A study of grass structure and function in response to drought and grazing

by

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B.S., Kansas State University, 2017

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

2021

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Abstract

Grass species have variable drought tolerance, which has been shown to contrast among grass lineages and between photosynthetic pathways (C_3,C_4). Knowledge of which structural or functional traits may allow certain grass species from different grass tribes to tolerate severe drought remain limited. In the first half of my thesis, I examined how several grass species responded to drought by measuring leaf-level physiology, and morphological traits of leaves and roots to address: 1) how do leaf-level gas-exchange responses to drought vary among photosynthetic pathways (C_3, C_4) and/or between grass tribes (Andropogoneae, Cynodonteae, Paniceae, and Danthonieae) over a time course of well-watered, to maximum drought, and following recovery? And 2) do morphological traits (e.g., leaf area, root length) vary between grass lineages and/or photosynthetic pathways? I found that grasses using the C_4 photosynthetic pathway were no more resilient than C_3 grasses under severe drought. This work also demonstrated that species from closely related groups shared common morphological characteristics and displayed similar physiological responses to drought, despite using different photosynthetic pathways, emphasizing the need to include phylogeny in ecophysiological research.

The second half of my work, I investigated belowground dynamics in response to grazing of two tallgrass prairie species (*Andropogon gerardii* and *Sorghastrum nutans*) from three sites in the Great Plains of North America. Grasses have co-evolved with disturbance (e.g., fire and grazing), resulting in large investments into belowground biomass. Roots and belowground storage organs (e.g., rhizomes) are responsible for acquiring limited resources, carbohydrate storage, and resprouting after disturbances. Grazing has the potential to alter growth of these belowground structures, yet few investigations examine root responses in paired

grazed/ungrazed locations or beyond just the superficial depths of the soil profile. Here, I explored how grazing altered grass root and rhizome structure and function by taking soil cores from grazed and ungrazed treatment areas in three native tallgrass prairies, where I compared root traits and non-structural carbohydrates (NSCs) by soil depth. I addressed two main questions: 1) does grazing alter root morphology (e.g., length, diameter) or root and rhizome biomass? 2) does grazing reduce non-structural carbohydrates? Overall, I observed root and rhizome biomass, root length, and starch (NSCs) were smaller in grazed treatment areas. All other root traits and non-structural carbohydrates were found to contrast between grazing treatments but varied in extent by location and soil depth. This investigation provides us a better understanding of how grazing can alter these belowground organs and reinforces the need for continued belowground ecological research, as changes in grassland belowground biomass and storage have the potential to negatively affect grass fitness and survival and grassland ecosystem services (e.g., carbon storage).

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Acknowledgements

I am so thankful for all of the opportunities I have been afforded while at Kansas State University. I am especially grateful to Jesse Nippert, for not only being an amazing advisor, but for giving me the opportunity to work in your lab as an undergrad and later as a graduate student. Those opportunities changed my life. I would also like to thank my committee members, Colby Moorberg and Mark Ungerer, for your patience and knowledge. Colby, you were a wonderful professor and Mark, I am so grateful for the opportunity to have worked on DIMBIO with you and your lab.

I am so thankful for my lab mates and mentors, Rory O'Connor, Seton Bachle, and Emily Wedel. You all are some of these most amazing people I have met. I could not have made it this far without all of you. I am so grateful for everything you have taught me, for always being willing to help me out in the field or in the lab, and for making research fun. Rory, thank you so much for 'stealing' me from the LTER and putting so much effort into teaching me to become a better field researcher, starting me on my path to R, and teaching me all you know about NSCs. Emily, thank you for making me laugh even on the hardest workdays. I will never forget almost losing the corer on the prairie with you. Seton, words cannot express how thankful I am for your help over the years, from teaching me R, being my co-teacher for Phys-lab, and spending hours upon hours digging up soil cores on the prairies.

I also want to thank every undergraduate technician I had an opportunity to work with and mentor. Lyndsey Swartz, Jeremiah Ruiz, Samuel Long, Madison Lofing, Sarah Inskeep, Chase Torla, Jessica Schauf, Makenna (Miller) Hotz, Greg Tooley, and the entire LTER clip crew. "My" work is actually "our" work, as none of this was possible without all of you. Despite the incredibly early mornings, scorching hot days, backbreaking work, ticks, and the hundreds of

hours spent bent over picking roots you all kept at it and made every day so much fun! I loved working with all of you!

And of course, thank you to all the amazing professors at the Division of Biology and the incredible staff and PIs at the Konza Prairie Long Term Ecological Research. Big thanks to Amanda Kuhl for her help and letting me borrow her crew for weeks. Thank you to Patrick O'Neal for teaching me how to use the soil corer, and to John Blair and John Briggs for allowing us to dig up Konza Prairie. Thank you to both Lydia Zeglin and Zhilong Yang for letting me use your lab space and equipment to run NSCs. Thank you to both Chris Helzer and Brian Obermeyer (of The Nature Conservancy) for allowing us to dig up Platte River Prairie and the Flint Hills Prairie Preserve. This work would never have been possible without the support from Kansas State University Division of Biology, Konza Prairie LTER, and the National Science Foundation.

Dedication

To my husband, I will never be able to thank you enough for supporting my dreams.

Chapter 1 - Introduction

Grassland ecosystems cover over 30% of the terrestrial surface globally and are dominated by species from the *Poaceae* (grass) family that are responsible for 20-25% of terrestrial productivity (Gibson 2008). Grassland ecosystems support habitat for wildlife, biodiversity, ecosystem services (e.g., biogeochemical cycling and carbon storage) and forage for native herbivores and domestic livestock production (Blair et al. 2014). Due to anthropogenic exploitations, grasslands are among the most endangered ecosystems in the world but are receiving the least conservation protection (Carbutt et al. 2017). Within North America, the tallgrass prairies of the Great Plains have been reduced to approximately 9% of the original land remaining as a result of conversion of land for agriculture, livestock production, and urbanization (Gibson 2008).

Grasses have co-evolved with fire, grazing, and drought, resulting in unique morphological and physiological characteristics that allow these plants to survive a range of environmental conditions (Knapp et al. 1998). One such evolutionary adaptation is the C_4 photosynthetic pathway in grasses. This adaptation evolved in response to lower CO_2 concentrations (at least 30 million years ago) but has since allowed grasses with this pathway to expand into and dominate warmer, drier regions due to key biochemical and spatial configurations within their leaves that decrease water loss while maintaining photosynthetic operations (Sage et al. 2012, Christin and Osborne 2014). Even though C_4 grasses have higher water use efficiencies on average than C_3 grasses, recent work has suggested that all grasses remain vulnerable to severe drought and tolerance to drought contrasts among different grass lineages (Nippert et al. 2007, 2009, Taylor et al. 2010, 2011). Grass species' belowground

structure (e.g., roots and rhizomes) and function (water and nutrient absorption) are responsible for their success and persistence in disturbed areas. Belowground biomass is estimated to average between 700-2100g m^{-2} (Rice et al. 1998) with the majority of biomass contained within the top 30 cm of soil (Nippert and Knapp 2007a, 2007b, Nippert et al. 2012). Early work from grassland ecologists in the 1900's demonstrated that roots and rhizomes were key adaptations to grassland plants tolerance during the drought of the 1930s and facilitated their recovery after the drought ended (Weaver 1968). Besides acquisition of soil resources, belowground plant organs (e.g., rhizomes) allow perennial grasses to resprout after fire and grazing disturbances, perpetuating the survival and dominance of perennial grass species in these regions (Benson and Hartnett 2006, Dalgleish and Hartnett 2009).

Global climate change is expected to increase dramatic climate events (e.g., drought), change precipitation patterns, and potentially intensify degradation in ecosystems, such as grasslands (IPCC 2014). Drought has the capability to decrease grassland productivity and alter plant species composition, resulting in a loss of wildlife habitat, changed biogeochemical cycles and carbon storage, and decreased livestock production (Craine et al. 2013). The interaction of grazing and drought could increase grassland invasibility of non-native species, increase albedo and evapotranspiration, further decreasing soil moisture (Asner et al. 2004), and increasing woody expansion (Ratajczak et al. 2012).

Despite their dominant presence in the second to largest biome on Earth (grasslands and savannas), grasses remain poorly represented in ecological studies (Craine et al. 2013). Research investigating how grasses respond to drought based on their photosynthetic pathway (C_3, C_4) has increased over the last few decades (Knapp 1993, Nippert et al. 2009), however we have only just begun to understand how drought tolerance in grasses vary according to their phylogeny and

morphological features (Taylor et al. 2010a). Grasses provide forage for sustaining livestock production, which is projected to increase with the growing human population (Asner et al. 2004). However the impact of cattle grazing on belowground plant traits of varying grass species remains rudimentary (Nippert et al. 2012, Laliberté 2017, Klimešová et al. 2018).

The broad objectives of this research were to 1) investigate how drought tolerance varies among grass species from different tribes and 2) understand how the effects of grazing alter belowground dynamics. In Chapter 2, I recorded changes in gas-exchange and measured morphological traits in order to understand how grass drought tolerance differs between photosynthetic pathway and phylogeny. In the face of future climate change, it is important to understand what characteristics (physiological or morphological) may allow grass species to tolerate or recover from severe drought. In Chapter 3, I investigated the effects of grazing on grass root traits (i.e., biomass, length, and diameter), rhizome biomass, and non-structural carbohydrates. This study allowed me to look at changes in root and rhizome biomass by soil depth and compare responses among three sites across the Great Plains of North America. This work will increase our fundamental knowledge of grass root traits and record how grazing may alter root structure and function within the tallgrass prairie.

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Chapter 2 - Drought response and morphological traits vary according to grass tribes

Introduction

Drought is expected to increase in severity and frequency across savannas and grasslands with future climate change (IPCC 2014). Grassland ecosystems cover over 30% of terrestrial land mass, provide 20-30% of annual terrestrial productivity, support biodiversity, sustain livestock production, and other various ecosystem services (e.g., biogeochemical cycles and carbon storage) (Gibson 2008). As the dominant species within these systems, grasses are responsible for the majority of this aboveground biomass and the ecosystem services provided by grasslands (Blair et al. 2014). Understanding what plant traits may allow grasses to cope with future drought is important for predicting not only grass plant productivity and survival, but the future of grassland ecosystem services and function.

Grasses have a range of drought tolerance strategies that are a combination of physiological and morphological traits allowing them to exist in a range of mesic to xeric landscapes (Tucker et al. 2011, Craine et al. 2013). The evolutionary adaptation, C_4 photosynthesis, has allowed some grass species to persist in warmer, drier environments without risk of photorespiration, unlike C_3 plants (Sage 2004). This adaptation has been associated with a conservative drought tolerance strategy due to a high-water use efficiency (*WUE*). This high *WUE* was previously suggested as being responsible for C_4 grasses' dominance in drier environments (Knapp 1993). However, C_4 drought tolerance has recently been shown to vary under different severities of drought and among different grass lineages (Nippert et al. 2007, 2009, Taylor et al. 2014). In light of future severe drought episodes, it is important to understand

which grasses can survive drought and identify if drought tolerance strategies differ among these grasses due to phylogeny and photosynthetic pathway and/or underlying morphological traits, such as fine root tissue abundance.

Over a decade of research has demonstrated that grass habitat, physiology, and morphology vary according to grass lineages (Christin et al. 2009, Liu et al. 2011, Edwards et al. 2007, Pau et al. 2013). The grass family consists of two major clades, BEP (Bambusoideae, Ehrhartoideae, and Pooideae) and PACMAD (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae), the former consisting of all C_3 grasses, and the later containing both C_3 and C_4 grasses (Aliscioni et al. 2012). Previous research has shown that habitats of grasses can vary between lineages within these grass clades and that the majority of C_4 grasses occupy drier regions with open canopies (Edwards and Smith 2010). Grasses from within the PACMAD clade have been shown to dominate in different regions that vary with precipitation, according to subfamilies (Liu et al. 2012.). For example, in North America, grasses from the subfamily Chloridoideae, such as *Bouteloua dactyloides* (buffalo grass), dominate the aridic to xeric short-grass prairies in the southwest and western regions. Whereas grasses from the subfamily Panicoideae, like *Andropogan gerardii* (big bluestem) dominate the ustic to udic, mesic tall grass prairies of the Great Plains. Several investigations using close and distantly related C_3 and C_4 grasses (within the PACMAD clade) have also demonstrated that photosynthetic rate (A), stomatal conductance to water vapor (g_s), and *WUE* varied between grass lineages and photosynthetic pathways in response to drought (Taylor et al. 2010, Ripley et al. 2010). Because evolutionary history influences many of these plant traits, characterizing which are common among closely related grass species may allow us to identify drought tolerance traits based on phylogenetic relatedness.

One major evolutionary adaptation within the PACMAD grass clade is the C_4 photosynthetic pathway. The C_4 pathway differs from C_3 photosynthesis by using a unique combination of morphological (i.e., Kranz anatomy) and biochemical (i.e. PEP-C enzyme) adaptations that allow them to succeed in dry warm environments while avoiding photorespiration and lowering water loss (Taiz and Zeiger 2010). CO_2 that diffuses through the stomates into mesophyll cells are turned into a four-carbon acid by the enzyme PEP-C. This conversion keeps the concentration of CO_2 inside the leaf (C_i) low, allowing for a high concentration gradient. This results in more diffusion of CO_2 into the leaves despite low stomatal conductance (g_s) (Ghannoum et al. 2003). After CO_2 has been converted into a four-carbon acid, it is moved into a specialized cell called the bundle sheath. This unique C_4 anatomy spatially separates the photosynthetic process. Once compartmentalized, RuBisCO has minimal contact with O_2 molecules, reducing the chances of photorespiration, and normal photosynthesis proceeds forward. Photorespiration is a by-product of RuBisCO's dual affinity for both CO_2 and O_2, which leads to reduced photosynthetic efficiency via loss of CO_2 and energy (Sage and Kubien 2007). The spatial separation in C_4 plants maximizes the concentration of CO_2 around RuBisCO, increasing the efficiency of the enzyme even when temperatures increase. Plants that use the C_4 pathway are frequently associated with warmer climates (Edwards and Still 2008), and having high photosynthetic rate (A), lower g_s, transpiration (E), and high water use efficiency (*WUE*, or *A/E*) (Nippert et al. 2007a, Taylor et al. 2010). C_3 plant species, however, have no special adaptations to isolate RuBisCO and lack the biochemical pathway to help alter the CO_2 concentrations within their leaves. This results in C_3 plants having increased chances of photorespiration, higher g_s and E. When C_3 plants respond to soil drying by altering their g_s, it reduces the flow of CO_2 into the leaves, limiting A which results in lower *WUE* (Ghannoum

2008). The increased risk of photorespiration in higher temperatures and reduction of *A* are why these plants are commonly associated with cooler, wetter landscapes and growing earlier in the season. Early researchers endorsed the notion that higher *WUE* in C_4 grasses imparted a high drought tolerance (and success) in warmer environments when compared to C_3 grasses (Knapp 1993, Sage and Monson 1999). Recent research, however, has revealed that *WUE* in C_4 grasses is typically reduced in response to severe drought (Nippert et al. 2007b, Ripley et al. 2010, Taylor et al. 2011), that C_4 grasses can be highly susceptible to this stress (Ghannoum 2008, Ripley et al. 2010) and this affects grasses differently based on lineage (Christin et al. 2009, Taylor et al. 2010). Since varying severities of drought affect grasses differently, we need research that includes both photosynthetic pathway and phylogeny, as well as traits, to better understand what does allow some grasses to persist (or recover) better than others.

Besides differences observed in physiological adaptations, grasses' morphology have been shown to vary between lineages (Liu et al. 2012, Frank et al. 2002, Liu and Osborne 2015). Morphological traits are closely linked with a plant's performance and survival in varying environments. Plants have adapted to survive a range of environments, but with limited resources available in most landscapes plants must choose where to invest their finite resources (Grime 1977, Chapin 1980). Plants are described as having to 'trade-off' between traits that acquire resources quickly and those that conserve resources ('plant economics spectrum', see Wright et al. 2004, Reich 2014). These traits have been used to identify plant strategies (how plants grow, survive, reproduce) and predict how plant will respond to their environment and resource gradients (Reich 2014). Plants categorized as having "fast" resource acquisition strategies typically have high growth rate, leaf area, and specific root length. These traits are considered to increase light and CO_2 capture, promote efficient water and nutrient uptake, and allow plants to

be competitive in productive habitats (Wright et al. 2004, Pérez-Ramos et al. 2013, Reich 2014). These traits have also been associated with plants using a "drought avoidance' strategy, where a high acquisition (uptake) of water allow them to avoid drought stress (Volaire et al. 2009, 2018). The trade-off for having larger investments into leaf or root surface area are 'cheaper', low quality plant tissues that may not last long or be durable to disturbance (Grime 1977, Wright et al. 2004). Plants that are associated with slow growth and a conservative resource strategy have small leaf and root areas, but larger investments into thick plant tissue. Thicker tissue purportedly allows plants to be less vulnerable to disturbances and possibly drought (Wright et al. 2004, Reich 2014, Volaire 2018). These plants are associated with a drought tolerance strategy, as they 'spend' less and conserve more resources in limiting environments (Balachowski et al. 2018, Volaire 2018). The trade-off, however, is low growth and less competitive ability when in non-limiting situations. Despite the extensive knowledge of these plant strategies and the evidence that links traits to evolutionary history, there is limited knowledge of how these traits vary among grasses or between grass lineages.

To understand how grasses may survive future climate change we need to investigate how grass species respond to severe drought and identify whether this differs between grass lineages in addition to photosynthetic pathway. Since drought tolerance involves a combination of physiological and morphological traits, we also need to investigate how morphology may vary between grass tribes, with the goal of identifying key plant functional traits that can be used for predictions and modeling. Here, I conducted a greenhouse experiment investigating several C_3 and C_4 grass species' response to drought. I asked the following questions. One, do leaf-level physiology responses to drought vary among pathways (C_3, C_4) and/or between grass tribes (Andropogoneae, Cynodonteae, Paniceae, and Danthonieae)? Two, do these differences vary

over time under well-watered conditions (initial response), drought (stress response), and after re-watering (recovery)? And three, do morphological traits (e.g., leaf area, root length) vary between grass lineages and/or photosynthetic pathways? To do this, I measured leaf level gas exchange under well-watered conditions, drought conditions, and two days after plants were rewatered. I then harvested and measured morphological traits on both above and belowground plant tissues after plants had recovered.

