

Resource-use strategies of woody plants in grassy ecosystems

by

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B.S., Bethel College, Kansas 2016
M.S., Kansas State University, 2019

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Grasslands and savannas contain diverse assemblages of herbaceous and woody species that contribute to ecosystem processes and maintenance of vegetation diversity and structural heterogeneity. The balance between these co-existing functional groups is maintained by fire, herbivory, and rainfall distribution. Woody species in grasslands and savannas are structurally and physiologically diverse and use a variety of strategies to establish and survive in these high-disturbance ecosystems. These strategies include deep root systems to mitigate drought stress, high belowground carbohydrate storage to resprout after disturbance, and dense canopies to shade out highly competitive grasses. Changes in climate and land management over the last century have favored woody plants and led to the rapid expansion of woody species into grassy ecosystems across the world in a process known as woody encroachment. In this dissertation, I used a broad suite of studies to assess the strategies used by trees and shrubs to grow and persist in lowveld savanna (Limpopo province, South Africa) and tallgrass prairie (eastern KS, USA).

In the first half of my dissertation, I used an *in situ* irrigation manipulation experiment in northeastern South Africa to (1) assess how the intensity, frequency, and timing of irrigation impacts the growth of six common savanna tree species (Chapter 2) and (2) understand how variation in water availability affects belowground processes such as root productivity and water partitioning among savanna trees and grasses (Chapter 3). Irrigation was used to manipulate the frequency, magnitude, depth, and timing of soil water availability for savanna tree saplings and co-existing grasses. I found few differences in above and belowground tree growth in response to variation in irrigation, but interspecific differences in tree growth reflected their leaf physiology and tree saplings used deeper soil water than grasses. Our results suggest that an increase in

water availability benefits tree growth regardless of the frequency or intensity of rainfall and root niche separation between trees and grasses occurs early in sapling establishment.

In the second half of my dissertation, I focused directly on the expansion of woody plants in grassy ecosystems. First, I explored the impacts of repeated shrub removal on community responses and shrub physiology in lowveld savanna (Chapter 4). Stems of the dominant woody species, *Colophospermum mopane*, were repeatedly cleared for 7 years in a semi-arid savanna. I found that 6-7 years of repeated clearing was necessary to induce large-scale shrub mortality. In addition, areas with reduced shrub cover had increased soil water availability, greater grass biomass, and attracted more herbivores compared to areas that were not cleared, suggesting dense woody vegetation reduces herbaceous biomass and alters ecosystem hydrology in this system. In my last study, I characterized the physiological traits and growth strategies of the most abundant woody encroaching species in tallgrass prairie (Chapter 5). I found that encroaching species encompass a spectrum of growth forms and leaf physiology, and two of the most abundant species fell at opposite ends of this spectrum. These results suggest niche complementarity promotes the encroachment of several woody species in tallgrass prairie. Together, my work highlights the diverse strategies used by woody plants to establish and persist in disturbance-prone grassy systems and the mechanisms that underlie the encroachment of the most abundant woody species.

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Approved by:

Major Professor
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Abstract

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Dedication

To my husband, Ryker.

Chapter 1 - Introduction

Grasslands and savannas are ancient ecosystems that contain diverse assemblages of herbaceous and woody species (Edwards et al., 2010; Stevens et al., 2022). These grass-dominated systems are often referred to as “open ecosystems” because they are composed of shade-intolerant species and rely on climate variability and frequent disturbance to maintain an open canopy (Bond, 2021). Positive feedbacks among grasses, fire, and herbivores maintain grassland structure and biodiversity by promoting grass growth while limiting the establishment and growth of woody plants (Bond, 2005; Lehmann et al., 2014; Charles-Dominique et al., 2016). Early biogeographers overlooked the importance of disturbance in these systems and classified biomes based on climate alone. However, many grasslands and savannas exist in regions that can support a closed canopy, leading to a mismatch between climate and vegetation structure (Whittaker 1970; Bond, 2021). Consequently, there has been a long-standing debate on the importance of top-down (fire and herbivory) vs. bottom-up (water and nutrients) processes in driving tree-grass coexistence in open ecosystems. More recently, scientists have acknowledged the interactive role of these processes (Staver et al., 2017; Holdo and Nippert, 2023), but the mechanisms driving plant responses to changes in these drivers remain a central focus of grassland and savanna ecology.

The top-down processes that shape vegetation structure are ultimately regulated by rainfall seasonality and variability. Vegetation growth in grassy ecosystems is highly seasonal (Lehmann et al., 2011). Dry or dormant seasons of 4-9 months determine the timing of plant growth and senescence both directly via water limitation (and temperature limitation in temperate systems) and indirectly by the curing of grasses that fuel fire (Lehmann et al., 2011; Archibald et al., 2019). Even herbivore impacts on savanna vegetation can be mediated by

rainfall variability as rainfall influences forage quantity and quality and the distribution of herbivores across precipitation gradients (Hempson et al., 2015; Staver et al., 2021). This environmental stochasticity causes tree cover to be highly variable in grassy ecosystems (Staver et al., 2011; Lehmann et al., 2014).

Maximum tree cover tends to increase with mean annual rainfall (MAR; Sankaran et al., 2005), but tree cover in areas with similar MAR can be highly variable due to differences in soil type, fire regimes, and rainfall distribution (Staver et al., 2017; D'Onofrio et al., 2019). Rainfall variability is a key regulator of soil hydrology and tree and grass cover across precipitation gradients (Synodinos et al., 2018; D'Onofrio et al., 2015). Savanna trees are long-lived and reliant on years with sufficient rainfall to overcome demographic bottlenecks due to fire, herbivory, and drought (Chesson, 2000, 2004; Higgins et al., 2000). High intra- and interannual rainfall variability makes predicting tree cover across savannas difficult as rainfall intensity, frequency, and growing season length can co-vary with changes in mean annual rainfall. Predicting vegetation responses to climate change will be exceedingly difficult without a mechanistic understanding of how precipitation frequency and intensity shape vegetation structure and savanna tree-grass competition.

Differences in tree and grass responses to precipitation variability are often attributed to differences in rooting depths and water-use strategies between these functional groups (Xu et al., 2015; D'Onofrio et al., 2019). Grasses are known to be intense competitors for water and nutrients in surface soils (Nippert et al., 2012) and can suppress tree growth even in well-watered conditions (February et al., 2013; Tomlinson et al., 2019). In contrast, woody plants have access to deeper soil water beyond the grass rooting zone, allowing them to avoid surface soil water deficits and competition with grasses (Schenk and Jackson, 2002; O'Keefe and Nippert, 2017;

Case et al., 2020a). Different water-use strategies among these functional types can alter vegetation responses to changes in precipitation regimes. For example, grasses are dominant competitors in semi-arid regions characterized by rainfall pulses (Sala et al., 1992). However, they are sensitive to changes in rainfall variability, as extended dry periods can reduce grass productivity, even if MAR does not change (Fay et al., 2002, 2003). Many grassy ecosystems are expected to experience more intense rainfall events with climate change (Westra et al., 2014 IPCC, 2022). Rainfall intensification increases water infiltration in well-drained soils, benefiting deep-rooted trees (Kulmatiski and Beard, 2013; Gherardi and Sala, 2015).

Woody species in grassy ecosystems are often analyzed as one functional group to generalize the responses of trees and grasses to climate variability and disturbance. However, woody species in grasslands and savannas are structurally and physiologically diverse and use various strategies to circumvent the challenges of frequent disturbance and water deficits. These strategies include deep root systems to mitigate drought stress (Zhou et al., 2020), thick bark and belowground structures to protect their buds (Hoffman et al., 2012; Charles-Dominique et al., 2015), and high carbohydrate storage to resprout after disturbance (Wigley et al., 2019; O'Connor et al., 2020). While many common traits facilitate the persistence of woody plants in disturbance-prone grassy ecosystems, interspecific differences among woody species can help explain variable responses to changes in fire, herbivory, and rainfall regimes. For example, trees with aggressive water-use strategies tend to have higher relative growth rates but are more sensitive to drought (Case et al., 2020b; Wargowsky et al., 2021). Ultimately, the resource-use strategies used by woody species will determine how species respond to changes in precipitation variability and land use, which could cause shifts in vegetation structure and community composition.

While woody plants are a natural component of many grassy ecosystems, grasslands and savannas around the world are experiencing an increase in the cover and abundance of woody plants – a process known as woody encroachment (Briggs et al., 2005; Archer et al., 2017; Stevens et al., 2017). Increased woody plant cover has converted open ecosystems into closed canopy shrublands and woodlands at the expense of the dominant herbaceous species (Ratajczak et al., 2012; Wieczorkowski et al., 2022). At local scales, fire frequency and grazing are the primary drivers of woody encroachment but increases in atmospheric [CO₂] and shifts in regional rainfall regimes have facilitated woody growth in grassy ecosystems worldwide. Woody encroachment has caused reduced grassland biodiversity, altered ecosystem water and carbon cycling, and billions of dollars in lost revenue for the cattle ranching industry (Archer & Predick, 2014; Morford et al., 2022). Once established, encroaching woody species are exceptionally difficult to manage because they are well equipped to deal with disturbance and alter the positive feedbacks that maintain an open ecosystem state. For example, in the tallgrass prairie of the Central Great Plains (USA), encroachment by clonal shrubs shade out herbaceous species and reduce fire intensity, further promoting their growth (Ratajczak et al., 2011). Differentiating growth and resource-use strategies among dominant encroaching species is necessary to predict woody encroachment in response to global change and develop effective management strategies to combat encroachment.

In this dissertation, I used a broad suite of studies to assess the strategies trees and shrubs use to grow and persist in lowveld savanna (Limpopo province, South Africa) and tallgrass prairie (eastern KS, USA). First, I used an *in situ* irrigation manipulation experiment to (1) assess how the intensity, frequency, and timing of irrigation impacts the growth of six common savanna tree species (Chapter 2) and (2) understand how variation in water availability affects

belowground processes such as root productivity and water partitioning among trees and grasses (Chapter 3). I then focused directly on woody encroachment by exploring the impacts of repeated shrub removal on community responses and shrub physiology in lowveld savanna (Chapter 4). Finally, I characterized the physiological traits and growth strategies of the most abundant woody encroaching species in tallgrass prairie to understand if the most rapidly encroaching species have unique carbon and water-use strategies compared to more slowly encroaching species (Chapter 5).

In **Chapter 2**, I utilized an *in situ* irrigation manipulation experiment to assess how savanna tree saplings respond to rainfall frequency, intensity, and seasonality at Wits Rural Facility, Limpopo, South Africa. In this experiment, irrigation was applied in either small, frequent pulses or large, infrequent pulses during the growing season (October – April). In a different treatment, irrigation was applied at 30 cm soil depth using buried irrigation lines to isolate the effects of deep soil water availability on tree growth. Finally, water availability was added during the dry season to assess the effects of rainfall seasonality on tree growth. I predicted trees would have the largest growth response to treatments that increase deep soil water (50 cm). Increases in deep soil water should be highest when irrigation is applied in large pulses and at 30 cm soil depth. I also predicted species would differ in their responses to irrigation, where faster-growing species with water-spending strategies will have greater responses to irrigation than slower-growing species. Tree leaf-level physiology (e.g., stomatal conductance and integrated water-use efficiency) should reflect interspecific growth differences among species.

In **Chapter 3**, I explored the belowground responses of savanna trees and grasses to irrigation manipulation using the same experimental design as Chapter 2. I used stable water

isotopes to measure depth of water uptake for tree saplings and grasses in the experimental plots. I focused on two tree species, *Acacia [Senegalia] nigrescens* and *Colophospermum mopane*, because these two species had the most divergent growth rates and leaf physiology (Chapter 2). I also used root ingrowth cores to assess the effects of rainfall frequency and intensity of belowground root productivity at 0 - 15 and 15 - 30 cm soil depths. I asked: (1) Do tree saplings consistently use deeper soil water than grasses and does depth of water uptake vary among tree species, and (2) How do rainfall intensity and frequency affect grass biomass, belowground productivity and nitrogen availability?

In **Chapter 4**, I present data from a 7-year tree clearing experiment in Mthimkhulu Game Reserve bordering Kruger National Park, South Africa. *Colophospermum mopane* stems and resprouting shoots were basally cut 2-3 times per year (2015-2022) in 3 pairs of treatment and control plots of 60 x 60 m. Permanent transects were used to monitor changes in grass cover and biomass, and herbivore activity via dung counts. Buried soil moisture probes were used to log changes in volumetric water content. I assessed the physiological responses of *C. mopane* to repeated cutting using non-structural carbohydrates and stable water isotopes to infer changes to energy storage and functional rooting depth, respectively. I predicted (1) repeated clearing would increase grass biomass, due to reduced competition between *C. mopane* and grasses, and subsequently increase grazer abundance due to increased forage and (2) repeated tree clearing would deplete belowground NSC storage, reducing the energy available for resprouting and shifting water uptake to shallow soils due to reduced investment in deep roots.

In **Chapter 5**, I characterized the growth and resource-use strategies of the most abundant encroaching woody species in tallgrass prairie. Over the past 40 years, Konza Prairie Biological Station (KPBS; Manhattan, KS) has undergone significant encroachment by several

woody species, but only three clonal shrubs have become dominant species across the landscape (Briggs et al., 2005; Ratajczak et al., 2014). The primary goal of this project was to identify whether clonal shrubs encroaching in tallgrass prairie have common physiological and functional traits or unique traits that differentiate species' carbon and water-use strategies. I measured a suite of carbon capture and drought tolerance traits for six encroaching clonal shrub species and one encroaching tree species at KPBS. I first used a 34-year dataset (PVC02; Hartnett et al., 2023) to assess the abundance of the most common encroaching species at KPBS. I then used allometry data to summarize leaf:stem mass ratios and leaf area of the focal woody species to understand aboveground carbon allocation. Finally, I compared traits related to gas exchange and drought tolerance among species to determine if the most abundant encroaching species have unique carbon and water-use strategies compared to more slowly encroaching species. Together, the chapters in this dissertation highlight the diverse strategies used by woody plants to establish and persist in disturbance-prone grassy ecosystems.

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Chapter 2 - Impacts of the amount, timing, and depth of water availability on savanna tree growth

Introduction

Water availability is the primary determinant of maximum tree cover in tropical savannas, systems co-dominated by a continuous layer of grasses and a discontinuous layer of trees (Sankaran et al., 2005; Lehmann et al., 2014; Xu et al., 2018). Savanna tree cover tends to increase with mean annual rainfall (MAR), where tree cover is primarily limited by water availability in semi-arid savannas ($\text{MAR} < 650 \text{ mm yr}^{-1}$) and fire frequency in humid savannas ($\text{MAR} > 650 \text{ mm yr}^{-1}$; Sankaran et al., 2005; Staver et al., 2011a). However, within a given MAR, tree cover can vary substantially across landscapes (Sankaran et al., 2005; Staver et al., 2011b; Lehmann et al., 2014). While the mechanisms driving variability in tree cover due to differences in fire regimes are well recognized (Higgins et al., 2000; Staver et al., 2011a; Archibald et al., 2019), variability in tree cover due to differences in rainfall regimes is poorly understood. Rainfall seasonality and stochasticity are known drivers of savanna tree cover across rainfall gradients and are often incorporated into models predicting tree-grass coexistence (Higgins et al., 2000; Van Wijk and Rodriguez-Iturbe, 2002; D'Onofrio et al., 2015; Xu et al., 2015), yet we lack a mechanistic understanding of the effects of variable rainfall regimes on tree cover. Given that rainfall events are expected to become less frequent and more intense in African savannas (IPCC, 2022), it is necessary to develop a mechanistic understanding of how savanna trees respond to rainfall intensity, intermittency, and seasonality.

African savannas occur in areas with high rainfall seasonality with dry seasons between 4-9 months that drive the timing of plant growth and senescence. Dry seasons limit tree growth directly via water limitation and indirectly by facilitating the curing of grass fuel loads that

support fire (Lehmann et al., 2011). In regions with higher MAR, longer growing seasons may contribute to increased tree cover because trees have more growth days and can potentially increase stores of carbohydrates and nutrients carried into the next growing season (Scholes and Archer, 1997). Additionally, trees are photosynthetically active for longer portions of the year than grasses, which reduces competition with grasses at the beginning and end of the growing season (Higgins et al., 2011; Ryan et al., 2017). While rainfall seasonality is generally predictable in savannas, the amount and timing of rainfall during the growing season can vary substantially within and among years. High intra- and interannual rainfall variability makes predicting tree cover across savannas difficult, and the effects of rainfall intensity and intermittency on tree cover are debated (Kulmatisk and Beard, 2013; Xu et al., 2015).

The two-layer model suggests deep-rooted trees benefit from rainfall regimes that increase deep soil water availability below the highly competitive grass rooting zone (Walker and Noy-Meir 1985). This model has been supported by experimental work showing that greater rainfall intensity increases deep soil water availability and tree growth (Kulmatisk and Beard, 2013; Berry and Kulmatiski, 2017). However, across large spatial scales, tree cover is lower in savannas with more intense but less frequent rainfall events (Good and Caylor, 2011; Xu et al., 2018). These contrasting results are likely due to complex interactions with soil type and rainfall intermittency. For example, while intense rainfall events may lead to rapid infiltration of water in sandy soils, the same rainfall events may lead to higher runoff on clayey soils if infiltration rates are exceeded (Hillel 1980; Case et al., 2018). Additionally, increased rainfall intensity is often associated with increased rainfall intermittency, and tree growth is limited by extended dry periods between rainfall events (Xu et al., 2015). This limitation is exacerbated by competition

with grasses, which are considered superior competitors for water in surface soils and can directly limit tree growth (Riginos, 2008; February et al., 2013).

In systems with stochastic disturbance events, like savannas, trees rely on years with sufficient rainfall for establishment and survival during the dry season (Higgins et al., 2000; Gignoux et al., 2009). Trees also face recruitment bottlenecks due to fire and browsing that increase sapling mortality or dieback (Sankaran et al., 2013; Holdo et al., 2014; Charles-Dominique et al., 2016; Staver et al., 2017). Temporary release from fire, browsing, and drought can induce large tree establishment and recruitment events, leading to long-lasting impacts on savanna community structure and ecosystem dynamics (Higgins et al., 2000; Staver and Bond, 2014). Consequently, changes in rainfall that favor tree growth may accelerate tree growth rates and transition into disturbance-resistant size classes, where adult individuals can persist for many decades. Thus, tree cover may vary across spatial gradients as a function of how stochastic events, including rainfall intensity and frequency, affect sapling growth.

Tree growth responses to rainfall variability will likely reflect interspecific resource-use strategies, which are determined by species adaptations to disturbance and climate, including rainfall intermittency and seasonality (Charles-Dominique et al., 2015; Archibald et al., 2021). Whether trees favor fast growth or resource conservation varies among species and is regulated by hydraulic architecture, stomatal regulation, and carbon allocation above vs. belowground (McDowell et al., 2008; Oliveira et al., 2021). Fast-growing species are resource-spenders and capitalize on increases in water availability to prioritize growing quickly above the highly competitive grass layer (Cory et al., 2022). In contrast, species from drier climate envelopes may grow slower and favor hydraulic safety (Oliveira et al., 2021; Wargowsky et al., 2023). Regardless of tree growth strategy, all savanna tree seedlings must invest in sufficient root

biomass and carbohydrate stores to survive the dry season and resprout after disturbance (Gignoux et al., 2009; O'Brian et al., 2014), and trees should benefit from greater deep soil water availability below the grass rooting zone (> 30 cm soil depth).

The primary objective of this study was to assess how the amount, frequency, and depth of water availability impacts the growth of common savanna tree species. We used an *in situ* irrigation experiment with five treatments to manipulate the frequency, intensity, depth, and timing of irrigation in a semi-arid savanna at Wits Rural Facility, South Africa. To assess the impacts of rainfall frequency vs. intensity on tree growth, we applied either small, frequent pulses or large, infrequent pulses of water to the soil surface during the growing season (October - April). In a third treatment, we isolated the effects of deep soil water availability by frequently irrigating at 30 cm soil depth using buried irrigation lines. Finally, we extended water availability into the dry season by irrigating from May – July. These treatments allowed us to assess the impacts of rainfall frequency, intensity, and timing within a single location and across multiple savanna tree species. Saplings of six common savanna tree species (*Acacia [Senegalia] nigrescens*, *Colophospermum mopane*, *Dichrostachys cinerea*, *Philenoptera violacea*, *Sclerocarya birrea*, and *Terminalia sericea*) were planted in each plot in 2017 (n = 25 plots). Grasses were seeded into the plots in 2018, and irrigation treatments began in 2020. We measured tree growth, stomatal conductance, and integrated water-use efficiency ($\delta^{13}\text{C}$) of the savanna tree species in response to each treatment. We predicted:

1. Trees would have the largest growth response to treatments that increase deep soil water at 50 cm. Increases in deep soil water should be highest when irrigation is applied in large pulses (pulse treatment) and at 30 cm soil depth (depth treatment).

2. Species would differ in their responses to irrigation, where faster-growing species with water-spending strategies will have greater responses to irrigation than slower-growing species. Tree leaf-level physiology (e.g., stomatal conductance and integrated water-use efficiency) should reflect interspecific growth differences and responses to the irrigation treatments.

Methods

Site description and experimental design

Wits Rural Facility – The experiment was established at Wits Rural Facility (WRF) in Limpopo Province, South Africa (-24°50'S, 31°09'E). WRF is a 350-ha lowveld savanna research site owned and managed by the University of Witwatersrand and located near the Kruger National Park Orpen Gate border. On average, the site receives around 680 mm of rainfall, most of which falls from October – April. The site is dominated by trees in the Combretaceae (genera *Terminalia* and *Combretum*) and Mimosoideae (genera *Sengalia*, *Vachellia*, and *Dichrostachys*) families. All vegetation was cleared, and rootstocks were removed before establishing the experimental plots. The area was fenced to prevent grazing and browsing by herbivores.

Irrigation experiment – In 2015, twenty-five 3 x 4.2 m plots were established using low-density polyethylene pipe drip irrigation lines. Within each plot, seven irrigation lines were placed 0.5 m apart at the soil surface or 30 cm below the soil surface. Two CS-616 soil moisture sensors (Campbell Scientific, Logan, UT, USA) were installed at 10 and 50 cm soil depths in every plot to assess soil volumetric water content (VWC; July 2017 – April 2023). Plots were assigned to five blocks and randomly assigned to one of five irrigation treatments (Table 2.1; n =

5 replicates per treatment). Irrigation was gravity-fed from large JoJo tanks near the experiment and controlled by watering each treatment for a constant amount of time on each watering day to achieve a total of ~350 mm of water per year. Each irrigation treatment received the same total amount of added water per year but was subject to different intensities, frequencies, or timing of watering events (Table 2.1). These treatments included increasing the frequency of irrigation either at the surface or 30 cm below the surface (surface and depth treatments), increasing the intensity of irrigation by irrigating heavily once per month (pulse treatment), or irrigating frequently during the dry season (season treatment). Watering of the surface and depth treatments occurred on the same days throughout the growing season (October - April).

In 2017, six savanna tree species (*Acacia [Senegalia] nigrescens*, *Colophospermum mopane*, *Dichrostachys cinerea*, *Philenoptera violacea*, *Sclerocarya birrea*, and *Terminalia sericea*) were germinated in pots at WRF and then transplanted into the experimental plots. Three saplings of each species were randomly planted within rows in each plot for a total of 18 saplings per plot spaced 0.5 m apart (450 saplings across the experiment; Fig. A.1). Grass seed of six common savanna grasses (*Aristida congesta*, *Eragrostis superba*, *Hyperthelia dissoluta*, *Panicum maximum*, *Pogonarthia squarrosa*, and *Perotis patens*) were randomly seeded in each plot in 2018 and clipped twice in 2019 to facilitate tree establishment. Irrigation treatments began in October 2020, giving nearly three years for soils to settle and tree seedlings to establish before treatments began. Tree seedlings that died before this time were replaced. For all analyses, we removed measurements from one plot with irrigation at depth because the irrigation was not working properly.

Tree growth

Tree height and diameter – To assess tree size and growth, we measured the height and basal diameter of each tree seedling at the end of each growing season from 2020-2023. Tree height was measured from the ground to the top of the apical bud. For species that branch and spread laterally (primarily *A. nigrescens*, *C. mopane*, and *D. cinerea*), we stretched the longest branch and measured the length from the ground to the meristem at the end of the branch. We rounded measurements for basal diameter to the nearest 0.5 mm and height to the nearest 0.1 cm. We calculated the change in height and diameter for each year. We excluded any trees that had a negative change in height that was > 2 cm or a negative change in diameter that was > 2 mm compared to the previous year. These individuals were likely resprouting trees that experienced dieback during the previous year.

Statistical analysis – For each individual, we averaged changes in height and diameter across all years. We used a linear mixed effects model to test if average growth for height and diameter differed among species and treatments using the *lme4* package in R (Bates et al., 2015). We included an interaction between treatment and species as fixed effects and Block as a random effect. Change in height and diameter were log transformed (+2) to meet the assumption of homogeneity of variance. We used the package *emmeans* (Lenth, 2019) to test pairwise comparisons among species and among treatments.

Modeled tree height

We modeled the tree height over time for each species in each irrigation treatment (following Wakeling et al., 2011 and Stevens et al., 2018). We used the growth equation from Higgins et al. (2000):

$$h = h_{y-1} + \left(1 - \frac{h_{y-1}}{h_{max}}\right) g_s$$

where h is the modeled tree height, h_{y-1} is the tree height in the previous year, h_{max} is the maximum tree height (10 m), and g_s is the growth rate of stems (cm yr^{-1}). The initial tree height was set to 15 cm, which was the mean height of all tree saplings across all irrigation treatments. To calculate g_s , we calculated the mean growth rate (cm yr^{-1}) of the fastest two individuals of each species in each treatment between 2021 and 2022 (Wakeling et al., 2011; Stevens et al., 2018). We used growth rates calculated from 2021-2022 because 2021 was the first year that the season treatment was applied by irrigating during the dry season (May – July). We ran the model for 50 years and compared the number of years it took for trees to reach 3 m height among treatments and species. Three meters is commonly used as a threshold height where trees escape top-kill by fire (Wakeling et al., 2011; Stevens et al., 2018).

Leaf physiology

Stomatal conductance – In 2022 and 2023, we measured stomatal conductance weekly from March-May using the Li-600 porometer (LI-COR Inc., Lincoln, NE, USA). We measured five tree species in each plot. We did not sample *D. cinerea* because the small leaflets do not fill the instrument chamber, making measurements unreliable. In 2022, we measured all plots on clear, sunny days between 800 and 1500 hours for eight sampling dates (March 15, 2022 – May 17, 2022). Measurements took place on irrigation days or the day after an irrigation event. In 2023, we measured g_{sw} once a week the morning after watering events (March 1, 2023 – April 28, 2023). Due to time constraints, we measured plots in the control, surface, and deep irrigation

plots every week and measured all plots every three weeks. This sampling resulted in 7 sampling dates for the control, surface, and deep irrigation treatments and four sampling dates for the pulse and season treatments. The season treatment is equivalent to the control until the start of the dry season (irrigated May – July) and the pulse treatment receives irrigation only once a month.

Carbon isotopes and foliar C:N – We collected 2-3 leaves from each tree species from three plots per treatment in 2022 and five plots per treatment in 2023. Leaves were dried at 60 °C for 72 hours and ground for carbon and nitrogen content and $\delta^{13}\text{C}$. Leaves were measured at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University using an Elementar vario Pyro cube coupled to an Elementar Vision mass spectrometer for isotope analysis. Isotopic abundance ratios were converted to δ notation using:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] * 1000$$

where R is the ratio of heavy to light isotopes for the sample and standard (Vienna-Pee-Dee Belemnite), respectively.

Statistical analysis – We analyzed 2022 and 2023 separately to test if g_{sw} increased with VWC at 10 cm soil depth. We used linear mixed effects models with VWC, species, and their interaction as fixed effects and treatment nested within day of year as a random effect. We used the `emtrends` function from the `emmeans` package to test if the slope of each species significantly increased with VWC. We then used pairwise comparisons to test if slopes significantly differed among species. To test for differences in average g_{sw} rates among treatments, we used linear mixed effects models with species, treatment, and their interaction as main effects and added day of year as a continuous predictor. We included tree ID nested within block as a random effect to account for repeated measures. We only tested for g_{sw} difference among treatments in 2023 because treatments were not sufficiently randomized throughout the day in 2022, which limited

our ability to compare g_{sw} across treatments. To test if $\delta^{13}C$ and foliar C:N differed among species and treatments, we included species, treatment, and their interaction as fixed effects and plot as a random effect for each year. We also tested the relationship between leaf %N and integrated water-use efficiency ($\delta^{13}C$) because foliar N concentrations are directly related to photosynthetic rates and can increase leaf water-use efficiency over time (Farquhar et al., 1989).

Results

Soil VWC

In general, irrigated plots had higher VWC than control plots (Fig. 2.1; Table A.1). During the growing season, irrigation at 30 cm depth increased VWC at 50 cm by 50 – 100 % relative to the control (Table A.2; $p < 0.05$) but did not significantly affect VWC at 10 cm. All other irrigation treatments increased VWC at 10 cm and 50 cm soil depths, but average differences among treatments and the control plots were not always significant (Fig. 2.1). For most treatments, VWC at 50 cm exceeded VWC at 10 cm even in plots frequently irrigated at the surface.

Tree growth

There was no significant interaction between treatment and species, suggesting treatments had similar effects on all species (Table A.3). In general, irrigation increased tree growth regardless of the frequency, depth, intensity, or timing of irrigation (Fig. 2.2a). Within each species, there were few significant differences in tree growth among treatments, but *A. nigrescens* and *P. violacea* showed greater height growth in the surface treatment than the control (Fig. A.2). Tree growth varied significantly among species. On average, *A. nigrescens*,

had greater height growth ($16.0 \pm 5.9 \text{ cm yr}^{-1}$; $P < 0.05$) than all other species and *C. mopane* and *T. sericea* had the lowest growth (Fig. 2.2c; 2.9 ± 0.4 and $2.9 \pm 0.13 \text{ cm yr}^{-1}$, respectively).

Modeled tree growth

We modeled tree growth over time to understand the effects of irrigation treatments on maximum tree growth rates and the number of years to escape the fire trap (critical threshold of 3 m tall; Fig. 2.3). *A. nigrescens* reached the escape height the fastest, ranging from 14 years in the surface treatment to 27 years in the extended season treatment. In contrast, *C. mopane* only reached the escape height in the surface and control treatments within the modeled 50 years (19 and 23 years, respectively). Across all species, the surface treatment had the lowest average years to escape height of 31 years, with four of the six tree species reaching the escape height the fastest in this treatment (ranging from 14 – 20 years; Fig. 2.2a). Species did not always reach the threshold height in the same order for each treatment, but species with the fastest average growth rates (Fig. 2.2c) also had the highest maximum growth rates. Interestingly, *S. birrea* is known to be extremely deep-rooted (Zhou et al., 2020) and responded the most to depth treatment with an escape time of 15 years compared to 41 years in surface treatment.

Leaf physiology

Stomatal conductance – The relationship between g_{sw} and VWC varied by species (interaction $P < 0.001$; Table A.4). In 2022, g_{sw} of all tree species, except for *C. mopane*, increased significantly with VWC at 10 cm (Fig. 2.4). In 2023, slopes were lower and g_{sw} of *C. mopane* and *P. violacea* did not increase significantly with VWC. In both years, *C. mopane* had the lowest g_{sw} rates among the tree species and showed little responses to irrigation treatments.

A. nigrescens, the species with the greatest growth rates, had the highest g_{sw} rates among species and had a steeper slope than *C. mopane* (2022 and 2023) and *P. violacea* (2023). In 2023, average g_{sw} varied by species ($P < 0.001$) and treatment ($P = 0.017$; Table A.5). Surprisingly, *A. nigrescens* was the only species with g_{sw} rates that responded significantly to irrigation, where g_{sw} in the surface and pulse treatments were greater than the control ($P = 0.02$ and 0.03 , respectively).

