *P. virgatum* had significantly lower ETR at Rockefeller in August ($P < 0.05$). Significant differences in ETR were observed between both sampling periods within all species of this study. *S. scoparium* had higher ETR at Albertson prairie in June and highest ETR at Konza in August ($P < 0.05$) (Figure 3.6). Albertson prairie *P. virgatum* and *S. nutans* exhibited significant changes over the growing

season ($P < 0.05$) (Figures 3.6-3.7). *A. gerardii* and *S. nutans* at Rockefeller prairie were found to be significantly different between June and August. *A. gerardii* was found to increase *ETR* between growing season measurement periods at the Konza location ($P < 0.01$) (Figure 3.6).

Leaf Morphology

Leaf micro-anatomical measurements derived from the cross-sectional images revealed that tissue measurements of mesophyll, bundle sheath, and vein tissue area varied according to geographic location and species measured. *P. virgatum* varied significantly from the other three species in nearly every micro anatomical trait measured here. The most eastern sample location (Rockefeller) typically had different responses from the other two locations for many traits ($P < 0.05$). *P. virgatum* contained significantly more bundle sheath cells than the other species ($P < 0.05$) in both June and August (Table A.8). Total bundle sheath area was also found to vary significantly when comparing data from Rockefeller to other research locations ($P < 0.05$). Mesophyll area differed significantly between species and location (Table A.8) ($P < 0.05$). *P. virgatum* had significantly fewer mesophyll cells compared to the other three species in both June and August. A significant interaction between research location and sample period existed for differences in mesophyll area. *P. virgatum* had a significantly higher BS:MS ratio compared to other species and varied in location (Table A.8). Vein area was found to be significantly different between species in both June and August ($P < 0.05$) (Tables A.5-A.8). Leaf microanatomical tissue measurements display that most leaf tissue is mesophyll, followed by bundle sheath cells, and vein area tissue with typically less than 20% (Tables A.5-A.8). Generally, my data show that most tissue types exhibit little change within a growing season compared to physiological measurements.

Anatomical traits derived from distance to stomata openings (D_m , BS_{thick} , and $Stomata_{xylem}$) revealed significant differences between species and location (Tables A.5-A.8). D_m (Distance through mesophyll layer), also varied between June and August ($P < 0.01$) (Tables A.5-A.8). D_m and g_s were found to relate positively with each other in *P. virgatum* only. Location of highest mean D_m was dependent on species during June sampling while August samples revealed that Hays had the highest D_m of all locations.

Bundle sheath thickness (BS_{thick}) did not differ between locations or months, but was significantly different ($P < 0.0001$) (Tables A.5-A.8) between species. All species varied significantly from each other ($P < 0.05$) except for *S. scoparium* and *A. gerardii* which were almost identical ($P = 0.99$). BS_{thick} was taken by measuring only the outside sheath of *P. virgatum*, which is shown to be significantly higher than the other species measured. $Stomata_{xylem}$ (distance from stomata to nearest xylem) was found to vary significantly among locations ($P < 0.05$) and species ($P < 0.001$). Over the growing season, differences were observed within a species at each location (Tables A.5-A.8).

Interveinal distance of each sample varied significantly between species ($P < 0.0001$) and month of the growing season ($P < 0.05$), but not between locations (Tables A.5-A.8). Mean IVD (Interveinal distance) was highest both June and August at the same location for each species, but in varying distances. *S. scoparium* and *A. gerardii* were the only species not significantly different from each other. Within subsampled veins (vascular bundles), species differed significantly in mean xylem area (Tables A.5-A.8). Significance was not observed across locations or sampling times, but *S. scoparium* xylem area was significantly smaller compared to other species (Figure 3.8) (Table A.7).

Discussion

The impacts of rainfall variation on physiology and leaf morphology of grassland species has been observed numerous times (Edwards *et al.* 2001; Fay *et al.* 2002; Tucker *et al.* 2011; Ocheltree, Nippert & Prasad 2012; Olsen *et al.* 2013; Schroeder-Georgi *et al.* 2016). However, there are few studies that document intra- and interspecific relationships between leaf-level physiology and morphology over the course of a growing season. The goal of this study was to examine physiological and morphological differences among four C₄ grass species across a spatial rainfall gradient over the course of the 2016 growing season. Using the natural precipitation gradient of Kansas facilitated these comparisons within a geographical region, and allowed me to determine the plasticity of leaf-level physiological and morphological traits. Findings from this research indicate that physiological measurements of gas exchange respond to short-term changes in soil moisture over the course of the growing season. In contrast, leaf morphology was more consistent across the growing season, but did vary significantly between the research locations and species of interest (Table A.5-A.8). Results from this study support previous research findings indicating a tight coupling between C₄ grasses physiological and morphological relationships in response to short-term changes in climate (Smith & Knapp 1999; Volder *et al.* 2010; Ocheltree *et al.* 2012; Christin *et al.* 2013).

Physiological gas exchange measurements varied significantly between species and across space and time (Figures 3.3-3.5). Konza Prairie physiological data collected in June had the highest rates of gas exchange compared to the other field sites (excluding *S. nutans* transpiration rate), while August measurements display a varied trend in gas exchange rates across sites. The disparity between locations and the sampling periods likely reflects differences among landscape fire treatments at Konza Prairie. Two watersheds on Konza were burned in the

spring, which could have allowed for an increase in nutrient availability (Baer *et al.* 2003) and a decrease in light competition from cool season grasses (Wilson 1988). However, Konza's g_s was likely driven by fire's removal of the top litter layer, allowing faster growth compared to the unburned locations: Albertson and Rockefeller (Gilliam, Seastedt & Knapp 1987).

Leaf-level morphological trait measurements were observed to vary significantly between species and location (Tables A.5-A.8). Significant statistical relationships between leaf morphological traits and physiological traits were not consistent among C_4 species in this study. However, there were species-specific relationships between microanatomical traits and corresponding physiological traits from the same measured individuals. Known relationships between cell types have varying effects on species physiology; for instance, increasing size of bundle sheath cells correlates with increased photosynthetic rate (Dengler *et al.* 1994). In the results shown here, the relationship between bundle sheath area (measured as a %) and maximum photosynthetic rate varied between *A. gerardii* and *P. virgatum* (Figure 3.9-3.10). *A. gerardii* is more sensitive to photosynthetic responses when small changes in bundle sheath area are present. Bundle sheath thickness behaved similarly within *A. gerardii* and *S. scoparium* species across locations (Figure 3.11), while *S. nutans* and *P. virgatum* did not show a trend. Bundle sheath cell thickness relates to its area and volume, and correlates with increased light absorption to chloroplast within the cells (Dengler *et al.* 1994; Maai, Miyake & Taniguchi 2011).

Differences in morphological and physiological relationships between species may reflect contrasting arrangements of decarboxylation tissues found in C_4 grasses (Rao & Dixon 2016). The species of interest included grasses that have NAD-dependent malic enzyme (NAD-ME, which use malate as a transported metabolite) and NADP-dependent malic enzyme (NADP-ME, which use aspartate as the transported metabolite) decarboxylating enzymes. *P. virgatum* is a

NAD-ME grass that holds similar arrangement of tissue as classical Kranz anatomy, but has two distinct layers of bundle sheath cells. *A. gerardii*, *S. nutans*, and *S. schizachyrium* are NADP-ME grasses that have one layer of both mesophyll and bundle sheath cells. While contributions from aspartate and malate are considered equal in their ability to transfer CO₂ for decarboxylation (Meister, Agostino & Hatch 1996; Rao & Dixon 2016), there may be differences in nitrogen-use efficiency due to the reduction of cellular aspartate in NAD-ME grasses (Bräutigam & Gowik 2016; Rao & Dixon 2016).

While grassland ecosystems in Kansas typically encounter periods of summer drought within most years, 2016 had above average rainfall for all locations measured in Kansas. Each site experienced abnormally high levels of rainfall in the latter half of the growing season. The impacts of higher late-season rainfall were most atypical for Albertson prairie (Hays, KS), which typically has hot dry late summer periods. Thus, the conditions at this site in 2016 has made results in species photosynthetic, stomatal conductance, and transpiration rates higher than most years (Maricle & Adler 2011).

The goal of this research was to determine the plasticity of leaf-level physiological and morphological traits among four dominant C₄ grasses. This project addressed three hypotheses which resulted in novel findings. Species' physiology was determined to vary significantly within Kansas rainfall gradient and across the growing season. While leaf morphological traits were observed to vary between species, most traits measured here remained static within species across time and location. I predicted that leaf morphological traits would significantly predict leaf physiology traits. The data showed significant relationships between morphology and physiology within species but not trends were not consistent among all species selected. The two main results gained from this research project are (1) morphological traits within a location and

species is fixed across the growing season. This finding suggests that leaf microanatomical structures produced early in the growing season will constrain the overall physiological response for the growing season. Therefore, early season growth of these species may be an indication of whole-season physiological potential. This suggests that late season droughts may have lower impact on potential physiological responses, than early-season droughts. (2) Because the selected dominant C₄ grasses have varying responses (physiological, morphological, and morphology predicting physiology), models estimating a “general C₄ grass response” are likely underestimating actual interspecific variability. The significance of missing the unique interspecific variability within the functional type (C₄ grass) has yet to be determined, and will require further research.