In this experiment, the C_3 grasses are expected to have higher g_s and *E*, lower *A* and *WUE,* when compared to C_4 plants under well-watered conditions and after drought stress is relieved (recovery) consistent with the literature (Nippert et al. 2007a, Taiz and Zeiger 2010). However, during peak drought stress, C_4 grasses are expected to have lowered *A* and *WUE*, similar to C_3 grasses, as observed in previous work (Ghannoum et al. 2003, Ghannoum 2008, Taylor et al. 2011, Nippert et al. 2012). Physiological responses to drought are expected to be similar among closely related groups (i.e., Andropogoneae and Paniceae tribes will have similar responses). And despite differences in their photosynthetic pathways (C_3, C_4), closer related Cynodonteae and Danthonieae tribes should behave similar as well. Morphological traits are expected to differ among grass tribes with closely related grasses having similar characteristics due to phylogenetic niche conservatism observed in grasses (Liu et al. 2012). According to current plant strategies, those grasses showing higher tolerance to drought (persisting longer), should have conservative characteristics such as low leaf and root surface areas. Those grasses that are associated with being fast resource acquirers (and drought avoiders) should have traits such as high specific leaf area (SLA), specific root length (SRL) and low tissue mass.

Materials and Methods

Plant Materials and Experimental Design

Grass Phylogeny and Seed Material

Grasses were chosen from the 'PACMAD' (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristodideae, and Danthonioideae) clade in the *Poaceae* family. Within the PACMAD clade, grasses were selected to represent four closely related tribes: Paniceae, Andropogoneae, Danthonieae, and Cynodonteae. Classification for tribes were based on current publication produced by the Grass Phylogeny Working Group (Aliscioni et al. 2012). Seeds of each species were acquired from the United States Department of Agriculture (USDA) National Plant Germplasm System. We selected three grass species from the tribe Paniceae (*Urochloa ruzziensis*, *Panicum virgatum*, and *Setaria viridis*), two species from the tribe Andropogoneae (*Sorghastrum nutans* and *Andropogon gerardii*), two species from the tribe Danthonieae (*Danthonia spicata* and *Rytidosperma semiannulare*). and two species from the tribe Cynodonteae (*Bouteloua dactyloides* and *Bouteloua gracillis*). Species within Danthonieae have the C_3 photosynthetic pathway while all other species use the C_4 photosynthetic pathway.

Experimental Conditions

Plants were first germinated in trays and then transferred to individually labeled pots in the greenhouse at Kansas State University, Manhattan, KS. All except two species were grown in 758 cm^3 soil volume, in potting soil with 10% sand mixed in. Two species (*B. gracillis* and *R. semiannulare*) were grown in 4590 cm^3 soil volume. Root characteristics were adjusted for density to account for soil volume. Daily light conditions in the greenhouse consisted of 16 hours light and 8 hours of dark, using lighting fixtures with 400-Watt high pressure bulbs. Plants were acclimated to greenhouse conditions for approximately one month before the drought

experiment began and were watered to saturation daily or as needed during this pre-treatment period.

The drought experiment was conducted over three trials, using different species each session, beginning June 2017, and concluding in February 2018. Between five to ten individuals in separate pots were used for each species. Plants were watered to saturation the day prior to physiological measurements (day zero), then water was withheld until plants showed evidence of low (or zero) photosynthetic rates and/or fluorescence performance (see leaf physiology section for details). Plants were also removed from drought treatment if signs of leaf senescence were observed across most leaves as leaf-level physiology measurements would be hindered. Once individual plants were considered extremely drought stressed, which was measured by stomatal closure, the plant was removed from drought, watered to saturation, and measured for physiological recovery exactly two days later.

Leaf Physiology

Gas Exchange

Leaf gas exchange was measured approximately every two days between the hours of 10:00 and 15:00 on individuals for all species. The measurements were taken on the healthiest (greenest, unrolled) leaf when possible. Leaves that had rolled from water stress were unrolled prior to gas exchange measurement. When possible, leaves with brown spots or senescence showing were not used. Leaf gas exchange measurements were taken with the LI-6400XT Portable Photosynthesis System (Li-Cor, Inc., Lincoln, NE, USA). Internal chamber CO_2 concentrations were adjusted (scrubbed) to match ambient levels of CO_2 and were supplied with 400 μmol CO_2 mol^{-1} air. Humidity was controlled by rate of flow and using a desiccant to maintain relative humidity (40-60%) within the chamber. Light intensity was set at 2000 μmol

m^{-2} s^{-1}. Each leaf was measured for area (cm^2) using the known length of the chamber and width of inserted leaves. When plant leaves were too narrow, multiple leaves were used to fill the chamber and the leaf area was adjusted appropriately. Measurements were taken after gas exchange stabilization, typically three minutes after inserting the leaf into the cuvette, although time to stabilization increased as the number of days into the drought treatments went longer. Gas exchange measurements conducted included light-saturated photosynthetic rate, (A_{sat}, μmols CO_2 m^{-2}), transpiration (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}), and intercellular CO_2 concentration (C_i, μmol CO_2 mol air^{-1}). Instantaneous water use efficiency (*WUE*) was calculated using A/E.

Chlorophyll fluorescence

The healthiest leaves from each species were measured for chlorophyll fluorescence (*ChlF*) every other day using a fluorometer (Mini-PAM, pulse-amplitude-modulated photosynthesis yield analyzer, Walz, Germany). Leaves were chosen from the healthiest looking leaves, inserted into the leaf clip holder, and then light-adapted chlorophyll fluorescence was measured. The Mini-PAM was programmed to take measurements in nine increments of increasing photosynthetically active radiation (PAR): 0, 450, 487, 498, 568, 679, 767, 923, 1087 μmol m^{-2-} s^{-1}. Each measurement included minimum fluorescence (F_o), followed by a saturating pulse, resulting in a maximum fluorescence (F_m). Yield (Y) was calculated as: $(F_m-F_o)/F_m = \Delta F/F_m'$. Electron transport (ETR) was simultaneously calculated using: Yield x PAR x 0.5 x ETR-factor (0.84).

Morphology

Aboveground Vegetation

At the completion of the drought experiment, individual plants from each pot were clipped above the crown tissue to measure aboveground morphology and biomass. Grass leaf area was measured using the LI-3100 Leaf Area Meter (LI-Cor, Lincoln, NE, USA). Subsamples of leaves were weighed (grams) for both wet weight and dry weight to calculate the leaf dry matter content (LDMC, mg/g). LDMC is calculated as: subsample wet weight (mg) divided by subsample dry weight (g). Leaf area was used to calculate total leaf area (m^2) and specific leaf area (SLA, leaf area (m^2) divided by weight of leaves (kg)). All samples were oven dried at approximately 60° C for 48 hours, then weighed. Aboveground biomass (kg) was totaled for each individual and averaged among grass tribes

Belowground Vegetation

After samples were clipped for aboveground measurements, remaining belowground tissue was removed from the pots and cleaned free of soil. The crown tissue was removed and weighed separately from the roots but was not included in total belowground biomass (g). The roots were measured for diameter (mm) and length (m) using WinRhizo, a digital root imaging program (WinRhizo, Regent Instruments Inc., Ontario, Canada). Before the analysis, roots were washed free of debris, floated in a small amount of water in clear acrylic trays (purchased through WinRhizo) and scanned. After the morphology analysis, roots were dried in an oven at 60° C for 48 hours, then weighed. Root weight density (RWD, g/m^3) was calculated using root dry weight (g) divided by soil volume (m^3). Root length density (RLD, m/m^3) was calculated as root length (m) divided by soil volume (m^3). Specific root length was calculated as root length (m) divided by root mass (g).

Statistics

Physiological responses were analyzed using a linear mixed-effects model with condition, tribe, and pathway as fixed effects and day as random effect using the ‘lme’ function in the “lme’ package in the statistical program R 4.0.4 (R Core Team 2021) For each tribe, mean values of each physiological response for each condition (initial, stress, and recovery) were calculated. Analysis of variance and post-hoc multiple comparisons were calculated using Tukey’s Honestly Significance Difference test. All morphology was averaged between grass tribes and photosynthetic pathways. All morphology data was analyzed using analysis of variance and post-hoc multiple comparisons were calculated using Tukey’s Honestly Significance Difference test.

Results

Leaf Physiology

Photosynthesis

Light-saturated photosynthesis (A_{sat}) exhibited a significant interaction between conditional day of treatment (initial, stress, and recovery) and tribe (p=0.004, Table 2.1,Figure 2.1). Cynodonteae's initial A_{sat} was significantly higher than Danthonieae and Paniceae ($p < 0.01$). During the peak of drought stress, every grass tribe had similar photosynthetic rates which were all significantly lower than their initial well-watered responses. Although Andropogoneae's recovery A_{sat} was higher than the other tribes after rewatering, the rates were not significantly different than the other grass tribes ($p \geq 0.12$). Each tribe, except for Cynodonteae, were able to recover their initial A_{sat} within two days after rewatering.

Comparisons between photosynthetic pathway (C_3, C_4) showed no significant interactions between A_{sat} and conditional day of treatment (Initial, Stress, and Recovery) (p=0.463, Figure 2.2,Table 2.2). Initial A_{sat} for both pathways showed significantly different rates which are typical of each pathway under well-watered conditions (p=0.01). However, this difference was lost during peak drought stress, when C_4 grasses' A_{sat} was found to be significantly lower than its' initial response ($p<0.001$). C_3 grasses' drought stress response was also significantly lower than its' initial response ($p<0.001$), however they were able to recover (after rewatering) to a A_{sat} that was no different than their initial response. Although the C_4 grasses' recovery rate (two days after watering) was significantly different than their peak drought stress response ($p<0.001$) they were unable to recover to pre-drought conditions (p=0.004) and their A_{sat} during recovery was found to be similar to that of the C_3 grasses (p=0.5).

Stomatal Conductance

When comparing stomatal conductance (g_s) between tribes and conditional days (initial, stress, and recovery) a significant interaction was present ($p < 0.001$, Table 2.1, Figure 2.1). Danthonieae was found to have significantly higher g_s initially than tribes Andropogoneae and Paniceae ($p<0.01$), which is a typical response of C_3 grasses. However, Danthonieae's *gs* did not differ significantly from its closely related 'sister-C_4 tribe', Cynodonteae. Cynodonteae differed from Paniceae ($p=0.003$). After drought progressed, none of the grass tribes differed in g_s during peak stress. Similar to their initial response, g_s in the 'recovery' time period for Danthonieae's g_s was higher than both Andropogoneae and Paniceae two days after rewatering ($p<0.0001$). Cynodonteae's recovery g_s was still significantly higher than Paniceae's ($p<0.001$), but a lower g_s than Danthonieae's after rewatering ($p=0.046$). All tribes recovered g_s similar to pre-drought conditions.

Like the differences found between grass tribes, g_s between photosynthetic pathways and conditional days (initial, stress, and recovery) also had a significant interaction ($p=0.004$, Figure 2.2, Table 2.2). Typical of C_3 plants, g_s was initially higher than the g_s of the C_4 grasses ($p=0.009$). However, during drought stress, the g_s of the C_3 grass reduced to a similar rate as the C_4 grass. Differences between the pathways were again evident after the grasses were rewatered with both groups recovering to pre-drought rates of g_s ($p<0.001$).

Internal CO_2 Concentration

There was a significant interaction between internal CO_2 concentration (C_i) between tribes and conditional (initial, stress, and recovery) day ($p=0.05$, Table 2.1,Figure 2.1). Andropogoneae, Paniceae, and Cynodonteae tribes all had significantly lower C_i than Danthonieae initially under well-watered conditions ($p \leq 0.05$). During peak drought stress,

however, Cynodonteae had similar high C_i similar to Danthonieae, its closely related 'sister tribe', despite the fact that this group of grasses use the C_4 pathway. All tribes had significantly higher C_i under drought stress than their initial responses ($p<0.001$). Two days after rewatering, all tribes recovered to their initial C_i, except for Cynodonteae, which had a significantly higher C_i than its response before the drought ($p<0.001$). This higher C_i was significantly different than the two other C_4 grass tribes, Andropogoneae and Paniceae ($p<0.001$), but not their C_3 'sister tribe', Danthonieae. Danthonieae had significantly higher C_i than both Andropogoneae and Paniceae even after rewatering ($p<0.01$).

There was not a significant interaction between photosynthetic pathway (C_3, C_4) and conditional days (initial, stress, and recovery) for the variable C_i. C_3 grasses had higher C_i than C_4 grasses under all conditions ($p \leq 0.05$, Figure 2.2,Table 2.2). Under drought stress, the C_4 grasses had a significantly higher C_i than its initial response ($p=0.01$) but were able to recover to pre-drought response after rewatering .

Transpiration

There was a significant interaction between condition (initial, stress, and recovery) and tribe for transpiration (*E*) ($p<0.01$, Table 2.1,Figure 2.1). Initially, Danthonieae had a significantly higher *E* than both Andropogoneae and Paniceae ($p<0.001$). Cynodonteae had a significantly higher *E* than Paniceae ($p<0.001$) but not significantly higher than Andropogoneae ($p=0.10$, Table 2.1). During peak drought stress, every grass tribe had similar *E* response which were significantly lower than their initial well-watered conditions ($p<0.01$). Two days after rewatering, the tribe Paniceae had recovered to its initial *E* rate and was found to be significantly lower than all other tribes ($p<0.01$). Although Danthonieae's *E* remained high after rehydration, it did not differ from its closely related 'sister tribe' Cynodonteae and was not significantly

different than Andropogoneae (p=0.09) despite using different photosynthetic pathways than both groups.

There was no significant interaction between photosynthetic groups (C_3, C_4) and condition (initial, stress, and recovery) for *E* but there were significant main effects ($p<0.01$, Figure 2.2, Table 2.2). Under well-watered conditions, C_3 grasses had a higher *E* than C_4 grasses ($p=0.05$). After succumbing to drought stress, C_4 grasses had similar *E* rates as C_3 grasses. Once rewatered, C_4 grasses' *E* rates were lower than C_3 grasses' *E* ($p=0.004$), and their own initial response. The C_3 grasses also recovered to their high *E* after rewatering.

Water Use Efficiency

There was not a significant interaction between tribe and condition (initial, stress, and recovery) for water use efficiency (*WUE*) ($p=0.12$, Table 2.1,Figure 2.1). Initially, the tribe Danthonieae had a significantly lower *WUE* than Paniceae ($p<0.001$). After drought stress had commenced, all tribes had significantly lower *WUE* than their initial well-watered response, except for Cynodonteae. After rewatering, Paniceae had higher *WUE* than all tribes, but was only significantly different than Danthonieae and Cynodonteae ($p<0.001$). Cynodonteae was unable to recover to predrought conditions and had a significantly lower *WUE* than Andropogoneae ($p<0.05$).

When comparing *WUE* between photosynthetic pathway (C_3, C_4) and condition (initial, stress, and recovery) there were no interactions but there were significant main effects ($p<0.01$, Figure 2.2, Table 2.2). Before drought began, C_4 grasses had expectedly higher *WUE* than C_3 grasses ($p=0.02$). Although the C_4 grasses' *WUE* was significantly lower than their initial response due to drought ($p=0.01$), even at the height of stress, their *WUE* remained lower than

the C_3 grasses ($p<0.01$). However, two days after being watered, the C_3 grasses were able to recover to a higher *WUE* but was not significantly different than that of the C_4 grasses.

Chlorophyll Fluorescence

There was no significant interaction between condition (initial, stress, and recovery) and tribe for chlorophyll florescence (*ChlF*) ($p= 0.5$, Table 2.1, Figure 2.1). Danthonieae had a higher *ChlF* but was not significantly different than the other tribes initially. All tribes had similarly low *ChlF* under drought stress. After rehydration, all tribes recovered *ChlF*, except the difference between Danthonieae and Paniceae was significantly different than predrought *ChlF* ($p= 0.018$).

Significant differences were found among the main effects, photosynthetic pathway (C_3,C_4) and condition (initial, recovery, and stress) but there was no interaction. The C_3 grasses had a higher *ChlF* than the C_4 grasses when well-watered ($p= 0.03$), however during peak drought stress the difference diminished (Figure 2.2, Table 2.2). Once the grasses were rewatered, the C_3 grasses resumed having a higher *ChlF* than the C_4 grasses ($p= 0.02$) and recovered its initial *ChlF* response. However, the C_4 grasses did not recover their predrought *ChlF*.

Morphology

Leaf dry matter content

Leaf dry matter content (LDMC, mg/g) differed significantly between grass tribes ($p<0.001$, Table 2.3, Figure 2.3). Paniceae had the smallest investment into LDMC, which differed from all other grass tribes ($p<0.001$), including Andropogoneae, its closest-related grass tribe. Both Danthonieae and Cynodonteae had similar LDMC, which could be expected between closely related tribes. However, Andropogoneae also had similar LDMC to both tribes, despite

being distantly related to either tribe. When comparing LDMC between photosynthetic pathways (C_3, C_4), grasses in the C_3 group were found to have a significantly higher investment into LDMC than the C_4 grasses (p=0.05, Figure 2.4, Table 2.4).

Aboveground biomass

Grass tribes did not differ significantly in their investment into above ground plant tissue (p=0.47, Table 2.3, Figure 2.3), despite grasses from each tribe achieving different heights and leaf sizes (personal observation, also see leaf area). There was also no difference between above ground biomass between photosynthetic types (p=0.5, Figure 2.4, Table 2.4).

Specific leaf area

Specific leaf area (SLA, $m^2\ kg^{-1}$) did not differ between grass tribes (p=0.352, Table 2.3, Figure 2.3). The tribes with the largest SLA (Paniceae and Andropogoneae) were similar (p=0.99) which may be attributed to those grass tribes being closely related. The other 'sister-tribes', Cynodonteae and Danthonieae, also had very similar lower SLA (p=0.99). When comparing SLA between photosynthetic pathways (C_3, C_4), neither group differed significantly from the other (p=0.3, Figure 2.4, Table 2.4).

Leaf area

Total leaf area (m^2) was found to differ significantly between tribes (p=0.03, Table 2.3,Figure 2.3). The largest difference was between the grass tribe Paniceae and Cynodonteae, with the former having the larger leaf area and the latter having the smallest leaf area (p=0.05). Similar to other morphological features, both sets of closely related 'sister-tribes' had similar investments into leaf area. The difference found between tribes however did not hold between photosynthetic pathways. There was no difference between leaf area of C_3 and C_4 grasses (p=0.21, Figure 2.4,Table 2.4).

