

Demography and leaf physiology of *Cornus drummondii* in response to disturbance in tallgrass prairie

by

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Abstract

The expansion of woody species into grasslands has altered community structure and ecosystem function of grasslands worldwide. Woody encroachment has led to a loss of floristic biodiversity and changes in ecosystem carbon, nitrogen, and water relations. In tallgrass prairie, the increased cover and abundance of woody species is typically attributed to human activities such as decreased fire frequency and increased grazing intensity. Clonal shrubs are of particular concern because of their ability to spread via vegetative reproduction and resprout after fire. The demographic mechanisms that underlie shrub responses to climate history and disturbance are unknown. Here, I measured ramet demography and leaf physiology of the clonal shrub *Cornus drummondii* in response to disturbance at the Konza Prairie Biological Station. I quantified the growth rate, reproduction, and ramet demography of *C. drummondii* in response to fire frequency, grazing, and simulated browsing. Fire and grazing increased *C. drummondii* ramet growth rates, but rates were low across all treatments in response to extreme drought during the summer in 2018. Browsing did not alter ramet growth rates, but it nearly eliminated sexual reproduction. Vegetative reproduction did not differ between treatments. These results highlight the population-level mechanisms of woody expansion in response to disturbance and emphasize the need for multiple pressures to control the spread of clonal shrubs into grasslands. Additionally, I investigated the effects of fire on leaf physiology. I compared gas exchange rates, water use efficiency, and leaf structure of *C. drummondii* shrubs immediately following burning to shrubs 1-year post-fire. Despite a severe drought in the year of this study, resprouting shrubs had higher photosynthetic rates, stomatal conductance, and transpiration rates. Additionally, burned shrubs had morphological differences with reduced leaf mass per area and decreased leaf dry matter content. These results suggest *C. drummondii* modifies leaf structure and physiology

in response to fire to maximize carbon gain and accelerate growth after fire. Taken together, these studies identify demographic and physiological mechanisms in response to multiple disturbances that contribute to the increased cover of *C. drummondii* in tallgrass prairie.

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Dedication

To my loving partner and soon to be husband, Ryker.

Chapter 1 - Introduction

Grasslands comprise as much as 40% of Earth's terrestrial surface (Chapin et al. 2001; Dixon et al. 2014) and are present on every continent except Antarctica. Grass-dominated ecosystems include African savannas, Eurasian steppes, South American pampas, and North American prairies. Historically, grasslands were maintained by interactions of fire, herbivory, and climate. The high inter- and intra-annual climate variability and frequent disturbances characteristic of grasslands created a highly responsive, productive, and diverse system. Grasslands provide a number of ecosystem services including livestock and crop production, biodiversity conservation, carbon storage, and water cycling.

Human activity has altered the drivers maintaining grasslands at the global scale (e.g. increased atmospheric CO₂ and N deposition) and regional scale (altered precipitation regimes). At the local and regional levels, changes in fire, grazing, and browsing frequency and intensity, and changes in land cover (e.g. urbanization and cultivation and conversion to crop agriculture) have also greatly altered natural grassland ecosystems. One major consequence of these altered drivers is increased cover and spread of woody species into grasslands and savannas worldwide, a process known as woody encroachment (Briggs et al. 2005; Van Auken 2009; Eldridge et al 2011; Myers-Smith et al. 2011; Devine et al. 2017; Archer et al. 2017; Stevens et al. 2017).

Woody encroachment has been shown to decrease grassland biodiversity and herbaceous productivity (Briggs et al. 2002; Lett & Knapp 2005; Ratajczak et al. 2012), shift ecosystem C and N storage (Hughes et al 2006; Knapp et al. 2008; McKinley & Blair 2008; Barger et al. 2011), alter ecosystem water cycling (Huxman et al. 2005; Logan & Brunsell 2015) and decrease forage for livestock (Anadón et al. 2014). The drivers, rates, and effects of woody encroachment vary by climate (e.g. arid to mesic, temperate to tropical), management techniques, and the

characteristics of the expanding woody species (Devine et al. 2017; Maestre et al. 2016; Bestelmeyer et al. 2018). For example, woody encroachment in arid sites is often attributed to overgrazing leading to reduced ground cover of grasses and increased shrub seed dispersal (Van Auken 2009), while woody encroachment in mesic sites is primarily attributed to reduced fire frequency (Briggs et al. 2005). Increased atmospheric [CO₂] may play a larger role in woody encroachment in temperate grasslands where C₃ plants are relatively more abundant. Importantly, drivers in all systems have complex interactions to determine rates of woody encroachment.

In fire prone systems, resprouting woody species (referred to as resprouters) are highly persistent across the landscape and contribute to the loss of grasslands. Resprouters have extensive root systems, rhizomes or other perennating organs, with high carbohydrate and nutrient storage, and a reserve population of belowground buds allowing them to resprout after disturbance. Shrubs resprouting after fire tend to regenerate aggressively with increased stem densities compared to unburned shrubs (Heisler et al. 2004; Heisler et al. 2007). Although fire may result in complete top-kill of shrubs, the negative effects of fire may be short-lived when shrubs are given enough time to recover.

In addition to fire, herbivory shapes grasslands by influencing community composition and plant architecture. Grazers increase floristic biodiversity by decreasing grass cover and releasing forbs from competition (Collins 1987; Hartnett et al. 1996; Knapp et al. 1999). Overgrazing can result in a major decrease in grass cover while influencing fire behavior by reducing fuel loads (Knapp et al. 1999) and allowing the survival of woody species after fire (Briggs et al. 2002; Van Auken 2009). Browsers are herbivores that feed on woody plants. Browsing controls woody populations (Midgley et al. 2010) and alters woody architecture

(Archibald & Bond 2003). A reduction in the abundance of browsers likely contributes to an increase in woody cover.

This thesis focused on woody encroachment in the tallgrass prairie of the Flint Hills in eastern Kansas. The North American tallgrass prairie is a highly fragmented and endangered ecosystem due to destruction by agriculture and crop production. The Flint Hills are the largest remaining tract of tallgrass prairie. This region only experienced mild cultivation due to the rocky terrain and steep slopes. Much of this region is now used for cattle grazing. The cattle industry is the most profitable agricultural sector in Kansas generating \$8.2 billion in 2017 (Kansas Department of Agriculture). Further loss of the tallgrass prairie is an ecological and economic threat in this region.

Rates of woody encroachment in the Flint Hills are reported as 1.4-2.7% increase in cover per year (Ratajczak et al. 2014) with 56% of the Flint Hills susceptible to conversion to woodland or shrubland due to infrequent fire (Ratajczak et al. 2016). Encroachment in this system typically manifests as nonlinear state transitions driven by fire frequency. Tallgrass prairie is susceptible to a shift to woodland or shrubland when burned less than every 3 years. The expansion of the deep rooted, clonal shrub *Cornus drummondii* (rough-leaf dogwood) is a major contributor to loss of the remaining mesic tallgrass prairie (Briggs et al. 2005; Ratajczak et al. 2011).

C. drummondii shrubs reproduce both sexually and vegetatively. Shrubs usually produce flowers in June and set fruit in July (Great Plains Flora Associations 1986). Vegetative reproduction occurs via rhizomes to produce new stems from belowground buds and results in the formation of discrete shrub “islands” (genet) made up of genetically identical stems (ramets). Clonal growth aids in mitigating competition (Hartnett & Bazzaz 1985), circumventing the

challenges of seedling establishment (Hartnett & Bazzaz 1983), increasing invasibility (Song et al. 2013; You et al. 2014; Liu et al. 2016), and prolonging successful genotypes. Additionally, clonal growth increases success of the genet by increasing resource acquisition and transfer of resources among ramets providing an environmental buffer (Liu et al. 2016). Ramets in the center of *C. drummondii* shrubs have deep root systems with access to deep soil water, which can be transferred to juvenile ramets with less developed root systems (Ratajczak et al. 2011). Once established, *C. drummondii* islands alter fire behavior by limiting herbaceous understory growth reducing fine fuel loads (Lett & Knapp 2003). Thus, fire cannot carry through established clones and the shrub survives (Ratajczak et al. 2011). This altered fire behavior creates a positive feedback for shrubs to develop new ramets and expand (Ratajczak et al. 2014).

The objectives of this thesis were to understand the effects of fire, browsing, and grazing on *C. drummondii* demography and the effects of fire on *C. drummondii* leaf physiology. The year this research was conducted was one of the driest years on record for the Konza Prairie and the tallgrass prairie region and drought became a large focus of the results. In Chapter 2, I explore the effects of fire, browsing, and grazing on *C. drummondii* ramet dynamics. It is essential to understand how shrub population dynamics and morphological plasticity respond to fire, browsing, and grazing to better predict land-cover changes and the consequences of woody cover for ecosystem function. In Chapter 3, I explore changes in leaf level physiology and leaf morphology of *C. drummondii* shrubs after fire and 1-year post-fire. Clonal shrub physiological responses to fire are important in understanding resource acquisition and use after disturbance. Taken together, these studies identify demographic and physiological mechanisms in response to multiple disturbances that contribute to the increased cover of *C. drummondii* in tallgrass prairie.

References

- Anadón, J. D., Sala, O. E., Turner, B. L., & Bennett, E. M. (2014). Effect of woody-plant encroachment on livestock production in North and South America. *Proceedings of the National Academy of Sciences*, 111(35), 12948–12953.
- Archer, S.R., Andersen, E.M., Predick, K.I., Schwinning, S., Steidl, R.J., Woods, S.R. (2017). Woody plant encroachment: Causes and consequences. In D.D. Briske (Ed.), *Rangeland systems* (pp. 25-84). New York, NY: Springer Science+Business Media.
- Archibald, S. & Bond, W.J. (2003). Growing tall vs growing wide: tree architecture and allometry of *Acacia karoo* in forest, savanna, and arid environments. *Oikos*, 102(1), 3-14.
- Barger, N. N., Archer, S. R., Campbell, J. L., Huang, C., Morton, J. A., & Knapp, A. K. (2011). Woody plant proliferation in North American drylands: A synthesis of impacts on ecosystem carbon balance. *Journal of Geophysical Research*, 116.
- Bestelmeyer, B.T., Peters, D.C., Archer, S.R., Browning, D.M., Okin, G.S., Schooley, R.L., & Webb, N.P. (2018). The grassland-shrubland regime shift in the southwestern United States: Misconceptions and their implications for management. *BioScience*, 68(9), 678-690.
- Briggs, J.M, Hoch, G.A., Johnson, L.C. (2002). Assessing the rate, mechanism and consequences of conversion of tallgrass prairie to *Juniperus virginiana* forest. *Ecosystems*, 5, 578-586.
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. L., Hoch, G. A., Lett, M. S., & McCarron, J. K. (2005). An Ecosystem in Transition: Causes and Consequences of the Conversion of Mesic Grassland to Shrubland. *BioScience*, 55(3), 243.
- Chapin, F.S, Sala, O.E., & Huber-Sannwald, E. eds. 2001. Global Biodiversity in a Changing Environment: Scenarios for the 21st Century. New York: Springer-Verlag.
- Collins, S.L. (1987). Interaction of disturbances in tallgrass prairie: A field experiment. *Ecology*, 68(5), 1243-1250.
- Devine, A. P., McDonald, R. A., Quaife, T., & Maclean, I. M. D. (2017). Determinants of woody encroachment and cover in African savannas. *Oecologia*, 183(4), 939–951.
- Dixon, A. P., Faber-Langendoen, D., Josse, C., Morrison, J., & Loucks, C. J. (2014). Distribution mapping of world grassland types. *Journal of Biogeography*, 41(11), 2003–2019.
- Eldridge, D. J., Bowker, M. A., Maestre, F. T., Roger, E., Reynolds, J. F., & Whitford, W. G. (2011). Impacts of shrub encroachment on ecosystem structure and functioning: Towards a global synthesis. *Ecology Letters*, 14(7), 709–722.
- Great Plains Flore Association (1986). ‘Flora of the Great Plains’. University Press of Kansas: Lawrence, KS.

- Hartnett, D.C. & Bazzaz, F.A. (1983). Physiological integration among intracloonal ramets in *Solidago canadensis*. *Ecology*, 64(4), 779-788.
- Hartnett, D.C. & Bazzaz, F.A. (1985). The integration of neighborhood effects by clonal genets in *Solidago canadensis*. *Journal of Ecology*, 73(2), 415-427.
- Hartnett, D. C., Hickman, K. R., & Walter, L. E. F. (1996). Effects of Bison Grazing, Fire, and Topography on Floristic Diversity in Tallgrass Prairie. *Journal of Range Management*, 49(5), 413–420.
- Heisler, J. L., Briggs, J. M., Knapp, A. K., Blair, J. M., & Seery, A. (2004). Direct and Indirect Effects of Fire on Shrub Density and Aboveground Productivity in a Mesic Grassland. *Ecology*, 85(8), 2245–2257.
- Heisler, J.L., Knapp, A.K., & Briggs, J.M. (2007). Fire and resource availability influence carbon and water relations of the C₃ shrub roughleaf dogwood in a mesic grassland. R.E. Masters & K.E.M Galley (eds.) Proceedings of the 23rd Tall Timbers Fire Ecology Conference: Fire in Grassland and Shrubland Ecosystems. Tall Timbers Research Station: Tallahassee, Florida.
- Hughes, R. F., Archer, S. R., Asner, G. P., Wessman, C. A., McMurtry, C., Nelson, J., & Ansley, R. J. (2006). Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. *Global Change Biology*, 12(9), 1733–1747.
- Huxman, T.E., Wilcox, B.P, Breshears, D.D., Scott, R.L., Snyder, K.A., Small, E.E., ... Jackson, R.B. (2005). Ecohydrological implications of woody plant encroachment. *Ecology*, 86(2), 308-319.
- Knapp, A. K., Blair, J. M., Briggs, J. M., Collins, S. L., Hartnett, D. C., Johnson, L. C., & Towne, E. G. (1999). The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes. *BioScience*, 49(1), 39–50.
- Knapp, A. K., Briggs, J. M., Collins, S. L., Archer, S. R., Bret-Harte, M. S., Ewers, B. E., ... Cleary, M. B. (2008). Shrub encroachment in North American grasslands: Shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology*, 14(3), 615–623.
- Lett, M. S., & Knapp, A. K. (2003). Consequences of shrub expansion in mesic grassland: Resource alterations and graminoid responses. *Journal of Vegetation Science*, 14(4), 487–496.
- Lett, M.S., & Knapp, A.K. (2005). Woody plant encroachment and removal in mesic grassland: production and composition responses of herbaceous vegetation. *American Midland Naturalist*, 153(2), 217-231.

- Liu, F., Liu, J., & Dong, M. (2016). Ecological consequences of clonal integration in plants. *Frontiers in Plant Science*, 7, 770.
- Logan, K. E., & Brunsell, N. A. (2015). Influence of drought on growing season carbon and water cycling with changing land cover. *Agricultural and Forest Meteorology*, 213, 217–225.
- Maestre, F. T., Eldridge, D. J., & Soliveres, S. (2016). A multifaceted view on the impacts of shrub encroachment. *Applied Vegetation Science*, 19(3), 369–370.
- McKinley, D. C., & Blair, J. M. (2008). Woody Plant Encroachment by *Juniperus virginiana* in a Mesic Native Grassland Promotes Rapid Carbon and Nitrogen Accrual. *Ecosystems*, 11(3), 454–468.
- Midgley, J. J., Lawes, M. J., & Chamaillé-Jammes, S. (2010). Savanna woody plant dynamics: The role of fire and herbivory, separately and synergistically. *Australian Journal of Botany*, 58(1), 1.
- Myers-Smith, I. H., Forbes, B. C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., ... Hik, D. S. (2011). Shrub expansion in tundra ecosystems: Dynamics, impacts and research priorities. *Environmental Research Letters*, 6(4), 045509.
- Ratajczak, Z., Briggs, J.M, Goodin, D.G., Luo, L., Mohler, R.L., Nippert, J.B., & Obermeyer, B. (2016). Assessing the potential for transitions from tallgrass prairie to woodlands: Are we operating beyond critical fire thresholds? *Rangeland Ecology & Management*, 69(4), 280-287.
- Ratajczak, Z., Nippert, J. B., & Collins, S. L. (2012). Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology*, 93(4), 697–703.
- Ratajczak, Z., Nippert, J. B., Hartman, J. C., & Ocheltree, T. W. (2011). Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. *Ecosphere*, 2(11), 121.
- Ratajczak, Z., Nippert, J. B., & Ocheltree, T. W. (2014). Abrupt transition of mesic grassland to shrubland: evidence for thresholds, alternative attractors, and regime shifts. *Ecology*, 95(9), 2633–2645.
- Song, Y.-B., Yu, F.-H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., & van Kleunen, M. (2013). United we stand, divided we fall: A meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia*, 171(2), 317–327.
- Stevens, N., Lehmann, C. E. R., Murphy, B. P., & Durigan, G. (2017). Savanna woody encroachment is widespread across three continents. *Global Change Biology*, 23(1), 235–244.
- Van Auken, O. W. (2009). Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management*, 90(10), 2931–2942.