References

- Adler, P.B. & Levine, J.M. (2007) Contrasting relationships between precipitation and species richness in space and time. *Oikos*, **116**, 221–232.
- Alderson, J. & Sharp, W.. (1994) *Grass Varieties in the United States*.
- Anderson, R.C. (2006) Evolution and origin of the Central Grassland of North America : climate , fire , and mammalian grazers. *Journal of the Torrey Botanical Society*, **133**, 626–647.
- Axelrod, D. (1985) Rise of the Grassland Biome , Central North America. *Botanical Review*, **51**, 163–201.
- Baer, S.G., Blair, J.M., Collins, S.L. & Knapp, A.K. (2003) Soil resources regulate productivity and diversity in newly established tallgrass prairie. *Ecology*, **84**, 724–735.
- Bailey, R.G. (1998) *Ecoregions*, 2nd ed. Springer Berlin Heidelberg, London.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of Climate Change on the Future of Biodiversity. *Ecol Lett.*, **12**, 365–377.
- Berry, J.O. & Patel, M. (2008) Kranz Anatomy and the C4 Pathway. *Encyclopedia of life sciences (ELS)*, 1–6.
- Borchert, J. (1940) The Climate of the Central North. *Annals of the Association of American Geographers*, **40**, 1–39.
- Borchert, J.R. (1950) The Climate of the Central North American Grassland. *Annals of the Association of American Geographers*, **40**, 1–39.
- Bräutigam, A. & Gowik, U. (2016) Photorespiration connects C3 and C4 photosynthesis. *Journal of Experimental Botany*, **67**, 2953–2962.
- Brown, W. (1975) Variations in Anatomy , Associations , and Origins of Kranz Tissue. *American Journal of Botany*, **62**, 395–402.

- Von Caemmerer, S. & Furbank, R. (2003) The C4 pathway: An efficient CO₂ pump. *Photosynthesis Research*, **77**, 191–207.
- Carmo-Silva, A.E., Francisco, A., Powers, S.J., Keys, A.J., Ascensão, L., Parry, M. a J. & Arrabaça, M.C. (2009) Grasses of different C4 subtypes reveal leaf traits related to drought tolerance in their natural habitats: Changes in structure, water potential, and amino acid content. *American Journal of Botany*, **96**, 1222–1235.
- Carpenter, R. (1940) The Grassland Biome. *Ecological Monographs*, **10**, 617–684.
- Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought - From genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.
- Christin, P.-A. & Osborne, C.P. (2014) The evolutionary ecology of C4 plants. *New Phytologist*, **204**, 765–781.
- Christin, P.-A., Osborne, C.P., Chatelet, D.S., Columbus, J.T., Besnard, G., Hodkinson, T.R., Garrison, L.M., Vorontsova, M.S. & Edwards, E.J. (2013) Anatomical enablers and the evolution of C4 photosynthesis in grasses. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 1381–6.
- Cook, B.I., Ault, T.R. & Smerdon, J.E. (2015) Unprecedented 21st century drought risk in the American Southwest and Central Plains. *Science Advances*, **1**, e1400082–e1400082.
- Cook, B.I., Smerdon, J.E., Seager, R. & Cook, E.R. (2014) Pan-Continental Droughts in North America over the Last Millennium. *Journal of Climate*, **27**, 383–397.
- Craine, J.M., Ocheltree, T.W., Nippert, J.B., Towne, E.G., Skibbe, A.M., Kembel, S.W. & Fargione, J.E. (2012) Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*, **2**, 1–5.
- Dengler, N.G., Dengler, R.E., Donnelly, P.M. & Hattersley, P.W. (1994) Quantitative leaf

- anatomy of C3 and C4 Grass (poaceae) Bundle sheath and Mesophyll surface area relationships. *Annals of Botany*, **73**, 241–255.
- Edwards, G.E., Franceschi, V.R., Ku, M.S., Voznesenskaya, E. V, Pyankov, V.I. & Andreo, C.S. (2001) Compartmentation of Photosynthesis in Cells and Tissues of C4 Plants. *Journal of experimental botany*, **52**, 577–590.
- Edwards, E.J., Osborne, C., Stromberg, C., Smith, S. & C4 Grass Consortium. (2010) The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, **328**, 587–590.
- Edwards, E. & Still, C. (2008) Climate, phylogeny and the ecological distribution of C4 grasses. *Ecology Letters*, **11**, 266–276.
- Edwards, E.J., Still, C.J. & Donoghue, M.J. (2007) The relevance of phylogeny to studies of global change. *Trends in Ecology & Evolution*, **22**.
- Ehleringer, J., Cerling, T. & Helliker, B. (1997) C4 Photosynthesis, Atmospheric CO₂ , and Climate. *Oecologia*, **112**, 285–299.
- Eissenstat, D.M. (2000) Root Structure and function in an ecological context. *New Phytologist*, **148**, 353–354.
- Eissenstat, D., Wells, C. & Yanai, R. (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, **147**, 33–42.
- Epstein, H.E., Lauenroth, W.K., Burke, I.C., Coffin, D.P., Ecology, S.P., Coffin, P. & Lauenroth, K. (1998) Regional Productivities of Plant Species in the Great Plains of the. *Plant Ecology*, **134**, 173–195.
- Eters, D., Tarks, P. & Hernandez, M. (2014) Functional response of U.S. grasslands to the early 21st-century drought. *Ecology*, **95**, 2121–2133.

- Fay, P., Carlisle, J.D., Danner, B.T., Lett, M.S., McCarron, J.K., Stewart, C., Knapp, A.K., Blair, J.M. & Collins, S.L. (2002) Altered rainfall patterns, gas exchange, and growth in grasses and forbs. *International Journal of Plant Sciences*, **163**, 549–557.
- Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M. & Collins, S.L. (2000) Altering rainfall timing and quantity in a mesic grassland ecosystem: Design and performance of rainfall manipulation shelters. *Ecosystems*, **3**, 308–319.
- Forrestel, E.J., Donoghue, M.J. & Smith, M.D. (2015) Functional differences between dominant grasses drive divergent responses to large herbivore loss in mesic savanna grasslands of North America and South Africa. *Journal of Ecology*, **103**, 714–724.
- Gilliam, F.S., Seastedt, T. & Knapp, A. (1987) Southwestern Association of Naturalists Canopy Rainfall Interception and Throughfall in Burned and Unburned Tallgrass Prairie. *The Southwestern Naturalist*, **32**, 267–271.
- Grass Phylogeny Working II, G. (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. *New Phytologist*, **193**, 304–312.
- Hatch, M. (1987) C4 Photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochimica et Biophysica Acta*, **895**, 81–106.
- Hoover, D., Duniway, M. & Belnap, J. (2015) Pulse-drought atop press-drought: unexpected plant responses and implications for dryland ecosystems. *Oecologia*, **179**, 1211–1221.
- Hoover, D., Knapp, A.K. & Smith, M.D. (2014a) Contrasting sensitivities of two dominant C4 grasses to heat waves and drought. *Plant Ecology*, **215**, 721–731.
- Hoover, D., Knapp, A. & Smith, M. (2014b) Resistance and resilience of a grassland ecosystem to climate extremes. *Ecology*, **95**, 2646–2656.
- Hoover, D.L. & Rogers, B.M. (2016) Not all droughts are created equal: the impacts of

- interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology*, **22**, 1809–1820.
- Houghton, J., Y, D., DJ, G., M, N., PJ, van der L., X, D., K, M. & C, J. (2001) Climate Change 2001: The Scientific Basis. *Climate Change 2001: The Scientific Basis*, 881.
- IPCC. (2007) *Climate Change 2007 Synthesis Report*.
- Kakani, V.G., Surabhi, G.K. & Reddy, K.R. (2008) Photosynthesis and fluorescence responses of C4 plant *Andropogon gerardii* acclimated to temperature and carbon dioxide. *Photosynthetica*, **46**, 420–430.
- Kellogg, E.A. (1999) Phylogenetic aspects of the evolution of C4 photosynthesis. *C4 Plant Biology* (eds R.F. Sage & R. Monson), pp. 411–444. Academic Press, San Diego.
- Kidder, D.L. (2005) Impact of Grassland Radiation on the Nonmarine Silica Cycle and Miocene Diatomite. *Palaios*, **20**, 198–206.
- Knapp, A.K. (1984) Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia*, **65**, 35–43.
- Knapp, A.K. (1993) Gas Exchange Dynamics in C3 and C4 Grasses: Consequence of Differences in Stomatal Conductance. *Plant physiology*, **74**, 113–123.
- Knapp, A.K. (2013) Effect of Fire and Drought on the Ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a Tallgrass Prairie Author (s): Alan K . Knapp Published by : Ecological Society of America Stable URL : <http://www.jstor.org/stable/1939184> . EFFECT OF FIRE. *Ecological society of America ESA*, **66**, 1309–1320.
- Knapp, A., Fay, P., Blair, J., Collins, S., Smith, M., Carlisle, J., Harper, C., Danner, B., Lett, M. & McCarron, J. (2002a) Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland. *Science*, **298**, 2202–2205.

- Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S. & McCarron, J.K. (2002b) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, **298**, 2202–2205.
- Kromdijk, J., Ubierna, N., Cousins, A.B. & Griffiths, H. (2014) Bundle-sheath leakiness in C₄ photosynthesis : a careful balancing act between CO₂ concentration and assimilation. *Journal of Experimental Botany*, **65**, 3443–3457.
- Lauenroth, W.K., Burke, I.C. & Gutmann, M.P. (1999) The Structure and Function of Ecosystems in the Central North American Grassland Region. *Great Plains Research*, **9**, 223–259.
- Lobell, D.B., Roberts, M.J., Schlenker, W., Braun, N., Little, B.B., Rejesus, R.M. & Hammer, G.L. (2014) Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science (New York, N.Y.)*, **344**, 516–9.
- Lundgren, M.R., Osborne, C.P. & Christin, P. (2014) Deconstructing Kranz anatomy to understand C₄ evolution. *Journal of Experimental Botany*, **65**, 3357–3369.
- Maai, E., Miyake, H. & Taniguchi, M. (2011) Differential positioning of chloroplasts in C₄ mesophyll and bundle sheath cells. *Plant Signaling & Behavior*, 1111–1113.
- Maricle, B.R. & Adler, P.B. (2011) Effects of precipitation on photosynthesis and water potential in *Andropogon gerardii* and *Schizachyrium scoparium* in a southern mixed grass prairie. *Environmental and Experimental Botany*, **72**, 223–231.
- Maxwell, K. & Johnson, G.N. (2000) Chlorophyll fluorescence--a practical guide. *Journal of experimental botany*, **51**, 659–668.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yezzer, E. a. (2008) Mechanisms of plant survival