$\delta^{13}C$ and foliar C:N – $\delta^{13}C$ and foliar C:N varied significantly by species, but not irrigation treatment in 2022 and 2023 (Table A.6). Average differences in $\delta^{13}C$ among species ranged from -31 to -29 ‰ and did not reflect differences in average g_{sw} . For example, *C. mopane* and *A. nigrescens* had similar $\delta^{13}C$ values (-30.5 ‰ and -30 ‰, respectively), but different average g_{sw} (0.2 and 0.8, respectively). *S. birrea*, a species known to have a water spending strategy (Warzowsky et al., 2021) had the lowest $\delta^{13}C$. All species had significantly different foliar C:N, except for *A. nigrescens* and *D. cinerea*. We found a strong positive correlation between %N and $\delta^{13}C$ (Fig. 2.5; $R^2 = 0.24$; $P < 0.001$).

Discussion

Tree cover in savannas tends to increase with mean annual rainfall, but we lack an understanding of whether this increase is driven by rainfall frequency, intensity, deep water infiltration, or longer growing seasons associated with greater mean annual precipitation. In this study, we assessed the effects of irrigation intensity, frequency, and timing on the growth of savanna tree saplings using an *in situ* irrigation experiment in lowveld savanna. We then modeled tree growth over time to assess how precipitation regimes may influence the rate at which juvenile trees grow out of disturbance-sensitive size classes. Surprisingly, we found few

differences in tree growth rates or stomatal conductance among the irrigation treatments in the first three years of this experiment. These results suggest increases in water availability, regardless of the frequency or intensity of rainfall, benefit tree growth, at least in semi-arid savanna with well-drained soils. However, when we modeled tree height over time, we found trees experiencing frequent rainfall events (i.e., the surface treatment) had higher maximum growth rates and reached critical size thresholds (3 m) faster than all other treatments for 4 of the 6 tree species. In addition, we found growth differences among species reflected their leaf-level physiology, where faster growing species had higher stomatal conductance than more slowly growing species. These results highlight the various water-use strategies of savanna trees use and reflect the time required to grow out of disturbance-prone size classes.

Our prediction that savanna trees, regardless of species, would benefit from deep soil water availability was partially supported. There were few differences in tree growth among treatments for each species (Fig. A.2). However, when species were averaged together, trees in the irrigated plots had greater growth rates than trees in the control plots (Fig. 2.2a). Savanna trees are known use deeper soil water than grasses to mitigate drought stress and competition with grasses in the surface soil layers (Chapter 3; Holdo et al., 2018; Case et al., 2020). This niche partitioning among trees and grasses is particularly pronounced in sandy soils where water infiltrates deeper into the soil profile (Case et al., 2020). While tree growth in the irrigation at depth treatment were higher than the control, the depth treatment did not differ from the other irrigation treatments. The soils at the research site are sandy and have high infiltration rates ($K_{fs} = 239 \pm 52.3 \text{ mm h}^{-1}$, *unpublished data*). Lack of growth differences among irrigation treatments within or across species was likely due to an increase in soil VWC at 50 cm depth regardless of

where or how the water was applied. Trees may be benefitting from soil water at depths > 30 cm in all irrigation treatments.

Differences in growth among tree species reflected interspecific leaf-level water-use dynamics. Species that have fast growth rates are typically resource-spending species and are considered more competitive for water, nutrients, and light than species with more conservative water-use strategies (Grime, 1977; Poorter et al., 2007). The species with the greatest growth rate, *A. nigrescens*, also had the highest g_{sw} rates that increased with increases in soil VWC. These results are consistent with experiments that observed water-spending strategies in saplings of other *Acacia* species (genera *Vachellia*) from both arid and mesic African savannas (Cory et al., 2022). Additionally, both *A. nigrescens* and *D. cinerea*, the fastest growing species, are considered drought sensitive at both the juvenile and adult life stages, suggesting these species favor growth over hydraulic safety (Case et al., 2020; Trotter et al., 2022). High stomatal conductance coupled with high root hydraulic conductivity (Wargowsky et al., 2021) make water-spending species effective competitors for capitalizing on frequent pulses in rainfall and rapidly grow out of the vulnerable size classes (Cory et al., 2022).

In contrast, the slowest growing species *C. mopane*, had low g_{sw} that did not respond to changes in VWC. *C. mopane* is a dominant species across semi-arid savannas in Southern African and has traits associated with drought tolerance, such as small xylem vessels and low root hydraulic conductivity (Wargowsky et al., 2021). Despite the lack of g_{sw} response to VWC, *C. mopane* still had a similar $\delta^{13}C$ to species with high g_{sw} , suggesting *C. mopane* always maintains low rates of stomatal conductance. Our results reinforce hypotheses on drivers limiting the range of *C. mopane*. Species that favor hydraulic safety are inherently slow growing (Oliveira et al., 2021). In this study, the growth of *C. mopane* had limited response to the

irrigation treatments, suggesting inherently slow growth rates likely limits the survival of this species in wetter savannas that are characterized by high grass biomass and more frequent fire (Stevens et al., 2018).

We found a strong linear relationship between foliar %N and integrated water-use efficiency ($\delta^{13}\text{C}$) across species (Fig. 2.5). High leaf N is associated with a greater capacity to fix CO_2 since most leaf N is allocated toward photosynthetic machinery (Field, 1986; Evans, 1989). Increased photosynthetic rates can reduce intercellular CO_2 concentrations, driving plants to decrease g_{sw} and increase water-use efficiency (Farquhar et al., 2002; Prentice et al., 2014). In our study, the tree species with higher foliar %N were all N-fixing species. *A. nigrescens* and *D. cinerea* are known N-fixing species, while *P. violacea* is reported as a facultative N-fixing species (Jacobs et al., 2007). In addition, these N-fixing species tended to have higher growth rates than non-fixing species (Fig. 2.2c). These higher growth rates could be due to higher photosynthetic rates and water-use efficiency (Vergutz et al., 2012; Adams et al., 2016; Byternowicz et al., 2023). In addition, alleviating water stress may increase available C to support N-fixing symbionts.

While we did not find an interaction among species and irrigation treatments, we did find treatment differences when we modeled tree height over time. For most species, time to fire escape height was reduced across irrigation treatments relative to the control. Trees experiencing frequent rainfall events (i.e., surface treatment) grew out of the fire trap the fastest in four of six tree species. These results reflect higher maximum growth rates in the surface treatment likely driven by elevated soil moisture at shallow and deep soil depths with less frequent or extreme dry-down events (Fig. 2.1). Higher maximum growth rates in the surface treatment vs. the depth treatment also indicate that shallow tree roots are still functional when water is available

(Kulmatiski and Beard, 2013). In combination with elevated VWC at 50 cm across all irrigation treatments, our results suggest that grasses do not ‘get all the water all the time’ despite their highly competitive resource-use strategy (Holdo and Brocato, 2015). Given the trends in growth rates among species and treatments, we expect divergent responses among species will grow as the experiment continues.

In conclusion, our results show that savanna tree species have variable growth strategies and that responses to changes in rainfall regimes will likely be constrained by the physiology of the dominant woody species. Trees are often analyzed as a single functional type to generalize tree-grass competitive dynamics. However, incorporating interspecific differences in water-use strategies into our understanding of tree-grass dynamics provides more mechanistic insight into tree-grass competition and responses to rainfall variability across precipitation gradients (Ketter and Holdo 2018). Future work on whether these traits are phylogenetically conserved (Zhou et al., 2020; Archibald et al., 2021) could allow for generalizing patterns of dominant woody species across precipitation gradients.

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Table 2.1 Description of irrigation treatments. All irrigated treatments received a total of ~350 mm additional water over the irrigation period.

Treatment	Irrigation period	Irrigation location	Irrigation frequency	# of water events yr ⁻¹	Duration of event
control (ambient)	none	none	none	none	none
surface	Oct - April	soil surface	3x per week	90	23 minutes
depth	Oct - April	30 cm below soil surface	3x per week	90	23 minutes
pulse	Oct - April	soil surface	1x per month	7	5 hours
season	May - July	soil surface	3x per week	40	46 minutes

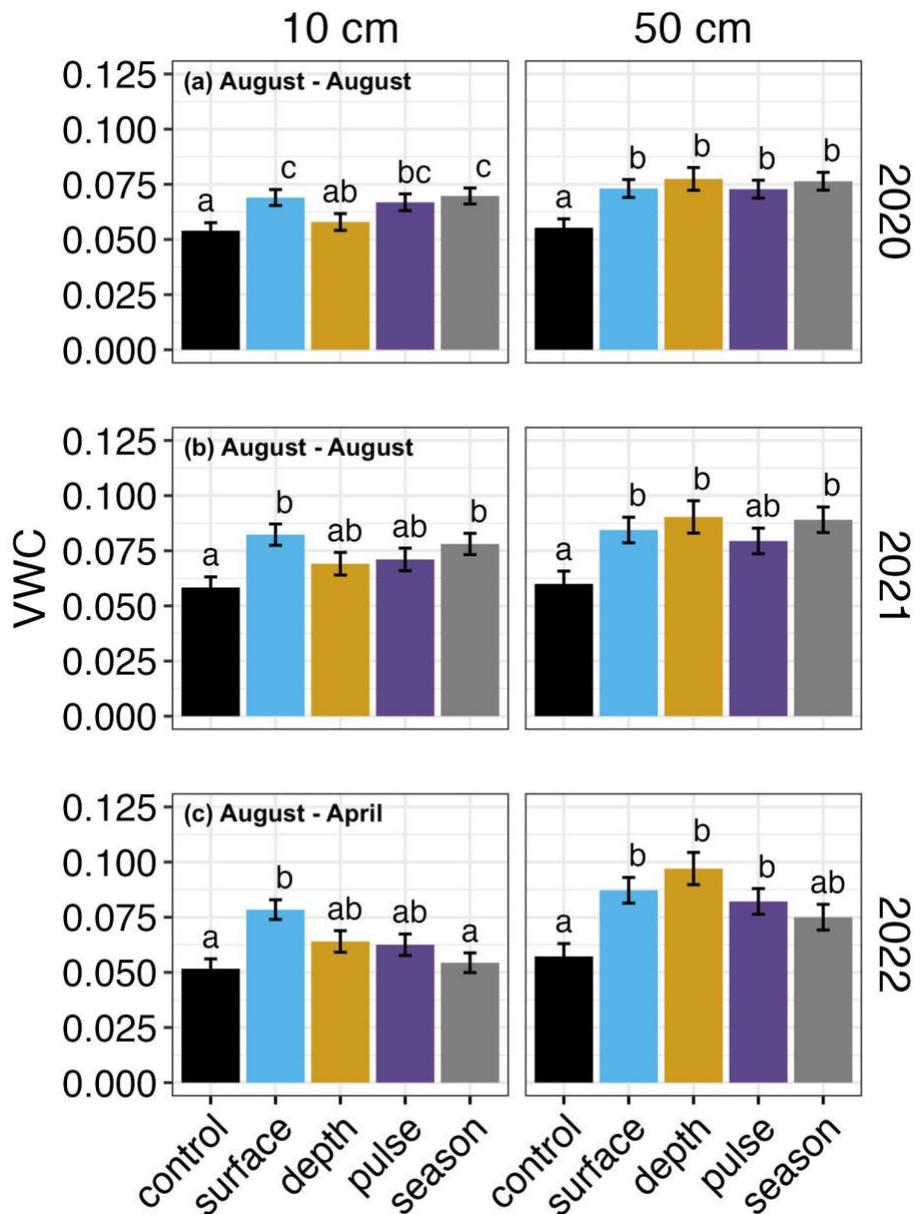


Figure 2.1 Average volumetric water content at 10 and 50 cm soil depths for hydrological years (a) 2020, (b) 2021, and (c) 2022. Notice 2022 is only calculated from August – April. Differences in VWC between the season and control treatments are likely underestimated in 2022 because of missing data from May – August 2023. Letters represent significant differences among treatments in each year at each soil depth ($P < 0.05$).

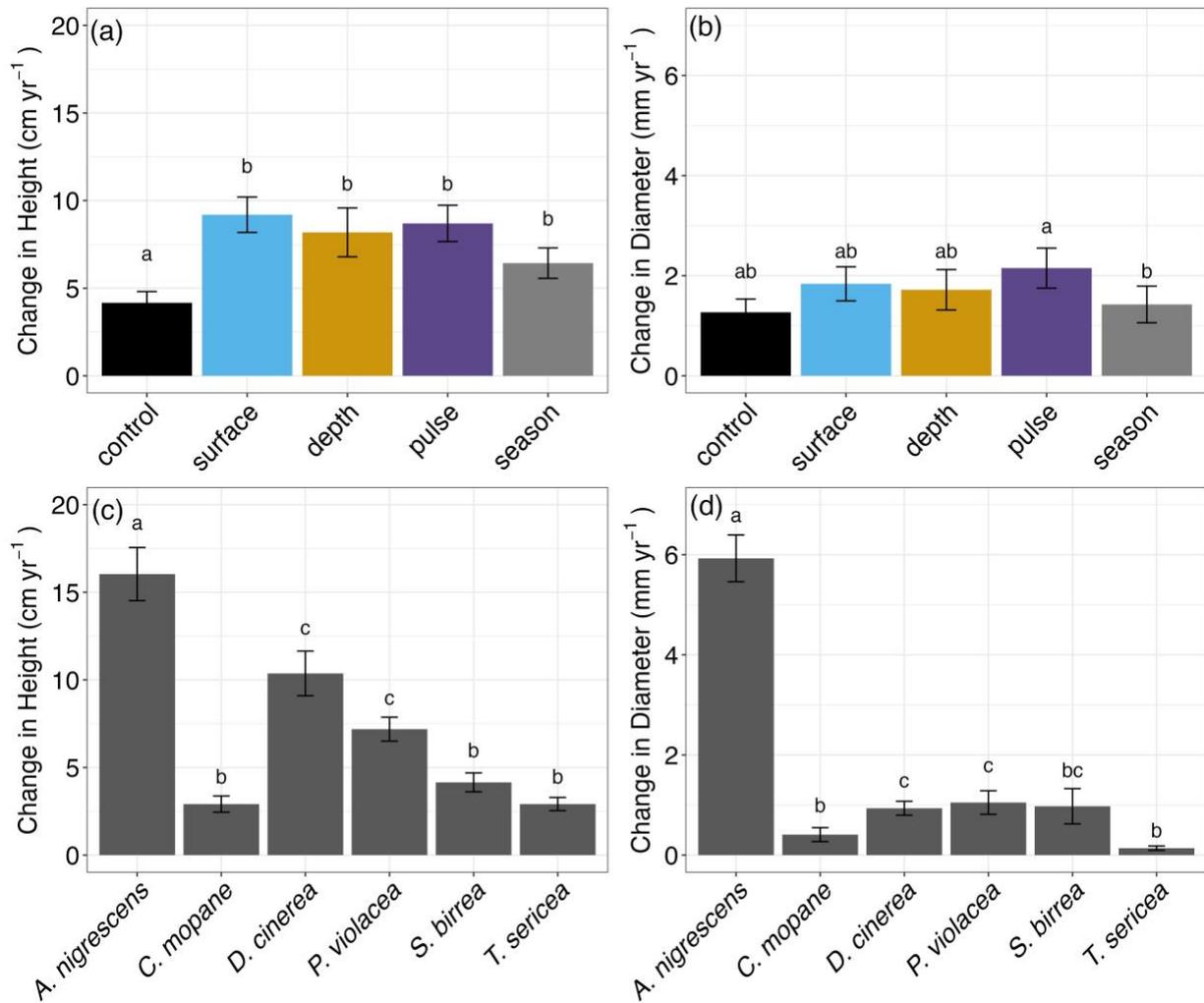


Figure 2.2 Change in height and diameter for each treatment (a and b) and each species (c and d). Bars represent mean \pm 1 SE. Values for each individual tree were calculated for each year (2020 – 2021, 2021 – 2022, and 2022 – 2023) and then averaged across years. Letters represent significant differences among each treatment or species ($P < 0.05$).

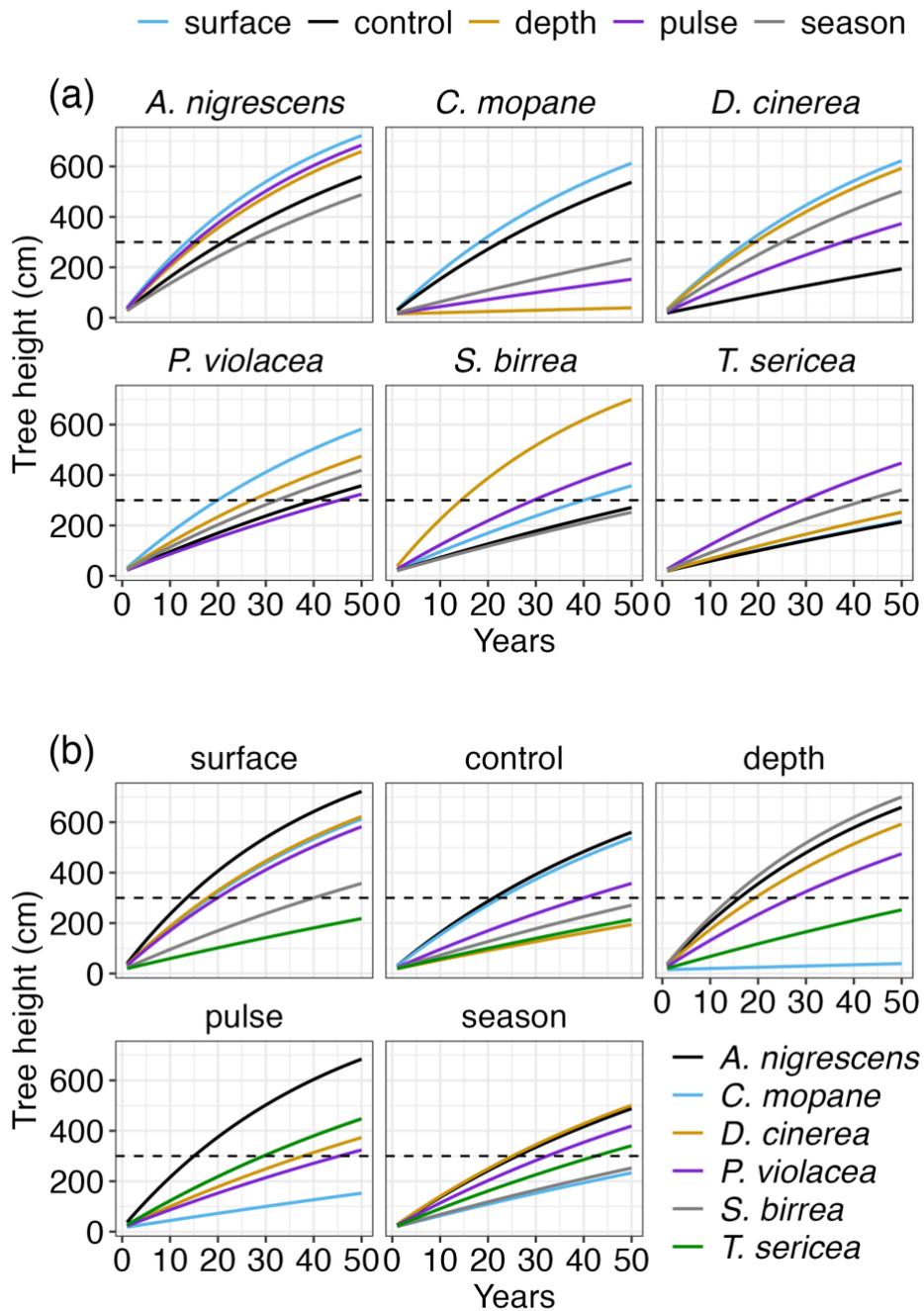


Figure 2.3 Modeled tree height over time for each species and treatment. Panel a compares modeled height over time among treatments for each species. Panel b compares modeled height over time among species within each treatment.

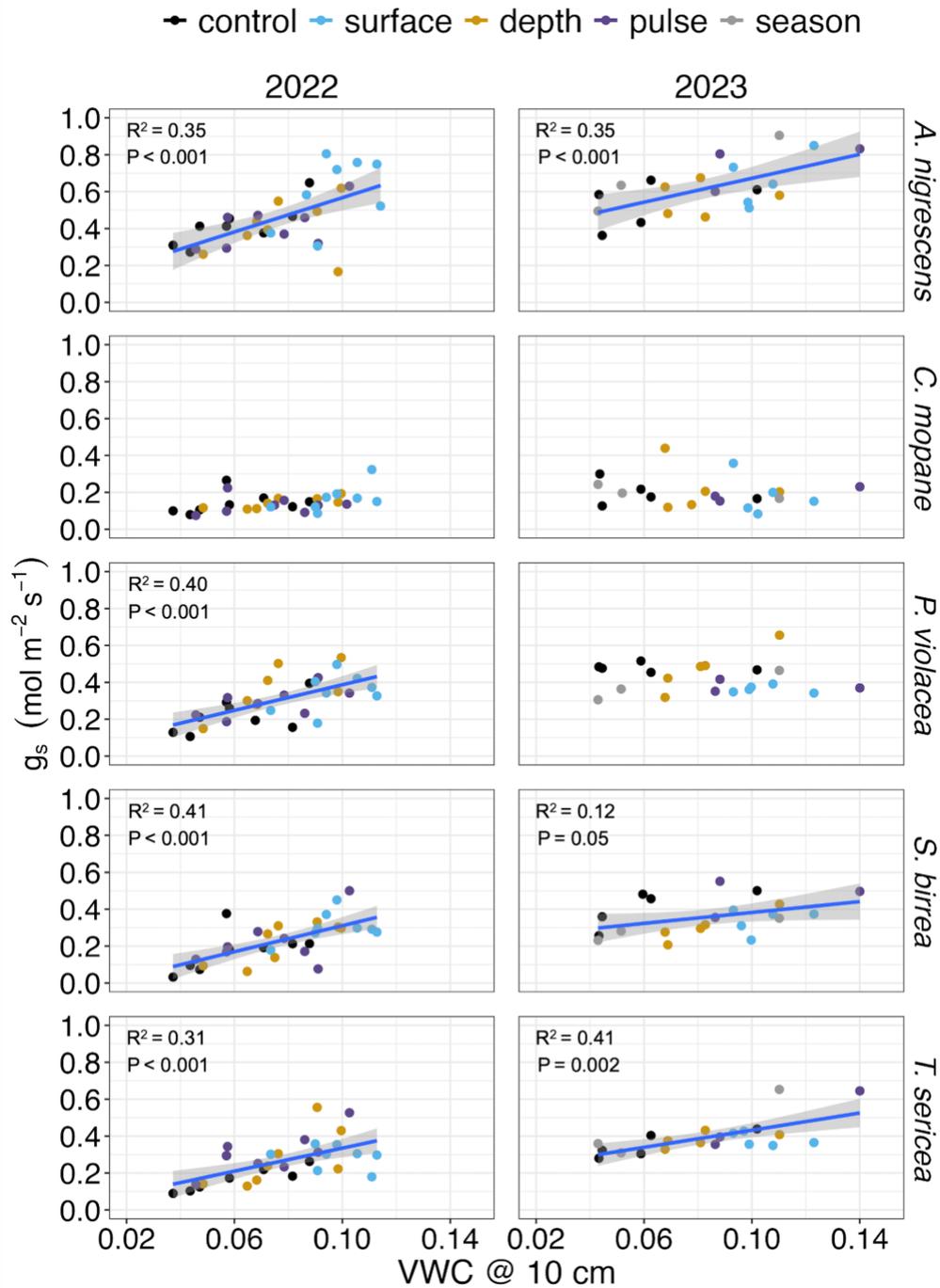


Figure 2.4 Relationship between soil volumetric water content at 10 cm and stomatal conductance for each species in 2022 and 2023. Points represent average g_{sw} for each treatment on each sampling date.

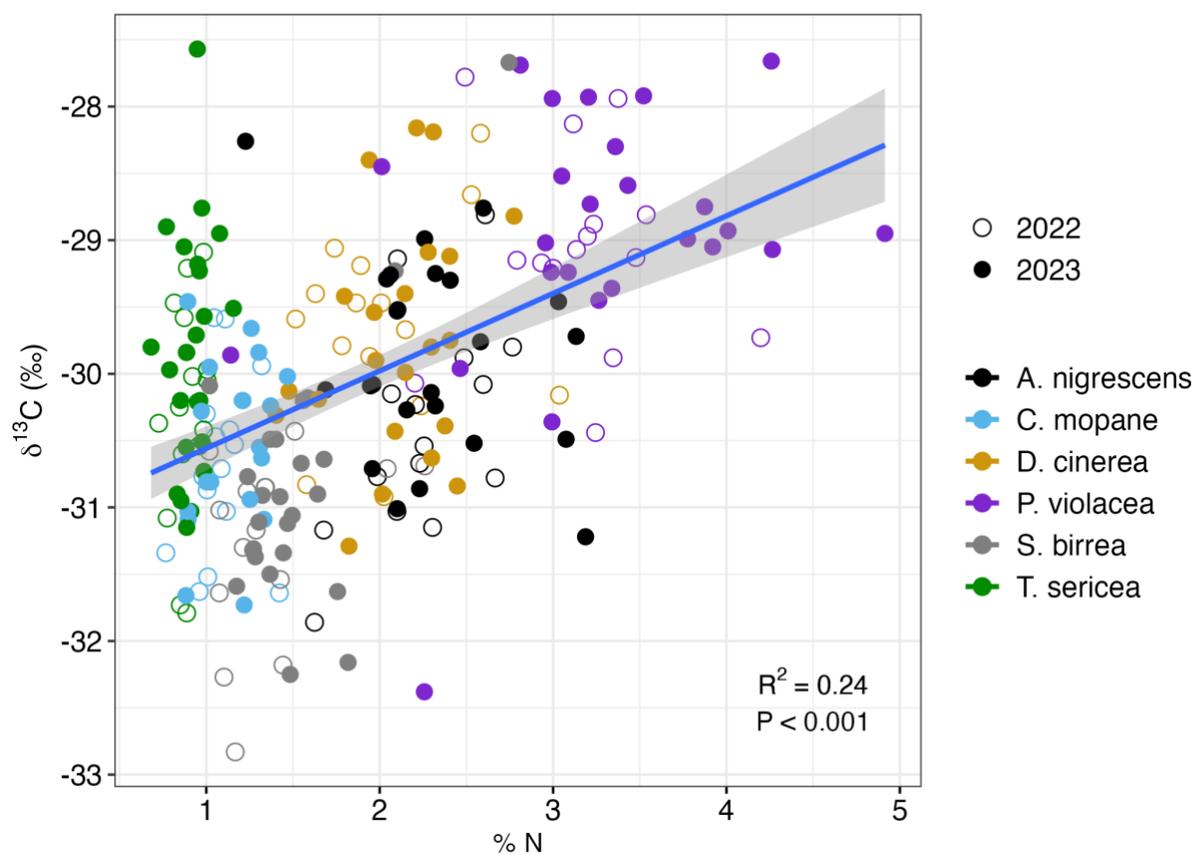


Figure 2.5 Relationship between foliar N (%) and $\delta^{13}\text{C}$ (‰). Points are individual trees sampled across irrigation treatments in 2022 (open) and 2023 (closed). Colors represent different species.

Chapter 3 - Effects of rainfall manipulation on belowground savanna dynamics

Introduction

The stable coexistence of trees and grasses is the defining characteristic of savanna ecosystems (Scholes and Archer, 1998; Sankaran et al., 2004). Water availability is a primary driver limiting productivity and tree cover in savannas (Rodríguez-Iturbe and D’Odorico, 1999; Bucini and Hanan, 2007). Belowground competition for water among trees and grasses shapes vegetation structure by limiting tree establishment and growth rates (Kraaij and Ward, 2006; February et al., 2013; Donaldson et al., 2022). Grasses are formidable competitors for resources in the surface soils and have dense root systems to quickly exploit pulses of water (Holdo and Bracato 2015; Belovitch et al., 2023). In contrast, trees have expansive root systems in shallow and deep soil layers, providing exclusive access to soil water beyond the grass rooting zone (Weltzin and McPherson 1997; Schenk and Jackson, 2002). Niche partitioning for water between these two functional types, known as Walter’s two-layer hypothesis, is one of the central explanations for savanna tree-grass coexistence (Walter, 1973; Walker and Noy-Meir, 1982; Holdo and Nippert, 2023). While many studies have shown that savanna trees use deeper soil water than grasses, there is growing recognition that tree-grass competition is more nuanced than complete separation of water uptake (Holdo and Nippert, 2023). For example, the degree of niche partitioning between trees and grasses varies by soil type, mean annual rainfall, and species (Holdo et al., 2018; Case et al., 2020). In addition, niche partitioning may occur over fine spatial scales (Holdo, 2013; Kulmatiski et al., 2020), and the depth of water uptake is likely plastic for some species based on water availability (Nippert and Knapp, 2007; Grellier et al., 2021).

Soil moisture availability depends on the frequency and intensity of rainfall events, which are notoriously variable in savanna systems (Good and Caylor, 2011; Lehmann et al., 2011; D’Onofrio et al., 2015). Moreover, rainfall patterns are expected to shift with climate change, where many savannas are predicted to experience increased precipitation intensity and variability (IPCC, 2022). The impact of altered rainfall regimes on tree and grass growth and competitive dynamics will depend on changes in shallow vs. deep soil moisture availability. In principle, small but frequent rainfall events should lead to chronically wet surface soils and less infiltration into deep soils, which should benefit highly competitive grasses (Schwinning and Sala, 2004; Griffin-Nolan et al., 2021). In this case, trees would need to have rapid resource acquisition strategies to compete with C₄ grasses in surface soils or invest in growth of deeper rooting systems that can access deep soil or groundwater (Nippert and Holdo, 2015). In contrast, rainfall intensification is often associated with increased intermittency, which can result in greater infiltration of water into the soil profile (Kulmatiski and Beard, 2013a), but also greater drying of surface soils between rain events (Fay et al. 2002, 2003; Knapp et al. 2008). These conditions benefit deep rooted trees (Kulmatiski and Beard, 2013a; Gherardi and Sala, 2015). In both scenarios, it would be advantageous for trees to shift their depth of water uptake from shallow to deeper soil layers as surface soils begin to dry (Kulmatiski and Beard, 2013b; Stahl et al., 2013).

Soil moisture also regulates nitrogen (N) availability through mineralization rates, leaching, and decomposition rates (Cui and Caldwell, 1997; Coetsee et al., 2012). Both C₄ grasses and C₃ trees are N-limited in savannas, and some studies have argued that the primary role of rainfall variability in savannas is to facilitate pulses in N availability (Scholes and Walker, 1993). Grasses have been shown to respond more to increases in N availability than trees, despite having lower N requirements and higher N-use efficiency (van der Waal et al.,

2009). Grass competition is suggested to increase under high resource availability, with N availability as a key regulator of savanna biomass (Sankaran et al., 2008; van der Waal et al., 2009; Cramer et al., 2007). However, over large landscape scales, nutrient availability co-varies with soil type, and tree cover does not correlate with soil nutrient concentrations (Sankaran et al., 2005).

Alteration to rainfall regimes can impact productivity and cause shifts in above vs. belowground biomass allocation, with implications for ecosystem-scale carbon cycling (Knapp et al., 2002; Wang et al., 2020). This is particularly important in grassland and savanna ecosystems that are dominated by C₄ grasses that allocate the majority of biomass belowground and substantially contribute to soil organic carbon stocks (Wigley et al. 2020; Zhou et al. 2023). Our understanding of aboveground responses to changes in rainfall patterns is substantially more complete than it is for belowground responses. For example, aboveground productivity is generally higher in wetter systems (Knapp and Smith, 2001), but more sensitive to changes in precipitation in drier ecosystems (Wilcox et al., 2017; Maurer et al., 2020). Changes in precipitation intensity or frequency can alter aboveground productivity even if mean annual rainfall does not change (Fay et al. 2002, 2003). For example, increased intermittency extends dry periods between rainfall events, reducing grass productivity (Fey et al., 2003). In contrast, belowground productivity has been shown to be insensitive to changes in water availability, particularly water additions, presumably because of increased carbon allocation to aboveground productivity (Wilcox et al., 2017; Zhang et al., 2021). While many studies have attempted to disentangle the effects of changing frequency, intensity, and magnitude of precipitation on aboveground plant productivity, the mechanisms behind those changes – which occur belowground – are not well understood.

