North American megafauna, friend or foe to woody encroachment across the Great Plains? Further exploring the American bison's ecological role on plant communities

by

Sidney Lake Noble

B.S., Valparaiso University, 2018
M.S., Miami University, 2020

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Abstract

Across the globe, grasslands are among the most threatened terrestrial ecosystems. Where grasslands remain intact, many face the threat of woody encroachment, which is the spread of woody vegetation into grassland systems. Within North America, woody encroachment causes habitat loss for grassland obligate species, economic loss of rangeland production, and altering the water cycle. The most intact grasslands left in North America are within the Great Plains and face widespread woody encroachment. Another large change in the Great Plains was the removal of large native herbivores, and replacing them with domesticated grazers or removing grazers altogether. Historically, the American Bison numbered between 30-60 million individuals across the Great Plains before their widespread expiration due to European colonization, market forces, and purposeful extermination. Bison reintroduction has become common across the Great Plains for conservation and ranching. This dissertation aims to further disentangle the role of bison on Great Plains' plant communities in the context of woody encroachment.

The effects of bison have been well studied within the mesic grasslands, or tallgrass prairies, of the Great Plains. In tallgrass prairies bison increase plant diversity and compositional heterogeneity. However, bison have been shown to increase woody plants within tallgrass prairie at high fire frequencies by creating low-fuel patches, but little work has been done exploring their effect in the near absence of fire. My second chapter examines the impact of bison on plant communities and woody encroachment in the near absence of fire. I found that bison continue to increase species richness at low fire frequencies, and bison reduce tree cover, especially *Juniperus virginiana*, one of the most common and impactful encroaching tree species in the Central Great Plains. My third chapter explores whether bison can help or hinder the reversal of encroachment within a woody-dominated state in the Central Great Plains at Konza Prairie

Biological Station. Returning frequent fire alone to encroached areas rarely reverses woody encroachment—a pattern known as hysteresis. After three years of annual fire and the establishment of bison exclosures, the woody plant area decreased, but there was no difference between bison presence and exclosures. Tree mortality was higher without bison, and plant community composition remains similar between bison grazed and ungrazed but with higher grass cover in the exclosures. My fourth chapter addresses the impact of bison on plant communities within mixed-grass prairies in the context of woody encroachment, contrasting with the well-documented influences in tallgrass prairies. We find that bison presence in mixed-grass prairies does not markedly increase plant species richness or diversity compared to areas grazed by cattle, in contrast to their effect in tallgrass prairies. Furthermore, bison have little effect on encroached communities. This research suggests that while critical for ecosystem restoration, bison reintroduction exhibits variable outcomes across grassland types, emphasizing the need for site-specific conservation tactics. They highlight the imperative for more expansive research on their ecological impacts beyond the tallgrass prairie, informing future conservation and management decisions as bison reintroduction efforts continue.

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Dedication

This dissertation is dedicated to the grasslands of North America and all life that depends and inhabits them.

Chapter 1 - Introduction

Grasslands, covering approximately 40% of the Earth's terrestrial surface, are critically important ecosystems increasingly threatened by human activity (Buisson et al. 2022). These biodiverse ecosystems are not only home to a wide array of flora and fauna but also provide essential ecosystem services such as carbon storage, soil erosion control, ranching, and support for pastoral communities (Curtin and Western 2008, Zhao et al. 2020, Chang et al. 2021). Despite their ecological value, grasslands across the globe have seen substantial conversion to agricultural use, leading to habitat fragmentation and loss (Scholtz and Twidwell 2022). Climate change compounds these pressures by altering precipitation patterns and increasing the frequency of extreme weather events, further altering these ecosystems (IPCC 2019, Prein and Mearns 2021). The conservation and restoration of grasslands are of paramount importance, requiring urgent attention and action to preserve their biodiversity and ecological resilience.

Where grasslands remain intact, woody encroachment, the expansion of woody plant species into grassland ecosystems, is one of the biggest threats to remaining grasslands (Van Auken 2009). This process fundamentally alters the ecosystem, converting open grassland into woodlands or shrublands (Coppedge et al. 2001, Ratajczak et al. 2014a, Archer et al. 2017). Woody encroachment alters key resource availability such as water, light, and nutrients (Huxman et al. 2005, Hughes et al. 2006, Brudvig et al. 2009). Furthermore, encroachment alters fire regimes and prevents fires that maintain some grassland systems (Archer et al. 2017). Woody plant encroachment is driven by several factors, including fire suppression, altered grazing patterns, increasing CO_2 concentrations, and climate change (Van Auken 2009, Kulmatiski and Beard 2013, Archer et al. 2017). As woody plants expand, they compete with native grasses and forbs for resources, leading to decreased biodiversity and changes in ecosystem services

provided by these grasslands (Archer et al. 2017). In North America, woody encroachment decreases plant species richness at the community scale by an average of 45% (Ratajczak et al. 2012). The consequences of woody encroachment are far-reaching, affecting not only biodiversity but also the ecosystem services they provide (Archer et al. 2017, Zhao et al. 2020). Further understanding the prevention and potential restoration of these encroached systems is necessary to keep grassland systems intact.

Within North America, the Great Plains contain some of the last remaining intact grasslands in the world (Scholtz and Twidwell 2022). Historically, the region was a vast expanse of uninterrupted grassland, characterized by a dominance of productive C_4 grasses in the more mesic eastern areas and shorter drought-tolerant C_4 grasses in the arid western regions. The central Great Plains presented a unique ecological interface where a diverse mix of C_3 and C_4 grasses prevailed, reflecting the transition between the eastern and western climatic conditions (Samson et al. 2004). After the last glacial maximum and the subsequent transition of these ecosystems from forest to grasslands, this diverse grassland matrix supported diverse flora and fauna, underpinning the ecological and evolutionary processes unique to the Great Plains. Over the past two centuries, the Great Plains have undergone significant changes due to European colonization, agricultural expansion, and urban development, leading to habitat loss and fragmentation (Scholtz and Twidwell 2022). The Great Plains is one of the least protected regions in North America (Comer et al. 2018), and only 30% of grassland remains across the entire Great Plains (Samson et al. 2004). Most of this decline is due to conversion to agriculture, with a rapid expansion during homesteading and continuing today at an estimated rate of 2 hectares or 5 acres of grassland per minute since 2014 (World Wildlife Fund 2020). However,

woody encroachment has become a persuasive threat to intact areas (Morford et al. 2022). Since 1990, tree cover has increased by 50% across the Great Plains (Morford et al. 2022).

In the Great Plains, fire exclusion is one of the primary drivers of woody encroachment (Van Auken 2009, Ratajczak et al. 2012). Before European colonization, long-term average fire intervals ranged from every 2-4 years in the eastern Great Plains or tallgrass prairies to 10-15 in the western Great Plains, caused by lightning and human ignition events (Allen and Palmer 2011; Guyette et al. 2012). Today, fire intervals across the Great Plains range from frequent (1-2 years) in managed tallgrass prairies to complete fire suppression (Samson et al. 2004; Ratajczak et al. 2016). Woody encroachment can eventually lead to an ecosystem transition from grass-dominated systems to shrub- or tree-dominated systems, leading to a loss of ecosystem services and diversity (Knapp et al. 2008, Ratajczak et al. 2012, Archer et al. 2017). Furthermore, there is evidence that woody encroachment within the Great Plains can represent alternative states (Ratajczak et al. 2014a, Ratajczak et al. 2014b).

While terrestrial ecosystems are inherently dynamic, they generally maintain stability within a spectrum of environmental conditions that vary across multiple temporal scales. When faced with disturbances or environmental change, many terrestrial ecosystems respond gradually and predictably (Cowles 1899, Odum 1969, Horn 1974). However, some ecosystems can undergo regime shifts, transitioning from one ecological state to another (Scheffer et al. 2001). Regime shifts can signify the presence of alternative states, a phenomenon where distinct ecosystem states coexist under identical environmental conditions (Beisner et al. 2003). The transition between these states occurs when disturbances push the system beyond its equilibrium threshold, leading to a significant shift to an alternative state. Additionally, the concept of

hysteresis highlights that restoring the original conditions that supported the initial state may not necessarily revert the ecosystem to its former state (Collins et al. 2021).

Within grasslands, woody encroached grasslands may represent alternative states (Ratajczak et al. 2014a, Wilcox et al. 2018, Pausas and Bond 2020). Within the tallgrass prairies of the Central Great Plains, alternative states exist based on fire frequency (Ratajczak et al. 2014a, Ratajczak et al. 2014b). The grassland state is maintained with a fire frequency of every 1 to 4 years; once a threshold of a 4-year fire return interval (FRI) is passed, these grasslands transition to shrublands. Additionally, increasing the FRI to every 8-10 years transitions the state to a woodland state. Furthermore, returning fire to these encroached states fails to revert them to grasslands, indicating hysteresis and thus the presence of alternative states (Collins et al. 2021). However, a large gap in the knowledge remains across the Great Plains, especially in relation to native megafauna, such as the American Bison (*Bison bison*)—the largest and most widespread grazing megafauna before European colonization.

Historically, bison occupied the entire Great Plains region, existing in vast herds. Estimates from one account in the Southern Great Plains estimate a herd of 12 million (Shaw 1995). With a total population size of 30-60 million across the entire Great Plains (Hornaday 1889, Shaw 1995). By the late 19th century, bison populations had reached the brink of extinction due to overhunting, with their numbers reduced to less than 1,000 individuals by 1890 (Isenberg 2000). Today, conservation efforts are reintroducing bison across all grassland types across the Great Plains (Sanderson et al. 2008). However, today's grasslands are very different from 150 years ago. Despite the recognized importance of bison in grassland ecosystems, there remains a considerable gap in our understanding of their specific effects on plant communities in the Great Plains, particularly concerning woody encroachment and grassland communities

outside the tallgrass prairie. To our knowledge, only a handful of studies investigate the influence of bison on plant communities outside tallgrass prairie in the Great Plains (Hillenbrand et al. 2019, see McMillan et al. 2019). While anecdotal and historical evidence suggests that bison play a crucial role in maintaining grassland communities and function, widespread and empirical studies are needed to quantify their impacts. This knowledge gap hinders our ability to effectively utilize bison as a tool for grassland restoration and conservation. Bridging this knowledge gap is crucial for crafting evidence-based management strategies that capitalize on the ecological influence of bison in curbing woody plant expansion, their impact on alternative states, and their role in shaping plant communities, particularly within C_3 grasslands.

This dissertation aims to investigate the effects of bison on grassland ecosystems, focusing on their impact on plant communities, woody encroachment, the dynamics of alternative states in the Central Great Plains, and how bison impacts on their environment affect their own habitat use. Furthermore, it aims to elucidate the role of bison in shaping plant communities in the Northern Great Plains and assess their influence on woody encroachment in those grasslands. Chapter two focuses on the effects of bison on woodland expansion within a tallgrass prairie using long-term plant data, a vegetation cover model, and a field experiment with tree seedlings. Chapter three focuses on the reversibility of woody encroachment within areas with long-term bison grazing and whether they promote hysteresis using a fire reversal experiment at Konza Prairie in NE, Kansas. Chapter four focuses on the effects of bison on woody plant expansion in the Northern Great Plains and their effect on plant communities using plant community data collected in the field. Through a combination of field observations, remote sensing, and long-term experiments, this research seeks to provide a detailed understanding of bison's ecological roles across these two distinct regions of the Great Plains. By doing so, this dissertation aims to

contribute valuable insights into the potential of bison reintroduction as a strategy for grassland restoration, offering a basis for informed management decisions aimed at conserving these critical ecosystems for future generations.

Chapter 2 - Native megafauna act as a surprising inhibitor of tree expansion in a Great Plains grassland

Sidney Noble, Zak Ratajczak, and Brynn Noble

Please note: This chapter is in submission at Ecological Applications.

Abstract

Within the Central Great Plains (CGP) of North America, changes in fire frequency can cause transitions from grasslands to shrublands or woodlands that are difficult to reverse. Fire plays a key role in mesic grasslands, affecting plant diversity and state transitions. However, how native grazers affect these transitions is largely unknown. Bison, categorized as grazers, typically negatively affect grasses and indirectly benefit woody plants. However, bison can also negatively impact woody plants through behaviors including horning, rubbing, and trampling. We report results from a 30-year experiment at Konza Prairie, a mesic grassland in the CGP, under fire suppression (20-year fire return intervals) and experimental presence/absence of bison. Based on remote sensing, deciduous tree cover was lower in bison-grazed areas (6% grazed vs. 16% ungrazed). Shrub land cover showed no difference (42% grazed vs. 41% ungrazed), while herbaceous land cover was higher in bison-grazed areas (51% grazed vs. 40% ungrazed). Evergreen tree cover (*Juniperus virginiana* L.), which decreases biodiversity and increases wildfire risk, was approximately 0% with bison compared to 4% without bison. In a seedling trial of *J. virginiana* L., we found eight times greater over-winter mortality in the bison treatment. Long-term plant community plots confirmed the absence of trees, with tree cover near zero over ~25 years with bison and increasing to ~30% without bison. Plant species richness was 197% and 138% higher in bison-grazed upland and lowland soils, respectively, compared to ungrazed areas. Species evenness and Shannon's index were higher in the bison treatment in upland soils, though these metrics were not statistically different between treatments in lowland soils. In both soil types, community composition differed significantly from the non-grazed treatment. Grazer reintroduction is often thought to favor woody plants by reducing competition with grasses and decreasing fire intensity. We found that bison had the opposite effect at low fire

frequencies. We argue that bison behaviors, including trampling and occasional browsing, account for this pattern. From a conservation perspective, bison might be useful for preventing tree expansion and increasing plant diversity in the absence of fire.

Keywords: alternative states, woody encroachment, fire, grasslands, grazers, diversity

Introduction

Woody encroachment—the expansion of woody plants—is one of the most acute threats to grasslands and savannas today, causing decreases in grassland biodiversity (Engle 2008), freshwater recharge (Huxman et al. 2005, Keen et al. 2022), and declines in forage for grazers (Anadon et al. 2014). The presence of large grazers is almost universally thought to enhance woody plant encroachment (Scholes and Archer 1997, Roques et al. 2001, Bond 2008) by increasing woody seed dispersal (Bakker et al. 2016) and reducing grass dominance, with cascading effects like less intense fires (Van Auken 2009; Holdo et al. 2009). Evidence for increasing woody encroachment with large grazers spans the globe (e.g., Scholes and Archer 1997, Van Auken 2009, Koch et al. 2022). In North American grasslands, most work has focused on the effects of domesticated cattle on woody encroachment (Madany and West 1983, Archer 1994, Briggs et al. 2002) rather than native megafauna, such as the American bison (*Bison bison*). This represents a knowledge gap in our understanding of these grasslands that coevolved with bison and other native megafauna (Flores 2016).

Climate, fire, and grazing are pivotal in shaping the structure and function of grasslands and savannas. Frequent fire plays a critical role by removing litter, favoring grasses over woody plants, altering plant community composition, and reducing woody plant encroachment (Van Auken, 2009). Grazers often have the opposite effect, reducing the abundance of palatable grasses, which reduces average fuel loads, allowing more woody plants to become established (Bond 2008, Holdo et al. 2009). Some grazers also increase heterogeneity by intensively grazing some plant areas, creating a mosaic of patches with low and high fuel loads and, therefore, fire intensity (Scholes and Archer 1997, Adler et al. 2001, Roques et al., 2001, Bond 2008). In some grasslands, fire and grazers effectively "compete" with each other—frequently burned areas

leave less forage for grazers, whereas heavily grazed areas often have inadequate fuel to carry fire (Archibald and Hempson 2016). But fires also tend to attract grazers in grasslands with high primary productivity (Fuhlendorf 2009). Understanding and managing these interactions between fire and grazers is crucial, particularly in the face of global environmental changes such as woody encroachment.

Since the last glacial maximum, fire and grazing have varied across the Great Plains of North America (Stambaugh et al. 2006, 2013, Guyette et al. 2015). Before European arrival, fire was common throughout much of the eastern Great Plains due to frequent ignitions by Native Americans to attract game and by natural causes such as lightning (Courtwright 2011, Roos et al. 2018, Stambaugh et al. 2013). In our specific study region, the Flint Hills of the Central Great Plains, fire intervals typically ranged from every 3 to 5 years (Allen and Palmer 2011, Stambaugh et al. 2013). Today, however, fire intervals vary from very frequent burning every 1-2 years to complete fire suppression, with the latter accounting for about half of the grassland area in the Flint Hills (Ratajczak et al. 2016).

Historically, megafauna are an important component of most grasslands, including the Great Plains (Ripple et al. 2015). Historically, bison were abundant across the Great Plains, especially in open habitats that supported high primary productivity and experienced less intense cold and heat stress (Shaw 1995, Wendt et al. 2022). In the late 1800s, bison were hunted nearly to extinction, with the population dwindling to fewer than 1,000 individuals by 1890 (Isenberg 2000). However, due to conservation efforts by private citizens, indigenous tribes, and government agencies, the bison population in North America now exceeds 500,000, with approximately 20,000 in conservation herds (Sanderson et al. 2008, Freese et al. 2007). As these reintroductions continue, we need empirical studies that can project their potential impacts in the

context of ecosystems that now exist in an altered Global Change context, including greater atmospheric CO_2 and increasing pressures from non-native species.

The interplay between fire frequency and grazing by bison plays a crucial role in shaping the ecological dynamics of the tallgrass prairies of North America. High fire frequency in the tallgrass prairies in the absence of grazers favors the dominant grasses (Collins and Calabrese 2012), but grazing by bison decreases grass dominance, which increases resource availability for other functional groups (Collins et al. 1998, Collins and Calabrese 2012, Koerner et al. 2018, Ratajczak et al. 2022). A similar mechanism exists in approximately half of grasslands worldwide with large grazers (Koerner et al. 2018). At our specific study site, plant diversity is maximized when fire frequency is infrequent (3 to 4-year fire intervals), and bison are present due to less competition from dominant grass species and a fire frequency that allows more fire intolerant species to establish (Collins and Calabrese 2012).

The effects of grazing and fire on mesic grasslands within North America have been well studied (Harnett et al. 1996, Collins and Smith 2006, Collins and Calabrese 2012). However, research on the interaction between grazers and woody plant encroachment remains limited, with most studies primarily concentrating on the effects of domesticated large grazers, such as cattle, within ecosystems subject to frequent burning (typical fire intervals ranging from one to four years). In grasslands, we have almost no studies on how native grazers impact grasslands in the absence or near absence of fire (see Briggs et al. 2005, Twidwell et al. 2013, and Ratajczak et al. 2014 for reviews). Up until recently, we might have assumed that bison and other large grazers would promote woody encroachment because their diet was thought to be almost purely graminoids in the Central Great Plains (Raynor et al. 2016, Griffith et al. 2017), and graminoid abundance generally reduces woody plant abundance (Scholes and Archer 1997). However, high

throughput sequencing has revealed that many non-graminoids, including woody plants, are found in the dung of grazers, including the bison at our study site (Bergmann et al. 2015, Craine et al. 2015, Craine 2021). These data suggest that although bison have been categorized as obligate grazers, they might supplement their diet with small amounts of forbs and woody plants. While graminoids are an overwhelming portion of bison diet (Raynor et al. 2016), this browsing could potentially limit the expansion of woody plants if this effect outweighs the competitive release from lower graminoid abundance through woody plant mortality caused by trampling, scratching, or rubbing (Coppedge and Shaw 1997). Finally, work in African savannas has shown that when grazers promote grazing lawns—areas of high grazer activity and low vegetation height—these tend to be areas of low tree recruitment (Voysey et al. 2021). These findings underscore the complex role of grazers in grassland ecosystems, highlighting the need for further research to fully understand their impact on plant community dynamics and woody encroachment.

This study aims to investigate the impact of a native grazer, the American Plains Bison, on plant community dynamics within the Central Great Plains, focusing on areas that have transitioned from infrequently burned grasslands to woody-dominated states. Understanding the net effects of megafauna takes large experimental treatments that play out over decades due to the movement of animals and the long lifespan of perennial plant species. Here, we take advantage of four replicated catchment basins (all >20 ha) where fire has been largely suppressed for over thirty years. Two of the catchment basins had bison reintroduced between 1987 and 1992, whereas the other two have remained ungrazed except by micrograzers (e.g., grasshoppers) and mesograzers (e.g., small rodents). We hypothesized that the net effect of fire suppression and grazing by bison would still increase plant community diversity and influence the composition of

woody plants, especially reducing the abundance of tree species possibly due to bison behaviors such as rubbing and browsing. However, we did not expect bison to reduce the cover of shrubs because most of the encroaching shrub species in the Central Great Plains can resprout after their aboveground stems are killed by fire, herbivory or trampling (Ratajczak et al. 2014).

Methods

Site

This study was conducted at the Konza Prairie Biological Station (KPBS), a 3,487 ha native tallgrass prairie located in northeastern Kansas, USA (39˚05' N, 96 ˚35' W). KPBS is a National Science Foundation long-term ecological research (LTER) in the Flint Hills ecoregion of the Central Great Plains, the largest expanse of unplowed tallgrass prairie in North America (Samson and Knopf 1994). The mean annual precipitation is 835 mm yr^{-1}, and the growing season temperature (May– September) is 32.6^{o} C, with the mean monthly maximum in July (36.2^{o}C). Typical grassland vegetation at Konza is dominated by four perennial C_4 grasses: *Andropogon gerardii* (Vitman) *Sorghastrum nutans* (L.) Nash, *Schizachyrium scoparium* (Michx.) Nash, and *Panicum virgatum* L.. In the absence of grazers, these four grass species become dominant and account for >90% of the aboveground net primary productivity (Smith et al. 2003). In contrast, forbs and other subdominant species make up the bulk of species richness in the absence of grazing (Towne 2002). Common perennial forbs include *Symphyotrichum* spp. (asters), *Eupatorium altissimum* L. (tall-joe pye weed*), Salvia azurea* Michx. (blue sage), and *Solidago* spp. (goldenrods). In frequently burned areas (every 1 to 4 years), bison promote tall *Solidago rigida* L. (rigid goldenrod) and *Verbena stricta* Vent. (hoary verbena), short annual species, and shorter grasses more typical of drier Great Plains grasslands (e.g., *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths and *Bouteloua dactyloides* (Nutt.) J.T. Columbus) (Ratajczak et al.

2022). Woody species are common along riparian areas and within the infrequently burned watersheds (4 or 20-year mean fire interval, Veach et al. 2014), with the most common shrub species being *Cornus drummondii* C.A. Mey. (rough-leaf dogwood) and *Rhus glabra* L. (smooth sumac).

Between the late 1970s to 1992 KPBS was divided along catchment boundaries into 64 fire management units, ranging from 12 to 136 ha in size. Replicate catchment units at KPBS have been experimentally burned at 1, 2, 3 to 4, and 20-yr fire intervals since 1983 (Knapp et al. 1998). In 1987, 30 bison were initially introduced and restricted to under half of its current spatial extent. In 1992, these bison were introduced to the remaining area, having access to 1012 ha, including catchment N20B (one of the watersheds in this study). The herd increased to an average of 270 adults to maintain a grazing rate of approximately 25% removal of aboveground production each year (Towne 1999). Changes in plant communities and woody encroachment have already been reported for more frequently burned catchments (e.g., Briggs et al. 2005, Ratajczak et al. 2014). Therefore, for this study, we only used catchments burned every 20 years. For this fire frequency, we had two catchments where bison were reintroduced and two catchments without any large grazers, referred to here as "ungrazed" from here on (see Fig. S 2.1 for treatments used at Konza).

Vegetation Sampling

Between 1983 to 1994, permanent plots were established in each grazing treatment, and two different topographic positions (uplands and lowlands) within each treatment. Plots were evenly spaced along 50-m transects, with five 10-m^2 circular plots per transect and eight transects per fire management unit, with four transects in uplands and four transects in lowlands (for a total of 20 plots per combination of fire, grazing, and topographic position). Upland soils are shallow,

rocky, and cherty silty clay loams typified by the Florence soil series. Lowland soils are deeper and non-rocky silty clay loams typified by the Tully soil series. Since data collection began, the cover of each species has been measured annually in each plot, recording species cover using a modified Daubenmire scale, with cover classes of 0–1%, 1–5%, 5–25%, 25–50%, 50–75%, 75–95%, or 95–100% aerial coverage (Bailey and Poulton 1968). Each plot was sampled in the spring (late April to early May) and autumn (late August) to capture within-season vegetation dynamics (e.g., some species are much more abundant in the spring, but senescent in the fall). For each year-by-plot combination, we used the maximum cover value for each species among these two sampling periods. To calculate approximate cover, we used the midpoint of the Daubenmire scale ranges. For species richness and diversity across the two treatments, we used the upland and lowland 10 m^2 plots located in the bison grazed and ungrazed catchment basins that are burned every twenty years (n=40 per treatment). We used the 1994 and 2020 sampling data to compare the two treatments directly. 1994 was used as the "before bison" comparison because it was the first year with equal sample sizes for both treatments, and 2020 was used as the "after-bison" comparison, because starting in 2021 we began a new experiment in the grazed treatment to test strategies for reversing woody encroachment.

Large-scale woody plant changes: remote sensing

Quantifying woody vegetation typically uses methods with a larger footprint than herbaceous species because woody species are much larger than other herbaceous plants. For instance, a single oak tree could take up an entire transect of the herbaceous species transects used in this study. Therefore, to quantify the larger scale extent of woody plant cover change, we used remote sensing that categorized the entire study area into grassland, shrub-dominated, evergreen tree-dominated (*Juniperus virginiana* L.), deciduous tree-dominated, and other (water, roads)

(Fig. S 2.1). This data-product has a 2 x 2 m resolution and was developed using a large training dataset, remote sensed inputs from low flying planes, and random forest models (Noble 2023, Noble, B. and Ratajczak *in revision*). Based on a hold-out dataset of 90,113 pixels that were not used to train the model, the overall model accuracy is 97%, producer accuracy is 96%, and user accuracy is 92%. We then used the package raster in R (Hijmans 2020) to determine the amount of cover for each vegetation class within the bison grazed 20 YR FRI and the ungrazed 20 YR FRI treatments. We masked riparian areas within 20 m (as we did in Keen et al. 2023, Dodds et al. 2023), because many riparian zones have deep soils and a wet microclimate that promotes woody vegetation regardless of fire or grazing.

Functional Plant Cover Over Time

We used the long-term plant community data from 1994-2020 to determine the cover of three different plant functional groups; tree cover, shrub cover, and grass cover. Each of these was the sum of the average cover in each treatment for each year.

Seedling Trial

Eastern red cedar, or *Juniperus virginiana* L., is one of the most abundant tree species in unburned or infrequently burned areas within the Flint Hills of Kansas (Nippert et al. 2021). In the autumn of 2020, we transplanted 40 *J. virginiana* L. seedlings into catchment basins with and without bison. Seedlings were 10 to 30 cm tall and were obtained from a *J. virginiana* L. woodland from a private property located 11 miles NNE of our study site, from a location similar soil to our sites. Along a fence-line contrast, twenty were planted in an area with bison access and twenty without bison access in November of 2020. Each treatment had two 100 m long transects placed 200 m apart. Along each transect, one seedling was planted every 10 meters along the 100 m transect (n=20 seedlings for bison grazed and n=20 for ungrazed). Seedlings

were revisited thrice, in December 2020, in January 2021, and in February 2021. With each visit, mortality and the apparent cause of mortality (browsed, trampling, and ripped out) were tracked. Seedling tracking was finished in February, as these treatments were scheduled to burn in the spring.

The choice to plant the seedlings in the late fall was strategic and informed by several factors. Most of the Konza Prairie Biological Station (KPBS) was scheduled for prescribed burning in the spring, necessitating that the transplanting be completed beforehand. Additionally, we aimed to have the treatment areas in close proximity to one another with the same soil and elevation. Also, we hypothesized that the time of most intense browsing by would be during the winter—by the late winter bison have reduced high protein forage throughout much of the site, and therefore, during this time the sole evergreen tree species (red cedar) would be a rare high protein forage site, despite its higher concentration of secondary defense compounds.