Root weight density

Investment into belowground biomass (RWD, g/m^3) differed significantly between tribes ($p<0.001$, Table 2.3, Figure 2.3). Andropogoneae had a significantly larger investment into RWD than all other tribes ($p<0.01$). Cynodonteae and Danthonieae had similar small RWD that was significantly different from the larger RWD of both Andropogoneae and Paniceae ($p<0.01$). Photosynthetic pathways were found to also have different RWD ($p<0.01$, Figure 2.4,Table 2.4). C_4 grasses when averaged together, had a larger RWD than the C_3 grasses ($p<0.001$).

Root length density

Grass tribes were found to have different root length density (RLD, m/m^3) ($p<0.01$, Table 2.3, Figure 2.3). Both Andropogoneae and Paniceae had similar large RLD, however, this was only significantly larger than Cynodonteae which had the smallest RLD ($p=0.01$). Despite Danthonieae having similar low RLD as Cynodonteae, the grasses RLD investment was not significantly different from any other tribe ($p=0.12$). This marginal difference was also reflected between C_3 and C_4 grasses. On average, C_4 grasses had larger RLD than the C_3 grasses, but these were not significantly different ($p=0.14$, Figure 2.4, Table 2.4).

Root diameter

Root diameter (mm) was significantly different between grass tribes ($p<0.001$, Table 2.3, Figure 2.3). Closely related tribes ,Andropogoneae and Paniceae, had similar larger diameters when compared to the other two tribes. Those tribes, Danthonieae and Cynodonteae, had similar diameters that were significantly smaller than the two larger ones ($p<0.001$). This difference was also reflected between photosynthetic pathways ($p<0.01$, Figure 2.4, Table 2.4). On average C_4 grasses had larger root diameters than the C_3 grasses.

Days in drought

The average time grasses survived during drought was found to differ significantly between all grass tribes ($p<0.001$, Table 2.5, Figure 2.5). Cynodonteae lasted on average 32 days, Danthonieae was 23 days, Andropogoneae survived 17 days, and Paniceae lasted 12 days on average. This difference was also present when compared between photosynthetic pathways. C_3 grasses on average lasted 23 days, which was longer than the C_4 grasses average 18 days ($p<0.03$, Table 2.6, Figure 2.6).

Discussion

Grass physiological traits

Under well-watered conditions, the C_4 grasses in this study responded as predicted with higher *A* and lower g_s, C_i, and *E*, than the C_3 grasses consistent with the literature. Once drought had progressed these differences among pathways diminished. Both *A*, g_s, and *E* had lower rates in response to drought, resulting in C_4 grasses having similar rates as C_3 (Table 2.2). *WUE* in C_4 grasses remained higher than C_3 grasses during drought stress. However, WUE had decreased by 40%, and upon rewatering had only recovered approximately 70% of initial *WUE* and was not found to be different than the C_3 grass *WUE* (Table 2.2). Interestingly, when analyzing differences between grass tribes, similarities in gas exchange between closely related tribes became apparent and the higher *WUE* rates for C_4 grasses was shown to be driven mainly by the high average *WUE* from tribe Paniceae. Under both well-watered and drought conditions neither g_s, *E*, or *WUE* differed between the C_4 tribe Cynodonteae and their closer related C_3 'sister' group, Danthonieae, despite using different photosynthetic pathways. Similarities between closely related C_4 tribes, Andropogoneae and Paniceae, were also found, supporting the hypothesis that closely related tribes would respond similar to drought. Whether well-watered or under drought, these tribes had similar rates for each physiological measurement except for transpiration upon rewatering.

Despite the adaptations that normally allow C_4 plants to conduct photosynthesis and achieve high *WUE* in mild to moderate drought, in this experiment drought was severe enough to reduce *A* and *WUE*. These results support previous work that demonstrates C_4 grasses can be as sensitive to varying severities of drought as C_3 grass species (Ghannoum 2005, Nippert et al. 2007b, Ripley et al. 2010, Taylor et al. 2011). The higher C_i observed in the C_4 grasses (during

peak drought stress) indicates that low g_s was not solely responsible for limiting assimilation rates Table 2.2). *WUE* decreased in C_3 grasses during drought due to a response of lowered g_s, which in turn reduced *A* because of a decrease in C_i (Ghannoum et al. 2003). Whereas in C_4 plants, C_i is not reduced by lower g_s (initially) because of their CO_2 concentrating mechanisms. As drought continues, reduced *A* has been shown as a result of low g_s, and biochemical and metabolic impairments (Ghannoum et al. 2003, Ripley et al. 2010, Taylor et al. 2014). This reduced *A* lowers *WUE* to rates similar of C_3 plants. After rewatering, most grasses were able to recover over 90% of their initial (well-watered) responses for all physiological measurements except for the tribe Cynodonteae. Those grasses were unable to recover their initial *A* rates but did have similar g_s. Since stomatal conductance resumed its previous rates, and C_i was not lowered after rewatering, it suggests that non-stomatal factors limited these grasses' ability to resume high *A* rates (Ghannoum 2008). These results reinforce the current knowledge that C_4 grasses are not necessarily robust to drought despite high *WUE* observed under mild or moderate drought (Taylor et al 2011, Ripley et al. 2010). The different responses observed in this experiment between grass tribes also suggest physiological traits are influenced by evolutionary history. Grasses in tribe Danthonieae belongs to subfamily Danthonioideae a 'sister' to the subfamily, Chloridoideae, which contains grasses from the tribe Cynodonteae. Tribes Andropogoneae and Paniceae are more closely related to each other as they belong within the subfamily Panicoideae. The observed similarities and differences between these groups were not apparent when measurements were averaged between photosynthetic pathways. It only becomes noticeable when controlling for phylogeny that similarities and differences between lineages were observed.

Grass morphology and plant strategies

In this experiment, there was a distinction between grasses that persisted longer during drought and those that succumbed earlier. Current drought strategy theories suggest that plants that are not desiccation tolerant, or use dormancy to avoid drought, use either drought avoidance strategies, drought tolerance strategies, or a combination of both to deal with water stress (Volaire 2008, 2018). The average number of days the grasses persisted during this experimental drought reveal that grasses have adapted a range of drought tolerance which varied between grass lineages (Figure 2.5, Figure 2.6). Here, we used the number of days in drought as a proxy for drought tolerance. While this metric is not the same as actual drought tolerance, it does provide evidence to interpret how grass strategies differed between tribes. When averaged between photosynthetic pathways, C_3 grasses persisted 23 days and C_4 grasses lasted 18 days (Table 2.6). Averaging between all C_4 grasses, however, blurs the division between tribes (and subfamilies). The grasses belonging to the tribes Cynodonteae and Danthonieae endured drought on average 32 and 23 days, respectively (Table 2.5). These grasses would be considered 'drought tolerators', which is supported by the knowledge that some of the grass species used in this experiment (within these tribes) dominate aridic and xeric habitats. Tribes Andropogoneae and Paniceae (subfamily Panicoideae) lingered for 17 and 12 days on average (Table 2.5). Based on this short persistence time during drought, these grasses could be considered 'drought avoiders'. Grass species from these tribes are known to reside in more ustic to udic, mesic habitats.

Specific leaf area (SLA, a parameter used to describe trade-offs between growth and conservation), and aboveground biomass (a component of SLA) did not vary significantly between grass tribes or photosynthetic pathway. However, total leaf area (LA, m^2) and LDMC did (Figure 2.3, Figure 2.4). Grass tribes Andropongoeae and Paniceae had similar high LA and

low LDMC opposite of those grasses within tribes Danthonieae and Cynodonteae. Differences in LDMC (an estimator for density of leaf tissue) and LA explains how differently these grasses are investing their resources despite similar dry aboveground biomass values. Higher LDMC and small leaf area are associated with plants growing in unproductive areas, with slower growth and longer living tissues (Reich 2014). These plants are suggested as having a higher tolerance during drought due to thicker cell walls (Wright et al. 2004, Comas et al. 2013, Reich 2014). Plants with low LDMC and high LA are associated with productive environments. According to plant strategies, these shared traits suggest those grass tribes Paniceae and Andropogoneae, may have higher resource acquisition, a benefit in productive areas where plants need to be competitive. The grass species in tribes Cynodonteae and Danthonieae would then be considered as resource conservative plants.

Belowground plant traits were found to differ between both photosynthetic pathway and grass tribes (Figure 2.3, Figure 2.4). C_3 grasses on average had thinner root diameters, higher specific root length (SRL), and lower root weight density (RWD) (Table 2.4). Once separated into grass tribes however it becomes clear that C_4 grasses also differed in root traits according to phylogenetic relatedness. Root traits were found to be similar between closely related groups for all root traits except RWD (Table 2.3). Grass from tribes Andropogoneae and Paniceae had higher RWD, root length density (RLD), and root diameter. Those grasses in tribes Danthonieae and Cynodonteae had higher SRL and thinner root diameters. According to plant strategies (Comas et al. 2013, Reich 2014, Prieto et al. 2015) species associated with fast resource acquisition and drought avoidance traits (tribes Andropogoneae and Panicoideae) should have high SRL, thin diameters, and those plants with conservative strategies and drought tolerance traits (tribes Cynodonteae and Danthonieae) should have low SRL and thicker diameters. Here,

contrary to previous root trait strategy theories, (root econmics spectrum; Roumet et al. 2016, Weemstra et al. 2016) the opposite was observed.

Grasses from tribes Cynodonteae and Danthonieae (in this experiment) were associated with conservative resource use according to their leaf traits. They also persisted longest during drought and recovered almost all physiological responses after rewatering (see physiology section). Some of the grasses used in this experiment within these tribes are found to dominate aridic to xeric areas in North America, have smaller stature, SLA, high LDMC, and small LA. Grasses from aridic habitats (i.e., *B. gracilis* used in this study) were identified in a recent study as having leaves with higher drought tolerance and roots that primarily rely on shallow soil water (Ocheltree et al. 2020). This reliance on shallow soil water was also observed in Craine et al. (2002) which found those short grasses to possess roots that are finer than those grasses from tribes Andropogoneae and Panicoideae (i.e., *A.gerardii* and *S.nutans* used in this study). Both studies observed that these grasses have roots that rely on shallow soil water, a necessity in regions with pulses of precipitation. Having a finer root diameter and higher SRL (cheaper carbon investment per root length) could allow these grasses to i) react quickly to episodes of precipitation (Balachowski et al. 2018), ii) explore more soil volume with thinner roots accessing smaller soil pores for water in dry habitats (Freschet et al. 2017) and iii) smaller root diameters may provide safety from water stress embolism (Fitter 1987, Wahl and Ryser 2000, Bristiel et al. 2019). Slower growing plants supposedly trade off high resource acquisition for longer living tissue, however if slower growing plants have less leaf area to produce photosynthates, it could be suggested that lower carbon investment roots (high SRL) that can be constructed quickly to react to rain events could be a better investment for plants in limiting environments, using their limited carbon resources in a way that maximizes the most limiting resource, water. Previous

work has associated thinner root diameters with higher tolerance to drought stress, as smaller diameters may have higher cavitation resistance (Richards and Passioura 1989, Wahl and Ryser 2000, Comas et al. 2013, Freschet et al. 2017) which would be a benefit for plants that exist in dry habitats. Finer roots were also found in more drought tolerant grasses of a Mediterranean grass population (Bristiel et al. 2019) which suggests this phenomenon is not isolated to North American grasses in xeric regions.

Grasses from tribes Andropogoneae and Paniceae are found to dominate more mesic areas, such as the ustic to udic tallgrass prairies of North America. This research found that grass traits varied from current plant trait strategies between above and belowground traits for these tribes as well. These grasses were found to have fast, resource acquisitive strategies according to their leaf traits, which agrees with plants growing in more rich resource environments (udic, mesic grasslands), where fast acquisition and competition are important drivers for grass dominance. Plants, such as those with 'acquisitive traits' should have parallel belowground traits such as high SRL and thinner root diameters, in order to acquire resources quickly. However, these grasses had larger investment into root weight and length density (per soil volume) and thicker root diameters. Craine et al. (2002) found complementary results as this, where tallgrass species such as big bluestem were found to have large density of roots with coarser diameters, which was suggested to facilitate soil water acquisition from a larger volume of soil. Although lower SRL and thicker diameters have been associated with slow growth and conservation, it could instead be suggested that plants with larger root diameters and high root density may be capable of moving larger volumes of water which would allow plants to achieve greater height (size) and faster growth in competitive environments (Wahl and Ryser 2000, Shenk and Jackson 2002). Grasses from the tallgrass prairies in North America, have been observed to have very

dense root network in the more shallow soil layers (Nippert and Knapp 2007) and mainly use water within the top 30 cm of soil, allowing grasses in this region to utilize pulses of precipitation. These grasses are known to have competitive, fast resource acquiring strategies, as growth in more mesic habitats require efficient resource capture. In order for belowground resource capture to match aboveground growth, roots would have to support a high resource acquisition of water and nutrients, while being competitive. Large investments into RWD and RLD would allow these grasses to exploit large volumes of soil while crowding out neighboring plant roots. These belowground traits combined with extremely low g_s (as observed in this experiment), would allow these grasses to access water when it is available and reduce water loss during periods of drought. In comparison to NA temperate locations, grasses from temperate and northern regions of France (Bristiel et al. 2019) were also found to have greater root biomass and densities that were associated with a higher water use and thicker roots. It was suggested that those roots with thicker diameter were able to take up more water than those with thinner roots.

Conclusion

This study aimed to demonstrate that drought tolerance varies among grasses, differing not only due to photosynthetic pathway, but evolutionary history and ecophysiological traits. The results observed here reinforce the current knowledge that grasses using the C_4 photosynthetic pathway are not necessarily more resilient in severe drought than grasses using the C_3 pathway. This work has also highlighted the need to incorporate phylogeny into ecophysiological research as the large differences observed between C_3 and C_4 grasses physiology were more likely due to divergence in phylogeny than differences between pathways. Morphological traits also identified commonality between closely related groups. Grasses in this study had both conservative and resource acquisitive traits that reflected both evolutionary history and current geographic

distribution. However, opposite of current belowground plant strategy theories, ‘conservative’ grasses from aridic and xeric regions had higher SRL, thinner roots, and ‘acquisitive’ grasses from ustic and udic, mesic areas had denser root mass and length, with thicker diameters. These observations are contradictory to current theories and suggest our understanding of belowground systems and strategies still need resolving, especially for finer roots of grass species.

Acknowledgements

I would like to thank Seton Bachle, Rory O’Connor, Lindsey Swartz, Makenna (Miller) Hotz, Jeremy Ruiz, Ryan Estes, Sam Sharpe, Fan Qui, Mark Ungerer, and Jesse Nippert for their help with the experimental set up, data collection, and processing. Funding for this project was provided by the National Science Foundation Dimensions in Biodiversity Award # 1342787.

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Tables and Figures

Table 2.1 Grass tribe physiological traits

Physiological traits under non-limiting water (Initial), drought conditions (Stress), and two days after rewatering (Recovery) between grass tribes. Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

A) Photosynthesis (A_{sat}, μmol CO_2 m^{-2} s^{-1})

Tribe	Initial	Stress	Recovery
Andropogoneae	17.116 ± 2.046ab	1.538 ±0.25a	15.503 ±1.71a
Cynodonteae	21.430 ± 2.38a	2.344 ± 1.26a	11.411 ±2.42a
Danthonieae	11.413 ± 1b	-0.534 ± 0.24a	9.012 ± 1.05a
Panicoideae	14.084 ± 0.71b	2.334 ±0.58 a	8.907 ±0.63a

B) Stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}

Tribe	Initial	Stress	Recovery
Andropogoneae	0.1101 ± 0.014ac	0.0307 ±0.006a	0.1124 ±0.01ab
Cynodonteae	0.1589 ±0.016ab	0.0499 ±0.008 a	0.1527 ±0.033ac
Danthonieae	0.1841 ±0.022b	0.0500 ±0.008a	0.2199 ±0.01c
Panicoideae	0.0917 ± 0.006c	0.0206 ±0.003a	0.0676 ±0.005b

C) Internal CO_2 concentration (C_i, μmol CO_2 mol air^{-1})

Tribe	Initial	Stress	Recovery
Andropogoneae	121.40±10.43a	281.05 ±16.25a	155.97 ±11.65a
Cynodonteae	160.70 ±9.68a	306.32 ±46.41b	328.49 ±70.8b
Danthonieae	276.06 ±4.7b	414.09 ± 17.67b	316.27 ±7.96b
Panicoideae	121.76 ±7.55a	257.61 ±28.5a	160.72 ±11.42a

D) Transpiration (E, mmol $H_2Om^{-2}s^{-1}$)

Tribe	Initial	Stress	Recovery
Andropogoneae	3.1 ±0.35ac	0.88 ±0.14a	3.15 ±0.34a
Cynodonteae	4.4 ±0.47bc	1.17 ±0.21a	3.36 ±0.73a
Danthonieae	4.51 ±0.42b	1.42 ±0.23a	4.53 ±0.31a
Panicoideae	2.02 ±0.12a	0.46 ±0.07a	1.43 ±0.09b

E)Water use efficiency (A (μmol CO_2 m-2 s^{-1}) / E (mmol $_{H2O}$ m^{-2} s^{-1})

Tribe	Initial	Stress	Recovery
Andropogoneae	5.54 ±0.2ab	2.09 ±0.32ab	5.01 ±0.34ab
Cynodonteae	4.94 ±0.21ab	2.21 ±1.4ab	1.22 ±0.28c
Danthonieae	2.56 ±0.1b	-0.83 ±0.5b	1.98 ±0.16bc
Panicoideae	7.12 ±0.19a	3.54 ±0.79a	6.3 ±0.29a

F) Chlorophyll fluorescence (ChlF, Δ F/Fm')

Tribe	Initial	Stress	Recovery
Andropogoneae	0.615 ±0.028a	0.342 ±0.045a	0.597 ±0.021ab
Cynodonteae	0.609 ±0.023a	0.451 ±0.065a	0.588 ±0.069ab
Danthonieae	0.73 ±0.01a	0.476 ±0.056a	0.712 ±0.019a
Panicoideae	0.647 ±0.017a	0.395 ±0.039a	0.626 ±0.024b