In this study, I used a 3-year *in situ* irrigation experiment to assess the effects of water availability on root production, nitrogen availability, and depth of water uptake of tree saplings and co-existing grasses in a semi-arid savanna. This experiment focused on trees at the sapling stage because it represents a critical demographic bottleneck in savannas, as saplings face intense grass competition due to water and light limitation during establishment (Cramer et al., 2007; Morrison et al., 2019; Donaldson et al., 2022). Irrigation was applied at different magnitudes, frequencies, and depths to increase precipitation by 350 mm (~ 50% of the mean annual rainfall). Treatments included increasing the frequency of irrigation either at the surface or 30 cm below the soil surface, increasing intensity by irrigating heavily once per month, or irrigating during the dry season (May – July). Irrigation at 30 cm soil depth was designed to isolate the effects of deep soil water availability. I used stable isotopes to assess the depth of water uptake for two tree species, *Acacia [Senegalia] nigrescens* and *Colophospermum mopane*, because these two species had contrasting growth rates and water-use strategies within this experiment (Chapter 2). I asked: (1) Do tree saplings consistently use deeper soil water than grasses and does depth of water uptake vary by species? and (2) How does rainfall intensity and frequency affect grass biomass, belowground productivity (BNPP), and nitrogen availability?

Methods

Experimental design

This study took place at the irrigation manipulation experiment at WRF (see Chapter 2). Briefly, the experiment was comprised of 25 plots (4.2 x 3 m) and five treatments (Fig. A.1; n = 5 plots per treatment) that altered the frequency, intensity, depth, and timing of irrigation to assess the effects of water availability on tree and grass growth. All plots received approximately

350 mm of additional water per year but were subject to different magnitudes, frequencies, and timing of watering events (Table 3.1). The five treatments included (1) control: ambient precipitation, (2) surface: increased frequency of irrigation at the surface, (3) depth: increased frequency of irrigation 30 cm below the surface, (4) pulse: increased intensity of irrigation (by watering once per month), and (5) extended season: irrigated frequently during the dry season. Irrigation was applied via gravity-fed drip line irrigation from large JoJo tanks near the site. The amount of water was controlled by watering each treatment for a constant amount of time on each watering day. All irrigation treatments were applied during the growing season from October – April, except for the extended season treatment, which was irrigated from May – July. Two CS-616 soil moisture sensors (Campbell Scientific, Logan, UT, USA) were installed at 10 and 50 cm soil depths in every plot to assess soil volumetric water content (VWC; July 2017 – April 2023).

In 2017, seedlings of six savanna tree species were transplanted into the experimental plots (*Acacia [Senegalia] nigrescens*, *Colophospermum mopane*, *Dichrostachys cinerea*, *Philenoptera violacea*, *Sclerocarya birrea*, and *Terminalia sericea*). Three seedlings per species were planted in each plot for a total of 18 trees per plot and 450 trees across the experiment (Fig. A.1). In 2018, six grass species were seeded into each plot (*Aristida congesta*, *Eragrostis superba*, *Hyperthelia dissoluta*, *Panicum maximum*, *Pogonarthia squarrosa*, and *Perotis patens*). Irrigation treatments began in October 2020, and any tree seedlings that died before this time were replaced.

Depth of water uptake for trees and grasses

Water isotopes – We used stable isotopes to measure the functional rooting depth of two tree species and two grass species in each treatment. We harvested non-photosynthetic tissue

from the branches of one *A. nigrescens* and one *C. mopane* individual in 3 (2022) to 5 (2023) plots per treatment, depending on the availability of saplings large enough for removal of stem tissue. This sampling involved removing the outer cambium / phloem from 5-10 cm long segments from 2-3 stems per individual. We chose to sample *A. nigrescens* and *C. mopane* because they have strikingly different leaf physiology and growth rates, and both species branch at the sapling stage, allowing us to harvest tissue without affecting tree height measurements (see Chapter 2). For grasses, we collected non-photosynthetic crown tissue from 3-4 individuals of *P. maximum* (2022 – 2023) and *H. dissoluta* (2023) per plot. Soil samples were sampled the same day as plant tissue from the surface soil, 10, 30, and 50 cm soil depths at least three days after a rainfall or irrigation event. Plant and soil samples were stored in Exetainer vials and frozen until processing. We used cryogenic vacuum distillation to extract xylem and soil water for isotopic analysis (Ehleringer & Osmond, 1989). Water samples were analyzed for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ on the Picarro L1102-I CRDS analyzer at Kansas State University and converted to δ notation using the equation:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] * 1000 \quad \text{Eq. 1}$$

where R is the ratio of heavy to light isotopes for the sample and standard (V-SMOW), respectively. Long-term precision of the analyzer was $< 0.3 \text{ ‰}$ for $\delta^2\text{H}$ and $< 0.15 \text{ ‰}$ for $\delta^{18}\text{O}$.

Statistical analysis – We used xylem $\delta^{18}\text{O}$ and $\delta^2\text{H}$ signatures to assess differences in functional rooting depth among tree and grass functional types and species. We first established that $\delta^{18}\text{O}$ and $\delta^2\text{H}$ decreased with soil depth for each year ($P < 0.001$). $\delta^{18}\text{O}$ and $\delta^2\text{H}$ were highly collinear (Fig. B.1), so we collapsed them into a single axis via PCA, where PC1 explained 95.9% of the variation in 2022 and 88.7% of the variation in 2023 (sensu Holdo et al., 2018;

Case et al., 2020). We ran separate mixed effects models for each year. (Bates et al., 2015) We tested whether PC1 declined significantly with log-transformed soil depth using plot as a random effect ($P < 0.001$ for both years). We then tested if PC1 differed between tree and grass species using plot as a random effect. To determine which species were significantly different from one another, we used the package *emmeans* (Lenth, 2023) for pairwise comparisons with Tukey's HSD adjustment.

Aboveground grass biomass

Grass biomass – To estimate aboveground grass biomass, we measured grass biomass at the end of each growing season using a disc pasture meter (DPM). Grass biomass for each plot was estimated by averaging nine DPM measurements per plot at the end of the growing season in 2022-2023. The DPM was calibrated outside of the experiment at WRF by harvesting, drying, and weighing the amount of grass biomass underneath the disc.

Statistical analysis – We used mixed effects models to assess differences in aboveground grass biomass among treatments and years. We included treatment, year, and their interaction as fixed effects and block as a random effect and used pairwise comparisons to determine which treatments were different from one another. We also calculated log response ratios to assess the magnitude of response in each treatment. Within each block, we paired the control plot to each treatment plot and calculated the log response ratios as:

$$\ln(RR) = \frac{Biomass_{control}}{Biomass_{treatment}} \quad \text{Eq. 2}$$

We tested if each response ratio was different from zero using a student's t-test.

Belowground root production

Belowground net primary productivity – To measure belowground net primary productivity, we installed two root ingrowth cores randomly into each plot (n = 50 cores) in May 2021 and March 2022. Cores were 5 x 30 cm and made of 2 mm mesh fiberglass screen. We placed cores 30 cm into the ground, filled each core with root-free soil from the respective plot, and gently packed the soil in each core to fill air spaces and achieve similar bulk density as the surrounding soil. We harvested the cores after approximately 1-year in March 2022 and March 2023. We then divided each core in half to 0-15 cm and 15-30 cm soil depths to assess root biomass by depth. Roots were removed by hand, cleared of any soil, dried at 60 °C for at least 48 h, and weighed to the nearest mg.

Root C isotopes – To separate root production into grass and tree biomass, we determined the carbon isotopic signature ($\delta^{13}\text{C}$) of roots at both depths (0-15 and 15-30 cm) of each BNPP core. After weighing, roots were finely ground using an 8000D Dual Mixer/Mill (SPEX SamplePrep, Metuchen, NJ). Roots were measured for carbon and nitrogen content and $\delta^{13}\text{C}$ at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University using an Elementar vario Pyro cube coupled to an Elementar Vision mass spectrometer for isotope analysis. Isotopic abundance ratios were converted to δ notation with equation 1 (Vienna-Pee-Dee Belemite standard).

Statistical analysis – We used mixed effects models to test if BNPP differed among treatments, soil depths, and years. We included treatment, soil depth, year, and their interactions as fixed effects and replicate nested within plot as the random effect. We then tested whether C_3 or C_4 root biomass differed among treatments or depth using linear mixed effects models. To estimate the proportion of C_3 and C_4 root biomass at each depth, we homogenized the roots from

each root core and depth for isotopic analyses. We used the average $\delta^{13}\text{C}$ signature of roots of five known trees and five known grasses for isotopic end members. We then estimated the proportion of roots from each functional type as:

$$\%C_3 = \frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_4}{\delta^{13}C_3 - \delta^{13}C_4} * 100 \quad \text{Eq. 3}$$

$$\%C_4 = 100 - \%C_3 \quad \text{Eq. 4}$$

where δ_{sample} is the isotopic signature of the sample from the root core and δ_{C_3} and δ_{C_4} are the average isotopic signatures of the C_3 and C_4 end members, respectively (Kirkels et al., 2022).

Negative proportions can occur when the isotopic signature of the sample is higher than the average C_4 end member or lower than the average C_3 end member. We set any negative proportions to 0 and any proportions above 100% to 100 (Case et al., 2020). We multiplied these proportions by the root biomass in each core to estimate C_3 and C_4 root biomass (g m^{-2}). We then analyzed C_3 and C_4 biomass separately to test for differences among treatments, depths, and their interaction, treating replicate nested within plot as a random effect.

N availability

Inorganic NH_4^+ and NO_3^- – We estimated total available inorganic nitrogen using resin bags buried 10 cm below the soil surface. We buried four mixed ion exchange resin bags in each plot to estimate NH_4^+ -N and NO_3^- -N concentrations following the Nutrient Network resin bag assembly and deployment protocol (<https://nutnet.org>). Resin bags were deployed at the end of the growing season in 2021 and 2022 and harvested at the end of the following growing season. Resin-sorbed NH_4^+ -N and NO_3^- -N were extracted in 2M KCl and quantified at the Soils Testing Lab at Kansas State University.

Statistical analysis – We tested for differences in $\text{NH}_4^+\text{-N}$, $\text{NO}_3\text{-N}$, and total inorganic N availability using mixed effects models, with treatment as a main effect and plot as a random effect. Inorganic nitrogen availability (N as $\mu\text{g g}^{-1}$ resin) was log transformed to meet the assumptions of normality and homogeneity of variance.

Results

Soil VWC

Irrigated plots tended to have higher VWC than the control plots (Fig. 3.1), but differences were greatest at 50 cm soil depth. Here, we report average % increase in soil VWC for 2021 hydrological year (August – August) because data was missing from May-August in 2023. The surface and season treatments resulted in the largest increases in VWC at 10 cm, with a 51% and 57% increase, respectively (Table A.2). All irrigation treatments, regardless of frequency, magnitude, or depth increased VWC at 50 cm. The surface treatment increased VWC at 50 cm by 48%, similar to the 59% increase in the irrigation at depth treatment. The season treatment increased both shallow and deep soil VWC the most relative to the control, by 57% and 63%, respectively, suggesting more water infiltrates during the dry season when grasses are dormant. During the growing season, percent increases in VWC compared to the control were similar or slightly higher for each treatment, except for the season treatment, which did not receive any additional water until May.

Grass biomass

Grass biomass increased from 2022 to 2023 (Fig. 3.2a), and both treatment and year had significant effects on grass biomass (Table B.1). In 2022, the surface and pulse treatments had

higher grass biomass than the control ($P = 0.07$ and 0.02 , respectively). In 2023, grass biomass within the irrigated plots was variable and only the surface treatment had significantly higher biomass than the control ($P = 0.01$). Log response ratios for grass biomass were only significant in 2022, where the surface, pulse, and season treatment had a response ratio significantly different from 0.

Belowground root production

BNPP differed among soil depths and years (Table B.2). In 2022, BNPP was significantly higher in shallow soils (0 – 15 cm) than in deeper soil (15 – 30 cm) in all treatments except for the season treatment (Fig. 3.2b). BNPP was lower overall in 2023 than in 2022 and did not differ between shallow and deep soil depths except in the control plot ($P = 0.02$). Within each year, there were no significant differences among treatments except the season treatment had significantly higher BNPP than the control and depth treatments in 2022 ($P = 0.02$ and < 0.001 , respectively). The ratio of shallow:deep root biomass was higher in 2022 than in 2023 ($P = 0.005$) but did not differ among treatments. Above:belowground biomass was lower in 2023 than 2022, but there were few differences among treatments (Fig. 3.2c; Table B.1). On average, C_4 roots made up 84% of total root biomass ($156 \pm 11.8 \text{ g m}^{-2}$), of which 65% was in the top 15 cm of soil (Fig. 3.3). C_4 root biomass was significantly higher in the surface soils than deep soils in all treatments. C_3 roots only made up 15% of total root biomass ($28 \pm 3.5 \text{ g m}^{-2}$) and did not differ by depth or treatment (Table B.3).

Water isotopes

In 2023, there was heavy rainfall the month leading up to isotope sampling, resulting in an approximate 27% higher VWC during sampling in March 2023 than in March 2022. Soil

isotopic composition declined with soil depth in 2022 and 2023, where shallow soil layers had isotopically enriched signatures (Fig. 3.4). PC1 values were used to infer differences in functional rooting depth among functional groups and species. Grasses used shallower soil water than trees in both 2022 and 2023 (Fig. 3.5). However, differences between trees and grasses were greater in 2022 when VWC was lower. *C. mopane* consistently used deeper soil water in both years, but *A. nigrescens* did not differ from grasses in 2023 (Fig. 3.5).

Resin bags

Frequent irrigation at the surface decreased NO_3^- and increased NH_4^+ availability compared to control plots (Fig. 3.6). However, irrigation did not affect total available inorganic N (Table B.4). $\text{NO}_3^- : \text{NH}_4^+$ ratios were highest in the control and depth treatments, which do not receive any additional water at the surface.

Discussion

Precipitation variability is a major driver of tree-grass coexistence in savannas (D'Onofrio et al., 2015; Synodinos et al., 2018). Intra-annual rainfall patterns can vary independently from total annual rainfall, making it difficult to isolate the impacts of intensity, frequency, and magnitude of precipitation on savanna tree-grass competition. Our current understanding of how these rainfall patterns influence savanna vegetation growth is primarily from an aboveground perspective, where grass productivity responds quickly to rainfall events compared to more slowly growing trees (Yu et al., 2017; Belovitch et al., 2023). Differences in rooting depth between these functional types plays a large role in how trees vs. grasses respond to pulses in water availability both above- and belowground (Schwinning & Sala 2004). In this study, we investigated the impacts of irrigation frequency, intensity, and timing on grass

biomass, belowground productivity, and tree-grass root niche partitioning. We focus this discussion on three main findings: (1) savanna saplings exhibit plasticity in depth of water uptake based on soil moisture availability, (2) this plasticity in water uptake varied among tree species, and (3) grass aboveground biomass was more sensitive to irrigation than belowground root biomass.

Grasses are considered superior competitors for water and nutrients in the surface soils (Nippert and Knapp, 2007; February et al., 2013). Here, C₄ grasses had high root productivity in the top 30 cm of soil, with 65% of grass roots concentrated in the upper 15 cm (Fig. 3.3). Concentrating fine root biomass in surface soils largely makes grasses reliant on surface soil water, which can dry out quickly via evapotranspiration. However, high fine root biomass also allows grasses to respond quickly to pulses in water availability (Sala and Lauenroth, 1982). In semi-arid systems, most precipitation events are small (< 5 mm) and quick uptake by grass roots can prevent water from infiltrating into deeper soil layers (Schinning and Sala, 2004; Holdo and Brocato, 2015). In contrast, large and/or compounding precipitation events infiltrate deeper into the soil profile and tree saplings should benefit from access to deeper soil water to reduce competition with grasses (Schwinning and Sala, 2004; Yu et al., 2017). In our study, water uptake by *C. mopane* was consistently deeper than grass water uptake, but *A. nigrescens* showed evidence of plasticity in depth of water uptake between years – this plasticity has been previously observed in *A. nigrescens* saplings in response to changes in soil water availability (Kulmatiski et al., 2013b). On the days we sampled for water isotopes, VWC in 2023 was, on average, 27% higher than in 2022. These results corroborate studies from several grassland systems that showed woody species tend to use shallow soil water when it is readily available but have the capacity to shift to deeper water uptake when surface soil water declines (Eggemeyer et

al., 2009; Wang et al., 2017; Keen, 2023). While we detected relatively little C₃ root productivity in the surface soils, this plastic water-use strategy highlights the importance of fine root investment in both shallow and deep soil layers for savanna trees (February et al., 2010). Furthermore, early research posited that variation in rainfall predominately drives vegetation growth by limiting the time for growth, rather than increasing plant growth rate itself (Scholes and Walker, 1993). We now recognize savanna tree growth rates are determined by complex interactions involving resource availability, competition, and above vs. belowground carbon allocation (Tomlinson et al., 2014, 2019). Nonetheless, plasticity in depth of water uptake should extend the growth window for savanna trees, as deeper soil layers retain water for longer than shallow layers.

Grasses are known to have higher water-use and higher relative growth rates compared to savanna trees under a wide range of soil moisture conditions (February et al., 2013; Belovitch et al., 2023). Our results here (in combination with Chapter 2), support the idea that savanna tree species that can capitalize on resource pulses, similar to the grasses, have an advantage in semi-arid savannas with high resource variability (Chesson et al., 2004; Synodinos et al., 2018; Cory et al., 2022). *A. nigrescens* has a number of traits that facilitate rapid water uptake and aboveground growth, including plasticity in depth of water uptake (Kulmatiski et al., 2013b), high stomatal conductance (Chapter 2), and high hydraulic conductivity (Wargowsky et al., 2021). In contrast, species like *C. mopane* with traits that favor drought tolerance – low hydraulic conductivity (Wargowsky et al., 2021) and stomatal conductance (Chapter 2), and consistent use of deep soil water (Holdo et al. 2018; Fig. 3.5) – may have slower overall growth rates but are able to function over a wider range of soil water potentials (Chesson et al., 2004). Together,

these results demonstrate two contrasting growth and resource acquisition strategies for savanna tree species.

Some studies have argued that the primary influence of water on tree growth is through mediating N availability via N mineralization and mass flow (Scholes and Walker, 1993). Under this argument, N is the primary driver of tree-grass competition, not water, and tree fine root biomass primarily correlates with N concentrations rather than soil water content (February and Higgins, 2010). In our study, we found no change in total inorganic N availability or shifts in foliar C:N ratios across treatments (Figs. B.2). Still, N is a limiting resource in savannas (Huntley et al., 1982), and increased water availability in irrigated plots could stimulate increased N-fixation by N-fixing tree species (*A. nigrescens*, *D. cinerea*, and *P. violacea*). N-fixation is energetically expensive, and alleviation of water stress may increase photosynthate supply to support N-fixing bacteria (Cramer et al., 2010). While the degree of N fixation in this experiment is unknown, we did observe evidence of N-fixation, including nodules in multiple root ingrowth cores and lower foliar $\delta^{15}\text{N}$ in N-fixing species (Fig. B.3; Cramer et al., 2007, 2010). Finally, we found lower $\text{NO}_3^- : \text{NH}_4^+$ ratios in the surface treatment compared to plots without surface irrigation. This response could reflect increased mobility of NH_4^+ , plant uptake of NO_3^- , or altered N transformations by soil microbes – though we lack the required data to determine the exact mechanisms. Together, these results suggest that the impacts of the irrigation treatments on vegetation growth are primarily driven by the direct effects of water, and less so through the indirect effects of water on soil inorganic N availability.

Aboveground grass biomass appeared to be more sensitive to irrigation treatments than belowground biomass. We saw higher total BNPP in 2022 than 2023, a dry year, mostly due to reduced BNPP in the top 15 cm of soil, suggesting that precipitation does have some influence

on belowground productivity. However, we did not see differences in BNPP among irrigation treatments within each year (Fig. 3.2b). BNPP has been shown to be more sensitive to extreme precipitation events than ANPP (Post and Knapp, 2020), but many studies have shown little or no response of belowground productivity to rainfall addition treatments (Wilcox et al., 2015; Zhang et al., 2021). Here, control plots had lower grass biomass and above:belowground biomass ratios compared to all of the irrigation treatments. These results support other studies that suggest BNPP responses may be buffered to rainfall additions by increasing carbon allocation aboveground rather than to root production (Wilcox et al., 2016; Zhang et al., 2021).

Generally, the ability to capitalize on resource pulses is described as a trait that makes grasses highly competitive in savanna systems. However, many studies have predicted that increased rainfall variability is expected to benefit trees over grasses in semi-arid savannas (D'Onofrio et al., 2015; Kulmatiski et al., 2013a; Yu et al., 2017). In our study, we found evidence of plasticity in depth of water uptake in a rapidly growing tree species, *A. nigrescens*. This plasticity is likely most advantageous in years with high rainfall intermittency, where trees can shift depth of water uptake to deeper soil water as the surface soils dry. In combination with a water-spending strategy, plasticity in source water makes savanna trees more competitive with the grass layer (Cory et al., 2022). Differences in water-use and rooting-depth strategies among tree species suggests that ecohydrological differences may explain differential responses of dominant species to changes in rainfall regimes. While changes in rainfall regimes may affect trees competitive dynamics belowground, we found little change in herbaceous BNPP across irrigation treatments. These results suggest the primary impact of precipitation regimes on grasses may be altered aboveground growth, which has ecosystem-level consequences for fire frequency and intensity and, ultimately, tree cover in savannas (D'Onofrio et al., 2018).

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Table 3.1 Description of each irrigation treatment. All irrigated treatments received a total of ~350 mm additional water over the irrigation period.

Treatment	Irrigation period	Irrigation location	Irrigation frequency	# of water events yr ⁻¹	Duration of each event
control (ambient)	none	none	none	none	none
surface	Oct - April	soil surface	3x per week	90	23 minutes
depth	Oct - April	30 cm below soil surface	3x per week	90	23 minutes
pulse	Oct - April	soil surface	1x per month	7	5 hours
season	May - July	soil surface	3x per week	40	46 minutes

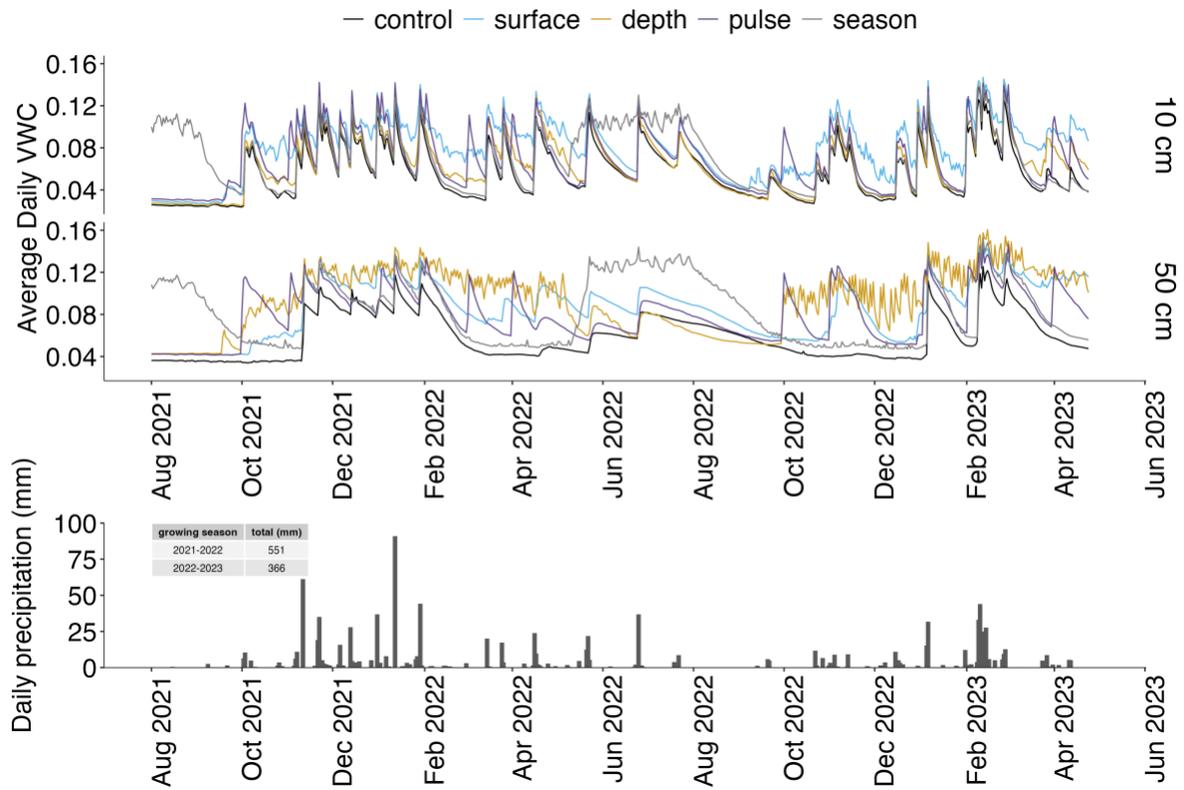


Figure 3.1 Mean daily volumetric water content at 10 and 50 cm soil depths for each treatment from August 2021 – April 2022. Bottom panel shows total daily precipitation for this period. Inset shows total growing season precipitation (October – April) for the 2021 and 2022 growing seasons.

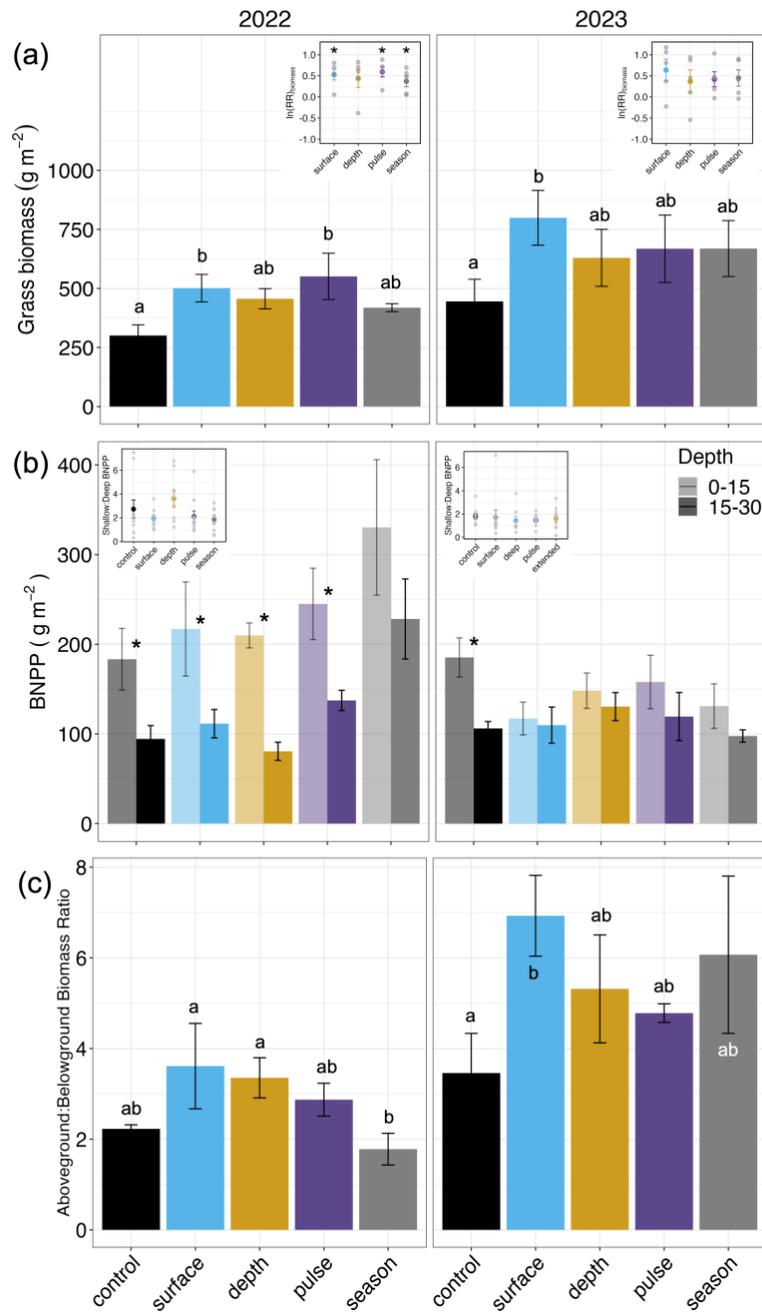


Figure 3.2 (a) Grass biomass, (b) belowground primary productivity, and (c) above:belowground biomass ratio for each treatment in 2022 and 2023. Bars represent mean \pm 1 SE. (a) Insets show log response ratio (mean \pm 1SE) and asterisks represent means were statistically different from 0 ($P < 0.01$). Points represent the log response ratio of each treatment block. (b) Asterisks represent significant different between shallow (0 - 15 cm) and deep (15 - 30 cm) soil depths in each treatment. Insets show mean shallow:deep BNPP in each year. Points represent the value for one ingrowth core. (c) Aboveground:belowground biomass ratio. Letters represent significant differences between treatments in each year ($P < 0.05$).

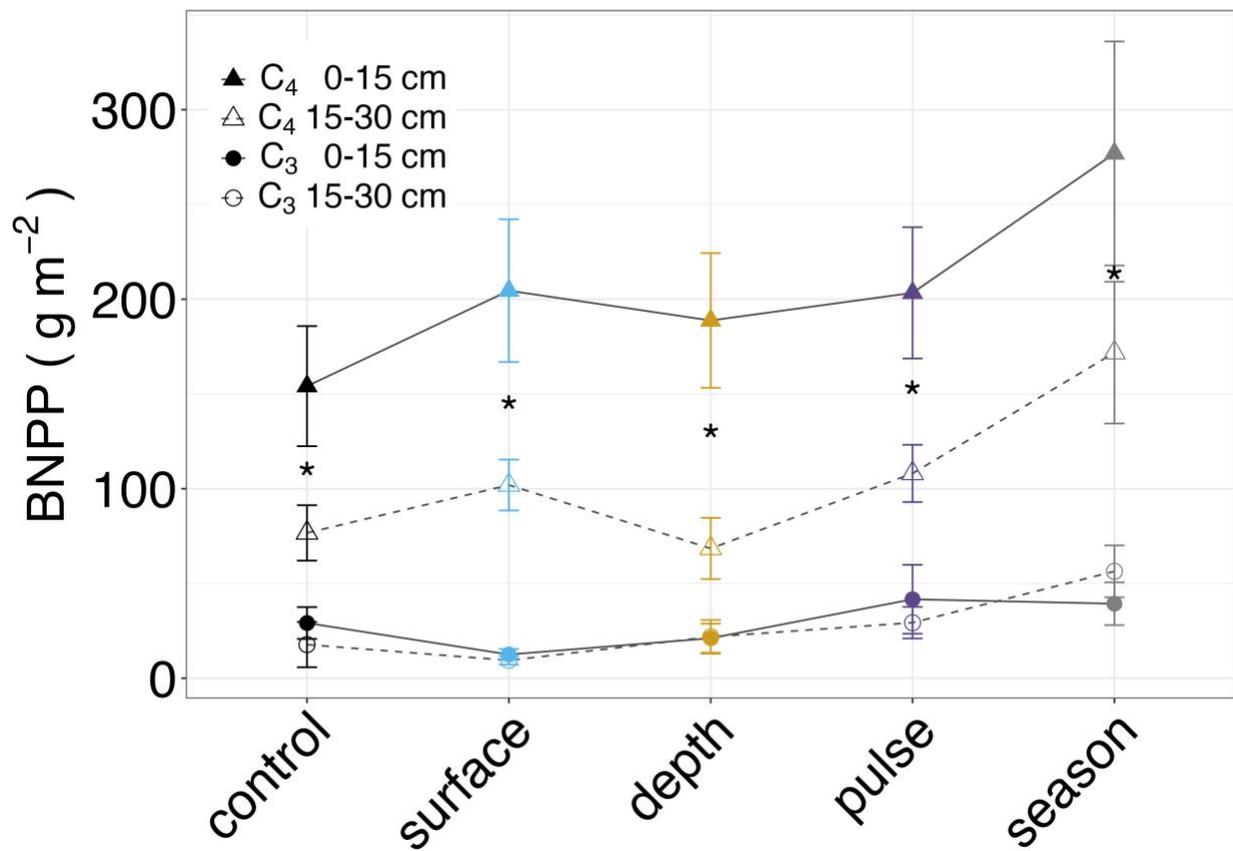


Figure 3.3 Estimated belowground net primary productivity (g m^{-2}) for C₄ (triangle) and C₃ (circle) roots at 0-15 cm (closed) and 15-30 cm (open) soil depths in each treatment (mean \pm 1 SE). Root cores were deployed in May 2021 and harvested in March 2022. Asterisks represent significant differences between surface and deep roots for C₄ grasses ($P < 0.05$).