Data analysis

To quantify the effect of bison on plant biodiversity, we calculated species richness, evenness, and species diversity, with a focus on alpha diversity (diversity metrics at the plot scale). Species richness is the total number of species found in each of the 80 10 m^2 plots. For species evenness, we calculated the Shannon's Diversity Index ($H = \Sigma(p_i \times \ln p_i)$ and then calculated the Shannon Equitability Index (Evenness = H/ln(S)), where S is the total number of unique species. Species diversity for each site was calculated and reported using the exponential Shannon's index, exp(H′ = $\Sigma(p_i \times \ln p_i)$, where p_i is the relative abundance of species *i*. An exponential Shannon's index (e^H) is the effective number of equivalently abundant species in a community, and generally more interpretable than a raw Shannon's Index value. For species richness, evenness, and diversity (e^H), we performed ANOVAs with time (the first and last year of data, 1994 and 2020,

respectively), grazing treatment (bison vs. ungrazed), soil type or topographic position (Florence soils and Tully soils) and their interactions as fixed effects. We performed post-hoc comparisons using the Tukey test in the R package multcomp (Hothorn et al. 2008) of all pairwise comparisons for year, grazing treatment, and soil type. Test were performed at an alpha level of 0.05, with an honest squares difference adjustment to *P* values. All analyses, packages, and those described hereon were run using the program R (v4.0.5; R Core Team 2021).

To describe how these three variables (species richness, evenness, and e^H) changed over time, we Generalized Additive Models (GAMs). GAMs are flexible semi-parametric models that allow for non-linear relationships between the response variable and predictor variables through the use of smooth functions. This flexibility is particularly useful for ecological data, which often exhibit non-linear patterns over time. For these GAMs, we performed independent analyses for each combination of response variable, grazing treatment, and soil type. Our modeling approach was implemented using the gam function provided by the mgcv package (Wood 2017).

To assess long-term plant community trajectories, we used a non-metric multidimensional scaling ordination (NMDS) based on a Bray-Curtis dissimilarity metric. Bray-Curtis is one of the most effective distance metrics for ecological data (Beals 1984; Ricotta and Podani 2017), and NMDS is a flexible ordination technique suitable for data that deviate from normality, which includes most vegetation data (Dexter et al. 2018). Species scores were used to determine what species drove the communities in the bison and ungrazed treatments. For this NMDS, species from functional groups (woody plants, grasses, forbs) were selected based on their statistical significance at the $\alpha = 0.05$ level, ensuring a focus on the most influential species. Subsequently, the envfit function in the package vegan (Oksanen et al. 2013) was employed to fit

environmental vectors onto the ordination diagram and to test the significance of the relationship between these species scores and environmental variables using a permutation test.

We used permutational multivariate analyses of variance (perMANOVA) with a Bray-Curtis dissimilarity metric using the package vegan (Oksanen et al. 2013) to determine if there were differences between the grazed and ungrazed treatments across soil types and the influence of time (increasing woody encroachment). A perMANOVA was used to determine any significant differences between the treatments across all years (1994-2020). Using separate perMANOVAs for both the first year (1994) and the last year (2020), we also compared the first and last years between the treatments to determine if the two communities were similar and thus comparable before grazing having an effect.

Our remote sensing was able to measure wall-to-wall land-cover for our two treatment units. We often use statistics because we can only measure a sub-set of a treatment, and therefore, need to generate a confidence interval for the average value of our measurements. In this case, we know that our measurements encompassed the entire community to ecosystem scale, negating the need for inferential statistical methods typically used in samples to infer about sub-samples. Our analysis focused on a descriptive and comparative approach. For the assessment of different vegetation types, such as shrub area, deciduous tree area, and herbaceous area, we converted into percent cover for each vegetation type to enhance the clarity and effectiveness of our graphical representations. This conversion allows for straightforward, visual comparisons of vegetation patterns across the examined areas, offering direct insights into the landscape's composition without the assumption of sampling error or variability. We recognize that this approach comes at the expense of replication, but argue that ecosystem-scale experiments, like this one, have an added benefit that they are much more realistic than plot-

based experiments, where a small location is altered by an experimental treatment but the surrounding meta-community is not. Others have made similar arguments (Carpenter 1996, Carpenter 1998, Schindler et al. 1998). In the case of megafauna treatments and their effects on trees (as in this study), large treatment areas are necessary, and this often means that replication is difficult.

For investigating plant functional group cover over time, we used Generalized Additive Models (GAMs) using the mgcv package in R (Wood 2017). For each vegetation cover type and treatment type (tree cover grazed, tree cover ungrazed, shrub cover grazed, shrub cover ungrazed, grass cover grazed, grass cover ungrazed), we fitted a separate GAM with the year as the predictor variable.

For the seedling trial, a 2-sample proportion test was used to determine if there were differences in mortality between the two treatments as of the final sampling.

Results

Species Diversity

In bison-grazed upland plots, species richness, evenness, and exponential Shannon diversity increased over time (Fig. 2.1A-C, Appendix S1: Table S1). Using Generalized Additive Models (GAMs), we observed a significant increase in species richness, with a substantial portion of the variance explained (adjusted $R^2 = 0.62$, $p < 0.001$). Evenness exhibited a minor increase, capturing a low amount of variance (adjusted $R^2 = 0.07$, $p < 0.001$). Similarly, Shannon diversity showed significant growth with notable explanatory power (adjusted $R^2 = 0.31$, $p < 0.001$).

In contrast, ungrazed upland plots saw only a minor increase in species richness with minimal variance explained (adjusted $R^2 = 0.06$, $p < 0.001$) (Fig. 2.1A). Evenness remained relatively steady with a small portion of the variance explained (adjusted $R^2 = 0.04$, $p = 0.002$)

(Fig. 2.1B). Exponential Shannon diversity changes were not significant and remained stable over the years. ($p = 0.06$) (Fig. 2.1C).

For lowland plots with bison grazing, species richness showed an increase, explaining a moderate portion of the variance (adjusted $R^2 = 0.356$, $p < 0.001$) (Fig. 2.1D). Evenness exhibited a minor but significant increase, with a small amount of variance explained (adjusted R-squared = 0.06, $p < 0.001$) (Fig. 2.1E). Exponential Shannon diversity also showed a significant increase with limited explanatory power (adjusted $R^2 = 0.05$, $p = 0.001$) (Fig. 2.1F).

Ungrazed lowland plots showed a significant increase in species richness, explaining a moderate portion of the variance (adjusted $R^2 = 0.20$, $p < 0.001$) (Fig. 2.1D). Evenness showed a significant increase, explaining a small portion of the variance (adjusted $R^2 = 0.105$, $p < 0.001$) (Fig. 2.1E). Shannon diversity also exhibited a significant increase, capturing a modest amount of variance (adjusted $R^2 = 0.11$, $p < 0.001$) (Fig. 2.1F).

The fixed effect ANOVA model revealed a significant effect of year, treatment (grazer presence), soil type, and their interactions on species richness, but soil type alone was insignificant for evenness and e^H (Table S 2.2). All three variables had no significant interaction for evenness (Table S 2.2). Species richness was significantly higher in the grazed treatment in 2020, for both soil types, 1994 grazed treatment data and all the ungrazed treatments (Fig 2.2A, see Table S 2.3 for means and SDs; see Table S 2.4 for all post-hoc comparisons). There were no differences in evenness across soil types and grazing treatment for 1994. In 2020, evenness in grazed upland plots was significantly higher than in the ungrazed upland plots (Fig 2.2B, Table S 2.2 & S 2.4), but not in lowlands. In the upland soils in 2020, e^H grazed was significantly higher than in all other year, treatment, and soil type combinations (Fig. 2.2C, Table S 2.2 & S 2.6). In

all other year (1994 and 2020), soil type, and grazing combinations were not significantly different.

Community composition

Initially, the grazed and ungrazed plant communities were close in NMDS (stress = 0.139) space, with slight differences along NMDS axis one based on soil type. Over time, the bison grazed communities have shifted upwards along NMDS axis two, with uplands shifting slightly to the left along NMDS axis one and lowlands shifting to the right along NMDS axis one. In contrast, ungrazed treatments changed little along the second NMDS axis. Still, in ungrazed areas both topographic positions shifted to the right on the first NMDS axis, with an especially large change in lowland plots (Fig. 3). In the grazed upland plots there was notable shift toward increased abundance of *Bouteloua spp.* and *Schizachyrium scoparium* (Michx.) Nash, indicative of a grazing lawn community due to bison grazing. Conversely, in ungrazed lowland soils, there is a slight movement toward *Juniperus virginiana* L. and *Gleditsia triacanthos* L., reflecting the presence of tree encroachment in the ungrazed plots. Grazed lowland plots, however, show a subtle trend towards *Prunus americana* Marsh., a thorny shrub species. Both grazed and ungrazed lowland plots have moved towards *Cornus drummondii* C.A. Mey., a clonal shrub.

A perMANOVA for 1994 revealed that grazing accounted for 5.4% of the variation (R^2 = 0.05, p = 0.001), soil type for 11.15% (R^2 = 0.11, p = 0.001), and their interaction for 4.42% (R^2 = 0.04, p = 0.001). In 2020, the influence of grazing increased to explain 10.8% of the variation (R^2 = 0.108, p = 0.001), soil type explained 18.12% (R^2 = 0.18, p = 0.001), and their interaction accounted for 6.15% (R^2 = 0.06, p = 0.001). The increase in the proportion of variation explained by these factors from 1994 to 2020 suggests that the impact of grazing and soil type on plant community composition has become more pronounced over time. A third perMANOVA of all

years revealed that year explained ~28% of the variance, grazers ~19%, soil type ~11 %, grazer and soil interaction ~8%, with all other interactions explaining ~8% of the variance (Table S 2.7).

Large-scale woody cover

The cover of herbaceous-dominated vegetation was 51% in the grazed treatment and 40% in the ungrazed plots (Fig. 2.4). Shrub cover was 42 and 41 percent for the grazed and ungrazed treatments, respectively. The grazed treatment had no *J. virginiana* L. (~0%) cover and lower deciduous tree cover (6%), compared to the ungrazed treatment, which had four percent *J. virginiana* L. cover and 16% deciduous tree cover. This lower amount of tree cover corresponds to a higher amount of herbaceous cover in the grazed treatment (~11% more).

Plant Functional Group Cover Over Time

The following data derive from long-term observation plots. The model for tree cover with grazers yielded a non-significant smooth term for Year (p=0.504). Tree cover has remained near zero for the duration of this study (Fig. 2.5A). For tree cover ungrazed, the model showed a highly significant smooth term for Year (p<0.001) with an adjusted R^2=0.93 with tree cover increasing (Fig. 5A). For shrub cover, both models indicated significant effects of Year. Shrub cover with bison, had an adjusted R^2=0.78 (p<0.001), while ungrazed, the model had an adjusted R^2=0.73 (p<0.001). Shrub cover has increased over time but now appears stable (Fig. 2.5B). Grass cover models also demonstrated significant smooth terms for Year in grazed (adj. R^2=0.6, p<0.001) and ungrazed (adj. R^2=0.63, p<0.001) treatments (See Table S 2.9 for each GAM model summary). Grass cover for both treatments have fluctuated over time with recent stabilization (Fig. 2.5C).

Seedling trial

Mortality of *J. virginiana* L. seedlings was significantly higher in the bison treatment (X^2 = 6.5333, df = 1, p = 0.01). In the grazed treatment, 45% of the seedlings were killed, 8 showing signs of browsing and being uprooted, and one trampled (see Fig. 2.6). In contrast, in the ungrazed treatment, only one out of twenty *J. virginiana* L. seedling died.

Discussion

Plains bison have been reintroduced to hundreds of sites across North America (USDA 2016), but to our knowledge, this is one of the first studies to report that bison inhibit tree expansion in the Great Plains. We are also unaware of studies finding that a nearly pure grazer (Raynor et al. 2016) can limit tree encroachment (Fig. 4). These results are surprising because grazers typically favor the expansion of woody species by reducing grass dominance (Anderies et al. 2002, Archer et al. 2017). Bison reintroduction also increased plant diversity and altered the plant community composition. This effect aligns with our hypotheses that bison would increase plant diversity and alter plant composition and the general understanding of how bison impact herbaceous tallgrass prairie communities (Collins and Calabrese 2012, Ratajczak et al. 2022).

How bison increase diversity are often in the context of frequent fires, which otherwise shifts plant communities to a homogenous state of high grass dominance in the absence of grazers (Fuhlendorf et al. 2006, Collins and Calabrese 2012). It was unclear if bison would increase diversity at low fire frequencies where grass dominance is lower, but the thirty years of data presented here show bison increased plant species richness in both upland and lowland soils. These findings support other work finding that bison and many grazers increase plant diversity across mesic grasslands across a gradient of fire frequencies (Collins et al. 1998, Collins and Calabrese 2012, Koerner et al. 2018). Moreover, this increase in diversity could mitigate the loss

of diversity due to woody encroachment (Ratajczak et al. 2012). The suppression of *J. virginiana* L. within the bison grazed areas could have long-term effects on species diversity, as *J. virginiana* L. expansion can reduce plant species richness by 95% (Briggs et al. 2002, Van Els et al. 2010). For instance, in one meta-analysis, *J. virginiana* L. decreased herbaceous plant diversity more than any other encroaching species in North America (Ratajczak et al. 2014).

When bison were first reintroduced, plant communities were similar in the grazed and ungrazed treatments. However, community composition has diverged over time, and these treatments now represent two distinct plant communities mediated by soil type (Fig 4). While the extent of shrub encroachment in both treatments has been similar, bison have prevented widespread tree establishment (Fig. 2.5A) and created a unique community more abundant short grass (e.g., *Bouteloua* spp.) and more forbs (Fig. 2.7). However, plant diversity (e^H) in the grazed lowlands was not significantly different from the ungrazed lowlands. This mostly reflects that the increase of small annual species that are present, but do not obtain higher cover values. Species richness did not diverge between the bison grazed lowlands and the ungrazed lowlands until one of the most severe droughts in four decades occurred in 2012. The divergence in species richness is probably due to the resilience of these grassland systems, because bison grazed areas tend to have more drought-adapted grasses and deep rooted forbs (Ratajczak et al. 2022).

Grazers are widely assumed to favor woody vegetation because they reduce grass abundance and, thereby, the effects of grasses on competition and the intensity of fires (Scholes and Archer 1997, Bond 2008). For instance, conventional cattle grazing in tallgrass prairie has been shown to increase tree establishment and decrease tree mortality from fires (Owensby et al. 1972, Hoch et al. 2002), but grazers have also reduced tree height due to browsing (Capozzelli et al. 2020). Although carbon isotopes indicate that bison overwhelmingly rely on grasses for

forage (Raynor et al. 2016), tree species' DNA has been found in bison dung and other large grazers (Craine et al. 2015). This matches work from African savannas that some species considered grazers can still have a proportion of woody plants in their diet (e.g. Kartzinal et al. 2015, Guyton et al. 2020). Although bison are unlikely to consume large amounts of woody vegetation, they could have an outsized impact on tree expansion if they browse trees when they are small. This effect would create a demographic bottleneck that prevents adult trees from becoming established (as in Higgins et al. 2000), which is the same mechanism that allows browsers and mixed-feeders to limit woody vegetation in African savannas (Staver et al. 2012, Sankaran et al. 2008). Our data show that bison potentially have prevented tree establishment and can kill a large proportion of small seedlings, and therefore, we argue this is one of the mechanisms by which bison are preventing *J. virginiana* L. woodland establishment in areas that are infrequently burned (~20 years). Additionally, bison in the Greater Yellowstone Ecosystem in the U.S.A. are suppressing aspen recruitment via herbivory, trampling, and breaking of aspen saplings (Beschta et al. 2020, Painter et al. 2023).

While our data suggest that bison may significantly impact the survival of young tree seedlings, it is important to consider the limitations of our *J. virginiana* L. transplant experiment. With a sample size of 20 saplings in each treatment, the findings are indicative rather than conclusive. The mortality rate of saplings was higher in the grazed plots, where 8 out of 20 died, compared to just one in the ungrazed plots. Direct evidence of bison feeding on or killing the saplings was not observed, although bison have been observed to browse woody vegetation at our site and in Yellowstone National Park, U.S.A. (Painter 2023). However, plant community data reveal an absence of *J. virginiana* L. in grazed areas, in stark contrast to its presence in ungrazed areas and its widespread encroachment elsewhere in the region (see Fig. 2.7),

supporting the hypothesis that the presence of bison suppress *J. virginiana* L. establishment. Additionally, in 2017 one of the ungrazed treatments underwent a prescribed burn where over half (758/1406) of the *J. virginiana* L. individuals within this treatment suffered mortality (Nippert et al. 2021). This further reinforces that bison probably inhibit *J. virginiana* L. as the cover comparison between the grazed and ungrazed treatments would have been higher if not for the prescribed burn; if we had performed remote sensing in 2017, we probably would have found almost twice as high *J. virginiana* L. cover in the ungrazed treatment.

In the absence of grazers, there is strong evidence that there are thresholds in fire frequency, beyond which tallgrass prairie transitions to shrublands or woodlands within the Central Great Plains of North America (Ratajczak et al. 2014). Mesic grasslands receive enough precipitation to support woodlands, but fire and competition from graminoids suppress woody plant recruitment (Briggs et al. 2005, Ratajczak et al. 2014, Twidwell et al 2013). However, without frequent fire, a self-reinforcing feedback can form where woody plants suppress grasses and lowering fire intensity (Ratajczak et al. 2011, Twidwell et al. 2013). This transition from a mesic grassland to shrublands and eventually woodlands is thought to represent a regime shift from one self-reinforcing state to another (Ratajczak et al. 2014, Ratajczak et al. 2016) that is difficult to reverse (Collins et al. 2021). Woodlands in the Central Great Plains are commonly dominated by eastern red cedar (*J. virginiana* L.) and deciduous trees (Briggs et al. 2002, Nippert et al. 2021). *J. virginiana* L. suppresses grass and forb growth due to their dense canopies, and in return, reduced surface fuel (Hoch et al. 2002). This reinforces the woodland state within this region by suppressing the shrub and understory layer and reducing the likelihood of low-intensity, frequent fires that maintain grasslands (Ratajczak et al. 2014).

The decreased tree cover, especially *J. virginiana* L., within the bison grazed treatment, has implications for the potential trajectory of the woodland. Bison may promote a shrub-dominated system with sparse trees and decrease the likelihood that a shrubland transitions to a tree-dominated system or greatly increase the time for transitioning into a woodland. We argue that bison break the transition from shrubland to woodland, due to the decrease in tree abundance. We propose an alternative conceptual model that includes bison (Fig. 2.8). Bison grazed mesic grasslands are significantly different in their species composition and vegetation structure (see Collins and Calabrese 2012) compared to ungrazed mesic grassland. Additionally, there is no transition to woodland, especially ERC dominated woodland—instead bison maintain a shrub-dominated system with sparse trees. This is more like the mosaic of mesic shrub thickets and sparse aggregates of savanna trees seen in some sub-tropical areas (e.g. Roques et al. 2001, Charles-Dominique et al. 2015).

One key feature of shrubland and woodland formation is the breaking of feedback loops that reinforce a grass-dominated state (Ratajczak et al. 2014), which makes these transitions difficult to reverse (Nippert et al. 2021, Collins et al. 2021). A major unknown is how bison affect the reversibility of woody encroachment. For instance, twenty years of annual burning in an encroached grassland without bison allowed an increase in grass cover (Collins et al. 2021). However, there are still areas dominated by shrub cover, indicating shrublands are resistant to reverting back to grasslands. Starting in the spring of 2021, the bison-grazed watersheds analyzed in this study began a fire reversal experiment where these areas are now burned annually. This experiment will provide the opportunity to understand the influence of bison on reversing transitions to alternative woody states.

Conclusion

We found that bison have decreased and altered the composition of tree species in mesic grassland in the Central Great Plains. This decrease is surprising because grazers are commonly thought to facilitate woody plant expansion by reducing the dominance of grasses. The possible mechanism behind this removal may relate to bison behavior, where bison will kill tree seedlings via trampling, browsing, and removal. Even small decreases or increases in tree cover can have large implications for grassland conservation and the maintenance of ecosystem services. For instance, grassland obligate birds, such as lesser prairie chickens, are incredibly sensitive to the presence of trees (Engle et al. 2008, Silber 2024). In the Red Hills to the Southwest of our study site, areas with a tree density of just two trees per hectare see almost no usage by lesser prairie chickens—an iconic and threatened grassland bird species (Lautenbach et al. 2017). Similarly, removing one isolated tree at our study site can create up to 14 ha of grassland bird habitat (Silber et al. 2024). Similar patterns have been seen with grassland birds in southern tallgrass prairie, where an increase in *Juniperus* spp. cover from 0 to 10% reduces bird abundance by 50% and an increase to 25% *Juniperus* spp. cover effectively removes grassland avifauna (Engle et al. 2008). Small increases in woody vegetation can also drive large decreases in freshwater recharge because woody vegetation tends to have higher transpiration rates than herbaceous species, and rely on deeper soil water sources, which are typically more closely connected to groundwater and streamflow (Keen et al. 2022). Because bison have such a strong negative effect on trees, we would expect they could impact the magnitude of these externalities of fire suppression. However, bison's impact on areas already dominated by mature trees may be limited. In such cases, additional management strategies, such as cutting or burning, might be necessary to complement the grazing impact of bison. Therefore, bison may be most effective in preventing

the expansion of species like *Juniperus* spp. or hindering their initial recruitment rather than reversing well-established woodlands.

Figures

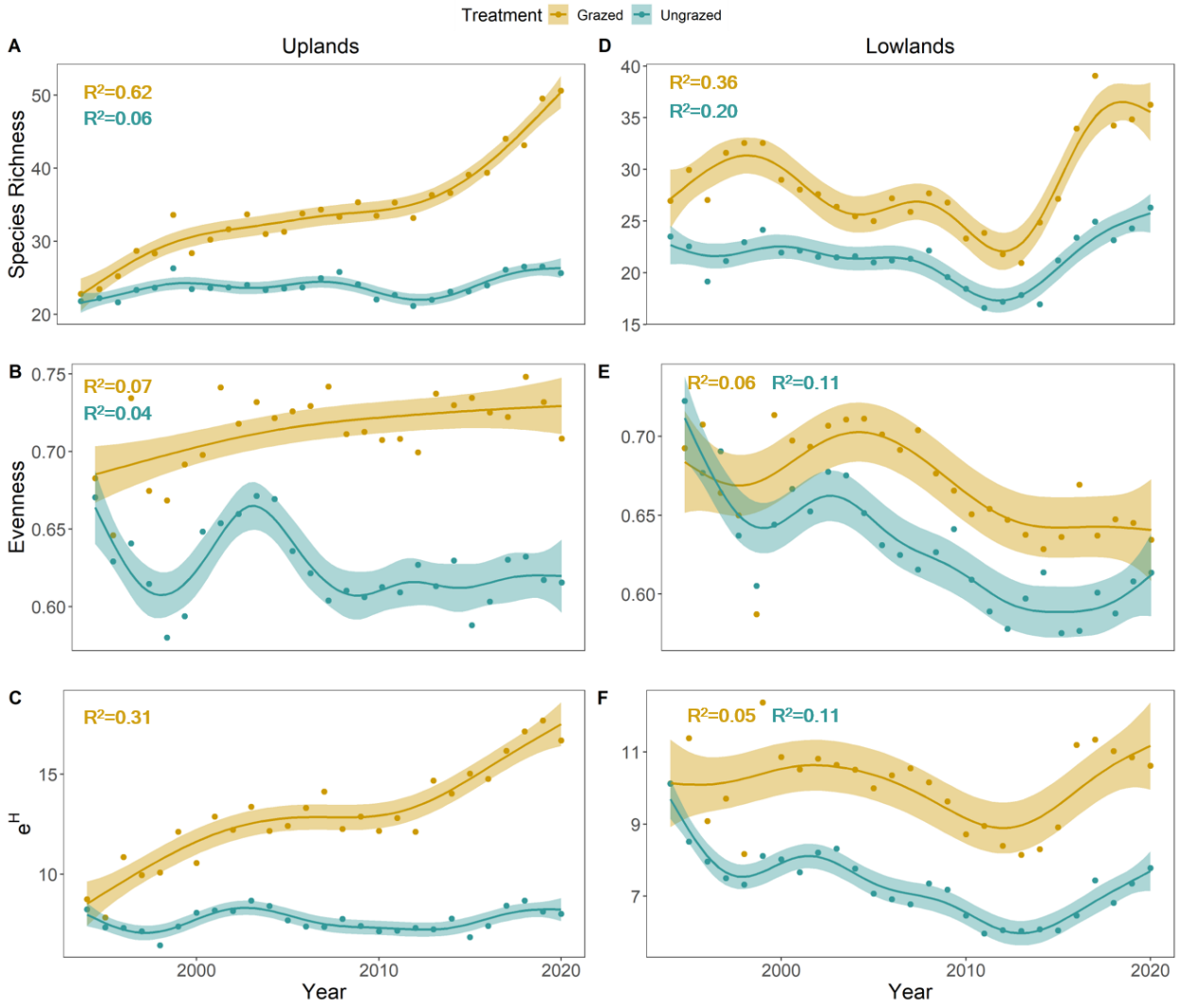

Figure 2.1: Time series of mean plant species richness per 10 m^2 plot (A), evenness (B), and mean equivalent species richness (e^H) (C) in upland (left panel) and lowland (right panel) soils in bison grazed and non-grazed woodlands from 1994 to 2020 (orange=grazed, blue = ungrazed). Points are annual averages and shaded areas span the 95th confidence interval of GAMs. All R^2 values are the adjusted R^2 and shown only for significant models ($p < 0.05$).

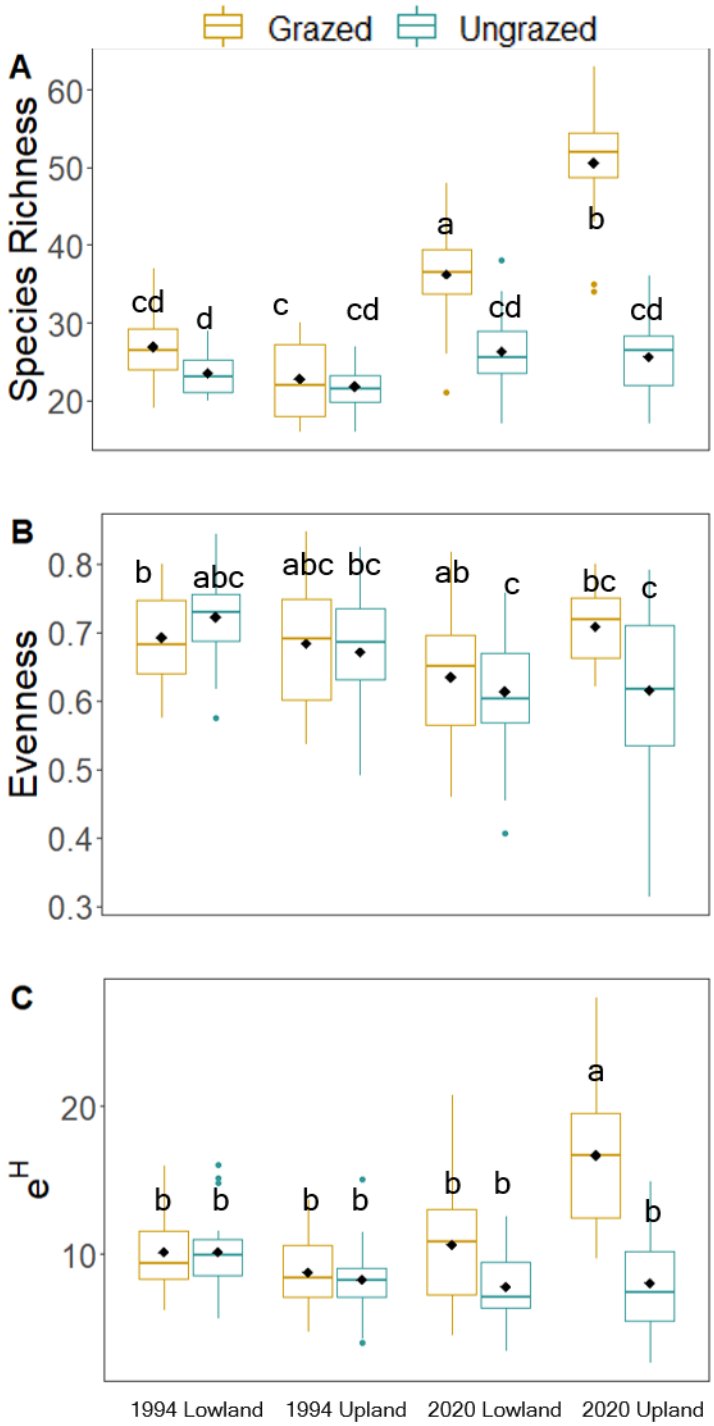

Figure 2.2: Plant species richness (A), evenness (B), and e^H(C) at 10m^2 for 1994 and 2020 (A) in grazed and ungrazed upland and lowland soils. Year, treatment, and soil type combinations that share the same lettering at the top of the graph did not have statistically significant differences at an alpha of 0.05. For all post-hoc comparisons see Table S 2.2-S 2.4. Solid lines indicate the median and black points represent the mean.