Table 2.2 Physiological traits by photosynthetic pathway

Physiological traits under non-limiting water (Initial), drought conditions (Stress), and two days after rewatering (Recovery) between photosynthetic pathways (C_3, C_4). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

A) Photosynthesis (A_{sat}, μmol CO_2 m-2 s^{-1})

Pathway	Initial	Stress	Recovery
C_3	11.410.99a	-0.533 ±0.24a	9.01 ±1.05a
C_4	17.5 ±1.3b	2.67 ±0.54a	12.38 ±1.25a

B) Stomatal conductance to vapor (g_s, mol m^{-2} s^{-1})

Pathway	Initial	Stress	Recovery
C_3	0.184 ±0.02a	0.05 ±0.007a	0.22 ±0.02a
C_4	0.12 ±0.01b	0.03 ±0.004a	0.11 ±0.01b

C) Internal CO_2 concentration (Ci, μmol CO_2 mol air^{-1})

Pathway	Initial	Stress	Recovery
C_3	276.05 ±4.69a	414.08 ±17.67a	316.27 ±0.96a
C_4	126.41 ±7.48b	259.32 ±22.11b	205.78 ±31.25b

D) Transpiration (E, mmol H_2O m^{-2} s^{-1}),

Pathway	Initial	Stress	Recovery
C_3	4.51 ±0.42a	1.42 ±0.23a	4.52 ±0.31a
C_4	3.19 ±0.28b	0.94 ±0.09a	2.72 ±0.31b

E)Water use efficiency (A/E)

Pathway	Initial	Stress	Recovery
C_3	2.56 ±0.09a	-0.83 ±0.49a	1.98 ±0.16a
C_4	5.89 ±0.23b	3.23 ±0.65b	4.34 ±0.95a

F) Chlorophyll fluorescence (ChlF, Δ F/F_m')

Pathway	Initial	Stress	Recovery
C_3	0.72 ±0.009a	0.475 ±0.06a	0.71 ±0.09a
C_4	0.59 ±0.01b	0.39 ±0.03a	0.56 ±0.03b

Table 2.3 Grass tribe morphological traits

Morphological traits (leaf dry matter (LDMC), aboveground biomass, specific leaf area (SLA), leaf area, root weight density (RWD), root length density (RLD), root diameter, and specific root length (SRL) compared between grass tribes. Values are mean ± 1 SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Tribe	Andropogoneae	Cynodonteae	Danthonieae	Panicoideae
Leaf traits				
LDMC (mg/g)	341.5 ±15.4a	370.1 ±17.3a	347.8 ±9.02a	264.9 ±10.5b
Above biomass (g)	1.08 ±0.1a	0.785±0.2a	1.21±0.3a	1.16 ±0.1a
SLA (m^2 kg^{-1})	8.56 ±0.43a	6.74 ±0.29a	6.99 ±0.84a	8.34 ±0.83a
Leaf area (m^2)	.0009 ±0.002ab	0.005 ±0.0012a	0.007 ±0.0012ab	0.011 ±0.0015b
Root traits				
RWD (g/m^3)	755.28 ±106.1a	102.39 ±11.8 b	136.47 ±4.94b	469.07 ± 31.42c
RLD (m/m^3)	48830 ±7868a	21364±2502b	29997±5738ab	45102 ±4161a
Root diameter (mm)	0.301 ±0.0118a	0.22 ±0.0025b	0.222 ±0.009b	0.279 ±0.0096a
SRL (m/g^{-1})	71.57 ±9.39a	214.25 ±13.7b	256.64 ±51.63b	105.01 ±10.62a

Table 2.4 Morphological traits compared between photosynthetic pathways

Morphological traits (leaf dry matter (LDMC), aboveground biomass, specific leaf area (SLA), leaf area, root weight density (RWD), root length density (RLD), root diameter, and specific root length (SRL) compared between photosynthetic pathways (C_3, C_4). Values are mean ± 1 SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

	Pathway	
	C_3	C_4
Leaf traits		
Leaf dry matter content (LDMC, mg/g)	347.78±9.02a	306.43±10.06b
Above biomass (g)	1.28 ±0.3a	1.06 ±0.09 a
Specific leaf area (SLA, m^2 kg^{-1})	6.99 ±0.84a	8.04 ±0.47a
Leaf area (m^2)	0.006 ±0.001a	0.009 ±0.0009a
Root traits		
Root weight density (RWD, g/m^3)	136.47 ±14.94a	457.099 ±43.56 b
Root length density (RLD, m/m^3)	29997 ±5738a	40774 ±3285a
Root diameter (Diam, mm)	0.22 ±0.009a	0.227±0.007b
Specific root length (SRL, m/g^{-1})	256.64 ±51.63a	121.01 ±9.97b

Table 2.5 Average days grass tribes survived drought

Average time (days) grass tribes survived drought stress. Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Tribe	Mean	SE
Andropogoneae	17.5a	0.93
Cynodonteae	32.3b	1.05
Danthonieae	23.5c	1.13
Panicoideae	12.0d	0.43

Table 2.6 Days in drought compared between photosynthetic pathways

Average time (days) grasses persisted during drought compared between photosynthetic pathway. Means reflect how long grasses survived drought stress. Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Pathway	Mean	SE
C_3	23.5a	1.13
C_4	18.02b	1.24

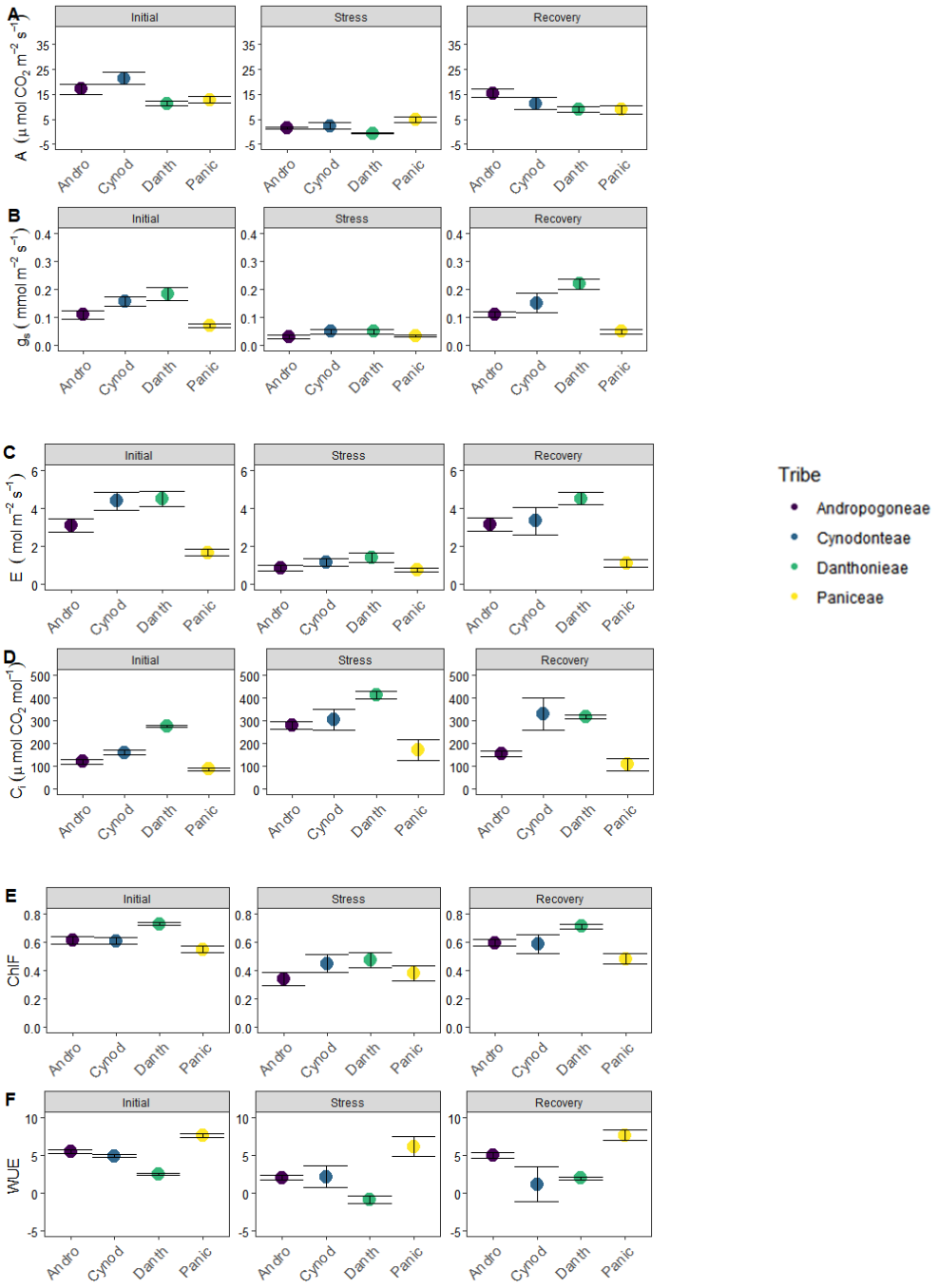

Figure 2.1 Physiological response compared between grass tribes.

Mean physiological response are categorized under well-watered conditions (Initial), drought (Stress), and two days after rewatering (Recovery). (A) photosynthesis, (B) stomatal conductance, (C), transpiration, (D) internal CO_2 concentration, (E) chlorophyll fluorescence, (F), water use efficiency. Response values are shown in mean ±1SE.

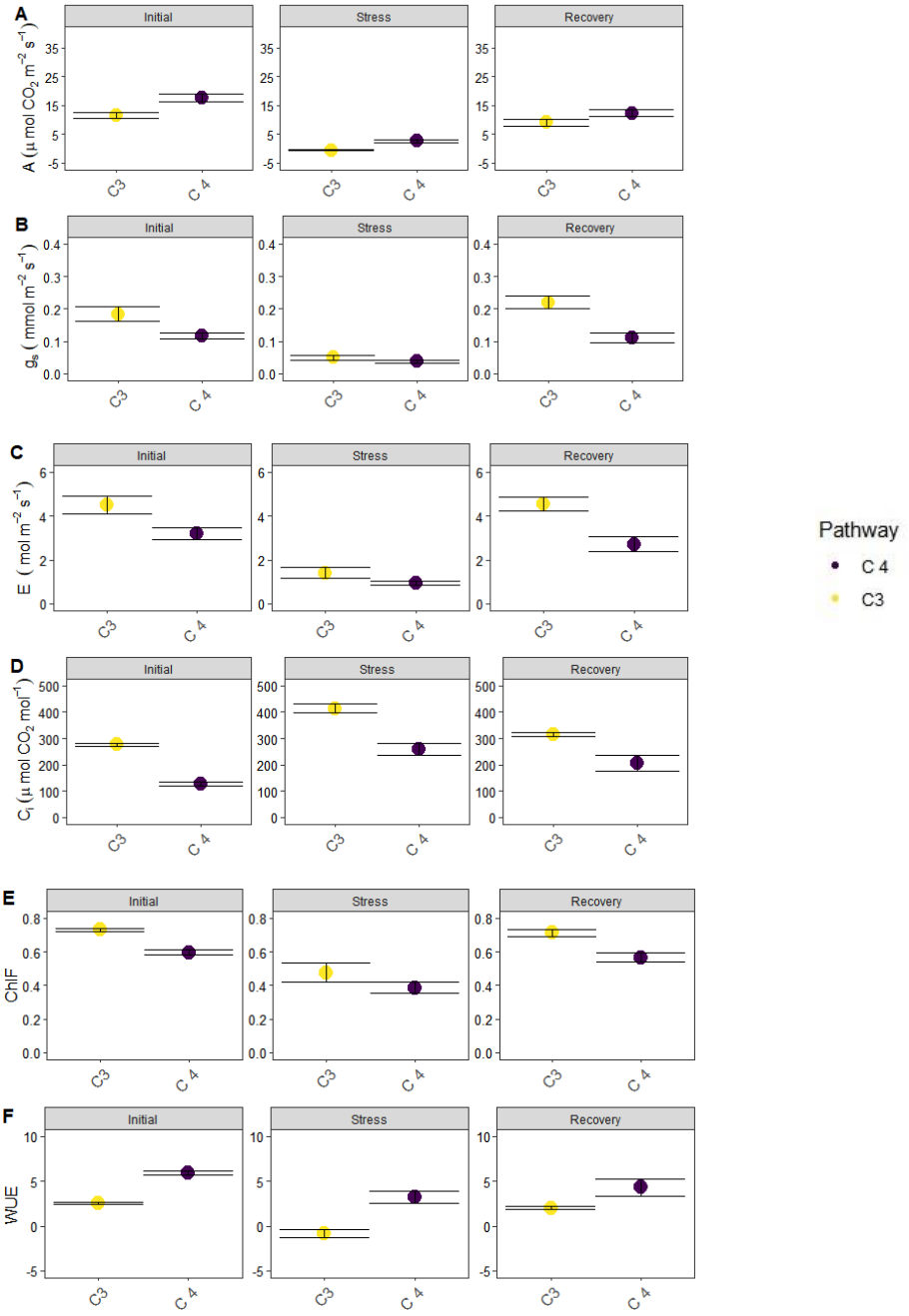

Figure 2.2 Physiological response compared between photosynthetic pathways.

Mean physiological responses are categorized under well-watered conditions (Initial), drought (Stress), and two days after rewatering (Recovery). (A) photosynthesis, (B) stomatal conductance, (C) transpiration,(D) internal CO_2 concentration, (E) chlorophyll fluorescence, (F), water use efficiency. Response values are shown in mean ±1SE.

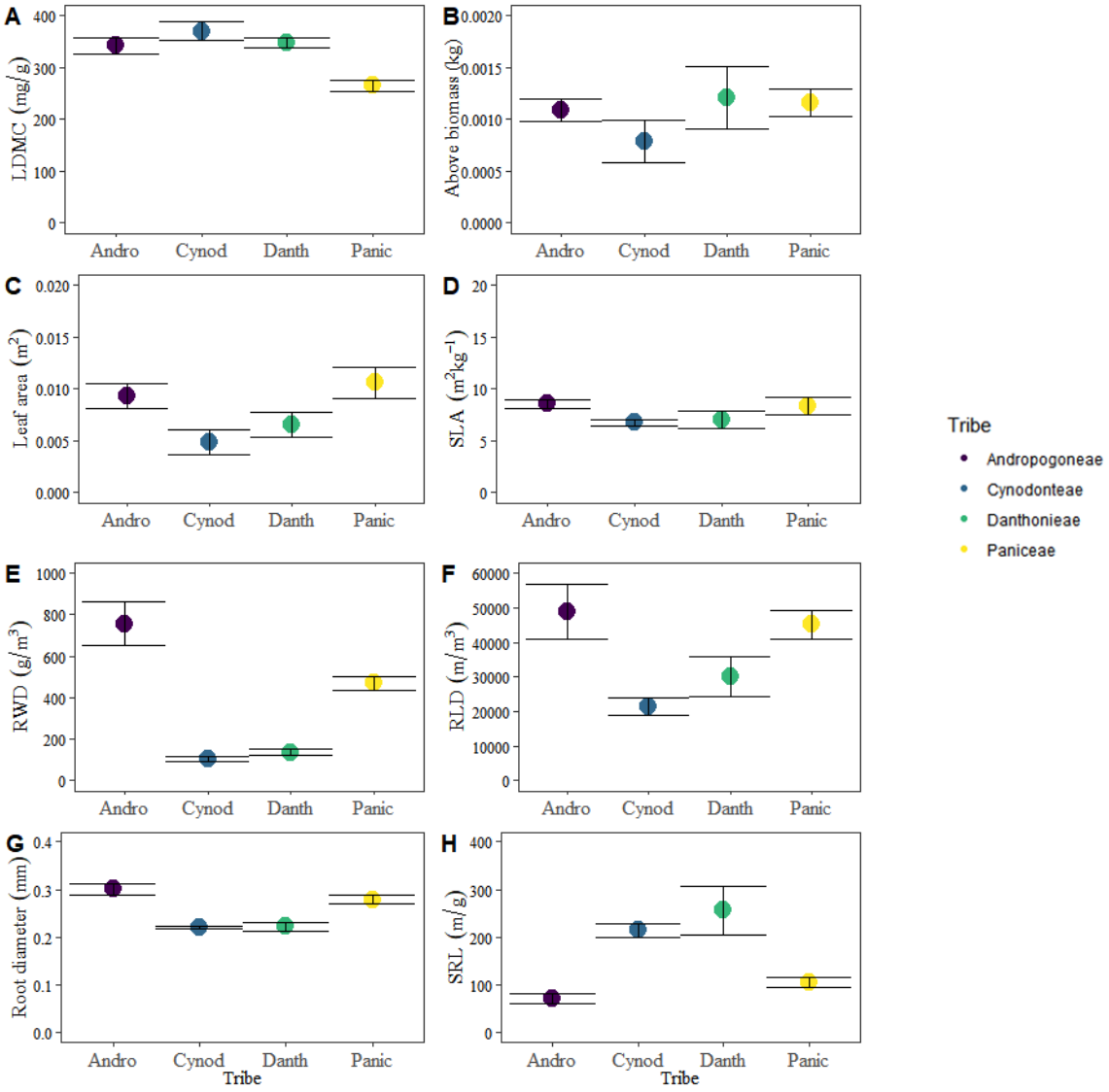

Figure 2.3 Morphology compared between tribes.

Comparison of morphological trait variation between grass tribes. (A) Leaf dry matter content (LDMC, mg/g), (B) above ground biomass (kg), (C) specific leaf area(SLA, $m^2\ kg^{-1}$), (D) total leaf area (m^2), (E) root weight density (RWD, g/m^3), (F) root length density (RLD, m/m^3), (G) root diameter(mm), (H) specific root length(SRL, m/g). Values are mean ± 1 SE.