You, W., Fan, S., Yu, D., Xie, D., & Liu, C. (2014). An invasive clonal plant benefits from clonal integration more than a co-occurring native plant in nutrient-patchy and competitive environments. *PLoS ONE*, 9(5): e97246.

Chapter 2 - Demography of *Cornus drummondii* in response to fire, browsing, and grazing in tallgrass prairie

Introduction

The increased cover and abundance of woody species in grasslands, referred to as woody encroachment, has altered ecosystem structure and function in grasslands worldwide (Roques et al. 2001; Briggs et al. 2005; Van Auken 2009; Stevens et al. 2017). Woody encroachment has resulted in decreased floristic diversity and graminoid productivity (Lett & Knapp 2005; Heisler et al. 2004; Ratajczak et al. 2012), increased aboveground biomass (Hughes et al. 2006; Knapp et al. 2008), changes in nutrient dynamics (Hughes et al. 2006; Knapp et al. 2008; Barger et al. 2011; Ward 2018), and decreased forage for livestock (Anadón et al. 2014). These alterations typically manifest as nonlinear state transitions in response to shifts in a number of drivers at the global, regional, and local level. These drivers may include increased atmospheric CO₂ and N deposition (global), altered precipitation patterns (regional), and changes in land use management (local) such as fire suppression and increased grazing intensity (Van Auken et al. 2009; D’Odorico et al. 2012; Ratajczak et al. 2014a; Devine et al. 2017).

Grasslands are maintained by interactions among fire, herbivory, and climate. In mesic grasslands and savannas, which receive enough precipitation to support woody species, woody cover is limited by fire and browsing. Frequent fire and browsing create demographic bottlenecks preventing the establishment of trees and their transition into the adult size class, referred to as the fire and browse trap (Higgins 2000; Sankaran et al. 2004; Briggs et al. 2005; Staver et al. 2009; Midgley et al. 2010, Ratajczak et al. 2011).

The effects of fire and browsing vary depending on the woody species growth form, life stage, and ability to resprout from belowground organs. Resprouters are more resistant to fire and

browsing than non-resprouters and are more likely to survive after disturbance due to established root systems, belowground perennating storage organs, and bud banks, facilitating regrowth after top-kill (Clarke et al. 2013; Dietze et al. 2014). Resprouting woody species persist on the landscape in the presence of fire and browsing and contribute to encroachment in arid and mesic systems.

The effects of disturbance on woody resprouter growth and demographics have been studied primarily in African savannas. Woody resprouters in these systems are adapted to fire and tend to have increased growth rates after fire as they recover. Growth rates decrease with time since fire as resources become more limited (Midgley et al. 2010). Conversely, browsing can reduce plant height and vertical growth due to removal of leaves and depletion of root reserves reducing resources available for growth (Midgley et al. 2010). Browsing may also induce compensatory growth, increasing investment in shoot growth to compensate for lost tissue (McNaughton 1981; du Toit et al. 1990).

The impacts of fire and browsing are largest in combination and prolong the time spent in the fire and browse trap (Staver et al. 2009; Midgley & Bond 2001; LaMalfa et al. 2019). Fire and browsing may also reduce seed production (Young & Augustine 2007; Goheen et al. 2007; da Silveira Pontes et al. 2016). The sapling stage is the most vulnerable, and once woody plants have out-grown the traps (reached a threshold minimum height and diameter that can survive surface fires) they are more resistant to the effects of fire and browsing.

Although studies in other systems provide good insight into woody plant response to disturbance, we lack an understanding of the effects of fire and browsing on woody species in the tallgrass prairie (TGP) of the Central Great Plains (CGP) in the United States. The TGP system has been identified as a tri-stable system, meaning three states (grasslands, shrubland, and

woodland) can coexist in the same system based on historic drivers. These states are largely determined by fire frequency and intensity (Ratajczak et al. 2014a). This hypothesis argues fire frequency of 1-3 years is required to maintain a grassland state and longer fire free intervals will promote the transition to a shrubland or closed canopy woodland. Suppression of fire for just 4 years allows establishment and growth of clonal shrubs, which can grow to a size resistant to fire (Figure 2.1).

Expansion of clonal shrubs into the tallgrass prairie is primarily due to increased cover due to lateral rhizomatous spread (Ratajczak et al. 2014b). Post-burn resprouting shrubs tend to have higher stem densities and increased growth rates than unburned shrubs (Heisler et al. 2004; Heisler et al. 2007). Although fire may result in complete top-kill of shrubs, the negative effects of fire may be short-lived if shrubs are given enough time to recover. The pressure of browsing on woody shrubs has been greatly reduced in the CGP since European settlement due to the decline in the diversity and abundance of large browsers (Conard et al. 2006) and the effects of browsing on encroaching shrubs are unknown. Thus, studying the ramet dynamics of these shrubs in response to disturbance is important for understanding woody encroachment because a major increase in shrub cover in this system is due to new ramet production of existing shrubs.

In addition to fire and browsing, grazing by large mammals has a large influence on grasslands of the CGP and other regions. Bison and cattle are the primary grazers in the Flint Hills of eastern Kansas in the CGP. Grazing by bison and cattle is typically associated with increased forb and shrub establishment due to suppression of dominant C₄ grasses and reduced fuel loads (Collins 1987; Hartnett et al. 1996; Briggs et al. 2002a; Zahner 2015). Ratajczak et al. (2014b) found annually burned grazed areas to have higher shrub establishment than annually burned ungrazed areas. However, unburned grazed sites had lower expansion rates than ungrazed

areas potentially due to decreased soil moisture or damage to shrubs by large grazers. Fire and grazing often have strong interactive effects on the plant community and their impacts on woody clonal dynamics are not well understood (Knapp et al. 1998).

The goals of my study were to assess fire, browsing, and grazing on the growth, reproduction, and ramet densities of a key encroaching clonal shrub species, *Cornus drummondii*. *C. drummondii* is the dominant encroaching woody species in tallgrass prairie. *C. drummondii* shrubs reproduce both sexually and vegetatively. Shrubs usually produce flowers in June and set fruit in July (Great Plains Flora Association 1986). *C. drummondii* reproduces vegetatively via rhizomes and produce new stems from belowground buds resulting in the formation of discrete shrub “islands” (genet) made up of genetically identical stems (ramets). Ramets grow between 2-6 m tall and ramet densities tend to increase with disturbance (Heisler et al. 2004). In this study, I compared ramet growth, reproduction, demography, and density within *C. drummondii* genets in response to fire, browsing, and grazing. Ramets in burned and unburned areas were subjected to simulated browsing to understand the impacts of fire and browsing and their interaction on ramet demography. Additionally, I compared ramet demography of shrubs in grazed and frequently burned areas to unburned, ungrazed control shrubs to understand the effects of a typical eastern Kansas management regime of grazing and biennial burns on ramet demographics.

I predicted fire to increase ramet densities and relative growth rates as genets allocate more growth to stem production and light capture after disturbance. I also expected fire to decrease flower production due to a trade off in resource allocation to aboveground growth over reproduction. Browsing may induce compensatory growth either through increased ramet growth or increased ramet densities. I predicted browsing would reduce ramet relative growth rates and

flower production because of the consistent removal of tissue and depleted belowground reserves from repeatedly investing in aboveground growth. Additionally, I expected browsing to increase ramet mortality due to the repeated removal of tissues, resulting in death of young ramets. Shrubs in grazed areas may have increased ramet relative growth rates by suppressing grass biomass resulting in decreased shrub-grass competition and increasing resource capture for shrubs. Additionally, I predicted shrubs in grazed areas to have higher ramet natality and stem densities due to reduced grass cover.

Methods

Study site

Data were collected at Konza Prairie Biological Station (KPBS; 39°05' N, 96°35' W) located 15 km south of Kansas State University in Manhattan, KS. The Konza Prairie is a 3,487-hectare native tallgrass prairie characterized by uplands with shallow limestone soils and lowlands with deep silty-clay loams separated by rocky hillslopes. The elevation varies from 320-444 m above sea level. The site is divided into 57 watersheds, each assigned to a specific annual spring fire frequency (1, 2, 4, or 20-year intervals) and grazing treatment (bison, cattle, or no large mammalian grazers). Mean annual precipitation (1982-2018) is 835 mm, with approximately 73% of annual precipitation falling during the growing season (April-September). Temperature means (1982-2018) range from a low of -1.2°C in January to a high of 26.1°C in July. C₄ warm-season grasses (*Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans*, and, *Schyzachyrium scoparium*) dominate the plant cover and annual productivity in portions of the site that are frequently burned. Areas subject to infrequent burns (≥ 4 years) are encroached

primarily by the woody shrubs *Cornus drummondii* and *Rhus glabra*. In areas of KPBS with 20-year burn frequencies, *Juniperus virginiana* (an evergreen tree) is abundant.

Study design and data collection

Research was conducted in 2018 on 3 experimental watersheds at KPBS which included 4 and 20-year burn frequencies without bison present and a 2-year burn frequency with bison present. Within each watershed, field assistants and I measured 20 *Cornus drummondii* shrubs (Table 2.1). We used shrubs exposed to a simulated browsing experiment established in 2015 (O'Connor 2019). Browsing was simulated by removing 50% of new (current year) aboveground shoot growth from each ramet within a given shrub island (genet) each month throughout the growing season (May-September). The 'browsed' tissues were removed from the site. Isolated shrubs were located in the lowlands of either a 4-year burn frequency or 20-year frequency, hereafter referred to as 'unburned' (n=20 in each burn treatment). Half of the shrubs in each burn frequency were randomly selected for the browsing treatment. In addition, I selected 20 shrub islands within the lowlands of a watershed with a 2-year burn frequency and grazed by bison. Bison were introduced to KPBS in 1987 and are located within an approximately 984 ha enclosure at KPBS. Stocking rate of about 300 bison results in approximately 25% consumption of graminoid biomass.

We established 0.25 m wide transects through the length of each shrub island. In May 2018, we tagged each ramet within the transect and recorded the number of living ramets. Ramets were counted again in September and used to calculate ramet density, mortality, and natality for each shrub island.

$$\text{stem density} = \# \text{ of live ramets within the transect} / \text{area of the transect}$$

mortality = # of dead ramets in September / # of live ramets in May

natality = # of new ramets in September / # of live ramets in May

sexual reproduction = # of flowering stems within transect / # of live ramets in May

Sexual reproduction was estimated as the number of flowering ramets within each transect during peak flowering in June. To estimate the flowering effort per ramet, we counted the number of inflorescence clusters on 5 ramets on the periphery and 5 ramets in the center of each shrub.

We used the center and peripheral ramets to measure ramet height and basal diameter in June and August. Height was measured to the last leaf-bearing node on the tallest axis. Individual shoot volume was estimated using its basal area and height and calculating the volume of a cone. Relative growth rates of the shoots were calculated as:

$$\frac{\ln(volume_2) - \ln(volume_1)}{\# \text{ of weeks}}$$

Shrub area was calculated using an ellipse area equation by measuring the maximum length and perpendicular width of each shrub. We multiplied shrub area by the ramet density of each shrub to estimate the total number of ramets per genet. Genet growth rates were estimated by multiplying the average ramet relative growth rate of each shrub by the estimated number of ramets in each shrub. Additionally, we compared leaf size among treatments. We measured leaf area (cm²) of 4 randomly selected leaves from each shrub and averaged by shrub before analysis.

Statistical analysis

All analyses were conducted using the statistical software R V3.6.0 (R Core Team 2019). Differences in ramet relative growth rate, natality, mortality, sexual reproduction, and leaf size among browsed shrubs and control shrubs were tested using two-way ANOVA with an

interaction between treatment and fire. A one-way ANOVA was used to compare shrubs in grazed areas with unburned control shrubs with treatment as the main effect. ANOVA tests were conducted using the car package (Fox & Weisburg 2019). Pairwise comparisons were made using the emmeans package (Lenth 2019) with Tukey's HSD adjustment. Graphics were made using the package ggplot2 (Wickham 2016).

Results

Growing season precipitation in 2018 (505 mm) was approximately 17% below the long-term average (608 mm) (Table 2.2). In June 2018, when *C. drummondii* was in peak flower, there was 71% less precipitation compared to long-term average. Approximately 45% of 2018 growing season precipitation fell within the last 2 weeks of August and 1st week of September (Figure 2.2).

Shrubs in burned sites had higher stem densities than those in unburned sites (Figure 2.3; $p < 0.001$). Browsing only reduced stem densities in the burned treatment ($p < 0.05$) and browsed shrub stem densities did not differ significantly between burned and unburned shrubs ($p > 0.05$). There was no significant difference in stem density between the beginning (May) and end (August) of the season within any treatment.

Ramet relative growth rates did not differ between ramets on the periphery and in the center of the shrub, so data were combined and position within the shrub was not included in the analyses. Ramet growth rate data were averaged by shrub before analysis. Ramet relative growth rates were extremely low in all treatments (Figure 2.4) and did not differ among browsing or fire treatments (Table 2.5). Ramets of shrubs in the grazed areas had significantly higher growth rates than the unburned control (not browsed or grazed) shrubs (Table 2.6).

Browsing nearly eliminated sexual reproduction (Figure 2.5). The burned control shrubs also had low flowering with an average of 2.5% of stems producing flowers. By contrast, the majority of stems flowered within the unburned control shrubs (72%), which had significantly higher proportion of flowering stems than all other treatments. There were no differences in vegetative reproduction (ramet natality) among browsed and control treatments. The burned control shrubs had the lowest ramet natality, which was significantly lower than the unburned control shrubs ($p < 0.05$). Shrubs in grazed areas did not differ in vegetative reproduction. Mortality of stems did not differ among treatments.

Genet area, estimated number of ramets per genet, and estimated genet growth rates were extremely variable within and between treatments (Table 2.4). Burned control shrubs and shrubs in grazed areas had the highest genet growth rates. In the burned treatment, browsed shrubs had reduced genet growth compared to the control, but minimal differences existed between unburned browsed and control shrubs. The unburned control shrubs had the lowest genet growth rates.

Leaf size of *C. drummondii* varied according to the grazing and browsing treatments. Browsed shrubs had ~86% smaller leaves than their respective controls (Figure 2.6). Shrubs in grazed areas had ~18% smaller leaves than the unburned controls. There were no differences between control shrubs in burned and unburned treatments.

Discussion

This study is the first to explore intra-clonal ramet demography on the most important encroaching woody species in the Flint Hills, *Cornus drummondii*, in response to disturbance. I examined the interactive effects of fire with simulated browsing and grazing by bison on *Cornus*

drummondii ramet growth rates, reproduction, and demography. The results show that fire and herbivory interact to alter ramet growth rates and ramet demography within *C. drummondii*.

Infrequent fires (< 4-year fire frequency) are known to promote shrub establishment and growth in tallgrass prairie, but the effects of fire with browsing and grazing were not well understood.

Shrub establishment and growth has been shown to be limited by above- and belowground competition with grasses (Scholes & Archer 1997; Clarke & Knox. 2009; Pierce et al. 2019). Shrub ramet relative growth rates were highest in the grazed area. These results suggest shrubs may experience a competitive advantage in response to grazing. Grazing suppresses grass biomass reducing their pre-emption of light and soil resources and their population level competitive effects on shrubs. Grazing reduces grass investment in roots (Johnson & Matchett 2001), which may increase shrub competitive ability belowground and increase growth rates of ramets, especially young ramets with less developed roots systems. Additionally, reduced above- and belowground grass biomass in grazed areas may increase deep water infiltration and increase water availability for ramets with deep roots systems. Although my results indicate that grazing may reduce competition and increase growth rates of shrub ramets, this effect may be context-specific as grazing has also been shown to reduce clonal shrub expansion rates due to decreased soil moisture causing higher soil evaporation compared to ungrazed areas (Ratajczak et al. 2014b).