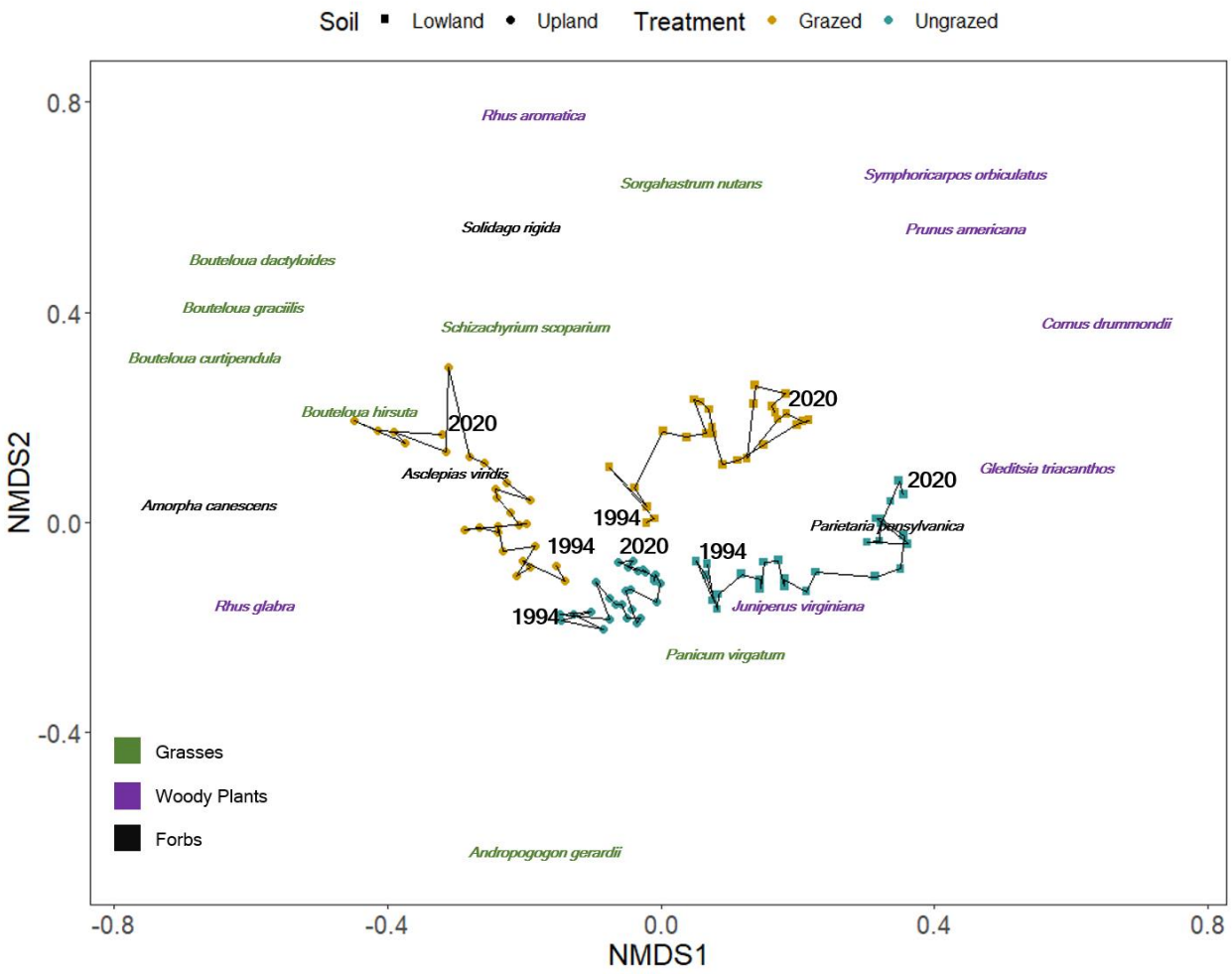

Figure 2.3: NMDS with a Bray-Curtis distance of the plant community composition data over time (1994-2020) across soil types in the grazed and ungrazed treatments. Ungrazed are blue and grazed are orange with lowland soils being represented by squares and upland soils by circles. Species are colored by functional group with purple representing forbs, green represents grasses, brown represents woody plants.

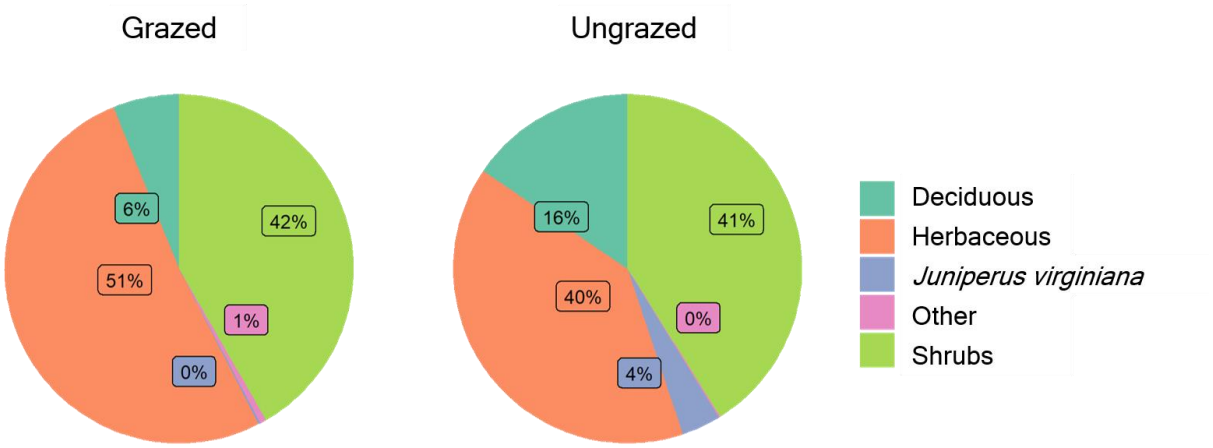

Figure 2.4: Pie charts of the different vegetation covers in the bison grazed and ungrazed treatments. The other category represents bare ground and water.

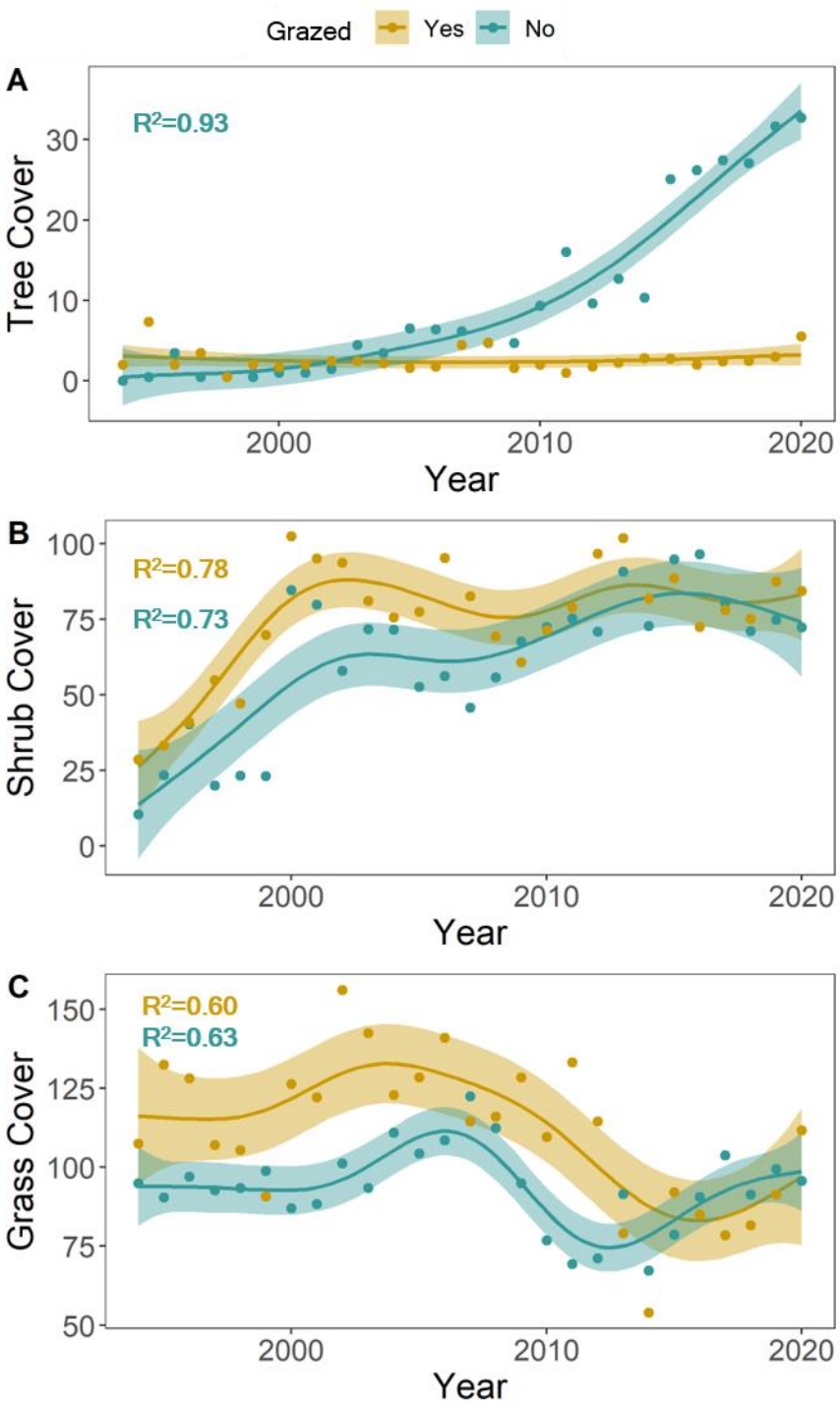

Figure 2.5: Tree cover, shrub cover, and grass cover as a function of time and grazing treatment (orange=grazed, blue = ungrazed). Points are annual averages and shaded areas span the 95th confidence interval of GAMs. Adjusted R^2 values are only shown for the significant GAMs ($p < 0.05$).

Figure 2.6: Photo of one of *Juniperus virginiana* seedlings ripped out in the bison grazed area at Konza Prairie Biological Station.

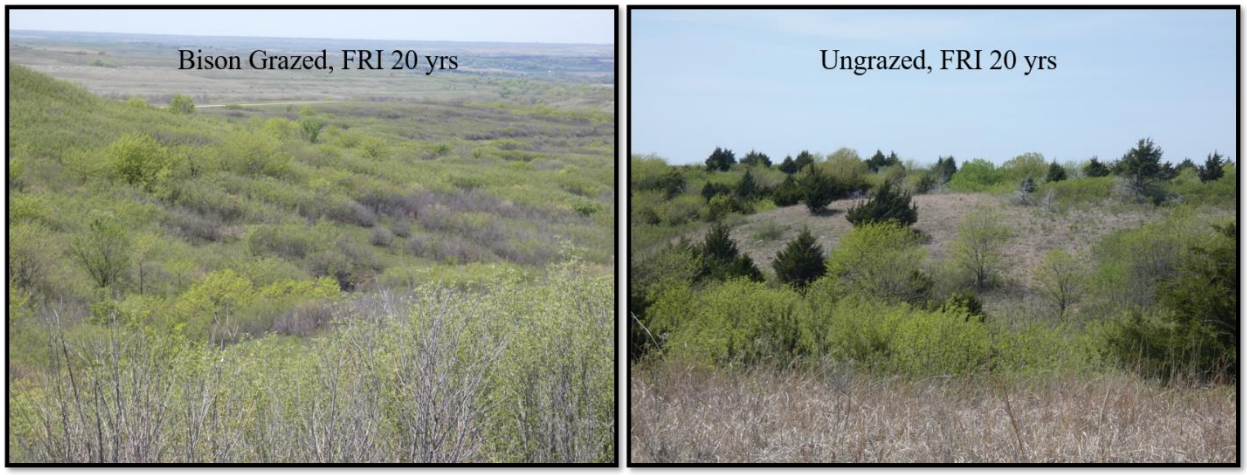

Figure 2.7: Photos of the bison grazed (left) and ungrazed (right) treatments burned approximately every 20 years. The ungrazed treatment contains *Juniperus virginiana* (the coniferous tree) while it is virtually absent in the bison grazed treatment.

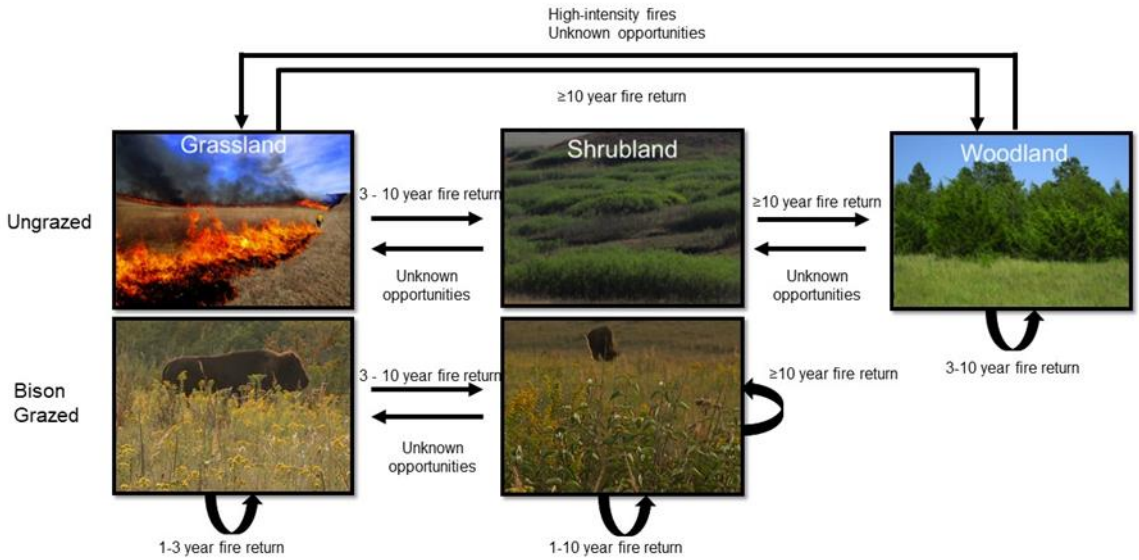

Figure 2.8: State and transition model of grassland, shrubland and woodland states including the presence and absence of bison adopted from Ratajczak et al. 2014. Square photos represent states and arrows represent processes that may allow a transition to another state. Selfing arrows represent hysteresis, where reintroducing conditions that maintained a grassland do not return the shrubland or woodland back to grassland. High intensity fires may transition an ERC dominated woodland back to a grassland (Bielski et al. 2021). The presence of bison prevents the transition of shrubland to ERC dominated woodland. Photo Credits: (Grassland: Eva Horne, Grassland with bison: Sidney Noble, Shrubland: Zak Ratajczak, Shrubland with bison: Sidney Noble, Woodland: John Blair).

Supplemental Figures and Tables

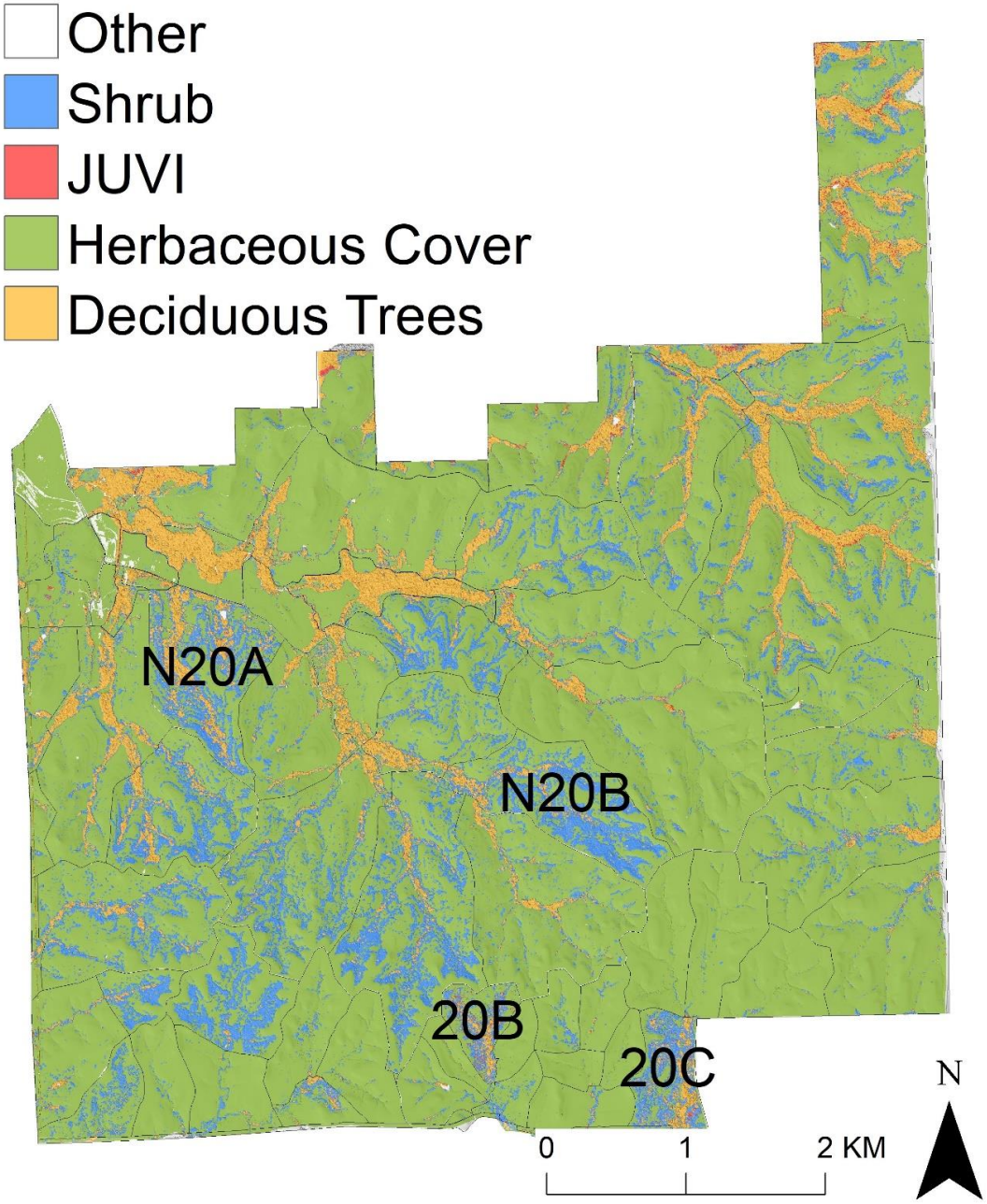

Figure S 2.1: Classified land use map of Konza Prairie Biological Station and treatments used in this study. N stands for native grazer (bison), 20 stands for fire return interval, and the following letter represents the replication. 20B and 20C are burned every twenty years with no grazers present. JUVI stands for *Juniperus virginiana.*

Model	Parametric Coefficient (Intercept)	Smooth Term (Year)	Adjusted R-squared	GCV
Species Richness (grazed, upland)	34.30 (0.22)	edf = 5.334, p < 0.001	0.622	26.444
Species Richness (ungrazed, upland)	23.7833 (0.2085)	edf = 6.723, p < 0.001	0.0611	23.818
Species Richness (grazed, lowland)	28.5185 (0.2404)	edf = 8.205, p < 0.001	0.356	31.752
Species Richness (ungrazed, lowland)	21.4019 (0.1889)	edf = 7.973, p < 0.001	0.199	19.592
Evenness (ungrazed, upland)	0.625573 (0.003913)	edf = 6.791, p < 0.001	0.0388	0.0083885
Evenness (grazed, upland)	0.714227 (0.002583)	edf = 5.132, p < 0.001	0.0726	0.0036444
Evenness (ungrazed, lowland)	0.629140 (0.004144)	edf = 6.473, p < 0.001	0.105	0.0094033
Evenness (grazed, lowland)	0.668934 (0.004136)	edf = 5.852, p < 0.001	0.0592	0.0093578
Exponential Shannon's (grazed, upland)	12.933 (0.145)	edf = 4.107, p < 0.001	0.306	11.465
Exponential Shannon's (ungrazed, upland)	7.6834 (0.1092)	edf = 5.855, p = 0.0561	0.0199	6.5196
Exponential Shannon's (grazed, lowland)	10.0489 (0.1488)	edf = 7.004, p < 0.001	0.0487	12.129
Exponential Shannon's (ungrazed, lowland)	7.3084 (0.1027)	edf = 6.503, p < 0.001	0.112	5.7705

Table S 2.1: Results of GAMs for species richness, evenness, and the exponential Shannon Index across soil types.

Table S 2.2: Results of the ANOVA for species richness, evenness, and the exponential Shannon Index, respectively for the first (1994 and last year (2020) of the bison grazed and ungrazed treatments.

	Species Richness					Evenness					Exponential Shannon Index				
Variable	Df	Sum of Squares	Mean Squares	F Value	P-value	Df	Sum of Squares	Mean Squares	F Value	P-value	Df	Sum of Squares	Mean Squares	F Value	P-value
Grazers	1	3871	3871	158.272	< 0.0001	1	0.0231	0.0231	3.048	0.08288	1	359.9	359.9	32.396	< 0.0001
Soil Type	1	154	154	6.299	0.0131	1	0.0005	0.0005	0.069	0.79385	1	23.6	23.6	2.125	0.14699
Year	1	4785	4785	195.646	< 0.0001	1	0.0967	0.0967	12.767	0.00047	1	86.0	86.0	7.744	0.00607
Grazers:Soil Type	1	394	394	16.099	< 0.0001	1	0.0326	0.0326	4.298	0.03985	1	99.7	99.7	8.979	0.00319
Grazers:Year	1	2318	2318	94.774	< 0.0001	1	0.0431	0.0431	5.694	0.01826	1	303.0	303.0	27.279	< 0.0001
Soil Type:Year	1	956	956	39.067	< 0.0001	1	0.0475	0.0475	6.275	0.01330	1	228.1	228.1	20.538	< 0.0001
Grazers:Soil Type:Year	1	761	761	31.125	< 0.0001	1	0.0022	0.0022	0.296	0.58749	1	71.2	71.2	6.408	0.01238
Residuals	152	3718	24	NA	NA	152	1.1513	0.0076	NA	NA	152	1688.4	11.1	NA	NA

Table S 2.3: The mean and standard deviation for species richness, evenness, and e^H for each year, treatment, and soil type combination.

Year, Treatment, and Soil Type	**Mean Species Richness and SD**	**Mean Evenness and SD**	**Mean e^H and SD**
1994 Grazed Upland	22.80 (4.96)	0.68 (0.09)	8.75 (2.76)
1994 Ungrazed Upland	21.80 (2.88)	0.67 (0.08)	8.26 (2.50)
1994 Grazed Lowland	26.95 (4.51)	0.69 (0.06)	10.12 (2.91)
1994 Ungrazed Lowland	23.50 (2.85)	0.72 (0.07)	10.12 (2.77)
2020 Grazed Upland	50.60 (7.18)	0.71 (0.05)	16.69 (4.77)
2020 Ungrazed Upland	25.65 (4.31)	0.62 (0.11)	8.03 (3.34)
2020 Grazed Lowland	36.25 (6.11)	0.63 (0.10)	10.62 (4.28)
2020 Ungrazed Lowland	26.30 (5.17)	0.61 (0.10)	7.78 (2.54)

Year, Treatment, Soil Type	diff	lwr	upr	p adj
1994 Grazed t-1994 Grazed f	4.15	-0.66	8.96	0.15
1994 Ungrazed f-1994 Grazed f	-1.00	-5.81	3.81	1.00
1994 Ungrazed t-1994 Grazed f	0.70	-4.11	5.51	1.00
2020 Grazed f-1994 Grazed f	**27.80**	**22.99**	**32.61**	**0.00**
2020 Grazed t-1994 Grazed f	**13.45**	**8.64**	**18.26**	**0.00**
2020 Ungrazed f-1994 Grazed f	2.85	-1.96	7.66	0.61
2020 Ungrazed t-1994 Grazed f	3.50	-1.31	8.31	0.34
1994 Ungrazed f-1994 Grazed t	**-5.15**	**-9.96**	**-0.34**	**0.03**
1994 Ungrazed t-1994 Grazed t	-3.45	-8.26	1.36	0.35
2020 Grazed f-1994 Grazed t	**23.65**	**18.84**	**28.46**	**0.00**
2020 Grazed t-1994 Grazed t	**9.30**	**4.49**	**14.11**	**0.00**
2020 Ungrazed f-1994 Grazed t	-1.30	-6.11	3.51	0.99
2020 Ungrazed t-1994 Grazed t	-0.65	-5.46	4.16	1.00
1994 Ungrazed t-1994 Ungrazed f	1.70	-3.11	6.51	0.96
2020 Grazed f-1994 Ungrazed f	**28.80**	**23.99**	**33.61**	**0.00**
2020 Grazed t-1994 Ungrazed f	**14.45**	**9.64**	**19.26**	**0.00**
2020 Ungrazed f-1994 Ungrazed f	3.85	-0.96	8.66	0.22
2020 Ungrazed t-1994 Ungrazed f	4.50	-0.31	9.31	0.08
2020 Grazed f-1994 Ungrazed t	**27.10**	**22.29**	**31.91**	**0.00**
2020 Grazed t-1994 Ungrazed t	**12.75**	**7.94**	**17.56**	**0.00**
2020 Ungrazed f-1994 Ungrazed t	2.15	-2.66	6.96	0.87
2020 Ungrazed t-1994 Ungrazed t	2.80	-2.01	7.61	0.63
2020 Grazed t-2020 Grazed f	**-14.35**	**-19.16**	**-9.54**	**0.00**
2020 Ungrazed f-2020 Grazed f	**-24.95**	**-29.76**	**-20.14**	**0.00**
2020 Ungrazed t-2020 Grazed f	**-24.30**	**-29.11**	**-19.49**	**0.00**
2020 Ungrazed f-2020 Grazed t	**-10.60**	**-15.41**	**-5.79**	**0.00**
2020 Ungrazed t-2020 Grazed t	**-9.95**	**-14.76**	**-5.14**	**0.00**
2020 Ungrazed t-2020 Ungrazed f	0.65	-4.16	5.46	1.00

Table S 2.4: Post-hoc Tukey comparisons for species richness. P-values of 0.00 are less than 0.01, bold indicates significance at an alpha of 0.05.