- and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist*, **178**, 719–739.
- Meister, M., Agostino, A. & Hatch, M. (1996) The roles of malate and aspartate in C4 photosynthetic metabolism of *Flaveria bidentis* (L.). *Planta*, **199**, 262–269.
- Monk, C. (1966) Ecological Importance of Root / Shoot Ratios. *Bulletin of the Torrey Botanical Club*, **93**, 402–406.
- Mueller, K., Blumenthal, M., Pendall, E., Carillo, Y., Dijkstra, F., Williams, D., Follet, R. & Morgan, J. (2016) Impacts of warming and elevated CO₂ on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters*, **19**, 956–966.
- Murchie, E.H. & Lawson, T. (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany*, **64**, 3983–98.
- Nelson, J.A., Morgan, J.A., LeCain, D.R., Mosier, A.R., Milchunas, D.G. & Parton, B.A. (2004) Elevated CO₂ increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado. *Plant and Soil*, **259**, 169–179.
- Nippert, J.B., Fay, P.A., Carlisle, J.D., Knapp, A.K. & Smith, M.D. (2009) Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. *Acta Oecologica*, **35**, 400–408.
- Nippert, J.B., Knapp, A.K. & Briggs, J.M. (2006) Intra-annual rainfall variability and grassland productivity: Can the past predict the future? *Plant Ecology*, **184**, 65–74.
- Ocheltree, T.W., Nippert, J.B. & Prasad, P.V. V. (2012) Changes in stomatal conductance along grass blades reflect changes in leaf structure. *Plant, cell & environment*, **35**, 1040–9.
- Olsen, J.T., Caudle, K.L., Johnson, L.C., Baer, S.G. & Maricle, B.R. (2013) Environmental and genetic variation in leaf anatomy among populations of *Andropogon gerardii* (Poaceae)

- along a precipitation gradient. *American Journal of Botany*, **100**, 1957–1968.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, a. F.M., Pronk, a., Vanguelova, E., Weih, M. & Brunner, I. (2007) Specific root length as an indicator of environmental change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, **141**, 426–442.
- Pérez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S.S., Cornwell, W.K.K., Craine, J.M.M., Gurvich, D.E.E., Urcelay, C., Veneklaas, E.J.J., Reich, P.B.B., Poorter, L., Wright, I.J.J., Etc., Ray, P., Etc., Díaz, S., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S.S., Cornwell, W.K.K., Craine, J.M.M., Gurvich, D.E.E., Urcelay, C., Veneklaas, E.J.J., Reich, P.B.B., Poorter, L., Wright, I.J.J., Ray, P., Enrico, L., Pausas, J.G., Vos, A.C. de, Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., Steege, H. ter, Heijden, M.G.A. van der, Sack, L., Blonder, B., Poschlod, P., Vaieretti, M. V., Conti, G., Staver, A.C., Aquino, S. & Cornelissen, J.H.C. (2013) New Handbook for standardized measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.
- Ramankutty, N. & Foley, J.A. (1999) Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles*, **13**, 997–1027.
- Rao, X. & Dixon, R.A. (2016) The Differences between NAD-ME and NADP-ME Subtypes of C4 Photosynthesis: More than Decarboxylating Enzymes. *Frontiers in plant science*, **7**, 1525.
- Rasband, W. (1997) ImageJ. *U.S National Institutes of Health*.
- Reekie, E.. & Bazzaz, F.. (1987a) Reproductive Effort in Plants. 1. Carbon Allocation to Reproduction. *The American Naturalist*, **129**, 876–896.

- Reekie, E.. & Bazzaz, F.. (1987b) Reproductive Effort in Plants. 2. Does Carbon Reflect the Allocation of Other Resources? *The American Naturalist*, **129**, 897–906.
- Ripley, B.S., Gilbert, M.E., Ibrahim, D.G. & Osborne, C.P. (2007) Drought constraints on C4 photosynthesis: Stomatal and metabolic limitations in C3 and C4 subspecies of *Alloteropsis semialata*. *Journal of Experimental Botany*, **58**, 1351–1363.
- Romm, J. (2011) Desertification: The next dust bowl. *Nature*, **478**, 450–451.
- Roumet, C., Urcelay, C., Díaz, S. & Diaz, S. (2011) Suites of root traits in species growing differ between the and perennial. *New Phytologist*, **170**, 357–367.
- Ruzin, S. (1999) *Plant Microtechnique and Microscopy*. Oxford University Press, New York.
- Sage, R.F. (2004) The evolution of C4 photosynthesis. *New Phytologist*, **161**, 341–370.
- Sala, A.O.E., Parton, W.J., Joyce, L. a & Lauenroth, W.K. (1988) Primary Production of the Central Grassland Region of the United States. *Ecological Monographs*, **69**, 40–45.
- Salinger, J., Sivakumar, M.V.K. & Motha, R.P. (2005) Increasing climate variability and change: Reducing the vulnerability of agriculture and forestry. *Climate Change*, **70**, 9–29.
- Schroeder-Georgi, T., Wirth, C., Nadrowski, K., Meyer, S.T., Mommer, L. & Weigelt, A. (2016) From pots to plots: Hierarchical trait-based prediction of plant performance in a mesic grassland. *Journal of Ecology*, **104**, 206–218.
- Smith, M.D. & Knapp, A.K. (1999) Exotic plant species in a C4 dominated grassland : invasibility , disturbance , and community structure. *Oecologia*, **120**, 605–612.
- Smith, M.D., Knapp, A.K. & Collins, S.L. (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology*, **90**, 3279–3289.
- Still, C.J., Berry, J.A., Collatz, G.J. & DeFries, R.S. (2003) Global distribution of C 3 and C 4

- vegetation: Carbon cycle implications. *Global Biogeochemical Cycles*, **17**, 6-1-6–14.
- Strömberg, C.A.E. (2005) Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11980–11984.
- Strömberg, C. a. E. (2011) Evolution of Grasses and Grassland Ecosystems. *Annual Review of Earth and Planetary Sciences*, **39**, 517–544.
- Taylor, S.H., Ripley, B.S., Martin, T., De-Wet, L.A., Woodward, F.I. & Osborne, C.P. (2014) Physiological advantages of C4 grasses in the field: A comparative experiment demonstrating the importance of drought. *Global Change Biology*, **20**, 1992–2003.
- Taylor, S.H., Ripley, B.S., Woodward, F.I. & Osborne, C.P. (2011) Drought limitation of photosynthesis differs between C3 and C4 grass species in a comparative experiment. *Plant, Cell and Environment*, **34**, 65–75.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature*, **418**, 671–7.
- Tucker, S.S., Craine, J.M. & Nippert, J.B. (2011) Physiological drought tolerance and the structuring of tallgrass prairie assemblages. *Ecosphere*, **2**, art48.
- Vico, G. & Porporato, A. (2008) Modelling C3 and C4 photosynthesis under water-stressed conditions. *Plant and Soil*, **313**, 187–203.
- Volder, A., Tjoelker, M.G. & Briske, D.D. (2010) Contrasting physiological responsiveness of establishing trees and a C4 grass to rainfall events, intensified summer drought, and warming in oak savanna. *Global Change Biology*, **16**, 3349–3362.
- Weaver, J.E. (1968) Prairie plants and their environment. *Agronomy and Horticulture Department*, 276.

Wilson, J. (1988) The Effect of Initial Advantage on the Course of Plant Competition. *Oikos*, **51**, 19–24.

Xu, Z. & Zhou, G. (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, **59**, 3317–3325.

Tables and Figures

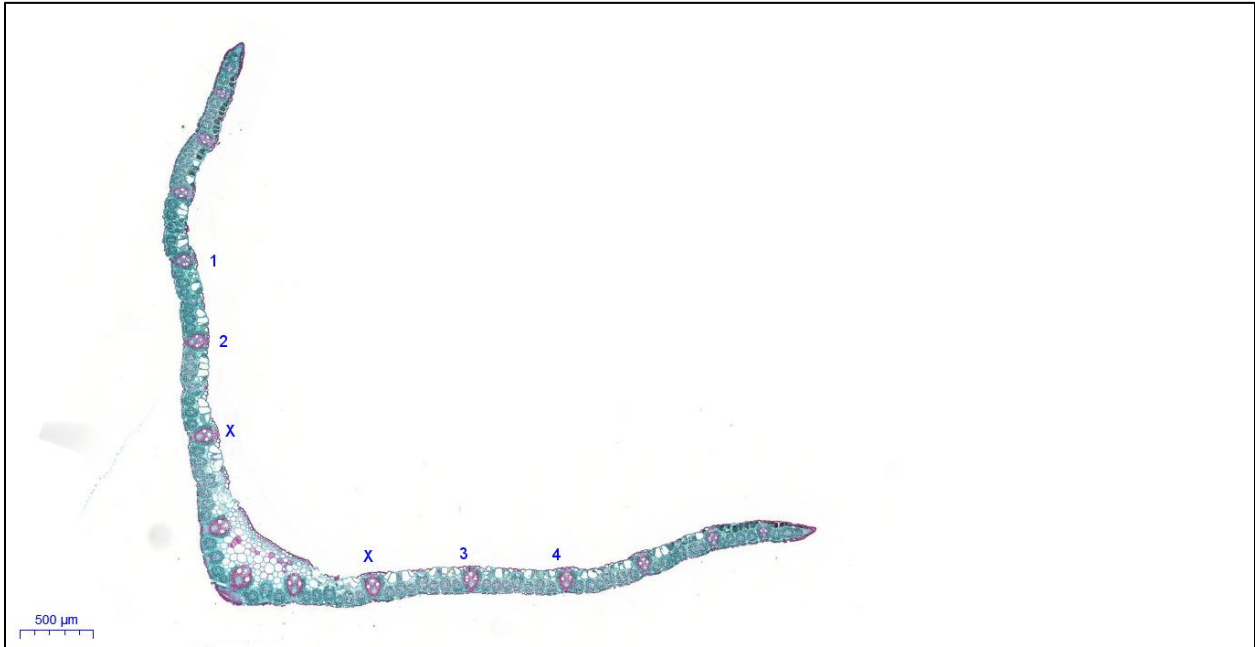


Figure 3.1 *Andropogon gerardii* leaf cross section from Konza Prairie, August of 2016 magnified 10X. Subsamples were taken between numbers 1 and 2; 3 and 4. Midrib was used as the reference point, X indicates a major vein that was unmeasured.