Shrubs in grazed areas had a lower proportion of flowering stems compared to unburned, ungrazed control shrubs. Additionally, shrubs in grazed areas did not produce as many inflorescence clusters per stem (mean of 1.2 clusters per stem) as the unburned shrubs (mean of 30.5 clusters per stem) probably due to the deeper soils and lack of disturbance in the unburned area. Shrubs in grazed areas had ~18% smaller leaves than unburned, ungrazed shrubs, but

differences were marginally significant and may be an artefact of deeper soils in the ungrazed area. Ramet natality and mortality did not differ between shrubs in the grazed area and unburned, ungrazed shrubs, but shrubs in the grazed areas had higher stem densities likely driven primarily by fire.

Shrubs in burned areas had higher stem densities. This is in agreement with previous research that showed that fire stimulates growth of rhizomatous and basal buds after top-kill (Heisler et al. 2004; Lett et al. 2004). Stem densities did not differ from the beginning and end of the season for any treatment due to the complete replacement of any stems that died during the growing season. This is similar to intra-clonal dynamics of *Rhus glabra*, another major encroaching species in the tallgrass prairie. Hajny et al. (2011) found stable ramet densities in *Rhus glabra* due to complete replacement of ramets after fire. Additionally, population growth in *R. glabra* was higher in burned than unburned sites (Hajny et al. 2011). In my study, genet level growth rates showed similar trends as the ramet level. Shrubs in burned areas had the highest genet growth rates (Table 2.4).

Compensatory growth is a common plant response to browsing. Simulated winter browsing has been shown to increase shoot growth during the following growing season in *Acacia* species and this was attributed to release from intraspecific competition of shoots for light (Du Toit et al. 1990; Gadd et al. 2001; Rooke et al. 2004). Results from my study did not show differences between browsed and control shrubs. Growth rates were extremely low in all treatments likely due to the summer 2018 drought and the effects of browsing on growth rates may be enhanced and better understood in a more average precipitation year, and certainly if measured cumulatively over multiple years. It is also possible that ramet growth rate in the browsed shrubs is limited by recovery time. Repeated growing season browsing may not give

shrubs enough time to recover by removing new growth. Additionally, browsed shrubs could be investing in the production of new ramets and basal resprouts over height growth.

Browsing did not result in increased ramet mortality or decreased ramet natality. This was surprising as these shrubs faced drought and repeated browsing throughout the growing season. The lack of ramet mortality and ability of shrubs to invest in new ramets during the drought emphasizes the persistence of clonal shrubs and the importance of belowground carbon and nutrient reserves, and belowground bud banks in stabilizing clonal plant population dynamics (Ott et al. 2019). Investing in new ramets during stress may be an invasive mechanism facilitated by clonal growth in attempt to colonize the area while other plant competitors are stressed. These data show that drought may affect the growth rates, but not necessarily survival of mature clonal shrubs.

Browsing with fire reduced ramet densities suggesting browsed shrubs did not produce as many ramets during resprouting as the burned control shrubs after fire in 2017. Genet growth rates in the browsed shrubs were also reduced in the burn treatment (Table 2.4). These results support other studies that the effects of browsing are strongest in combination with fire (Staver et al. 2009; Midgley et al. 2010). The difference in ramet densities, but not single-year ramet mortality or natality between treatments indicates that fire and browsing have a longer-term effect on ramet population dynamics that were not captured within one growing season.

Browsing has been shown to change shrub architecture by removing apical dominance resulting in increased branching and changes in leaf size. Browsing resulted in some ramets producing 2 main shoot axes (*personal observation*), however differences in branching between browsed and control shrubs seemed minimal. Browsing in this study caused variation in leaf size. Leaves on browsed shrubs were on average ~86% smaller than their respective controls. The

highly reduced leaves seemed to be more abundant during the drought year than previous browsing seasons (O'Connor, *personal communication*). Replacement of leaves is energetically costly and repeated investment likely reduced the belowground C and nutrient reserves. Browsed shrubs may reduce leaf size to invest in more leaves and fill out the canopy faster than investing in fewer large leaves (Hartnett et al. 2012). Other studies of simulated browsing have shown increased leaf area (Rooke et al. 2004). However, these studies either implemented browsing during the winter or browsed less often than my study. Additionally, reduced leaf size may reduce the damage by future browsing by reducing the amount of tissue lost per bite.

Shrubs continually invest in aboveground growth during repeated browsing during the growing season. Repeated browsing results in reduced shrub cover as the plant cannot fully recover. Additionally, constant resprouting results in reduced belowground nonstructural carbohydrate (NSC) storage (O'Connor 2019), which potentially reduces the shrubs' ability to invest in growth the following growing season. Concentrated and repeated growing season browsing may be an effective management technique to suppress clonal shrub growth and increase grass cover (da Silveira Pontes et al. 2016; O'Connor 2019). Additionally, in my study simulated browsing eliminated flower and seed production due to the removal of flowering meristems. These data suggested that browsing during the growing season was an effective time to control for shrub reproduction to prevent or minimize contributions to the seed bank. These results also suggest drought may be a good time to invest in shrub removal strategies (brush cutting, herding browsers, herbicides) as shrubs have limited growth and seed production. Understanding the growth rates and demographics of clonal shrubs is important for developing models to project shrub expansion in response to different disturbance regimes. More data are

needed to understand how these factors change in response to interannual rainfall and climate variability.

References

- Anadón, J.D., Sala, O.E., Turner II, B.L., & Bennett, E.M. (2014.) Effect of woody-plant encroachment on livestock production in North and South America. *Proceedings of the National Academy of Sciences of the United States of America*, 111(35), 12948-12953.
- Barger, N. N., Archer, S. R., Campbell, J. L., Huang, C., Morton, J. A., & Knapp, A. K. (2011). Woody plant proliferation in North American drylands: A synthesis of impacts on ecosystem carbon balance. *Journal of Geophysical Research*, 116.
- Bond, W. J., & Midgley, J. J. (2001). Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology & Evolution*, 16(1), 45–51.
- Briggs, J. M., Hoch, G. A., & Johnson, L. C. (2002a). Assessing the Rate, Mechanisms, and Consequences of the Conversion of Tallgrass Prairie to *Juniperus virginiana* Forest. *Ecosystems*, 5(6), 578–586.
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J.L., Hoch G.A., Lett, M.S., & McCarron, J.K. (2005). An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *Bioscience*, 55(3), 225-236.
- Briggs, J.M., Knapp, A.K., & Brock, B.L. (2002b). Expansion of woody plants in tallgrass prairie: a fifteen-year study of fire and fire-grazing interactions. *The American Midland Naturalist*, 147(2), 287-294.
- Clarke, P.J. & Knox, K.J.E. (2009). Trade-offs in resource allocation that favour resprouting affect the competitive ability of woody seedlings in grassy communities. *Journal of Ecology*, 97, 1374-1382.
- Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., Enright, N.J., & Knox, K. J. E. (2013). Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist*, 197(1), 19–35.
- Collins, S. L. (1987). Interaction of Disturbances in Tallgrass Prairie: A Field Experiment. *Ecology*, 68(5), 1243–1250.
- Conard, J. M., Gipson, P. S., & Peek, M. (2006). Historical and Current Status of Elk in Kansas, 7.

- Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D., & Vargas, R. (2014). Nonstructural carbon in woody plants. *Annual Review of Plant Biology*, 65(1), 667–687.
- D’Odorico, P., Okin, G. S., & Bestelmeyer, B. T. (2012). A synthetic review of feedbacks and drivers of shrub encroachment in arid grasslands. *Ecohydrology*, 5(5), 520–530.
- Devine, A. P., McDonald, R. A., Quaife, T., & Maclean, I. M. D. (2017). Determinants of woody encroachment and cover in African savannas. *Oecologia*, 183(4), 939–951.
- Du Toit, J. T., Bryant, J. P., & Frisby, K. (1990). Regrowth and Palatability of Acacia Shoots Following Pruning by African Savanna Browsers. *Ecology*, 71(1), 149–154.
- Fox, J. & Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage.
- Gadd, M. E., Young, T. P., & Palmer, T. M. (2001). Effects of Simulated Shoot and Leaf Herbivory on Vegetative Growth and Plant Defense in *Acacia drepanolobium*. *Oikos*, 92(3), 515–521.
- Goheen, J.R., Young, T. P., Keesing, F., & Palmer, T.M. (2007). Consequences of herbivory by native ungulates for the reproduction of a savanna tree. *Journal of Ecology*, 95(), 129–138.
- Hartnett, D. C., Hickman, K. R., & Walter, L. E. F. (1996). Effects of Bison Grazing, Fire, and Topography on Floristic Diversity in Tallgrass Prairie. *Journal of Range Management*, 49(5), 413–420.
- Hartnett, D., Ott, J., Sebes, K., & Dithlago, M. (2012). Coping with herbivory at the juvenile stage: Responses to defoliation and stem browsing in the African savanna tree *Colophospermum mopane*. *Journal of Tropical Ecology*, 28(2), 161–169.
- Heisler, J.L., Briggs, J.M., Knapp, A.K., Blair, J.M., & Seery, A. (2004). Direct and indirect effects of fire on shrub density and aboveground productivity in a mesic grassland. *Ecology*, 85(8), 2245–2257.
- Heisler, J.L., Knapp, A.K., & Briggs, J.M. (2007). Fire and resource availability influence carbon and water relations of the C₃ shrub roughleaf dogwood in a mesic grassland. R.E. Masters & K.E.M Galley (eds.) Proceedings of the 23rd Tall Timbers Fire Ecology Conference: Fire in Grassland and Shrubland Ecosystems. Tall Timbers Research Station: Tallahassee, Florida.
- Higgins, S. I., Bond, W. J., & Trollope, W. S. W. (2000). Fire, resprouting and variability: a recipe for grass–tree coexistence in savanna. *Journal of Ecology*, 88(2), 213–229.
- Hughes, R. F., Archer, S. R., Asner, G. P., Wessman, C. A., McMURTRY, C., Nelson, J., & Ansley, R. J. (2006). Changes in aboveground primary production and carbon and

- nitrogen pools accompanying woody plant encroachment in a temperate savanna. *Global Change Biology*, 12(9), 1733–1747.
- Johnson, L.C. & Matchett, J.R. (2001). Fire and grazing regulate belowground processes in tallgrass prairie. *Ecology*, 82(12), 3377–3389.
- Knapp, A. K., Blair, J. M., Briggs, J. M., Collins, S. L., Hartnett, D. C., Johnson, L. C., & Towne, E. G. (1999). The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes. *BioScience*, 49(1), 39–50.
- Knapp, A.K., Briggs, J.M., Collins, S.L., Archer, S.R., Bret-Harte, M.S., Ewers, B.E, Peters, D.P., Young, D.R., Shaver, G.R., Pendall, E., & Cleary, M.B. (2008). Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology*, 14(3), 615–623.
- Knapp, A.K., Briggs, J.M., Hartnett, D.C., & Collins, S.L., editors. (1998). Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York, New York, USA.
- LaMalfa, E. M., Kimuyu, D. M., Sensenig, R. L., Young, T. P., Riginos, C., & Veblen, K. E. (2019). Tree resprout dynamics following fire depend on herbivory by wild ungulate herbivores. *Journal of Ecology*, 0(0), 1–10.
- Lett, M.S., & Knapp, A.K. (2005). Woody plant encroachment and removal in mesic grassland: production and composition responses of herbaceous vegetation. *American Midland Naturalist*, 153(2), 217–231.
- Lett, M. S., Knapp, A. K., Briggs, J. M., & Blair, J. M. (2004). Influence of shrub encroachment on aboveground net primary productivity and carbon and nitrogen pools in a mesic grassland. *Canadian Journal of Botany*, 82(9), 1363–1370.
- Midgley, J. J., & Bond, W. J. (2001). A Synthesis of the Demography of African Acacias. *Journal of Tropical Ecology*, 17(6), 871–886.
- Midgley, J. J., Lawes, M. J., & Chamaillé-Jammes, S. (2010). Savanna woody plant dynamics: the role of fire and herbivory, separately and synergistically. *Australian Journal of Botany*, 58(1), 1–11.
- O'Connor, R.C. (2019). Drivers, mechanisms, and thresholds of woody encroachment in mesic grasslands. (Doctoral dissertation). Retrieved from K-State Research Exchange.
- Ott, J. P., Klimešová, J., & Hartnett, D. C. (2019). The ecology and significance of below-ground bud banks in plants. *Annals of Botany*, 123(7), 1099–1118.
- Pierce, N. A., Archer, S. R., & Bestelmeyer, B. T. (2019). Competition suppresses shrubs during early, but not late, stages of arid grassland-shrubland state transition. *Functional Ecology*, 0, 1–11.

- R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ratajczak, Z., Nippert, J.B., Briggs, J.M., & Blair, J.M. (2014a). Fire dynamics distinguish grasslands, shrublands and woodlands as alternative attractors in the Central Great Plains of North America. *Journal of Ecology*, 102(6), 1374-1385.
- Ratajczak, Z., Nippert, J.B., & Collins, S.L. (2012). Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology*, 93(4), 697-703.
- Ratajczak, Z., Nippert, J. B., Hartman, J. C., & Ocheltree, T. W. (2011). Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. *Ecosphere*, 2(11), 121.
- Ratajczak, Z., Nippert, J. B., & Ocheltree, T. W. (2014b). Abrupt transition of mesic grassland to shrubland: evidence for thresholds, alternative attractors, and regime shifts. *Ecology*, 95(9), 2633–2645.
- Rooke, T., Bergström, R., Skarpe, C., & Danell, K. (2004). Morphological Responses of Woody Species to Simulated Twig-Browsing in Botswana. *Journal of Tropical Ecology*, 20(3), 281–289.
- Roques, K. G., O'Connor, T. G., & Watkinson, A. R. (2001). Dynamics of shrub encroachment in an African savanna: relative influences of fire, herbivory, rainfall and density dependence. *Journal of Applied Ecology*, 38(2), 268–280.
- Sankaran, M., Hanan, N. P., Scholes, R. J., Ratnam, J., Augustine, D. J., Cade, B. S., ... Zambatis, N. (2005). Determinants of woody cover in African savannas. *Nature*, 438(7069), 846–849.
- Scholes, R.J. & Archer, S.R., (1997). Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics*, 28, 517-544.
- Staver, A. C., Bond, W. J., Stock, W. D., van Rensburg, S. J., & Waldram, M. S. (2009). Browsing and fire interact to suppress tree density in an African savanna. *Ecological Applications*, 19(7), 1909–1919.
- Stevens, N., Lehmann, C. E. R., Murphy, B. P., & Durigan, G. (2017). Savanna woody encroachment is widespread across three continents. *Global Change Biology*, 23(1), 235–244.
- da Silveira Pontes, L., Magda, D., Gleizes, B., & Agreil, C. (2016). Shrub control by browsing: Targeting adult plants. *Acta Oecologica*, 70, 121–128.
- Van Auken, O. W. (2009). Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management*, 90(10), 2931–2942.

- Ward, D., Trinogga, J., Wiegand, K., du Toit, J., Okubamichael, D., Reinsch, S., & Schleicher, J. (2018). Large shrubs increase soil nutrients in a semi-arid savanna. *Geoderma*, 310, 153–162.
- Young, T. P., & Augustine, D. J. (2007). Interspecific Variation in the Reproductive Response of Acacia Species to Protection from Large Mammalian Herbivores. *Biotropica*, 39(4), 559–561.
- Zahner, A. (2015). Plant responses to grazer-mediated habitat alterations in tallgrass prairie. (Master's thesis). Retrieved from K-State Research Exchange.

Table 2.1 Shrub island sample sizes among fire frequency, simulated browsing, and grazing treatments.

Treatment	Fire Frequency	Years Since Last Burn	Simulated Browsing	Bison Present	n
Unburned Control	20	6	N	N	10
Unburned Browsed	20	6	Y	N	10
Burned Control	4	1	N	N	10
Burned Browsed	4	1	Y	N	10
Grazed	2	1	N	Y	20

Table 2.2 Long term (1982-2017) mean and 2018 growing season precipitation (mm) for each month. Historical values are means $\pm$ 1 SE.