Year, Treatment, Soil Type	diff	lwr	upr	p adj
1994 Grazed t-1994 Grazed f	0.01	-0.07	0.09	1.00
1994 Ungrazed f-1994 Grazed f	-0.01	-0.10	0.07	1.00
1994 Ungrazed t-1994 Grazed f	0.04	-0.04	0.12	0.84
2020 Grazed f-1994 Grazed f	0.03	-0.06	0.11	0.98
2020 Grazed t-1994 Grazed f	-0.05	-0.13	0.04	0.65
2020 Ungrazed f-1994 Grazed f	-0.07	-0.15	0.02	0.23
2020 Ungrazed t-1994 Grazed f	-0.07	-0.15	0.02	0.20
1994 Ungrazed f-1994 Grazed t	-0.02	-0.11	0.06	0.99
1994 Ungrazed t-1994 Grazed t	0.03	-0.05	0.11	0.96
2020 Grazed f-1994 Grazed t	0.02	-0.07	0.10	1.00
2020 Grazed t-1994 Grazed t	-0.06	-0.14	0.03	0.41
2020 Ungrazed f-1994 Grazed t	-0.08	-0.16	0.01	0.10
2020 Ungrazed t-1994 Grazed t	-0.08	-0.16	0.01	0.08
1994 Ungrazed t-1994 Ungrazed f	0.05	-0.03	0.14	0.56
2020 Grazed f-1994 Ungrazed f	0.04	-0.05	0.12	0.87
2020 Grazed t-1994 Ungrazed f	-0.04	-0.12	0.05	0.89
2020 Ungrazed f-1994 Ungrazed f	-0.06	-0.14	0.03	0.49
2020 Ungrazed t-1994 Ungrazed f	-0.06	-0.14	0.03	0.44
2020 Grazed f-1994 Ungrazed t	-0.01	-0.10	0.07	1.00
2020 Grazed t-1994 Ungrazed t	**-0.09**	**-0.17**	**-0.00**	**0.03**
2020 Ungrazed f-1994 Ungrazed t	**-0.11**	**-0.19**	**-0.02**	**0.00**
2020 Ungrazed t-1994 Ungrazed t	**-0.11**	**-0.19**	**-0.02**	**0.00**
2020 Grazed t-2020 Grazed f	-0.07	-0.16	0.01	0.13
2020 Ungrazed f-2020 Grazed f	**-0.09**	**-0.18**	**-0.01**	**0.02**
2020 Ungrazed t-2020 Grazed f	**-0.09**	**-0.18**	**-0.01**	**0.02**
2020 Ungrazed f-2020 Grazed t	-0.02	-0.10	0.07	1.00
2020 Ungrazed t-2020 Grazed t	-0.02	-0.11	0.06	0.99
2020 Ungrazed t-2020 Ungrazed f	-0.00	-0.09	0.08	1.00

Table S 2.5: Post-hoc Tukey comparisons for evenness. P-values of 0.00 are less than 0.01, bold indicates significance at an alpha of 0.05.

Year, Treatment, Soil Type	diff	lwr	upr	p adj
1994 Grazed t-1994 Grazed f	1.37	-1.86	4.61	0.90
1994 Ungrazed f-1994 Grazed f	-0.49	-3.73	2.75	1.00
1994 Ungrazed t-1994 Grazed f	1.37	-1.87	4.61	0.90
2020 Grazed f-1994 Grazed f	**7.94**	**4.70**	**11.18**	**0.00**
2020 Grazed t-1994 Grazed f	1.87	-1.37	5.11	0.64
2020 Ungrazed f-1994 Grazed f	-0.72	-3.96	2.52	1.00
2020 Ungrazed t-1994 Grazed f	-0.97	-4.21	2.27	0.98
1994 Ungrazed f-1994 Grazed t	-1.87	-5.11	1.37	0.64
1994 Ungrazed t-1994 Grazed t	-0.00	-3.24	3.24	1.00
2020 Grazed f-1994 Grazed t	**6.57**	**3.33**	**9.81**	**0.00**
2020 Grazed t-1994 Grazed t	0.50	-2.74	3.74	1.00
2020 Ungrazed f-1994 Grazed t	-2.10	-5.34	1.14	0.49
2020 Ungrazed t-1994 Grazed t	-2.34	-5.58	0.90	0.34
1994 Ungrazed t-1994 Ungrazed f	1.87	-1.37	5.10	0.64
2020 Grazed f-1994 Ungrazed f	**8.43**	**5.19**	**11.67**	**0.00**
2020 Grazed t-1994 Ungrazed f	2.36	-0.88	5.60	0.33
2020 Ungrazed f-1994 Ungrazed f	-0.23	-3.47	3.01	1.00
2020 Ungrazed t-1994 Ungrazed f	-0.47	-3.71	2.76	1.00
2020 Grazed f-1994 Ungrazed t	**6.57**	**3.33**	**9.81**	**0.00**
2020 Grazed t-1994 Ungrazed t	0.50	-2.74	3.74	1.00
2020 Ungrazed f-1994 Ungrazed t	-2.10	-5.34	1.14	0.49
2020 Ungrazed t-1994 Ungrazed t	-2.34	-5.58	0.90	0.35
2020 Grazed t-2020 Grazed f	**-6.07**	**-9.31**	**-2.83**	**0.00**
2020 Ungrazed f-2020 Grazed f	**-8.66**	**-11.90**	**-5.43**	**0.00**
2020 Ungrazed t-2020 Grazed f	**-8.91**	**-12.15**	**-5.67**	**0.00**
2020 Ungrazed f-2020 Grazed t	-2.60	-5.83	0.64	0.22
2020 Ungrazed t-2020 Grazed t	-2.84	-6.08	0.40	0.13
2020 Ungrazed t-2020 Ungrazed f	-0.24	-3.48	3.00	1.00

Table S 2.6: Post-hoc Tukey comparisons for e^H. P-values of 0.00 are less than 0.01, bold indicates significance at an alpha of 0.05.

Table S 2.7: Results of the perMANOVA for 1994, 2020, and all years of the bison grazed and ungrazed treatments.

	1994						2020						1994-2020					
Variable	Df	Sum of Squares	Mean Squares	F. Model	R^2	P-value	Df	Sum of Squares	Mean Squares	F. Model	R^2	P-value	Df	Sum of Squares	Mean Squares	F. Model	R^2	P-value
Grazers	1	0.6295	0.6295	5.1971	0.054	0.0001	1	2.1765	2.1765	12.6493	0.11	0.0001	1	2.7655	2.7655	71.594	0.19	0.0001
Soil	1	1.2993	1.2993	10.7259	0.11	0.0001	1	3.6503	3.6503	21.2151	0.18	0.0001	1	1.7157	1.7157	44.417	0.12	0.0001
Year	1	N/A	N/A	N/A	N/A	N/A	1	N/A	N/A	N/A	N/A	N/A	1	4.1021	4.1021	106.197	0.28	0.0001
Grazers:Soil	1	0.5149	0.5149	4.2510	0.04	0.0001	1	1.2385	1.2385	7.1981	0.06	0.0001	1	1.1101	1.1101	28.739	0.08	0.0001
Grazers:Year	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0.3330	0.3330	8.6200	0.02	0.0001
Year:Soil	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0.5419	0.5419	14.029	0.04	0.0001
Grazers:Year:Soil	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0.3556	0.3556	14	0.02	0.0001
Residuals	76	9.2062	0.12113	N/A	0.79	N/A	76	13.0767	0.1721	NA	0.65	NA	100	3.8627	3.8627	NA	0.26	NA

Table S 2.8: Species scores for significant species at an alpha of 0.05. The first six letters of the genus and first five letters of specific epithet are given.

Species	NMDS1	NMDS 2	p-values
acalyp.virgi	0.40	-0.08	0.00
achill.mille	-0.08	0.56	0.00
agerat.altis	0.38	0.31	0.00
ambros.artem	0.09	0.30	0.00
ambros.psilo	-0.37	0.25	0.00
amorph.canes	-0.76	0.03	0.00
androp.gerar	-0.22	-0.65	0.00
antenn.negle	-0.66	0.34	0.00
apocyn.canna	0.15	-0.25	0.00
asclep.steno	-0.19	-0.25	0.00
asclep.sulli	0.22	-0.22	0.00
asclep.syria	0.14	-0.27	0.00
asclep.tuber	0.12	0.49	0.00
asclep.verti	0.16	0.32	0.00
asclep.virdf	-0.57	-0.04	0.00
asclep.virds	-0.27	0.13	0.01
astrag.crass	-0.65	0.21	0.00
baptis.austr	-0.12	-0.54	0.00
baptis.bract	-0.17	-0.59	0.00
botryc.virgi	0.10	0.27	0.00
boutel.curti	-0.68	0.35	0.00
boutel.dacty	-0.57	0.47	0.00
boutel.graci	-0.60	0.39	0.00
boutel.hirsu	-0.37	0.24	0.00
bricke.eupat	-0.37	-0.52	0.00
bromus.arven	0.03	0.48	0.00
bromus.inerm	-0.33	-0.48	0.00
callir.invol	0.06	0.54	0.00
calyst.macou	0.03	0.30	0.01
carex.austr	-0.08	0.49	0.00
carex.brevi	0.30	0.52	0.00
carex.grise	0.13	0.38	0.00

carex.inops	-0.68	0.13	0.00
carex.meadi	0.29	-0.20	0.00
ceanot.herba	-0.47	-0.53	0.00
celtis.occid	0.46	0.16	0.00
chlori.verti	-0.31	0.40	0.00
cirsiu.altis	0.11	-0.32	0.00
cirsiu.undul	-0.49	0.32	0.00
comand.umbel	0.09	0.51	0.00
conyza.canad	-0.05	0.22	0.04
cornus.drumm	0.75	0.39	0.00
cratae.molli	0.10	0.30	0.00
croton.monan	-0.04	0.25	0.02
cuscut.indec	0.07	0.25	0.01
cynanc.laeve	0.08	0.26	0.01
cyperu.lupul	-0.47	0.45	0.00
cyperu.xmeso	-0.26	0.26	0.00
dalea.candi	-0.62	-0.13	0.00
dalea.purpu	-0.66	-0.03	0.00
delphi.carol	-0.12	-0.35	0.00
descur.pinna	-0.29	-0.06	0.01
desmod.panic	0.24	0.00	0.02
desmod.perpl	0.23	0.34	0.00
desmod.sessi	0.25	-0.25	0.00
dichan.oligo	-0.53	0.24	0.00
dichan.ovale	0.23	-0.14	0.01
draba.cunei	-0.29	0.30	0.00
draba.repta	-0.29	0.25	0.00
echina.angus	-0.61	-0.22	0.00
eleoch.compr	0.28	-0.24	0.00
elymus.canad	0.03	0.26	0.01
elymus.virgi	0.11	0.36	0.00
eragro.spect	-0.27	0.27	0.00
erecht.hiera	0.30	-0.09	0.00
eriger.annuu	0.01	0.26	0.02
eriger.phila	0.09	0.25	0.01

eriger.strig	-0.59	0.27	0.00
eupato.altis	0.39	0.14	0.00
euphor.cyath	0.13	0.40	0.00
euphor.glypt	-0.39	0.32	0.00
euphor.macul	-0.23	0.26	0.00
euphor.margi	0.26	-0.19	0.00
euphor.nutan	0.02	0.48	0.00
euphor.prost	-0.18	0.16	0.03
euphor.spath	-0.28	-0.40	0.00
fallop.scand	0.36	0.07	0.00
galium.circa	0.10	0.24	0.01
geum.canad	0.33	0.46	0.00
glandu.bipin	-0.17	0.28	0.00
gledit.triac	0.53	0.08	0.00
grinde.squar	-0.34	0.32	0.00
hackel.virgi	0.28	0.08	0.00
hedeom.hispi	-0.42	0.11	0.00
helian.pauci	-0.22	0.22	0.00
hordeu.pusil	-0.41	0.42	0.00
hybant.verti	-0.62	0.32	0.00
hymeno.scabi	-0.54	0.19	0.00
juncus.inter	0.34	-0.00	0.00
junipe.virgi	0.16	-0.18	0.03
koeler.macra	-0.64	0.30	0.00
kummer.stipu	-0.13	0.41	0.00
lactuc.ludov	0.09	-0.27	0.01
lactuc.serri	0.02	-0.30	0.00
lepidi.densi	-0.68	0.29	0.00
lesped.capit	-0.32	0.14	0.00
lesped.cunea	0.09	0.25	0.01
lesped.viola	0.45	0.54	0.00
liatri.punct	-0.23	0.28	0.00
linum.sulca	-0.70	0.29	0.00
lithos.incis	-0.51	0.38	0.00
Lithos.occid	0.17	0.58	0.00

lomati.foeni	-0.61	0.05	0.00
lonice.maack	0.21	0.07	0.04
medica.lupul	0.13	0.35	0.00
mimosa.quadr	0.15	-0.65	0.00
monard.fistu	0.12	0.50	0.00
muhlen.cuspi	-0.29	-0.53	0.00
muhlen.racem	0.18	0.51	0.00
oenoth.glauc	-0.22	0.11	0.04
oenoth.macro	-0.57	0.30	0.00
oenoth.serru	-0.61	0.18	0.00
ophiog.vulga	0.16	0.33	0.00
opunti.macro	-0.59	0.29	0.00
oxalis.dille	-0.13	0.37	0.00
oxalis.stric	-0.37	-0.05	0.00
packer.platt	-0.66	0.23	0.00
pariet.pensy	0.34	0.02	0.00
parthe.quinq	0.25	-0.00	0.02
paspal.setac	-0.18	0.19	0.01
pediom.escul	-0.52	-0.16	0.00
pediom.tenui	-0.27	0.26	0.00
penste.cobae	-0.41	-0.02	0.00
penste.grand	-0.40	0.08	0.00
physal.pumil	-0.24	-0.46	0.00
planta.patag	-0.49	0.27	0.00
planta.rhodo	-0.24	0.20	0.00
planta.virgi	-0.24	0.44	0.00
poa.prate	0.24	-0.74	0.00
prunus.ameri	0.49	0.59	0.00
pyrus.calle	0.29	0.10	0.00
ratibi.colum	-0.60	0.10	0.00
rhus.aroma	-0.13	0.81	0.00
rhus.glabr	-0.61	-0.18	0.00
ribes.misso	0.31	0.10	0.00
rosa.arkan	0.31	-0.25	0.00
rubus.occid	0.31	0.12	0.00

rubus.pensi	0.61	-0.27	0.00
ruelli.humil	0.25	-0.54	0.00
salvia.azure	-0.76	-0.23	0.00
sanicu.canad	0.13	0.37	0.00
schedo.panic	-0.46	0.48	0.00
schiza.scopa	-0.27	0.35	0.00
scutel.parvu	0.13	0.37	0.00
securi.varia	0.42	0.07	0.00
senna.maril	0.10	0.42	0.00
silene.antir	-0.39	0.08	0.00
sisyri.campe	-0.25	0.20	0.00
smilax.tamno	0.28	0.01	0.01
solanu.carol	0.29	-0.39	0.00
solida.altis	0.46	-0.27	0.00
solida.canad	0.19	0.64	0.00
solida.gigan	0.11	0.37	0.00
solida.misso	-0.16	-0.41	0.00
solida.rigid	-0.22	0.54	0.00
sorgha.nutan	-0.00	0.62	0.00
sparti.pecti	0.39	-0.03	0.00
spermo.inerm	-0.25	0.33	0.00
spheno.obtus	0.10	0.22	0.04
sporob.compo	0.29	0.33	0.00
sporob.crypt	-0.36	0.32	0.00
sporob.heter	0.05	-0.76	0.00
sporob.vagin	-0.38	0.36	0.00
stenar.nigri	-0.45	0.35	0.00
stroph.leios	0.05	0.23	0.03
sympho.orbic	0.44	0.70	0.00
symphy.drumm	0.20	0.55	0.00
symphy.oblon	-0.25	-0.54	0.00
symphy.seric	-0.52	-0.28	0.00
teucri.canad	0.47	-0.18	0.00
torili.arven	0.17	0.26	0.00
toxico.radic	0.57	-0.11	0.00

tragia.beton	-0.47	0.08	0.00
tragia.ramos	-0.37	0.33	0.00
tragop.dubiu	-0.40	-0.27	0.00
triden.flavu	0.29	0.57	0.00
trioda.lepto	-0.11	0.24	0.02
triost.perfo	0.17	0.40	0.00
ulmus.ameri	0.53	0.12	0.00
ulmus.rubra	0.45	0.10	0.00
verben.stric	-0.30	0.45	0.00
verben.urtic	0.40	-0.07	0.00
vernon.baldw	-0.33	0.56	0.00
veroni.arven	0.11	0.35	0.00
viola.misso	0.09	0.26	0.01
viola.nephr	0.06	0.32	0.01
viola.pedat	-0.46	0.26	0.00
vitis.ripar	0.14	0.21	0.02
vulpia.octof	-0.51	0.45	0.00
zantho.ameri	0.15	0.42	0.00

Table S 2.9: Summary of GAMs for each treatment and vegetation cover type.

Model	Parametric Coefficient (Intercept)	Smooth Term (Year)	Adjusted R-squared	GCV	Scale Est.	n
Tree Cover (grazed)	2.609 (0.271)	edf = 2.054, p = 0.504	0.0422	2.2362	1.9832	27
Tree Cover (ungrazed)	10.138 (0.532)	edf = 3.1, p < 0.001	0.934	9.011	7.6428	27
Shrub Cover (grazed)	75.066 (1.807)	edf = 7.59, p < 0.001	0.776	129.35	88.196	27
Shrub Cover (ungrazed)	61.405 (2.415)	edf = 5.839, p < 0.001	0.73	210.89	157.47	27
Grass Cover (grazed)	111.172 (2.854)	edf = 6.335, p < 0.001	0.604	302	219.95	27
Grass Cover (ungrazed)	93.61 (1.53)	edf = 6.659, p < 0.001	0.632	88.243	63.213	27

Chapter 3 - Possible Hysteresis in a Woody Dominated State Grazed by Bison (*Bison bison*) Following a Fire Reversal Experiment

Abstract

Within the grasslands of the Central Great Plains (CGP), fire suppression has led to a phenomenon known as woody encroachment. Based on fire return intervals (FRI), alternative states form in the CGP, including grassland, shrubland, and woodland. Returning frequent fire alone to encroached areas rarely reverses these transitions—a pattern known as hysteresis. This study investigates the influence of the formerly most widespread native megafauna in North America, Plains Bison (*Bison bison*), on the reversibility of a woody-dominated state after the frequent fire has been returned to a tallgrass prairie (20 yr FRI to 1 yr FRI, fire reversal). This study occurred at Konza Prairie Biological Station, where bison have access to a 985-ha area divided into separate fire treatments. Additionally, bison exclosures were established in the 20-year FRI treatments before the reintroduction of frequent fire. After four years of data collection, bison usage increased only after the first year of reintroduced fire. The shrub area has decreased in areas with and without bison access. However, shrub area loss was highest after the first fire. Tree mortality is higher without bison, and plant community composition remains similar between bison grazed and ungrazed but with higher grass cover in the exclosures. Furthermore, compared to a historically 1-year FRI bison grazed treatment, there is significantly more shrub cover in the fire reversal treatment, bison usage remains lower, and trees are more abundant and not only confined to riparian areas. Our results suggest that there has been some success in reversing woody encroachment. However, the pace of this reversal appears to be slow, and therefore, full reversal of encroachment will likely take decades and there is still the possibility that it will eventually stall as shown by a previous experiment.

Introduction

Grasslands, vital for their biodiversity and ecosystem services, are increasingly recognized as one of the most imperiled terrestrial ecosystems globally (Hoekstra et al. 2005). One of the most pervasive threats to grasslands that remain is woody plant encroachment (WPE). WPE is the invasion of woody plants into open, grass-dominated systems, converting them into shrublands, savannas, or woodlands (Briggs et al. 2005, Archer 2017). WPE occurs due to multiple factors, including increasing CO_2 concentrations, changes in climate, intensive grazing, and fire suppression (Buitenwerf et al. 2012, Archer et al. 2017). The conversion of grassland systems into shrublands/woodlands leads to profound changes in ecosystem functions and services, including loss of forage for grazing, decreased stream flow, and loss of grassland-adapted species (van Auken 2000; Kulmatiski & Beard 2013; Wilcox et al. 2018; Keen et al. 2023).

Within North American grasslands, tree cover has increased by an estimated 6.9 million ha from 1990-2019 (Morford et al. 2023). This rapid expansion of woody plants has had a large economic impact on rangeland production, with an estimated loss of 4.1-5.6 billion dollars in cattle production (Morford et al. 2023). This rapid expansion has prompted widespread investments in preventing and reversing woody encroachment, especially in the Central Great Plains (Twidwell et al. 2014). However, when management does not prevent encroachment, several studies suggest that woody encroached mesic grasslands represent alternative states, making them difficult to reverse (Ratajczak et al. 2014, Collins et al. 2021). This property is often referred to as hysteresis: 1) first, a change in drivers initiates a large change in state; 2) second, after transition occurs (such as an expansion of woody plants), the driver variable is reversed in an attempt to reverse the change in ecosystem state, and 3) third, despite this reversal

of drivers, the ecosystem fails to return to its previous state. Hysteresis has been demonstrated in ecosystems ranging from lakes to coral reefs, deserts, and sub-tropical savannas (Fung et al. 2011, Staver et al. 2011, D'Odorico et al. 2012). Several mechanisms can generate hysteresis, but one of the most common is that a newly dominant species or functional group establishes self-reinforcing feedbacks that resist changes in key drivers (Suding et al. 2004). In the case of woody plant encroachment, these feedbacks are often shrub-soil interactions (in semi-arid grasslands, D'Odorico et al. 2011, D'Odorico et al. 2012) or a localized decrease in fire intensity (in more mesic grasslands) (Twidwell et al. 2013b, Ratajczak et al. 2014a).

In the mesic grasslands of the Central Great plains (our study region), fire frequency significantly influences WPE. In mesic grasslands or tallgrass prairies, there is strong evidence for three alternative attractors based on fire frequency, with grasslands being maintained by 1-3 yr fire return intervals (FRI), shrublands forming with 3-10 yr FRI, and woodland with >10 yr FRI (Ratajczak et al. 2014, Fogarty et al. 2021). Once these mesic grasslands transition to shrubland or woodland, returning fire alone is not enough to return them to grasslands, suggesting hysteresis (Collins et al. 2021). This is because shrubs reduce the amount of fuel for fires (Ratajczak et al. 2011) and store carbohydrates in their roots, allowing them to resprout vigorously even in the rare event that fire kills their aboveground stems (Janicke and Fick 1998, O'Connor et al. 2020). For instance, an experiment at Konza Prairie Biological Station in the Flint Hills ecoregion failed to fully revert a woody-dominated state to mesic grassland following a 20-year fire reversal from a 20 yr FRI to 1 yr FRI (Collins et al. 2021). Some experiments using "extreme fire"—which combine high fuel loads and fire that occur during windy dry conditions, have had some success at reversing encroachment (Bielski et al. 2021). But all of

these experiments occurred in the absence of a key evolutionary component of grassland ecosystems—native megafauna.

Most grasslands coevolved with large grazers (megagrazers from hereon), and contemporary and paleo studies suggest that in mesic grasslands, megagrazers are important for maintaining plant diversity (Towne et al. 2005, Collins and Calabrese et al. 2012). Prior to European colonization, bison numbered in the millions across the Great Plains, but they collapsed to less than a thousand individuals by the end of the 19th century due to hunting and purposeful extermination (Sanderson et al. 2008, Flores 2016). Today, bison are being reintroduced across much of the Great Plains, including many mesic grasslands or tallgrass prairie (Freese et al. 2007, Eyheralde 2015, Blackburn et al. 2020, Ratajczak et al. 2022). In the absence of large grazers within frequently burned mesic grasslands, C4 grasses dominate (Collins et al. 1998, Knapp et al. 1999, Collins and Calabrese 2012). However, bison preferentially consume C4 grasses, increasing light availability and decreasing their competitive ability, allowing other less competitive plant species to establish (Hartnett et al. 1996, Collins and Calabrese 2012). Additionally, bison create large amounts of habitat heterogeneity through wallowing, defecation, and trampling, increasing habitat patchiness and diversity at small scales, thus increasing overall diversity (Knapp et al. 1999, Lueders et. 2006, Allred et al. 2011).

Bison effects on woody plants are now just coming to light with recent bison reintroductions, reflecting a renewed interest in bison effects; this includes private and NGO investments (e.g., the American Prairie, the Nature Conservancy), the formation of the intertribal buffalo council for the transfer of bison from federal to tribal groups (US Department of Interior 2023), and recent calls that bison reintroduction could enhance the food sovereignty to some indigenous group (Shamon et al. 2022). As these reintroductions occur, we need data-driven

approaches to estimate the long-term impacts. Bison kill tree species through trampling, rubbing, and horning (Coppedge and Shaw 1994). In Yellowstone National Park, U.S.A., bison possibly prevent aspen recruitment due to trampling and browsing (Beschta et al. 2020, Painter et al. 2023). At our research site, Konza Prairie, bison have prevented the widespread establishment of the tree species eastern red cedar (*Juniperus virginiana*), possibly due to preventing seedling establishment and also reducing other tree species (Ch. 2). However, bison do increase woody plants in grasslands burned 1-4 years due to increased heterogeneity in fuels, reducing the likelihood of woody plant mortality, and do not affect shrub cover in areas burned every 20 years (Collins and Calabrese 2012; Ch. 2).

A relative unknown is the effect of a large grazer, in this case, Plains bison, on the hysteresis of woody plant expansion, as occurred in an ungrazed fire reintroduction experiment at our research site (Collins et al. 2021). Starting in 2021, two catchments with infrequent fire (~one fire per 20 years for 40 years) and grazed by bison were "reversed" by reintroducing fire annually to these treatments. At the time of the reversal, woody encroachment was extensive (Fig. S 3.1). Additionally, bison exclosures were established in these treatments to measure the interactive effects of bison and reintroducing frequent fire. This study aims to determine if hysteresis occurs after bison and fire reintroduction and measure the magnitude of bison effects on hysteresis. For the purposes of this study, we define hysteresis as failure to revert to a herbaceous-dominated state from a woody-dominated state. Due to the infrequency of fire before the start of the reversal, we hypothesized that bison would use these treatments more following the reversal, especially after the first fire due to increased light availability and increased foliar nitrogen content following the change from infrequent fire to frequent fire (Blair 1997, Raynor et al. 2015, Raynor et al. 2017). This potential increase in bison usage could allow for more

trampling of woody vegetation, potentially increasing mortality. However, we hypothesize due to bison grazing, there will be less fuel availability, decreasing the probability of woody plant mortality in the long term. We also assessed how plant communities respond to increased fire with and without bison grazing. We hypothesize that bison grazing will continue to allow for high species diversity in areas absent of woody plants, but that ungrazed exclosures will become less diverse over time, shifting to a grass-dominated state in areas without encroachment.