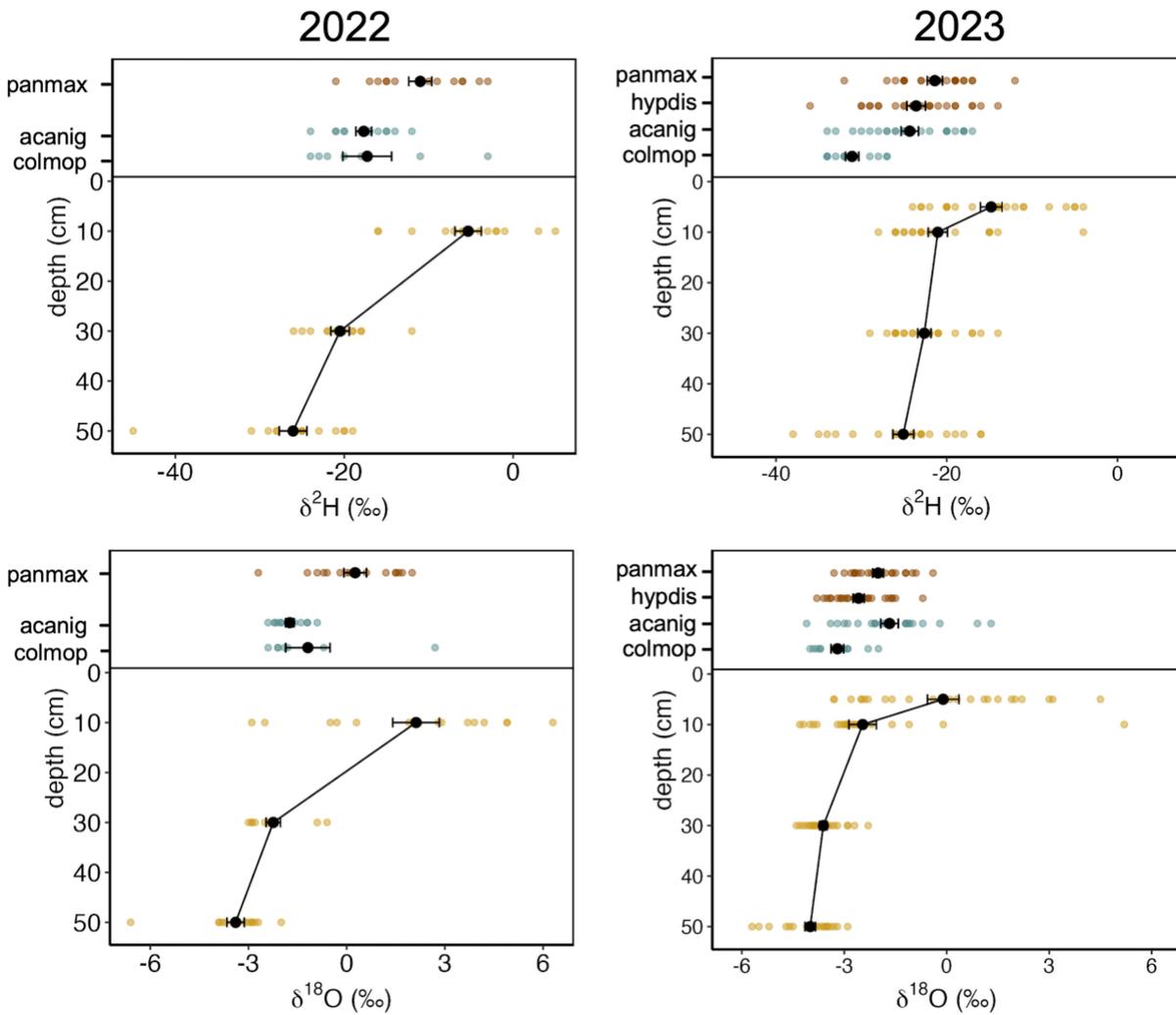


Figure 3.4 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for grasses (red), trees (blue), and soil (yellow) in 2022 and 2023. Black circles represent the mean \pm 1SE for each species and soil depth. Species codes for grasses are panmax = *Panicum maximum* and hypdis = *Hyperthelia dissoluta*. Species codes for trees are acanig = *Acacia nigrescens* and colmop = *Colophospermum mopane*.

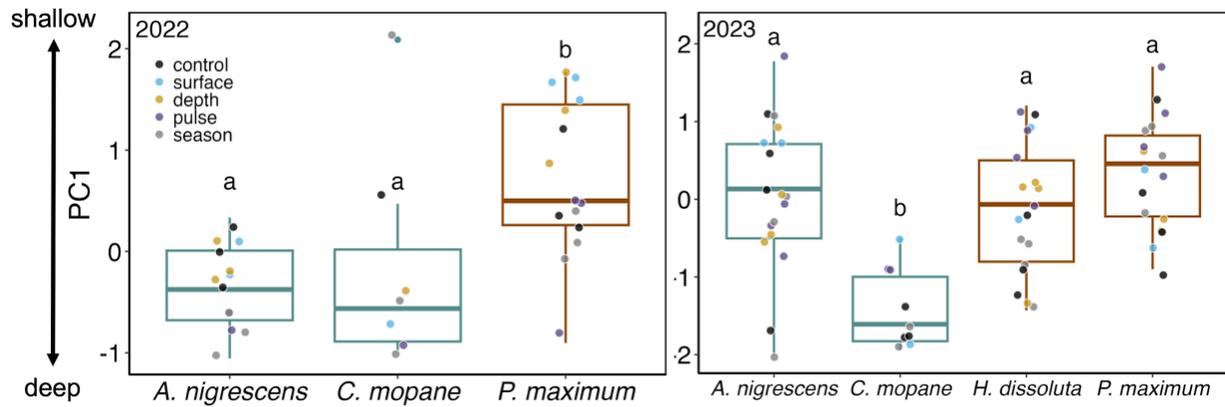


Figure 3.5 Boxplot of PC1 isotope scores for tree saplings, *Acacia nigrescens* and *Colophospermum mopane*, and C₄ grasses, *Hyperthelia dissoluta* and *Panicum maximum*, in the experimental plots in 2022 (left) and 2023 (right). PC1 axis represents both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values, where lower PC1 values correspond with deeper soil water uptake. Color of points represent individual samples in each irrigation treatment. Letters represent significant differences among species within each year ($P < 0.05$).

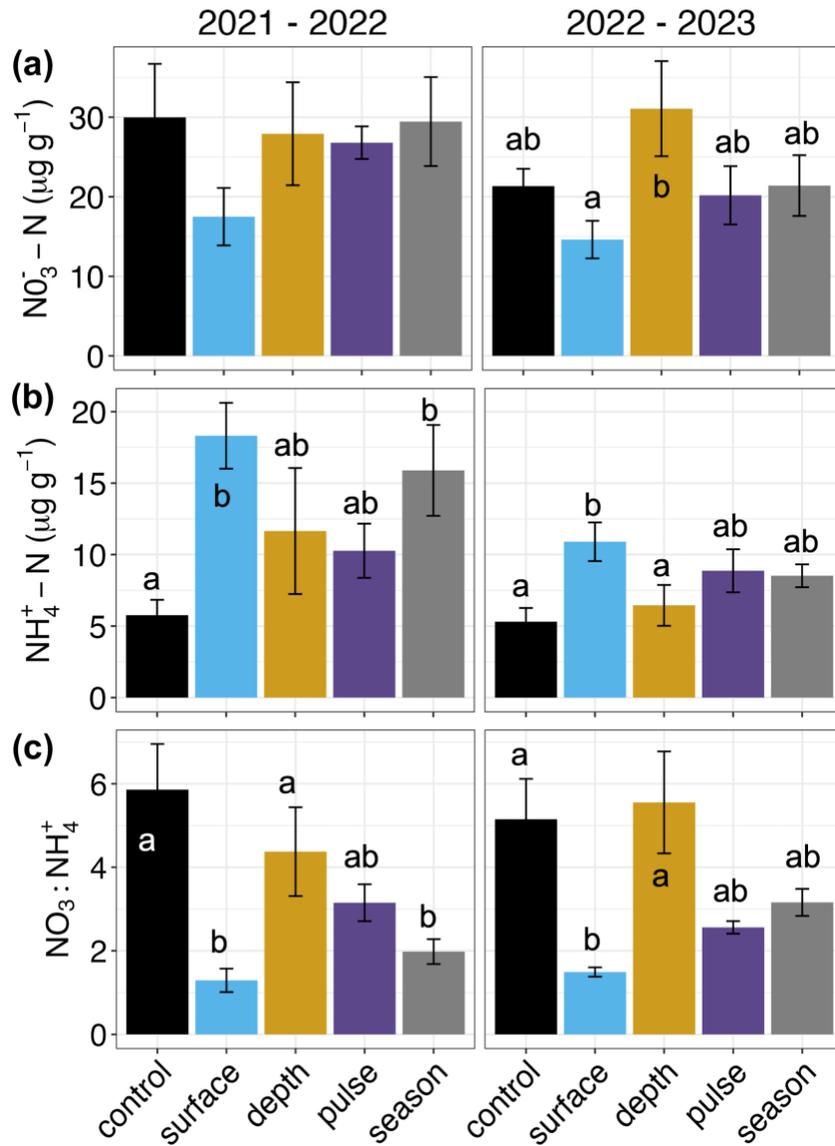


Figure 3.6 Estimated concentrations of (a) $\text{NO}_3^- \text{-N}$ and (b) $\text{NH}_4^+ \text{-N}$ sorbed to resin bags buried in each plot for 1 year (March – March). (c) ratio of $\text{NO}_3^- \text{-N} : \text{NH}_4^+ \text{-N}$. Bars represent mean \pm 1SE. Letters represent significant differences among treatments within each year ($P < 0.05$).

Chapter 4 - Repeated bush clearing as a mechanism for savanna recovery following bush encroachment

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Introduction

Woody plants in African savannas provide beneficial services for human livelihoods including timber and fuelwood, food sources for domestic and wild browsers and carbon storage (Makhado et al., 2014). While woody plants are integral components of savanna ecosystems, tree and shrub cover has increased over the past century in savannas worldwide, a process known as bush encroachment (Venter et al., 2018). Bush encroachment often results in negative economic and ecological shifts including decreased grass biomass used for livestock production (Archer and Predick 2014), decreased biodiversity (Ratajczak et al., 2012; Wiczorkowski & Lehmann, 2022), and depleted soil moisture via increased rainfall interception and transpiration (Honda & Durigan, 2016). In southern African savannas, bush encroachment is associated with a shift from tall trees to short-statured shrubs (Zhou et al., 2021), which can make it difficult to see the charismatic animals that attract tourists, possibly resulting in a loss of tourism revenue for protected areas (Gray & Bond, 2013; Luvuno et al., 2022).

Frequent or high intensity fire is often proposed as the most effective way to prevent bush encroachment at large scales (Smit et al., 2016; Scholtz et al., 2022). However, historical fire frequencies may no longer limit the expansion of woody species (Case & Staver, 2017), and fire is infrequent in semi-arid savannas. As an alternative, bush clearing has become a common management technique to mitigate the negative effects of bush encroachment (Smit, 2005; Ding & Eldridge, 2019). This typically involves the removal of small-statured trees and shrubs while

maintaining large trees to preserve habitat heterogeneity, shade for livestock and wildlife, and the gradients of nutrient and water availability associated with large trees (Schmitt et al., 2022; Treydte et al., 2010). Bush clearing is labor intensive and expensive (Luvuno et al., 2022) and has variable ecosystem effects dependent on functional traits and management goals of the woody species to be removed (Ding & Eldridge, 2019; Eldridge & Ding, 2021).

Long-term reductions in tree and shrub cover require either the use of herbicide or repeated clearing efforts, as most savanna woody species are well-adapted to disturbance and resprout following the loss of their above-ground parts (Bond & Midgley, 2001). These species use nonstructural carbohydrates (NSC) stored in their trunks and belowground organs to regrow aboveground tissues following disturbance (Wigley et al., 2019). Single or infrequent disturbances only have short-term effects on tree size and cover because these species typically have sufficient NSC stores to recover. In addition, resprouting often results in the transformation of tall, single-stemmed trees into short, multi-stemmed shrubs which can make game viewing more difficult and contradict the initial goals of bush clearing efforts (Fisher et al., 2014). Repeated cutting has the potential to deplete NSC reserves in woody species to the point of mortality – especially in conjunction with other pressures like fire or browsing – leading to long-term reductions in woody plant cover (Smit, 2004).

In this study, we assessed the consequences of repeated bush clearing on grass biomass, herbivore presence, and woody plant physiology in a South African savanna dominated by *Colophospermum mopane* (Kirk ex Benth.) J. Léonard (hereafter ‘mopane’). Mopane covers ~35% of Southern African savannas and is often reported as a woody encroaching species (O’Connor et al., 2014; Stevens, 2021) and the proportion of short-statured mopane shrubs has increased within the study region over the past several decades (Zhou et al. 2021). Mopane can

grow as a tall tree, but often grows as a short multi-stemmed shrub (< 4 m) after top-kill by elephants or fire (Stevens, 2021). Given the widespread dominance of mopane across Southern Africa, it is necessary to assess the potential costs and benefits of clearing this species, particularly in protected areas as most research on the effects of bush clearing have been conducted in rangelands (Smit et al., 2013).

For this experiment, mopane shrubs < 4 m tall were cut repeatedly for 7 years. We assessed the effects of cutting on grass biomass and monitored herbivore presence via dung counts. We predicted that repeated clearing would increase grass biomass, due to reduced competition between mopane and grasses, and increased grazers abundances, due to both increased forage and predator avoidance (Burkepile et al., 2013). We also examined the effects of cutting on mopane belowground NSC storage and functional rooting depth, to assess the mechanisms by which repeated cutting may eventually lead to mortality and long-term alteration of woody plant abundance. We predicted that repeated bush clearing would deplete belowground NSC storage, reducing the energy available for resprouting and shifting water uptake to shallower soils due to reduced investment in deeper roots.

Methods

Study site

Mthimkhulu Game Reserve is a rural 7500-hectare community-owned reserve that shares an open border with Kruger National Park, South Africa (23°31'46' S; 31°06'12' E). The reserve is managed by the Mthimkhulu Tribal Authority. Since 2016, the South African Environmental Observation Network has been working with this authority on long-term, socio-ecological research that can both improve ecosystem services and create employment for rural people living

adjacent to the reserve. The tribal authority identified eco-tourism as the most desirable land use for the reserve, and bush clearing was suggested to enhance tourism, as has typically been done in privately-owned game reserves in the region.

The site receives 467 mm mean annual rainfall, most of which falls between November and April (Mahlangeni Ranger station, 14 km south of the site, 1968 – 2010). Summers are hot and humid, with maximum temperatures typically above 30 °C, while minimum temperatures in winter rarely fall below 10 °C. The region has granite-derived, nutrient poor soils but the experimental plots were located on an old alluvial terrace of the Klein Letaba River with soils at least 1 m deep. Soil texture is approximately 62% sand, 20% clay, and 17% silt (Table C.1). The site falls within the Lowveld Mopaneveld vegetation type of Mucina and Rutherford (2006), a semi-arid savanna characterized by a dense cover of mopane shrubs, sparsely scattered trees, and low grass cover. The plots contained dense monotypic mopane stands with occasional *Combretum imberbe* Wawra and *Vachellia tortilis* (Forssk.) Gallaso & Banfi trees and *Grewia bicolor* Juss. shrubs. The grass layer was sparse and consisted mostly of annual *Aristida* species, with scattered tufts of perennial grasses *Urochloa mosambicensis* (Hack.) Dandy and *Panicum maximum* Jacq. Most of the ground cover consisted of bare soil and mopane leaf litter. Fire is infrequent in the region (~10 years; Smit et al., 2013), but the site burned in 2014, the year before our study began, resulting in top-kill of most mopane shrubs <2 m (*personal observation*).

Experimental design and community sampling

In 2015, we established three pairs of 60 x 60 m plots, spread equidistant across an alluvial terrace, with specific locations selected to ensure that tree and shrub cover was similar in each pair. One plot of each pair was randomly assigned to a repeated mopane clearing treatment

(n = 3 cleared and 3 control plots). From 2015-2022, mopane shrubs < 4 m tall were cut at the base 2-3 times per year and cut material removed from the plots (Fig. 4.1). For most response variables, sampling began in 2017 at the end of the growing season. In every plot, volumetric soil water content (VWC) was measured every hour (Oct. 18, 2017 – June 13, 2022) using CS655 time-domain reflectometry probes (Campbell Scientific Inc., Logan, UT, USA) at 10, 30 and 80 cm depths. To assess the effects of mopane clearing on the plant and animal community, we randomly established two 50 m transects within each plot. Transects were nonoverlapping and at least 20 m apart and 10 m from the nearest plot boundary. Grass and shrub cover were estimated using the point-step method (Evans & Love, 1957; Hardy & Tainton, 1993). At each 1 m point along each transect we measured the distance to the nearest perennial grass tuft, the width of the tuft, and the presence of mopane cover directly above each 1 m point. Mopane individuals were categorized into four height classes: < 0.5 m, 0.5-2 m, 2-5 m, and > 5 m. The number of mopanes in each height class was summed across the two transects in each plot. Frequency of each height class was calculated as the percentage of points with tree or shrub cover directly overhead. At the end of each growing season, herbaceous biomass (g m^{-2}) was clipped in four – six 0.5 x 0.5 m quadrats randomly located in each plot. Biomass samples were dried at 80 °C for two days, sorted into current and past season material, and weighed. We used each transect line as a 4 m wide belt transect to estimate animal visitation by counting species dung piles approximately every 1-2 months from April 2016 – August 2022.

Mopane physiology

At the end of each growing season, mopane mortality was surveyed in each treatment plot by walking from a random cut mopane to its nearest dead or alive neighbor until 100-250

mopane individuals were recorded. Individuals were considered dead if no green resprouting tissue was present. As the stumps of dead individuals persisted for many years, this provided a viable means of estimating mortality over time.

To assess the effects of repeated clearing on depth of water uptake, we used stable water isotopes to measure mopane functional rooting depth (Holdo et al., 2018). In March of 2015, 2017, and 2018, we collected non-photosynthetic tissue from mopane and dominant grass species within each plot to collect xylem water. We collected soil samples from 10, 30, and 50 cm depths from soil pits. All samples were placed in exetainer vials, placed on ice, and frozen until processing. We used cryogenic vacuum distillation to extract water from plant and soil samples for isotopic analysis. Extracted water was analyzed on the Picarro L1102-I CRDS analyzer (Picarro, Inc., Santa Clara, CA) at Kansas State University. Samples were referenced to V-SMOW and converted to delta notation using:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

Where R is the ratio of the heavy to light isotope. ChemCorrect software was used to flag samples that had organic contamination, which were removed from any subsequent analyses.

We measured NSC concentrations of mopane belowground stems to assess changes in carbon storage. Sections of mopane stems/boles were collected 10-20 cm below the surface at the same time as samples for isotopic analysis. Samples were washed to remove soil, microwaved for 90 sec to stop enzymatic activity, dried for 72 h, and ground to a fine powder. NSC extraction and analysis followed the procedure outlined in O'Connor et al. (2020).

Statistical analysis

We used R V4.2.1 (R Core Team, 2022) for all statistical analyses. Repeated measures ANOVAs using the *lme4* and *car* packages (Bates et al., 2015; Fox & Weisburg, 2019) were used to test the effects of mopane clearing on soil VWC, mopane and grass responses, and mopane NSC concentrations, and the package *emmeans* (Lenth, 2022) for Tukey's HSD pairwise comparisons. For soil VWC, distance to perennial grass tuft, width of grass tuft, and mopane NSC analyses we included an interaction between treatment and year as fixed effects and plot as a random effect. For soil VWC, we only included data during the growing season (November – April). We ran the same model for grass biomass, but included quadrat nested within plot as a random effect to account for variability due to multiple quadrats taken within each plot (n = 4-6 quadrats per plot). For the frequency of mopane size classes, we included an interaction between treatment and size class as fixed effects, with plot nested within year as random effects. We used a (log+1) transformation for mopane frequency, distance to the nearest perennial grass tuft, and the width of the grass tuft to meet the assumptions of normality and homogeneity of variance.

Dung counts were analyzed following the protocol used in Voysey et al. (2021). Since the length of transects used for dung counts varied from 50 – 100 m, we standardized dung counts by transect length and summed the number of dung piles per species across the two transects within each plot. We then divided the number of dung piles by the number of days between sampling dates. For each species, we averaged the standardized dung counts across all sampling dates to estimate an average deposition per day. To assess if this dung metric significantly differed for each species between cleared and control plots, we used a generalized mixed effects model with a Tweedie distribution using the *glmmTMB* function with a log link function in the *glmmTMB* package (Brooks et al., 2017). The Tweedie distribution can account for zero-inflated data

common in herbivore dung surveys. We calculated herbivore preference for cleared vs. control plots by dividing the number of dung piles in cleared plots by the total number of dung piles in cleared and control plots for each sampling date, where 1 is complete preference for cleared plots and 0 is complete preference for control plots (Voysey et al., 2021; Donaldson et al., 2018).

To assess differences in functional rooting depth among grasses and mopane in the cleared and control treatments, we analyzed each year (2015, 2017, and 2018) separately. Since δD and $\delta^{18}\text{O}$ were collinear and varied similarly with soil depth (Figs S1-S2), we collapsed δD and $\delta^{18}\text{O}$ into a single axis using PCA to analyze both water isotopes in a single analysis (sensu Holdo et al., 2018 and Case et al., 2020). The PCA approach is a useful alternative to isotope mixing models when plant isotopic signatures fall outside of the range of sampled soil isotopic signatures, indicating plants are using water from deeper sources than the soil sampled.

Results

Soil moisture

Differences in soil moisture between cleared and control treatments were greatest in the surface soils, where cleared plots had significantly higher VWC than control plots at 10 cm soil depth during the growing seasons of 2017 – 2022 ($P < 0.001$; Fig. 4.2a; Table C.2). The mean of differences in mean daily VWC was 39% higher at 10 cm, 10% at 30 cm, and 18% at 80 cm. From 2020-2021, when rainfall was above-average (Fig. 4.2b), the difference at 10 cm was nearly 60%.

Vegetation responses

Mopane mortality was low in the first few years of clearing, then increased steadily up to 80% after 7 years (Table 4.1). The frequency of mopane taller than 0.5 m was significantly lower

in cleared plots than control plots (Fig. 4.3; Table C.3). In cleared plots, most mopane were less than 0.5 m tall, while most mopane in control plots were between 2-5 m tall with few small trees.

Grass biomass was higher in cleared plots than control plots in 2020-2022 (Fig. 4.4a; Table C.4). The mean distance to the nearest perennial grass tuft was shorter in the cleared treatment in 2021, suggesting higher average grass density in cleared plots (Fig. 4.4b). The widths of perennial grass tufts did not differ among treatments, except in 2017, where tufts in the cleared plots were larger than those in the control plots (Fig. 4.4c). Differences in distance to perennial grass tufts and widths of grass tufts among years were variable and likely due to differences in precipitation (Fig. 4.2b), where 2018 was an unusually dry year resulting in larger distances to grass tufts and smaller tufts compared to 2021, a wet year.

Animal dung counts

Between April 2016 and August 2022, dung from 11 herbivore species were recorded (Table C.5). Dung of impala, buffalo, elephant (*Loxodonta africana*), and giraffe (*Giraffa camelopardalis*) were the most common and present in all years, while many herbivores had low dung counts and were not present in all years (Fig. C.3). Total dung counts across years were higher in cleared than control plots, mostly driven by buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*; Table C.5). These species had significantly higher dung deposition per day in the cleared plots than control plots (Fig. 4.5; Table C.6).

Mopane physiology

Glucose concentrations were unaffected by cutting, as were sucrose concentrations in most years (Fig 7a, b). In contrast, stem starch concentrations were significantly lower in cut

than control mopane in 2016-2018 (Fig. 4.7c; Table C.7). This difference increased with repeated cutting, with average starch concentrations 1.8, 2.6, and 7.8 times greater in control than cut shrubs in 2016, 2017 and 2018, respectively.

Soil water isotopic composition represented by PC1 declined with depth in all years where shallow soil layers had isotopically enriched signatures (Fig. C.4). Mopane used deeper soil water than grasses in cleared and control plots (Fig. 4.8; Table C.8). In 2017 and 2018, cleared mopane shrubs tended to use shallower soil water on average than control shrubs, but these differences were not significant (Fig. 4.8).

Discussion

Repeated clearing of a dominant woody species initially appeared to have only minor effects on reversing bush encroachment, resulting in top-kill but minimal mortality of shrubs (5% after 2-3 years of clearing). However, eventually clearing resulted in high shrub mortality (80% after 6-7 years of clearing) and altered ecosystem functioning, with increased surface soil water availability, greater grass biomass, and altered herbivore activity. This experiment demonstrates that bush clearing through repeated cutting can be an effective management tool for mitigating the negative effects of bush encroachment, at least in mopane-dominated, semi-arid savannas. While some ecological responses occurred rapidly (after only a few cuts), multiple cuttings over many years were required to induce significant mortality and long-term reductions in woody cover.

Woody plants have been hypothesized to have facilitative effects on grass growth and biodiversity in semi-arid savannas through amelioration of heat and water stress and nutrient enrichment under canopies (Dohn et al., 2013; Moustakas et al., 2013). However, woody plants in high densities can reduce grass biomass and diversity through shading (Pilon et al., 2020). Our

results indicate that mopane trees have strong competitive effect on the grass layer, and that reducing soil water in the upper soil layers is likely to be a mechanism of competition. Higher woody cover can lead to decreased soil moisture at surface and deep soil layers through increased transpiration, canopy interception of rainfall, and reduced infiltration (Smit & Rethman, 2000; Aldworth et al., 2022). There appeared to be a lagged responses of 5 years after initial clearing before grass biomass increased significantly in the cleared plots, although this was confounded with differences in annual rainfall (the 1st three years of the study period had below-average rainfall, and the 2nd three years above-average). Changes in perennial grass tuft size and ‘point-to-tuft’ distance over time suggest that while perennial tufts in the cleared treatment were able to increase in size after the initial cuts, they only began to increase in density after two years of cutting. This increase in density presumably created substantial increases in biomass over the entire sward. The large interannual fluctuations in ‘point-to-tuft’ distances suggest that the dominant perennial species (i.e., *Urochloa mossambicensis*) had population fluctuations in response to changes in annual rainfall, but with greater recruitment in the cleared plots. This is consistent with the results of Wilcox et al (2020) who found a rapid recolonization by this species following a severe drought at a nearby site.

The opening of the canopy that facilitated increased grass biomass also created areas of preferred habitat for impala and buffalo that utilize open spaces to mitigate predation risk by increasing visibility (Burkepile et al., 2013). We saw few differences in habitat preferences of other herbivore species, which was not surprising as predator avoidance is unlikely to affect feeding behavior in large species such as elephant and giraffe, while mesobrowsers such as kudu may not find sufficient forage in the cleared plots. For other species, low dung counts also limited our ability to draw general conclusions on herbivore feeding guilds (Table C.5). Further

research is needed to test whether these patterns would hold if clearing were implemented over larger scales. However, at landscape scales, it is likely that the density of grazers would either be unaffected or increase, given the greater supply of forage, provided that sufficient areas of high woody cover remained for herbivores that require shade, such as buffalo. Browser density could decline if vast areas were cleared, and ideally the management of the reserve should aim to create a mosaic of cleared and uncleared landscapes. This is also suggested by Schmitt et al. (2022), who found herbivore species richness and abundance were highest in areas of intermediate woody cover, and lowest in areas of low habitat heterogeneity (similar to the monodominant mopane stands in the control plots of this study).

In addition to creating open areas that increase habitat heterogeneity, resprouting shrubs from cleared areas may be more attractive to browsers and even increase browse availability for small to medium-sized herbivores (e.g., impala). Resprouting mopane shrubs are shorter and tend to have lower C:N than un-damaged trees (Kohi et al., 2010). Adult mopane trees tend to have high tannin concentrations, but repeated browsing and clearing may reduce investment in tannins (Kohi et al., 2010), making resprouts more attractive and more susceptible to lower NSC concentrations, growth rates, and seed production associated with browsing (Staver et al., 2009; Stevens, 2021).

Despite repeated loss of all above-ground parts, most mopane individuals persisted for many years. This demonstrates the remarkable resprouting ability of mopane and highlights the difficulty of creating long-term reductions in shrub density. The cut shrubs used substantial starch reserves to maintain adequate supplies of glucose and sucrose. This likely allowed shrubs to maintain deep roots, and access to deeper soil water than grasses. The continued use of deep soil water by repeatedly cut shrubs suggests that this source of water is critical for their survival.

Mopane invests most fine roots in in the top 40 cm and coarse roots between 40-60 cm of soil (Smit & Rethman, 1998). This shallow and sprawling root system suggests differences in water uptake depth between trees and grasses may be small when water is available (Kulmatiski et al., 2020) and hydrological niche partitioning exists even when shrubs have been depleted of belowground reserves. Reduced starch reserves weaken the ability of mopane shrubs to recover following disturbance and the combination of multiple disturbances (e.g, cutting with drought, fire, or herbivory) may increase shrub mortality and long-term, large-scale reductions of woody cover (O'Connor et al 2020; Staver et al., 2009).

A transition of a savanna ecosystem to a stable open state requires internal feedbacks to maintain that state after ceasing removal treatments (Ratajczak et al., 2018) and would likely require bush clearing at large scales. Scholes (1990) found that mopane stands cut and treated with herbicide returned to their pre-cleared density within 14 years, suggesting that one-time bush clearing is unlikely to reduce mopane cover over decadal time scales, and clearing is required about every 10 years. However, in that study, the application of herbicide was estimated to cause only 40% mortality. We found 80% mortality after 7 years of repeated cutting, and return to original densities may therefore take much longer. While the method used for this study is labour-intensive and requires greater short-term investment, it may prove to be more cost-effective in the long term. Rates of seedling recruitment will play a key role in determining effective control, as seed sources from uncleared areas could facilitate large recruitment events in years with ideal environmental conditions. Clearing larger areas, with lower perimeter:area ratios could be beneficial in reducing seed establishment from surrounding areas. In our study, no cut mopane were observed to flower and substantial recruitment from shrubs inside repeatedly cleared areas seems unlikely if starch reserves are reduced enough to prevent flowering.

Additionally, recruitment events may be limited by increases in grass biomass that limits woody plant establishment and growth rates, particularly in wet years when grass biomass is high (February et al., 2013; Riginos, 2009).