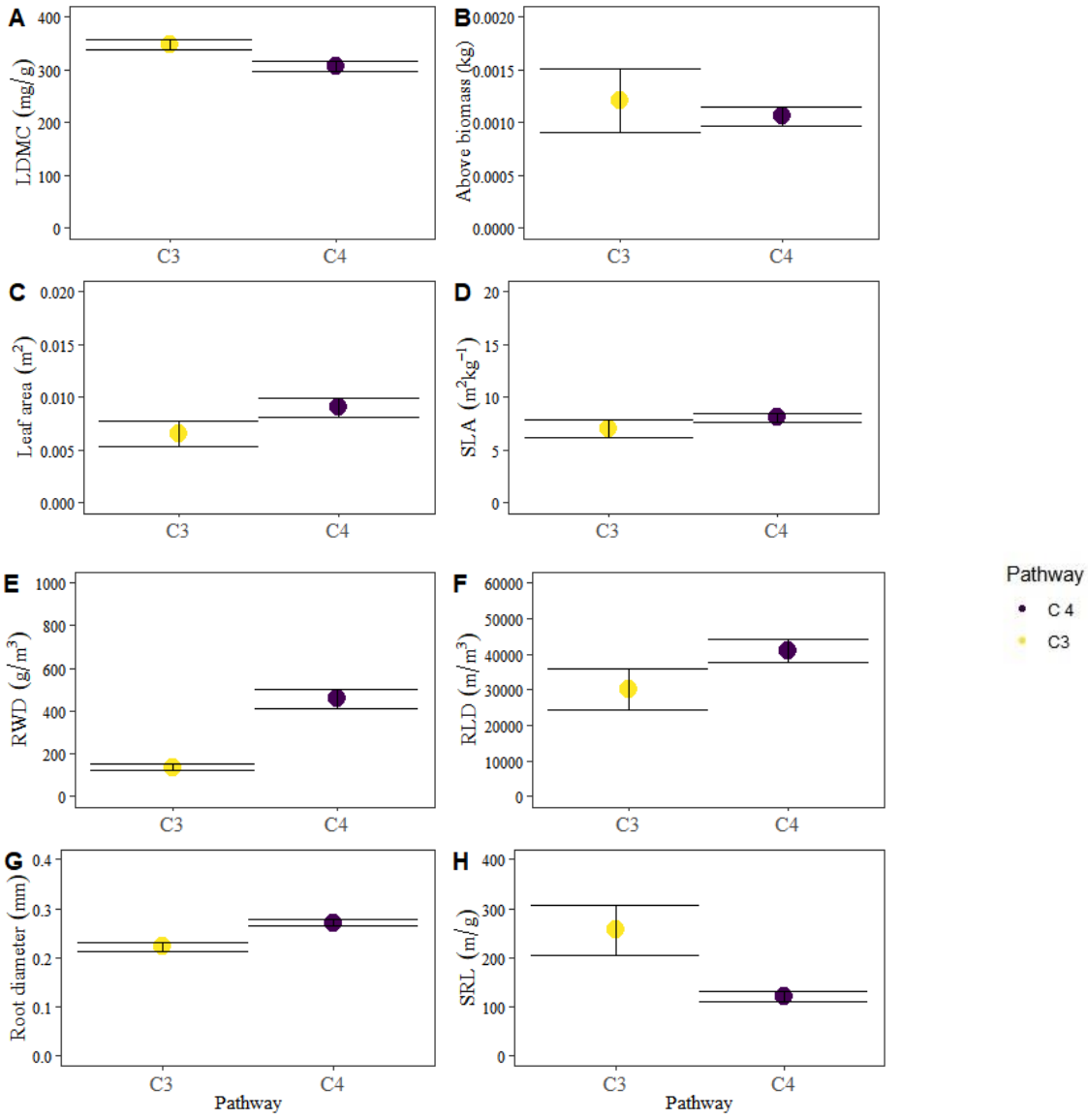

Figure 2.4 Morphology compared between photosynthetic pathways.

Morphological trait variation between photosynthetic pathway (C_3, C_4). (A) Leaf dry matter content (LDMC, mg/g), (B) above ground biomass (kg), (C) specific leaf area (SLA, $m^2\,kg^{-1}$), (D) total leaf area (m^2), (E) root weight density (RWD, g/m^3), (F) root length density (RLD, m/m^3), (G) root diameter (mm), (H) specific root length (SRL, m/g). Values are mean ± 1 SE.

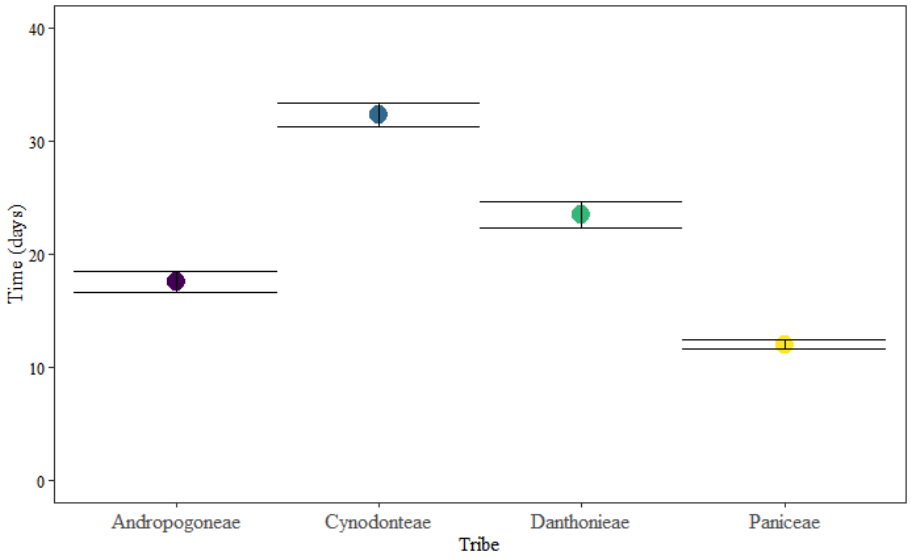

Figure 2.5 Average days in drought compared between grass tribes.

Mean time (days) each grass tribe persisted during drought. Values are shown in mean ±1SE.

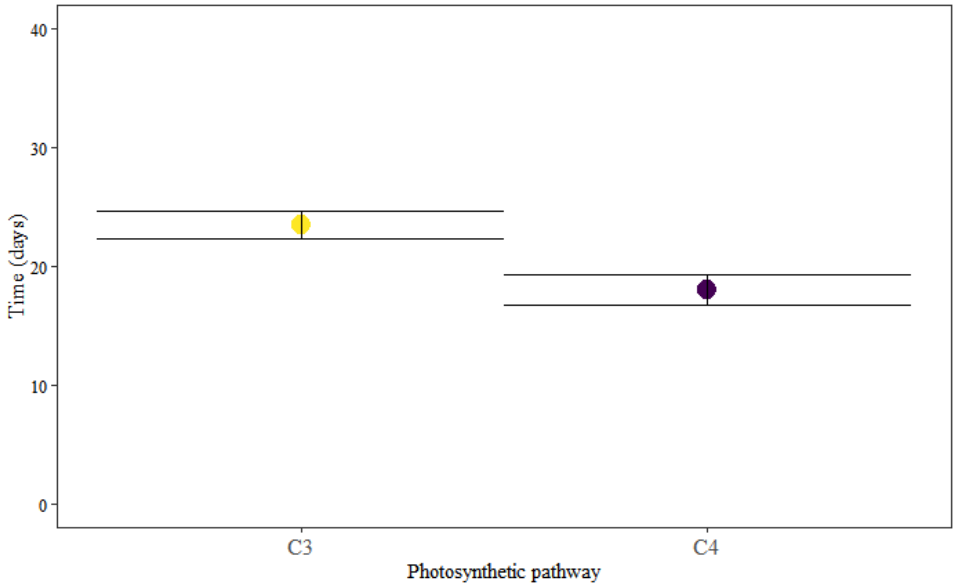

Figure 2.6 Average days in drought compared between photosynthetic pathways.

Mean time (days) grasses belonging to different photosynthetic pathways survived drought stress. Values are shown in mean ±1SE.

Chapter 3 - Grazing alters root traits, rhizome biomass, and non-structural carbohydrates at varying soil depths

Introduction

Grassland plants invest in growth, biomass, and energy storage belowground disproportionately more than plant species in other ecosystems (Weaver 1968). However, grassland research traditionally focuses on how grazing impacts aboveground productivity, ignoring this critical component. Grassland perennial plants have co-evolved with grazing and fire, devoting a large portion of their photosynthate and energy reserves into roots and rhizomes. (Gibson 2008). These belowground organs are responsible for water and nutrient acquisition, carbohydrate storage, and reproduction mechanisms that allow them to resprout after disturbances. Grazing has the potential to reduce allocation to root and rhizome biomass resulting in negative effects on future resource acquisition, growth, and survival (VanderWeide and Hartnett 2015). Yet, investigations including roots and rhizomes are seldom included due to the difficulty and destruction involved in observing belowground dynamics (Biswell and Weaver 1933).

Early research by grassland ecologist J. Weaver observed that increased grazing intensity had a negative (decreasing) effect on belowground grass biomass in the Great Plains region of North America (Weaver 1968). In a simulated grazing experiment (i.e., clipping), Biswell and Weaver (1933) showed a reduction in both belowground root biomass and length and a failure to produce new rhizomes in response to repeated defoliation. This pivotal work influenced

ecologists' interests in belowground dynamics. Since these early studies, further research in the Great Plains has shown varied responses to grazing and simulated grazing (i.e., clipping or mowing). Compared to our understanding of grazing impacts on aboveground grass biomass and productivity, research investigating belowground processes has been limited. Investigations by McNaughton et al. (1998) in the Serengeti found no evidence that grazing reduced productivity or biomass annually. However, the same year Biondini et al. (1998) found heavy grazing by cattle (after 8 years) reduced root biomass in North Dakota, USA. In the Great Plains (Kansas, USA), Johnson and Matchett (2001) used root ingrowth soil cores to inspect changes in roots to grazing and found that root productivity decreased and heavily grazed (*B. bison*) lawns had half the amount of root biomass as ungrazed treatment areas. Frank et al. (2002) found grazing by native ungulates in Yellowstone (Wyoming, USA) stimulated an increase in root productivity. Kitchen et al. (2009) found simulated grazing (mowing) to decrease root biomass. Nippert et al. (2012) compared grazed (*B. bison*) and ungrazed treatment areas using deep soil cores where they found more biomass and root length in ungrazed areas as compared to grazed. These confounding investigations limit our understanding of how grazing alters root traits and suggest further investigations of root dynamics are warranted.

Environmental factors (e.g., climate, soil) can alter available water and nutrients in grasslands. Root traits (e.g., biomass, root length density, and diameter) and rooting depth affect a plants ability to acquire these resources (Schenk and Jackson 2002). Roots of tallgrass prairie species have been shown to rely on shallow soil water and those shallow root systems have been found to be impacted from grazing (Nippert et al. 2012, Johnson and Matchett 2001, Nippert and Knapp 2007). Root length density (RLD) can influence how plants are able to compete for soil resources, as increasing RLD has been shown to provide competitive success (Schenk and

Jackson 2002, Ravenek et al. 2016). Disturbances have been shown to influence exotic species cover in grassland sites (Smith and Knapp 1999) thus, alterations to root traits (e.g., RLD) from grazing has the potential to negatively impact a plant's capabilities to forage for water and nutrients, alter its competitive ability, and may influence grassland invasibility.

Rhizomes (belowground stem organs used mainly for sites of reproduction and carbohydrate storage) are responsible for spring growth, resprouting following disturbance (e.g. fire and grazing) and account for large portions of belowground biomass (Klimešová et al. 2018). The ability of grassland plants to tolerate disturbance depends in part on their investment into belowground storage and structures (e.g., rhizomes and non-structural carbohydrates, NSCs). Reproduction in grasslands is mainly through vegetative processes, as seedlings tend to be more vulnerable to grazing, fire, and drought (Benson and Hartnett 2006). NSCs (e.g., sucrose, glucose, and starch) are stored in both roots and rhizomes and are posited to play a large role in resprouting of perennial plants (Klimesova and Klimes 2007). Carbohydrate storage is used by plants when leaves senesce seasonally or photosynthetic tissues are removed (e.g., grazing and fire) and when photosynthesis cannot supply growth and maintenance (Chapin et al. 1990, Janeček and Klimešová 2014, Martínez-Vilalta et al. 2016) Previously, Dalgleish et al. (2009) showed that over 10 years of grazing caused a reduction in belowground rhizomes and buds. Dong et al. (2012) and more recently, Ottaviani et al. (2021) demonstrated that regardless the type of disturbance (i.e. grazing or mowing) increased intensities and frequencies of disturbance resulted in a decrease of rhizome biomass. Carbohydrate reserves have been shown to fluctuate seasonally, decreasing in the spring and increasing in the fall (Janeček et al. 2015) as well as decreasing in response to resprouting after disturbance (Janeck and Klimešová 2014). Although studies investigating rhizomes and carbohydrates are limited, these observations

suggest that allocation to aboveground growth after defoliation reduces belowground biomass and carbohydrates (Chapin et al. 1990).

Incorporating belowground structure and function is imperative to understanding whole plant processes, fitness, and future survival (Laliberté 2017, Klimešová et al. 2018). However, research has been limited to contradictory conclusions and rarely includes other belowground storage structures (i.e., rhizomes) or carbohydrate components and concentrations in root tissues. The broad objective of this research was to investigate how grazing (by cattle) affects grass root structure and function. I aimed to answer these two main questions, 1) how does grazing impact investment into grass root morphology and root and rhizome biomass? and 2) does grazing reduce root non-structural carbohydrates? I did this by specifically looking at changes in total root length, biomass, specific root length, diameter, rhizome biomass, and root and rhizome NSCs. Comparisons were made between grazed and ungrazed treatment areas at three locations in the Great Plains of North America where samples were taken from two co-dominate grass species (*A. gerardii* and *S. nutans*) and compared across different soil depths.

Materials and Methods

Site Descriptions

This study took place at three different locations within the Great Plains region of North America. Platter River Prairie is tallgrass prairie with approximately 4,609 acres in Wood River, Nebraska, USA (40°44′, 98°34′W). Mean annual precipitation is 730 mm. Average high temperatures are between the months of May to August (16.2-3.5 °C). Flint Hills Tall Grass Prairie Preserve is located in Cassody, Kansas, USA (38°02′,96°37′W). This location covers 2,188 acres. Average high temperatures are between the months of April to October (20-32.7 °C). Mean annual precipitation is 870 mm. Konza Prairie Biological Station is a 3,487-ha prairie in Manhattan, KS, USA (39°05′,96°35′W). Mean annual precipitation is 900 mm and average high temperatures are in the months of May to September (25-32 °C).

Experimental Design

Soil cores were taken using a Geoprobe Systems hydraulic push corer (Salina, KS, USA) in June 2018, at the three locations listed above. The core had a diameter of approximately 3 cm and reached to a depth of 2 m when possible. Soil cores were removed from grazed and ungrazed treatment areas within each location. Each site was accessed prior to data collection to ensure similar topography, fire frequency, grazing history, and identify the dominant grass species within each location. Random plots were used within treatment areas, but soil cores were positioned over one of two dominant tallgrass species used in this analysis: *Andropogon gerardii* (big bluestem) and *Sorghastrum nutans* (Indian grass). Four cores of each species were removed from both grazed and un-grazed locations, totaling 16 cores removed from each site. Once removed, each core was divided into a depth class (0-10 cm, 11-20 cm, 21-35 cm, 36-50 cm, 51-

75 cm, 75-100 cm, 101-150, 150+ cm (when possible). A portion of soil was saved from each depth, grazing treatment, and location, which was analyzed for soil texture at the Soil Testing Lab at the Department of Agronomy, Kansas State University (Manhattan, KS, USA, Table 3.21). Soil texture class was calculated using the United States Department of Agricultural Natural Resources Conservation Service soil texture calculator. Each core was then washed free of soil and debris, using deionized water, tweezers, and 2 mm mesh sieves.

Root Morphology

Roots were analyzed by depth for length (cm) and diameter (mm) using WinRhizo, a digital root imaging program (WinRhizo, Regent Instruments Inc., Ontario, Canada). This involved floating clean roots in a clear acrylic tray (purchased from WinRhizo) and imaging the roots on a flatbed Canon scanner. Root morphology was analyzed for root length (cm) and diameter (mm). After root morphology was complete, the roots were dried in an oven at 60 °C for 48 hours and weighed for dry biomass (g).

Non-structural carbohydrates

Roots and rhizomes that were washed free of debris were microwaved before root analysis to stop enzymatic activity, then oven dried after morphological analysis as described above. Dried root and rhizome biomass was homogenized and finely ground using a Wig-L-Bug ball mill grinder (Dentsply Sirona; York, PA,USA). Root biomass was insufficient for NSC analyses for depths below 35 cm. Thus, to have enough sample, I combined root samples at deeper depths (36-100 cm) for NSC analysis. Approximately 20 mg of finely ground root tissues were combined with 80% ethanol in 2.5 ml microcentrifuge tubes. All tubes were placed in a dry

bath incubator for 20 minutes at 80 °C. Tubes were centrifuged and supernatant was removed into separate (labeled) microcentrifuge tubes. This process of ethanol extraction and heating was repeated two more times. (Hendrix 1993, Landhausser et al., 2018). The supernatant removed from the tubes were used to quantify sucrose and glucose using the Total Starch Assay kit from Megazyme Co. (Wicklow, Ireland). Next, 20 µl of extract were pipetted into 3 wells (sucrose, glucose, and control) on a 96 well plate. Three replications were made for each individual sample. The plates were placed in a drying oven uncovered at 55 °C to evaporate the ethanol and then rehydrated with 20 µl of dH_2O (deionized water). Samples processed for sucrose had 10 µl of invertase to each well. These plates were incubated on a plate shaker for 15 minutes at 37 °C at 300 Hz. Sample wells (sucrose and glucose) had 200 µl GOPOD solution (glucose oxidase/peroxidase reagent with O-dianisidine), blank wells had 200 µl dH_2O added. Trays were incubated at room temperature for 20 minutes. Absorbance (at 510 nm) was read in the spectrophotometer. Glucose and sucrose standard curves were used to determine concentrations. Leftover tissue sample from the ethanol extraction was rehydrated with 1 ml of dH_2O and autoclaved for 1 hour at 135°C. Afterwards they were dried overnight at 65 °C. The Total Starch Assay kit from Megazyme was used to digest the samples. The dried tissues had 1 ml of α-amylase added to the microcentrifuge tubes, vortexed and then heated for 30 minutes at 85 °C. Samples were cooled then vortexed at 13,000 g for 1 minute. 15 µl of amyloglucosidase solution was added and then the tube was mixed by vortex again. The tubes were heated in a shaking water bath at 50°C for 45 minutes. The samples were cooled before micropipeting. 20 µl of each sample were replicated three times on a 96 well plate, with 200 µl of GOPOD solution added. Trays were incubated for 20 minutes, then absorbance was read in the spectrophotometer at 510 nm. Starch standard curve was made from known starch concentrations (maize).