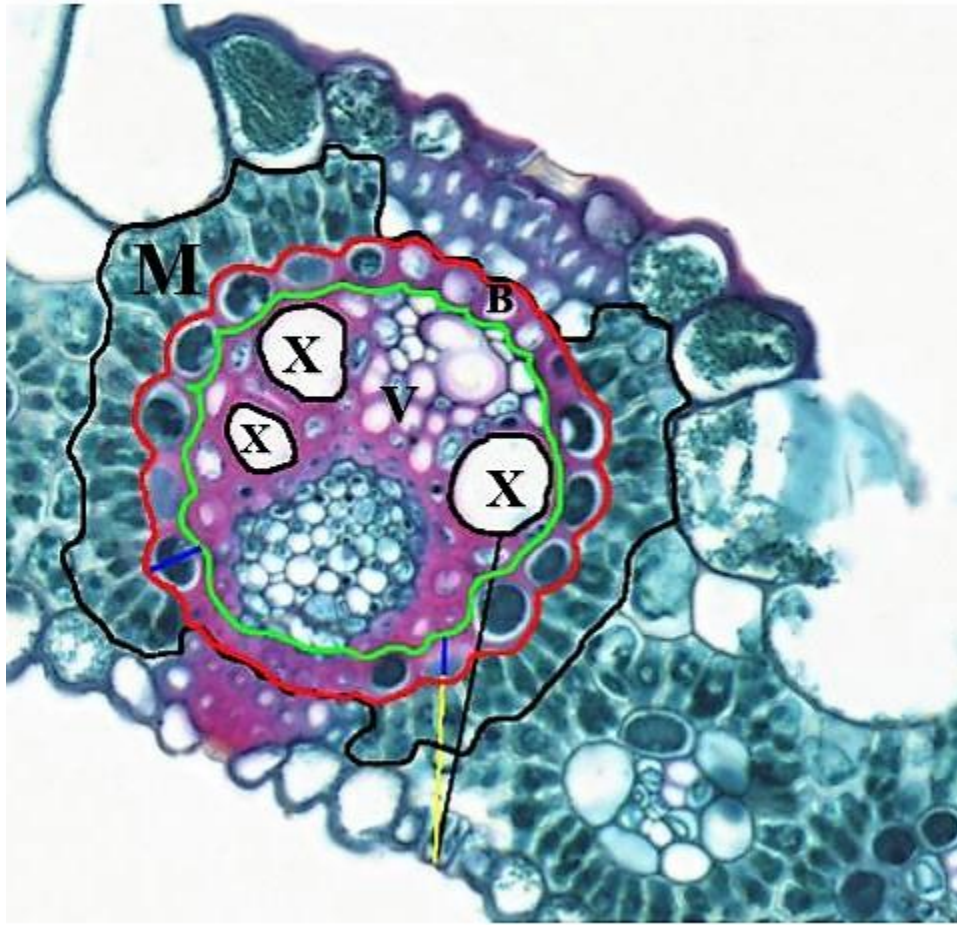


Figure 3.2 Cross section of *Andropogon gerardii* from Konza Prairie, August of 2016 magnified 40X. Mesophyll cells (M), Bundle sheath cells (B), Vein area (V), Xylem area (X), yellow line (AVE D_m), blue line (AVE BS_{Thick}), black line (Stomata_{xylem}).

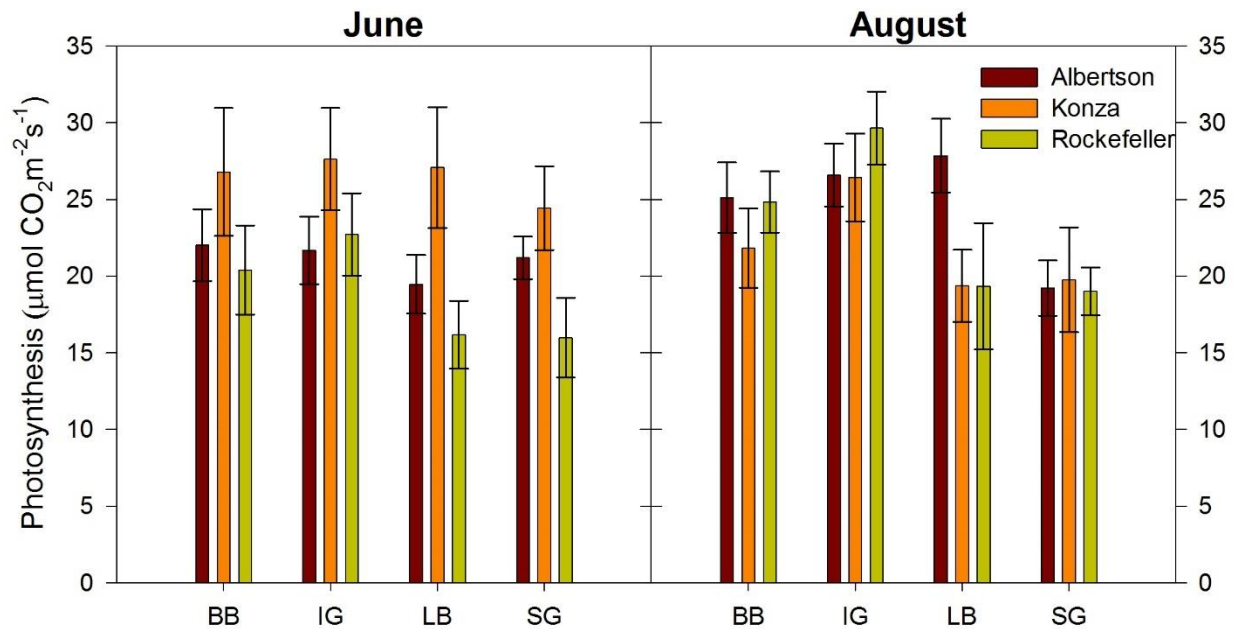


Figure 3.3 Mean maximum photosynthetic rate A_n of each species at all sampling locations. BB: *A. gerardii*; IG: *S. nutans*; LB: *S. scoparium*; SG: *P. virgatum*.

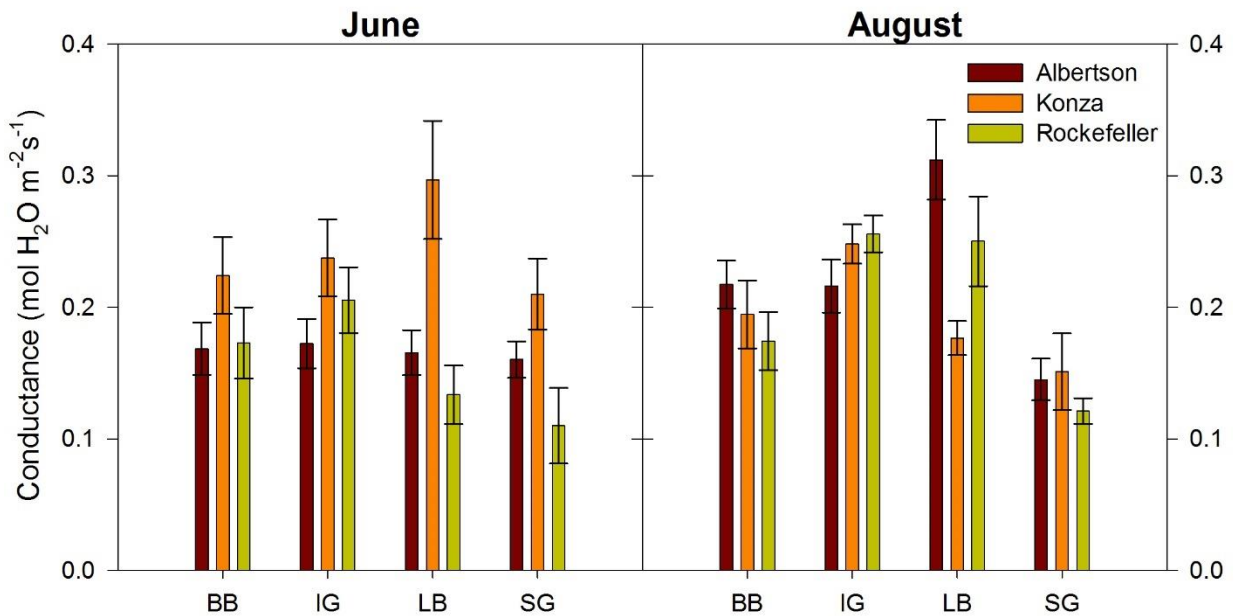


Figure 3.4 Mean stomatal conductance rate g_s of each species at all sampling locations. BB: *A. gerardii*; IG: *S. nutans*; LB: *S. scoparium*; SG: *P. virgatum*.