The economic value of bush clearing encroached savannas in South Africa has been estimated to be US \$2.1 billion (Stafford et al., 2017). This estimate included the potential benefits of restoring soil water recharge and grazing capacity and using harvested wood for electricity, fuel, and wood composite products. This presents potential industrial economic benefits of clearing bush encroached areas on a large scale. Additionally, bush clearing may benefit local individuals at smaller scales by using targeted woody biomass for firewood and fencing. However, we recognize bush clearing has variable effects dependent on climate and the woody species to be removed, and proper ecosystem management will vary depending on the goals of local communities, ecologists, and economists. The method used in this study was notably more expensive (~US \$22,000 ha⁻¹ for seven years of repeat cutting or ~US \$3,100 ha⁻¹ yr⁻¹) than the widely used method of a one-off cut followed by herbicide application (~US \$4,300 ha⁻¹ for one year). However, the use of herbicides may be undesirable due to the toxicity of the chemicals to humans and wildlife and the potential effects on non-target plant species. Furthermore, our method created regular employment in an area where poverty and unemployment are high. The social benefits must therefore outweigh financial costs, particularly if donor funding can be used to support management objectives. Finally, more intensive clearing methods may be necessary in the future, if the drivers of encroachment, such as elevated atmospheric CO₂, continue to increase. Targeted and frequent management practices are increasingly necessary in a changing climate and fragmented landscapes where the return of

disturbance (fire or browsers) to the system is insufficient to limit bush encroachment (Case et al., 2017; Collins et al., 2021).

In summary, our results highlight that repeated, targeted bush clearing can increase soil moisture, grass biomass, and ungulate habitat in semi-arid savannas. Crucially, repeated clearing was necessary to reduce starch reserves to levels that prevent resprouting and induce tree mortality. While this approach was more expensive than conventional methods, it had positive ecological effects, created social benefits in the form of temporary employment, and may produce longer-lasting reductions in shrub densities. We recommend bush-clearing projects need to have decadal-scale management plans that include repeated clearing to increase the return on investment and successfully mitigate the negative impacts of bush encroachment in the long term.

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Statement on Inclusion

Our study took place on Mthimkhulu Game Reserve, a community-owned game reserve in Limpopo, South Africa. Residents from Phalaubeni village, adjacent to the reserve, were employed as labourers for bush-clearing and assisted with data collection. Local authors and community members were involved in the planning and execution of this experiment.

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Table 4.1 Average percent mortality ($\pm 1SE$) of mopane shrubs in cleared plots ($n = 3$). Bush clearing began in 2015. In each plot, we recorded whether the nearest neighbor of a randomly selected mopane was dead or alive until 100-250 shrubs were recorded.

Year	% Mortality	Total shrubs surveyed
February 2018	4 ± 2	442
July 2019	18 ± 5	631
May 2020	45 ± 5	537
May 2021	50 ± 5	411
March 2022	80 ± 8	449



Figure 4.1 (a) Control plots at Mthimkhulu Game Reserve. *Colophospermum mopane* forms thick, monodominant stands. (b) Plots cleared of all mopane trees and shrubs < 4 m tall. (c) resprouting mopane shrub. Photos taken in March 2020.

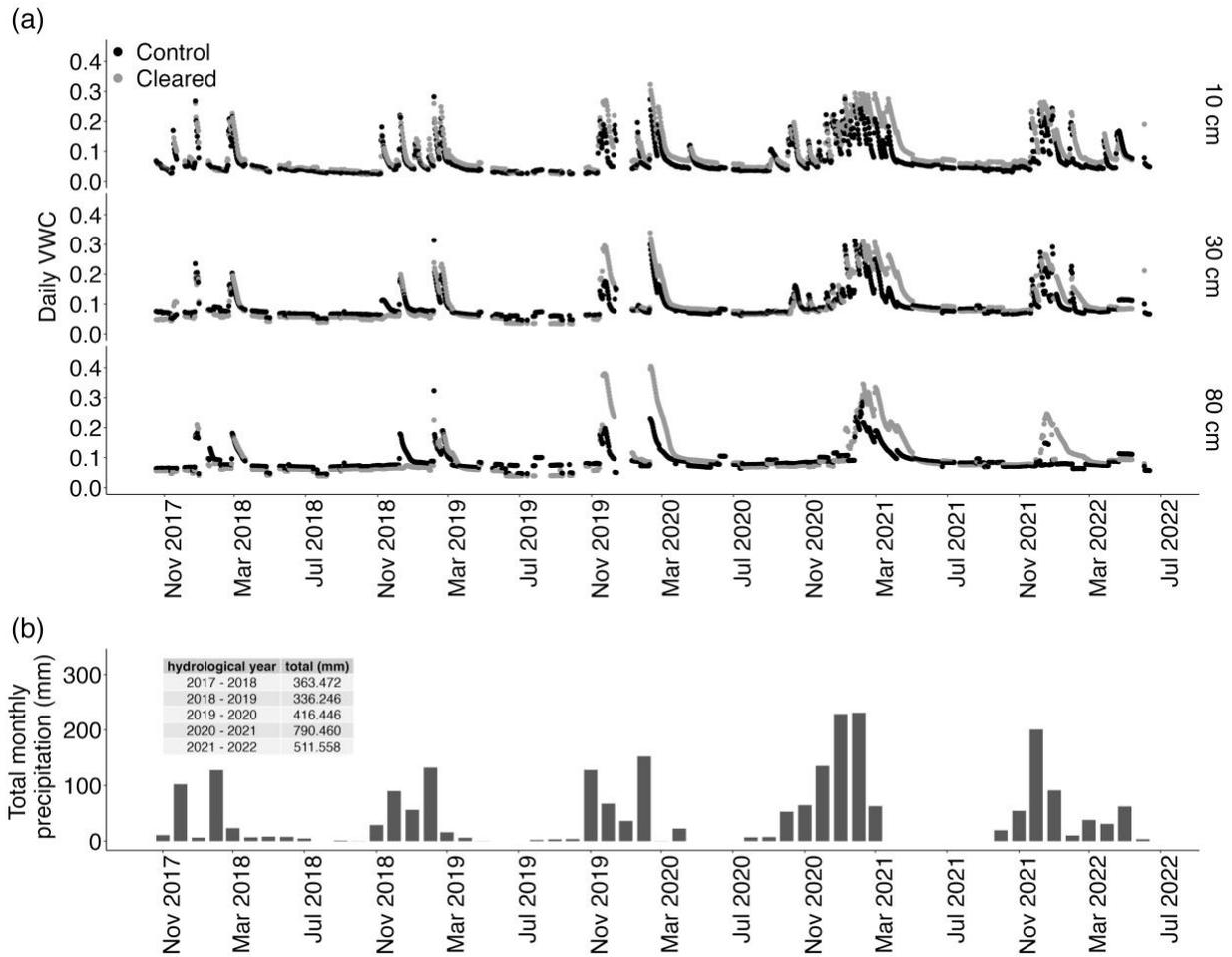


Figure 4.2 (a) Mean daily volumetric water content for cleared and control treatments at 10, 30, and 80 cm soil depths for November 2017 – July 2022. Growing season spans November – April. (b) Total monthly precipitation (mm) from November 2017 – July 2022. Inset shows total precipitation (mm) for each hydrological year (July 1 – June 30).

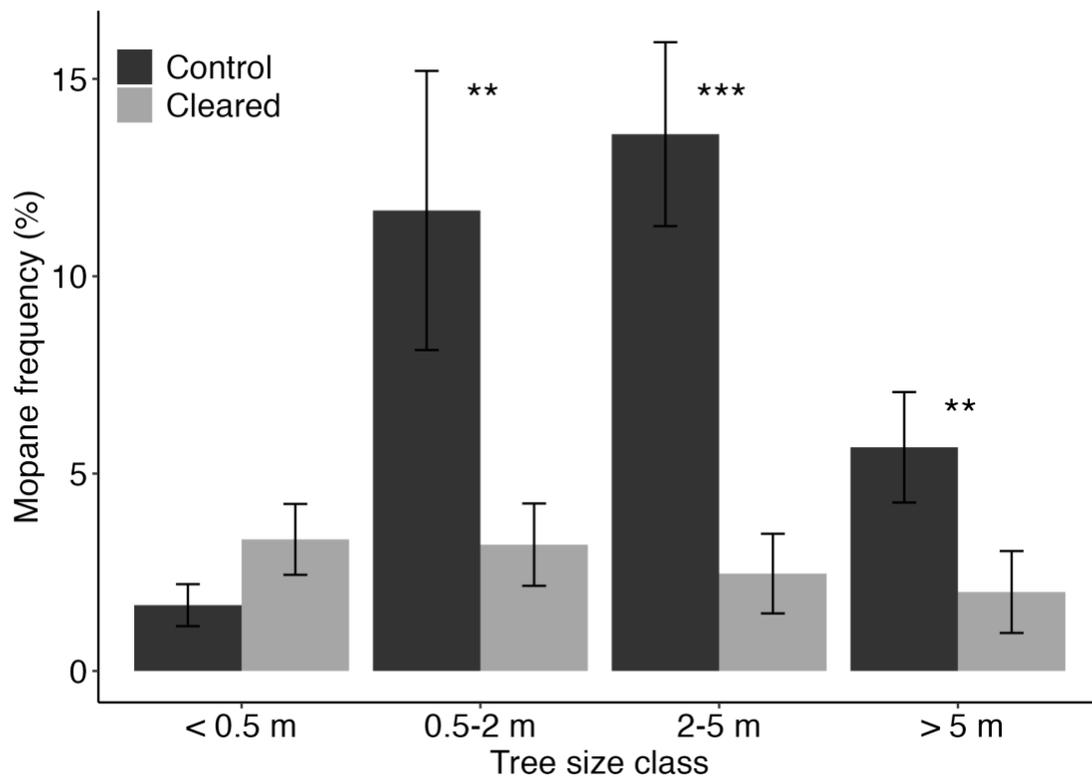


Figure 4.3 Mean (\pm 1SE) frequency (%) of mopane tree size classes averaged across years (2017-2022). Bush clearing began in 2015. The height of every mopane tree at each meter along two 50 m long transects was measured and categorized into one of four size classes: < 0.5 m, 0.5-2 m, 2-5 m, or > 5 m. Mopane frequency was summed across transects. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

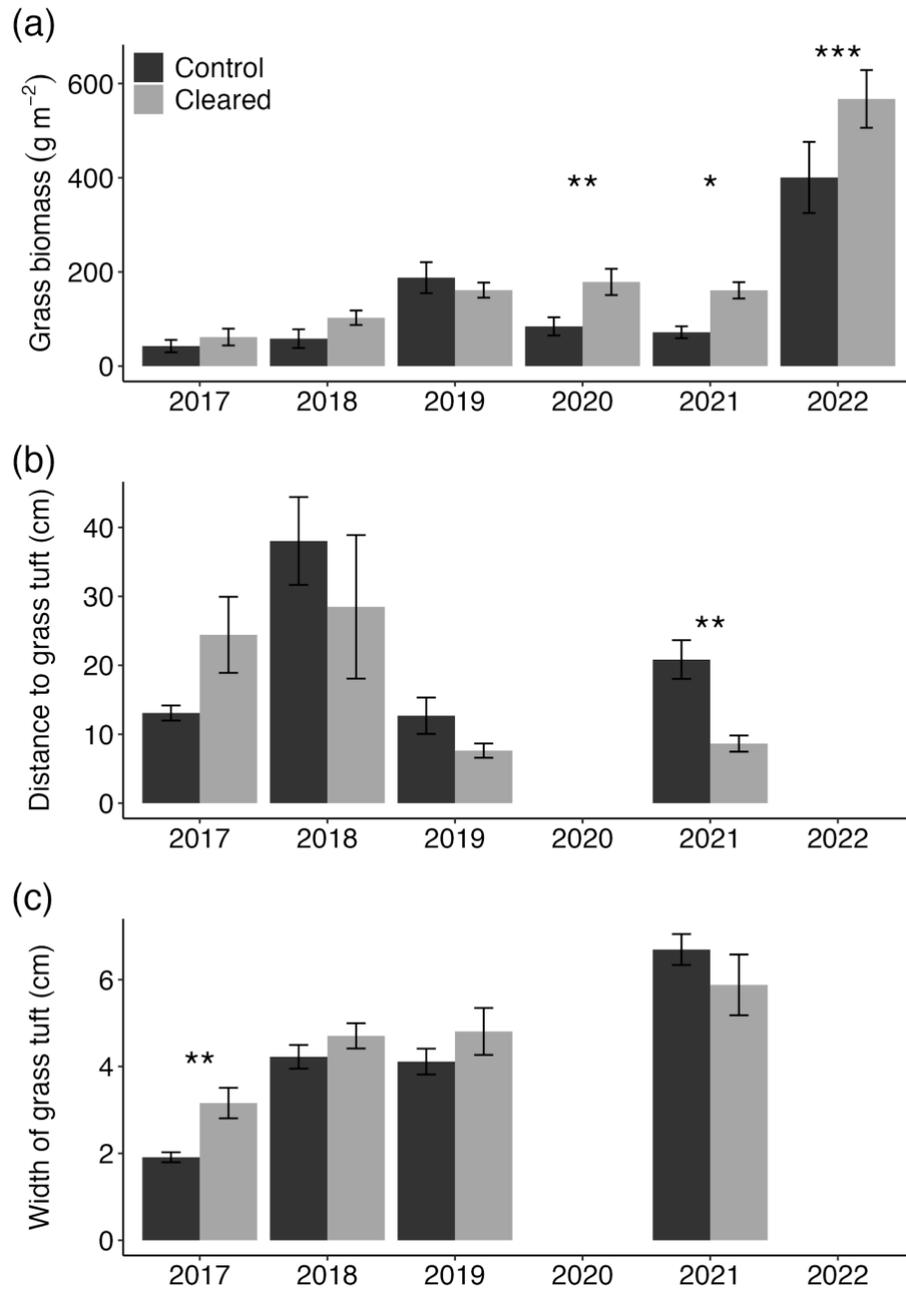


Figure 4.4 Mean (± 1 SE) (a) grass biomass (g m^{-2}), (b) distance from transect to nearest perennial grass tuft (cm), and (c) width of nearest perennial grass tuft (cm) in cleared and control plots. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Grass data from 2020 and 2022 are missing due to sampling constraints.

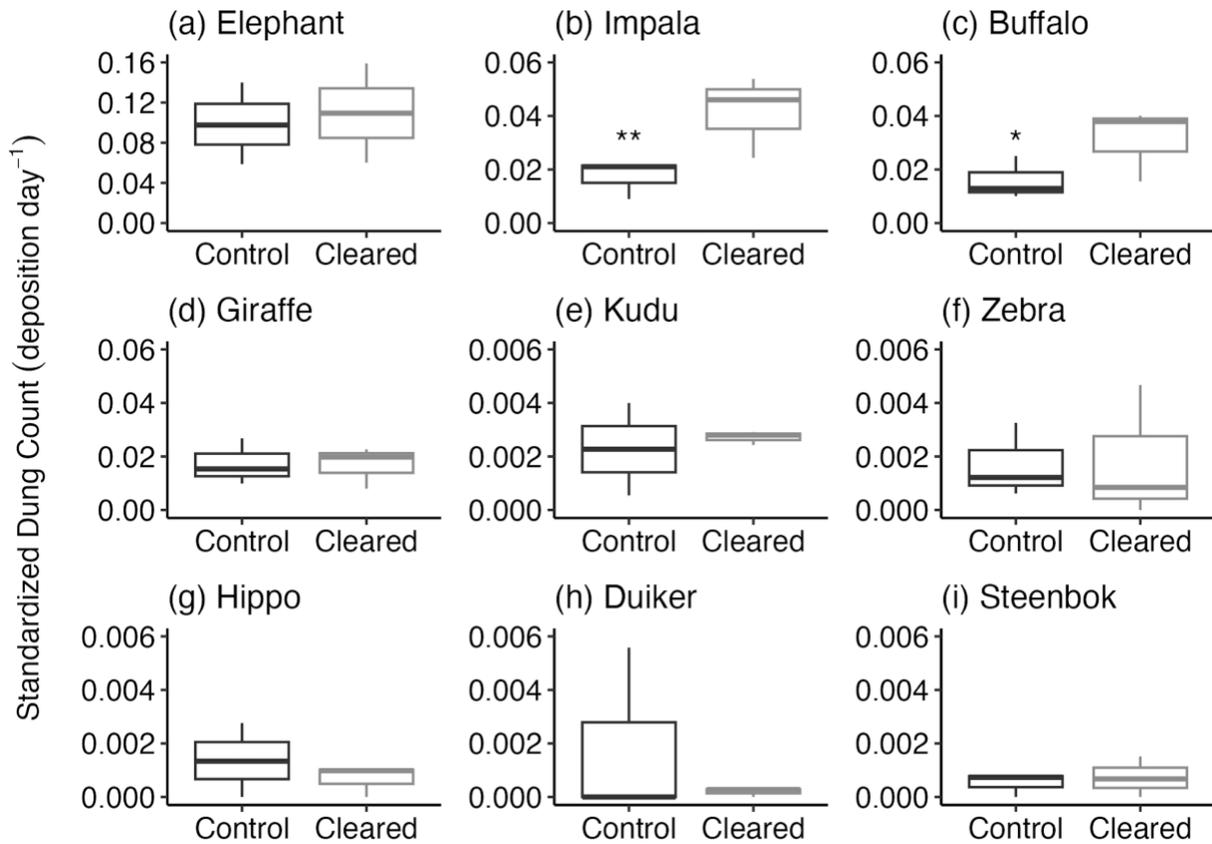


Figure 4.5 Boxplot of estimated dung deposition per day for various herbivore species (a-h). Species are ordered from the highest total dung counts recorded during the study to the lowest. Standardized dung counts were averaged for each species in each plot across all sampling dates ($n = 3$). Note panels (b-d) are an order of magnitude higher than panels (e-i). Panel (a) has a unique scale. * $P < 0.05$, ** $P < 0.01$.

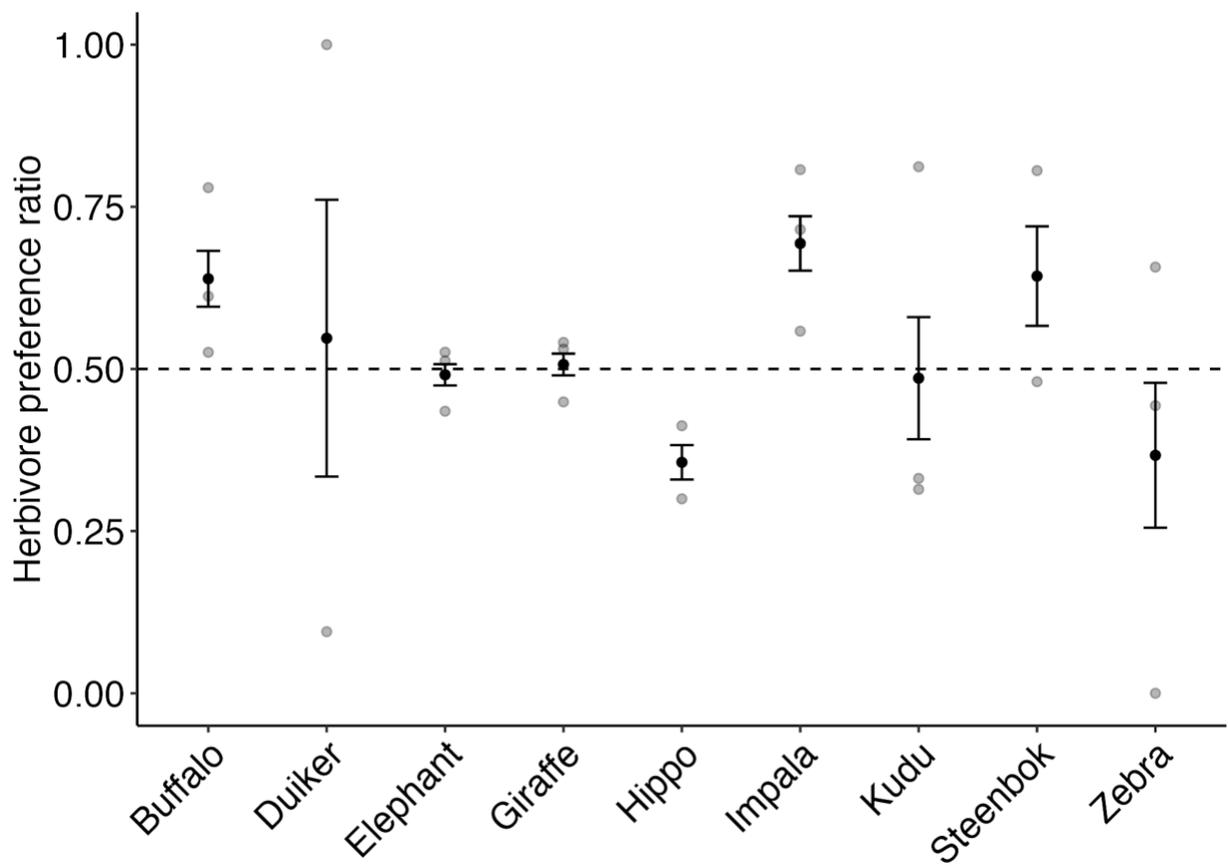


Figure 4.6 Habitat preference ratio of various herbivores (mean \pm 1SE). Preference ratio was calculated from the total standardized dung counts for each species in each plot across all sampling dates. Preference ratio ranges between 0 and 1, where 1 is complete preference for cleared plots and 0 is complete preference for control plots. Grey points are the preference ratio for each plot (n = 3).

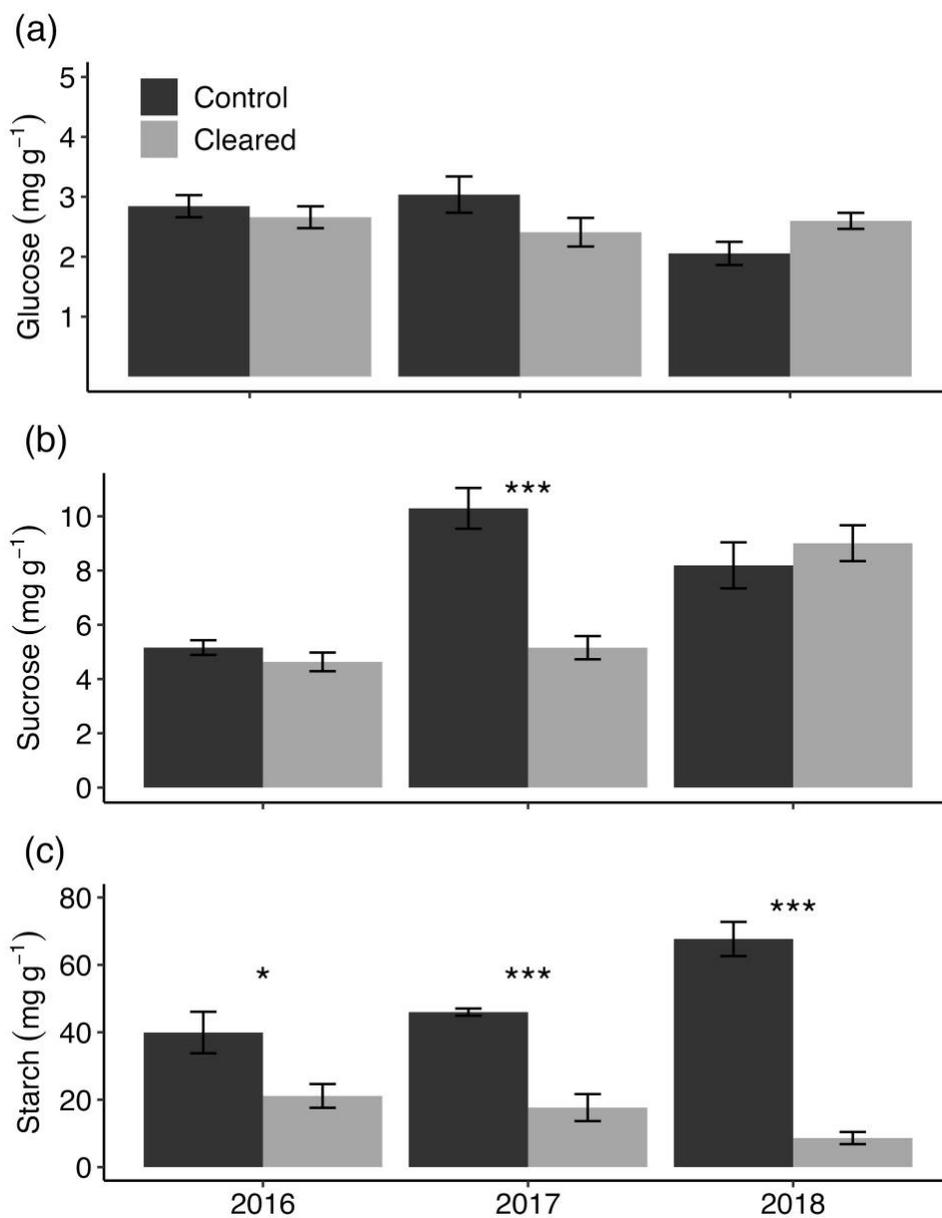


Figure 4.7 . Mean (\pm 1SE) (a) glucose, (b) sucrose, and (c) starch concentrations (mg g⁻¹) of mopane trees in cleared and control plots. * P < 0.05, ** P < 0.01, *** P < 0.001.

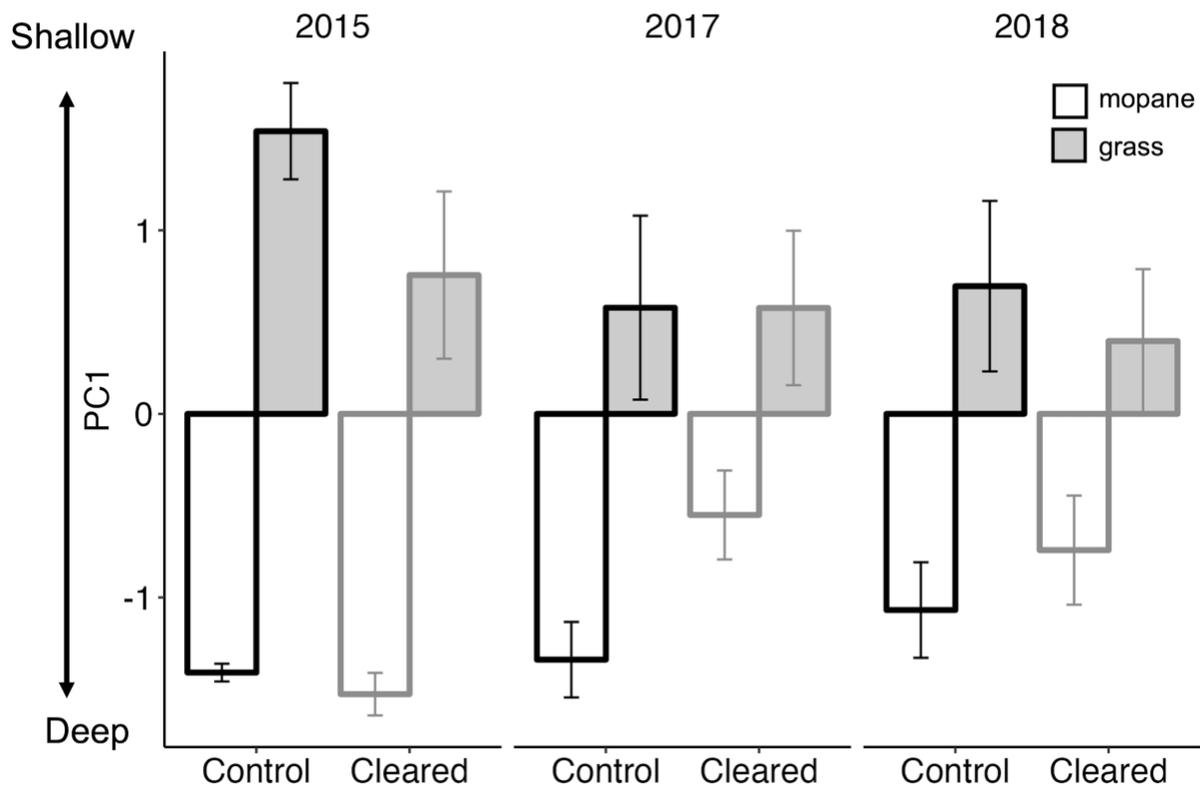


Figure 4.8 Mean PC1 isotope scores (\pm 1SE) in cleared and control plots in 2015, 2017, and 2018. The PC1 axis represents both δ D and δ^{18} O values. Lower PC1 values correspond with deeper soil water.

Chapter 5 - Woody encroaching shrubs show divergent resource-use strategies in tallgrass prairie

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Introduction

Grasslands often contain diverse assemblages of non-grassy species that coexist with highly abundant grasses. These non-grassy species contribute to plant diversity, and ecosystem heterogeneity, and support a diversity of pollinators and supplemental food for livestock (Bråthen et al., 2021). Over the past half-century, woody species native to grasslands have increased in abundance, replacing herbaceous species (Wieczorkowski & Lehmann, 2022). The encroachment of native woody plants has resulted in reduced grassland biodiversity, altered ecosystem water and carbon cycling, and billions of dollars in lost revenue for the cattle ranching industry (Archer & Predick, 2014; Ratajczak et al., 2012; Morford et al., 2022). Woody encroachment is driven by complex interactions among changes to local disturbance regimes (e.g., suppression of fire and browsing) and global change drivers (e.g., increased atmospheric CO₂ concentrations) that favor C₃ woody plants over the C₄ grasses that dominate warm temperate to tropical grasslands (Venter et al., 2018). Despite the common drivers that favor woody plants, some woody species native to grasslands have rapidly increased in abundance, while other coexisting woody species have not (Ratajczak et al., 2014a; Zhou et al., 2021). In this study, we characterized the growth and resource-use strategies of encroaching woody species in tallgrass prairie (eastern Kansas, USA) that span an order of magnitude in abundance to determine if rapidly encroaching shrubs have unique carbon and water-use strategies compared to coexisting native shrubs that are not changing in abundance.

Many combinations of traits determine resource-use strategies that may promote the expansion of some encroaching woody species over others. Land use changes that favor woody species may select for particular traits and promote the encroachment of functionally similar species. If environmental filtering or competition with herbaceous plants selects for certain suites of traits, then the most abundant woody encroaching species should occupy similar niches (Grime, 2006; Scheffer and Van Nes, 2006; Mason et al., 2011). When niche overlap is substantial, species can still coexist if fitness differences between species are small (Godoy and Levine, 2014). For example, encroaching shrubs are often clonal and have high belowground nonstructural carbohydrate storage, facilitating rapid resprouting after top-kill due to fire or mechanical removal (Bond & Midgely, 2003; O'Connor et al., 2020, 2022). Alternatively, limiting similarity predicts that coexistence among species requires resource partitioning, and the most abundant encroaching species should occupy different niches (MacArthur and Levins, 1967). High functional trait diversity among encroaching species would suggest that niche complementarity drives the coexistence of multiple encroaching woody species.

Both filtering (i.e., similar niches) and niche complementarity (i.e., different niches) are mechanisms of species coexistence that interact through environmental variability and species functional tradeoffs (Angert et al., 2009; Hallett et al., 2019). We might expect that a community shows filtering on one niche axis (e.g., disturbance traits) and complementarity on another (e.g., resource acquisition traits; Mason et al., 2011). For example, fast-growing woody species can quickly grow above the grass canopy to size classes less susceptible to fire and browsing (Higgins et al., 2000; Staver et al., 2009). However, species that are shallow-rooted or have “risky” water use strategies (i.e., anisohydric) may be more susceptible to drought-induced mortality than slower-growing species (Case et al. 2020). Woody species with similar or

different niches can be characterized using physiological and functional traits that reflect interspecific carbon and water-use strategies.