Methods

Site

This study was conducted at the Konza Prairie Biological Station (KPBS), a 3,487-ha native tallgrass prairie located in northeastern Kansas, USA (39˚05' N, 96 ˚35' W). KPBS is a National Science Foundation long-term ecological research (LTER) in the Flint Hills ecoregion of the Central Great Plains, the largest expanse of unplowed tallgrass prairie in North America (Samson and Knopf 1994). The mean annual precipitation is 835 mm yr^{-1}, and the growing season temperature (May– September) is 32.6^{o} C, with the mean monthly maximum in July (36.2^{o}C). The topography is complex: upland soils are shallow, rocky, and cherty silty clay loams typified by the Florence soil series. Lowland soils are deeper and non-rocky silty clay loams typified by the Tully soil series. Steep hills and flat benches of shallow soil connect these two topographic positions. Typical grassland vegetation at Konza is dominated by four perennial C_4 grasses: *Andropogon gerardii* (Vitman), *Sorghastrum nutans* (L.) Nash, *Schizachyrium scoparium* (Michx.) Nash, and *Panicum virgatum* L.. In the absence of grazers, these four grass species become dominant and account for >90% of the aboveground net primary productivity (Smith et al. 2003). In contrast, forbs and other subdominant species make up the bulk of species richness in the absence of grazing (Towne 2002). Common perennial forbs include *Symphyotrichum* spp.

(asters), *Eupatorium altissimum* L. (tall-joe pye weed*), Salvia azurea* Michx. (blue sage), and *Solidago* spp. (goldenrods). In frequently burned areas (every 1 to 4 years), bison promote tall *Solidago rigida* L. (rigid goldenrod) and *Verbena stricta* Vent. (hoary verbena), short annual species, and shorter grasses more typical of drier Great Plains grasslands (e.g., *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths and *Bouteloua dactyloides* (Nutt.) J.T. Columbus) (Ratajczak et al. 2022). Woody species are common along riparian areas and within the infrequently burned watersheds (4 or 20-year mean fire interval, Veach et al. 2014), with the most common shrub species being *Cornus drummondii* C.A. Mey. (rough-leaf dogwood).

Between the late 1970s to 1992 KPBS was divided along catchment boundaries into 64 fire management units, ranging from 12 to 136 ha in size. Replicate catchment units at KPBS have been experimentally burned at 1, 2, 3 to 4, and 20-yr fire intervals since 1983 (Knapp et al. 1998). In 1987, 30 bison were initially introduced and restricted to under half of its current spatial extent. In 1992, these bison were introduced to the remaining area, with access to 1012-ha. The herd increased to an average of 270 adults to maintain a grazing rate of approximately 25% removal of aboveground production each year (Towne 1999).

Fire Reversal Experiment and Sampling

In the spring of 2021, two treatment catchments grazed by bison burned every ~20 years began an annual fire frequency treatment. These treatments have now been burned in 2021, 2022, and 2023. In the spring of 2021, 8 bison exclosures, 15x15m in size, were established in each catchment for a total of 16 exclosures. Additionally, 16 plot pairs grazed by bison were established in 2020.

Tracking bison usage

To track bison usage within each of our grazed plots, we tracked bison dung counts at the end of the growing season (late August) using a classification system developed for our research site (Noble and Ratajczak 2023). All dungs within our plots were counted and put into five classes: Class 1: Fresh, <48 hours old, dark brown, no crust yet, wet; Class 2: Dark to medium brown, crust on outside, inside still soft, mostly or fully intact; Class 3: Light brown to gray, fully dry and hard, maybe starting to fall apart; Class 4: Fully gray, possibly breaking down into chunks; Class 5: Decayed, only tiny pieces remaining, mostly dirt or grass left (see Noble and Ratajczak 2023). In general, bison dung decays slowly at our site (over 150 days), and therefore, to track near-term usage, we analyzed data using classes 1 to 4.

Is there greater woody plant area and mortality with or without bison grazing?

To determine if there is an effect of bison on woody plant cover and mortality, we tracked shrub area, shrub stem mortality and resprouts, and tree top kill and resprouts in each of our 15 x 15 m plots. For shrub area, we estimated cover each sampling year 2020, 2021, 2022, and 2023 using high precision Trimble GPS units (an accuracy of around 0.5 m), walking around continuous shrubs that we term “islands.” The island's edge was determined by a height of ≥ 0.5 m and a density of at least three ramets within 0.5 m of each other. Shrub stem mortality was tracked, establishing three random shrub transects on the inside of the island, the edge, and the outside of the island. We tagged all shrub stems ≥ 0.5 m in height, measured diameter, and identified species. All trees ≥0.5 m in each plot were tagged, the diameter measured at 0.5 m, and identified. We tracked whether shrub stems and trees suffered mortality/topkill each year. Additionally, we tagged any additional resprouts repeating the measurements above. All sampling occurred at the end of the growing season in late August and early September.

For determining whether there was higher shrub stem resprouting and survival we compared the proportions of resprouting and survival between the two treatments (bison vs. exclosures) across the study period. Proportions were calculated by dividing the number of resprouting or surviving shrubs by the total number of shrub stem in the previous year. The comparison of resprouting rates between bison and exclosures was performed using a two-sample test for equality of proportions with a continuity correction. This test evaluates if the observed proportion of resprouting shrubs in each treatment is significantly different. Similarly, survival rates were compared using the same statistical method to determine if the proportion of surviving shrubs differed significantly between treatments.

For comparing shrub area between the treatments, the areas were log-transformed to meet the assumptions of normality and homogeneity of variances required for ANOVA. A mixed-design ANOVA was performed to analyze the effects of time (year) and treatment condition (exclosure vs. bison present) on shrub area. Following the ANOVA, post-hoc tests were conducted to compare mean shrub areas between specific years and treatments where significant effects were identified.

For comparing tree topkill between the treatments, we applied generalized linear models (GLMs) to evaluate the impact of time (yearly changes) and treatment (exclosure vs. bison) on tree mortality. We only analyzed the species *Gleditsia triacanthos* as this accounted for ~95% of our tree composition. The response variable, mortality, was treated as a binary outcome (dead or alive), and logistic regression was utilized to model the probability of tree mortality as a function of the predictors.

Plant species diversity and community differences between bison grazed and exclosures

To determine changes in plant diversity and plant communities in our grazed and exclosure treatments, we established a 15 m transect down the middle of our 15x15 m plots. Plant community composition was measured at 1 m, 5 m, 9 m, and 13 m with a 1 m^2 quadrat in 2022 and 2023. Each species was identified, and species cover was recorded using a modified Daubenmire scale, with cover classes of 0–1%, 1–5%, 5–25%, 25–50%, 50–75%, 75–95%, or 95–100% aerial coverage (Bailey and Poulton 1968). To calculate approximate cover, we used the midpoint of the Daubenmire scale range. All vegetation sampling was done at the end of the growing seasons (late August- early September).

To quantify the effect of exclosures and bison on plant diversity, we calculated species richness and species diversity. Species richness is the total number of species found in each of the 15x15 m plots. Species diversity for each site was calculated and reported using the exponential Shannon's index, $\exp(H' = \Sigma(p_i \times \ln p_i)$, where p_i is the relative abundance of species *i*. An exponential Shannon's index (e^H) is the effective number of equivalently abundant species in a community, and generally more interpretable than a raw Shannon's Index value. For species richness and diversity (e^H), we performed ANOVAs with year (2022 and 2023), exclosures, and their interaction as fixed effects. We performed post-hoc comparisons using the Tukey test in the R package multcomp (Hothorn et al. 2008) of all pairwise comparisons for year. Test were performed at an alpha level of 0.05, with an honest squares difference adjustment to *P* values.

We employed a non-metric multidimensional scaling ordination (NMDS) based on a Bray-Curtis dissimilarity metric to determine if species composition diverged. Bray-Curtis is one of the most effective distance metrics for ecological data (Beals 1984; Ricotta and Podani 2017), and NMDS is a flexible ordination technique suitable for data that deviate from normality, such as vegetation data (Dexter et al. 2018). We used permutational multivariate analyses of variance

(perMANOVA) with a Bray-Curtis dissimilarity metric using the package vegan (Oksanen et al. 2013) to determine if significant differences existed between the exclosures and bison grazed. To determine what species drove the changes in composition, we used indicator species analysis (SIMPER).

Results

Bison Usage

There was a significant effect of the year on dung density—our proxy for bison activity (Table 3.1, Fig. 3.1A), including a significant increase in dung density from 2020 to 2021 (mean difference = 0.073 m^2, 95% CI [0.024, 0.122], $p < 0.001$). In the third (2022) and fourth (2023) year there was a decrease from this high, with values that fall between the pre-fire reintroduction sampling and the fire year of fire reintroduction (2022: mean difference = 0.027 m^2, 95% CI [-0.022, 0.075], $p = 0.488$; 2023: mean difference = 0.046 m^2, 95% CI [-0.003, 0.094], $p = 0.075$). This suggests that the initial increase in bison usage did not continue to rise at the same rate in the following years. When comparing the year 2021 with 2022 and 2023, the differences were not significant (2021-2022: mean difference = -0.046 m^2, 95% CI [-0.095, 0.002], $p = 0.069$; 2021-2023: mean difference = -0.027 m^2, 95% CI [-0.076, 0.021], $p = 0.468$), indicating that the usage remained relatively stable after the significant increase in 2021.

Bison usage was the similar in 2023 between treatments that have been burned annually and our fire reversal treatments (Fig. 3.1B). The Welch two-sample t-test yielded a t-value of 1.4 with 75.993 degrees of freedom, resulting in a p-value of 0.166. This indicates that there is not a statistically significant difference in the mean dung density between the two treatments at the alpha 0.05 level. The 95 percent confidence interval for the difference in means ranged from -0.012 to 0.071 m^2 of dung, further illustrating the lack of a significant difference. The mean dung

density in the annually burned treatments was 0.160 m², compared to the fire reversal treatments which had a mean of 0.130 m².

Woody plant area bison grazed vs exclosures

Over time shrub area has shrank (Fig. 3.2). The log-transformed shrub area data demonstrated a significant main effect for Year ($F = 8.029$, $p < 0.001$), indicating that shrub cover changed over the years following the fire regime reversal (Table 3.2). Additionally, there was a significant main effect of exclosure ($F = 11.247$, $p < 0.001$). However, the interaction effect between Year and exclosure was not significant ($F= 0.021$, $p = 0.9958$). Over the years, shrub area decreased significantly, with the greatest reduction observed from 2020 to 2023 (mean difference = -1.122, $p < 0.001$). This corresponds to an average shrinkage of ~27 m^2 per shrub island. In the exclosures, shrub areas did not significantly change from 2020 to any other year; $p>0.05$ for all comparisons. However, outside the exclosures shrub area had significantly declined from 2020 compared to 2023 (mean difference = -1.116, $p = 0.001$). Comparing exclosure versus non-exclosure areas, there was a significant difference in shrub area (mean difference = 0.695, $p < 0.001$). However, there was no difference in shrub area at the start of this study in 2020.

Shrub Stem Resprouts and Survival

In 2022, the proportion of shrubs resprouting in exclosures was significantly higher than in bison areas, with 82.61% in exclosures compared to 38.11% in bison areas ($X^2 = 100.6$, $p < 0.001$, Fig. 3.3). In 2023, a similar pattern was observed, where exclosures again showed a higher proportion of resprouting at 84.54%, compared to 68.72% in bison areas ($X^2 = 16.76$, $p < 0.001$, Fig. 3.3). The shrub stem survival rates in 2022 also exhibited significant differences between the treatments. Bison areas had a survival rate of 70.44%, compared to 22.83% observed in

exclosures ($X^2 = 16.76$, $p < 0.001$). However, in 2023, the difference in survival rates between bison (49.15%) and exclosures (43.81%) was not statistically significant ($X^2 = 1.36$, $p = 0.243$).

Tree topkill

The overall mortality of *Gleditsia triacanthos*, the most common tree, showed a significant year effect, with a marked decrease in 2023 ($z = -3.067$, $p = 0.002$), as well as a significant interaction between exclosure status and year ($z = 4.349$, $p < 0.001$). While the status of being grazed showed a tendency toward lower mortality rates, this difference was not statistically significant ($z = -1.692$, $p = 0.09$) (Table 3.3). In 2022, the proportion of mortality in ungrazed areas was lower (43%) compared to grazed areas (58%). In 2023, this trend was amplified, with mortality rates dropping to 28% in grazed areas, while in exclosures, the mortality increased to 77% (Fig. 3.4).

Plant species diversity

There was a significant effect of year and bison presence (exclosures) on species richness and a plant diversity index (Table 3.4). There was a significant decrease in species richness from 2022 to 2023, with a mean difference of -5.41 species ($p < 0.001$). The presence of bison was associated with an increase in species richness, where plots with bison showed a mean increase of 2.41 species compared to exclosures ($p = 0.018$). The interaction between year and bison presence indicated varying effects over the two years. From 2022 to 2023, exclosures and bison present plots experienced a significant reduction in species richness (exclosures=-4.44 species, $p = 0.012$, bison = -6.38 species, $p < 0.001$). However, the difference between exclosures and bison present were not statistically significant (Fig. 3.5 A, Table S 3.1).

There was a notable decrease in the exponential Shannon index from 2022 to 2023 (mean difference: -5.69, $p < 0.001$). Bison presence led to an increase in the exponential Shannon index

by an average of 3.38 equivalent species (p = 0.007). In 2022, plots with bison had a significantly higher exponential Shannon index compared to those without bison (4.85, p = 0.03). The transition from 2022 to 2023 in plots with bison saw a significant decrease in the exponential Shannon index (-7.16 units, p = 0.001) (Fig. 3.5 B, Table S 3.2).

Plant community composition in the bison grazed vs exclosures

The perMANOVA revealed significant effects of bison presence and watershed on plant community, indicating that both these factors play roles in shaping community composition (Table 3.5). Bison presence and watershed each explained ~8% of the variance. The factor 'Year' also had a significant effect on the plant community ($F = 1.949$, $R^2 = 0.026$, $p = 0.022$), suggesting that plant community composition has changed from 2022 to 2023. However, the interactions between bison and watershed, bison and year, watershed and year, as well as the three-way interaction were not significant (Table 3.5).

The non-metric multidimensional scaling (NMDS) ordination plot visually supports these perMANOVA findings (Fig. 3.6). The plot depicts distinct clustering of plant communities based on bison presence, as indicated by the separation of points along the NMDS1 axis. Plant communities in areas with bison are separated from those without bison which corresponds to the significant perMANOVA result for bison presence. The points also show a temporal shift from 2022 to 2023, although the spread suggests some overlap, reflective of the lower R^2 value for the year effect.

Discussion

After three years of annual fire reintroduction, woody plants still dominate the treatments. After the first fire, bison usage increased significantly compared to pre-fire reversal. However, it was not significantly different from the pre-fire reversal usage in the following years with annual

fire. This is supported by other work at Konza, that bison prefer areas that have been recently burned after being unburned for several years (Joern et al. 2015, Raynor et al. 2017). Furthermore, we have seen an initial decrease in shrub size following the fire reversal, but this appears to be leveling off or even growing for some species, as many of these species have the ability to resprout following fire. Tree topkill was high with and without bison. However, this has declined with bison, probably due to lower fuel loads (Fig. 3.7, Fig. S 3.2B). The plant communities are shifting, especially with the exclusion of bison. Plant species richness and diversity remain similar with and without bison. However, there was decrease from 2022 to 2023, likely due to a drought (USDM 2023). Interestingly, our most abundant woody species, *Cornus drummondii*, is one of the species driving the change in bison exclosures. The increase in *C. drummondii* cover in the bison exclosures is probably due to the higher mortality of stems and the resprouting of these stems in our vegetation plots (Fig. 3.3, Fig. S 3.2A).

Bison habitat utilization in tallgrass prairies is influenced by a dynamic interplay of fire, forage quality, and topographical variations, aligning with known behaviors of large grazers (Eby et al. 2014, Raynor et al. 2017). At the Konza Prairie Biological Station (KPBS), bison prefer areas that have recently experienced fire, which, coupled with varying fire-return intervals, creates a shifting mosaic of vegetation that affects their foraging behavior (Raynor et al. 2017). Bison are particularly drawn to treatments that have undergone burning within recent years, showcasing a tendency to utilize these areas from spring through autumn (Raynor et al. 2017). This attraction is thought to be driven by the availability of high-quality forage in these areas, which is further enhanced by soil nitrogen levels that are boosted following fires. The pattern of bison space use within the experimental design is not controlled, which is noteworthy because it reflects the natural behavior of these animals in a dynamic ecosystem. Although the

KPBS bison unit is smaller than the realistic migratory range before colonization. Still, bison historical participants in this environment, "vote with their feet" by moving towards areas that meet their needs, effectively mimicking historical responses to fluctuating fire regimes (Shaw and Carter 1990, Raynor et al. 2017, McMillian et al. 2021). This uncontrolled aspect of their habitat usage is a strength of the study, as it provides genuine insights into how bison naturally interact with and adapt to the changing availability of resources. Despite the constraints posed by variable climatic events and experimental conditions, this approach allows for a more authentic understanding of how grazers may react to reintroducing fire on a landscape scale.

While there has been some woody plant reduction following our four-year study, it appears likely that bison will not affect hysteresis in this system, and woody plants will probably still dominate in the long term. Another experiment at Konza Prairie with no grazers has failed to revert a woody-dominated state to grassland (Collins et al. 2021). Furthermore, with the presence of bison, we expect that there will be less woody plant mortality and topkill, as bison reduce graminoid abundance in these systems, which is the primary fuel source (Towne et al. 2005). We have noticed that in our exclosures, there appears to be a more topkill of shrubs and trees, with more cover of *Cornus drummondii* from resprouts in the exclosures (Fig. 3.3, Fig. S 3.2A). Thus, bison may even promote more woody cover and hinder reversal. However, this study is in its infancy, and further monitoring is warranted; ecosystems dominated by perennial plants are prone to long transient behavior of very slow change, punctuated by rapid changes (Bestelmeyer et al. 2011, Hastings 2016, Ratajczak et al. 2017).

The dominant woody plant species in our woody-dominated state with bison are *Cornus drummondii* for shrubs and *Gleditsia triacanthos* for trees. Interestingly, past work at Konza has found that bison prevent the establishment of the dominant tree encroacher in the Central Great

Plains, *Juniperus virginiana* (Ch 2). However, it is less so with *G. triacanthos* and not all with *Cornus drummondii*. This is probably due to the resprouting nature of these plants, which makes it difficult to impossible to rid them with fire once they begin to dominate the landscape. Once established, they form dense clonal thickets, reducing herbaceous plant cover to near zero, with only a few species existing in the understory of these shrub islands (eg., *Symphoricarpos orbiculatus, Parietaria pensylvanica*). Historically, these species were probably limited in our systems via browsing in addition to fire (O'Connor et al. 2020). We are seeing that the cover of these species can be reduced, but it is unclear just how much their expansion can be reversed in the long-term.

Woody encroachment has been difficult to reverse in other grassland and savanna systems. In Kruger National Park, South Africa, efforts to employ higher-intensity fires as a strategy to counter woody encroachment have similarly failed to achieve the desired reversal of vegetation changes (Strydom et al. 2023). The persistent presence and even proliferation of woody plants despite aggressive fire management suggest a possible hysteresis effect within these systems, where the ecological state has shifted to a new equilibrium that is resistant to traditional management interventions (Nippert et al. 2021, Collins et al. 2021, Bielski et al. 2021). This resistance indicates that once thresholds are crossed, returning to a previous plant community composition may require more than simply modifying fire regimes. Instead, it underscores the need for a multi-faceted approach that might include integrating other management practices like mechanical removal or targeted grazing alongside fire (Twidwell et al. 2021). In a mixed-grass prairie in Nebraska, the Loess Canyons, extreme fire restored herbaceous cover but was quickly reinvaded without fire continuation (Bielski et al. 2021, Fogarty et al. 2022). Moreover, this scenario highlights the importance of understanding the

underlying ecological mechanisms driving woody encroachment, which may be influenced by factors such as altered fire frequencies, climate change, and changes in herbivore populations (Venter et al. 2018). As in Kruger National Park and the Loess Canyons, where high-intensity fires have not simplified the task of managing woody species, it becomes clear that adaptive management strategies, informed by ongoing research and tailored to specific ecological contexts, are crucial for addressing complex conservation challenges like woody encroachment.

Conclusions

Bison responses to reintroducing fire reiterate the interesting capability of bison to sense high quality forage (Fortin 2003, Raynor et al. 2015). After three years of annual burning in a heavily encroachment grassland, we have found some reductions in shrub cover, high tree topkill, and the beginning of some shifts in the plant community. If these trends continue, encroachment could potentially be reversed after more than a decade. However, we expect this trend to slow, as it has in the exclosures, as smaller less resistant shrubs and trees die first, and larger woody plants remain. Our results underscore that, at best, woody encroachment can be partially reversed with prescribed fires, but at best, this is likely to be a long process. This underscores that need for creative adaptive management approaches to reverse encroachment at the faster time scale that production systems usually follow. We plan to continue this experiment for at least two decades, with the intent that we will see if other factors, such as interactions with climate change, affect the trajectory of woody plants, resulting in new strategies to reverse shrub encroachment.

Tables

Factors	Sum of Squares	Degrees of Freedom	F-Statistic	P-Value
Year	0.1139	3	5.387	0.001487
Residual	1.0993	156	-	-

Table 3.1: ANOVA table for bison dung density and effect of year.

Factors	Degrees of Freedom	Sum of Squares	F-Statistic	P-Value
Year	3	48.24	7.98	0.00004
Exclosure	1	22.67	11.25	0.000907
Year * Exclosure	3	0.13	0.02	0.995786
Residual	281	566.36	-	-

Table 3.2: ANOVA table for log-transformed shrub area and the factors Year, Exclosure, and their interaction.

Factor	Estimate	Std. Error	z value	P-value
Intercept	0.3083	0.2195	1.404	0.16018
Exclosure	-0.5766	0.3407	-1.692	0.09057
Year	-1.2777	0.4166	-3.067	0.00216
Exclosure:Year	2.7624	0.6352	4.349	1.37e-05

Table 3.3: The results from a generalized linear model (GLM) analyzing the effect of exclosure status and year on mortality for *Gleditsia triacanthos*, using a binomial distribution to model the response variable.

Factors	Degrees of Freedom	Sum of Squares	Mean Square	F Value	P-Value
Species Richness					
Year	1	467.6	467.6	29.927	<0.001
Bison	1	92.6	92.6	5.929	0.018
Year:Bison	1	15.0	15.0	0.961	0.331
Residuals	60	937.6	15.6		
Exp Shannon's					
Year	1	517.6	517.6	22.192	<0.001
Bison	1	183.1	183.1	7.849	0.007
Year:Bison	1	34.5	34.5	1.479	0.228
Residuals	60	1399.4	23.3		

Table 3.4: Table for the results of an ANOVA examining the effects of Year, Bison (Exclosure), and their interaction on plant species richness and a diversity index (e^H).

Factor	Df	Sum of Squares	R^2	F	P-value
Bison	1	1.0565	0.0821	6.0830	0.001
Year	1	0.3384	0.0263	1.9485	0.022
Watershed	1	1.0047	0.0781	5.7845	0.001
Bison:Year	1	0.1258	0.0098	0.7242	0.743
Bison:Watershed	1	0.2075	0.0161	1.1947	0.259
Year:Watershed	1	0.3001	0.0233	1.7278	0.044
Bison:Year:Watershed	1	0.1067	0.0083	0.6141	0.885
Residual	56	9.7264	0.7560		
Total	63	12.8661	1.0000		

Table 3.5: perMANOVA table evaluating the influence of Bison, Year, and Watershed, as well as their interactions on plant community composition.

Figures

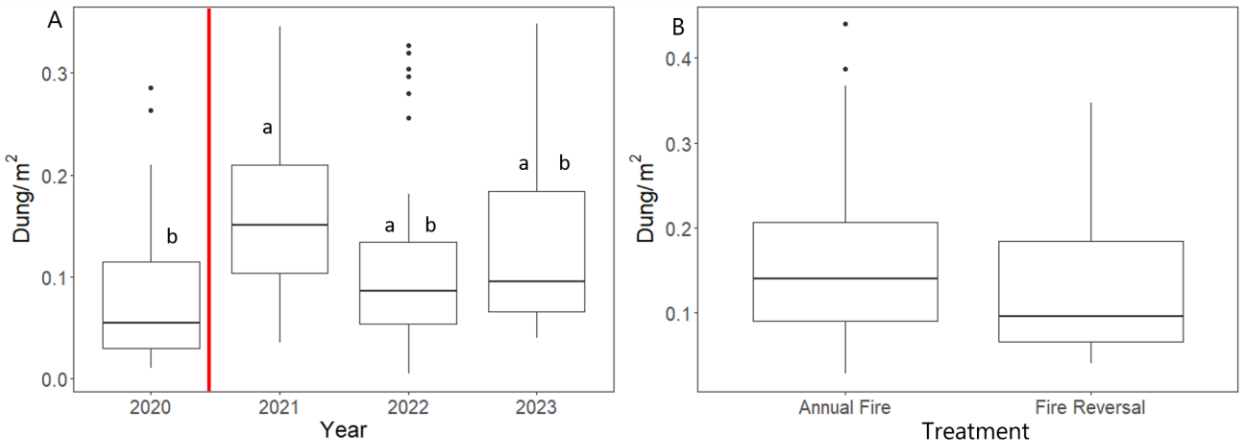

Figure 3.1: A) Bison dung pile density from 2020-2023. Years that share a letter are not significantly different from each other ($p > 0.05$). **B)** Comparison between dung density in the annual fire treatment vs the fire reversal treatment in 2023. Dung density was not significantly different between the treatments ($p > 0.05$).

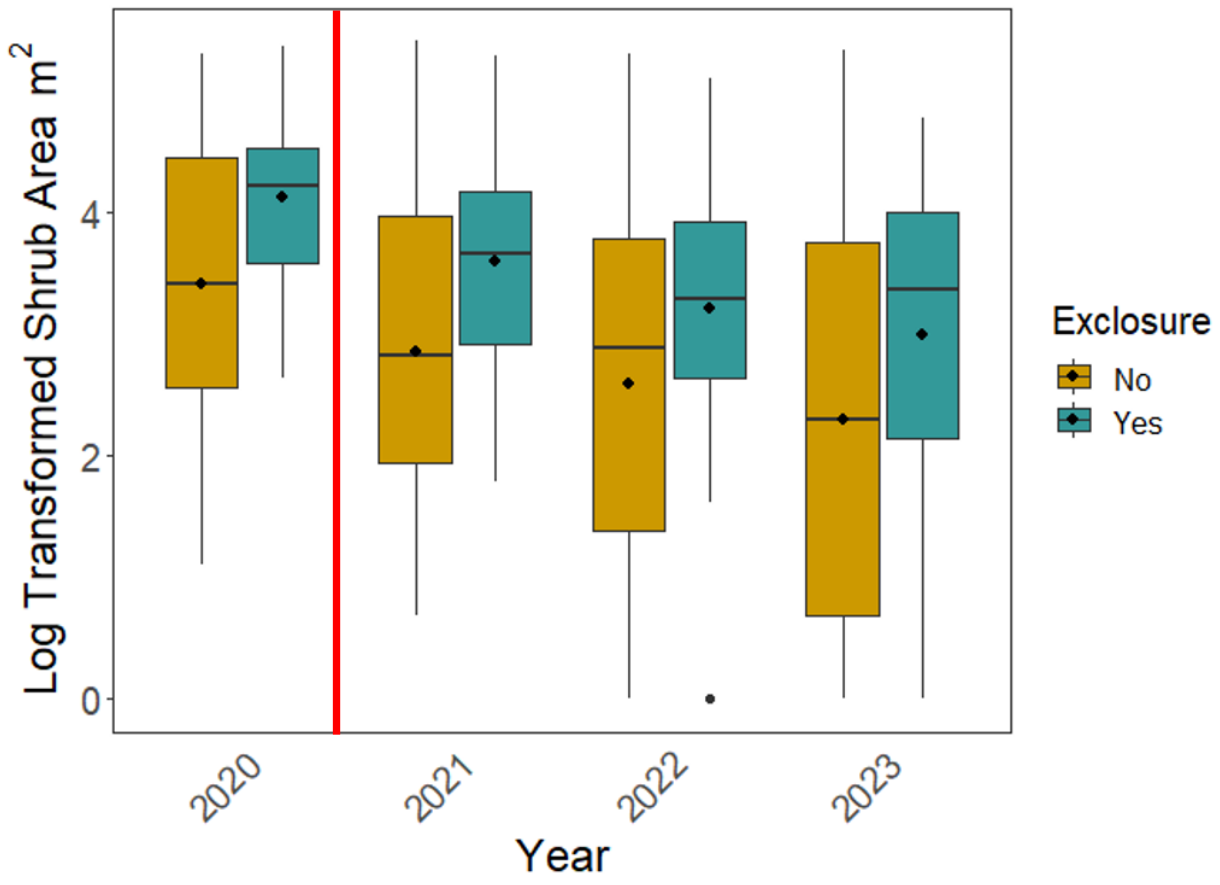

Figure 3.2: Boxplot of the log transform shrub area in exclosures (blue) and with bison (orange). The red line represents the start of the fire reversal. Black dots represent the mean.