Month	Historical 1982-2017	2018	% Change
April	83.3 $\pm$ 47.7	41.3	-50
May	110.4 $\pm$ 79.1	80.4	-27
June	131.5 $\pm$ 67.7	37.9	-71
July	101.1 $\pm$ 78.6	86.6	-14
August	110.0 $\pm$ 52.6	140.3	+22
September	72.0 $\pm$ 41.4	125.3	+43
Total	608.7 $\pm$ 65.2	504.9	-17

Table 2.3 Mean height (cm) and diameter (mm) of ramets in the center and periphery for each treatment. All values are means $\pm$ 1 SE.

Treatment	Stem Height		Stem Diameter	
	Center	Periphery	Center	Periphery
Unburned Control	175.38 $\pm$ 7.26	91.78 $\pm$ 5.64	18.43 $\pm$ 1.18	10.23 $\pm$ 0.56
Unburned Browsed	118.22 $\pm$ 6.13	66.62 $\pm$ 2.97	13.55 $\pm$ 0.84	8.10 $\pm$ 0.40
Burned Control	92.53 $\pm$ 5.25	66.13 $\pm$ 2.54	7.95 $\pm$ 0.57	6.81 $\pm$ 0.28
Burned Browsed	39.05 $\pm$ 1.82	43.78 $\pm$ 1.59	4.36 $\pm$ 0.18	4.84 $\pm$ 0.19
Grazed	94.70 $\pm$ 3.97	59.86 $\pm$ 2.36	7.41 $\pm$ 0.33	6.69 $\pm$ 0.25

Table 2.4 Mean shrub area (m²), stem density (m⁻²), estimated number of ramets, and estimated genet relative growth rates (mm³ mm⁻³ week⁻¹) of shrubs for each treatment. The number of ramets per shrub were estimated by multiplying shrub area and stem density. Genet relative growth rates were estimated by multiplying the estimated number of ramets by the average ramet relative growth rate for each shrub. All values are means $\pm$ 1 SE.

Treatment	Area	Stem Density	# Ramets	Genet Growth
Unburned Control	40.79 $\pm$ 15.30	14.88 $\pm$ 1.99	615.24 $\pm$ 370.08	9.93 $\pm$ 6.24
Unburned Browsed	39.09 $\pm$ 23.17	18.19 $\pm$ 2.20	663.16 $\pm$ 334.47	13.46 $\pm$ 9.39
Burned Control	88.78 $\pm$ 41.59	37.12 $\pm$ 2.78	3150.81 $\pm$ 1582.27	79.37 $\pm$ 48.72
Burned Browsed	41.30 $\pm$ 37.90	22.52 $\pm$ 3.93	843.97 $\pm$ 595.24	13.31 $\pm$ 8.49
Grazed	45.60 $\pm$ 43.98	31.19 $\pm$ 2.01	1448.62 $\pm$ 1292.81	63.56 $\pm$ 82.60

Table 2.5 ANOVA results for browsing, fire, and grazing treatments for stem densities. Browsed and control shrubs in the burned and unburned watersheds were compared. Shrubs in the grazed area were compared to unburned control shrubs. Alpha = 0.05.

Comparison		DF	F	P
Browsing and Fire	Treatment	1	5.1051	0.030
	Fire	1	17.7703	<0.001
	Treatment*Fire	1	7.16	0.011
Grazed vs. Unburned Control	Treatment	1	26.237	<0.001

Table 2.6 ANOVA results for browsing, fire, and grazing treatments for ramet relative growth rates. Browsed and control shrubs in the burned and unburned watersheds were compared. Shrubs in the grazed area were compared to unburned control shrubs. Alpha = 0.05.

Comparison		DF	F	P
Browsing and Fire	Treatment	1	0.0004	0.985
	Fire	1	1.0898	0.303
	Treatment*Fire	1	1.6487	0.207
Grazed vs. Unburned Control	Treatment	1	26.237	<0.001

Table 2.7 ANOVA results for browsing, fire, and grazing treatments for the and ramet natality, mortality, and flowering stems. Browsed and control shrubs in the burned and unburned watersheds were compared. Shrubs in the grazed area were compared to unburned control shrubs. Alpha = 0.05.

Comparison		DF	F	P
Natality	Browsing and Fire	Treatment	1	0.324
		Fire	1	3.918
		Treatment*Fire	1	5.533
	Grazed vs. Unburned Control	Treatment	1	0.5072
Mortality	Browsing and Fire	Treatment	1	0.388
		Fire	1	1.024
		Treatment*Fire	1	0.003
	Grazed vs. Unburned Control	Treatment	1	0.219
Flowering Stems	Browsing and Fire	Treatment	1	152.66
		Fire	1	126.15
		Treatment*Fire	1	126.52
	Grazed vs. Unburned Control	Treatment	1	71.517

Figure 2.1 Maintenance of grasslands and transition to alternative stable states adapted from Ratajczak et al. 2014a. A) Grasslands are maintained at a fire frequency of 3 or less years. B) Fire suppression leads to a woodland state transition. C) Infrequent fire allows shrub establishment, growth, survival, and clonal expansion leading to a shrubland state.

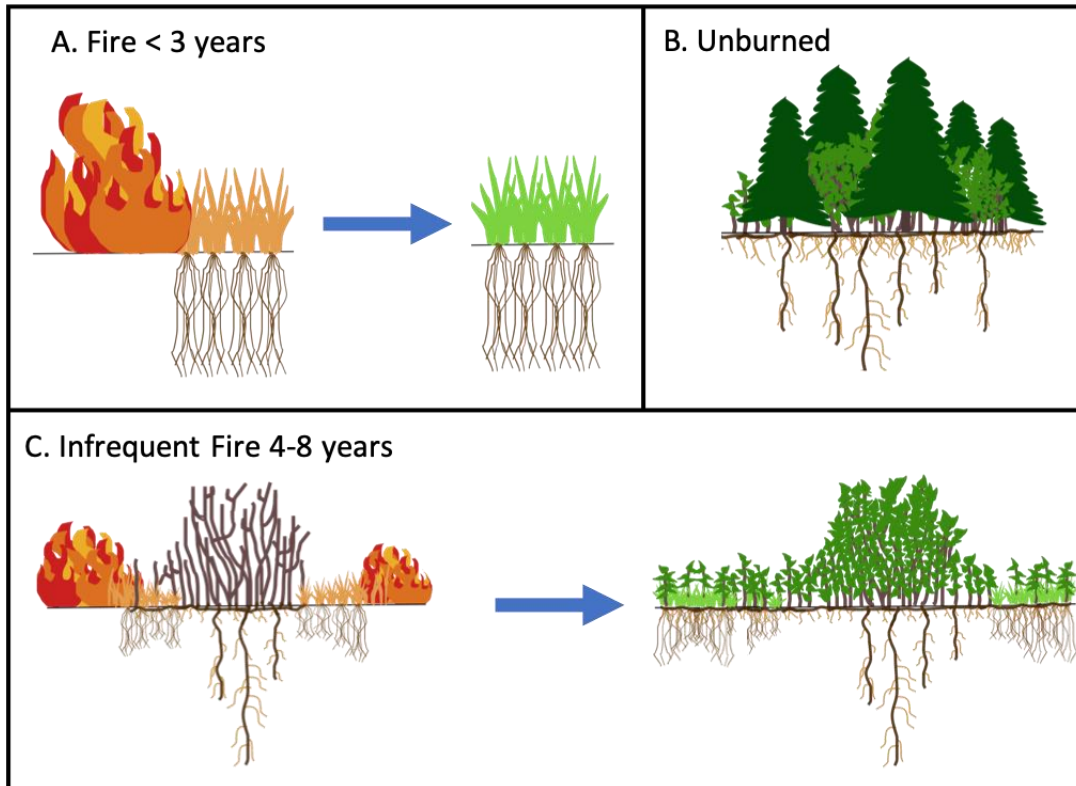


Figure 2.2 Daily growing season precipitation (mm) April – September. All rainfall events shown are greater than 5 mm since more than 5 mm must fall to reach the soil surface. Day of Year 121, 152, 182, 213, and 244 correspond to the first of each month the growing season (May, June, July, August, and September, respectively).

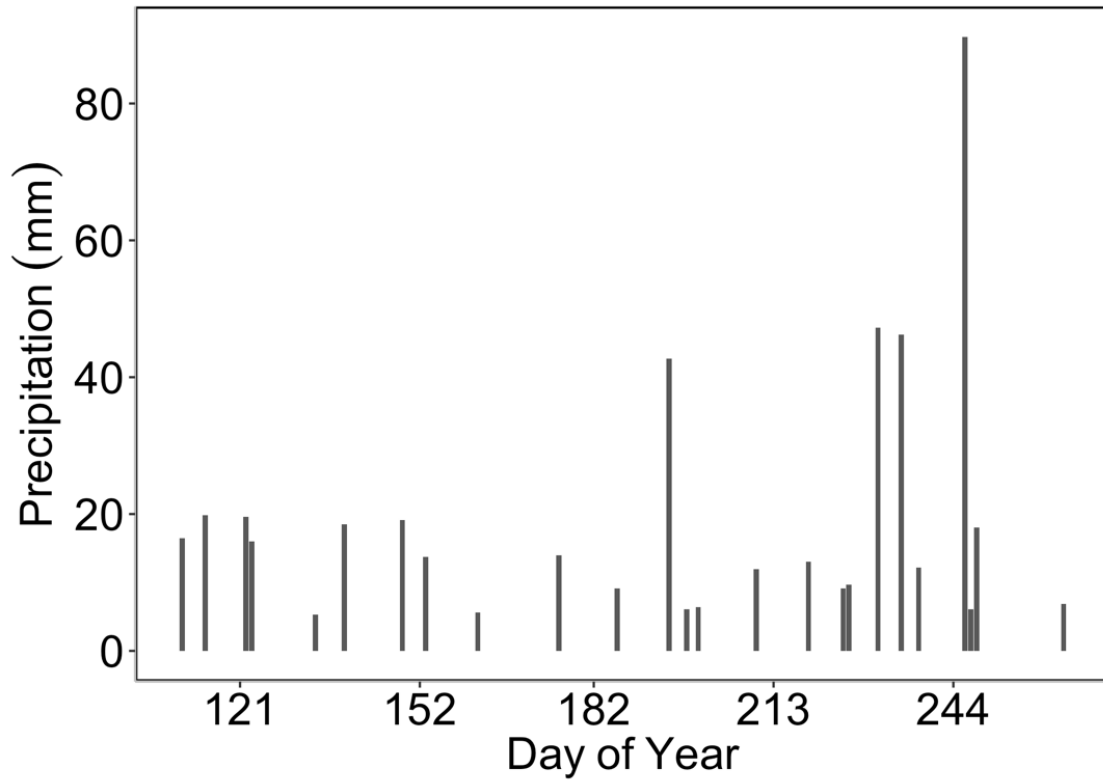


Figure 2.3 Average initial and final stem density (# stems m^{-2}) for each treatment. All values are means $\pm$ 1 SE. UB = unburned, B = burned

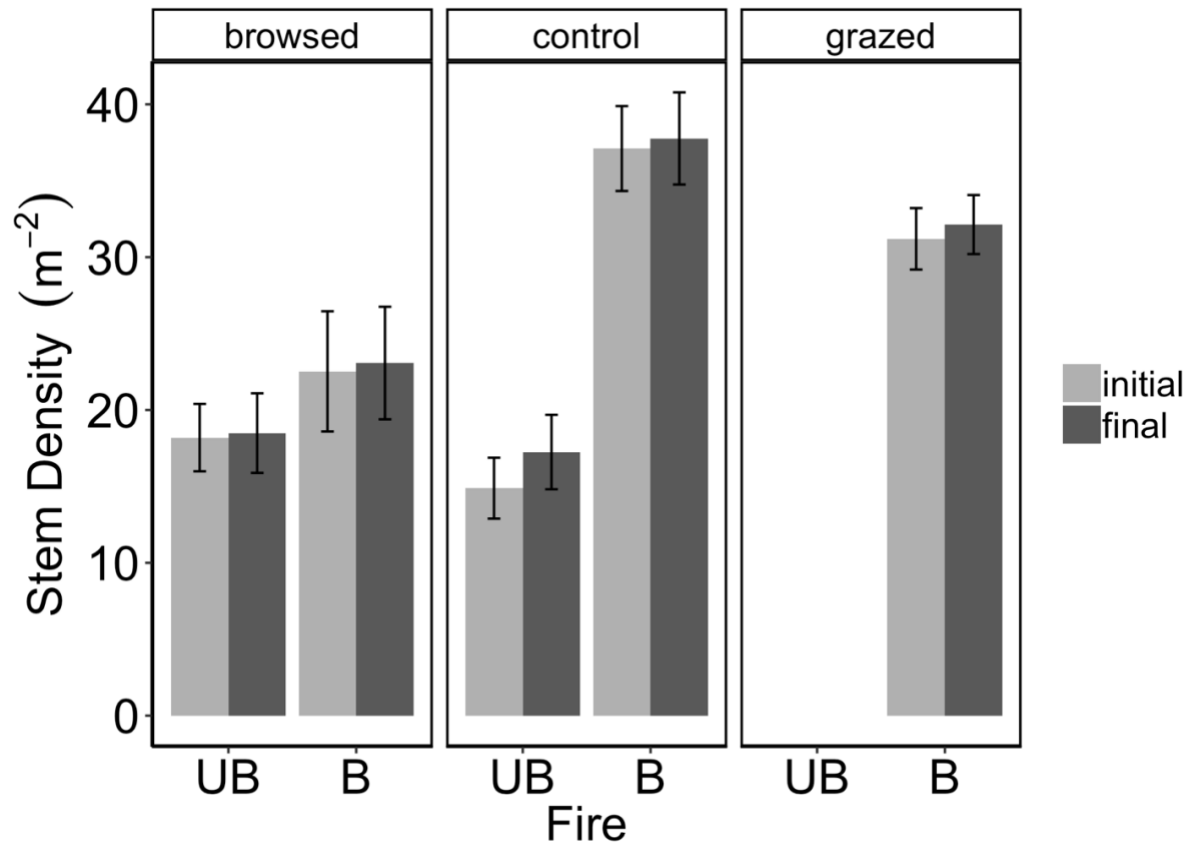


Figure 2.4 Average ramet relative growth rates ($\text{mm}^3 \text{mm}^{-3} \text{week}^{-1}$) for each treatment. UB = Unburned, B = Burned. The grazed area is burned every 2 years and the ungrazed, burned area is burned every 4 years. All values are means $\pm$ 1 SE.