For several decades, trait-based ecology has focused on characterizing resource-use strategies using easily measured functional traits to draw inferences on conservative vs. acquisitive plant growth strategies and describe vegetation dynamics from communities to landscape scales (Lavorel & Garnier, 2002; Wright et al., 2004; Funk et al., 2017). Leaf-level functional traits can strongly correlate with growth and environmental variables, but the mechanisms driving these relationships can be difficult to identify, particularly in grasslands where frequent disturbance increases inter- and intraspecific trait variability (Wigley et al., 2016). Coupling functional traits with detailed leaf-level physiological measurements can give more mechanistic insight into species' carbon and water-use strategies related to growth-tolerance tradeoffs and, ultimately, abundance across landscapes (Cornwell and Ackerly, 2010; Higgins et al., 2012; Poorter et al., 2012). Understanding the traits and physiology of the most abundant encroaching species can help identify the mechanisms driving shifts in ecosystem-level carbon and water cycles because the traits of the most abundant woody encroaching species will likely have the largest impacts on these fluxes (Grime 1998; Eldridge & Ding 2021). For instance, in African savannas, encroachment by the N-fixing species *Dichrostachys cinerea* increases soil N content and accelerates litter decomposition rates, causing altered soil C dynamics (Blaser et al., 2014; Mogashoa et al., 2021). In tallgrass prairie, encroachment by a deeply rooted shrub with high transpiration rates (*Cornus drummondii*) has led to increased evapotranspiration and depletion of soil water stores (O'Keefe et al., 2020; Keen et al., 2023), which probably would not be the case if these same shrubs were shallow rooted or had conservative water use.

Like many mesic grasslands and savannas, fire suppression is the primary driver of woody encroachment in the tallgrass prairie of the Central Great Plains (USA), and clonal shrubs are the primary species driving woody encroachment (Ratajczak et al., 2014a; Charles-Dominique et al., 2015; Case et al., 2017, 2020). Some clonal shrubs, but not all, have dense growth forms that shade out herbaceous species and alter ecosystem fire dynamics, further promoting their growth (Ratajczak et al., 2011; Tooley et al., 2022). Past research in this system has focused on functional type differences between woody plants and grasses to understand different physiological strategies that drive shrub-grass competition (O’Keefe et al., 2020; Keen, 2023), but little work has been done to identify interspecific variation in shrub physiology and biomass allocation (e.g., leaf:stem ratios) or to link functional traits with the expansion of encroaching species.

Here, we assessed whether clonal shrubs encroaching in tallgrass prairie have common physiological and functional traits or unique traits that differentiate species' carbon and water-use strategies. We measured a suite of physiological and functional traits related to carbon capture and drought tolerance (Table 5.1) of six encroaching clonal shrub species (*Cornus drummondii*, *Prunus americana*, *Rhus aromatica*, *Rhus glabra*, *Rubus pensilvanicus*, and *Zanthoxylum americanum*) and one encroaching tree species (*Gleditsia triacanthos*) at Konza Prairie Biological Station (KPBS, Manhattan, KS, USA). These species were chosen because they grow above the grass canopy and, as a group, comprise over 63% of woody cover across the entire site (excluding sub-shrubs; Fig. D.1). All the measured woody species have increased in abundance at KPBS over the past four decades but are encroaching at vastly different rates, and only three species have become dominant species on the landscape. This study has three parts: First, we identified the trends in relative cover, frequency, and dominance of the focal woody species

using a 34-year species composition dataset (1988-2022). Second, we used allometry data to summarize leaf:stem mass ratios and leaf area of the focal woody species to understand aboveground carbon allocation. Finally, we compared traits related to gas exchange and drought tolerance among species to determine if the most abundant encroaching shrubs have unique carbon and water-use strategies compared to more slowly encroaching shrubs.

Methods

Study Site

Konza Prairie Biological Station (KPBS) is a 3,487-ha native tallgrass prairie site located in the Flint Hills of eastern Kansas (39°05' N, 96°35' W). The site is an experimental landscape divided into areas with various fire frequencies (1, 2, 4, or 20-yr fire return intervals) and grazing regimes (bison, cattle, or no large mammalian grazers). KPBS has rocky uplands with shallow soils, steep slopes, and lowlands with deeper soils (~2 m). Mean annual precipitation is 846 mm (1982-2022), 75% of which falls from April – September. Areas with a 4-yr fire return were historically dominated by C₄ grasses but have undergone significant encroachment by clonal woody shrubs in the past three decades (Briggs et al., 2005; Ratajczak et al., 2014).

Long-term species cover

We assessed long-term woody cover for seven focal species using permanent community composition plots at KPBS (Hartnett et al., 2023). These species included six encroaching clonal shrubs (*Cornus drummondii* C.A. Mey, *Prunus americana* Marshall, *Rhus aromatica* Aiton, *Rhus glabra* L., *Rubus pensilvanicus* Poir., and *Zanthoxylum americanum* Mill.) and one nonclonal tree (*Gleditsia triacanthos* L.). We focused on two watersheds with a 4-year burn

frequency, one grazed by bison and one ungrazed. Plots were established in 1983, and bison were introduced into the grazed area between 1987 and 1991. Each watershed included four 50 m long transects with five 10 m² plots (n = 20 plots per watershed). Species cover was estimated in each plot using a modified Daubenmire scale (1 = 0-1% cover, 2 = 2-5% cover, 3 = 6-25% cover, 4 = 26-50% cover, 5 = 51-75% cover, 6 = 76-95% cover, 7 = 96-100% cover). For this study, all Daubenmire scale values were converted to the midpoint values for analysis (0%, 0.5%, 3.5%, 15%, 32.5%, 62.5%, 85%, or 97.5% cover). We only used cover in the lowlands because woody cover is limited in the uplands. We calculated relative cover for the focal species as the cover of each species was divided by the total cover of species in the watershed, including shrubs, trees, and herbaceous species. We calculated species frequency as the percentage of plots where each species occurred. We then used the average relative cover and frequency to calculate a species dominance indicator index, where higher values indicate a species has a high relative cover and is distributed across the sampling plots (Avolio et al., 2019). We used the average species dominance indicator index over the last 10 years (2012 – 2022) to rank the shrub species from the most to least abundant in all the figures.

Shrub aboveground biomass allocation

Leaf area, stem mass, and leaf mass for the focal woody species, excluding *G. triacanthos*, were opportunistically sampled in the summer of 2020. These measurements were sampled across eight watershed units at KPBS, including 2-year and 4-year burn frequencies in bison grazed and ungrazed areas (see Table D.1 for sample sizes and locations). Stems of each species were harvested, placed in a bucket of water, and immediately transported to an air-conditioned lab. Stem height, stem diameter at 0.50 m height, and leaf area were measured for

each stem within 6 hours of harvest. The cumulative leaf area for each stem was measured using a Li-3100C (Li-COR Biosciences, Lincoln, NE). A limited number of stems were measured for *Z. americana* and *R. pensilvanicus* because these species have thorns on their leaves, which could damage the Li-3100C. However, the stems that were measured for these species spanned the full range of size classes encountered in field surveys. For *C. drummondii*, we used stems harvested in a similar way as part of another study (Tooley et al., 2022). Leaves and stems were dried at 60 °C for at least four days and weighed for stem and leaf dry mass. We used these measurements to calculate leaf:stem mass ratios, height:diameter ratios, and the ratio of leaf area to the aboveground biomass.

Statistical analysis – We tested for differences in leaf:stem mass and height:diameter ratios among species using a Kruskal-Wallis test. We used this non-parametric test because of the largely unequal sample sizes among species, particularly *C. drummondii*, for which we had three times as many stems as the other species (Table D.1). We then used a Dunn's test with a Bonferroni correction to assess which species had significantly different biomass ratios.

Physiological and functional traits

In 2022, we sampled physiological and functional traits related to carbon gain and drought tolerance for the seven focal woody species. Precipitation in 2022 was average, with 601 mm between April and September. We sampled individuals in the lowlands of bison grazed and ungrazed areas with a 4-year fire return interval near the long-term species composition plots. Bison grazing is not expected to impact shrub growth and physiology directly but can impact soil compaction and chemistry, which may indirectly affect shrub water-use dynamics (Greenwood & McKenzie, 2001; O'Keefe and Nippert, 2017). Shrubs of the same species were a minimum of

10 m from one another to ensure the independence of replicates. Areas were last burned in April of 2021, resulting in top-kill of most individuals (*personal observation*), so we sampled resprouting stems of shrubs 1-yr post fire. *G. triacanthos* is a tall tree, and not all individuals were top-killed in 2021.

Measurements included leaf-level physiological traits derived from pressure-volume curves, A-C_i curves, light response curves, and functional traits that are commonly used as metrics of plant growth at larger scales (Table 5.1). We measured eight replicates per species for parameters derived from response curves (n = 4 individuals per watershed) and ten replicates per species for functional trait measurements (n = 5 individuals per watershed). Functional traits were measured following the guidelines in Pérez-Harguindeguy et al. (2016). See Appendix E for detailed methods on how traits were measured.

Statistical analysis – All statistical analyses were performed in R V4.2.1 (R Core Team, 2022). We used Principal Component Analysis (PCA) to summarize trait correlations among species using the *prcomp* function in the stats package (R Core Team, 2022). Trait data were normalized, and log transformed to linearize relationships among variables and to ensure each trait had equal weight in the analysis. One replicate of π_{tip} was missing for *Cornus drummondii*, so we used the average of all other replicates to fill in the missing value. We used a type II one-way ANOVA to assess if average PC1 and PC2 coordinates differed among species using the car package (Fox and Weisberg, 2019).

We tested for trait differences among species with linear mixed models using the *lmer* function in the lme4 package (Bates et al., 2015). We included species as a fixed effect and replicate nested within watershed as a random effect. Light response and A-C_i curves were taken over multiple days due to time restraints, and we included replicate nested within date as a

random effect. A_{sat} and SLA were log transformed to meet the assumptions of homogeneity of variance and normality. We used the *emmeans* package to test for pairwise comparisons among species (Lenth, 2023).

Results

Long-term species cover

Over 34 years, the relative cover of the focal woody species — as a community — increased from approximately 0 to 45% in the ungrazed watershed and 0 to 20% in the bison-grazed watershed (Fig. 5.1 a-b). This increase was primarily driven by three species: *C. drummondii*, *P. americana*, and *R. glabra*. Other species, *G. triacanthos*, *R. aromatica*, and *R. pensilvanicus* had a much lower cover than the other species but are present in up to 20% of the plots (Fig. 5.1 c-d). The primary encroacher, *C. drummondii*, has become present in over 80% of sampled plots in the ungrazed area. *Z. americanum* did not appear in any plots until 2020, but this species is present at comparable densities to *R. pensilvanicus* and *R. aromatica* across areas burned every 4-years (Ratajczak, *unpublished*; Fig. D.2).

Species biomass allocation

C. drummondii had significantly lower leaf:stem mass ratios than all other species, except for *P. americana*, indicating a lower investment in leaf mass for a given stem mass than the other focal species (Table 5.2). *C. drummondii* also had a higher height:diameter ratio than other species, except for *R. pensilvanicus*. Although the maximum height of *R. pensilvanicus* is much lower. *R. pensilvanicus* and *R. glabra* showed increased leaf investment with the highest

leaf:stem mass and leaf area:aboveground biomass ratios than the other species, but these were only significantly higher than *C. drummondii* and *P. americana* ($P < 0.05$; Table 5.2).

Physiological and functional traits

PCA – PC1 and PC2 accounted for 28.55% and 21.56% of the variation in traits, respectively (Fig. 5.2). Traits related to leaf photosynthetic capacity (A_{sat} , J_{max} , and V_{Cmax}) divided species along PC1, where J_{max} and V_{Cmax} contributed the most to PC1 (25% for both variables). Traits related to leaf structure and chemistry (LDMC, foliar C:N, $\delta^{13}\text{C}$) divided species along PC2, with foliar C:N and LDMC contributing the most to PC2 (31% and 19%, respectively). LCP contributed to 17% of the variation in PC2, which was most likely driven by *G. triacanthos* that has a higher LCP than the other species (Fig. 5.3b). Traits related to drought tolerance (i.e., π_{tlp} and WD) contributed less to the first two PC axes than traits related to carbon capture, but π_{tlp} was associated with *C. drummondii*, *R. pensilvanicus*, and *Z. americanum*, which have higher π_{tlp} than other species (Fig. 5.4a). Two of the most abundant encroaching species, *C. drummondii* and *R. glabra*, fell at opposite ends of PC1 and their mean PC1 coordinate value was significantly different from all other species ($P < 0.05$). The only nonclonal species, *G. triacanthos*, was isolated from the other species on PC2 and had higher variance along both PC1 and PC2 than the other species. The two least abundant species, *R. pensilvanicus* and *Z. americanum*, had different PC2 scores than most other species (*C. drummondii*, *R. aromatica*, and *R. glabra*), likely driven by their lower foliar C:N than the other species (Fig. 5.5a).

Light response and A-Ci response curves – Parameters from photosynthetic response curves varied significantly among species. A_{sat} , V_{Cmax} , and J_{max} were highly correlated and showed similar trends among species, where the most abundant shrub, *C. drummondii*, had

significantly lower values than most other species (Fig. 5.3). The third most abundant shrub, *R. glabra*, had the highest $V_{c_{max}}$ and J_{max} values and were nearly 2-fold higher than *C. drummondii* (36.79 ± 2.03 to 72.47 ± 3.87 and 76.88 ± 2.46 to 156.8 ± 9.10 , respectively). LCP and ϕ did not differ among species, except *G. triacanthos* had higher LCP than most other species (Figure 2b-c).

Water-use / drought tolerance traits – π_{tlp} , $\delta^{13}C$, and woody density varied significantly among species. *C. drummondii* and *R. pensilvanicus* had higher end of growing season π_{tlp} than all other species except for *Z. americanum* (Figure 3a). Variability for wood density was very low, and all species differed significantly from one another except for the species with the lowest wood densities (*R. glabra*, *R. pensilvanicus*, and *Z. americanum*). *P. americana*, *R. aromatica*, and *R. glabra* had higher $\delta^{13}C$ than most other species, but only by $\sim 0.5 - 1 \text{ ‰}$. The tree, *G. triacanthos*, had the lowest $\delta^{13}C$, indicating lower leaf-level water-use efficiency.

Leaf functional traits – Foliar C:N ratios varied more among species (on average 19 to 34) than SLA or LDMC, which were similar for most species (80 to $118 \text{ cm}^2 \text{ g}^{-1}$ and 303 to 388 mg g^{-1} , respectively). *G. triacanthos*, *R. pensilvanicus*, and *Z. americanum* had lower C:N than all other shrub species. The lower C:N ratio in these species was primarily driven by higher leaf nitrogen content, typically associated with high photosynthetic and leaf turnover rates. C:N ratios are reflected in *R. pensilvanicus*, *Z. americanum*, and *G. triacanthos* position on the PCA. *C. drummondii* and *R. glabra*, the two shrub species with the largest differences in photosynthetic capacity, had similar C:N ratios, but *C. drummondii* had $\sim 7\%$ lower leaf carbon content than other species.

Discussion

After conversion to row crop agriculture, encroachment by trees and shrubs is the greatest threat to remaining grasslands worldwide (Wiezorkowski & Lehmann, 2022). Woody encroachment can push grasslands into a woody-dominated alternative stable state that is difficult to reverse (Staver et al., 2011; Collins et al., 2021). Despite this conservation threat, it is unclear why some native woody species have increased substantially in abundance while others have barely increased in abundance. In this study, we provided detailed physiological and functional trait data for the most common shrub species in tallgrass prairie. Our results show that encroaching species encompass a spectrum of growth forms and leaf physiology that reflect differences in resource-use strategies. Some of the most commonly used functional traits, such as SLA and LDMC, showed very little difference between species. However, for almost all traits with meaningful variation between species, the dominant encroacher, *C. drummondii*, had the most extreme values, such as very high height:diameter ratios and very low leaf-level photosynthetic capacity. These interspecific differences differentiate the most abundant encroaching species from the less abundant species and suggest that niche complementarity promotes the encroachment of multiple woody species in mesic grasslands.

No single characteristic differentiated encroaching vs. non-encroaching species, as the ability to encroach reflects an amalgamation of physiological tolerances, seed production and dispersal, phenotypic plasticity, biomass allocation, growth rates, and characteristics of the environment. The same has been seen in several other open ecosystems (Cornwell and Ackerly, 2010, Higgins et al., 2012, Charles-Dominique et al., 2015b). We found strong clustering by species in multivariate space, suggesting each species has a unique suite of traits that differentiate it from other species. Most notably, the most abundant encroaching species, *C.*

drummondii, was isolated from all other species along PC1 (Fig. 5.2). PC1 was primarily driven by differences in photosynthetic response curve parameters, where *C. drummondii* had the lowest $V_{C_{max}}$, J_{max} , and A_{sat} values. Low leaf-level photosynthetic capacity is reflected in previous studies showing this species has low instantaneous photosynthetic rates (McCarron & Knapp, 2001; Wedel et al., 2021). This seemingly low leaf-level assimilation capacity is compensated for by dense canopies (Tooley et al., 2022), high apparent quantum yield (Fig. 5.3c), and deep root systems (Ratajczak et al., 2011; Keen, 2023). Access to deep soil water likely allows the species to buffer changes in growth due to environmental variability (Nippert et al., 2013), resulting in slow but steady carbon capture throughout the growing season.

These results suggest there is a spectrum of woody growth forms: at one end are species that invest in stems to grow tall above the grass canopy (*C. drummondii* and *P. americana*), and at the other end are shorter species that allocate more aboveground biomass to leaves (*R. glabra* and *R. pensilvanicus*). The two most abundant species, *C. drummondii* and *P. americana*, have an aggregated growth form (i.e., phalanx), where stems grow close together. These species invest in height and then form dense canopies (LAI = 8), which has been shown to further promote shrub growth by reducing fuel loads and suppressing fire intensity (Ratajczak et al., 2011; Tooley et al., 2022). At the other end of the spectrum are species that have high leaf:stem mass ratios and exhibit a disperse growth strategy (i.e., guerilla), where stems grow further apart. While shrubs in this category have lower stem densities and relative cover, they have a similar frequency to *P. americana* in ungrazed areas (Fig. 5.1c). These opposing strategies are typically related to nutrient and light acquisition (Fischer & Van Kleunen, 2001; Bittebiere et al., 2020) but also reflect differences in how these growth forms respond to and alter fire regimes. Short species with disperse clonal growth forms have lower LAI values (LAI = 2-3), similar to the

dominant C₄ grasses, and are less effective at shading out herbaceous species (Knapp 1986). Higher leaf:stem mass and greater % foliar carbon suggests that *R. glabra* invests in fewer but larger leaves with higher photosynthetic capacity, as reflected in the PCA (Fig. 5.2; Fig. 5.3). *R. glabra* might also invest more in belowground carbon storage because it thrives in frequent fire despite having to resprout following most fires (Hajny and Hartnett, 2011).

Less dominant species, such as *R. pensilvanicus* and *Z. americanum*, tended to cluster together in the PCA and had similar carbon and water-use traits that differentiated them from other species. These two species had traits associated with a favoring growth over drought tolerance, including higher SLA and π_{lp} and lower wood density and $\delta^{13}C$ (Fig. 5.4 and 5.5). Frequent drought may keep these species at lower relative abundances, particularly if they have more shallow root systems than other woody species.

The relative advantage of these different growth strategies likely shifts across disturbance regimes and resource gradients. Rapid growth rates are a key variable predicting species- and landscape-level woody cover in savanna systems (Wakeling et al., 2011). *R. glabra* likely confers an advantage in more frequently burned grasslands because this species can coexist with grasses and persist at heights similar to the dominant grasses and forbs. This reflects resprouting woody species in frequently burned savannas (fire every 1-2 years) of the Cerrado and lowveld savanna, where short growth forms with high belowground storage and bud protection are common (Charles-Dominique et al., 2015a; Chiminazzo et al., 2023). In contrast, growing tall has allowed *C. drummondii* and *P. americana* to dominate areas burned every 4-years, and any co-dominance with *R. glabra* may be transient through time (Fig. 5.1a,e) as dense canopies may potentially out compete smaller statured shrubs with high light requirements. This mirrors the thicket forming species in the Coastal United States (Brantley and Young, 2007) and South

Africa (Charles-Dominique et al., 2015b), which also form dense canopies and typically do not coexist with herbaceous species. Even within similar growth strategies, species vary in their resource-use and drought tolerance. Difference in π_{tlp} between the two most abundant species, *C. drummondii* and *P. americana*, likely reflects differences in drought tolerance (low π_{tlp}) vs. avoidance (access to deep soil). *C. drummondii* is known to rely on deep soil water (> 30 cm), while *P. americana* relies more on surface soils (McCarron & Knapp, 2001) and is likely more sensitive to extended droughts. These two species provide an excellent example of how filtering and niche complementarity may act on different niche axes, where disturbance and herbaceous competition has filtered for resprouting species with dense canopies, but these species have contrasting drought tolerance strategies.

We found surprisingly little variation in the common plant functional traits SLA and LDMC across species (Fig. 5.5c-d). While some interspecific differences in these traits were significant, variation across species was low compared to coexisting woody species in other systems (Case et al., 2020). These results suggest that these commonly measured leaf traits are either (1) strongly filtered or (2) do not capture meaningful differences among species growth strategies for the shrubs in this system. We did not find expected correlations among SLA, LDMC, and other measured traits. For example, LDMC is a commonly measured trait that is often linked to π_{tlp} (Blumenthal et al., 2020; Laughlin et al., 2020). However, in this study, species showed similar LDMC despite having very different π_{tlp} . Similarly, SLA is typically assumed to be positively correlated with assimilation and growth rates (Reich et al., 1992), but SLA and A_{sat} were orthogonal in multivariate space. While we were limited by the number of species in this study, we certainly wouldn't have forecast key interspecific differences in photosynthetic capacity or leaf-level drought tolerance by examining SLA or LDMC alone.

We posited that we might see evidence of filtering for some traits (resulting in small trait differences between) versus niche partitioning for others (resulting in larger trait variation between species). Based on classic plant functional traits, we would conclude that niche partitioning plays a limited role and instead community-level filtering occurs, because SLA and LDMC values showed marginal variation across species. Other traits show more evidence of niche partitioning, specifically for the most extreme trait values, with *C. drummondii* and *P. americana* on one end of the spectrum, and *R. glabra* on the other. Two of the most abundant species (*C. drummondii* and *R. glabra*) had the most extreme values for leaf:stem mass, height:diameter, maximum photosynthetic rate, and wood density. But interestingly, these two sets of species were extreme in opposite directions, with *C. drummondii* investing more in vertical growth at the expense of maximum photosynthetic rate. Others have noted null, linear and concave down relationships between species traits and species abundance (Cornwell and Ackerly, 2010; Higgins et al., 2012), but to our knowledge, this might be relatively rare example of a concave upwards relationship between trait values and abundance.

Conclusion

We suggest that niche complementarity promotes encroachment of several woody encroaching species in tallgrass prairie. Niche complementarity is a well-cited mechanism driving grassland diversity-stability relationships by increasing ecosystem productivity and resistance to perturbations (Isbell et al., 2015). Across large spatial scales, woody species have been shown to increase plant diversity and productivity, particularly in wetter grasslands compared to drier grasslands (Soliveres et al., 2014). This positive relationship may, in part, be driven by wetter grasslands supporting more woody species than drier grasslands through

increased resource availability and niche complementarity. However, mechanisms maintaining ecosystem function in an encroached state, such as the portfolio effect, may make finding management regimes that suppress an entire community of woody encroaching species more challenging (Loreau et al., 2021). Ultimately, the diversity of encroaching woody species in mesic grasslands may make it more challenging to reach management goals that aim to combat encroachment and restore encroached grasslands.

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Table 5.1 Traits and number of replicates per species measured in this study. We measured seven different woody species in two different watersheds.

	Abbreviation	Trait	Unit	Reps (per watershed)
Functional	SLA	specific leaf area	mg cm ⁻²	10 (5)
	LDMC	leaf dry matter content	mg g ⁻¹	10 (5)
	Foliar C:N	leaf carbon : nitrogen ratio		10 (5)
	wd	wood density	g cm ⁻³	10 (5)
Physiological	$\delta^{13}\text{C}$	leaf water use efficiency	‰	10 (5)
	π_{TLP}	leaf turgor loss point at the end of the growing season	MPa	8 (4)
	A_{sat}	light saturated photosynthetic rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$	8 (4)
	LCP	light compensation point	$\mu\text{mol m}^{-2} \text{s}^{-1}$	8 (4)
	ϕ	apparent quantum yield	mol CO ₂ mol incident photon ⁻¹	8 (4)
	J_{max}	maximum rate of electron transport	$\mu\text{mol m}^{-2} \text{s}^{-1}$	8 (4)
	V_{Cmax}	maximum rate of carboxylation	$\mu\text{mol m}^{-2} \text{s}^{-1}$	8 (4)

Table 5.2 Mean \pm 1 SE and median values for leaf : stem mass, leaf area : aboveground biomass, and height : diameter ratios for each focal species. Letters indicate significant differences ($P < 0.05$) among species using the Dunn's test with a Bonferroni correction.

Species	Growth form	Leaf : stem mass		Leaf area : aboveground biomass		height : diameter	
		mean	median	mean	median	mean	median
<i>Cornus drummondii</i>	aggregate	0.32 \pm 0.01	0.31 ^a	3.73 \pm 0.14	3.42 ^a	2.31 \pm 0.07	2.23 ^a
<i>Prunus americana</i>	aggregate	0.44 \pm 0.04	0.44 ^{abe}	3.59 \pm 0.26	3.91 ^{ab}	1.66 \pm 0.12	1.56 ^b
<i>Rhus glabra</i>	disperse	1.16 \pm 0.13	1.08 ^c	4.99 \pm 0.30	4.79 ^{bc}	1.32 \pm 0.14	1.15 ^b
<i>Rubus pensilvanicus</i>	disperse	0.97 \pm 0.07	1.02 ^{cd}	8.49 \pm 0.67	7.89 ^c	1.84 \pm 0.10	1.77 ^{ab}
<i>Rhus aromatica</i>	intermediate	0.62 \pm 0.07	0.53 ^{bd}	3.88 \pm 0.23	4.03 ^{ab}	1.49 \pm 0.10	1.42 ^b
<i>Zanthoxylum americana</i>	intermediate	0.65 \pm 0.05	0.63 ^{bcde}	4.53 \pm 0.18	4.62 ^{abc}	1.65 \pm 0.19	1.28 ^b
<i>Gleditsia triacanthos</i>	Single-stemmed tree	-	-	-	-	-	-

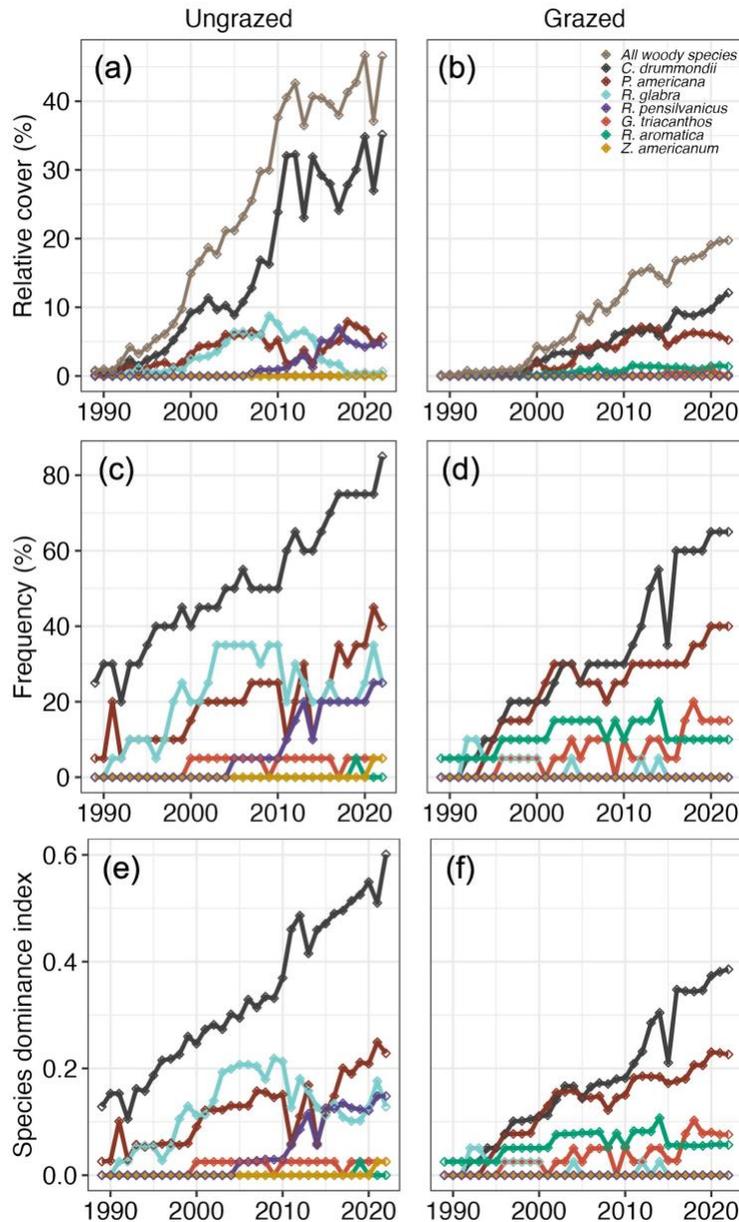


Figure 5.1 Relative cover (a-b), frequency (c-d), and species dominance index (e-f) of for the focal woody species from 1989 – 2022. Relative cover of each species was calculated using all species, woody and herbaceous, present in the plots ($n = 20$ plots per ungrazed and grazed areas). The tan line in panels a and b is the total relative cover of all woody species, including all trees and shrubs that grow above the grass canopy. Frequency was calculated as the percentage of plots where each species occurred. Species dominance index is calculated as the average relative cover and frequency, where an index closer to 1 means the species has high cover and is found in a high number of plots (Avolio et al., 2019).

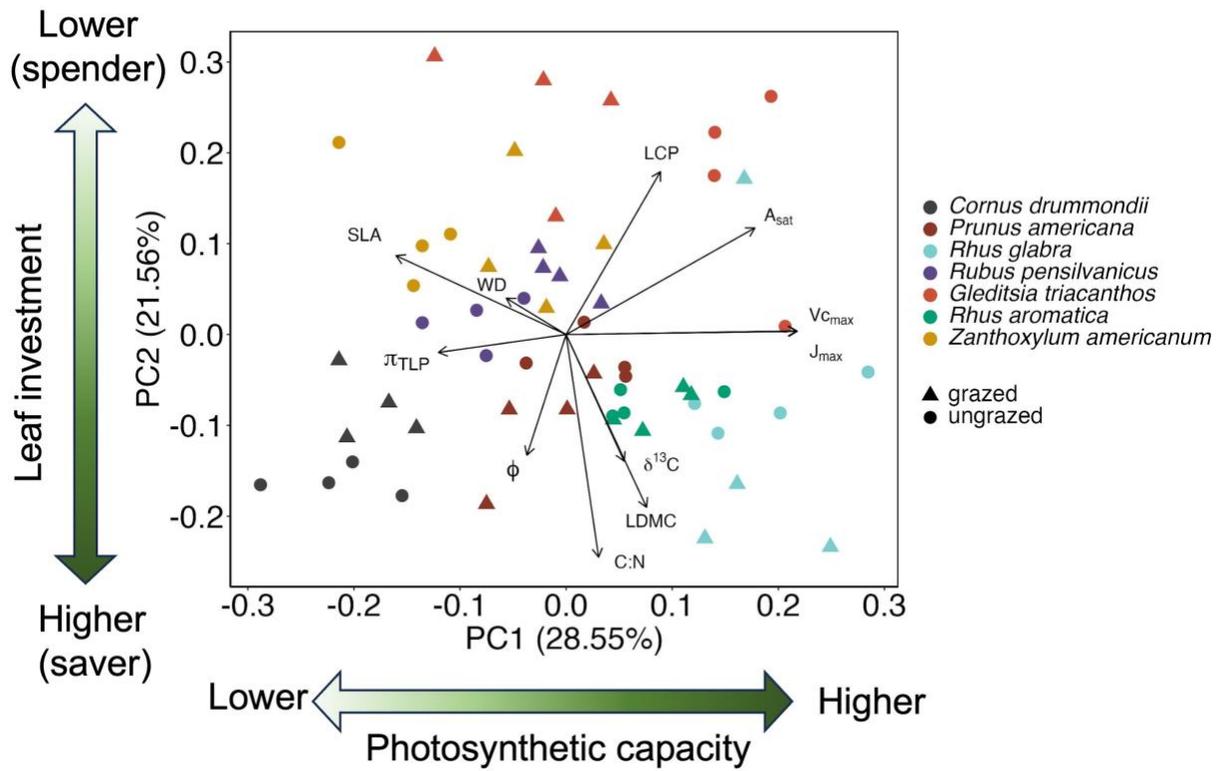


Figure 5.2 Principal components analysis of the seven focal species and measured physiological and function traits (n = 8 per species). Colors represent species and shapes represent the two locations sampled, one grazed (triangles) and one ungrazed (circles).