Statistics

Morphology and NSCs were analyzed using a linear mixed-effects model with location, treatment, species, and depth as fixed effects and sample ID as random effect using the ‘lme’ function in the “lme’ package in the statistical program R 4.0.4 (R Core Team 2021). Analysis of variance and post-hoc multiple comparisons were calculated using Tukey’s Honestly Significance Difference test.

Results

Morphology

Root and rhizome weight density

Root weight density (RWD, g/cm^3) was found to vary significantly for all main effects (location, species, grazing treatment, and soil depth) but there were no significant interactions (P<0.05, Table 3.1). The Konza Prairie location had the lowest RWD when compared to the Flint Hills Prairie, which had the highest RWD (p=0.05, Figure 3.1). RWD was higher in the ungrazed treatments than the grazed treatment (p = 0.0002, Figure 3.2, Table 3.2). *A. gerardii* had significantly higher RWD than *S. nutans* (Figure 3.3, Table 3.2). RWD for the shallowest layer (0-10 cm) and rhizomes were significantly higher than all other depths (p < 0.0001, Table 3.2, Figure 3.4).

Root length density

Root length density (RLD, cm/cm^3) varied significantly by depth (p < 0.0001) and had a significant interaction between location and grazing treatment (p = 0.036, Table 3.3). The Flint Hills Prairie ungrazed RLD was significantly higher than all other locations and treatments (p<0.05, Table 3.4, Figure 3.1). Average RLD for the ungrazed treatments were found to be significantly higher than the grazed treatments (p<0.01, Figure 3.2, Table 3.4). The highest RLD was found at the shallowest depth, (0-10 cm) which differed from all other depths (p<0.05,Table 3.4,). The next depth (11- 20 cm) had the second highest RLD, which differed significantly from all other depths below. The RLD for depths 21-35 cm and 36-55 cm did not differ from each other, but did differ from everything below 56 cm. There were no differences between species for RLD (Figure 3.3, Table 3.2).

Specific root length

Specific root length (SRL, m/g) had significant differences for all main effects but no interactions ($p<0.05$, Table 3.7). The Konza Prairie had higher SRL than both locations ($p=0.003$, Table 3.8, Figure 3.1). The grazed treatments had higher SRL than the ungrazed treatments ($p=0.02$, Table 3.8, Figure 3.2). *S.nutans* had higher SRL than *A.gerardii* ($p=0.009$, Figure 3.3, Table 3.8). The highest SRL was found at depths 56-75 cm and 76-100 cm, which differed from all other soil depths ($p<0.001$, Table 3.8, Figure 3.4).

Non-structural carbohydrates

Starch

Differences in the non-structural carbohydrate starch (ug/mg dry weight) were found between location, species, and soil depth ($p<0.05$, Table 3.9) but there were no significant interactions. Starch was highest at the Konza Prairie but did not differ significantly from the Platte River Prairie starch (Table 3.10, Figure 3.6). Starch was significantly higher for *A.gerardii* than *S.nutans* ($p<0.001$, Figure 3.8, Table 3.10). Starch on average was much lower in the rhizomes when compared to the soil depths 0-10 cm and 11-55 cm ($p=0.0004$. Table 3.10, Figure 3.9).

Sucrose

There was a significant interaction found between location and species ($p=0.02$, Table 3.11) for sucrose (ug/mg dry weight). Sucrose for the grass species *A .gerardii* at the Platte River was significantly lower than all other combinations of locations and species ($p<0.05$, Table 3.12 Figure 3.6). Overall significance was found between location and treatment ($p=0.047$, Table 3.11), however Tukey's HSD for multiple comparison did not find any significant differences between location and grazed combinations for sucrose (Table 3.12).

Glucose

Glucose (ug/mg dry weight) was found to be significantly different for location ($p<0.001$, Table 3.13). Platte River Prairie had the highest glucose but was not significantly different than the Flint Hill Prairie location (Table 3.14, Figure 3.6).

Konza Prairie deep soil depths (up to 125 cm)

Konza Prairie was the only location with enough root tissue below 55 cm to combine and test for non-structural carbohydrates. Results for starch show the only differences were between depth and no other main effects. Starch (ug/mg) was lowest at the deepest depth (56 -125 cm) and was significantly different than the highest starch found at soil depths (0-10 cm and 11-55cm) ($p<0.05$, Table 3.15, Figure 3.9). Sucrose (ug/mg) was found to also differ significantly for soil depth ($p=0.003$, Table 3.17). Sucrose found at the soil depth; 11-55 cm had the highest mean value which differed from the lowest sucrose found at the soil depth 56-125 cm (Figure 3.9). Glucose was found to differ between soil depths ($p=0.001$) and species ($p=0.001$, Table 3.19). *A.gerardii* had higher glucose than *S.nutans* ($p<0.01$, Table 3.20, Figure 3.9). Similar to the results as sucrose, glucose average values were found to be highest at the soil depth 11-55 cm, which was significantly higher than the deepest depth, 56-125 cm ($p<0.001$, Table 3.19, Figure 3.9).

Discussion

Root traits have been shown to have variable responses to grazing. Previous studies have observed an increase, no difference, and decrease in root biomass, length, and productivity (McNaughton et al. 1998, Johnson and Matchett 2001, Frank et al. 2002). This study reported reduced root weight density (RWD) and root length density (RLD) in response to grazing, similar to previous work in the Great Plains region of North America (Johnson et al. 2001, Kitchen et al. 2009, Nippert et al. 2012). Regardless of grazing treatment, all root mass and root length were shown to decrease significantly by depth (Figure 3.4). Over 90% of RWD (roots and rhizomes) and RLD was found in the top 0-35 cm of soil. This is consistent with recent work which has demonstrated that dominate C_4 grasses within this region depend largely on the shallow soil layers for resources and invest a large portion of their biomass to form a dense mat of tissue at these depths (Nippert and Knapp 2007a, 2007b, Nippert et al. 2012). Competition in the prairies exist belowground for space in addition to water and nutrient capture. Grazing has been shown to increase the abundance of non-dominant grass and forb species (Hartnett et al. 1996). Not only does a high density of roots allow these grass to be competitive for limiting soil resources, the dense network of roots and rhizomes help form a barrier to invasive species (Schenk and Jackson 2002, Ravenek et al. 2016). These decreases in roots hint that even moderate (annual) grazing has the potential to impact resource acquisition and alter grass species' competitive success. Both grass species (*A. gerardii*, big bluestem and *S. nutans*, Indian grass) used in this study showed a decrease in biomass due to grazing, however, there was a large difference between RWD and diameter between species (Figure 3.3). This could imply that either grazing affects grass species differently, similar to how fire causes differential

physiological responses in some grass species (*A. gerardii*, Knapp 1985) or grass species, such as big bluestem, have an adaptive response to invest in larger belowground organs to improve their fitness and persistence on the landscape. The observed decrease in rhizome biomass was similar to previous work that showed rhizomes decreased under heavy grazing and frequent mowing (Dong et al. 2012, Janeček and Klimešová 2014) and grazing decreased all belowground reproductive organs (sensu 'bud bank', Benson and Hartnett 2006, Dalgleish and Hartnett 2009). Although the prairies used in this study are not managed intensely, these results show that even moderate grazing has the capability to negatively affect rhizome production. Reduction of rhizome biomass is suggested to be a trade-off for the short term benefit of resprouting (Hartnett 1989), however it may result in a decrease of future reproductive success.

Perennial plants store a large portion of their biomass into roots and rhizomes, especially in environments with high disturbance, such as fire, drought, and grazing (Gomes et al. 2016). Within these belowground organs, carbohydrates are stored to be used seasonally or daily for various functions when photosynthesis is unable to supply enough carbon (e.g., maintenance at night, respiration, Martínez-Vilalta et al. 2016). Non-structural carbohydrates (NSCs) were found to vary between grazing treatments; however, the largest differences were found for starch (Figure 3.7). The largest concentration of starch was located in the roots of the top 0-10 and 11-55 cm of soil, which suggests that carbohydrate storage (and biomass) is invested preferentially to shallow soil layers (Figure 3.9). However, starch found within the rhizomes were extremely low in comparison. This could reflect a large decrease of NSC from rhizomes in response to spring regrowth, as samples were taken in June after plants were actively growing, or less investment of NSC initially in rhizomes in comparison to NSC stored in roots. Similar to the RWD results, big bluestem was found to have a larger starch concentration than Indian grass

(Figure 3.8). Again, this could either be attributed to different response to grazing pressures or an evolutionary trait, as carbohydrate composition (e.g., starch, sucrose, or fructose) in leaves have been found to contrast among grasses in different subfamilies (Moraes et al. 2013, Gomes et al. 2016), but studies comparing and contrasting root NSC composition between grass species have yet to be initiated.

Although this study took place in three locations within the tallgrass prairies of North America and differed in soil texture (see Table 3.21), there was an effect of grazing on RWD, RLD, diameter, and SRL, though differences varied in magnitude depending on location and root trait measured (Figure 3.1). RWD was consistently smaller in grazed treatment areas, regardless of location. Grasses in the grazed locations invested into higher SRL than those measured in the ungrazed treatment areas. SRL is considered an 'economic plant strategy' (Wright et al. 2004) that shows how a plant invests limited resources. The grazed grasses had limited biomass that required a "cheaper investment" in tissue per length of biomass. Although total sucrose and glucose did not vary between grazing treatments, there was a varied response when analyzed between locations. This may be in part because soluble sugars (i.e., sucrose and glucose) are used for both immediate functions and storage, which may overshadow grazing effects (Martínez-Vilalta et al. 2016). Starch was also found to have varied concentrations between locations, which could be due to site specific differences, grazing legacy, and the time of year plant tissue was collected (NSC could be increasing due to resupplies from current photosynthesis).

Conclusion

Grasslands are among the most threatened ecosystems in the world due to anthropogenic activities such as conversion to agriculture and overgrazed rangelands for domestic livestock production (Gibson 2008). One of the challenges to conserving and managing these systems is allowing for sustainable livestock production while preserving future grass success and survival in a changing climate. A complete knowledge of the effects of grazing is lacking, hindering our ability to make informed management decisions. This current work showed that grazing has the potential to alter belowground root and rhizome traits and non-structural carbohydrates. These changes have the potential to alter current grass resource acquisition, future vegetative reproduction, and competitive success. My work also illustrates that belowground ecological research in grasslands is thoroughly lacking. A continued focus on root dynamics will give us a better understanding of how grazing may impact grass species below ground structure and function, and future grassland production.

Acknowledgements

I would like to thank Seton Bachle, Rory O'Connor, Emily Wedel, Makenna (Miller) Hotz, Jessica Schauf, Chase Torla, Samuel Long, Sarah Inskeep, Madison Lofing, Greg Tooley, Jesse Nippert, Patrick O'Neal, Chris Helzer, Brian Obermeyer, Paula Matile, Lydia Zeglin, Zhilong Yang, Amanda Kuhl, and the LTER clip crew. This work would never have been possible without the incredible help from all of you.

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Tables and Figures

Table 3.1 Root weight density ANOVA

ANOVA for root weight density (g/cm^3) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and species (*A.gerardii* and *S.nutans*). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	3.80	0.0316	*
Trt	1	36	16.63	0.0002	*
Spp	1	36	4.80	0.0350	*
Loc:Trt	2	36	0.749	0.4800	
Loc:Spp	2	36	0.488	0.6179	
Trt:Spp	1	36	0.290	0.5934	
Loc:Trt:Spp	2	36	0.865	0.4296	
	NumDF	DenDF	F value	Pr(>F)	
Depth	7	169	23.98	<.0001	*

Table 3.2 Mean root weight density.

Average root weight density (g/cm^3) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*), and soil depth (cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Location	Mean	SE
Flint Hill Prairie	0.00098a	0.00011
Konza Prairie	0.00059b	0.00011
Platte River Prairie	0.00097a	0.00015
Grazing treatment	**Mean**	**SE**
Grazed	0.00057a	0.00007
Ungrazed	0.00114b	0.00012
Species	**Mean**	**SE**
A. gerardii	0.00098a	0.00012
S. nutans	0.00068b	0.00007
Soil Depth	**Mean**	**SE**
Rhizomes	0.00166a	0.00027
0-10 (cm)	0.00178a	0.00017
11-20 (cm)	0.00062b	0.00005
21-35 (cm)	0.00034b	0.00006
36-55 (cm)	0.00013b	0.00002
56-75 (cm)	0.00007b	0.00002
76-100 (cm)	0.00004b	0.00001
101-200 (cm)	0.00001b	0.00001

Table 3.3 Root length density ANOVA.

ANOVA root length density (cm/cm^3) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*) and depth (cm). Significance at the $\alpha=0.05$ level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	7.13	0.001	*
Trt	1	36	6.85	0.009	*
Spp	1	36	0.177	0.676	
Loc:Trt	2	36	3.35	0.036	*
Loc:Spp	2	36	0.495	0.613	
Trt:Spp	1	36	0.227	0.636	
Loc:Trt:Spp	2	36	0.572	0.569	
	NumDF	DenDF	F value	Pr(>F)	
Depth	6	157	182.17	<0.0001	*
Spp	1	44	0.002	0.962	
Trt	1	44	2.96	0.092	
Depth:Spp	6	157	0.042	0.999	
Depth:Trt	6	157	0.28	0.945	
Spp:Trt	1	44	0.168	0.683	
Depth:Spp:Trt	6	157	0.23	0.965	

Table 3.4 Mean root length density.

Average root length density (cm/cm^3) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and soil depth (cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

	Grazed		Ungrazed	
Location	Mean	SE	Mean	SE
Flint Hills Prairie	1.35a	0.22	2.97b	0.4
Konza Prairie	1.12a	0.26	1.29a	0.25
Platte River	2.17a	0.36	2.49a	0.44

Soil depth	Mean	SE
0-10 (cm)	4.93a	0.22
11-20 (cm)	2.01b	0.12
21-35 (cm)	0.85c	0.07
36-55 (cm)	0.44c	0.05
56-75 (cm)	0.29d	0.04
76-100 (cm)	0.13d	0.02
101-200 (cm)	0.06d	0.01

Grazing Treatment	Mean	SE
Ungrazed	2.07a	0.15
Grazed	1.45b	0.21

Table 3.5 ANOVA for root diameter.

ANOVA for root diameter (mm) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*) and depth (cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)
Loc	2	36	1.32	0.28
Trt	1	36	3.08	0.08
Spp	1	36	1.22	0.27
Loc:Trt	2	36	1.05	0.35
Loc:Spp	2	36	0.28	0.75
Trt:Spp	1	36	0.19	0.66
Loc:Trt:Spp	2	36	1.40	0.25

	NumDF	DenDF	F value	Pr(>F)
Depth	6	171	0.16	0.98

Table 3.6 Mean root diameter.

Average root diameter (mm) estimated for grazing treatment (grazed and ungrazed), and species (*A. gerardii* and *S. nutans*). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Grazing Treatment	Mean	SE
Ungrazed	0.425a	0.028
Grazed	0.354a	0.008

Species	Mean	SE
A. gerardii	0.414a	0.026
S. nutans	0.357a	0.008

Table 3.7 Specific root length ANOVA.

ANOVA for specific root length (m/g) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*) and depth (cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	6.75	0.003	*
Trt	1	36	5.94	0.019	*
Spp	1	36	7.43	0.009	*
Loc:Trt	2	36	0.45	0.63	
Loc:Spp	2	36	1.13	0.33	
Trt:Spp	1	36	2.14	0.15	
Loc:Trt:Spp	2	36	2.08	0.14	

	NumDF	DenDF	F value	Pr(>F)	
Depth	6	138	5.56	<.0001	*

Table 3.8 Mean specific root length.

Average specific root length (m/g) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*), and soil depth (cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Location	Mean	SE
Flint Hills Prairie	3.49a	0.20
Konza Prairie	5.30b	0.47
Platte River Prairie	3.85a	0.28

Grazing treatment	Mean	SE
Grazed	4.85a	0.34
Ungrazed	3.69b	0.26

Species	Mean	SE
A. gerardii	3.75a	0.36
S. nutans	4.89b	0.37

Soil depth	Mean	SE
0-10 (cm)	3.48a	0.24
11-20 (cm)	3.82a	0.27
21-35 (cm)	3.79a	0.29
36-55 (cm)	4.68a	0.59
56-75 (cm)	7.53b	1.47
76-100 (cm)	7.37b	3.03
101-200 (cm)	5.68a	1.34

Table 3.9 ANOVA for starch.

ANOVA for starch (ug/mg) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and species (*A.gerardii* and *S.nutans*) and soil depth(cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	5.17	0.006	*
Trt	1	36	4.04	0.15	
Spp	1	36	9.64	0.0001	*
Loc:Trt	2	36	0.62	0.26	
Loc:Spp	2	36	1.07	0.07	
Trt:Spp	1	36	1.68	0.43	
Loc:Trt:Spp	2	36	1.09	0.27	
Depth	2	68	0.87	0.0004	*
Trt	1	46	3.82	0.15	
Depth:Trt	2	68	0.58	0.95	

Table 3.10 Mean starch.

Average starch (ug/mg) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*), and soil depth (rhizomes, 0-10 cm, and 11-55 cm). Values are mean ±1SE. Letters indicate significant difference as determined by Tukey's post hoc tests ($p \leq 0.05$).

Location	Mean	SE
Flint Hill Prairie	8.03a	1.19
Konza Prairie	15.04b	1.92
Platte River Prairie	13.64b	2.06
Species	Mean	SE
A. gerardii	15.94a	1.63
S. nutans	8.26b	1.09
Grazing treatment	Mean	SE
Grazed	10.39a	1.40
Ungrazed	13.36a	1.48
Soil Depth	Mean	SE
Rhizomes	13.61a	1.76
0-10 (cm)	14.77b	1.62
11-55 (cm)	5.82b	1.37

Table 3.11 Sucrose ANOVA.