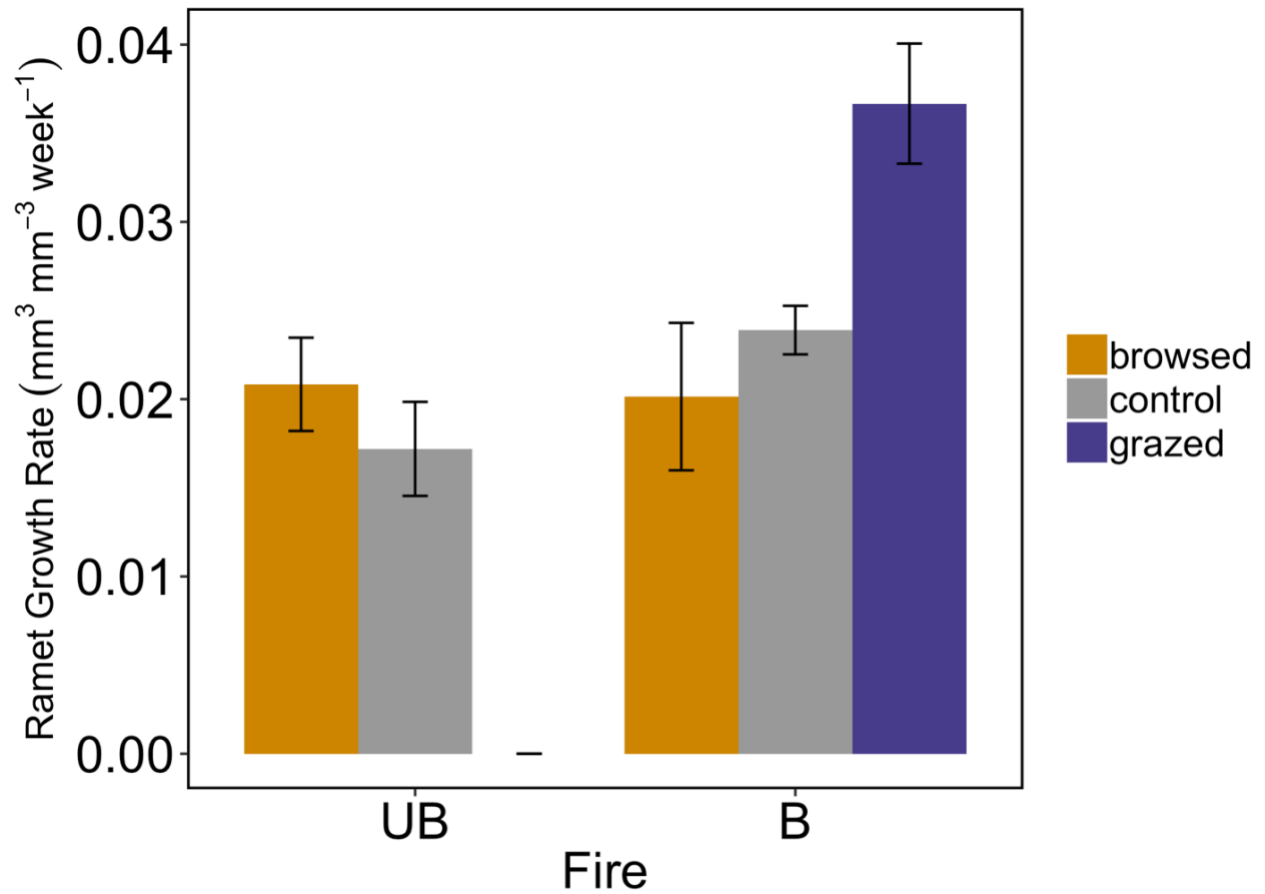


Figure 2.5 Boxplot of the proportion of stems in transects that produced flowers (sexual), are new (natality), or died (mortality) for each treatment. UB = Unburned, B = burned.

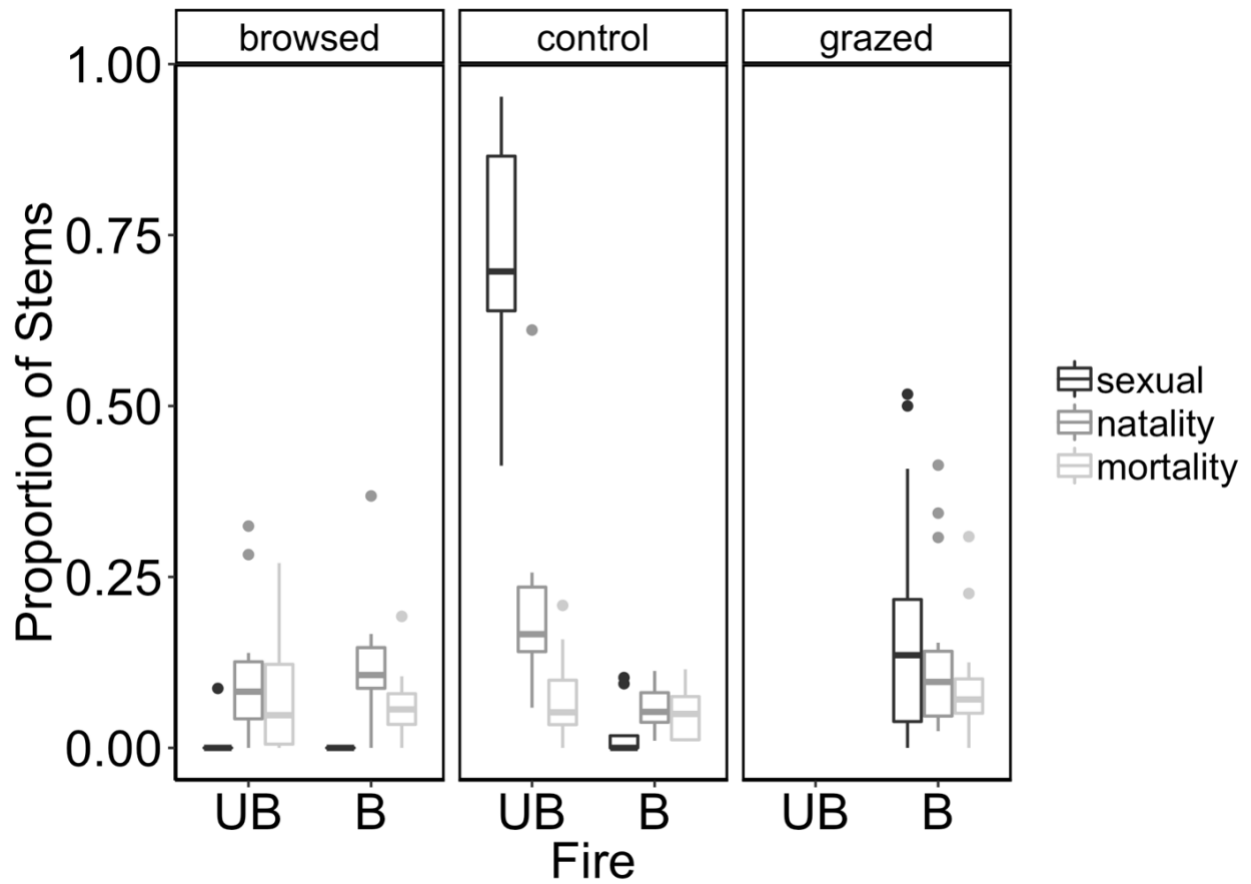
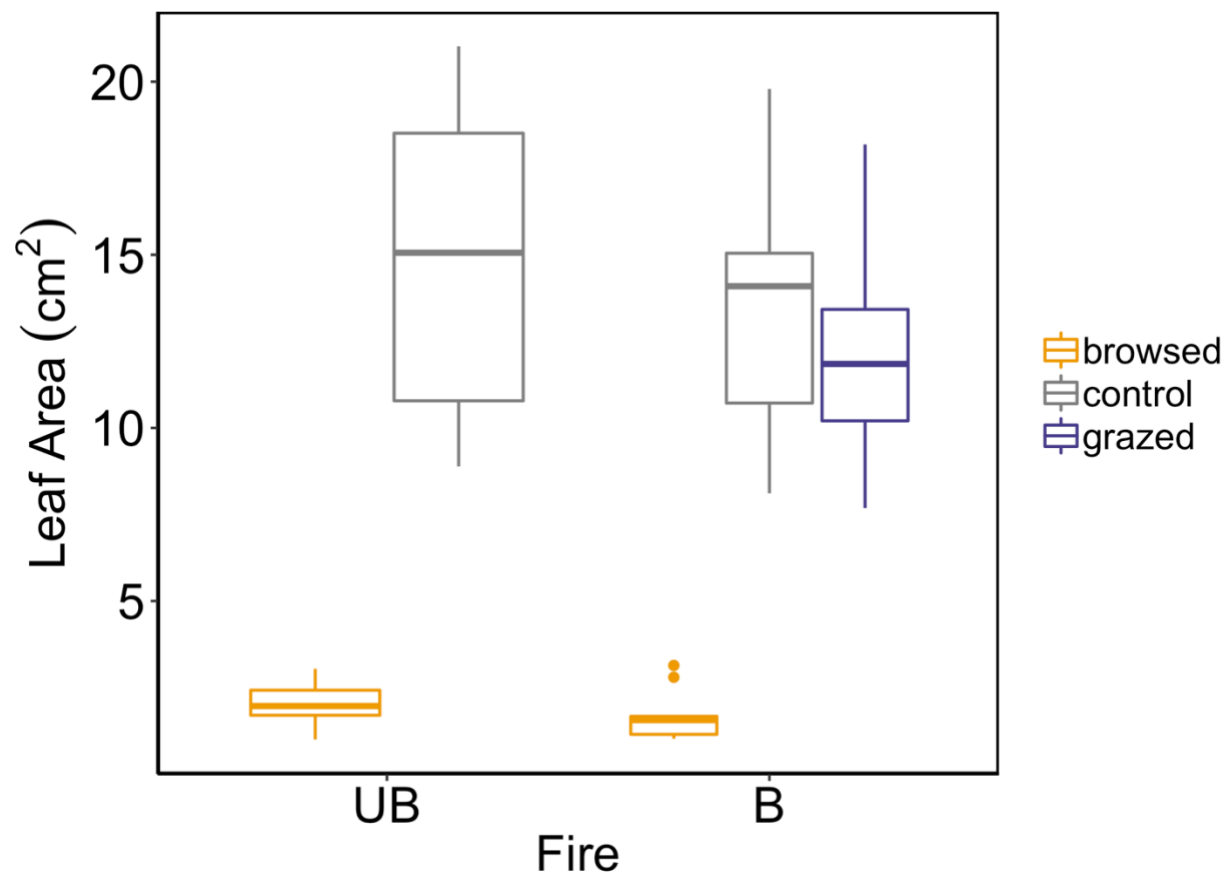


Figure 2.6 Boxplot of leaf area (cm²) for each treatment. UB = unburned, B = burned.



Chapter 3 - Effects of fire on *Cornus drummondii* leaf physiology during drought

Introduction

Fire is a major driver of tallgrass prairie ecosystem structure, nutrient cycling, and resource availability. Fire drives the suppression of woody species to maintain a grass dominated system (Briggs et al. 2002; Briggs et al. 2005). Fire also results in a volatilization of N, and increased light availability for regrowing plants (Hulbert 1988; Knapp & Seastedt 1986; Blair 1997). Grassland plant species have become adapted to survive fire by producing belowground buds that regenerate aboveground shoot populations after disturbance (Clarke et al. 2013; Ott et al. 2019). For many woody species, resprouting after fire is a key trait required for survival in grasslands and the ability to persist across the landscape (Bond & Midgley 2001). Typically, fire kills woody saplings, preventing establishment. However, at infrequent fire intervals (< 4-year fire frequency) woody plants can grow large enough to survive fire and develop large underground root systems with stored carbon and nutrient reserves to facilitate regrowth after fire (Dietze et al. 2014).

Plant shoots regenerating after fire (referred to as resprouts) tend to have higher growth rates and productivity than plants in unburned areas due to increased resource availability (Castell et al. 1994; McCarron & Knapp 2003). This increase in productivity in response to changes in the limiting resources is a result of the transient maxima hypothesis. The accumulation of N in infrequently burned grasslands is readily taken up by resprouts after fire due to a transient release from light limitation (Seastedt & Knapp 1993; Blair 1997). High N content is positively correlated with photosynthetic rate because the majority of leaf N is used to build photosynthetic machinery (Field and Mooney 1986; Evans 1989) resulting in resprouts

having higher photosynthetic rates and growth rates than plants in unburned locations (DeSouza et al. 1986; Castell et al. 1994; Fleck et al. 1998; Goorman et al. 2011). Increased photosynthetic rates of woody resprouts can also be due to increased root:shoot ratio resulting in improved leaf level water and nutrient availability (DeSouza et al. 1986; Peña-Rojas et al. 2004).

The expansion of the native, clonal shrub *Cornus drummondii* is contributing to a major loss of the mesic tallgrass prairie of the Central Great Plains (Ratajczak et al. 2011). *C. drummondii* shrubs form distinct islands that limit light availability and shade out herbaceous species in the understory (Lett & Knapp 2003). The immediate effects of fire on *C. drummondii* include increased stem densities due to activation of belowground buds (Ott et al. 2019) but reduced shrub productivity (Heisler et al. 2004; Heisler et al. 2007). Previous research has shown *C. drummondii* has deep root systems that mitigate competition for water from grasses and decouple shrub leaf physiology from environmental variability (Ratajczak et al. 2011; Nippert et al. 2013). Studying the response of *C. drummondii* leaf structure and physiology to fire will help further our understanding of the mechanisms clonal shrubs use to expand and succeed in tallgrass prairie.

I examined the post-fire leaf physiology on resprouting stems of *Cornus drummondii* resprouts. During the summer of 2018, I compared shrubs burned in a prescribed fire earlier in 2018 to shrubs burned in 2017. I compared leaf level gas exchange, water use efficiency (WUE), and photosynthetic nitrogen use efficiency (PNUE) between burn year treatments. Additionally, I used leaf traits such as leaf mass per area (LMA) and leaf dry matter content (LDMC) to help explain differences between the burn year treatments. I addressed the following questions: 1) How does fire influence leaf level physiology and leaf structure of *C. drummondii* ramets (i.e. 2018 vs. 2017 burn treatment)? 2) How do responses vary with position within shrub islands

(center vs. periphery)? 3) If differences exist between treatments what is the most likely mechanism(s) to explain the physiological response to fire?

Methods

Study site

Data were collected at Konza Prairie Biological Station (KPBS; 39°05' N, 96°35' W) located 15 km south of Kansas State University in Manhattan, KS. KPBS is a 3,487-hectare native tallgrass prairie characterized by uplands with shallow limestone soils and lowlands with deeper silty-clay loams separated by rocky hillslopes. The elevation varies from 320-444 m above sea level. The site is divided into 57 watersheds that are each assigned to a specific annual fire frequency (1, 2, 4, or 20-year burns) and grazing treatment (bison, cattle, or no large mammalian grazers). Mean annual precipitation (1982-2018) is 835 mm, with approximately 73% of annual precipitation falling during the growing season (April-September). Temperature means (1982-2018) range from an average daily low of -1.2°C in January to a high of 26.1°C in July. C₄ warm-season grasses (*Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans*, and, *Schyzachyrium scoparium*) dominate the plant cover and annual productivity in portions of the site that are frequently burned. Areas subject to infrequent burns ($\leq$ 4-year fire frequency) are encroached primarily by the woody shrubs *Cornus drummondii* and *Rhus glabra*. In areas of KPBS with 20-year burn frequencies, *Juniperus virginiana* (an evergreen tree) is abundant.

Study design and data collection

Research was conducted in 2018 at KPBS on 2 watersheds with a 4-year burn frequency (4A, 18.84 ha and 4B, 54.5 ha). 4A was burned in spring 2018 and 4B burned in spring 2017. I

randomly sampled 40 mature *C. drummondii* shrubs located in the lowlands of each watershed. Shrubs had an average aboveground area of 44 m². Fire penetrated through all the shrub islands in the 2018 burn treatment, therefore only resprouts were measured within this burn year treatment. There were four sampling dates throughout the summer (2018 day of year – 162, 183, 205, 229). Day of year 162 corresponds with June 11, 2018. Leaf-level gas exchange measurements (light-saturated photosynthetic rate, A_{max} ; transpiration, E ; and stomatal conductance to vapor, g_s) were made on one fully expanded, sun-exposed leaf at the periphery and in the center of each shrub ($N=40$ /watershed) using the Li-6400 open-system gas analyzer (Li-Cor, Inc., Lincoln, NE). Cuvette conditions were set to $[CO_2] = 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$, relative humidity = 40 – 60%, and photosynthetically active radiation (PAR) = $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Leaves were allowed to stabilize to chamber conditions before recording the measurement. Gas exchange measurements were collected between 10:00 and 16:00 h. Sampling order was random for each sampling date to minimize variability contributed by diurnal effects.

The leaf used for gas exchange and three other leaves from the periphery and center of each shrub were collected, placed in a moist plastic bag and stored in a cooler until brought back to the lab for processing. June leaves were collected after gas exchange sampling (on day of year [DOY] 179) and all other leaves were collected during the gas exchange measurements. Since N content and leaf functional traits vary with leaf age, I collected the youngest fully expanded sun-exposed leaves which typically were collected from the 3rd node down. Leaves were stored in a refrigerator (at 2 °C) and measured for leaf area and wet weight within 24 hours of collection. Leaves were dried at 53 °C (for 72 hours) and subsequently weighed for dry mass. I calculated leaf mass per area (LMA) and leaf dry matter content (LDMC) from the measured leaf traits. Leaf relative water content (RWC) was calculated as the difference of leaf wet and dry weight

corrected for leaf dry weight. LMA, LDMC, and RWC were calculated for each leaf and then averaged by position for each shrub before analysis.