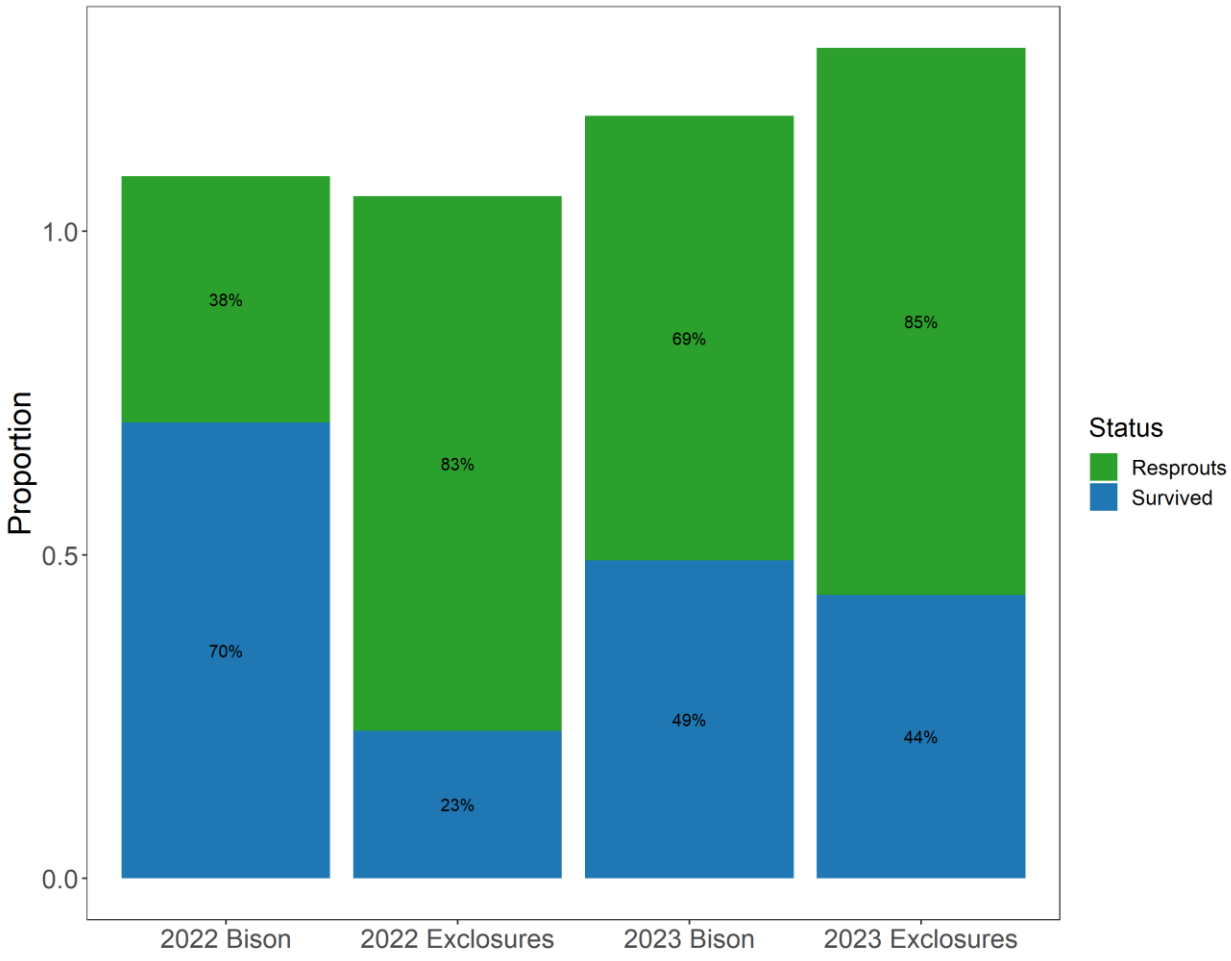

Figure 3.3: Proportion of shrub stem resprouts and survival across the years and treatments based on the previous year's shrub stem count. Proportions can exceed one due to resprouts.

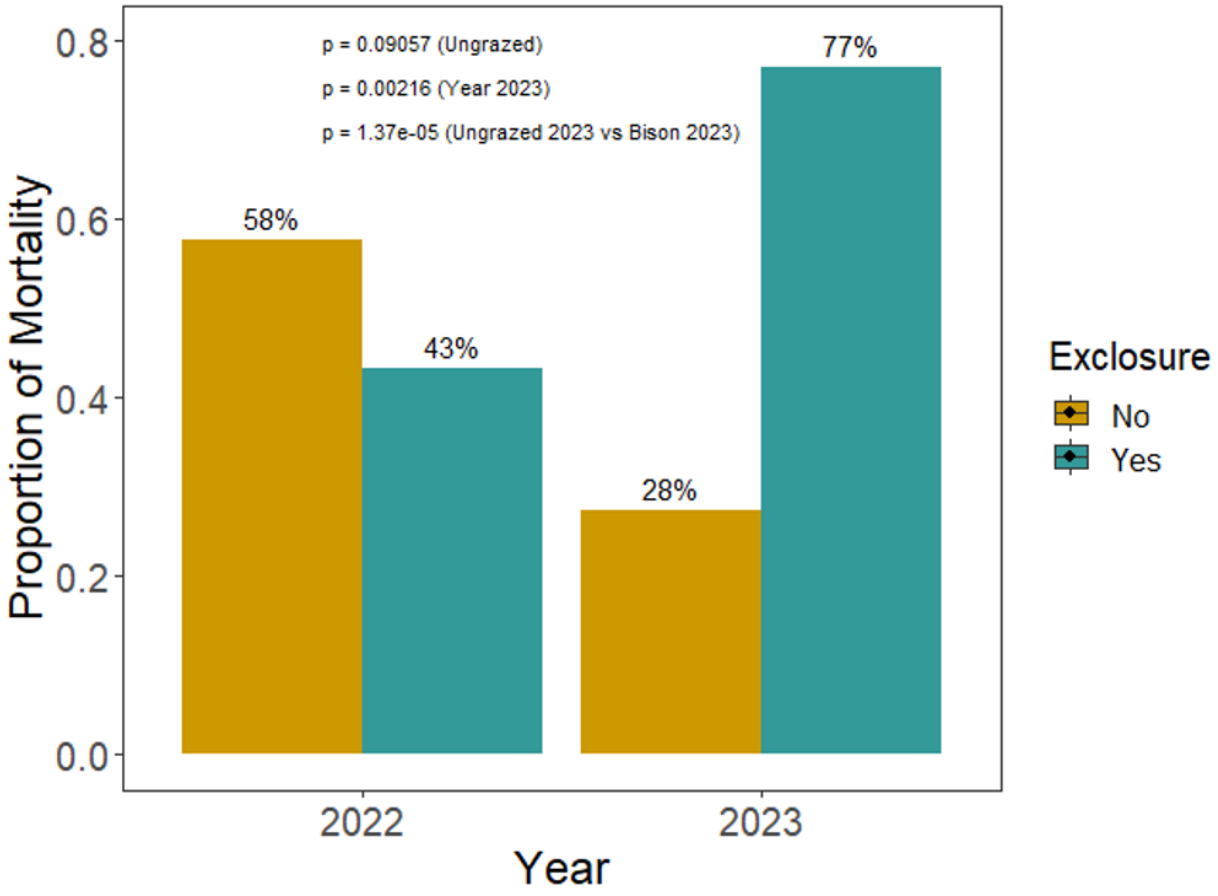

Figure 3.4: The bar graph presents the proportion of tree (*Gleditsia triacanthos*) topkill with bison and in exclosures following a fire reversal treatment for the years 2022 and 2023, with the comparison between areas with bison (teal bars) and without bison (orange bars). In 2022, the mortality rate was 58% without bison and decreased to 43% with bison presence. The following year, tree mortality dropped to 28% in areas without bison, but surged to 77% where bison grazed. Statistical analysis reveals this increase to be highly significant (p = 1.37e-05), suggesting a strong impact of bison on tree topkill.

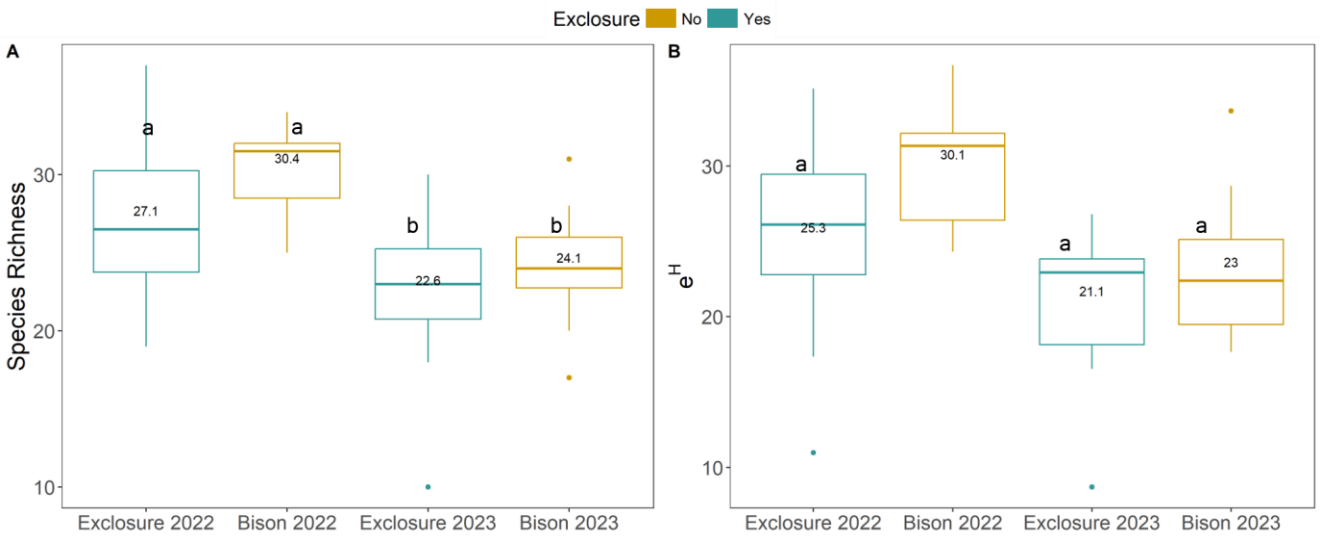

Figure 3.5: Species richness (A) and an exponential Shannon index (e^H) (B) in exclosures and with bison for 2022 and 2023. Numbers represent the mean. Boxplots that share lettering at the did not have statistically significant differences at an alpha of 0.05.

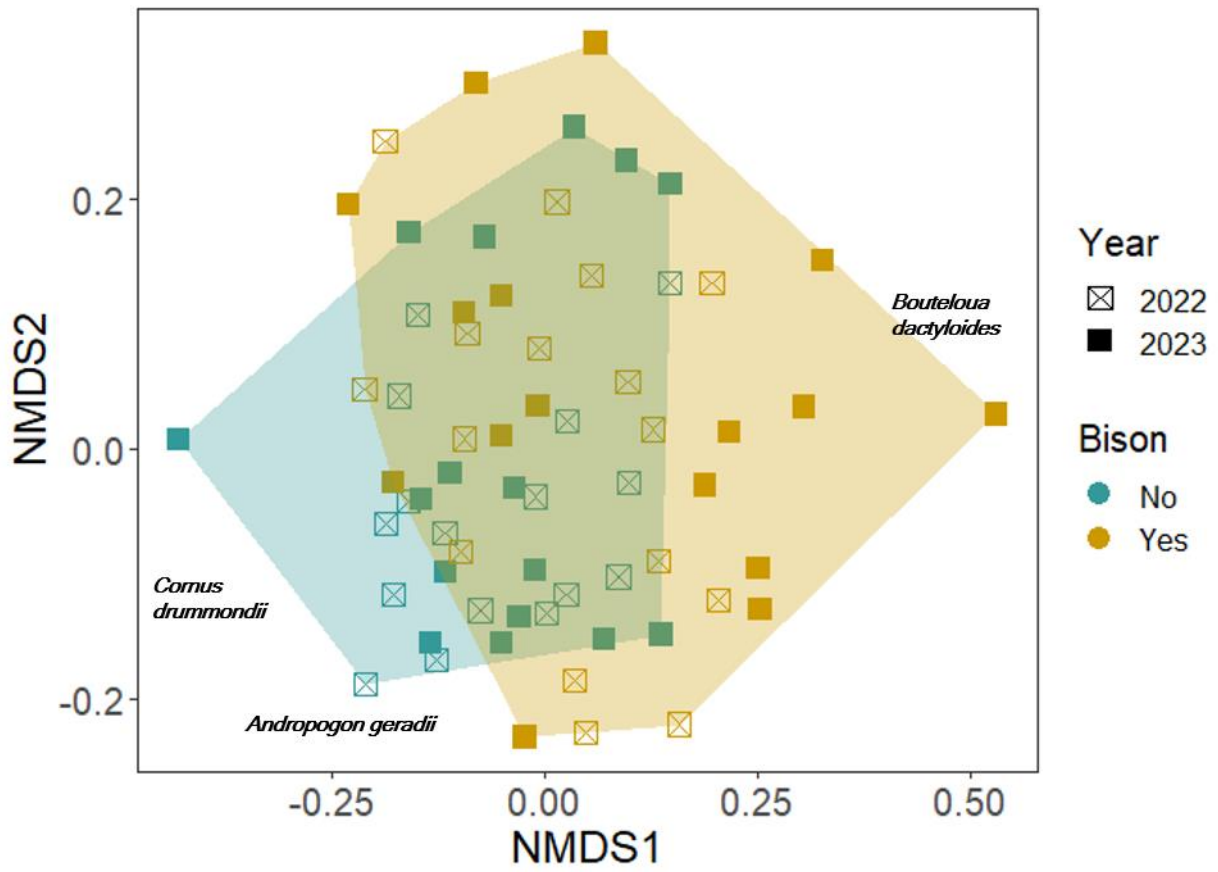

Figure 3.6: NMDS of the plant within exclosures and with bison in the years 2022 and 2023 with three selected species based on SIMPER analysis and significant species scores at the alpha level of 0.05.

Figure 3.7: Photo of exclosure fence line with bison grazed on the left side and the exclosure on the right in August of 2023. Bison visibly decrease graminoid cover, preventing fire from traveling across the landscape.

Supplemental Figures and Tables

Figure S 3.1: Photo of one of the bison-grazed fire reversal treatments, dominated by woody vegetation.

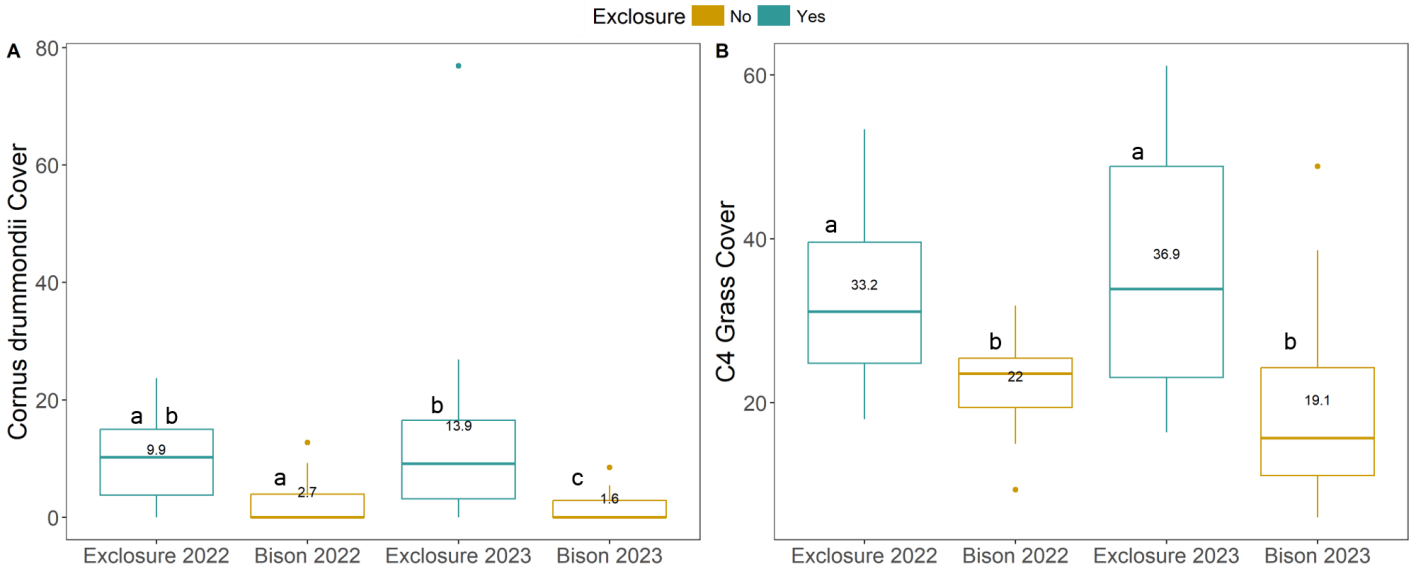

Figure S 3.2: *Cornus drummondii* (A) and C4 grass cover (B) in exclosures and bison present plots. Numbers represent the mean cover. Boxplots that share lettering at the did not have statistically significant differences at an alpha of 0.05.

Comparison	Difference	Lower Confidence Interval	Upper Confidence Interval	Adjusted p-value
Yearly Differences				
2023 vs. 2022	-5.40625	-7.383034	-3.429466	9e-07
Bison Presence				
Yes vs. No	2.40625	0.4294663	4.383034	0.0178876
Year and Bison Interaction Differences				
2023:No vs. 2022:No	-4.4375	-8.1306587	-0.7443413	0.0123261
2022:Yes vs. 2022:No	3.3750	-0.3181587	7.0681587	0.0851078
2023:Yes vs. 2022:No	-3.0000	-6.6931587	0.6931587	0.1503677
2022:Yes vs. 2023:No	7.8125	4.1193413	11.5056587	<0.00001
2023:Yes vs. 2023:No	1.4375	-2.2556587	5.1306587	0.7334540
2023:Yes vs. 2022:Yes	-6.3750	-10.0681587	-2.6818413	0.0001476

Table S 3.1: Table of all Tukey HSD comparisons for species richness.

Comparison	Difference	Lower Confidence Interval	Upper Confidence Interval	Adjusted p-value
Yearly Differences				
2023 vs. 2022	-5.687655	-8.102745	-3.272564	1.51e-05
Bison Presence				
Yes vs. No	3.382612	0.967522	5.797703	0.0068342
Year and Bison Interaction Differences				
2023:No vs. 2022:No	-4.219256	-8.7312882	0.2927762	0.0749396
2022:Yes vs. 2022:No	4.851011	0.3389789	9.3630433	0.0303948
2023:Yes vs. 2022:No	-2.305042	-6.8170745	2.2069899	0.5353004
2022:Yes vs. 2023:No	9.070267	4.5582349	13.5822993	<0.00001
2023:Yes vs. 2023:No	1.914214	-2.5978185	6.4262459	0.6780714
2023:Yes vs. 2022:Yes	-7.156053	-11.6680856	-2.6440212	0.0005257

Table S 3.2: Table of all Tukey HSD comparisons for a diversity index (e^H).

Chapter 4 - The Surprisingly Muted Impact of Bison (*Bison bison*) on Plant Communities in the Northern Great Plains

Abstract

In the Great Plains of North America, where some of the world's last intact grassland systems reside, the historical dominance of grasslands is being challenged by widespread habitat loss, leaving only 30% of the original ecoregion. Against this backdrop, conservation efforts have prioritized the reintroduction of the American bison (*Bison bison*), the continent's largest native grazer, whose numbers once ranged between 30-60 million. Our study delves into the impact of bison on plant communities within mixed-grass prairies, in the context of woody encroachment, contrasting with the well-documented influences in tallgrass prairies. By analyzing vegetation surveys and dung counts across sites with a history of bison presence and varying degrees of woody plant encroachment, we seek to unravel the nuances of bison's ecological role. We found that bison presence in mixed-grass prairies did not increase plant species richness or diversity when compared to areas grazed by cattle, in contrast with tallgrass prairie. We also found that bison potentially increase the abundance of invasive species at one of our sites, underscoring the complex dynamics between grazing practices and invasive species management. Woody encroachment is a major threat to grassland conservation. We found that some encroached areas displayed higher species richness, which appears to be influenced by moderate canopy cover rather than bison activity. This research suggests that bison reintroduction, while critical for ecosystem restoration, exhibits variable outcomes across grassland types, emphasizing the need for site-specific conservation tactics. Our findings highlight the need for more expansive research on bison’s ecological impacts throughout the Great Plains to inform future conservation and management decisions as bison reintroduction efforts continue.

Introduction

The Great Plains of North America contains some of the world's last intact grassland systems (Scholtz and Twidwell 2022). Historically, these grasslands comprised ~3.3 million km^2 with three major grassland types based on a precipitation gradient. In the mesic east these grasslands were dominated by tall C_4 grasses (*Andropogon geradii*, *Sorghastrum nutans*, *Schizachyrium scoparium*, and *Panicum virgatum*), with a mix of C_3 (*Pascopyrum smithii*, *Nassella viridula*, and *Hesperostipa* spp.) and C_4 grasses in the Central Great Plains, and short, drought tolerant, C_4 grasses (*Bouteloua* spp.) in the arid western great plains (Samson et al. 2004, Augustine et al. 2021). Today, about 70% of the Great Plains ecoregion has been lost (Samson et al. 2004). Within these last remaining intact grasslands, efforts have been underway to protect and restore these systems, including reintroducing the most abundant former grazer, the American bison (*Bison bison*) (Sanderson et al. 2008, Department of Interior 2023).

The American bison is North America's largest grazer, and prior to their widespread extirpation, their numbers ranged from 30-60 million and had large impacts on the landscape (Hornaday 1889, Hart 2001). Following their widespread extermination, conservation efforts beginning in the 1890s have allowed for the recovery of the American bison from less than 1000 individuals to ~26,000 in conservation herds and 500,000 raised as livestock (Sanderson et al. 2008). With this, bison are continually being reintroduced onto their former landscape on NGO lands, state and federal lands, tribal lands, and private ranches (USDA 2016, US Department of Interior 2023); although, they still only occupy less than 1% of their pre-colonization range (Sanderson et al. 2008). However, most bison research comes from just one grassland type on the Great Plains, the tallgrass prairie.

Bison reintroduction has been well studied on the tallgrass prairie due to work at several long-term sites throughout the Flint Hills, which stretch through eastern Kansas to Northern Oklahoma (Coppedge et al. 1998a, Coppedge et al. 1998b, Knapp et al. 1999, Allred et al. 2011, Collins and Calabrese 2012). Within tallgrass prairie, bison consume the dominant C_4 grasses (Towne et al. 2005, Ratajczak et al. 2022). These grasses are highly competitive and obtain high biomass compared to other grasses and forbs. However, bison preferentially consume these warm-season grasses, allowing for an increase in resource availability, especially by increasing light availability (Fahnestock and Knapp 1994). Bison have been shown to increase plant diversity by 103% at small scales and 86% at the landscape scale within tallgrass prairie (Ratajczak et al. 2022). Additionally, bison create habitat heterogeneity via patch grazing and wallowing (Fuhlendorf and Engle 2001). Furthermore, bison's horning and rubbing behavior can girdle trees, potentially preventing widespread tree establishment (Coppedge and Shaw 1997; Ch 2).

The effects of bison on plant communities within grasslands of the Great Plains outside the tallgrass prairie have been understudied. We know of only a handful of studies that investigate the effects of bison on plant community composition and plant diversity in shortgrass and mixed-grass prairie (Cid et al. 1991, Fahnestock and Detling et al. 2002, Hillerbrand et al. 2019, McMillian et al. 2019, Yu et al. 2023). Bison are typically considered a keystone species based on their effects in tallgrass prairie, especially on plant communities (Knapp et al. 1999). A study in the mixed-grass prairie showed mixed evidence that bison have a keystone effect, following a 10-year bison reintroduction did not increase plant species diversity (Shannon’s Index and Simpson Index) or plant structural heterogeneity (McMillan et al. 2019). However, there was higher plant species richness and compositional heterogeneity (McMillan et al. 2019).

In a grassland in Yellowstone National Park, USA, bison have decreased plant species diversity and increased invasive species along riparian areas (Kauffman et al. 2022). For instance, the extent of threat from invasive species varies across sites and theoretically, the effects of keystone herbivory should be highest in more productive environments, like the central Great Plains, and lower in less productive grasslands, such as the western and northern Great Plains (Bakker et al. 2006).

Bison reintroduction is taking place in face of widespread global change. Intact grasslands in the Great Plains are quite different today than two centuries ago, including anthropogenic climate change, invasive species, and woody encroachment. Woody encroachment is one of the most persuasive threats to remaining intact grasslands in the Great Plains (Symstad and Leis 2017, Gaskin et al. 2021, Morford et al. 2022). Woody plant encroachment is the invasion of woody plants into grasslands, potentially transitioning the grass dominated state to woodlands/shrublands (Ratajczak et al 2014a, Archer et al. 2017). In April of 1805 near present day Williston, ND, USA Merriweather Lewis wrote,

> I asscended to the top of the cutt bluff this morning, from whence I had a most delightfull view of the country, the whole of which except the vally formed by the Missouri is void of timber or underbrush, exposing to the first glance of the spectator immence herds of Buffaloe, Elk, deer, & Antelopes feeding in one common and boundless pasture (Devoto 1953).

Today, these surrounding grasslands in western North Dakota have undergone widespread *Juniperus scopulorum* (Rocky Mountain juniper) encroachment (Symstad and Leis 2017, Allred et al. 2021). Woody encroachment typically results in a decrease in species diversity, a loss of herbaceous production, and degradation of habitat for grassland species (Ratajczak et al. 2012,

Morford et al. 2022, Silber et al. 2024). Avoiding woody encroachment is increasingly difficult, because of increased atmospheric CO_2, nitrogen deposition, and increasing woody plan seed rain (Archer et al. 1995, Köchy and Wilson 2001, Prather et al. 2017). Thus, it is vital to not only understand the influence of bison on plant communities but also in the context of woody plant encroachment.

It is generally believed that grazers promote the proliferation of woody plants by diminishing grass cover, thereby lessening both the competitive pressure from grasses and the severity of fires (Scholes and Archer 1997; Bond 2008). For example, traditional cattle grazing in tallgrass prairies has been documented to enhance tree colonization (Owensby et al. 1972; Hoch et al. 2002), although grazing activities can also lead to decreased tree heights due to browsing (Capozzelli et al. 2020). Despite carbon isotope analysis suggesting a strong preference for grasses among bison (Raynor et al. 2016), DNA from tree species has been detected in bison dung as well as that of other large herbivores (Craine et al. 2015). This supports findings from African savannas where it is observed that animals typically classified as grazers sometimes consume woody vegetation (e.g., Kartzinal et al. 2015; Guyton et al. 2020). Within the tallgrass prairie bison have been found to potentially suppress *Juniperus virginiana* establishment in infrequently burned grasslands (Ch 2). While bison are not major consumers of woody plants, their selective browsing on young trees could significantly hinder tree maturity, creating a demographic bottleneck that impedes the establishment of adult trees—a similar process noted in browsers and mixed-feeders in limiting tree growth in African savannas (Higgins et al. 2000; Staver et al. 2012; Sankaran et al. 2008). Bison have also been found to suppress *Populous tremuloides* (quaking aspen) recruitment within Yellowstone National Park, USA (Beschta et al.