ANOVA for sucrose (ug/mg) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and species (*A. gerardii* and *S. nutans*) and soil depth (cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	0.54	0.64	
Trt	1	36	0.003	0.92	
Spp	1	36	0.03	0.76	
Loc:Trt	2	36	3.18	0.047	*
Loc:Spp	2	36	3.64	0.02	*
Trt:Spp	1	36	1.54	0.42	
Loc:Trt:Spp	2	36	0.002	0.58	
Depth	2	68	0.13	0.58	
Spp	1	46	0.00	0.96	
Depth:Spp	2	68	1.26	0.19	

Table 3.12 Mean sucrose.

Average sucrose (ug/mg) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and species (*A. gerardii* and *S. nutans*). Values are mean ±1SE. Letters indicate significant difference as determined by Tukey's post hoc tests ($p \leq 0.05$).

	A.gerardii		*S.nutans*	
Location	Mean	SE	Mean	SE
Flint Hills Prairie	4.55a	0.54	4.45a	0.87
Konza Prairie	5.25a	0.87	3.40a	0.41
Platte River Prairie	2.57b	0.70	4.99a	0.77

	Grazed		Ungrazed	
Location	Mean	SE	Mean	SE
Flint Hills Prairie	5.45a	0.79	3.56a	0.58
Konza Prairie	3.41a	0.72	5.05a	0.66
Platte River Prairie	3.56a	0.88	4.08a	0.73

Table 3.13 Glucose ANOVA.

ANOVA for glucose (ug/mg) comparing location (Flint Hills Prairie, Konza Prairie, Platte River Prairie), grazing treatment (grazed and ungrazed), and species (*A. gerardii* and *S. nutans*) and soil depth(cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Loc	2	36	9.1	<.001	*
Trt	1	36	0.008	0.98	
Spp	1	36	0.14	0.65	
Loc:Trt	2	36	0.74	0.48	
Loc:Spp	2	36	1.08	0.38	
Trt:Spp	1	36	0.20	0.68	
Loc:Trt:Spp	2	36	1.88	0.17	
Depth	2	60	2.23	0.10	
Loc	2	42	9.10	<.001	*
Spp	1	42	0.09	0.71	
Depth: Loc	4	60	0.99	0.38	
Depth:Spp	2	60	0.65	0.42	
Loc:Spp	2	42	1.12	0.38	
Depth:Loc:Spp	4	60	1.16	0.32	

Table 3.14 Mean glucose.

Average glucose (ug/mg) estimated for location (Flint Hills Prairie, Konza Prairie, Platte River Prairie) and soil depth (rhizomes, 0-10 cm, and 11-55 cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Location	Mean	SE
Flint Hills Prairie	4.48ab	0.24
Konza Prairie	3.89a	0.11
Platte River Prairie	5.11b	0.20

Soil Depth	Mean	SE
Rhizomes	4.12a	0.21
0-10(cm)	4.74a	0.21
11-55(cm)	4.50a	0.20

Table 3.15 Starch ANOVA for Konza deep soil.

ANOVA for starch (ug/mg) using only Konza Prairie data. Analysis shown for grazing treatment (grazed and ungrazed), species (*A.gerardii* and *S.nutans*) and soil depth (rhizomes. 0-10 cm, 11-55 cm, and 56-125 cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Trt	1	16	0.46	0.5	
Spp	1	16	0.87	0.36	
Trt:Spp	1	16	0.001	0.96	
	NumDF	DenDF	F value	Pr(>F)	
Depth	3	15	3.75	0.03	*

Table 3.16 Mean starch for Konza deep soil.

Average starch (ug/mg) for Konza Prairie data only by soil depth (cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Soil Depth	Mean	SE
Rhizomes	9.46b	3.02
0-10 (cm)	17.45a	3.1
11-55 (cm)	15.51a	3.35
56-125 (cm)	2.28b	0.57

Table 3.17 Sucrose ANOVA for Konza deep soil.

ANOVA for sucrose (ug/mg) using only Konza Prairie data. Analysis shown for grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*) and soil depth (rhizomes. 0-10 cm, 11-55 cm, and 56-125 cm). Significance at the α=0.05 level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Trt	1	16	0.89	0.35	
Spp	1	16	2.83	0.11	
Trt:Spp	1	16	0.12	0.72	
	NumDF	DenDF	F value	Pr(>F)	
Depth	3	15	6.92	0.003	*
Trt	1	18	2.49	0.13	
Depth:Trt	3	15	2.76	0.07	

Table 3.18 Mean sucrose for Konza deep soil.

Average sucrose(ug/mg) using Konza Prairie soil depth (rhizomes. 0-10 cm, 11-55 cm, and 56-125 cm). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Soil Depth	Mean	SE
Rhizomes	2.69abc	0.67
0-10 (cm)	3.69abc	0.66
11-55 (cm)	5.82b	0.9
56-125 (cm)	2.43c	0.63

Table 3.19 Glucose ANOVA for Konza deep soil.

Anova for glucose (ug/mg) using only Konza Prairie data. Analysis shown for grazing treatment (grazed and ungrazed), species (*A. gerardii* and *S. nutans*) and soil depth (rhizomes. 0-10 cm, 11-55 cm, and 56-125 cm). Significance at the $\alpha=0.05$ level is indicated with an asterisk.

	NumDF	DenDF	F value	Pr(>F)	
Trt	1	16	0.16	0.69	
Spp	1	16	7.45	0.01	*
Trt:Spp	1	16	0.42	0.52	

	NumDF	DenDF	F value	Pr(>F)	
Depth	3	18	8.18	0.001	*
Trt	1	18	1.67	0.21	
Depth:Trt	3	18	0.66	0.58	

Table 3.20 Mean glucose for Konza deep soil.

Average glucose (ug/mg) using only Konza prairie data for soil depth (rhizomes, 0-10 cm, 11-55 cm, 56-125 cm) and species (*A. gerardii* and *S. nutans*). Values are mean ±1SE. Letters indicate significant differences determined by Tukey's post hoc tests ($p \leq 0.05$).

Soil Depth	Mean	SE
Rhizomes	3.65ab	0.15
0-10 (cm)	3.85ab	0.18
11-55 (cm)	4.09b	0.19
56-125 (cm)	2.85c	0.17

Species	Mean	SE
A. gerardii	4.02a	0.17
S. nutans	3.43b	0.12

Table 3.21 Soil texture.

Soil texture for shallow soil depths (0-10, 11-20, 21-35, and 36-55 cm) by location (Flint Hills Prairie, Platte River Prairie, and Konza Prairie) and grazing treatments (grazed and ungrazed).

			Soil texture			
Location	Treatment	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Class
Flint Hills Prairie	Grazed	0 -10	18	58	24	Silt loam
		11-20	14	46	40	Silty clay
		21-35	10	36	54	Clay
		36-55	8	36	56	Clay
Flint Hills Prairie	Ungrazed	0 -10	24	50	26	Silt loam
		11-20	22	48	30	Clay loam
		21-35	16	44	40	Silty clay
		36-55	12	36	52	Clay
Platte River Prairie	Grazed	0 -10	48	32	20	Loam
		11-20	48	32	20	Loam
		21-35	64	26	10	Sandy loam
		36-55	94	2	4	Sand
Platte River Prairie	Ungrazed	0 -10	60	24	16	Sandy loam
		11-20	48	34	18	Loam
		21-35	66	18	16	Sandy loam
		36-55	90	2	8	Sand
Konza Prairie	Grazed	0 -10	14	38	48	Clay
		11-20	8	44	48	Silty clay
		21-35	8	42	50	Silty clay
		36-55	8	44	48	Silty clay
Konza Prairie	Ungrazed	0 -10	10	52	38	Silty clay loam
		11-20	10	38	52	Clay
		21-35	6	38	56	Clay
		36-55	4	36	60	Clay

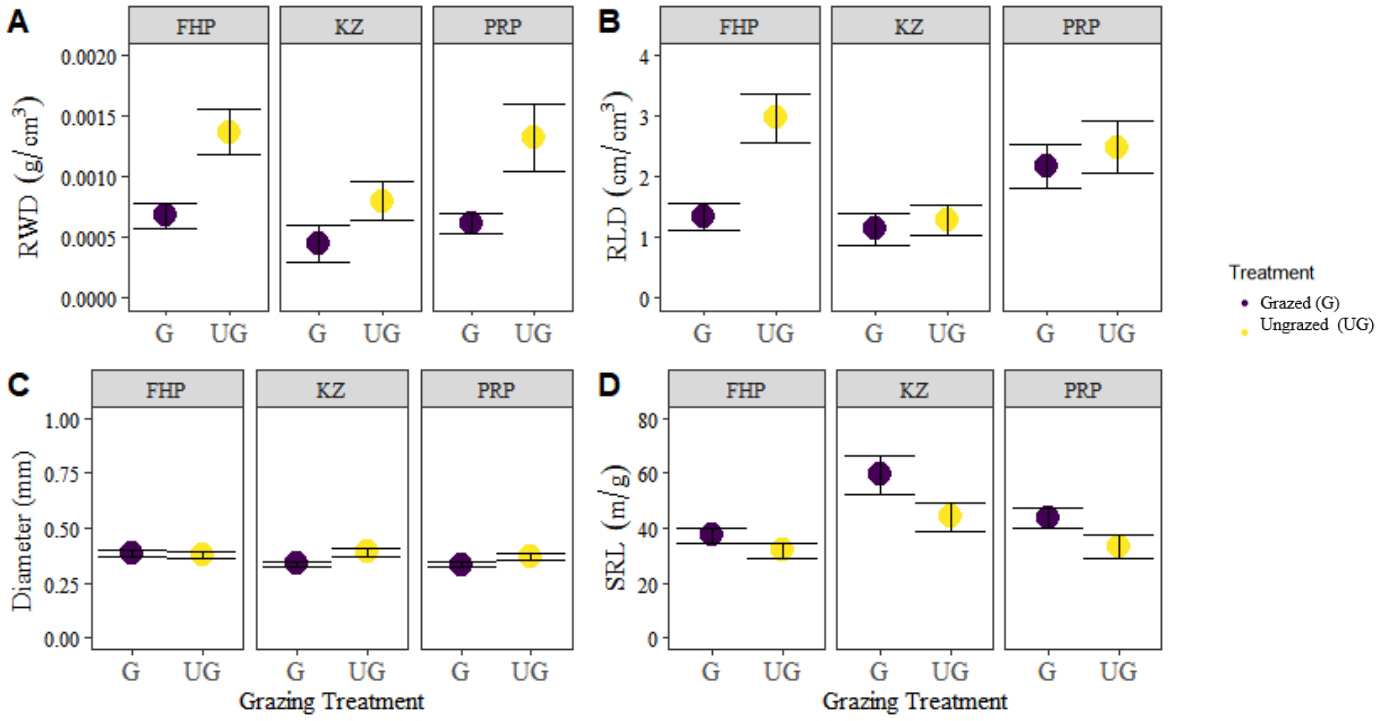

Figure 3.1 Mean morphology traits between locations and grazing treatments.

Average morphological traits: A)= root weight density (g/cm^3), B) root length density (cm/cm^3), C) root diameter (mm), D) specific root length (SRL, m/g). Comparisons shown between locations (FHP =Flint Hills Prairie, KZ=Konza Prairie, PRP=Platte River Prairie) and grazing treatment (UG=ungrazed, G=grazed). Trait values are shown in mean±1SE.

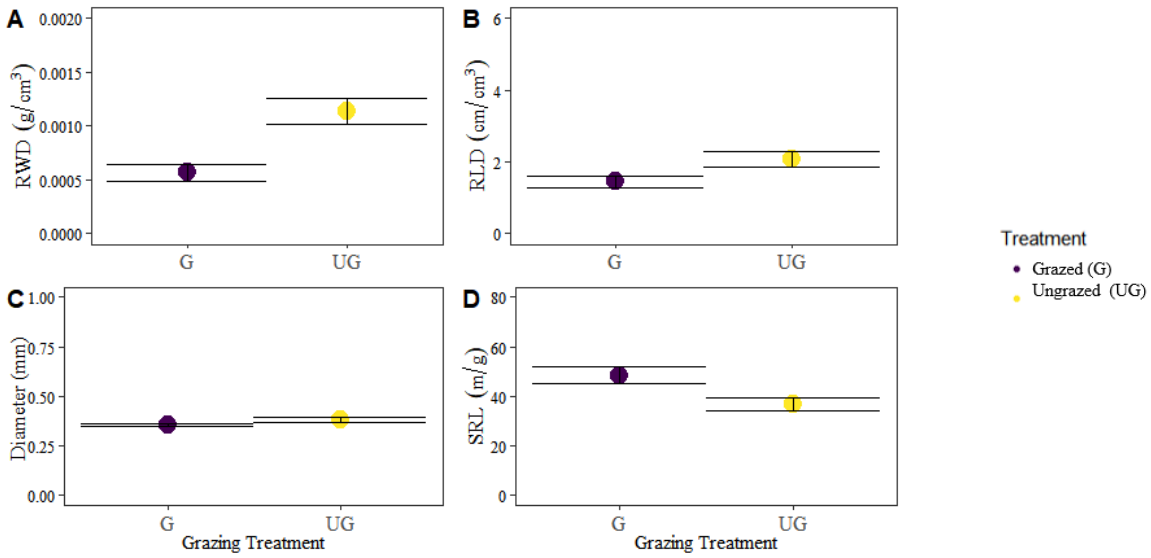

Figure 3.2 Mean morphology traits between grazing treatments.

Average morphological traits: A)= root weight density (g/cm^3), B) root length density (cm/cm^3), C) root diameter (mm), D) specific root length (SRL, m/g). Comparisons shown between grazing treatments (UG=ungrazed, G=grazed). Trait values are shown in mean±1SE.

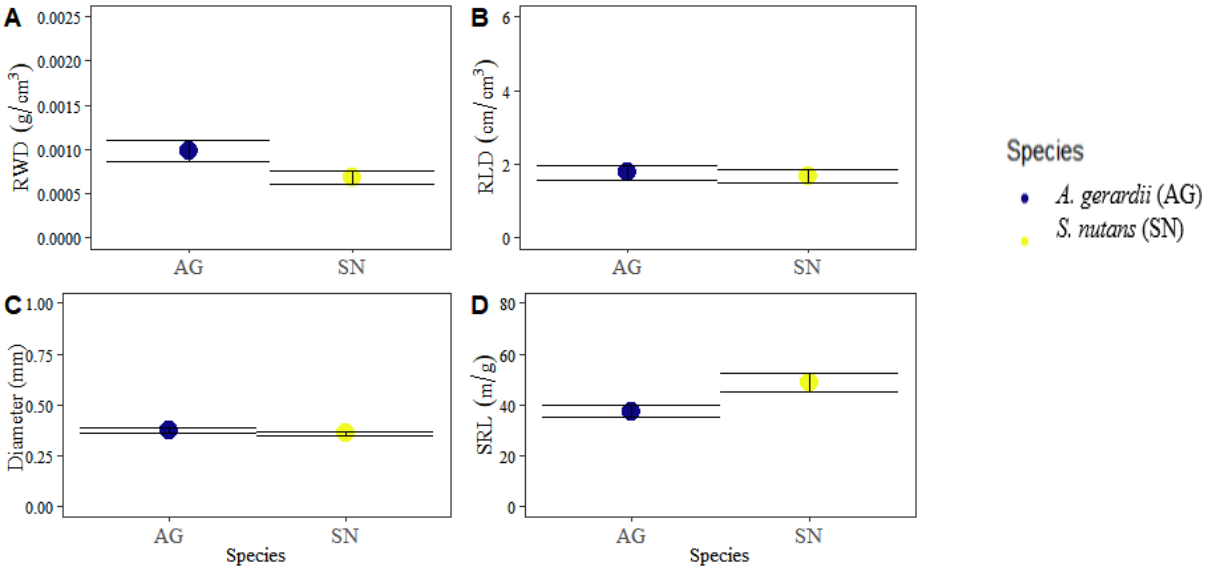

Figure 3.3 Mean morphological traits between grass species.

Average morphological traits: A)= root weight density (g/cm^3), B) root length density (cm/cm^3), C) root diameter (mm), D) specific root length (SRL, m/g). Comparisons shown between grass species (AG=*A.gerardii* and SN= *S.nutans*). Trait values are shown in mean±1SE.

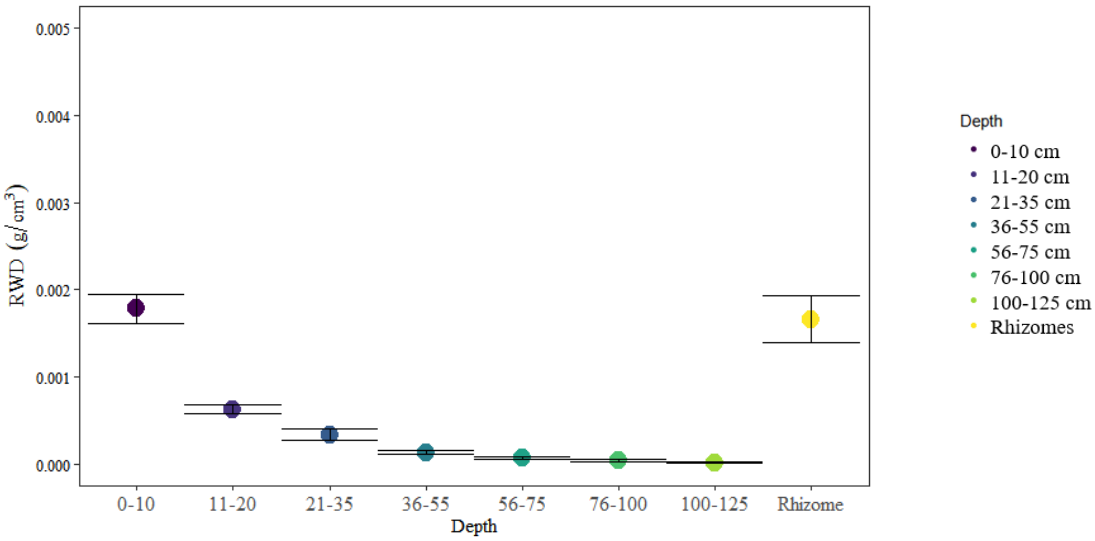

Figure 3.4 Average root weight density by soil depth.

Mean root weight density (RWD, g/cm^3) for soil depths (cm) and rhizomes. Values are shown in mean±1SE.