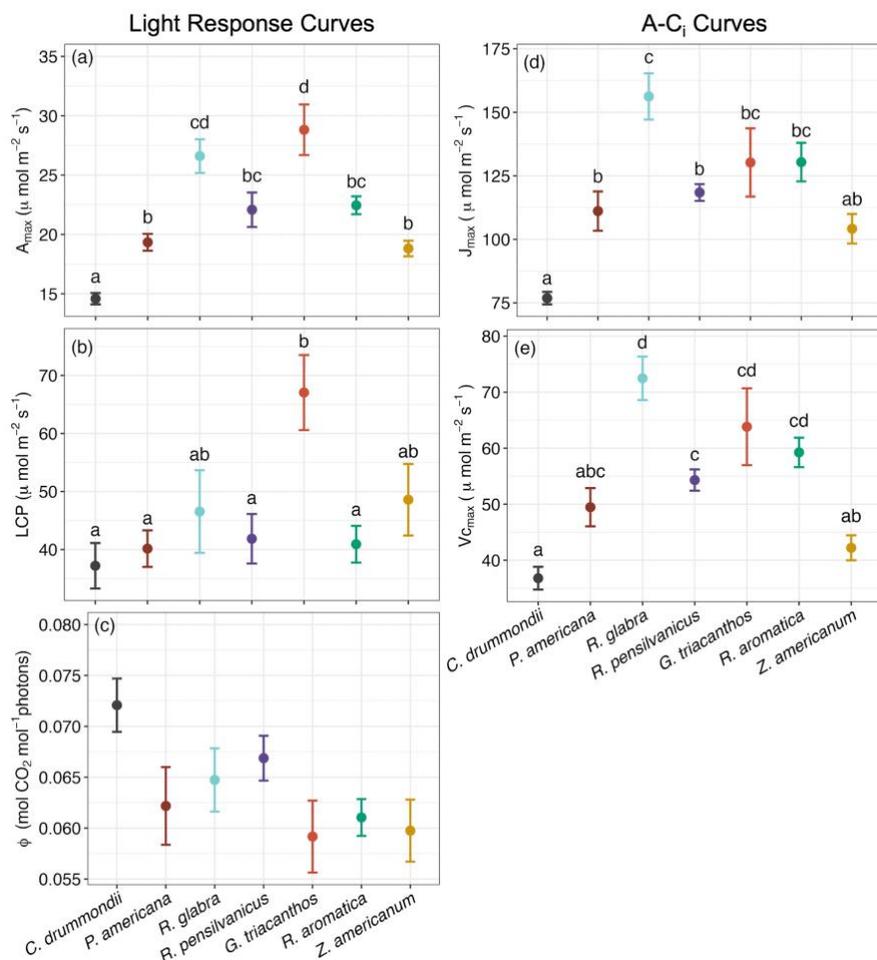


Figure 5.3 Parameters from light response (a-c) and A-C_i curves (d-e; mean \pm 1SE) of the seven focal shrub species (n = 8 curves per species). (a) light saturated photosynthetic rates, (b) light compensation point, (c) apparent quantum yield, (d) maximum electron transport rate, and (e) maximum rate of carboxylation. Letters represent significant differences among species (P < 0.05).

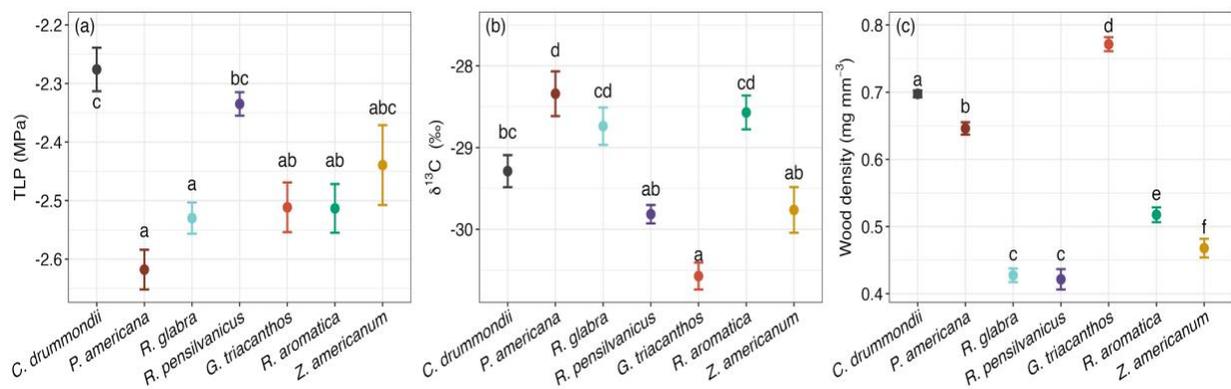


Figure 5.4 Traits related to water-use and drought tolerance of the seven focal woody species. (a) turgor loss point, (b) integrated leaf water-use efficiency, and (c) wood density. Letters represent significant differences among species ($P < 0.05$).

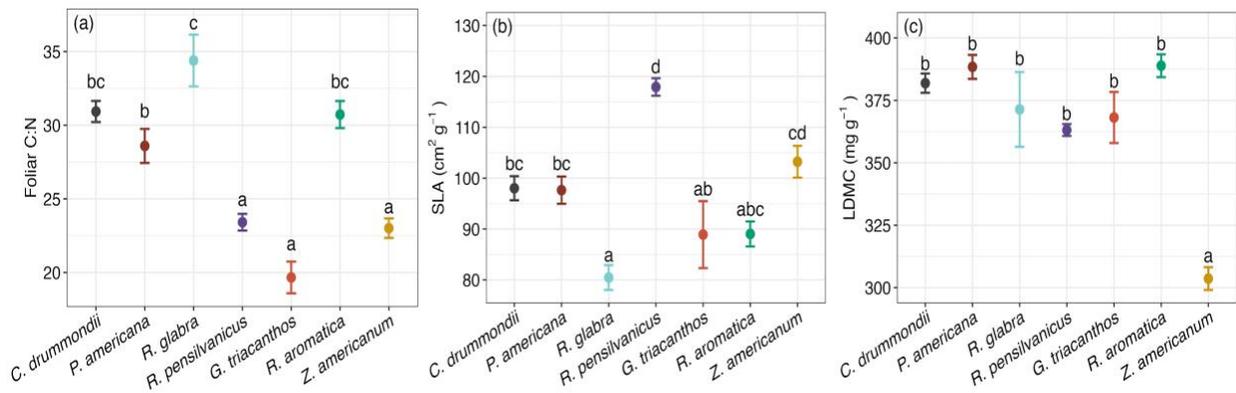


Figure 5.5 Leaf functional traits of the seven focal woody species. (a) foliar C:N, (b) specific leaf area, and (c) leaf dry matter content. Letters represent significant differences among species ($P < 0.05$).

Chapter 6 - Conclusion

Grasslands and savannas are highly diverse and heterogeneous ecosystems shaped by environmental stochasticity and the interactions between trees and grasses (Scholes and Archer, 1997). Trees and grasses are highly divergent growth forms and are often (appropriately) analyzed as singular functional types to generalize ecological patterns and processes across large spatial and temporal scales. Nevertheless, woody plants native to grassy ecosystems are diverse and use various strategies to establish, grow, and persist in these disturbance-prone ecosystems (Bond and Midgley, 2001; Bond, 2004; Charles-Dominique et al., 2015). For example, variation in rooting depth and hydraulic architecture can explain species responses to drought and competition with grasses (Zhou et al., 2020; Wargowsky et al., 2021). Recognizing the diverse strategies used by woody plants is necessary for understanding the mechanisms driving tree-grass interactions and predicting the consequences of climate and land-use change across resource gradients. In this dissertation, I compared the resource-use strategies of common woody species in lowveld savanna and tallgrass prairie to achieve three main objectives: (1) understand how rainfall variability impacts savanna tree growth and competition with co-existing grasses (Chapters 2 and 3), (2) explore the effectiveness and community-level impacts of repeated tree clearing in response to woody encroachment (Chapter 4) and (3) characterize the growth strategies of the most abundant encroaching woody species in tallgrass prairie (Chapter 5).

In the first half of my dissertation, I investigated how rainfall distribution impacts above and belowground savanna dynamics using an *in situ* irrigation experiment in lowveld savanna (Limpopo Province, South Africa). In this experiment, irrigation was used to manipulate the frequency, magnitude, depth, and timing of water availability. I found that irrigation increased soil water content at 10 and 50 cm soil depths, regardless of how it was distributed. This increase

in soil water beyond the grass-rooting zone benefited deep-rooted trees and increased tree growth in all irrigated plots, no matter the frequency or intensity of irrigation. However, when I modeled tree growth over time, trees that experienced frequent irrigation, representing small but frequent rainfall events, grew into adult-size classes the fastest in four of six tree species. Additionally, we found that interspecific differences in leaf-physiology reflected growth differences among tree species. Most notably, the fastest growing species, *A. nigrescens*, and the slowest growing species, *C. mopane*, had divergent water-use strategies both above and belowground. *A. nigrescens* had high stomatal conductance that responded to changes in soil moisture more so than the rates of other species. Moreover, this species shifted water uptake from deep to shallow soil depths when soil moisture was higher in surface soils. Together the results from Chapters 2 and 3 suggest that grasses do not ‘get all the water, all the time,’ at least in savannas with sandy soils. This benefits deep rooted tree saplings and highlights the importance of niche partitioning in sapling establishment. Finally, the results from these two chapters show that water-spending tree species are likely to be more competitive for water and confer an advantage in pulse driven ecosystems, such as semi-arid savannas.

In the second half of my dissertation, I focused directly on the expansion of woody plants into grassy ecosystems (i.e., woody encroachment). Woody encroachment is one of the largest threats to grassland and savannas worldwide (Venter et al., 2018; Wieczorkowski and Lehmann, 2022). Once established, the traits of the dominant woody encroachers make encroachment extremely difficult to reverse (Ratajczak et al., 2014; Collins et al., 2021). In **Chapter 4**, I assessed the effectiveness of repeated tree clearing as a tool to mitigate bush encroachment in a semi-arid savanna in South Africa (Mthimkhulu Game Reserve, Limpopo Province). Clearing reduced tree belowground starch concentrations but had limited effects on tree survival in the

first 2-3 years after initial clearing. However, 80% of trees were dead after 7 years. In addition, cleared plots had greater soil moisture and grass biomass than the control treatment and dung counts showed impala and buffalo visited the cleared treatment more frequently. These results highlight the resilience of resprouting woody plants to even highly intensive management practices, and the need for decadal-scale management plans to successfully manage woody cover and the negative impacts of woody encroachment.

In **Chapter 5**, I characterized the growth and resource-use strategies of the most abundant encroaching woody species in tallgrass prairie. I found evidence that these encroaching species span a spectrum of growth forms and resource-use strategies, with two of the most abundant species falling at opposite ends of this spectrum. Moreover, the most abundant encroaching woody species, *Cornus drummondii*, had the most extreme trait values, with very high height:diameter ratios and very low leaf-level photosynthetic capacity. These results suggest that niche complementarity facilitates the encroachment of several woody species in mesic grasslands, and this diversity of resource-use strategies may make it more challenging to reach management goals that aim to combat woody encroachment.

Together, the chapters in this dissertation contribute to a growing body of research aimed at understanding the influence of interspecific differences among woody plants on grassland vegetation structure responses to climate and land use change. Compared to the herbaceous species, woody plants in grassy ecosystems respond slowly to environmental change. However, my research suggests that some woody species respond quickly to environmental variability, a characteristic likely associated with woody encroaching species. Trees with high growth rates can capitalize on resource pulses, facilitating rapid growth and long-term persistence on the landscape. These ‘windows of opportunity’ (as described in Yu et al., 2017) allow for the

establishment and rapid expansion of woody species, and consequently change disturbance regimes to promote their growth (Ratajczak et al., 2014; Nackley et al., 2017). In sum, recognizing the various resource-use strategies of the dominant woody species in grassy ecosystems will help us understand variations in woody cover across precipitation gradients and how these ecosystems will respond to global change.

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Appendix A - Chapter 2 supplementary material

Table A.1 Soil volumetric water content linear mixed model results for each treatment at 10 cm and 50 cm depths. Separate models were run for each year and depth.

Model: VWC ~ Treatment + (1|Block)

Depth	Predictor	2020		2021		2022	
		<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
10 cm	intercept	14.959	<0.001	12.102	<0.001	11.583	<0.001
	surface	4.978	<0.001	5.476	<0.001	5.427	<0.001
	depth	1.216	0.24406	2.296	0.0375	2.339	0.0345
	pulse	3.972	0.0013	2.71	0.0168	2.058	0.0585
	season	5.212	<0.001	4.512	<0.001	0.552	0.5898
50 cm	intercept	13.694	<0.001	10.358	<0.001	9.839	<0.001
	surface	3.626	0.0026	3.521	0.0033	4.41	<0.001
	depth	3.838	0.0016	3.717	0.002	4.974	<0.001
	pulse	3.566	0.003	2.804	0.0138	3.665	0.0024
	season	4.295	<0.001	4.188	<0.001	2.616	0.0201

Table A.2 Mean % increase in VWC for each treatment relative to the control plots for each hydrological year and growing season. % increase was calculated for block and then averaged across blocks. Hydrological year is missing for 2022 because of missing data from May – August.

	Treatment	Hydrological Year (%)		Growing season (%)	
		August 1 – July 31		October 1 – May 1	
		10 cm	50 cm	10 cm	50 cm
2020	surface	42.5	37.5	47.5	38.8
	depth	11.8	41.2	6.0	50.0
	pulse	35.4	38.1	40.4	44.0
	season	63.0	55.8	1.8	15.4
2021	surface	50.8	48.2	66.0	55.1
	depth	24.1	59.2	30.2	89.2
	pulse	30.6	45.0	37.7	62.7
	season	56.9	63.8	8.5	23.5
2022	surface	--	--	76.8	71.4
	depth	--	--	26.8	100
	pulse	--	--	29.3	64.8
	season	--	--	5.5	22.7

Table A.3 Output for change in height (cm) and diameter (mm) of tree saplings in the irrigation experiment.

Model: $\log(\text{change} + 2) \sim \text{Species} * \text{Treatment} + (1|\text{Block})$

Predictor	Change in height (cm)		Change in diameter (mm)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	7.55	<0.001	2498	<0.001
Species	31.65	<0.001	76.36	<0.001
Treatment	7.29	<0.001	2.72	0.029
Species*Treatment	1.04	0.403	1.31	0.165

Table A.4 Output for stomatal conductance (g_{sw}) in response to changes in VWC at 10 cm soil depth.

Model: $g_{sw} \sim \text{VWC} * \text{Species} + (1|\text{DOY:Treatment})$

Predictor	2022		2023	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	3.61	0.058	36.93	<0.001
VWC @ 10 cm	44.66	< 0.001	24.84	<0.001
Species	1.11	0.356	7.36	<0.001
VWC*Species	7.34	< 0.001	4.67	<0.001

Table A.5 Output for stomatal conductance in response to treatments in 2023.

Model: $\text{sqrt}(g_{sw}) \sim \text{Species} * \text{Treatment} + \text{DOY} + (\text{Block:TreeID})$

Predictor	2023	
	<i>F</i>	<i>P</i>
Intercept	429.43	< 0.001
Treatment	3.06	0.017
Species	17.73	< 0.001
DOY	72.48	< 0.001
Treatment*Species	0.96	0.513

Table A.6 Output for $\delta^{13}\text{C}$ and foliar C:N in 2022 and 2023.
 Model: Response ~ Species * Treatment + (1|Plot)

Response	Predictor	2022		2023	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
$\delta^{13}\text{C}$	Intercept	11873	< 0.001	17185	< 0.001
	Species	17.66	< 0.001	14.03	< 0.001
	Treatment	0.920	0.483	0.40	0.802
	Species*Treatment	1.19	0.300	1.36	0.161
C:N	Intercept	2431	< 0.001	38893	< 0.001
	Species	164.49	< 0.001	142.10	< 0.001
	Treatment	0.64	0.639	0.22	0.919
	Species*Treatment	1.25	0.250	1.04	0.421

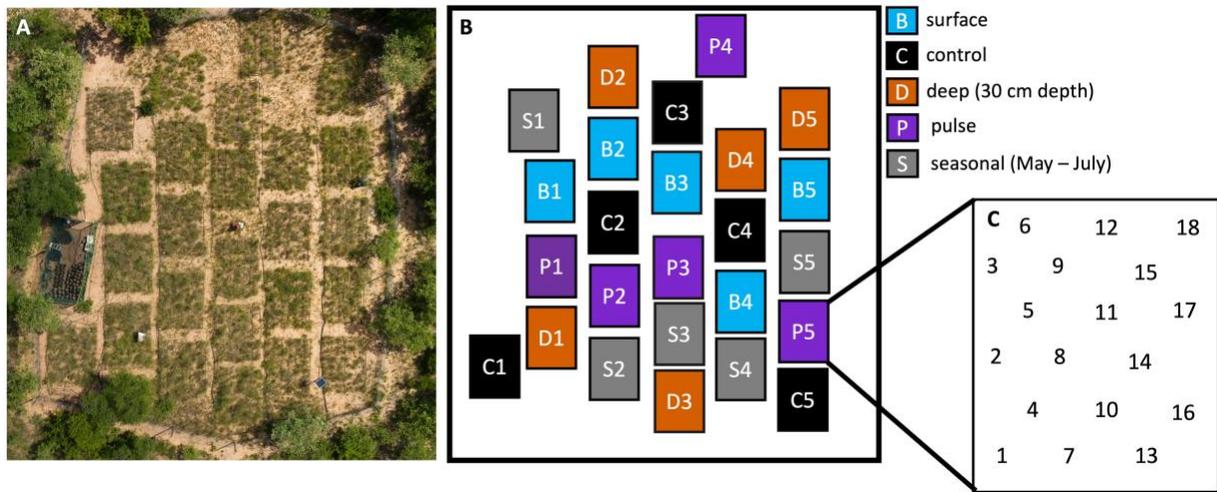


Figure A.1 (a) Aerial view of irrigation experimental plots taken in 2021. Each plot is 4.2 x 3 m. (b) Experiment plot setup. Plots were randomly assigned to one of 5 irrigation treatments (N = 25 plots). Irrigation treatments are identified by the color and letter within each plot (B = surface; C= control; D= depth; P=pulse; S=season). Plots are arranged in 5 blocks identified by the number in the plot ID. (c) Schematic of an individual plot with 18 trees planted. Three replicates of six tree species were planted within each plot in 2017 (N = 450 trees). Grasses were seeded among trees in 2018.

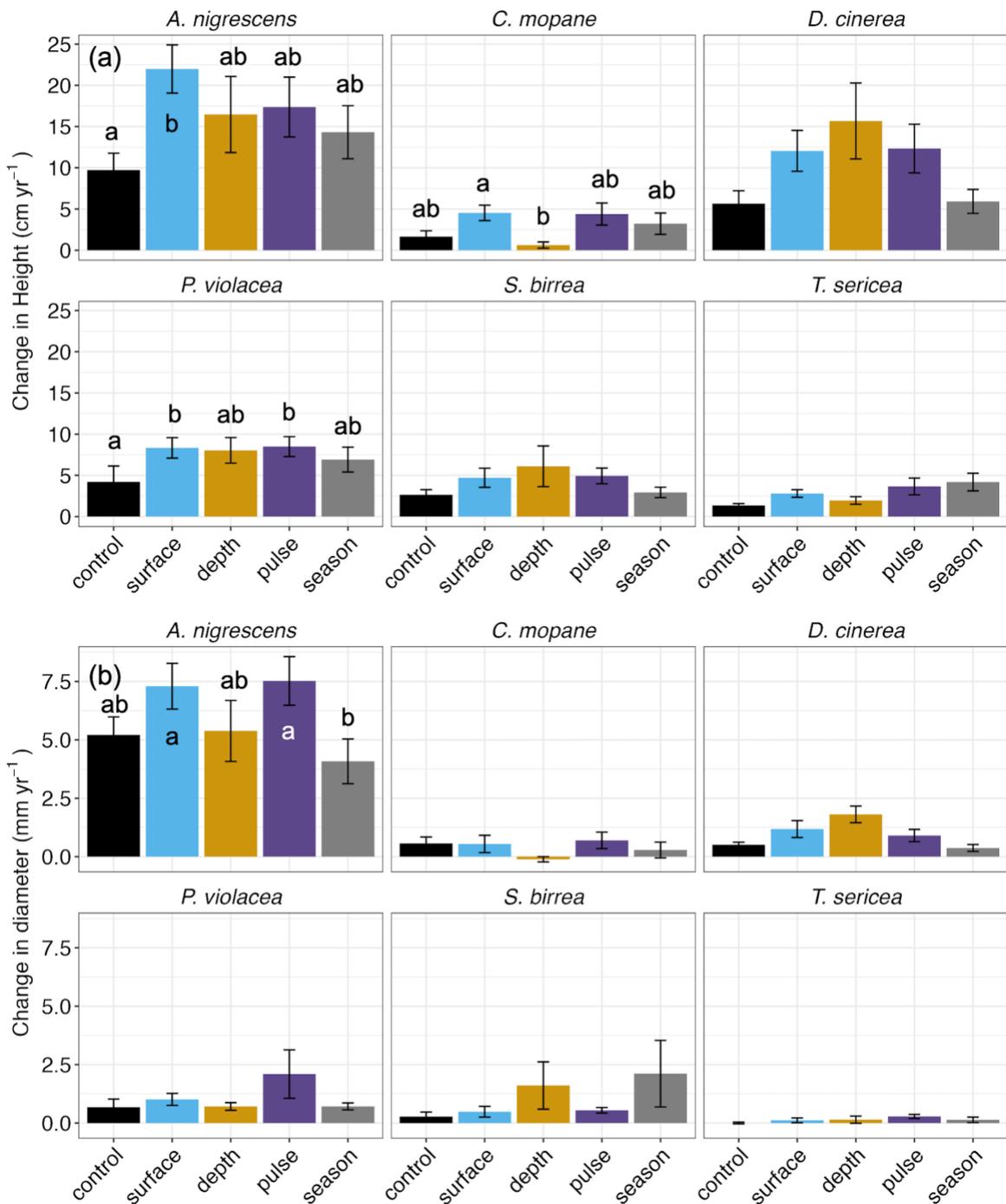


Figure A.2 (a) Change in height and (b) diameter for each species in each treatment. Bars represent mean ± 1 SE. Values for each individual tree were calculated for each year (2020 – 2021, 2021- 2022, 2022-2023). And then averaged across years. Letters represent significant differences among treatments for each species ($P < 0.05$).

Appendix B - Chapter 3 supplementary material

Table B.1 Output for grass biomass, shallow : deep root biomass ratio, and above : belowground biomass ratio for 2022 and 2023

Model: $\log(\text{biomass}) \sim \text{Treatment} * \text{Year} + (1|\text{Plot})$

Model: $\text{ratio} \sim \text{Treatment} * \text{Year} + (1|\text{Plot})$

Predictor	Grass biomass		shallow:deep		above:belowground	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	6763	< 0.001	21.27	0.085	130.31	< 0.001
Treatment	5.30	0.002	0.644	0.632	15.46	0.003
Year	14.20	< 0.001	8.033	0.005	37.53	< 0.001
Treatment*Year	0.34	0.843	1.22	0.307	8.72	0.068

Table B.2 Output for belowground net primary productivity in 2022 and 2023.

Model: $\text{BNPP} \sim \text{Treatment} * \text{Depth} * \text{Year} + (1|\text{Plot:Replicate})$

Predictor	F	DF	P
Intercept	8260	1	<0.001
Treatment	0.9002	4	0.472
Depth	42.7704	1	< 0.001
Year	9.6336	1	0.002
Treatment*Depth	0.6402	4	0.634
Treatment*Year	6.0430	4	< 0.001
Depth*Year	6.3156	1	0.0132
Treatment*Depth*Year	1.1108	4	0.3543

Table B.3 Output for C₃ and C₄ root biomass estimated from root ingrowth cores in 2021-2022.
Model: log(biomass+1) ~ treatment * depth + (1|Plot : Replicate)

Predictor	C ₃		C ₄	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	64.71	<0.001	526.60	<0.001
Treatment	1.47	0.217	1.03	0.396
Depth	10.66	0.002	9.78	0.003
Treatment*Depth	2.14	0.091	0.96	0.439

Table B.4 Output for inorganic N availability ($\mu\text{g g}^{-1}$ resin) measured with resin bags in 2021-2022 and 2022-2023.

Model: log(N) ~ treatment + (1|Plot)

Year	Predictor	NO ₃ ⁻ - N		NH ₄ ⁺ - N		NO ₃ ⁻ : NH ₄ ⁺		Total N	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
2021-2022	Intercept	223.03	<0.001	63.20	<0.001	49.84	<0.001	360.28	<0.001
	Treatment	1.72	0.189	4.54	0.01	9.790	<0.001	0.067	0.990
2022-2023	Intercept	309.29	<0.001	45.42	<0.001	61.95	<0.001	704.20	<0.001
	Treatment	3.67	0.022	7.87	<0.001	10.95	<0.001	0.806	0.536

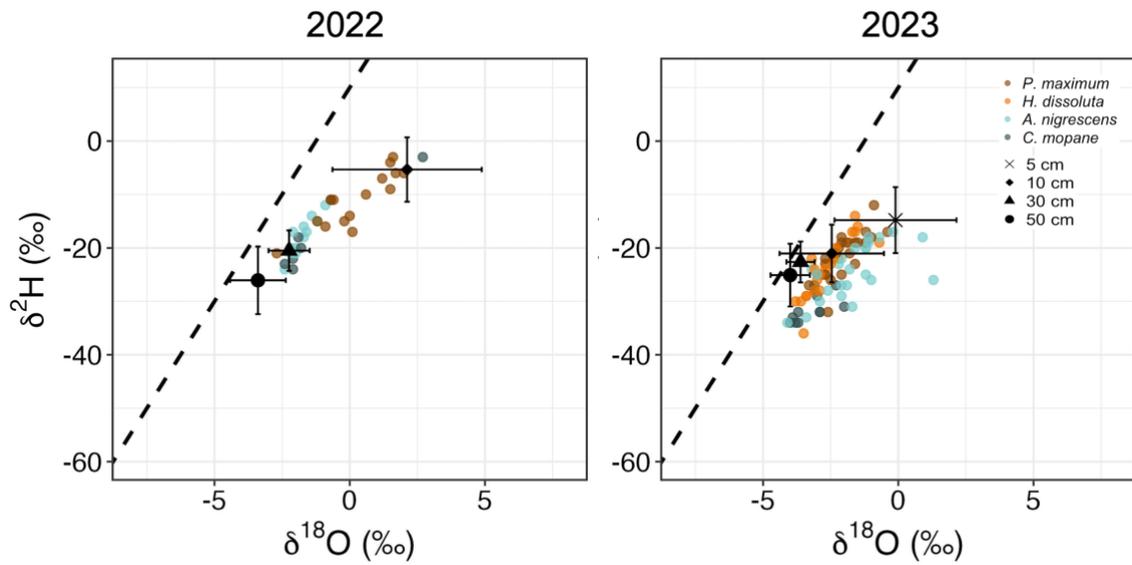


Figure B.1 δD and $\delta^{18}O$ values for C_4 grass species (red and orange), tree saplings (light and dark blue) from the experimental plots in 2022 and 2023. Shapes represent average \pm 1SE isotopic values for each soil depth soils.

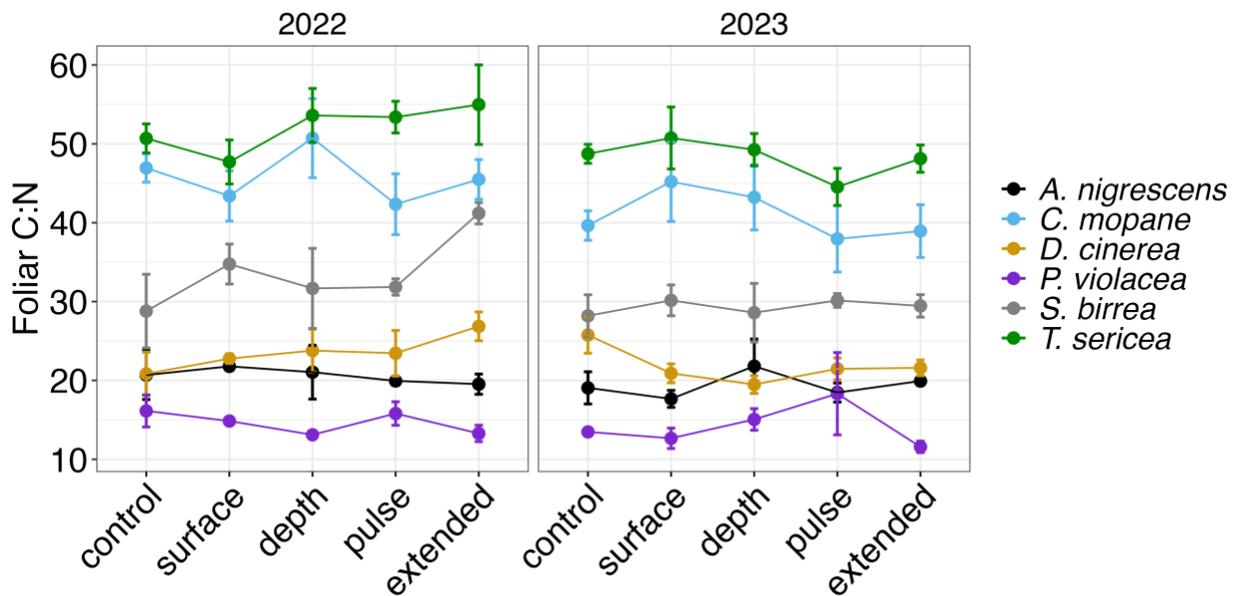


Figure B.2 (a) Foliar C:N concentrations for each species in each treatment in 2022 and 2023 (mean \pm 1 SE).

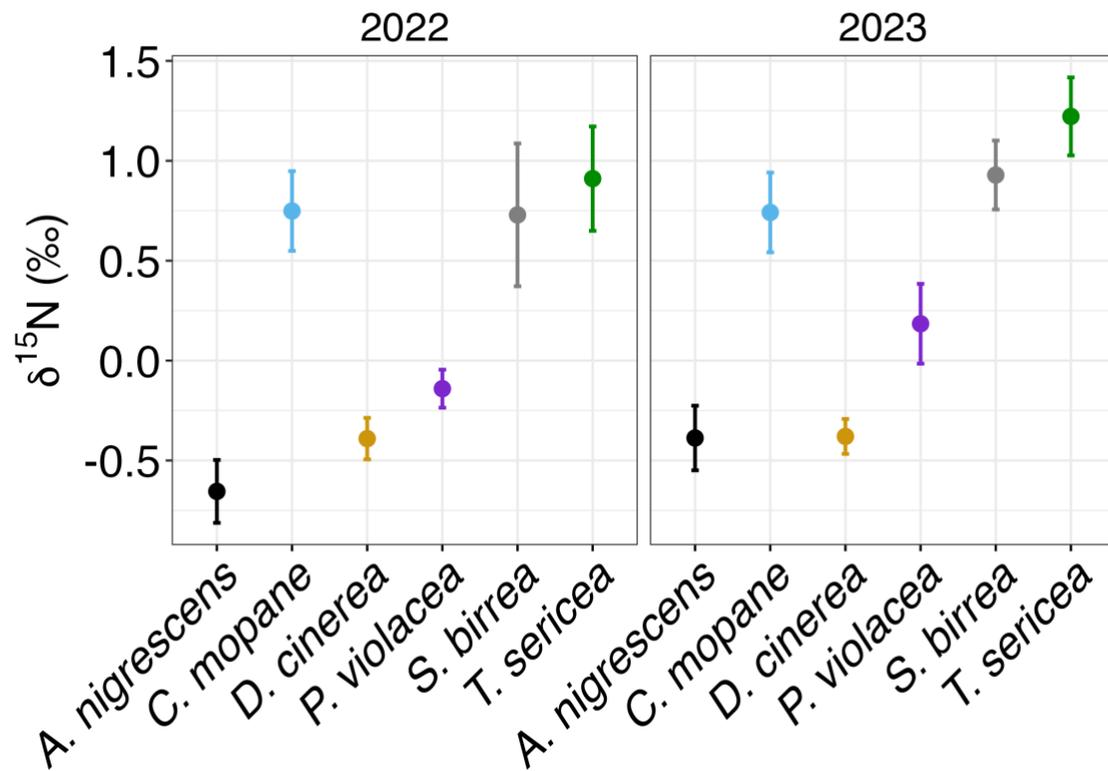


Figure B.3 Foliar $\delta^{15}\text{N}$ for each species in each treatment in 2022 and 2023 (mean \pm 1 SE). *A. nigrescens*, *D. cinerea*, and *P. violacea* are all N-fixing species.