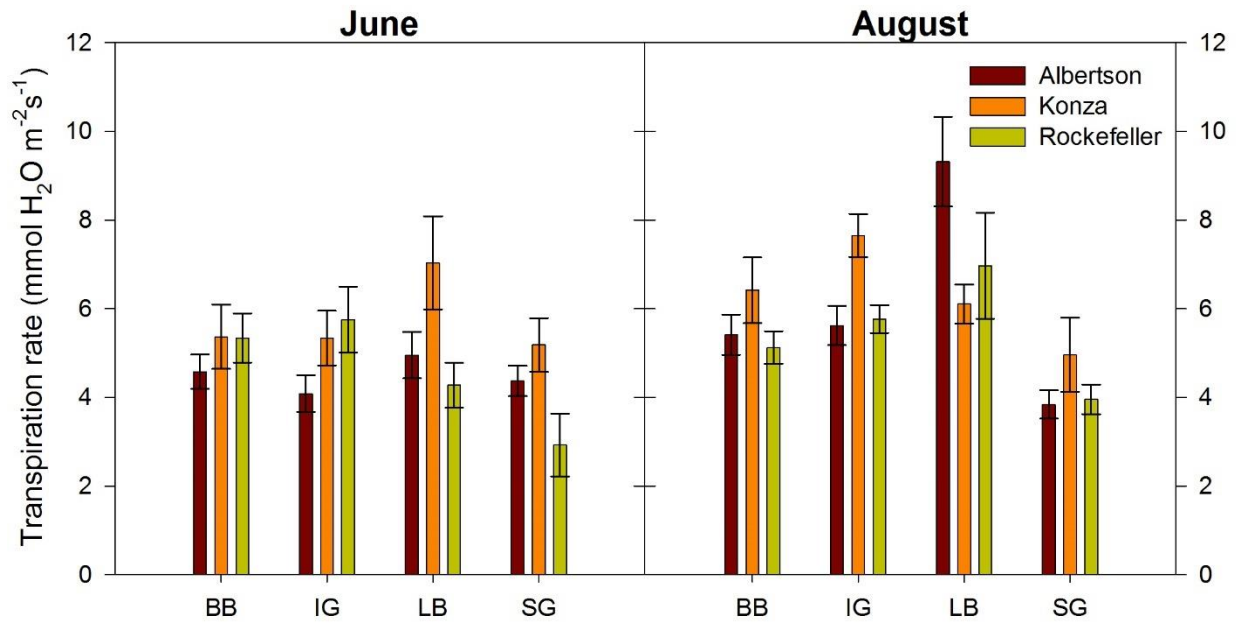


Figure 3.5 Mean transpiration rate E of each species at all sampling locations. BB: *A. gerardii*; IG: *S. nutans*; LB: *S. scoparium*; SG: *P. virgatum*.

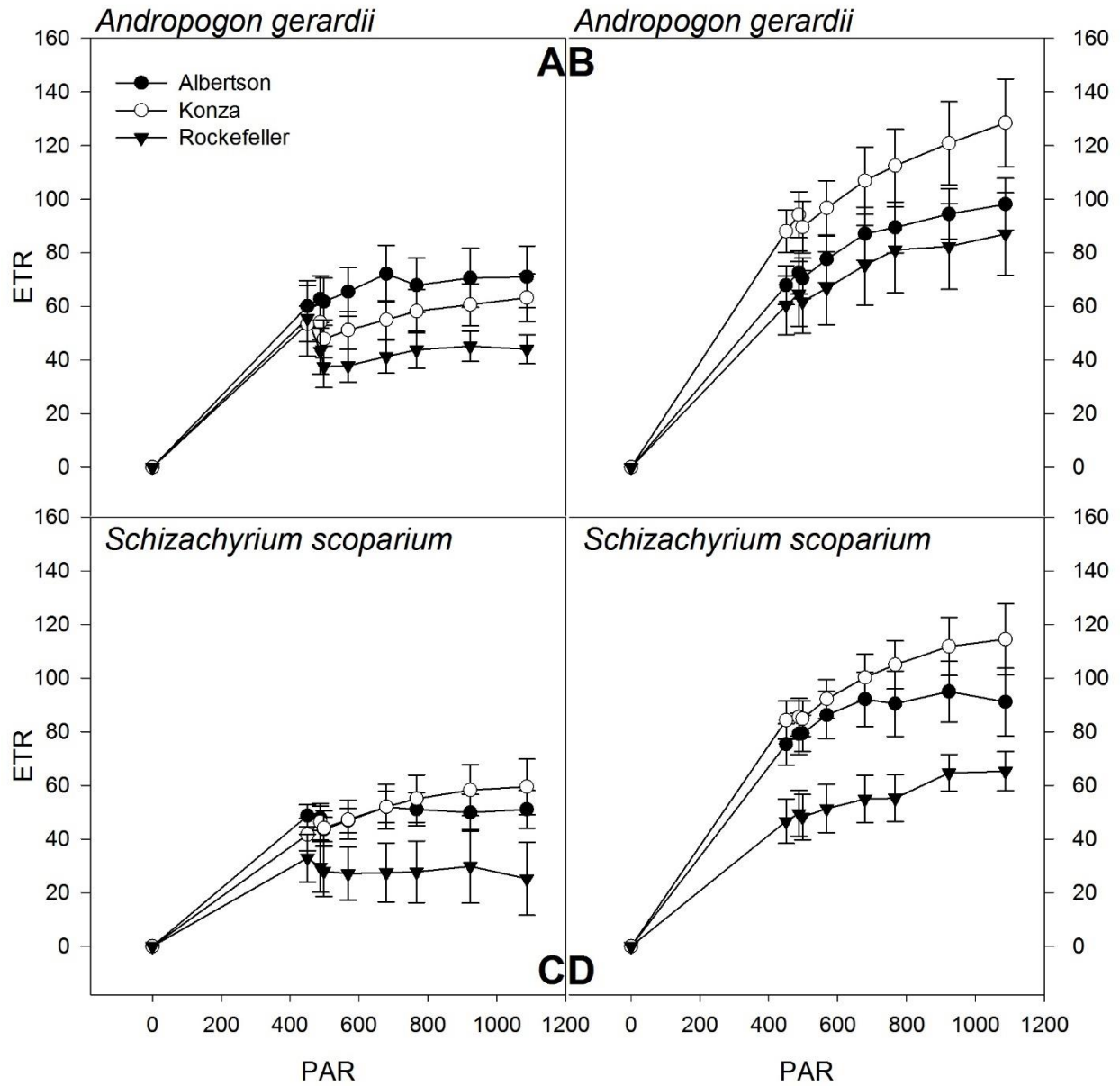


Figure 3.6 Light response curves at all sampling locations of June (A)(C) and August (B)(D). Photosynthetically available radiation (PAR). Values represent means, +/- 1 SE.

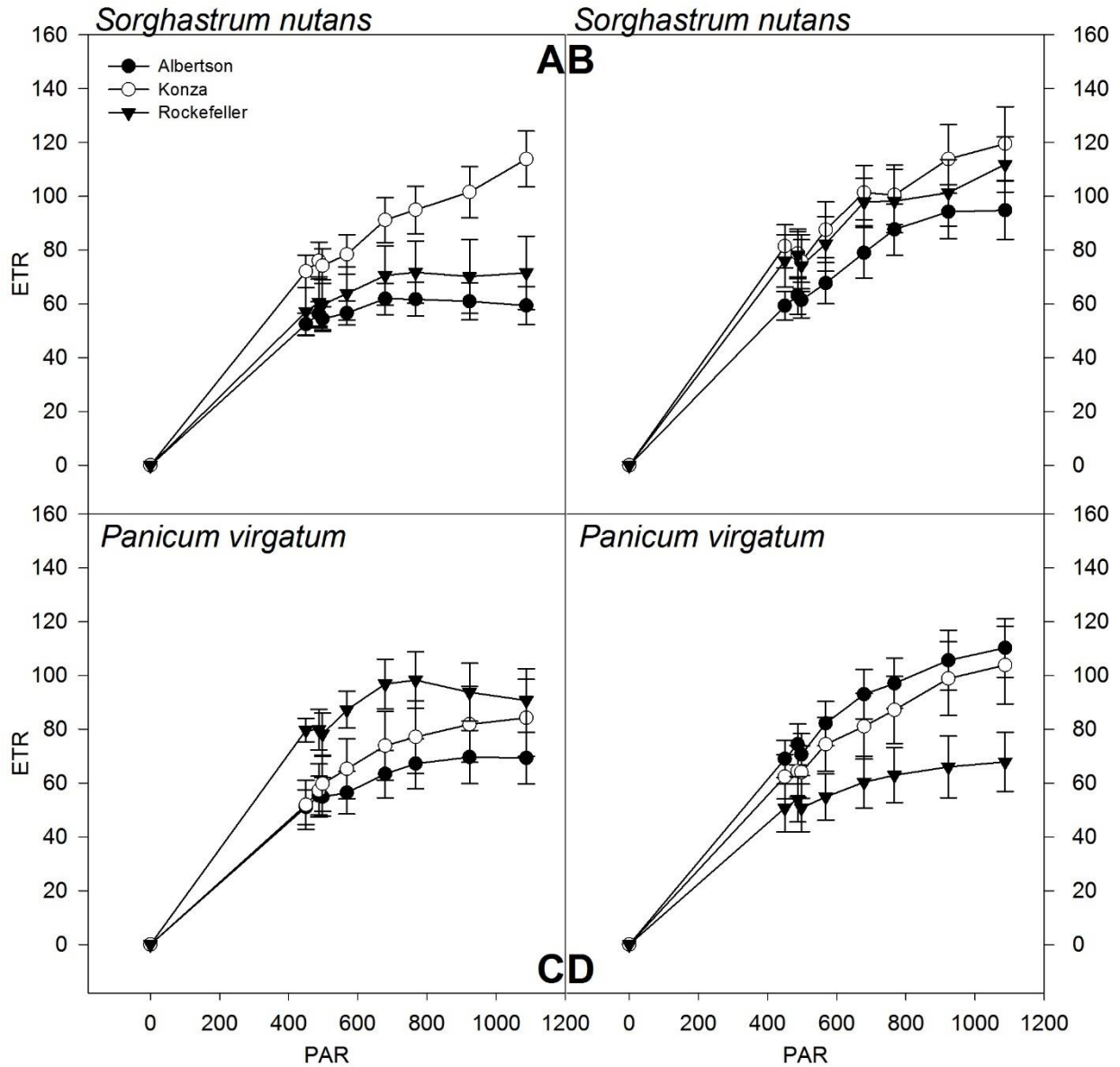


Figure 3.7 Light response curves at all sampling locations of June (A)(C) and August (B)(D). Photosynthetically available radiation (PAR). Values represent means, ± 1 SE.