2020, Painter et al. 2023). However, this phenomenon has not been well documented in other grasslands.

The study had two objectives: 1) Understand the effects of bison on plant communities outside of tallgrass prairie. For this study we used sites with bison located in the mixed-grass prairie in western South Dakota and North Dakota, USA. These sites have had bison present on the landscape for the past 60-65 years, with a gap of ~80 years when bison were not present due to their expiration. We hypothesized that bison would increase plant species diversity, due to a decrease in graminoid abundance. However, this effect would be smaller than in tallgrass prairies as the mixed-grass prairies in the Northern Great Plains are more arid and cooler than tallgrass prairie, thus the dominant grasses do not achieve the biomass seen in tallgrass prairie, where the dominant grasses out shade many other plant species. 2) Compare encroached plant communities to grassland communities, and disentangle whether bison had any effect on the encroached communities. We hypothesized that bison would have a negligible effect on encroached plant communities as they would select and spend more time in open grassland where there is higher quantity and quality forage.

Methods

Sites

This study took place across three sites utilizing National Parks where bison have been reintroduced and National Grasslands where bison are absent. We selected Badlands and Theodore Roosevelt National Parks, located in the mixed-grass prairie region of the Great Plains. Badlands National Park is situated in the northern Great Plains in southwestern South Dakota, USA (Lat 43°51′00″N, Long 102°20′00″W). The area experiences a semiarid climate characterized by brief, warm summers (22°C average) and cold winters (−4°C average). The

average annual precipitation (1990-2020) is 49.8 cm, with approximately 75% occurring from April to September. During the study period, annual precipitation reached 36.45 cm in 2022 and 54.23 cm in 2023. The park's landscape is predominantly composed of western wheatgrass (*Pascopyrum smithii*), green needlegrass (*Nassella viridula*), and little bluestem (*Schizachyrium scoparium*). The park currently manages for a population of 1000-1200 bison that have access to ~32,000ha (U.S. Department of the Interior 2023). Bison were first reintroduced into the park in 1963. The bison consume about 12% of plant productivity in a normal-precipitation year (Licht 2016). Additionally, we used a neighboring Buffalo Gap National Grassland to compare grasslands and encroached areas without bison.

Theodore Roosevelt National Park is located in western North Dakota. The park consists of a South Unit (Lat 46°58′00″N, Long 103°30′00″W) immediately north of Medora, North Dakota, and a North Unit (Lat 47°36′00″N, Long 103°23′00″W) 21 km south of Watford City, North Dakota and 70 km north of the South Unit. For the purpose of this study, we treated the South and North Unit as separate sites. The South Unit has about 18,400 ha available to bison and the North Unit has about 9,600 ha. The habitat within the two units consists of a mosaic of rugged and barren badlands topography intermixed with areas of mixed-grass prairie. Dominant vegetation in the prairie includes needle-and-thread (*Hesperostipa comata*), green needlegrass, and western wheatgrass. Average precipitation is about 38 cm mostly falling between April and July, with the South Unit receiving 42 cm and 31 cm in 2022 and 2023, respectively. The North Unit received 55 cm and 33 cm in 2022 and 2023, respectively. Bison were first reintroduced into the South Unit in 1956 and 1962 for the North Unit. The park manages for a population of 300-500 bison in the South Unit and 100–300 in the North Unit, with bison removing about ~7% of the aboveground biomass. Additionally, there are other grazing ungulates in the South Unit

which include about 360 elk (*Cervus canadensis*), a mixed feeder, and 100 feral horses (*Equus caballus*), a primarily grazer (Licht 2016). We used the neighboring Little Missouri National Grassland to compare sites without bison.

Plot Location Selection and Field Methods

Vegetation surveys were conducted in factorial combination of 1) open grassland and within encroached areas, and 2) areas with and without native megafauna. Sampling occurred in July of 2022 and July 2023. Thirty plots (15 open and 15 encroached) were established in each combination (Theodore Roosevelt NP North Unit, Little Missouri National Grassland North Unit, Theodore Roosevelt NP South Unit, Little Missouri National Grassland South Unit, Badlands NP, and Buffalo Gap National Grassland) for a total of 180 vegetation plots (See Fig. 4.1, 4.2, 4.3 for plot locations). Each open or grassland plot was paired with an encroached or woodland plot between 200-400 meters away. Plot locations were decided on ArcMap, selecting open grassland sites and encroached/woody sites with satellite imagery (ESRI 2020). Additionally, we avoided badland features, as these are dominated by bare ground (Perkins et al. 2019). Vegetation plots were 25 x 25 m with one 25 m transect down the center of the plot. For plant community sampling we established 5 1 m^2 understory vegetation sampling points along the transect every five meters (5 sample points per plot). In each 1-meter quadrat, we estimated plant cover of each species using a modified Daubenmire scale, with cover classes of 0–1%, 1–5%, 5–25%, 25–50%, 50–75%, 75–95%, or 95–100% aerial coverage (Bailey and Poulton 1968). To calculate approximate cover, we used the midpoint of the Daubenmire scale range. Within each plot we estimated canopy cover using a spherical densitometer in the center of each quadrat at 1.5m in height by the same observer. There is some overestimation in canopy cover via this method, however, canopy cover via a densitometer is ecologically relevant for understory

vegetation due to its angular measurement (Nuttle 1997). Canopy cover was used as a proxy for light availability within these plots. To determine recent bison usage in National Parks and cattle usage in National Grasslands, we counted dung piles within each plot.

Comparing Plant Community Composition

For comparing plant community composition between bison present grasslands and encroached areas vs bison absent/catt present, we used a non-metric multidimensional scaling ordination (NMDS) based on a Bray-Curtis dissimilarity metric. Bray-Curtis is one of the most effective distance metrics for ecological data (Beals 1984; Ricotta and Podani 2017), and NMDS is a flexible ordination technique suitable for data that deviate from normality, such as vegetation data (Dexter et al. 2018). Species scores were used to determine what species drove the communities in the bison and ungrazed treatments. For this NMDS, species from functional groups (woody plants, grasses, forbs) were selected based on their statistical significance at the $\alpha = 0.05$ level, ensuring a focus on the most influential species. Subsequently, the envfit function in the package vegan (Oksanen et al. 2013) was employed to fit environmental vectors onto the ordination diagram and to test the significance of the relationship between these species scores and environmental variables using a permutation test. We used permutational multivariate analyses of variance (perMANOVA) with a Bray-Curtis dissimilarity metric using the package vegan (Oksanen et al. 2013) to determine if there were differences between the bison present and bison absent plots and the encroached and grassland plots. Furthermore, in order to further the effects of bison, we also performed perMANOVAs for only the grassland plots and only the encroached plots.

We also performed an indicator species analysis (SIMPER) using the simper function from the vegan package, to determine the species contributing most significantly to

dissimilarities between the bison-grazed grasslands and bison absent grasslands, and separately between the encroached communities and traditional grasslands. For each pairwise comparison, the analysis provided a breakdown of the percentage contribution of each species to the total dissimilarity observed.

Plant Species Richness and Diversity

To quantify the effect of bison on plant diversity, we calculated species richness and species diversity. Species richness is the total number of species found for each 25 m transect (5 $1m^2$ quadrats). Species diversity for each site was calculated using the exponential Shannon's index, $\exp(H' = \Sigma(p_i \times \ln p_i)$, where p_i is the relative abundance of species *i*. An exponential Shannon's index (e^H) is the effective number of equivalently abundant species in a community. For species richness and diversity (e^H), we performed ANOVAs with year (2022 and 2023), bison presence, and plot type (grassland vs encroached). We performed post-hoc comparisons using the Tukey test which adjusts p-values for multiple comparisons. We used the package multcomp (Hothorn et al. 2008) for all pairwise comparisons for year, bison presence, and plot type at an alpha level of 0.05, with an honest squares difference adjustment to *P* values.

Encroachment Effect on Diversity

For the effects of encroachment on plant diversity we fitted both linear (Species Richness/ $e^H = \beta 0 + \beta 1 \cdot CC + \beta 2 \cdot Bison + \epsilon$) and quadratic models (Species Richness/$e^H = \beta 0 + \beta 1 \cdot AVG + \beta 2 \cdot CC^2 + \beta 3 \cdot Bison + \epsilon$) to assess the relationship between species richness, e^H, and the predictors (canopy cover (CC) and bison presence). Data were initially screened for normality and homogeneity of variances. The Akaike Information Criterion corrected for small sample sizes (AICc) was employed to ensure robustness against overfitting. The model with the lower

AICc value was selected as it indicates a better fit to the data with an appropriate number of parameters, adjusting for the size of the dataset.

Results

Bison and Cattle Dung Counts

Dung pile counts averaged between 4.3 dung piles to 57.1 dung piles across are sites. Dung counts were much lower in encroached plots than grassland plots across all of our units. This suggests minimal usage by bison or cattle within the encroached plots. Bison and cattle dung counts were comparable at each individual unit, suggesting similar grazing intensities between our National Park sites and National Grassland sites (Figure 4.1).

Plant Community Composition BADL and BG

The NMDS plot (Figure 4.2, stress=0.15) illustrates distinct clustering of plant communities between Badlands National Park and Buffalo Gap National Grassland, based on the presence of bison and encroachment. Plant communities in areas with bison show a separate clustering compared to those without bison, across both encroached and grassland habitats. With more distinct clustering with those with bison. The temporal aspect also shows a pattern, with the 2023 communities clustering differently from 2022. The perMANOVA revealed a significant effect of bison presence ($R^2 = 0.052$, $F = 7.487$, $p = 0.001$), year ($R^2 = 0.032$, $F = 4.571$, $p = 0.001$), and encroachment ($R^2 = 0.093$, $F = 13.316$, $p = 0.001$) on plant community composition. However, the interaction between bison and year ($F = 1.108$, $p = 0.308$), year and encroachment ($F = 1.099$, $p = 0.332$), and the three-way interaction between bison, year, and encroachment ($F = 0.825$, $p = 0.642$) were not significant. Notably, the interaction between bison presence and encroachment was significant but explained little of the variation ($R^2 = 0.017$, $F = 2.484$, $p = 0.003$), suggesting that the impact of bison on plant communities is marginally influenced by encroachment.

The SIMPER analysis comparing encroached and grassland sites highlighted that certain plant species were particularly influential in characterizing the dissimilarity in plant community composition between these two habitat types. The cumulative contributions of the five most influential species are as follows: *Pascopyrum smithii* (10.8%, a C_3 grass), *Bromus japonicus* (20 %, an invasive C_3 grass), *Carex filifolia* (28.9%, a sedge), *Bouteloua curtipendula* (35.9%, a C_4 grass), and *Bromus tectorum* (41.5%, an invasive C_3 grass). These results suggest that the presence of these species is highly indicative of the ecological shifts between encroached and grassland habitats, as all of these species are graminoids that occur in open grassland. While, encroached habitats had very little understory herbaceous vegetation cover.

The perMANOVA conducted on the grassland sites to assess the influence of bison presence and temporal changes on plant community composition showed significant main effects for both factors. Bison presence had a significant effect on plant community structure ($F = 6.95$, $p = 0.001$), explaining 10% of the variation in the data. Similarly, the year also had a significant effect ($F = 4.58$, $p = 0.001$), accounting for ~7% of the variation. However, the interaction between bison presence and year was not significant ($F = 1.09$, $p = 0.351$), indicating that the effect of bison on plant communities did not differ between the two sampling years. The significant main effects suggest that bison presence is a strong determinant of plant community composition in grassland sites, independent of the year sampled. The lack of a significant interaction between bison presence and year implies that the impact of bison is consistent across the temporal scale of this study. The SIMPER analysis showed that several plant species contributed differentially to the dissimilarity between plant communities with and without bison in grasslands. The cumulative contributions of the five most influential species are as follows: *Bromus japonicus* (11.3%, an invasive C_3 grass), *Pascopyrum smithii (*22.2%, a C_3 grass), *Carex*

filifolia (32.5%, a sedge), *Bromus tectorum* (40.6%, an invasive C_3 grass), and *Bouteloua curtipendula* (47.1%, a C_4 grass). This indicates that these species are most indicative of the changes in plant communities associated with bison presence. Two of these species *Bromus japonicus* and *B. tectorum* are highly invasive cool-season grasses.

Plant Community Composition Theodore Roosevelt NP South Unit and Little Missouri NG South Unit

The NMDS plot (Figure 4.3, stress = 0.14) illustrates the separation in plant community composition between Theodore Roosevelt National Park South Unit with bison and the Little Missouri National Grassland South Unit without bison. The distribution of points suggests that both bison presence and encroachment contribute to community variation, with differences apparent between 2022 and 2023 samples as well.

The perMANOVA analysis revealed significant effects of bison presence ($R^2 = 0.025$, $F = 3.505$, $p = 0.001$), encroachment ($R^2 = 0.144$, $F = 20.511$, $p = 0.001$), and year ($R^2 = 0.016$, $F = 2.231$, $p = 0.008$) on plant community composition. The interaction between bison presence and encroachment was also significant ($R^2 = 0.019$, $F = 2.758$, $p = 0.001$), indicating that the effect of bison on plant communities differs between encroached and grassland areas. However, the interactions involving the year were not significant, suggesting that while there is an annual variation, it does not significantly alter the effects of bison presence and encroachment.

The SIMPER analysis further detailed the specific species that most significantly contribute to the differences between encroached and grassland habitats. The cumulative contributions of influential species include: *Juniperus horizontalis* (7.59%, an evergreen shrub), *Pascopyrum smithii* (13.4%, a C_3 grass), *Schizachyrium scoparium* (18.9%, a C_4 grass),

Symphoricarpus occidentalis (24.1%, a sub-shrub), and *Juniperus scopulorum* (28.8%, an evergreen tree).

The perMANOVA analysis for the grassland only plots revealed significant effects of bison presence ($R^2 = 0.066$, $F = 3.467$, $p = 0.001$). However, year and the interaction between bison and year were not significant, suggesting that there was no significant annual variation. The SIMPER analysis further detailed the specific species that most significantly contribute to the differences between bison present grasslands and bison absent grasslands. The cumulative contributions of the five most influential species include: *Pascopyrum smithii* (11%, a C_3 grass), *Schizachyrium scoparium* (21.9%, a C_4 grass), *Carex filifolia* (29.2%, a sedge), *Poa pratensis* (33.8%, an invasive C_3 grass), and *Euphorbia virgata* (38.4%, an invasive forb).

Plant Community Composition Theodore Roosevelt NP North Unit and Little Missouri NG North Unit

The NMDS plot (Figure 4.4, stress = 0.15) reveals patterns of plant community composition within Theodore Roosevelt National Park North Unit in comparison to Little Missouri National Grassland North Unit. Bison presence, year, and encroachment status appear to influence plant community distribution, with some separation observed along NMDS Axis 1 and Axis 2. Particularly, communities in encroached areas display more variation along Axis 1 compared to grassland areas.

The perMANOVA results indicated significant effects of bison presence ($R^2 = 0.026$, $F = 3.624$, $p = 0.001$), encroachment ($R^2 = 0.141$, $F = 19.921$, $p = 0.001$), and year ($R^2 = 0.013$ $F = 1.831$, $p = 0.026$) on plant community composition. While the interaction between bison presence and encroachment approached significance ($R^2 = 0.012$, $F = 1.702$, $p = 0.042$), interactions involving year were not significant, suggesting that the influence of bison and

encroachment on plant communities is not strongly dependent on the year sampled within the scope of this study.

The SIMPER analysis further identified which species contributed most to the dissimilarity between encroached and grassland plant communities within Theodore Roosevelt NP North Unit and Little Missouri National Grassland North Unit. The cumulative contributions of the most influential species are as follows: *Symphoricarpos occidentalis* (12.2%, a sub-shrub), *Pascopyrum smithii* (20.4%, a C_3 grass), *Poa pratensis* (27.1%, an invasive C_3 grass), *Juniperus horizontalis* (32.4%, an evergreen shrub), and *Rhus aromatica* (36.6%, a shrub).

The perMANOVA, conducted on grassland sites to assess the impact of bison presence and year, demonstrated that bison had a significant effect on plant community composition ($F = 4.3975$, $p = 0.001$), explaining 7 % of the variation. The year did not significantly affect the plant communities ($F = 1.2442$, $p = 0.255$), nor did the interaction between bison and year ($F = 0.7177$, $p = 0.726$). This indicates that while bison significantly shape the plant community structure, the temporal factor, within the years considered, does not.

The SIMPER analysis determined the contribution of individual plant species to the dissimilarity between grassland sites with and without bison. The cumulative contributions of the most influential species are as follows: *Pascopyrum smithii* (13.2%, a C_3 grass), *Poa pratensis* (23.9%, an invasive C_3 grass), *Bouteloua gracilis* (31.1%, a short C_4 grass), *Bromus inermis* (36.7%, an invasive C_3 grass), and *Nassella viridula* (41.9%, a C_3 grass).

Plant Species Richness and Diversity

Analysis of variance revealed that the presence of bison in Badlands NP vs Buffalo Gap NG significantly influences species richness ($F = 36.454$, $p < 0.001$). While the main effects of both

year ($F = 3.348$, $p = 0.070$) and encroachment type ($F = 3.348$, $p = 0.070$) were not statistically significant.

Significant interactions were noted; particularly, the interaction between encroachment and bison presence was statistically significant ($F = 6.561$, $p = 0.012$), indicating that the effect of bison on species richness varies by encroachment. However, other interaction terms showed no significant effects.

Post-hoc comparisons using Tukey's Honest Significant Difference indicated marked differences within and across interaction categories. Specifically, plots with bison presence consistently demonstrated lower species richness compared to those without bison. Notably, the difference in species richness between bison-present and bison-absent conditions was -5.5 species (95% CI: -7.305 to -3.695, $p < 0.001$). Furthermore, detailed interactions such as year and bison presence and bison and encroachment showed significant differences in species richness adjustments depending on the specific combinations of year and habitat type, illustrating the complex dynamics between temporal factors, encroachment, and bison presence (Table S 4.1).

The ANOVA for the exponential Shannon index in Badlands NP and Buffalo Gap NG revealed a significant effect of bison presence, encroachment, and the interaction between bison and encroachment ($p<0.001$ for all). Encroached plots had lower diversity compared to grassland plots (mean difference = -7.276, $p < 0.001$). The presence of bison was associated with a significant decrease in the exponential Shannon index compared to plots with cattle (mean difference = -4.033, $p < 0.001$). The post-hoc Tukey HSD test revealed that 2022 and 2023 grassland plots with bison had lower diversity than plots without bison ($p < 0.001$).

An ANOVA for species richness in Theodore Roosevelt NP South Unit and Little Missouri NG South Unit revealed that only encroachment had significant impact on species richness ($p < 0.001$). There were 3.3 less species in the grassland plots than the encroached plots across all years ($p < 0.001$). However, a post-hoc Tukey HSD revealed that only all grassland plots in 2023 vs encroached plots in 2023 were significantly different from each other ($p = 0.008$). There was no difference in species richness based on year or the presence of bison. The ANOVA on the exponential Shannon index revealed that only encroachment had significant effect on diversity ($p < 0.001$). Diversity was 3.5 equivalent species higher in all grassland plots vs all encroached plots across all years ($p < 0.001$).

The ANOVA for species richness in Theodore Roosevelt NP North Unit and Little Missouri National Grassland North Unit had similar results to Theodore Roosevelt NP South Unit and Little Missouri NG South Unit . The only significant predictor was encroachment status, with 5.5 less species in all grassland plots vs all encroached plots ($p < 0.001$). The ANOVA for the exponential Shannon index revealed that year ($p = 0.018$) and the encroachment bison interaction had significant effect on diversity ($p = 0.039$) The post-hoc comparison revealed that diversity was lower in 2023 than in 2022. Additionally, that diversity was higher in grassland bison present plots ($p = 0.03$).

Plant Diversity and Canopy Cover

Species richness in Badlands NP and Buffalo Gap NG was best modeled via a quadratic model. The model indicated quadratic effects of canopy cover ($p < 0.001$) and a negative effect of bison presence on species richness, with a 5.38 decrease in species richness when bison were present ($p < 0.001$). The model explained 43.11% of the variance in species richness (Adjusted $R^2 = 0.4311$). In terms of Shannon diversity, the linear model provided the best fit and demonstrated a

significant negative relationship with canopy cover ($p < 0.001$) and bison presence ($p < 0.001$), explaining 53.88% of the variation (Adjusted $R^2 = 0.5388$).

In Theodore Roosevelt NP South Unit and Little Missouri NG South Unit , the quadratic model for species richness was significant but explained only 4% of the variance ($p = 0.033$, Adjusted $R^2 = 0.04$). Bison presence was removed as it was not significant. Similarly, for exponential Shannon diversity in Theodore Roosevelt NP South Unit, bison presence was not significant and removed. The linear model was the best fit and showed a significant negative linear effect of canopy cover but only 8% of the variance was explained ($p < 0.001$, Adjusted $R^2 = 0.083$).

A quadratic model for Theodore Roosevelt NP North Unit and Little Missouri National Grassland North Unit showed a significant relationship, with the model explaining 25% of the variance ($p < 0.001$, Adjusted $R^2 = 0.25$). Both canopy cover ($p < 0.001$) and canopy cover squared ($p < 0.001$) were significant, suggesting a strong nonlinear effect of canopy cover on species richness. Bison presence was not significant in explaining species richness and removed from the model. For Shannon diversity, the quadratic model was the best fitting model with canopy cover ($p = 0.038$), canopy cover squared ($p = 0.009$), and bison presence ($p = 0.01$) all significant. However, only about 8% of the variance was explained by the model (Adjusted $R^2 = 0.083$, $p = 0.004$).

Discussion

Our findings suggest that bison have small effects on plant communities in mixed-grass prairies, particularly when compared to areas subjected to light to moderate cattle grazing. While the presence of bison does influence plant community composition, this effect is relatively subdued, especially when contrasted with that in tallgrass prairies. Moreover, our research

provides no evidence to support the notion that bison enhance plant species richness and diversity in these ecosystems over areas grazed by cattle. In fact, in Badlands National Park, bison presence is associated with reduced species richness and diversity, potentially due to an increase in invasive plant species. Interestingly, some sites within encroached areas exhibited higher species richness, which seems to be linked to intermediate canopy cover. Overall, our results indicate that bison may not exert a keystone herbivory effect on plant communities in these mixed-grass prairies.

At all our study sites—Badlands NP, Theodore Roosevelt NP South Unit, and Theodore Roosevelt NP North Unit—bison significantly modified community composition, which was more pronounced in grassland areas where bison spend more time, as evidenced by our dung counts (Fig. 4.1). However, these changes were not substantial, particularly in comparison with tallgrass prairies (Collins and Calabrese et al. 2012, Ratajczak et al. 2022). This variance in community composition likely stems from greater composition heterogeneity, as indicated by broader dispersion in our NMDS plots, corroborating other research on bison effects in mixed-grass prairies (Lueders et al. 2006, McMillan et al. 2019).

One of the largest differences between our study and studies in tallgrass prairie was that there was no positive effect of bison plant species richness and diversity. In one reintroduction bison have increased plant species richness by 103% at local scales ($10m^2$) in 30 years, with increases still happening (Ratajczak et al. 2022). Furthermore, at one of our sites, Badlands National Park, there was a noticeable decline compared to without bison in species richness and diversity, possibly due to bison increasing disturbance for invasive species. Bison areas had more *Bromus japonicus* and *B. tectorum.* Additionally, invasive plant cover was higher in the presence of bison than without bison (Fig. S 4.1) These graminoid species are highly invasive in the

Northern Great Plains and are a top concern to land managers in this region (Symstad and Leis et al. 2017, Symstad 2022). It is possible that year-round disturbance by bison is increasing invasive species, as this has been seen in Yellowstone National Park, USA (Kauffman et al. 2023). Surprisingly, there was no difference in species richness or diversity at our Theodore Roosevelt National Park sites. This is surprising, especially since bison have been reintroduced on these landscapes for over 60 years. This greatly contradicts long term bison reintroduction in tallgrass prairie (Knapp et al. 1999, Ratajczak et al. 2022). In fact, bison have been on this landscape for ~30 years longer than at research that has been conducted at Konza Prairie Biological Station located in the tallgrass prairie in east-central Kansas. However, at Konza Prairie bison are stocked at a rate to remove ~25% of aboveground biomass, while in Badlands and Theodore they remove ~12% and ~7%, respectively (Towne 1999, Licht 2016). McMillan et al. found that bison did increase species richness in comparison to cattle grazing but not species diversity after 10 years of bison reintroduction (2019). In areas of lower productivity, large herbivores tend to have a minimal impact on diversity, and may even contribute to a decrease in plant diversity (Bakker et al. 2006).

One surprising result, regardless of bison presence, was that there was higher species richness within our encroached sites at Theodore Roosevelt NP South Unit/Little Missouri National Grassland South Unit and Theodore Roosevelt NP North Unit/Little Missouri National Grassland North Unit . However, once we accounted for canopy cover the reason behind this was clear. Similar to oak savannas in the Midwestern United States, there is higher species richness at intermediate canopy covers (Peterson and Reich 2008, Noble and Bauer 2022). This probably due to a greater representation of different plant functional groups and plant species that can tolerate lower light conditions (Noble and Bauer 2022). This might be because our measurements

largely reflect the beta to gamma scale of diversity—a combination of communities—and intermediate canopy cover sites combine a mix of different community types, with some herbaceous dominated patches and some woodland dominated patches (as in Peterson and Reich 2008). Species diversity did follow a negative linear relationship at two of our sites (Badlands NP, Theodore Roosevelt NP South Unit This is probably due to the fact that plant cover was dominated by a few species, such as the mat forming *Juniperus horizontalis* and there is more bareground underneath higher canopy covers (personal observation).

Bison are considered keystone species in the tallgrass prairies of the Great Plains, especially due to their effects on plant community composition and plant diversity (Knapp et al. 1999, Ratajczak et al. 2022). Our results show that the presence of bison on the landscape in Badlands and Theodore Roosevelt National Parks has not greatly altered the plant communities in relation to light to moderate cattle grazing, which a majority of the intact rangeland left in the Great Plains is grazed by cattle. These results are supported by a recent reintroduction in Montana where bison have had a more subdued effect on plant communities and diversity when compared to tallgrass prairie (McMillan et al. 2019). However, there is evidence from other grasslands across the Great Plains that bison do have positive effects compared to cattle. For example, a bison reintroduction in Montana, USA passively restored riparian vegetation, increasing bird diversity and cervid occupation (Boyce et al. 2022). Additionally, at one of our study sites, Theodore Roosevelt National Park, bison have created more heterogeneity in plant cover types (Lueders et al 2006). Furthermore, our results are observational and not experimental in nature. While, our results show that bison may not have as large impact on mixed-grass prairies as tallgrass prairie, it is not conclusive. There is a lack of studies that investigate the effects of bison on plant communities outside of tallgrass prairie. This underscores the need for

more targeted research to fully understand the ecological roles of bison in non-tallgrass prairie settings across the Great Plains, which is crucial as bison reintroduction continues to expand.

Conclusion

Research on the impacts of bison within tallgrass prairies is extensive and is now beginning to expand into other ecosystems. Unlike in tallgrass prairies, our study, along with others, suggests that the keystone plant effects typically observed in tallgrass environments may be muted in other grasslands within the Great Plains. It is important to note, however, that our analysis focused solely on plant communities. Other significant keystone effects, such as the creation of habitat through bison wallows and the development of grazing lawns—areas of high forage quality that boost bison productivity—were not covered. These effects are particularly critical as bison reintroductions are increasingly carried out on private and tribal lands, where there is a dual focus on conservation and enhancing bison productivity. To fully understand the broad spectrum of impacts that bison and other megafauna have on ecosystems, we advocate for the adoption of standardized measurement techniques across various bison reintroduction projects.