Appendix C - Chapter 4 supplementary material

Table C.1 Average percent of sand, silt, clay, total inorganic nitrogen, and total organic carbon in cleared and control plots at 10, 30, 50, and 100 cm soil depths. Samples were taken in March 2020 (n = 3 per plot).

Treatment	Depth (cm)	Sand	Silt	Clay	N	TOC
Cleared	10	70.6	16.6	12.6	0.06	0.58
	30	68.0	15.3	16.6	0.06	0.43
	50	62.0	18	20	0.04	0.36
	100	65.3	14	20.6	0.05	0.29
	average	66.9 ± 24.6	15.8 ± 1.0	17.2 ± 3.5	0.05 ± 0.008	0.48 ± 1.42
Control	10	63.3	19.3	17.3	0.06	0.72
	30	60.6	18.6	20.6	0.05	0.51
	50	52.6	19.3	28.0	0.09	0.42
	100	53.3	18.6	28.0	0.04	0.39
	average	57.5 ± 17.2	19.0 ± 0.5	23.5 ± 8.2	0.06 ± 0.01	0.45 ± 2.0

Table C.2 ANOVA output for soil volumetric water content at 10, 30, and 80 cm soil depths. Models only included data during the growing season (November- April). Growing season refers to each wet season from November – April. Block refers to paired control and cleared plots and accounts for spatial variability among blocks.

Model: VWC ~ treatment * growing season + (1|block)

Soil Depth	Predictors	Df	<i>F</i>	<i>P</i>
10 cm	intercept	1	98.32	0.003
	treatment	1	10.23	0.001
	growing season	4	157.40	<0.001
	treatment * growing season	4	14.48	<0.001
30 cm	intercept	1	46.15	0.014
	treatment	1	2.49	0.114
	growing season	4	155.45	<0.001
	treatment * growing season	4	15.81	<0.001
80 cm	intercept	1	16.42	0.051
	treatment	1	0.56	0.455
	growing season	4	104.91	<0.001
	treatment * growing season	4	23.53	<0.001

Table C.3 ANOVA output for frequency of mopane tree size classes.

Model: $\log(\text{frequency}+1) \sim \text{treatment} * \text{size class} + (1|\text{year}:\text{plot})$

Predictors	Df	F	P
intercept	1	9.89	0.002
treatment	1	22.39	<0.001
size class	3	1.04	0.380
treatment*size class	3	6.10	<0.001

Table C.4 ANOVA output for grass biomass, distance to nearest perennial grass tufts, and width of perennial grass tuft.

Models: $\text{grass biomass} \sim \text{treatment} * \text{year} + (1|\text{plot}:\text{quadrat})$

$\log(\text{distance} + 1) \sim \text{treatment} * \text{year} + (1|\text{plot})$

$\log(\text{width} + 1) \sim \text{treatment} * \text{year} + (1|\text{plot})$

Response	Predictors	Df	F	P
grass biomass	intercept	1	5.30	0.023
	treatment	1	0.50	0.480
	year	5	29.32	<0.001
	treatment*year	5	2.60	0.027
distance to nearest perennial grass tuft	intercept	1	194.14	<0.001
	treatment	1	2.45	0.145
	year	3	9.91	<0.001
	treatment*year	3	5.66	0.003
width of perennial grass tuft	intercept	1	400.99	<0.001
	treatment	1	11.82	0.001
	year	3	8.79	<0.001
	treatment*year	3	3.82	0.018

Table C.5 Total standardized dung counts of herbivore species. Transects were surveyed approximately every 1-2 months from 2016-2022. The lengths of the transects varied in a few control plots and all dung counts were standardized to a 50 m transect.

Species	Control	Cleared
Elephant	271.8	261.7
Impala	80.3	187.2
Buffalo	79.4	151
Giraffe	82.5	80.5
Kudu	16.8	11.7
Zebra	6.6	9.6
Hippo	7.91	4.3
Duiker	8.08	1.7
Steenbok	2	5.01
Waterbuck	1.78	3.67
Wildebeest	0.76	0
Total	555.39	712.71

Table C.6 Generalized linear mixed effects model for herbivore dung counts. In each plot, dung counts were standardized to 50 m transects and summed across two transects. We estimated dung deposition per day, by dividing the standardized dung counts by the number of days in between sampling events and then averaged the dung counts across all sampling dates for each plot.

Models: estimated deposition per day ~ treatment + (1|plot), family=tweedie(link= "log")

Species	Predictors	Estimate	Std Error	Z	P
Elephant	intercept	-2.354	0.217	-10.842	<0.001
	Cleared treatment	0.093	0.306	0.303	0.762
Impala	intercept	-4.086	0.214	-19.079	<0.001
	Cleared treatment	0.879	0.281	3.133	0.0017
Buffalo	intercept	-4.165	0.244	-17.082	<0.001
	Cleared treatment	0.661	0.327	2.023	0.0431
Giraffe	intercept	-4.076	0.236	-17.291	<0.001
	Cleared treatment	-0.031	0.329	-0.093	0.926
Kudu	intercept	-5.585	0.383	-14.59	<0.001
	Cleared treatment	-0.324	0.572	-0.567	0.571
Zebra	intercept	-6.431	0.585	-10.989	<0.001
	Cleared treatment	0.014	0.803	0.018	0.986
Hippo	intercept	-6.633	0.050	-13.154	<0.001
	Cleared treatment	-0.688	0.696	-0.988	0.323
Duiker	intercept	-7.161	1.629	-4.395	<0.001
	Cleared treatment	-1.582	1.942	-0.815	0.415
Steenbok	intercept	-7.569	0.655	-11.564	<0.001
	Cleared treatment	0.372	0.853	0.437	0.662

Table C.7 ANOVA output for mopane stem nonstructural carbohydrate concentration, including glucose, sucrose, and starch.

Model: NSC ~ treatment * year + (1|plot)

Response	Predictors	Df	<i>F</i>	<i>P</i>
glucose	intercept	1	149.383	< 0.001
	treatment	1	0.173	0.683
	year	2	0.406	0.668
	treatment*year	2	2.833	0.066
sucrose	intercept	1	93.267	< 0.001
	treatment	1	0.403	0.532
	year	2	15.535	< 0.001
	treatment*year	2	15.160	< 0.001
starch	intercept	1	47.712	< 0.001
	treatment	1	11.695	0.002
	year	2	2.619	0.080
	treatment*year	2	12.039	< 0.001

Table C.8 ANOVA output for functional rooting depth of mopane trees and grasses. Years were analyzed separately.

Model: PC1 ~ treatment * growth form + (1|plot)

Year	Predictors	Df	<i>F</i>	<i>P</i>
2015	intercept	1	31.923	<0.001
	growth form	1	35.691	<0.001
	treatment	1	0.094	0.763
	growth form * treatment	1	1.520	0.235
2017	intercept	1	2.038	0.211
	growth form	1	9.537	0.004
	treatment	1	1.615	0.259
	growth form * treatment	1	1.860	0.181
2018	intercept	1	3.726	0.092
	growth form	1	5.885	0.021
	treatment	1	0.330	0.583
	growth form * treatment	1	0.851	0.367

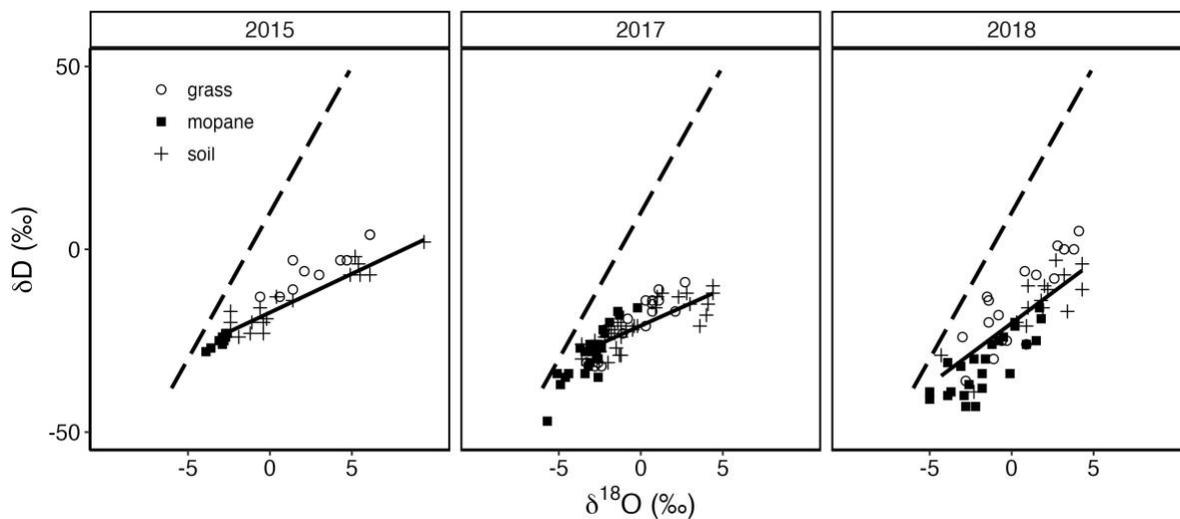


Figure C.1 δD and $\delta^{18}O$ values for C₄ grasses (open circles), mopane trees (closed squares), and soil (plus symbols) in 2015, 2017, and 2018. Dashed line represents the meteoric water line, and the solid line is the regression line for the soil samples.

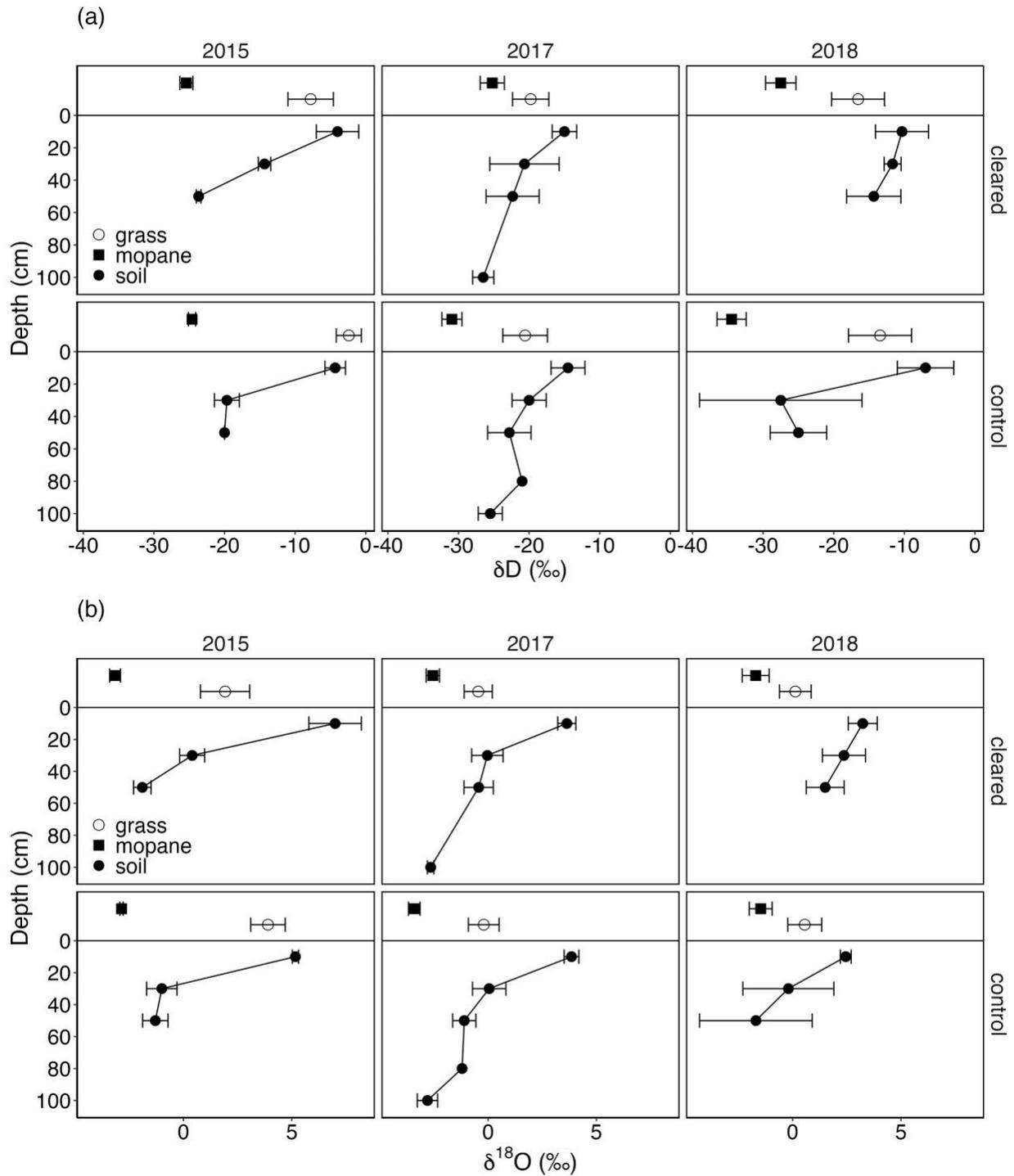


Figure C.2 (a) δD and (b) $\delta^{18}O$ values for C_4 grasses (open circles), mopane trees (closed squares), and soil (closed circles) by depth in 2015, 2017, and 2018.

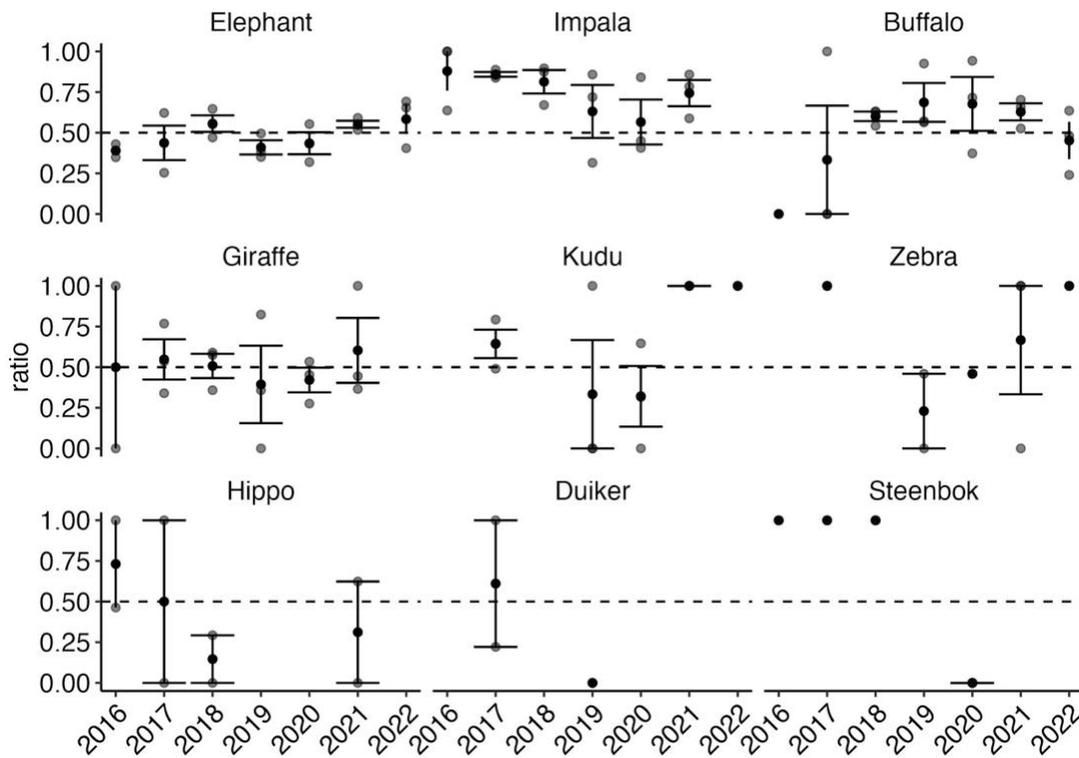


Figure C.3 Habitat preference of various herbivores from 2016-2022. Preference ratio was calculated from the total standardized dung counts for each species in each plot (n=3). Grey points are the preference ratio for each plot and black points are the mean \pm 1SE. Preference ratio ranges from 0 to 1, where 1 is complete preference for cleared plots and 0 is complete preference for control plots.

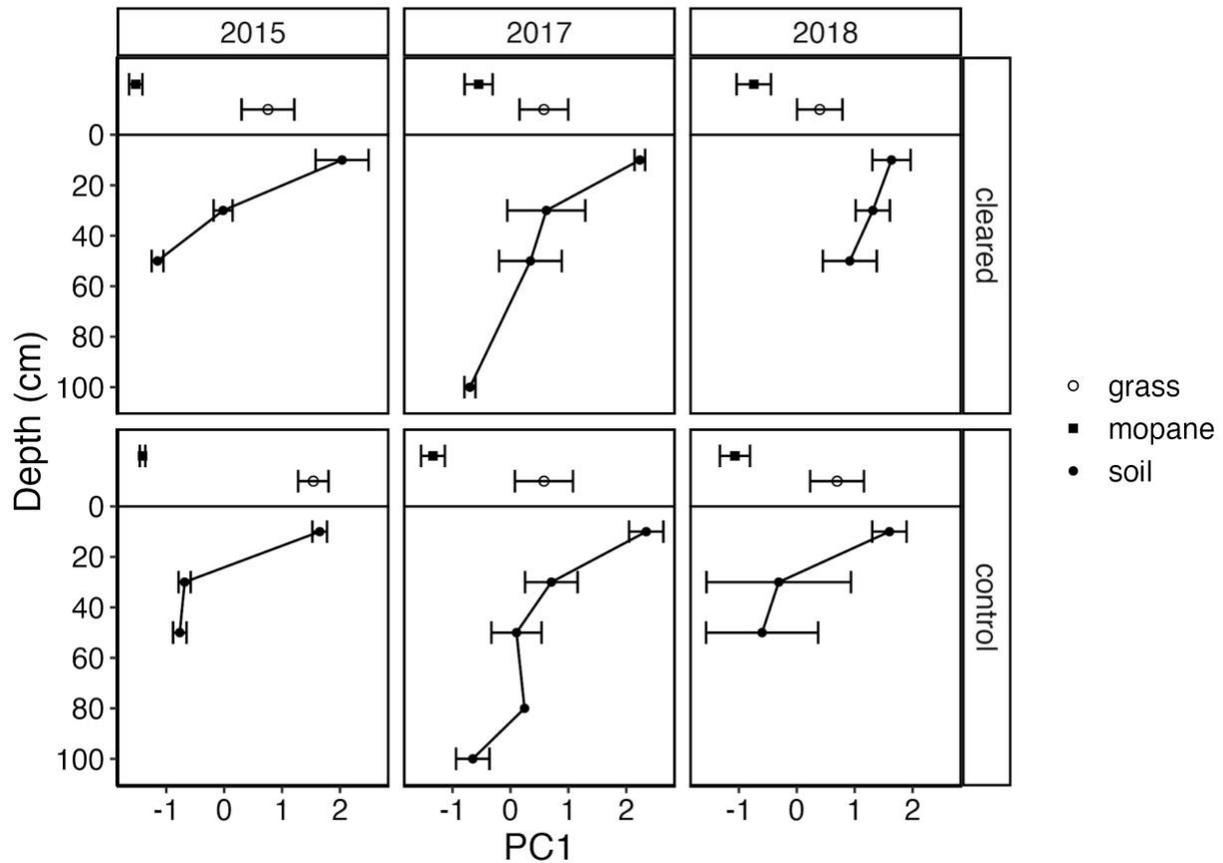


Figure C.4 Average PC1 values \pm 1SE for C4 grasses (open circles), mopane trees (closed squares) and soil samples (closed circles) in cleared and control plots in 2015, 2017, and 2018. PC1 of soil samples decreased with depth. PC1 represented 96.76, 92.74, and 92.46% of variation in 2015, 2017, and 2018, respectively.

Appendix D - Chapter 5 supplementary material

Table D.1 Total number of stems and locations for each species sampled for leaf area, stem mass, and leaf mass. Watershed ID refers to specific watershed names on Konza Prairie, where the number represents the fire frequency and watersheds that start with “N” are grazed by bison.

Species	# of stems	Fire Frequency	Watershed ID
<i>Cornus drummondii</i>	76	4	K4A, N4D
<i>Prunus americana</i>	26	2, 4	N2B, 4B, 4F, N4D
<i>Rhus aromatica</i>	23	1,2,4	N2A, N2B, 4B, N4D, Headquarters
<i>Rhus glabra</i>	20	2	N2B, 4B, N4D, N1A
<i>Rubus pensilvanicus</i>	16	2,4	N2B, 4B, 4F
<i>Zanthoxylum americanum</i>	11	2,4	N2B, 4B

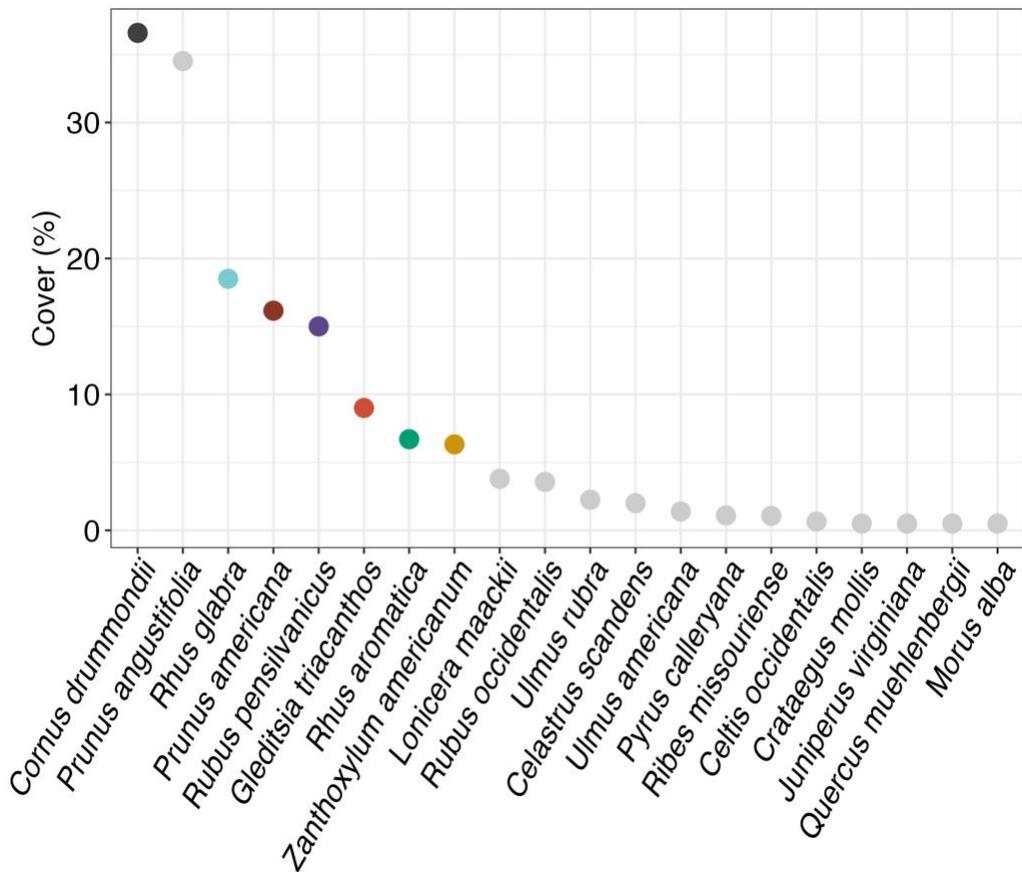


Figure D.1 Cover of all woody species in the long-term species cover transect across the entire site. Cover was averaged for each species over the last 10 years (2012 – 2022) across 1-, 4-, and 20-year fire frequencies. Sub-shrubs and vines were removed from the dataset and only woody species that grow above the grass canopy layer are shown. Gray points represent species present in the species composition plots that were not measured in this study.

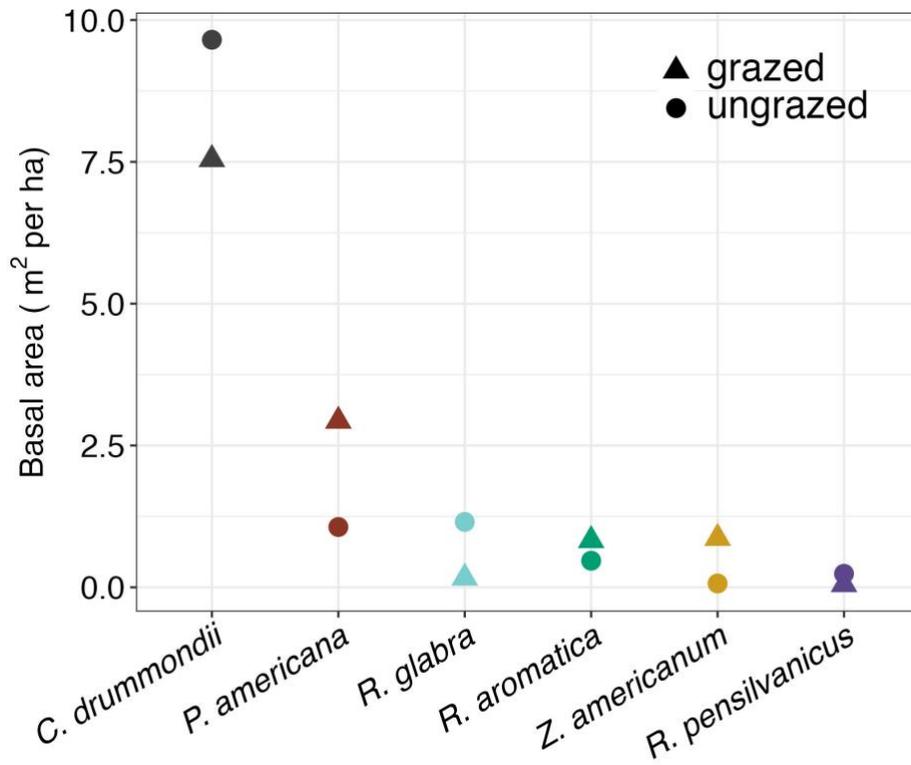


Figure D.2 Basal area ($\text{m}^2 \text{ha}^{-1}$) estimated across watersheds burned every 4 years on Konza Prairie. Field surveys were performed in 2020 across 48 plots in grazed and ungrazed areas. Within each plot, three 20 m long transects were walked and every 2 m the diameter of every woody stem above 0.5 m was measured within a 0.25 m^2 area.

Appendix E - Chapter 5 detailed methods

Physiological and functional traits methods

Rapid light response curves – We sampled light response curves in July of 2022 using the Li-6400XT photosynthesis system (Li-Cor, Inc., Lincoln, NE). We sampled four replicates of each species in grazed and ungrazed areas ($n = 8$ per species). Light intensities were set to 2000, 1600, 1200, 800, 400, 200, 100, 50, 30, 15, and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$. CO_2 inside the chamber was set to 400 ppm and relative humidity was maintained between 40-50%. Each leaf was allowed 90-200 seconds to stabilize between light intensities. We derived quantum yield (ϕ), light compensation point (LCP), light saturated photosynthetic rate (A_{max}) using the *photosynthesis* package in R (Stinziano et al. 2023).

A-Ci response curves – In July 2022, we sampled A-C_i response curves on the same shrubs as light response curves using the Li-6800 photosynthesis system (Li-Cor, Inc., Lincoln, NE). We used the dynamic assimilation response curve method (DAC; Saathoff & Welles, 2021). DAC uses dynamic equations to continuously change CO_2 concentrations from 1600 ppm to 40 ppm. Leaves were allowed to stabilize to chamber conditions before starting each curve. Initial chamber conditions were set to: $\text{CO}_2 = 400$ ppm, temperature = 30 C, relative humidity = 50%, light = $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$. We derived J_{max} and V_{Cmax} using the *plantecophys* package in R (Duursma 2015). J_{max} and V_{Cmax} are parameters that represent maximum electron transport rate and carboxylation capacity, respectively.

Pressure-volume curves – Pressure-volume curves were sampled in August of 2022 on the same shrubs used for photosynthetic response curves using the bench dehydration method ($n = 8$ curves per species; Tyree & Hammel, 1972; Bartlett et al., 2012). Terminal sections of

branches were collected before sunrise between 400 and 530 hr, placed in a plastic bag with a moist paper towel, and stored in a refrigerator until measuring. All branches were collected on the same morning as they were measured. To start each curve, a portion of the stem with the youngest developed leaves were cut underwater with a razor blade, placed in a bag, and weighed (Ohaus Pioneer, Ohaus Corporation, Parsippany, NJ, USA). Leaf water potential was measured using a Scholander Pressure Chamber (PMS Instrument Company, Albany, OR, USA). The leaf was weighed again immediately after the water potential measurement, allowed to dry down on the bench top for 2 minutes, placed in the plastic bag to equilibrate for 20 minutes and then water potential was measured again. This process was repeated with dry down periods of 2 minutes to 2 hours until a pressure of 3.0 – 3.5 MPa was reached. Each leaf was dried at 60 °C for 48 hours and weighed. We derived turgor loss point (π_{TLP}) for each sample.

Leaf functional traits – Specific leaf area (SLA) and leaf dry matter content (LDMC) were measured in late July of 2022 (sensu Perez-Harguindeguy et al., 2016). Briefly, five leaves from a total of 10 shrubs per species were used for leaf functional trait measurements (n = 350 leaves). Leaves were collected, placed in a moist plastic bag, and stored in a cooler until processing. We measured fresh leaf area using imageJ. Leaves were then rehydrated overnight and measured for water saturated mass. We dried the leaves for 48 h at 60 °C and weighed them for leaf dried mass. SLA and LDMC were calculated for each individual leaf and then averaged for each individual shrub. Leaves for each replicate shrub were combined, ground, and measured for foliar C:N and $\delta^{13}\text{C}$. C, N, and $\delta^{13}\text{C}$ were measured at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University using an Elementar vario Pyro cube coupled to an Elementar Vision mass spectrometer. Isotopic abundance was converted to δ notation using:

$$\delta = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \times 1000$$

where R is the ratio of heavy to light isotopes ($^{13}\text{C}:^{12}\text{C}$) for the sample and standard. Standards were calibrated to the international standard, Vienna Pee-Dee Belemnite for $\delta^{13}\text{C}$. Within and across run variability of the working standard was $< 0.05 \text{ ‰}$.

Woody density – Wood density, a metric of drought tolerance, was measured as described in Perez-Harguindeguy et al. (2016). Briefly, a 10 cm long section of stem was cut 10 cm above the ground and measured for fresh volume. Stem sections were then dried at $60 \text{ }^\circ\text{C}$ for 72 hours and weighed to calculate stem density (mg cm^{-3} ; $n = 10$ per species).

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