For each shrub, four leaves from each position (periphery and center) for each shrub were combined and ground. For each watershed and sampling date, 20 homogenized samples from the periphery and 20 from the center were used for stable isotope analysis. I measured N and C content (% dry mass) and the stable isotopic composition of leaves at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. Ground samples were combusted using an Elementar vario Pyro cube. An Elementar Vision mass spectrometer was used for isotope analysis. The δ notation for isotopic ratios was calculated as:

$$\delta = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000$$

Where R is the ratio of heavy to light isotopes for the sample and standard (Vienna-Pee-Dee Belemnite), respectively. $\delta^{13}\text{C}$ was used as a proxy for integrated water-use efficiency (iWUE). Larger values (enriched) of leaf $\delta^{13}\text{C}$ indicate a higher iWUE. iWUE is typically used as a proxy for intrinsic WUE (A/g_s). I therefore expected trends to be the same for these 2 measurements with differences reflecting iWUE as a longer-term measurement incorporating daily WUE over the life span of the leaf while intrinsic WUE is an instantaneous measurement.

Photosynthetic nitrogen-use efficiency was calculated as:

$$PNUE = \frac{A \text{ } (\mu\text{mol m}^{-2}\text{s}^{-1})}{N_{\text{area}} \text{ } (g \text{ m}^{-2})}$$

N content per leaf area (N_{area} ; g/m²) was determined as the N content of the homogenized leaf samples (g/g) multiplied by the average leaf area (m²).

Statistical analysis

Statistical analyses were performed in R V3.6.0 (R Core Team 2019). Daily precipitation data were obtained through the Climate and Hydrology Database Projects collected from the KPBS headquarters. I used linear mixed effects repeated measure ANOVA models using the lmer function in the lmerTest package (Kuznetsova et al. 2017) to test for differences in gas exchange rates, WUE, PNUE, and leaf structure. Fixed effects for all models included burn year (2017 and 2018) and sampling date (DOY 162, 183, 205, 229). Position (center or periphery) nested within shrub ID was the random effect. Pairwise comparisons were made using the emmeans package (Lenth 2019) with Tukey's HSD adjustment. Graphics were made using the package ggplot2 (Wickham 2016).

Results

Precipitation during the sampling period was approximately 41% below the long-term average (Table 3.1). In addition, the early- and mid-growing season precipitation was minimal and approximately 45% of 2018 growing season precipitation fell after sampling was complete. Gas exchange rates did not differ significantly ($p > 0.05$) between positions (center and periphery) within measured shrubs in the 2017 burn treatment. In the 2018 burn treatment, leaves in the center of the shrub had marginally higher transpiration and stomatal conductance than those at periphery, but only for the first two sampling periods. No position differences existed

thereafter. Because shrub position data were largely insignificant, "position" was not included as a main effect in subsequent analyses.

Shrubs in the 2018 burn treatment had higher gas exchange rates than those in the 2017 burn treatment throughout the growing season, including A_{max} , E , and g_s (Fig. 3.2). Differences in gas exchange between burn year treatments were greatest at the beginning of the season and differences diminished through time. The interaction between sampling date and burn year was significant ($p < 0.001$) for all gas exchange variables indicating the magnitude of the difference between burn year treatments varied significantly through time (Table 3.3). Burn year treatments had similar trends in A_{max} following those expected in response to precipitation (Figure 3.2A). Average A_{max} values on DOY 183 were nearly half of those on DOY 162 and increased again on DOY 205 after the site received multiple days with rainfall. Differences in A_{max} between burn year treatments were significant for the first 3 sampling dates. E and g_s were highest at the beginning of the season in the 2018 burn treatment and declined by DOY 183 where they remained stable through the remainder of the growing season (Figure 3.2 B & C). E and g_s did not vary across the growing season in the 2017 burn treatment. Differences between burn year treatments were significant for all sampling dates for E and the first 2 sampling dates for g_s . Gas exchange variability within sampling date was highest on the first sampling date especially in the 2018 burn treatment and variability was reduced for the remainder of the growing season.

Intrinsic WUE and $\delta^{13}C$ showed similar trends between burn year treatments (Figure 3.3). The 2018 burn treatment had lower WUE (for both metrics of WUE: intrinsic WUE and $\delta^{13}C$) at the beginning of the season than the 2017 burn treatment and differences declined through the growing season. Intrinsic WUE and $\delta^{13}C$ varied significantly across the growing season in the 2018 burn treatment ($p < 0.001$), but not in the 2017 burn treatment. The 2018 burn treatment had

significantly lower intrinsic WUE on DOY 162, but differences between burn year treatments declined across the growing season (Figure 3.3A). No differences in $\delta^{13}\text{C}$ were present between burn year treatments on any sampling date. In the 2018 burn treatment, leaves sampled on DOY 162 and 183 had lower intrinsic WUE than DOY 205 and 229. $\delta^{13}\text{C}$ was significantly lower on DOY 176 and 183 than DOY 229.

Plants in the 2018 burn treatment had significantly higher %N than the 2017 burn treatment across the growing season ($p < 0.001$). Average %N decreased significantly on DOY 205 in both the burn year treatments ($p < 0.05$). Differences in N_{area} (g m^{-2}) were not significant between burn year treatments when adjusted for leaf area ($p > 0.05$) and there was no significant interaction between burn year and sampling date (Table 3.5). The 2018 burn treatment had higher PNUE with significant differences between burn year treatments for the first 3 sampling dates ($p < 0.05$). N_{area} and PNUE covary as N_{area} was used to calculate PNUE.

Leaf traits varied significantly between watersheds and over the course of the growing season (Figure 3.5). Resprouts from the 2017 burn treatment had significantly higher LMA and LDMC than shrubs in the 2018 burn treatment, regardless of sampling date (Figure 3.5). LMA and LDMC increased significantly until DOY 205 for both burn year treatments.

Discussion

This study gives insight into the physiological mechanisms used by a major encroaching shrub to survive in tallgrass prairie after fire and during drought. Shrubs in the 2018 burn treatment (resprouts) had higher photosynthetic rates, transpiration rates and stomatal conductance to water vapor than shrubs in the 2017 burn treatment across the growing season. Increased gas exchange in resprouts is consistent with other studies (DeSouza et al. 1986;

McCarron & Knapp 2003; Goorman et al. 2011). For the first 2 sampling dates, resprouts had mean photosynthetic rates over 2x higher than shrubs in the 2017 burn treatment and transpiration rates nearly 4x higher than the 2017 burn treatment. Differences between treatments diminished through time with no differences in photosynthetic rates or stomatal conductance by DOY 229. Higher gas exchange rates in resprouts compared to shrubs in the 2017 burn treatment is likely due to a combination of possible factors, such as increased root:shoot ratio and leaf N content.

Increased gas exchange rates are likely related to increased root:shoot ratio resulting in increased leaf level water availability (Castell et al. 1994; Peña-Rojas et al. 2004). Resprouts had ~30% higher leaf relative water (RWC) content than shrubs in the 2017 burn treatment. Differences in leaf RWC decreased across the growing season to ~10% lower RWC in the 2017 burn treatment than the 2018 burn treatment on DOY 229. Mature *C. drummondii* shrubs have deep, established root systems with access to deep soil water (>30cm; Ratajczak et al. 2011; Nippert et al. 2013). Deep roots provide a consistent water source allowing new aboveground growth to exert high transpiration rates resulting in reduced intrinsic WUE at the beginning of the season (Figure 3.3A). However, burn year treatments had only minor effects on intrinsic and integrated WUE ($\delta^{13}\text{C}$) and differences decreased across the growing season. Resprouts increased WUE later in the season, which is likely related to reduced g_s .

Resprouts with increased transpiration and photosynthetic rates could also be due to the ability of leaves to regulate their microclimate by increasing the latent heat and thereby decreasing leaf temperatures after fire (Gates 1968; Michaletz 2015). Previous work has shown *C. drummondii* lowers leaf temperature relative to air temperature after fire, creating a favorable microclimate for photosynthesis (O'Connor 2019). In addition, O'Connor (2019) reported that

leaf temperature regulation was strongest following fire, but not during years without fire. While leaf temperature data were not measured as part of this study, increased stomatal conductance and transpiration rates are likely to impact leaf thermal energy budget, and supports the assertion that post-fire resprouts have altered physiological processes than leaves from unburned locations.

In addition to increased root:shoot ratio and transpiration, increased gas exchange rates in the 2018 burn treatment may be due to higher %N in leaves as leaf N concentration is positively correlated with photosynthetic rates (Field and Mooney 1986; Evans 1989). Fire resulted in a pulse in leaf N content as resprouts had higher %N across the growing season and a trend of higher N_{area} , supporting the transient maxima hypothesis. McCarron & Knapp 2003 also showed support for the transient maxima hypothesis in *C. drummondii* as infrequently burned shrubs had higher %N and photosynthetic rates than unburned shrubs. However, N_{area} differences between burn year treatments were not significant. Leaves of resprouts also had increased PNUE. At a given irradiance, PNUE is typically negatively correlated with N content (Lambers 2008). Although resprouts had higher % N, N_{area} did not differ between burn year treatments. Thus, lower PNUE in the 2017 burn treatment is likely driven by low stomatal conductance and photosynthetic rates.

These results and all the measured physiological responses to fire (increased gas exchange rates, lower WUE, and higher PNUE) correspond with changes in leaf structure. LMA and LDMC may vary within species in response to a plant's environment (Poorter et al. 2009). Resprouts had lower LMA and LDMC than shrubs in the 2017 burn treatment. LMA is typically negatively correlated with photosynthetic rate, leaf N concentration, protein content, and PNUE (Poorter et al. 2009). My results show resprouts produced leaves with higher %N and less structural investment (i.e. cell walls and lignin) than leaves from the 2017 burn treatment.

LDMC is often positively correlated with leaf lifespan also suggesting the shrubs in the 2017 burn treatment were investing more in leaf structure than resprouts. These results suggest that resprouts are investing in growth and likely have greater leaf turnover than the 2017 burn treatment. Greater leaf turnover also helps explain the trend of resprouts with lower $\delta^{13}\text{C}$ earlier in the season. Lower LMA and LDMC suggests resprouts modified leaf structure to maximize C gain and minimize leaf C investment and indicates resprouts were investing in shoot growth after fire, which restores height to maximize light capture. Growth investment may be an important mechanism in competing with vigorous grass growth after fire and to replenish belowground reserves used for resprouting.

Lack of gas exchange differences between the center and periphery of the shrub may be due to clonal integration, the ability of shrubs to translocate water and resources among ramets. Previous research indicated no difference occurred in gas exchange between leaves measured at the center and periphery of *C. drummondii* shrubs (O'Keefe & O'Connor, unpublished).

Although ramets on the periphery and center are experiencing different microclimates, leaf level physiology remains fairly uniform throughout the upper canopy. The transfer of resources among ramets increases physiological uniformity, biomass production, and competitive ability of the genet (Hartnett & Bazzaz 1983; Song et al. 2013). Any differences seen in gas exchange by position were on the first sampling date for the 2018 burn treatment and disappeared thereafter (data not shown). The higher A_{max} , E , and g_s in the center of resprouting shrubs may be due to closer proximity to the center taproot and thus more immediate water availability for the center ramets.

The sampling period during the summer 2018 was one of the driest and hottest on record at KPBS. Average photosynthetic and transpiration rates were slightly lower than those reported

for *C. drummondii* in other studies (McCarron & Knapp 2003; Muench et al. 2016) suggesting some evidence of leaf level drought stress. My study found similar photosynthetic rates in a previous study that averaged rates across the growing season during drought (Heisler et al. 2007). Heisler et al. (2007) found unburned shrubs had lower photosynthetic rates than burned shrubs during a dry year, but higher photosynthetic rates than burned shrubs during a wet year. My results also show lower photosynthetic rates in unburned shrubs during a dry year suggesting differences in gas exchange rates are driven mostly by water stress resulting in lower stomatal conductance. Heisler et al. (2007) attribute lower photosynthetic rates of burned shrubs during a wet year to shrub age. They focused on shrubs 3-5 years old, which were smaller and not yet considered mature and dominate. Thus, these shrubs may have had less developed roots systems and experience hotter, drier soil conditions than unburned shrubs. Peña-Rojas et al. (2004) found *Quercus ilex* resprouts to have higher photosynthetic rates than control trees during drought but found no differences between resprouts and controls when water was readily available. Additionally, Peña-Rojas et al. (2004) reported that control trees had a larger decrease in stomatal conductance and photosynthesis than resprouts and found stomatal conductance to be the main limiting factor limiting photosynthetic rates.

Although gas exchange rates were lower than reported in other studies, gas exchange, WUE, and PNUE in the 2017 burn treatment remained stable across the growing season. Deep rooted, clonal shrubs such as *C. drummondii* have a consistent water source and are generally thought of as insensitive to growing season climatic variability, which helps explain stability across the growing season (Nippert et al. 2013). $\delta^{13}\text{C}$ values are similar to those reported in other studies suggesting *C. drummondii* maintains relatively constant stomatal control within and among growing seasons (Nippert et al. 2013; O'Connor 2019).

These results indicate *C. drummondii* changes leaf structure in response to fire in order to maximize C gain, but leaf physiology remains relatively stable across the growing season, even during drought. In general, the effects of fire on *C. drummondii* are similar to those observed for resprouting shrubs in other systems such as increased gas exchange rates and leaf %N. These post-fire physiological traits are likely attributes that help clonal shrubs respond to disturbance, increase competitive success with grasses and ultimately promote long-term persistence and expansion in high-disturbance environments like the tallgrass prairie.

References

- Blair, J. M. (1997). Fire, N Availability, and Plant Response in Grasslands: A Test of the Transient Maxima Hypothesis. *Ecology*, 78(8), 2359–2368.
- Bond, W. J., & Midgley, J. J. (2001). Ecology of sprouting in woody plants: The persistence niche. *Trends in Ecology & Evolution*, 16(1), 45–51.
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. L., Hoch, G. A., Lett, M. S., & McCarron, J. K. (2005). An Ecosystem in Transition: Causes and Consequences of the Conversion of Mesic Grassland to Shrubland. *BioScience*, 55(3), 243.
- Briggs, J.M., Knapp A.K., & Brock, B.L. (2002). Expansion of woody plants in tallgrass prairie: A fifteen-year study of fire and fire-grazing interactions. *The American Midland Naturalist*, 147(2), 287-294.
- Castell, C., Terradas, J., & Tenhunen, J. D. (1994). Water Relations, Gas Exchange, and Growth of Resprouts and Mature Plant Shoots of *Arbutus unedo* L. and *Quercus ilex* L. *Oecologia*, 98(2), 201–211.
- Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., Enright, N.J., & Knox, K. J. E. (2013). Resprouting as a key functional trait: How buds, protection and resources drive persistence after fire. *New Phytologist*, 197(1), 19–35.
- DeSouza, J., Silka, P. A., & Davis, S. D. (1986). Comparative physiology of burned and unburned *Rhus laurina* after chaparral wildfire. *Oecologia*, 71(1), 63–68.
- Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooh, J.A., Richardson, A.D., & Vargas, R. (2014). Nonstructural carbon in woody plants. *Annual Review of Plant Biology*, 65(1), 667-687.