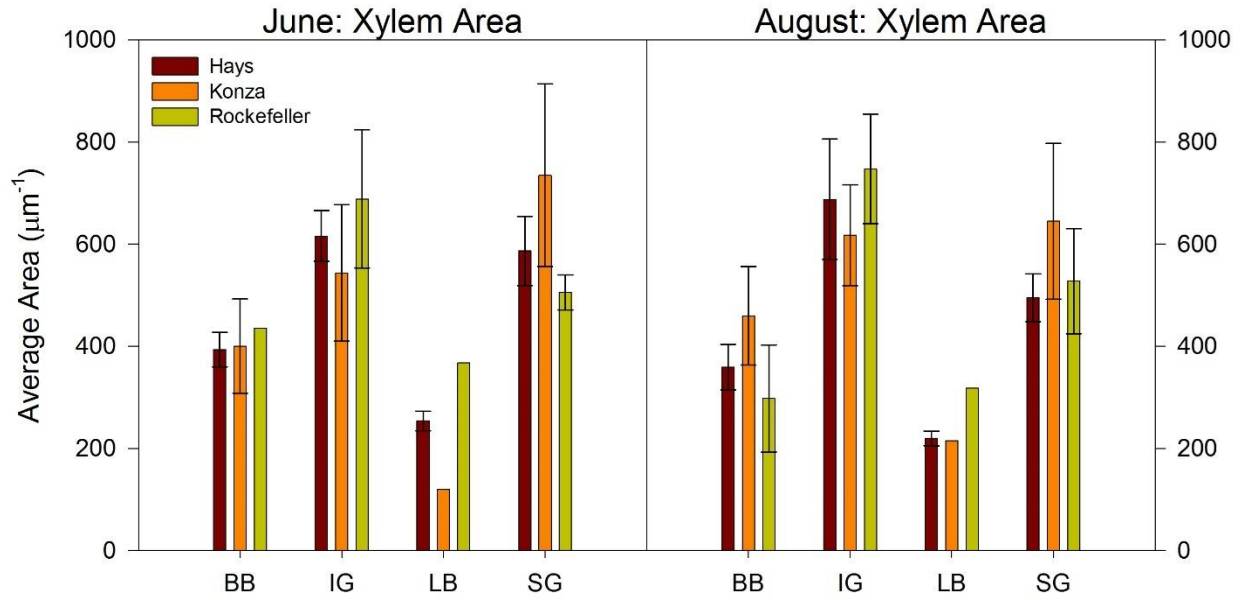


Figure 3.8 Mean xylem area for each species at all three research location in both June and August.

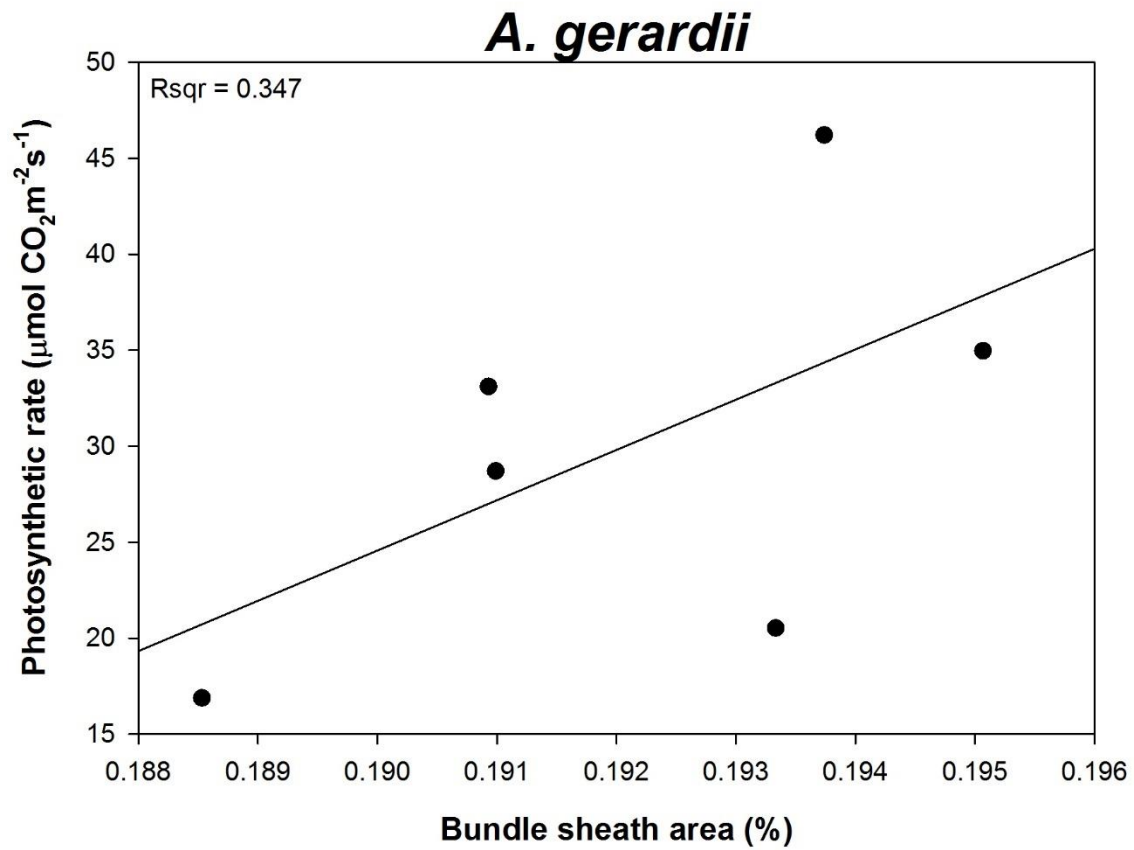


Figure 3.9 Correlation of bundle sheath area (expressed as a % of subsample) and maximum photosynthetic rate (A_n) for *A. gerardii*.

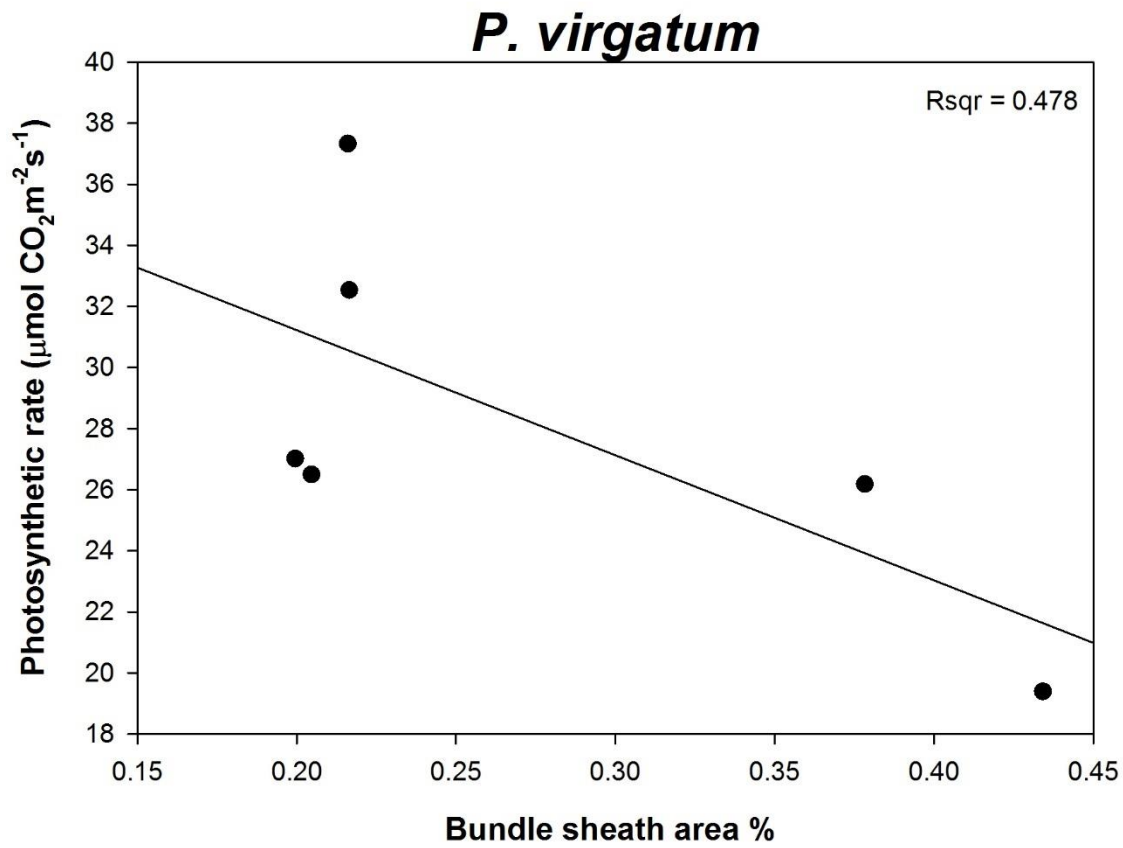


Figure 3.10 Correlation of bundle sheath area (expressed as a % of subsample) and maximum photosynthetic rate (A_n) for *P. virgatum*.