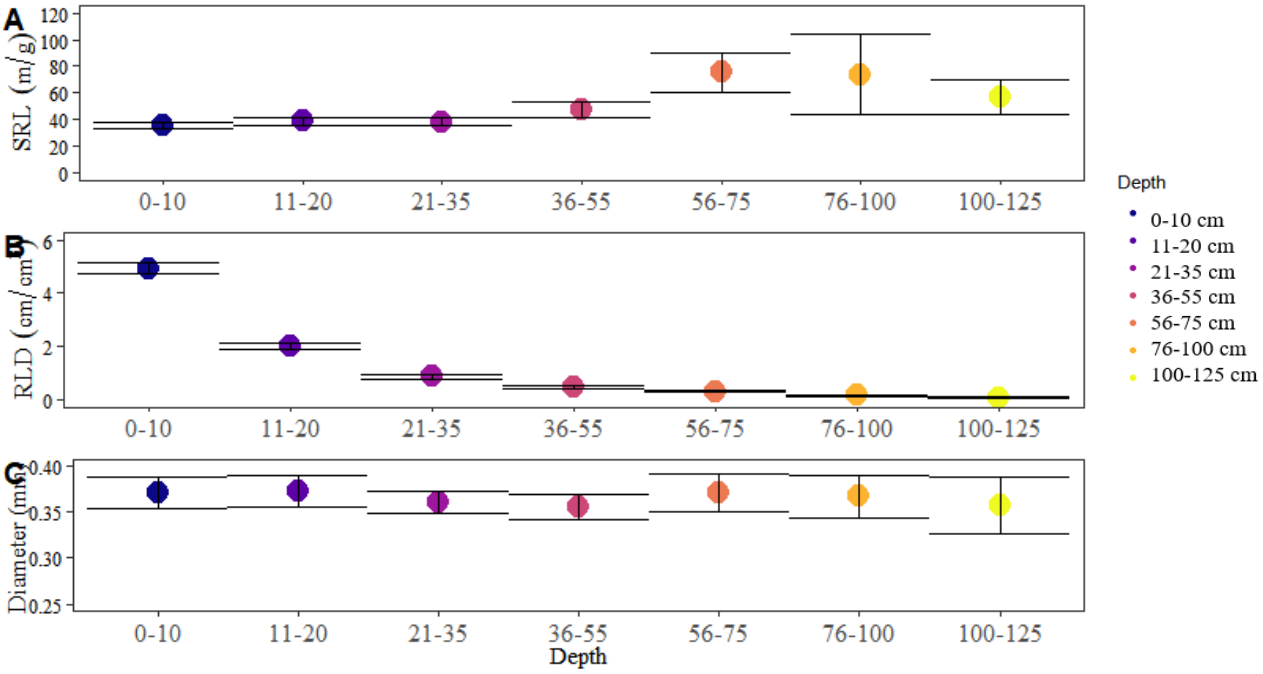

Figure 3.5 Mean morphological responses by soil depth.

Average morphological response for SRL (specific root length, g/g), RLD (root length density, cm/cm^3), and diameter(mm). Shown are mean values ±1SE for each soil depth (cm).

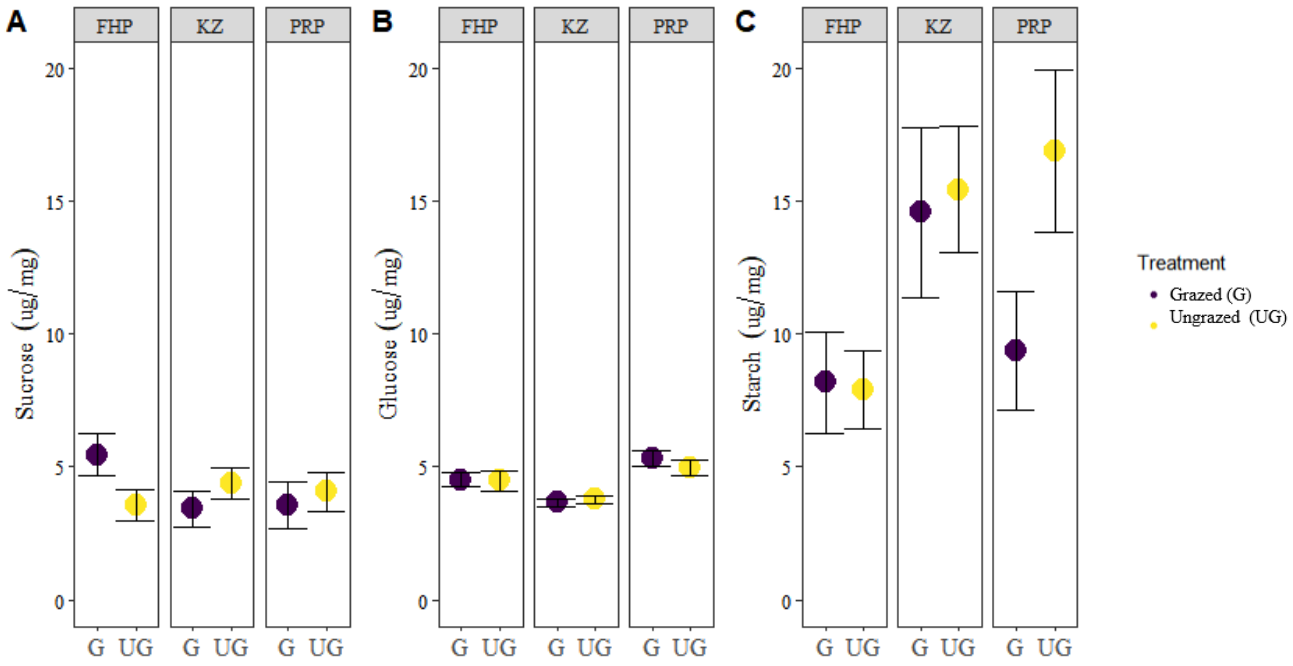

Figure 3.6 Mean non-structural carbohydrates by location and treatment.

Average non-structural carbohydrate (A) sucrose, B) glucose, and C) starch) compared between grazing treatments (G=grazed, UG=ungrazed) and locations (FHP=Flint Hills Prairie, KZ=Konza Prairie, PRP=Platte River Prairie). Response values are shown in mean±1SE.

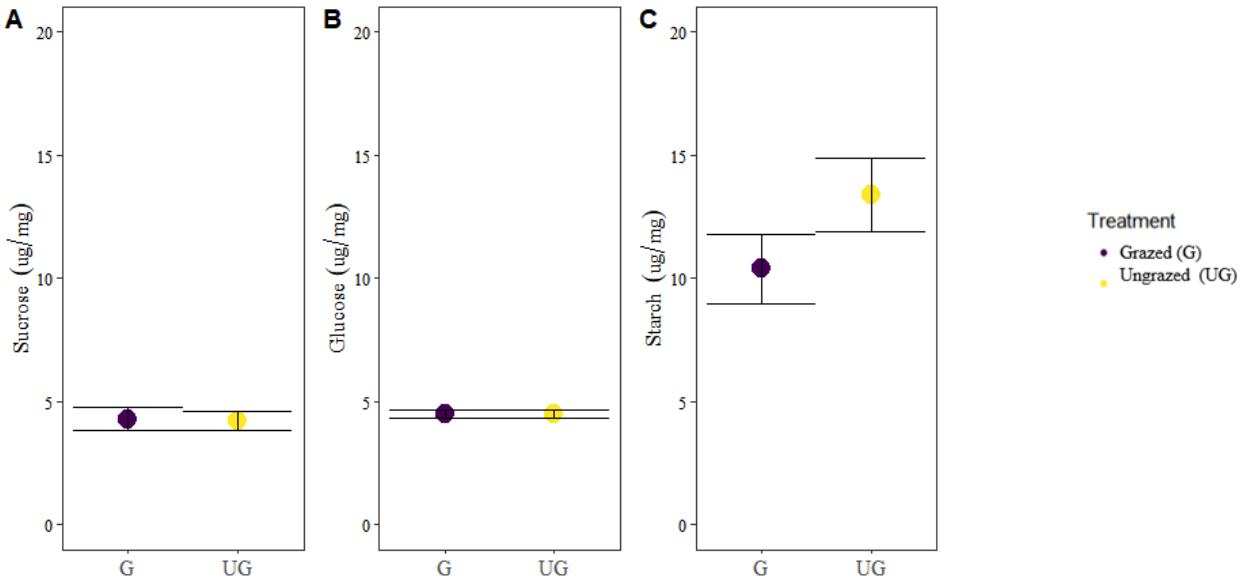

Figure 3.7 Mean non-structural carbohydrate between grazing treatments.

Average non-structural carbohydrate (A) sucrose, B) glucose, and C) starch) compared between grazing treatments (G=grazed, UG=ungrazed). Values are shown in mean±1SE.

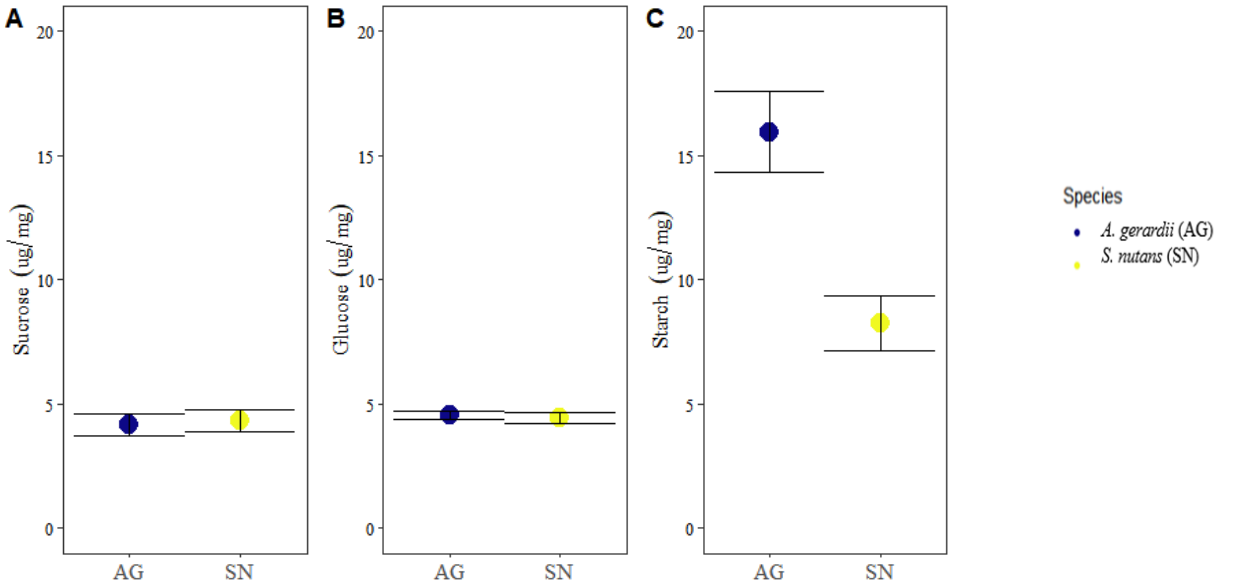

Figure 3.8 Mean non-structural carbohydrate between grass species.

Average non-structural carbohydrate (A) sucrose, B) glucose, and C) starch) differences between grass species (AG= *A.gerardii* and SN= *S.nutans*). Values are shown in mean±1SE.

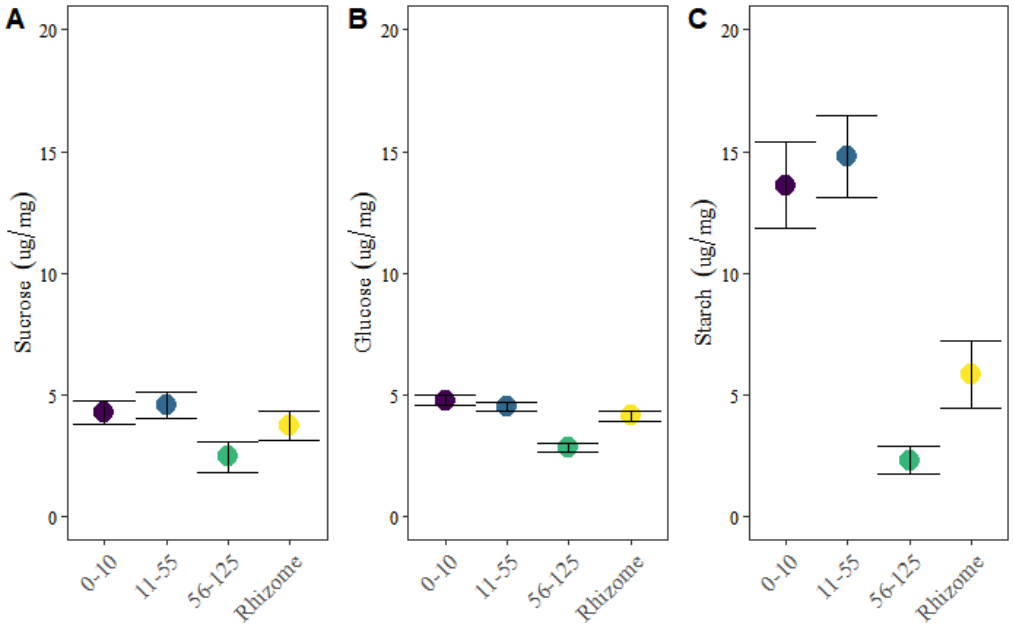

Figure 3.9 Mean non-structural carbohydrates by soil depth.

Average non-structural carbohydrates (A) sucrose, B) glucose, and C) starch) compared between soil depths (0-10 , 11-55, and 56-125 cm) and rhizomes. Values are ±1SE.

Chapter 4 - Conclusion

As the dominant species in grassland ecosystems, grasses are responsible for large portions of aboveground primary productivity and belowground biomass, which fluctuate with varying precipitation, altered fire regimes, and grazing pressures (Briggs and Knapp 1995, Rice et al. 1998). Climate change has the potential to alter precipitation patterns and increase temperature, severity of drought, and negative effects of disturbances (Asner et al. 2004, IPCC 2014). Reductions in productivity of either above or belowground biomass can have drastic consequences on wildlife food and shelter, biogeochemical cycles, non-native herbaceous and woody expansion, and decreased livestock production (Smith and Knapp 1999, Ratajczak et al. 2012, Craine et al. 2013). In response to varying environments, unpredictable disturbances (e.g., grazing, fire, and drought), and competition for limited soil resources (e.g., water and nutrients), grasses have evolved unique physiological and morphological adaptations to persist and dominate these systems.

In Chapter 2, I investigated C_3 and C_4 grass species response to drought by measuring gas-exchange and morphological traits in several congeneric grass species. C_4 grasses have high water use efficiency (*WUE*) when compared to C_3 grasses, which may have benefited their expansion and success into drier environments (Sage and Monson 1999). However, at the peak of drought stress in this experiment, their high *WUE* diminished, which suggests that the role of high inherent WUE as a mechanism of drought tolerance in C_4 grasses may not be as significant of an adaptive trait as originally expected during severe drought. Grasses from the closer related tribes Danthonieae and Cynodonteae persisted longer during drought (23 and 32 days) and had similar gas-exchange (stomatal conductance to water vapor, g_s, transpiration, E, and water use

efficiency, *WUE*) despite using different photosynthetic pathways (C_3 and C_4). They also had smaller leaf areas, lower leaf dry matter content (LDMC), higher specific root length (SRL), and thinner roots, adaptations that allow them to persist and dominate in aridic and xeric habitats. Grasses from the subfamily Panicoideae (tribes Andropogoneae and Paniceae) had higher *WUE*, lower g_s and *E*, but survived drought on average 17 and 12 days. These grasses had larger leaf areas, lower LDMC, low SRL, but larger investments into root weight and length. The large root systems and leaf areas allow these grasses to be competitive in ustic to udic, mesic prairie environments. Similarities in these aboveground and belowground morphological traits suggest that local adaptations and evolutionary history, in addition to photosynthetic pathway, allow these grasses to be successful in their varying environments.

In Chapter 3, I extracted soil cores from grazed and ungrazed treatment areas at three tallgrass prairies in the Great Plains, in order to examine changes in belowground traits in response to grazing. Root, rhizome, and non-structural carbohydrate (NSC) samples were collected from two dominant grass species (*A. gerardii* and *S. nutans*) and compared by grazing treatment, location, and soil depth. This study showed that root and rhizome biomass, root length density, and starch were reduced in response to grazing. In addition, these root characteristics all decreased by soil depth. All root, rhizome, and NSC results varied slightly among locations, likely as a result in small differences between precipitation, soil characteristics, grazing legacy, and time of year.

The potential for both drought and grazing to alter grass success and change aboveground and belowground productivity require further investigations in the face of future climate change. This involves understanding how grass species with varying drought and grazing tolerances differ in structural and functional traits, in addition to phylogeny and photosynthetic pathways.

Despite many advantages of using the C_4 photosynthetic pathway, my work demonstrates that this pathway alone was not responsible for persistence during drought, as grasses using the C_3 pathway survived drought longer than half of the C_4 grasses within this experiment. This suggests that our understanding of how grass populations may (or may not) survive future drought scenarios is incomplete. Grassland systems rely heavily on the dominant species, where a shift in grass species abundance and composition has the potential to affect entire grassland productivity and ecosystem services (Briggs and Knapp 1995, Craine et al. 2013). In addition, grasses that were characterized as having a drought tolerance strategy (were resilient longer during drought) had conservative aboveground traits, but belowground features differed from current plant strategy theories (Reich 2014). These findings suggest that our understanding of root traits and drought tolerance strategies of grass species is also limited (Laliberté 2017).

Furthermore, my work examining belowground structure and function in the tallgrass prairies demonstrated that root trait investigations in grazing systems is incomplete. My work showed that grazing can reduce belowground root biomass, length, and diameter, which have the potential to alter species resource acquisition and future competitive success (Dalgleish et al. 2009). This work also showed that grazing alters rhizome biomass and non-structural carbohydrates, knowledge that is critically lacking in the literature (Klimešová et al. 2018). Collectively, my work highlights the value of investigating drought tolerance in grass species, emphasizes the need to include phylogeny, and shows that our knowledge of grass root traits is severely lacking, despite the implications that reduced belowground structure and function can negatively affect future vegetative reproduction, productivity, and ecosystem services (Benson and Hartnett 2006, Nippert et al. 012, Craine et al. 2013).

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