- Evans, J. R. (1989). Photosynthesis and Nitrogen Relationships in Leaves of C₃ Plants. *Oecologia*, 78(1), 9–19.
- Field, C. & Mooney, H.A. 1986. The photosynthesis-nitrogen relationship in wild plants. *On the Economy of Plant Form and Function* (ed. R. Givnish), pp. 25-55. Cambridge University Press, Cambridge.
- Fleck, I., Hogan, K. P., Llorens, L., Abadia, A., & Aranda, X. (1998). Photosynthesis and photoprotection in *Quercus ilex* resprouts after fire. *Tree Physiology*, 18(8–9), 607–614.
- Gates, D.M. (1968). Transpiration and leaf temperature. *Annual Review of Plant Physiology*, 19, 211-238.
- Goorman, R., Bartual, A., Paula, S., & Ojeda, F. (2011). Enhancement of photosynthesis in post-disturbance resprouts of two co-occurring Mediterranean *Erica* species. *Plant Ecology*, 212(12), 2023–2033.
- Hartnett, D.C. & Bazzaz, F.A. (1983). Physiological integration among intracolonial ramets in *Solidago canadensis*. *Ecology*, 64(4), 779-788.
- Heisler, J. L., Briggs, J. M., Knapp, A. K., Blair, J. M., & Seery, A. (2004). Direct and Indirect Effects of Fire on Shrub Density and Aboveground Productivity in a Mesic Grassland. *Ecology*, 85(8), 2245–2257.
- Heisler, J.L., Knapp, A.K., & Briggs, J.M. (2007). Fire and resource availability influence carbon and water relations of the C₃ shrub roughleaf dogwood in a mesic grassland. Pages 86-93 in R.E. Masters and K.E.M. Galley (eds.). Proceedings of the 23rd tall timbers fire ecology conference: fire in grassland and shrubland ecosystems. Tall timbers research station, Tallahassee, Florida, USA.
- Hulbert, L. C. (1988). Causes of Fire Effects in Tallgrass Prairie. *Ecology*, 69(1), 46–58.
- Knapp, A. K., & Seastedt, T. R. (1986). Detritus Accumulation Limits Productivity of Tallgrass Prairie. *BioScience*, 36(10), 662–668.
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82(13), 1-26.
- Lambers, H., Chapin, F.S., & Pons, T.L. (2008). *Plant Physiological Ecology*, pp. 58. Springer Science + Business Media, New York, NY.
- Lenth, R. (2019). emmeans: Estimated marginal means, aka least-squares means. R package version 1.3.4.
- Lett, M. S., & Knapp, A. K. (2003). Consequences of shrub expansion in mesic grassland: Resource alterations and graminoid responses. *Journal of Vegetation Science*, 14(4), 487–496.

- McCarron, J. K., & Knapp, A. K. (2003). C3 shrub expansion in a C4 grassland: Positive post-fire responses in resources and shoot growth. *American Journal of Botany*, 90(10), 1496–1501.
- Michaletz, S.T., Weiser, M.D., Kaspari, M., Helliker, B.R., & Enquist, B.J. (2015). Plant thermoregulation: Energetics, trait-environment interactions, and carbon economics. *Trends in Ecology & Evolution*, 30(12), 714-724.
- Muench, A.T., O’Keefe, K., & Nippert, J.B. (2016). Comparative ecohydrology between *Cornus drummondii* and *Solidago canadensis* in upland tallgrass prairie. *Plant Ecology*, 217(3), 267-276.
- Nippert, J. B., Ocheltree, T. W., Orozco, G. L., Ratajczak, Z., Ling, B., & Skibbe, A. M. (2013). Evidence of Physiological Decoupling from Grassland Ecosystem Drivers by an Encroaching Woody Shrub. *PLoS ONE*, 8(12), e81630.
- O’Connor, R.C. (2019). Drivers, mechanisms, and thresholds of woody encroachment in mesic grasslands. (Doctoral dissertation). Retrieved from K-State Research Exchange.
- Ott, J. P., Klimešová, J., & Hartnett, D. C. (2019). The ecology and significance of below-ground bud banks in plants. *Annals of Botany*.
- Peña-Rojas, K., Aranda, X., & Fleck, I. (2004). Stomatal limitation to CO₂ assimilation and down-regulation of photosynthesis in *Quercus ilex* resprouts in response to slowly imposed drought. *Tree Physiology*, 24(7), 813–822.
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565–588.
- R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ratajczak, Z., Nippert, J.B., Hartman, J.C., & Ocheltree, T.W. (2011). Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. *Ecosphere*, 2(11), 121.
- Song, Y.-B., Yu, F.-H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., & van Kleunen, M. (2013). United we stand, divided we fall: A meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia*, 171(2), 317–327.
- Wickman, H. (2016). ggplot2: Elegant graphics for data analysis. Springer.

Table 3.1 Long term (1982-2017) mean and 2018 growing season precipitation (mm) for each month.

Month	Historical 1982-2017	2018	% Change
April	83.3 ± 47.7	41.3	-50
May	110.4 ± 79.1	80.4	-27
June	131.5 ± 67.7	37.9	-71
July	101.1 ± 78.6	86.6	-14
August	110.0 ± 52.6	140.3	+22
September	72.0 ± 41.4	125.3	+43
Total	608.7 ± 65.2	504.9	-17
Sampling Period	244.4 ± 17.4	144.2	-41

Table 3.2 Leaf relative water content of shrubs burned in 2017 and shrubs burned in 2018 and the % difference between burn year treatments. All values are means ± 1 SE.

DOY	2017	2018	% Difference
173	1.28 ± 0.10	1.85 ± 0.22	-30
183	1.18 ± 0.10	1.44 ± 0.17	-18
205	1.09 ± 0.06	1.37 ± 0.12	-20
229	1.08 ± 0.08	1.21 ± 0.11	-10

Table 3.3 Linear mixed effects model results of gas exchange in response to burn year treatment and sampling date as fixed effects and position within the shrub as a random effect.

		NumDF	DenDF	F	P
A	Burn Year	1	79.437	63.8436	<0.001
	Date	3	211.429	34.6776	<0.001
	Burn Year*Date	3	211.429	6.6441	<0.001
E	Burn Year	1	79.734	148.849	<0.001
	Date	3	208.253	56.309	<0.001
	Burn Year*Date	3	208.253	41.768	<0.001
g_s	Burn Year	1	79.726	59.805	<0.001
	Date	3	208.932	28.838	<0.001
	Burn Year*Date	3	208.932	28.635	<0.001

Table 3.4 Linear mixed effects model results of WUE in response to burn year treatment and sampling date as fixed effects and position within the shrub as a random effect. Intrinsic WUE was calculated as A/g_s and $\delta^{13}C$ is often used as an integrated proxy for WUE.

		NumDF	DenDF	F	P
intrinsic WUE	Burn Year	1	79.072	27.4563	<0.001
	Date	3	212.9	13.7014	<0.001
	Burn Year*Date	3	212.9	9.5854	<0.001
$\delta^{13}C$	Burn Year	1	78.252	0.8483	0.359
	Date	3	233.889	15.1382	<0.001
	Burn Year*Date	3	233.889	4.93	0.002

Table 3.5 Linear mixed effects model results of %N, leaf N content ($g\ m^{-2}$), and PNUE ($\mu\ mol\ g^{-1}\ s^{-1}$) in response to burn year treatment and sampling date as fixed effects and position within the shrub as a random effect.

		NumDF	DenDF	F	P
% N	Burn Year	1	78.143	101.519	<0.001
	Date	3	205.223	83.190	<0.001
	Burn Year*Date	3	205.223	15.323	<0.001
N ($g\ m^{-2}$)	Burn Year	1	78.813	4.195	0.044
	Date	3	211.683	14.934	<0.001
PNUE	Burn Year	1	72.464	59.6223	<0.001
	Date	3	208.335	41.6088	<0.001
	Burn Year*Date	3	208.335	5.6477	<0.001

Table 3.6 Linear mixed effects model results of LMA ($g\ m^{-2}$) and LDMC ($mg\ g^{-1}$) in response to burn year treatment and sampling date as fixed effects and position within the shrub as a random effect.

		NumDF	DenDF	F	P
LMA	Burn Year	1	77.985	88.877	<0.001
	Date	3	232.441	301.987	<0.001
	Burn Year*Date	3	232.441	17.963	<0.001
LDMC	Burn Year	1	77.967	194.149	<0.001
	Date	3	232.629	265.514	<0.001
	Burn Year*Date	3	232.629	45.747	<0.001

Figure 3.1 Daily growing season precipitation (mm) April – September. All rainfall events shown are greater than 5 mm since more than 5 mm must fall to reach the soil surface. Day of Year 121, 152, 182, 213, and 244 correspond to the first of each month throughout the growing season (May, June, July, August, and September, respectively).

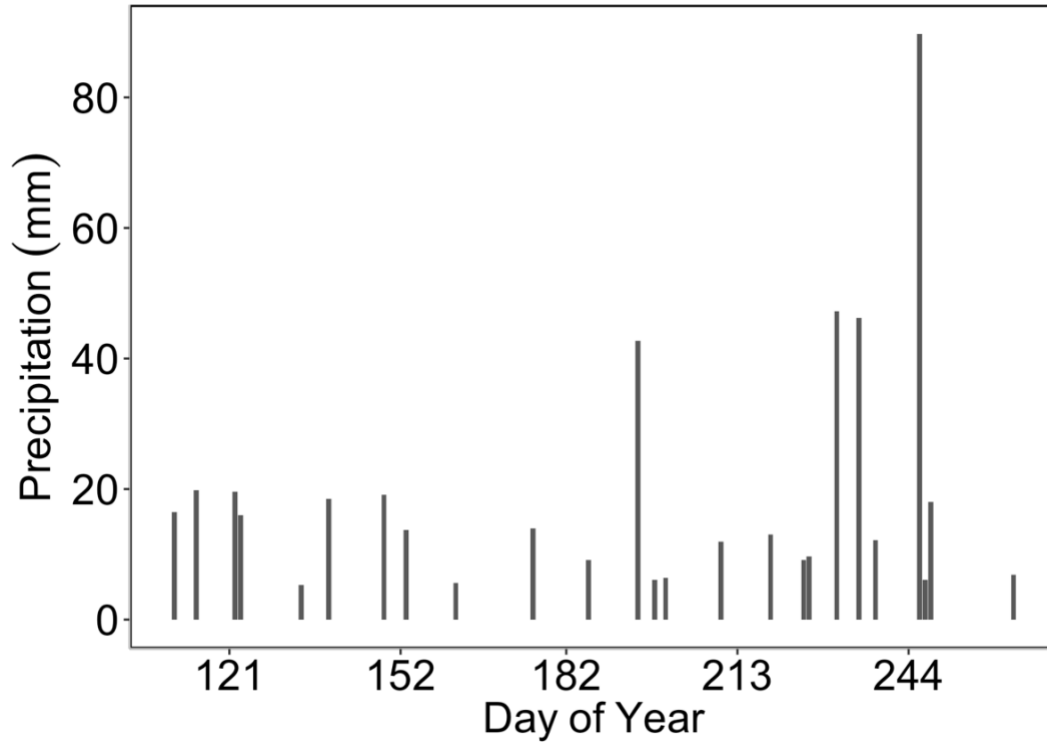


Figure 3.2 Leaf level gas exchange measurements for *C. drummondii* throughout the growing season. A) CO₂ assimilation rate, A_{max} . B) transpiration rate, E . C) stomatal conductance, g_s . Values shown are the mean $\pm$ 1SE. Asterisks indicate significant differences among burn treatments within sampling date. Alpha = 0.05.

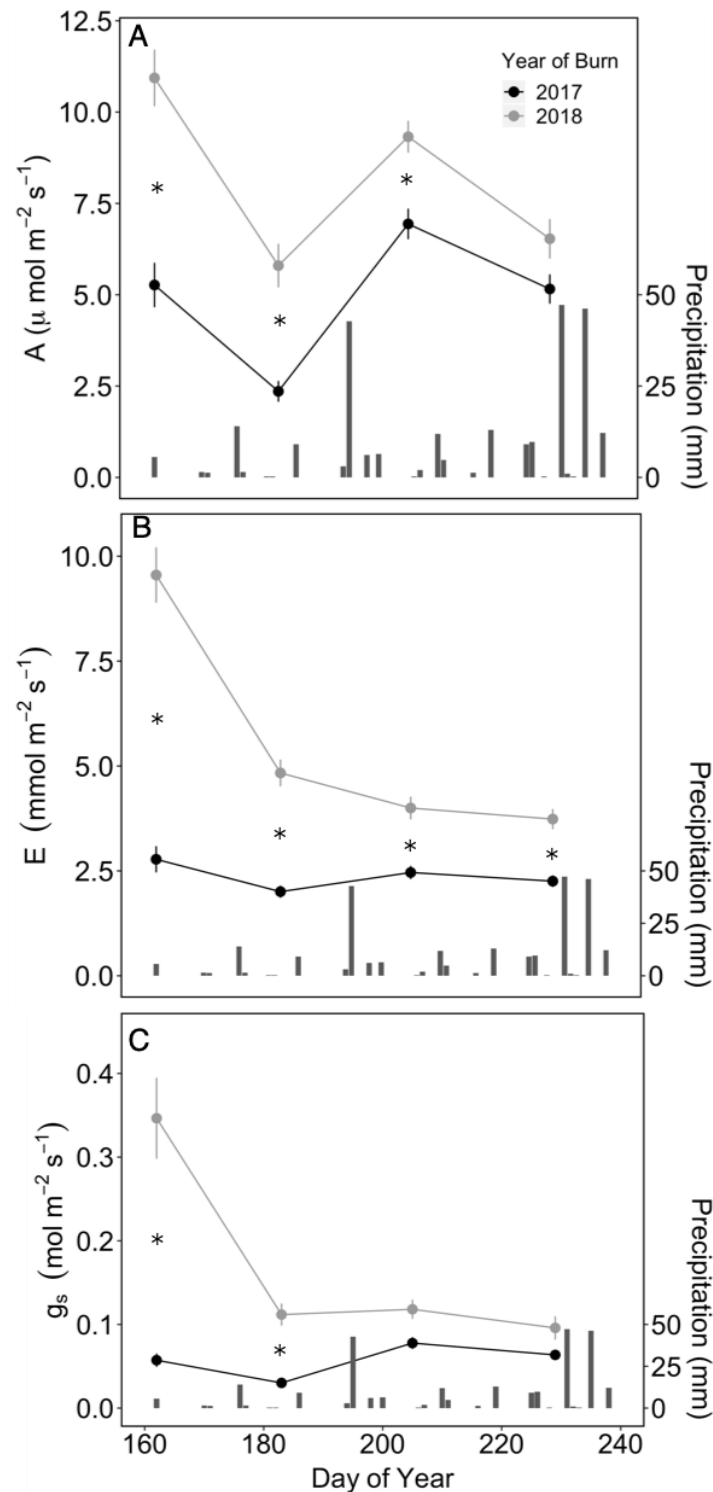


Figure 3.3 Intrinsic WUE and $\delta^{13}\text{C}$ measurements for *C. drummondii* throughout the growing season. A) Intrinsic WUE calculated as A/gs from gas exchange measurements. B) $\delta^{13}\text{C}$ measurements from homogenized leaf samples from each shrub. Leaves in June were collected on DOY 173. Values shown are the mean $\pm$ 1 SE. Asterisks indicate significant differences among burn treatments within sampling date. Alpha = 0.05.