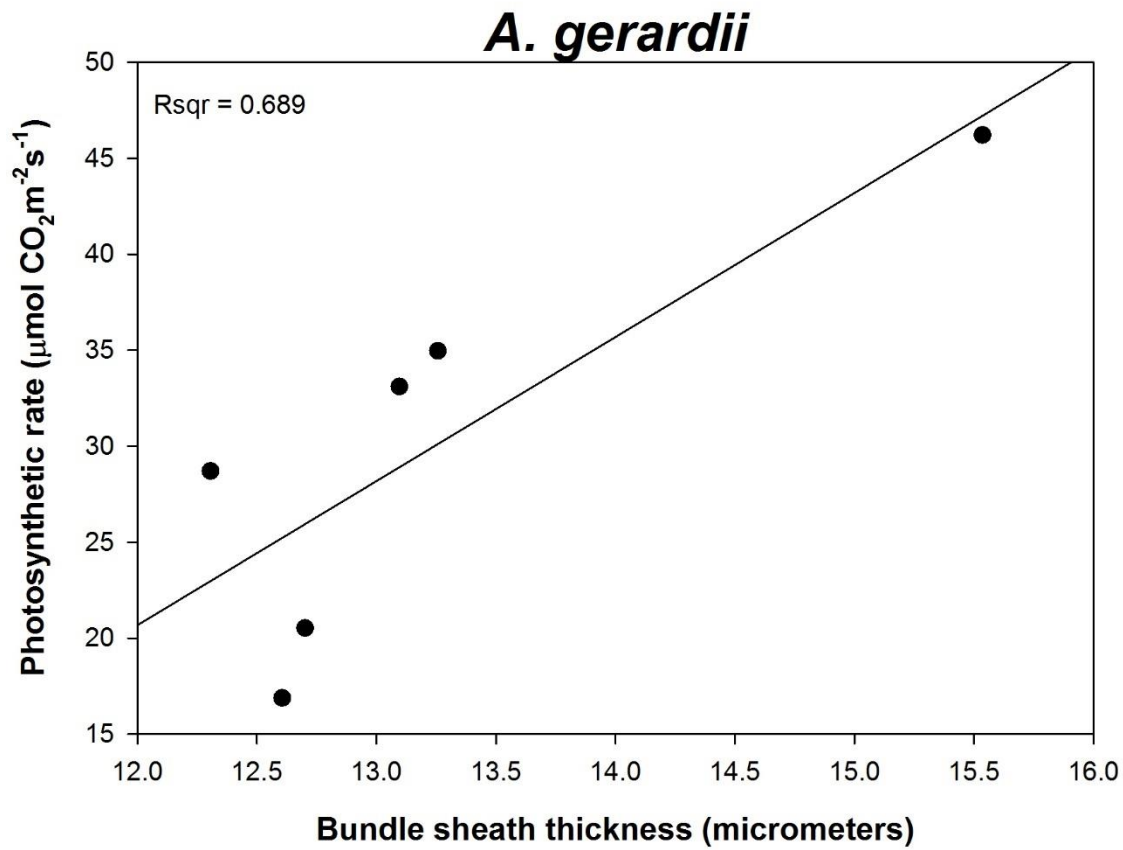


Figure 3.11 Correlation of bundle sheath thickness (μm) and maximum photosynthetic rate (A_n) for *A. gerardii*.

Chapter 4 - Conclusions

Grasslands are a dominant biome that span 25% of the Earth's land surface (Ramankutty & Foley 1999), and contribute to ecological complexity and hold substantial species diversity. However, grasslands commonly contain 'dominant species' – species with disproportionally large impacts on productivity and energy, nutrient, and water budgets (Smith, Knapp & Collins 2009). Dominant species can also have disproportional influence on human diet (Tilman *et al.* 2002), ecological processes (Knapp *et al.* 2002a), and grazer proliferation (Forrestel *et al.* 2015). The grassland biome is characterized by high climate variability which is projected to become even more variable in future climate scenarios over the coming century, likely impacting energy and nutrient/hydrological cycling in grassland ecosystems (Sage 2004; Kidder 2005; Edwards *et al.* 2010). Further research that explores unique phylogenetic, physiological, and morphological traits among grass species responding to climate variability is necessary (Grass Phylogeny Working II 2012).

Climate change is projected to alter current environmental conditions for grassland systems, leading to increased temperature and rainfall variability (IPCC 2007). Increased variability can lead to drought conditions which will negatively affect growth of numerous grassland and agricultural species (Salinger, Sivakumar & Motha 2005; Mueller *et al.* 2016). Although grassland species are able to cope with current climate variability, further understanding of how grasses respond to future intensified climate change scenarios is needed. The goal of this thesis was to characterize the physiological and morphological response of C₃ and C₄ grasses to across a range of water availabilities representing optimal to extreme drought conditions.

In chapter 2, I conducted a greenhouse experiment that compared physiological responses of C₃ and C₄ congeners to dry-down conditions (100% water reduction). Two genera were used in the experiment (*Festuca*: C₃ and *Paspalum*: C₄), and drought tolerant traits were measured daily. Leaf-level physiological traits included A_{max} , g_s , E , and chlorophyll fluorescence; and root-level morphological traits included root length, mass, fine-root proportion, fine-root length, root:shoot ratio, and specific root length (*SRL*). C₄ species typically respond with continued optimal photosynthesis during drought conditions, however, my results indicate an opposite pattern. *Festuca* species (C₃), responded by maintaining optimal gas exchange rates for nearly twice the time compared to *Paspalum* (C₄). Results from the root-level analysis indicate that *Festuca* species have a higher specific root length (*SRL*), a trait that is an indicator of species response to precipitation variability (Ostonen *et al.* 2007). Results from this experiment suggest that leaf and root-level traits also determine a species response to altered water availability.

In chapter 3, I examined the variability of dominant C₄ grass species physiology and morphology in response to varying levels of water availability (across the Kansas rainfall gradient) over a growing season. Four C₄ grasses were examined due to their dominance of Kansas' prairies systems: *A. gerardii*, *S. scoparium*, *S. nutans*, and *P. virgatum*. Results from this experiment indicate that leaf-level physiology is variable between species over spatial and temporal scales, while leaf-level morphology is significantly different between species but static within species. There was also evidence of correlations between morphological (such as bundle sheath area) and physiological traits (photosynthetic rate). Interestingly, statistical relationships among species varied with regard to strength and nature of association. A_{max} for *A. gerardii* increased with more bundle sheath area present, while A_{max} decreased with increasing bundle sheath area in *P. virgatum*. This suggests that although C₄ grasses utilize the same general

photosynthetic pathway, research estimating a “general C₄ grass response” may be missing inter-specific variability existing in grasses.

Within the central Great Plains of North America, the mesic grassland biome will likely be effected by increased climate variability resulting from climate change. Increased temperature and decreased precipitation will be major drivers of species physiological and morphological responses (Eissenstat, Wells & Yanai 2000; Fay *et al.* 2002; Schroeder-Georgi *et al.* 2016). This research has provided a first approximation linking physiological and morphological responses for several grass species at leaf and root-level and provides mechanistic insight valuable for forecasting changes in future novel environments.

References

- Edwards, E.J., Osborne, C., Stromberg, C., Smith, S. & C4 Grass Consortium. (2010) The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, **328**, 587–590.
- Eissenstat, D., Wells, C. & Yanai, R. (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, **147**, 33–42.
- Fay, P., Carlisle, J.D., Danner, B.T., Lett, M.S., McCarron, J.K., Stewart, C., Knapp, A.K., Blair, J.M. & Collins, S.L. (2002) Altered rainfall patterns, gas exchange, and growth in grasses and forbs. *International Journal of Plant Sciences*, **163**, 549–557.
- Forrestel, E.J., Donoghue, M.J. & Smith, M.D. (2015) Functional differences between dominant grasses drive divergent responses to large herbivore loss in mesic savanna grasslands of North America and South Africa. *Journal of Ecology*, **103**, 714–724.
- Grass Phylogeny Working II, G. (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. *New Phytologist*, **193**, 304–312.
- IPCC. (2007) *Climate Change 2007 Synthesis Report*.
- Kidder, D.L. (2005) Impact of Grassland Radiation on the Nonmarine Silica Cycle and Miocene Diatomite. *Palaios*, **20**, 198–206.
- Knapp, A., Fay, P., Blair, J., Collins, S., Smith, M., Carlisle, J., Harper, C., Danner, B., Lett, M. & McCarron, J. (2002) Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland. *Science*, **298**, 2202–2205.
- Mueller, K., Blumenthal, M., Pendall, E., Carillo, Y., Dijkstra, F., Williams, D., Follet, R. & Morgan, J. (2016) Impacts of warming and elevated CO₂ on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters*, **19**, 956–966.

- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M.R., Lõhmus, K., Majdi, H., Metcalfe, D., Olsthoorn, a. F.M., Pronk, a., Vanguelova, E., Weih, M. & Brunner, I. (2007) Specific root length as an indicator of environmental change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, **141**, 426–442.
- Ramankutty, N. & Foley, J.A. (1999) Estimating historical changes in global land cover: Croplands from 1700 to 1992. *Global Biogeochemical Cycles*, **13**, 997–1027.
- Sage, R.F. (2004) The evolution of C4 photosynthesis. *New Phytologist*, **161**, 341–370.
- Salinger, J., Sivakumar, M.V.K. & Motha, R.P. (2005) Increasing climate variability and change: Reducing the vulnerability of agriculture and forestry. *Climate Change*, **70**, 9–29.
- Schroeder-Georgi, T., Wirth, C., Nadrowski, K., Meyer, S.T., Mommer, L. & Weigelt, A. (2016) From pots to plots: Hierarchical trait-based prediction of plant performance in a mesic grassland. *Journal of Ecology*, **104**, 206–218.
- Smith, M.D., Knapp, A.K. & Collins, S.L. (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology*, **90**, 3279–3289.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. (2002) Agricultural sustainability and intensive production practices. *Nature*, **418**, 671–7.

Appendix A - Additional Information

Table A.1 Precipitation data (mm) of each research site. Percentage of annual rainfall received, and average soil moisture of location Acquired from local weather and airport stations.

	May-June Precipitation	% of Annual Rainfall	Soil Moisture	July-August Precipitation	% of Annual Rainfall	Soil Moisture
Albertson	78.99	13.2	0.17	130.81	21.9	0.23
Konza	149.35	16.6	0.34	120.65	13.4	0.35
Rockefeller	217.42	21.4	0.40	127.76	12.6	0.32

Table A.2 ANOVA results for species, location, month, and interaction effects for photosynthetic rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean Square</i>	<i>F value</i>	<i>P value</i>
Location (L)	2	205.7	3.090	.04777
Species (S)	3	306.6	4.607	.00389
Month (M)	1	28.5	0.428	.51362
L x S	6	48.0	0.722	.63267
L x M	2	451.9	6.791	.00142
S x M	3	54.2	0.814	.48731
L x S x M	6	42.2	0.634	.70257

Table A.3 ANOVA results for species, location, month, and interaction effects for stomatal conductance rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean Square</i>	<i>F value</i>	<i>P value</i>
Location (L)	2	0.03405	6.376	0.00209
Species (S)	3	0.06126	11.471	0.000000598
Month (M)	1	0.01003	1.878	0.17222
L x S	6	0.00640	1.198	0.30880
L x M	2	0.06253	11.710	0.0000160
S x M	3	0.01168	2.187	0.09099
L x S x M	6	0.01739	3.256	0.00453

Table A.4 ANOVA results for species, location, month, and interaction effects for transpiration rate. Numbers in bold show significance ($P < 0.05$).

	<i>DF</i>	<i>Mean square</i>	<i>F value</i>	<i>P value</i>
Location (L)	2	23.76	6.597	0.001696
Species (S)	3	46.40	12.883	0.000000106
Month (M)	1	54.04	25.004	0.000147
L x S	6	6.13	1.702	0.122471
L x M	2	4.47	1.240	0.291787
S x M	3	9.13	2.584	0.054587
L x S x M	6	13.68	3.798	0.001343

Table A.5 Mean values for each measured physiological and morphological trait of *A. gerardii*. Missing values indicate trait could not be measured for sample.

	Albertson June		Konza June		Rockefeller June		Albertson August		Konza August		Rockefeller August	
	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>A. gerardii</i>	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>A_{max}</i>	34.96	3.76	46.19	5.81	20.51		33.10	5.60	16.87	1.29	28.70	0.73
<i>G_s</i>	0.27	0.03	0.35	0.04	0.16		0.25	0.03	0.19	0.03	0.20	0.01
<i>E</i>	6.61	0.64	8.23	0.96	5.86		6.64	0.77	6.53	1.04	5.72	0.35
<i>Soil Moisture</i>	0.22	0.01	0.24	0.11	0.45		0.24	0.01	0.35	0.03	0.32	0.01
<i>Max ETR</i>	109.30	11.13	96.50	12.84	98.80		131.90	4.88	156.10	19.35	89.90	17.55
<i>IVD</i>	95.48	4.07	99.15	8.02	85.31		85.13	3.26	91.87	4.98	85.47	1.80
<i>AVE Xylem Area (μm²)</i>	393.16	33.77	615.69	92.24	253.47		358.92	44.55	687.42	96.50	219.31	104.89
<i>% Vein Area</i>	0.17	0.01	0.150	0.01	0.16		0.17	0.01	0.19	0.01	0.17	<0.00
<i>% Bundle Sheath area</i>	0.20	0.01	0.19	0.02	0.19		0.19	0.018	0.19	0.01	0.19	0.01
<i>Mesophyll Area (%)</i>	0.64	0.01	0.64	0.01	0.63		0.64	0.02	0.65	0.01	0.66	<0.00
<i>BS : MS</i>	0.31	0.02	0.35	0.03	0.38		0.30	0.03	0.31	0.01	0.36	0.01
<i>AVE D_m (μm)</i>	49.42	3.37	46.45	5.88	40.51		45.45	4.42	39.68	1.15	41.75	5.35
<i>AVE BS_{thick} (μm)</i>	13.26	0.37	15.54	1.26	12.70		13.10	0.85	12.61	0.31	12.31	0.61
<i>Stomata_{xylem} (μm)</i>	77.23	5.42	73.98	7.29	65.88		79.82	6.88	69.54	3.93	78.44	2.74

Table A.6 Mean values for each measured physiological and morphological trait of *S. nutans*.

	Albertson June		Konza June		Rockefeller June		Albertson August		Konza August		Rockefeller August	
	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>S. nutans</i>	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>A_{max}</i>	34.73	3.60	47.31	6.78	29.61	2.17	35.89	3.80	25.83	4.28	38.38	3.36
<i>G_s</i>	0.27	0.03	0.37	0.05	0.23	0.01	0.28	0.04	0.23	0.01	0.31	0.02
<i>E</i>	6.31	0.71	8.35	0.53	8.02	0.86	6.62	0.89	6.82	0.48	6.91	0.42
<i>Soil Moisture</i>	0.10	0.03	0.22	< 0.00	0.43	0.02	0.17	0.02	0.34	<0.00	0.28	0.01
<i>Max ETR</i>	70.8	12.53	168.5	19.60	149.3	23.87	155.2	17.55	195.9	34.00	143.8	14.27
<i>IVD</i>	130.32	4.50	118.72	12.79	113.46	11.08	127.55	7.30	117.96	8.01	112.09	2.65
<i>AVE Xylem Area (μm²)</i>	586.44	49.42	400.19	133.29	543.35	135.07	494.67	117.84	459.37	98.94	616.96	107.56
<i>% Vein Area</i>	0.17	0.01	0.161	0.02	0.18	0.01	0.16	0.01	0.19	0.01	0.21	0.01
<i>% Bundle Sheath area</i>	0.36	0.01	0.22	0.01	0.17	0.01	0.35	0.01	0.19	0.02	0.19	< 0.00
<i>Mesophyll Area (%)</i>	0.51	0.01	0.63	0.01	0.67	< 0.00	0.52	0.01	0.62	0.01	0.62	0.01
<i>BS : MS</i>	0.30	0.01	0.26	0.02	0.32	0.02	0.29	0.01	0.31	0.03	0.35	0.01
<i>AVE D_m (μm)</i>	49.20	4.22	44.46	3.44	38.85	1.90	46.44	1.42	38.03	2.96	35.31	3.99
<i>AVE BS^{thick} (μm)</i>	17.89	1.00	16.08	0.61	15.61	0.51	23.71	6.48	14.89	0.41	15.37	0.54
<i>Stomata_{xylem} (μm)</i>	80.38	4.65	74.61	4.20	60.72	2.02	81.81	4.35	71.50	4.41	70.35	8.05

Table A.7 Mean values for each measured physiological and morphological trait of *S. scoparium*. Missing values indicate trait could not be measured for sample.

	Albertson June		Konza June		Rockefeller June		Albertson August		Konza August		Rockefeller August	
<i>S. scoparium</i>	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>A_{max}</i>	19.85	2.14	33.82		20.58		39.78	7.88	16.51		20.64	
<i>G_s</i>	0.12	0.01	0.27		0.11		0.37	0.09	0.16		0.35	
<i>E</i>	3.65	0.38	6.73		4.22		12.31	2.93	4.63		7.19	
<i>Soil Moisture</i>	0.16	0.02	0.38		0.33		0.24	0.04	0.36		0.31	
<i>Max ETR</i>	75.3	16.28	35.7		42.6		151.1	18.49	117.4		75	
<i>IVD</i>	77.47	3.02	74.77		99.74		73.28	1.68	71.73		85.47	
<i>AVE Xylem Area (μm²)</i>	119.43	19.09	734.75		407.94		214.82	14.26	644.41		297.66	
<i>% Vein Area</i>	0.18	0.01	0.15		0.16		0.15	<0.00	0.19		0.15	
<i>% Bundle Sheath area</i>	0.21	<0.00	0.37		0.23		0.20	0.02	0.38		0.22	
<i>Mesophyll Area (%)</i>	0.650	0.01	0.532		0.606		0.619	0.02	0.512		0.608	
<i>BS : MS</i>	0.31	0.01	0.31		0.35		0.29	0.04	0.32		0.34	
<i>AVE D_m (μm)</i>	40.39	3.80	51.98		40.77		44.81	1.57	35.85		39.04	
<i>AVE BS_{thick} (μm)</i>	14.69	1.92	16.80		12.80		13.47	1.60	10.85		12.64	
<i>Stomata_{xylem} (μm)</i>	63.28	4.45	80.49		61.69		66.12	3.87	62.14		60.52	

Table A.8 Mean values for each measured physiological and morphological trait of *P. virgatum*.

	Albertson June		Konza June		Rockefeller June		Albertson August		Konza August		Rockefeller August	
	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE	Mean	STE
<i>P. virgatum</i>												
<i>A_{max}</i>	27.02	2.41	32.53	4.86	26.18	3.79	26.49	3.72	37.33	7.63	19.39	0.91
<i>G_s</i>	0.25	0.03	0.30	0.05	0.21	0.03	0.21	0.03	0.35	0.10	0.16	0.02
<i>E</i>	6.18	0.61	7.49	1.38	4.43	0.32	4.86	0.67	10.64	2.94	4.23	0.38
<i>Soil Moisture</i>	0.17	0.03	0.26	0.04	0.42	0.01	0.23	0.03	0.39	0.02	0.32	0.01
<i>Max ETR</i>	79.9	7.68	57.1	6.3	163.4	9.66	125.1	9.61	54.8	1.60	143	25.20
<i>IVD</i>	171.57	10.33	187.22	5.85	159.06	7.23	158.05	2.77	165.60	23.24	144.01	9.23
<i>AVE Xylem Area (μm²)</i>	688.22	67.67	367.11	178.78	505.56	34.17	747.00	46.46	318.14	152.42	527.28	143.29
<i>% Vein Area</i>	0.12	<0.00	0.10	0.01	0.10	<0.00	0.13	0.02	0.11	<0.00	0.14	0.02
<i>% Bundle Sheath area</i>	0.20	0.01	0.22	<0.00	0.38	<0.00	0.21	0.02	0.22	0.03	0.43	0.07
<i>Mesophyll Area (%)</i>	0.62	0.01	0.62	0.01	0.52	<0.00	0.58	<0.00	0.63	0.04	0.43	0.06
<i>BS : MS</i>	0.71	0.03	0.71	0.01	0.73	0.01	0.67	0.04	0.76	0.12	1.171	0.39
<i>AVE D_m (μm)</i>	37.54	3.05	43.39	2.34	35.59	2.55	35.30	1.41	33.71	3.39	31.54	1.17
<i>AVE BS_{thick} (μm)</i>	27.79	3.81	26.60	0.23	24.08	1.28	24.07	0.34	22.66	0.93	21.64	1.01
<i>Stomata_{xylem} (μm)</i>	88.39	9.37	91.27	7.76	78.38	3.89	81.46	4.56	78.22	0.73	70.52	3.11