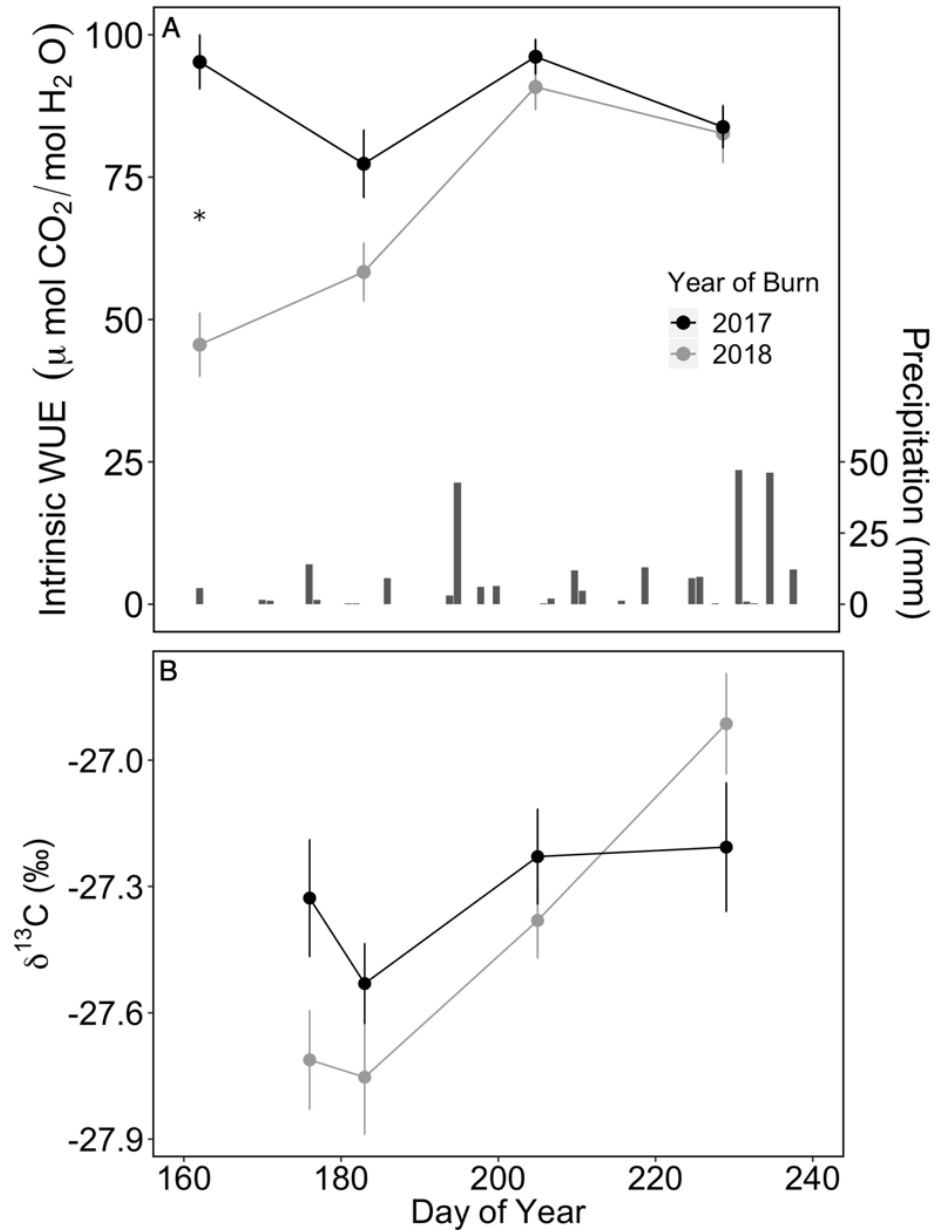


Figure 3.4 Leaf %N, N content, and photosynthetic N use efficiency (PNUE) for *C. drummondii* throughout the growing season. A) %N from homogenized leaf samples from each shrub. B) Leaf N content (g m^{-2}) calculated from %N and mean leaf area of the homogenized leaf samples. C) Photosynthetic N use efficiency calculated as $A_{\text{max}} / \text{leaf N content}$. Leaves in June were collected on DOY 173. Values shown are the mean ± 1 SE. Asterisks indicate significant differences among burn treatments within sampling date. Alpha = 0.05.

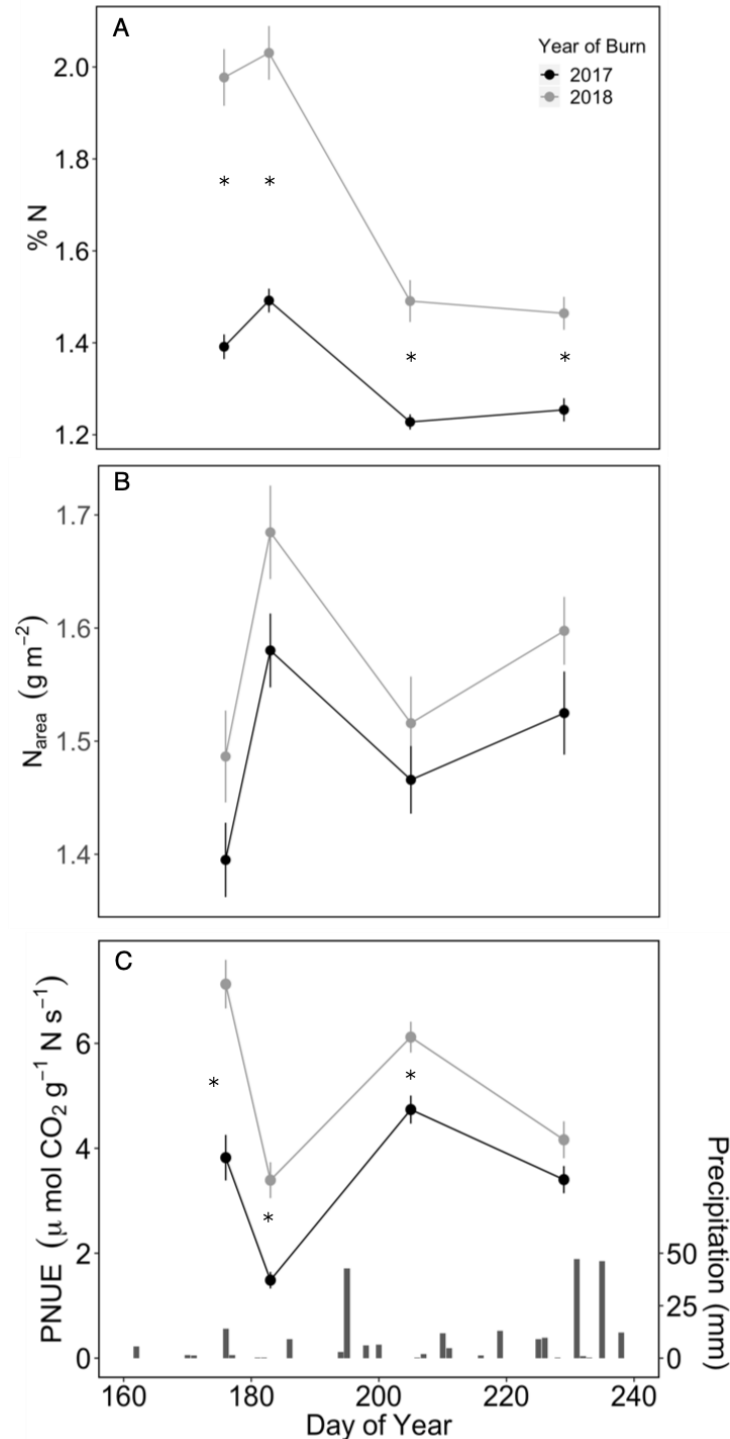
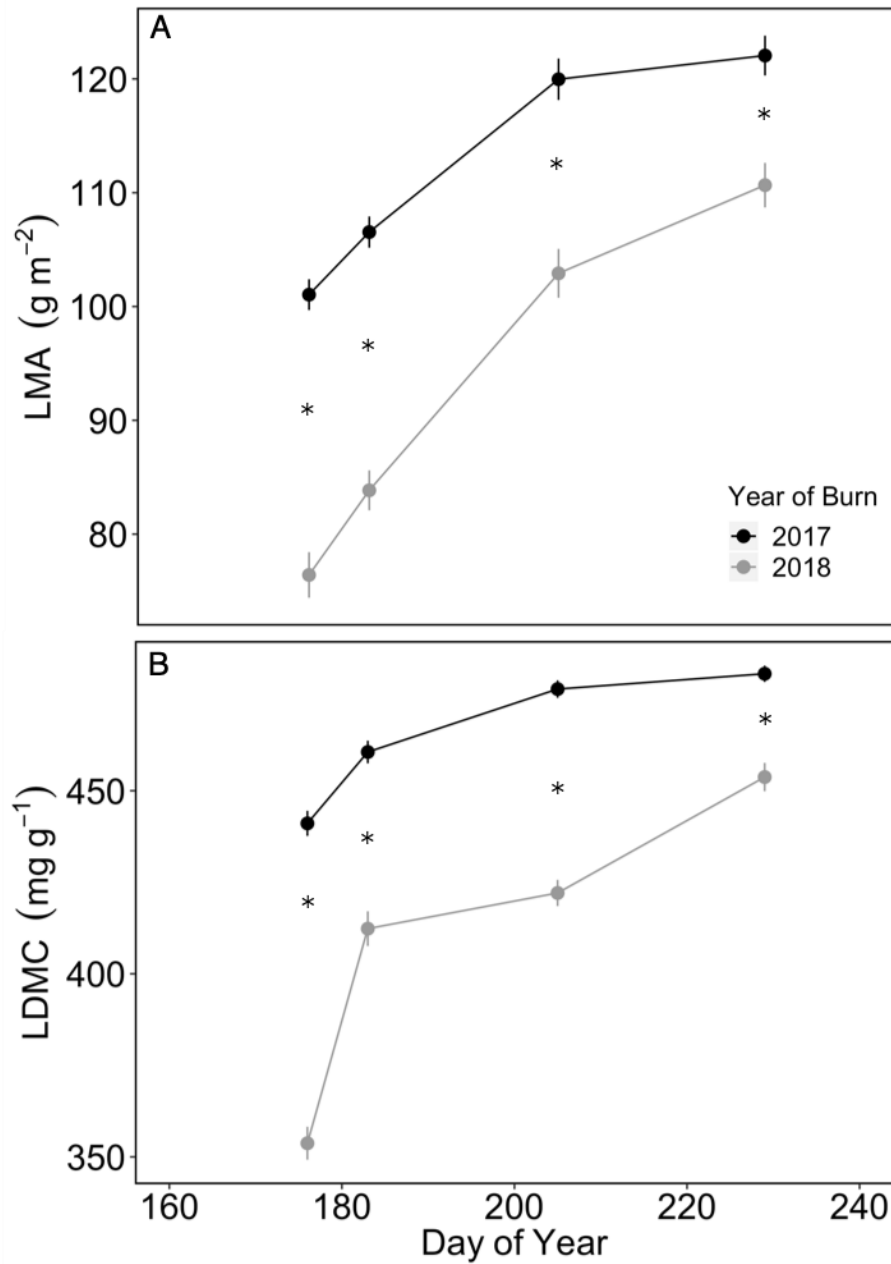


Figure 3.5 Leaf traits for *C. drummondii* throughout the growing season. Measurements were taken on 8 leaves per shrub and averaged before analysis. A) Leaf dry mass per unit area (LMA). B) Leaf dry matter content (LDMC). Values shown are the mean $\pm$ 1 SE. Asterisks indicate significant differences among burn treatments within sampling date. Alpha = 0.05.



Chapter 4 - Conclusion

Woody encroachment in grasslands is a global phenomenon widespread in North America, South America, Africa, and Australia (Briggs et al. 2005; Van Auken 2009, Eldridge et al. 2011). Woody expansion into open grasslands has resulted in a loss of biodiversity (Ratajczak et al. 2012), alterations in ecosystem C and N storage (Hughes et al. 2006; McKinley & Blair 2008; Barger et al. 2011), and loss of forage for livestock (Anadón et al. 2014). Woody species are often suppressed in grasslands due to frequent disturbance and the establishment and expansion of woody species is often attributed to human altered drivers such as reduced fire frequency and increased grazing intensity (Briggs et al. 2005; Van Auken 2009).

In the tallgrass prairie of North America, the prevention of transition from grassland to woodland is often attributed to frequent fires (Daubenmire 1968; Briggs et al. 2005). However, once established, clonal woody species are able to resprout and thrive under infrequent fire regimes (< 4-year fire frequency). *Cornus drummondii* is the most dominant woody species encroaching in the Flint Hills of eastern Kansas, USA. *C. drummondii* contributes to a major loss of tallgrass prairie through the formation of distinct shrub islands that limit light availability and nearly eliminate all open grassland herbaceous species from under the canopy (Lett & Knapp 2003). This thesis aimed to understand the demographic and physiological mechanisms contributing to increased *C. drummondii* cover in tallgrass prairie.

In chapter 2, I quantified intra-clonal ramet growth rate, reproduction, and demography of *C. drummondii* in response to fire frequency, grazing, and simulated browsing. We used shrubs located within 3 experimental watersheds which included 4 and 20-year burn frequencies without bison present and a 2-year burn frequency with bison present. Half of the shrubs in the 4 and 20-year burn frequencies were subject to simulated browsing. Summer 2018 was one of the

driest on record for Konza Prairie Biological Station. Extreme drought resulted in low ramet growth rates in all treatments. Ramets in the grazed area had the highest growth rates likely due to reduced grass biomass increasing shrub competitive ability for resources. Ramets growth rates did not differ among browsed and control shrubs in either burn treatment. Additionally, browsed and control shrubs did not differ in ramet natality suggesting browsed shrubs may be investing in new stems over ramet growth. However, more data is needed to understand resource allocation in response to browsing and variation in response to precipitation. Browsing nearly eliminated sexual reproduction and browsed shrubs had significantly lower ramet densities than control shrubs. These results highlight the effects of browsing are strongest in conjunction with fire and suggest drought may be an effective time to control for woody species as shrubs are not investing in growth and have limited sexual reproduction.

In chapter 3, I examined *C. drummondii* leaf level physiology across the growing season in response to fire. I compared shrubs burned in 2017 to shrubs burned in 2018. I measured leaf level gas exchange (A_{max} , E , g_s), intrinsic WUE, $\delta^{13}C$, and various leaf traits (RWC, LMA, LDMC) to assess differences between resprouts and shrubs 1-year post-fire. Leaf physiology remained relatively stable across the growing season even during drought. Little variation in leaf physiology emphasizes the importance of the deep root systems providing a consistent water source to buffer shrubs from environmental variability (Nippert et al. 2013). In response to fire, resprouts had higher photosynthetic and transpiration rates and photosynthetic nitrogen use efficiency across the growing season. Higher transpiration rates in resprouts resulted in reduced intrinsic water use efficiency at the beginning of the season. Differences between burn year treatments for all measurements decreased across the growing season. These results correspond with differences in leaf structure between burn year treatments. Resprouts had lower LMA with

higher %N compared to shrubs burned in 2017. LMA is typically negatively correlated with photosynthetic rate, leaf N concentration, protein content, and PNUE (Poorter et al. 2009). These data indicate *C. drummondii* changes leaf structure in response to fire in order to maximize C gain to accelerate growth.

The shifts in ecosystem structure and function caused by woody expansion has contributed to a major loss of tallgrass prairie (Ratajczak et al. 2016). Understanding the demographic and physiological mechanisms in response to disturbance is essential for predicting woody cover in the future. This research contributed to a demographic and physiological understanding of the mechanisms that contribute to the increased cover of *C. drummondii* in tallgrass prairie.

References

- Anadón, J. D., Sala, O. E., Turner, B. L., & Bennett, E. M. (2014). Effect of woody-plant encroachment on livestock production in North and South America. *Proceedings of the National Academy of Sciences*, 111(35), 12948–12953.
- Barger, N. N., Archer, S. R., Campbell, J. L., Huang, C., Morton, J. A., & Knapp, A. K. (2011). Woody plant proliferation in North American drylands: A synthesis of impacts on ecosystem carbon balance. *Journal of Geophysical Research*, 116.
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. L., Hoch, G. A., Lett, M. S., & McCarron, J. K. (2005). An Ecosystem in Transition: Causes and Consequences of the Conversion of Mesic Grassland to Shrubland. *BioScience*, 55(3), 243.
- Daubenmire, R. (1968). Ecology of fire in grasslands. *Advances in Ecological Research*, 5, 209-266.
- Eldridge, D. J., Bowker, M. A., Maestre, F. T., Roger, E., Reynolds, J. F., & Whitford, W. G. (2011). Impacts of shrub encroachment on ecosystem structure and functioning: Towards a global synthesis. *Ecology Letters*, 14(7), 709–722.
- Lett, M. S., & Knapp, A. K. (2003). Consequences of shrub expansion in mesic grassland: Resource alterations and graminoid responses. *Journal of Vegetation Science*, 14(4), 487–496.

- McKinley, D. C., & Blair, J. M. (2008). Woody Plant Encroachment by *Juniperus virginiana* in a Mesic Native Grassland Promotes Rapid Carbon and Nitrogen Accrual. *Ecosystems*, 11(3), 454–468.
- Nippert, J. B., Ocheltree, T. W., Orozco, G. L., Ratajczak, Z., Ling, B., & Skibbe, A. M. (2013). Evidence of Physiological Decoupling from Grassland Ecosystem Drivers by an Encroaching Woody Shrub. *PLoS ONE*, 8(12), e81630.
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytologist*, 182(3), 565–588.
- Ratajczak, Z., Briggs, J.M, Goodin, D.G., Luo, L., Mohler, R.L., Nippert, J.B., & Obermeyer, B. (2016). Assessing the potential for transitions from tallgrass prairie to woodlands: Are we operating beyond critical fire thresholds? *Rangeland Ecology & Management*, 69(4), 280-287.
- Ratajczak, Z., Nippert, J. B., & Collins, S. L. (2012). Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology*, 93(4), 697–703.
- Van Auken, O. W. (2009). Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management*, 90(10), 2931–2942.