Tables

Factor	Df	Sum Sq	Mean Sq	F value	P-value
Year	1	83.3	83.3	3.348	0.0700 .
Encroached	1	83.3	83.3	3.348	0.0700 .
Bison	1	907.5	907.5	36.454	2.08e-08***
Year:Type	1	4.0	4.0	0.162	0.6881
Year:Bison	1	58.8	58.8	2.362	0.1271
Type:Bison	1	163.3	163.3	6.561	0.0118*
Year:Type:Bison	1	0.8	0.8	0.033	0.8552
Residuals	112	2788.1	24.9		

Table 4.1: Table for the results of a factorial ANOVA examining the effects of Year, Type (Grassland vs Encroached), and Bison presence on species richness at Badlands National Park and Buffalo Gap National Grassland.

Factor	Df	Sum Sq	Mean Sq	F value	P-value
Year	1	5.0	5.0	0.256	0.613563
Type	1	1588.4	1588.4	80.942	6.80e-15***
Bison	1	488.0	488.0	24.866	2.26e-06***
Year:Type	1	26.1	26.1	1.329	0.251408
Year:Bison	1	52.2	52.2	2.662	0.105579
Type:Bison	1	228.5	228.5	11.644	0.000897***
Year:Type:Bison	1	2.0	2.0	0.101	0.751575
Residuals	112	2197.8	19.6		

Table 4.2: Table for the results of a factorial ANOVA examining the effects of Year, Encroachment, and Bison presence on a diversity index (e^H) at Badlands National Park and Buffalo Gap National Grassland.

Factor	Df	Sum Sq	Mean Sq	F value	P-value
Year	1	19	19.2	0.476	0.49175
Type	1	327	326.7	8.096	0.00528**
Bison	1	3	2.7	0.067	0.79636
Year:Type	1	120	120.0	2.974	0.08738 .
Year:Bison	1	5	4.8	0.119	0.73082
Type:Bison	1	21	20.8	0.516	0.47393
Year:Type:Bison	1	11	10.8	0.268	0.60594
Residuals	112	4519	40.4		

Table 4.3: Table for the results of a factorial ANOVA examining the effects of Year, Type (Grassland vs Encroached), and Bison presence on species richness at Theodore Roosevelt National Park South Unit and Little Missouri National Grassland South Unit.

Factor	Df	Sum Sq	Mean Sq	F value	P-value
Year	1	13.4	13.4	0.490	0.4855

Type	1	370.5	370.5	13.576	0.000355
Bison	1	0.6	0.6	0.022	0.8831
Year:Type	1	182.4	182.4	6.682	0.011024
Year:Bison	1	0.4	0.4	0.015	0.9024
Type:Bison	1	118.0	118.0	4.323	0.039890
Year:Type:Bison	1	13.0	13.0	0.478	0.4908
Residuals	112	3056.6	27.3		

Table 4.4: Table for the results of a factorial ANOVA examining the effects of Year, Type (Grassland vs Encroached), and Bison presence on a diversity index (e^H) at Theodore Roosevelt National Park South Unit and Little Missouri National Grassland South Unit.

Factor	Df	Sum Sq	Mean Sq	F value	P-value
Year	1	170	170.4	3.722	0.0562
Type	1	859	858.7	18.756	0.0000325***
Bison	1	4	3.7	0.080	0.7775
Year:Type	1	63	63.1	1.378	0.2430
Year:Bison	1	4	3.7	0.080	0.7775
Type:Bison	1	49	49.4	1.079	0.3011
Year:Type:Bison	1	0	0.2	0.005	0.9463
Residuals	112	5127	45.8		

Table 4.5: Table for the results of a factorial ANOVA examining the effects of Year, Type (Grassland vs Encroached), and Bison presence on species richness at Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	1	171	171.48	5.719	0.0184*
Type	1	0	0.21	0.007	0.9331
Bison	1	115	114.83	3.830	0.0528 .
Year:Type	1	27	27.47	0.916	0.3405
Year:Bison	1	10	10.41	0.347	0.5569
Type:Bison	1	131	131.31	4.379	0.0386*
Year:Type:Bison	1	4	3.52	0.118	0.7324
Residuals	112	3358	29.98		

Table 4.6: Table for the results of a factorial ANOVA examining the effects of Year, Type (Grassland vs Encroached), and Bison presence on a diversity index (e^H) at Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit.

Figures

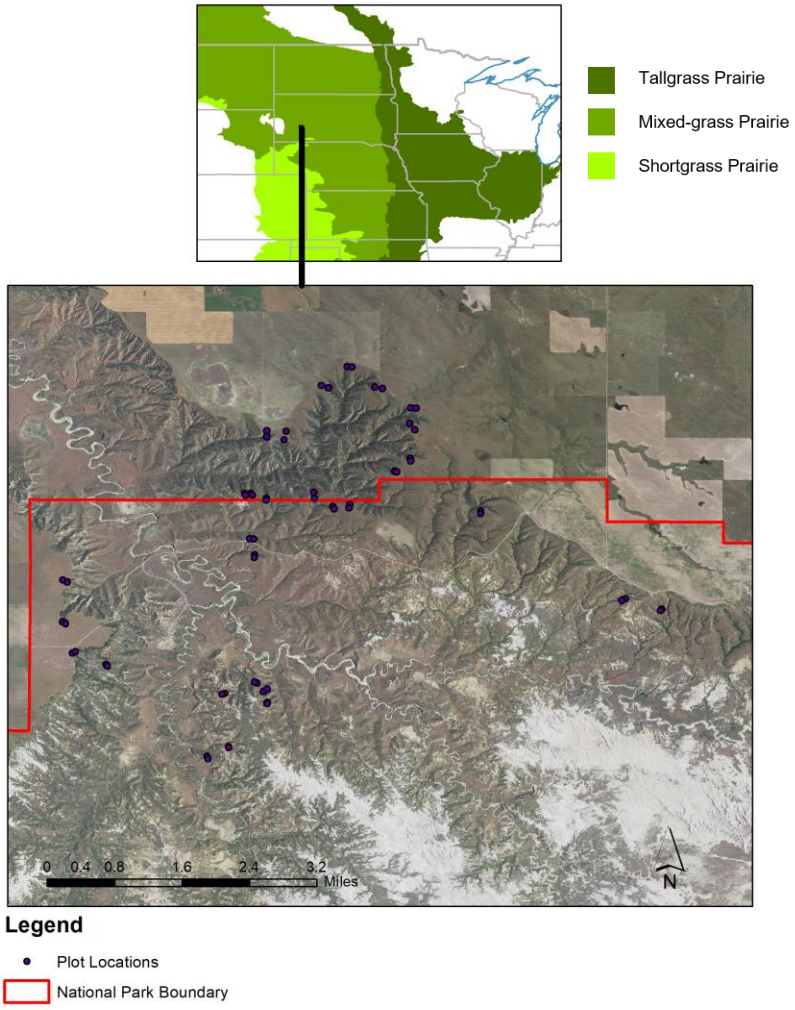

Figure 4.1: Map of plot locations in Badlands National Park and Buffalo Gap National Park.

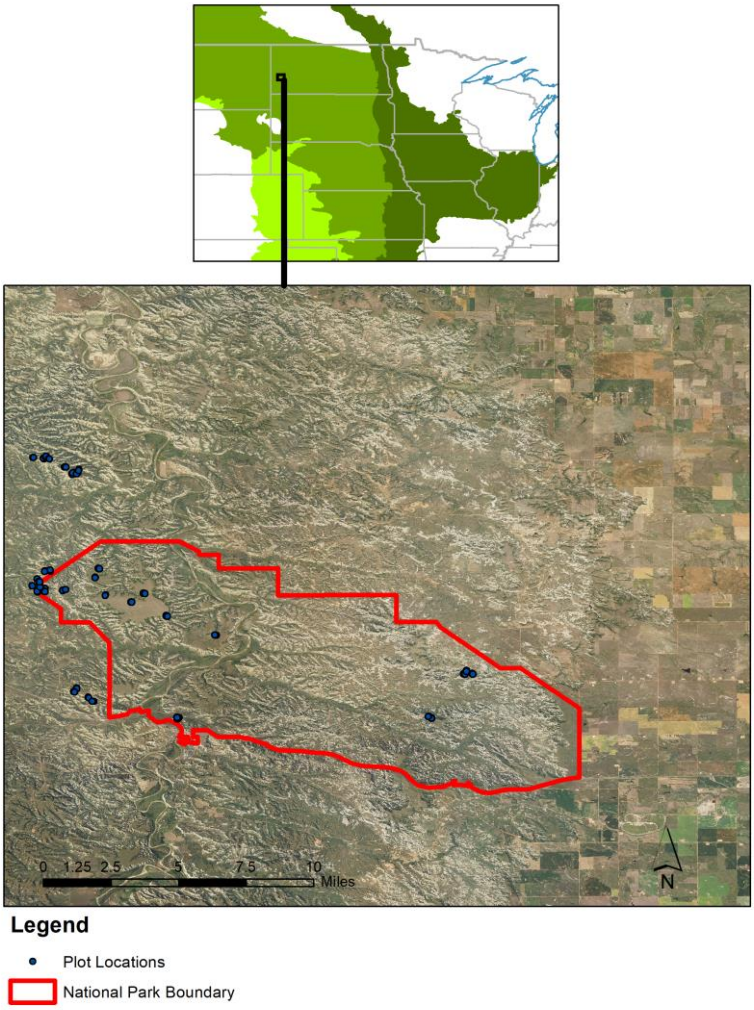

Figure 4.2: Map of plot locations in Theodore Roosevelt National park South Unit and Little Missouri National Grassland South Unit.

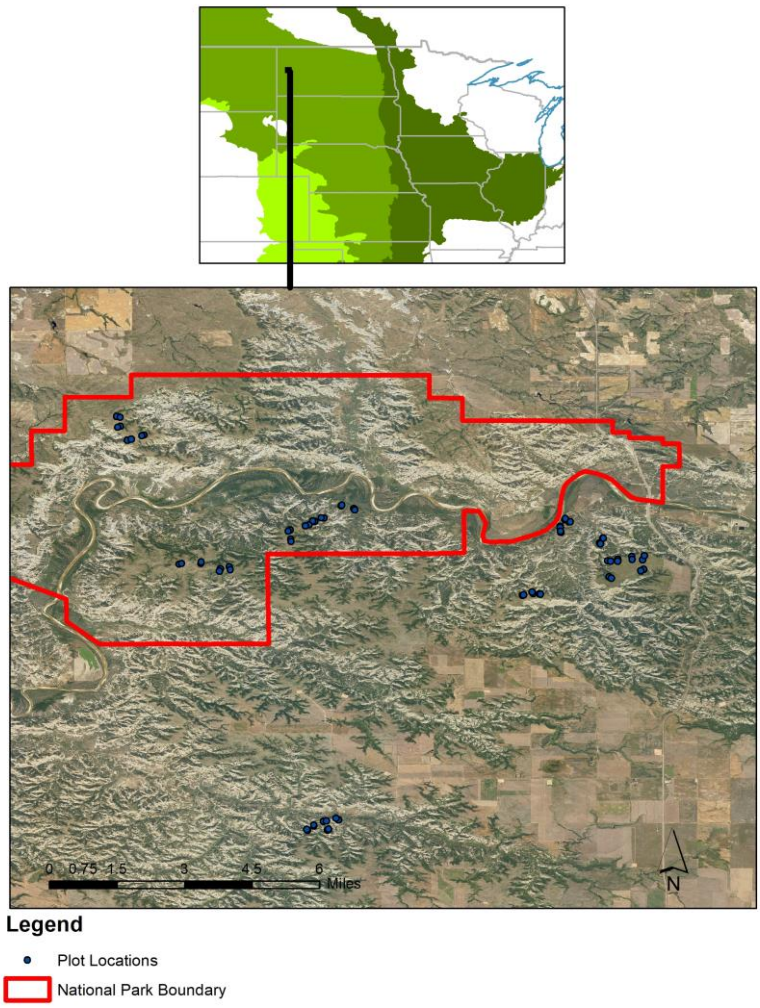

Figure 4.3: Map of plot locations in Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit.

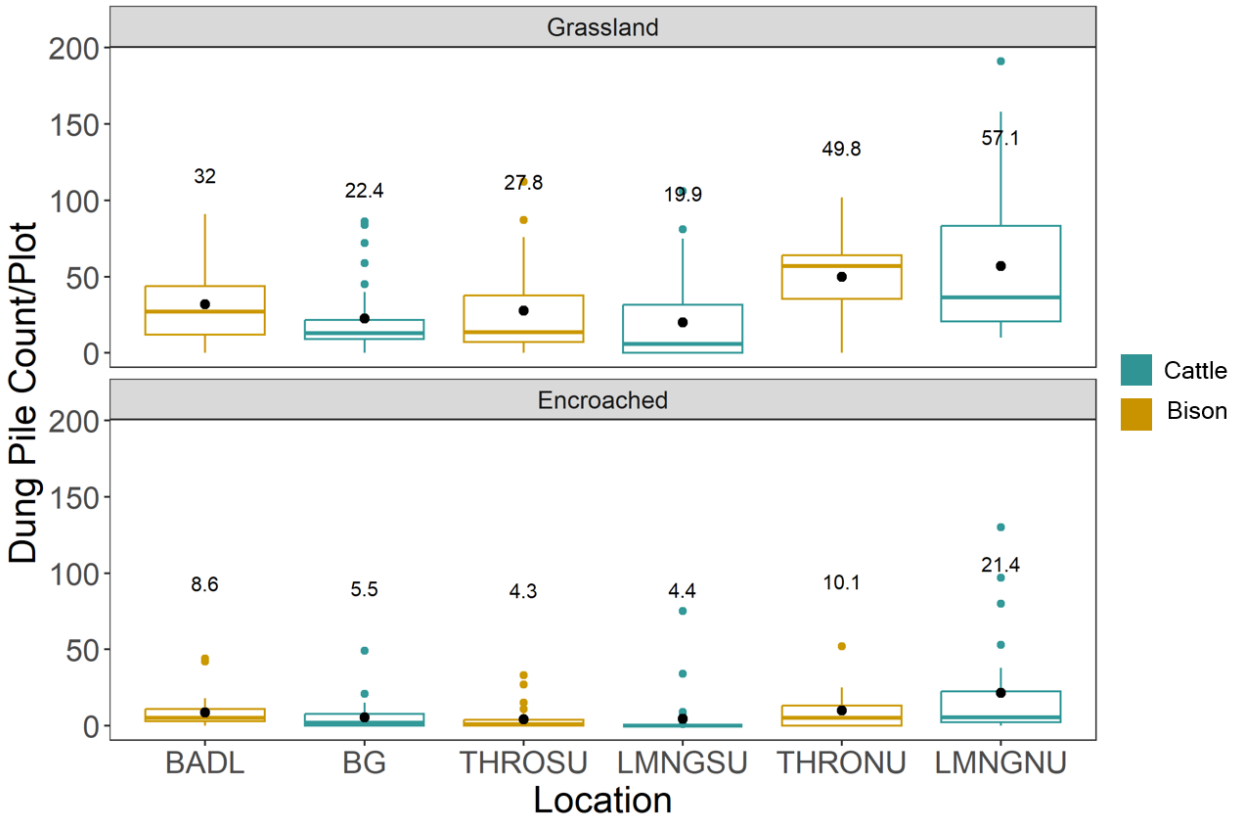

Figure 4.4: Dung counts of bison and cattle dung across our sites. Black dots and numbers signify the mean.

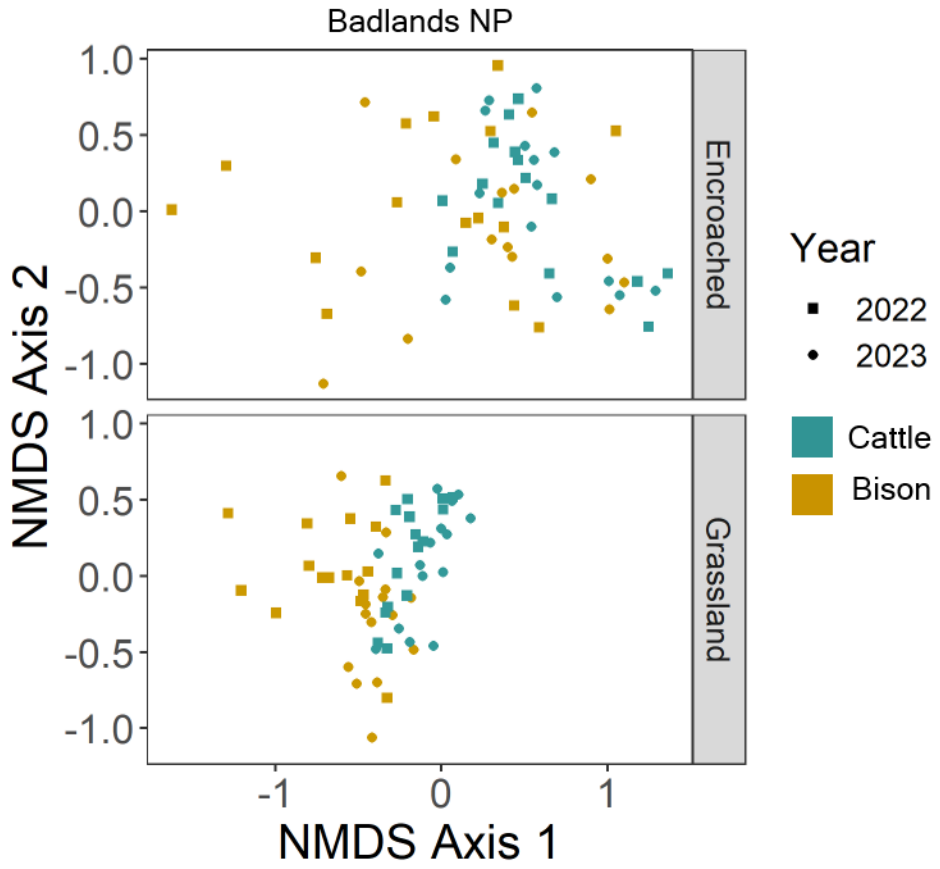

Figure 4.5: NMDS of Badlands National Park and Buffalo Gap National Grassland plant communities.

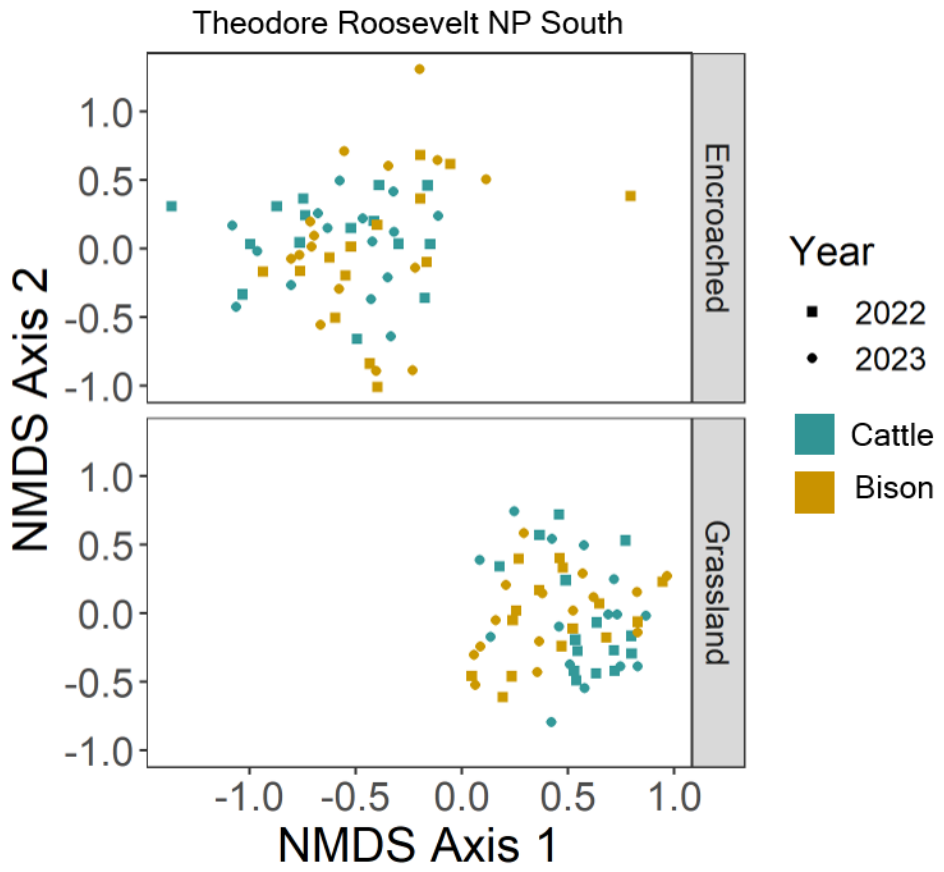

Figure 4.6: NMDS of Theodore Roosevelt National Park South Unit and Little Missouri National Grassland South Unit plant communities.

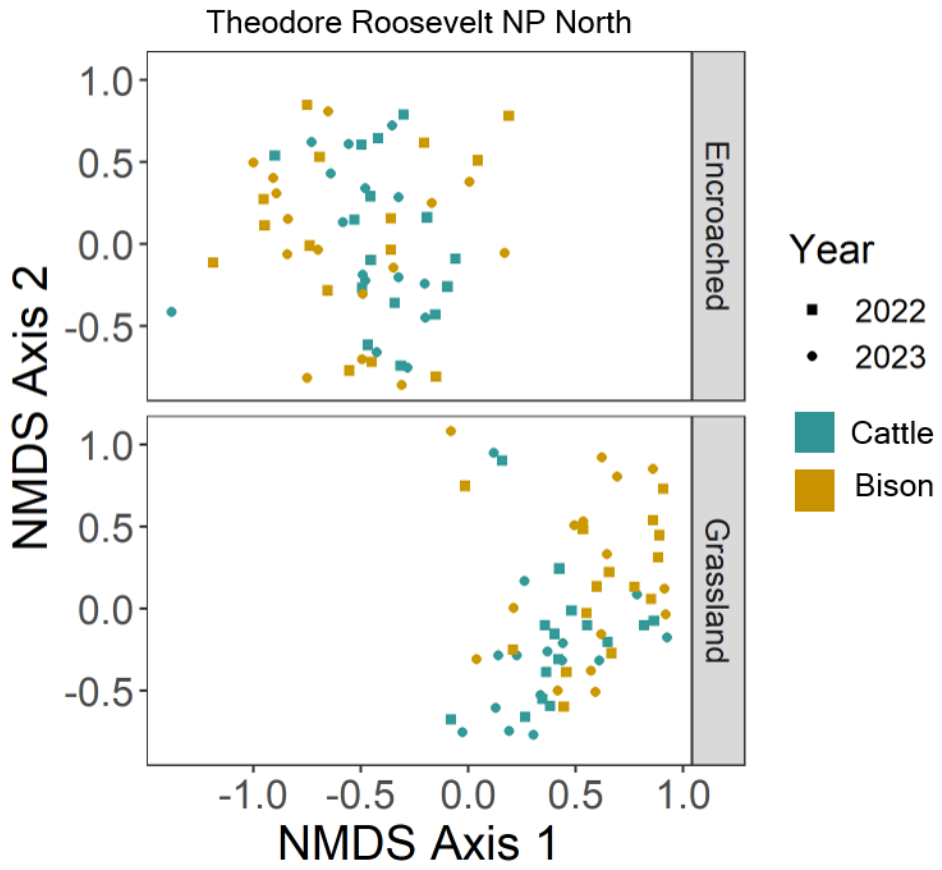

Figure 4.7: NMDS of Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit plant communities.

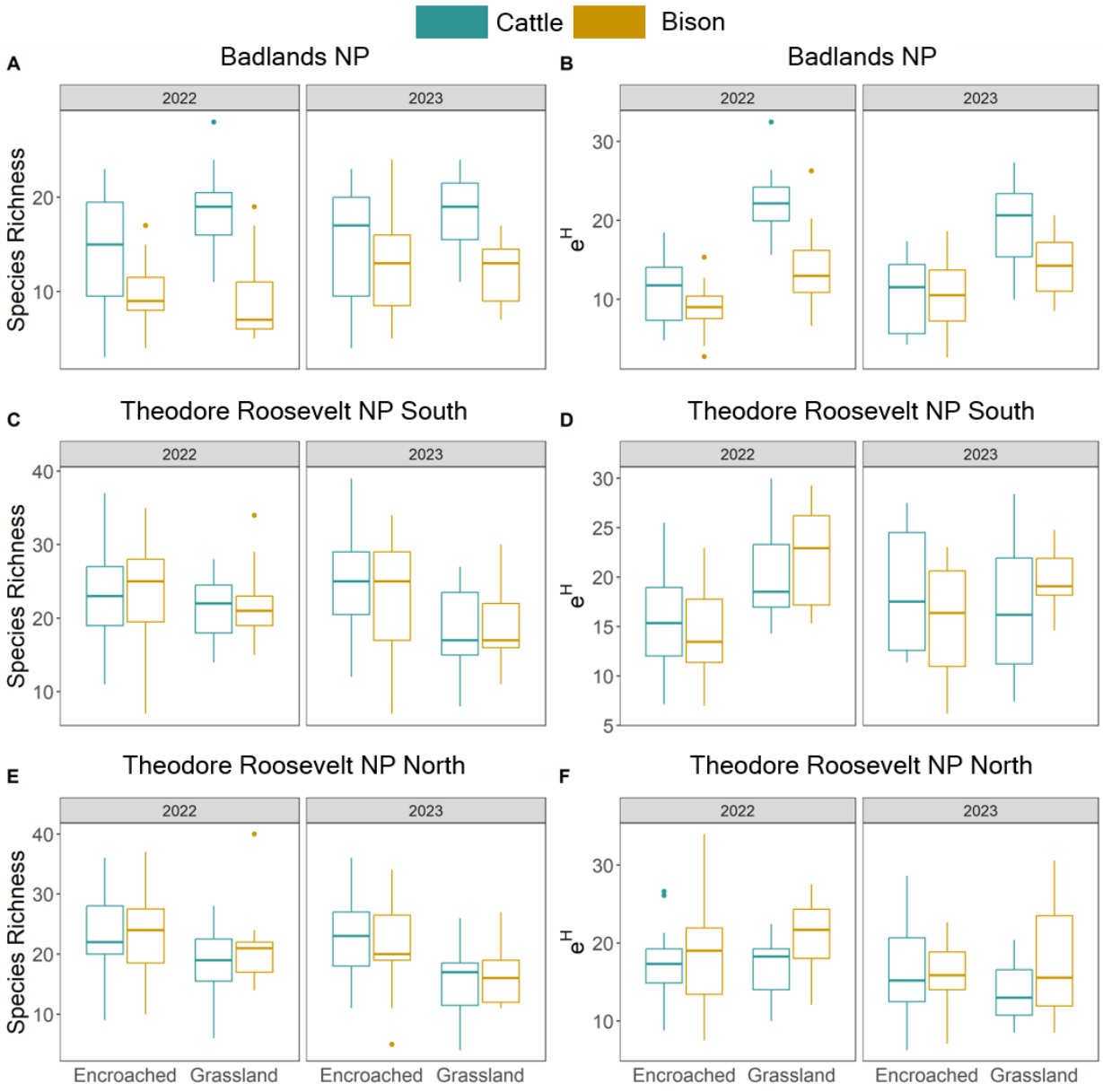

Figure 4.8: Boxplots of species richness and a diversity index across sites. Year and encroachment status have been separated out.

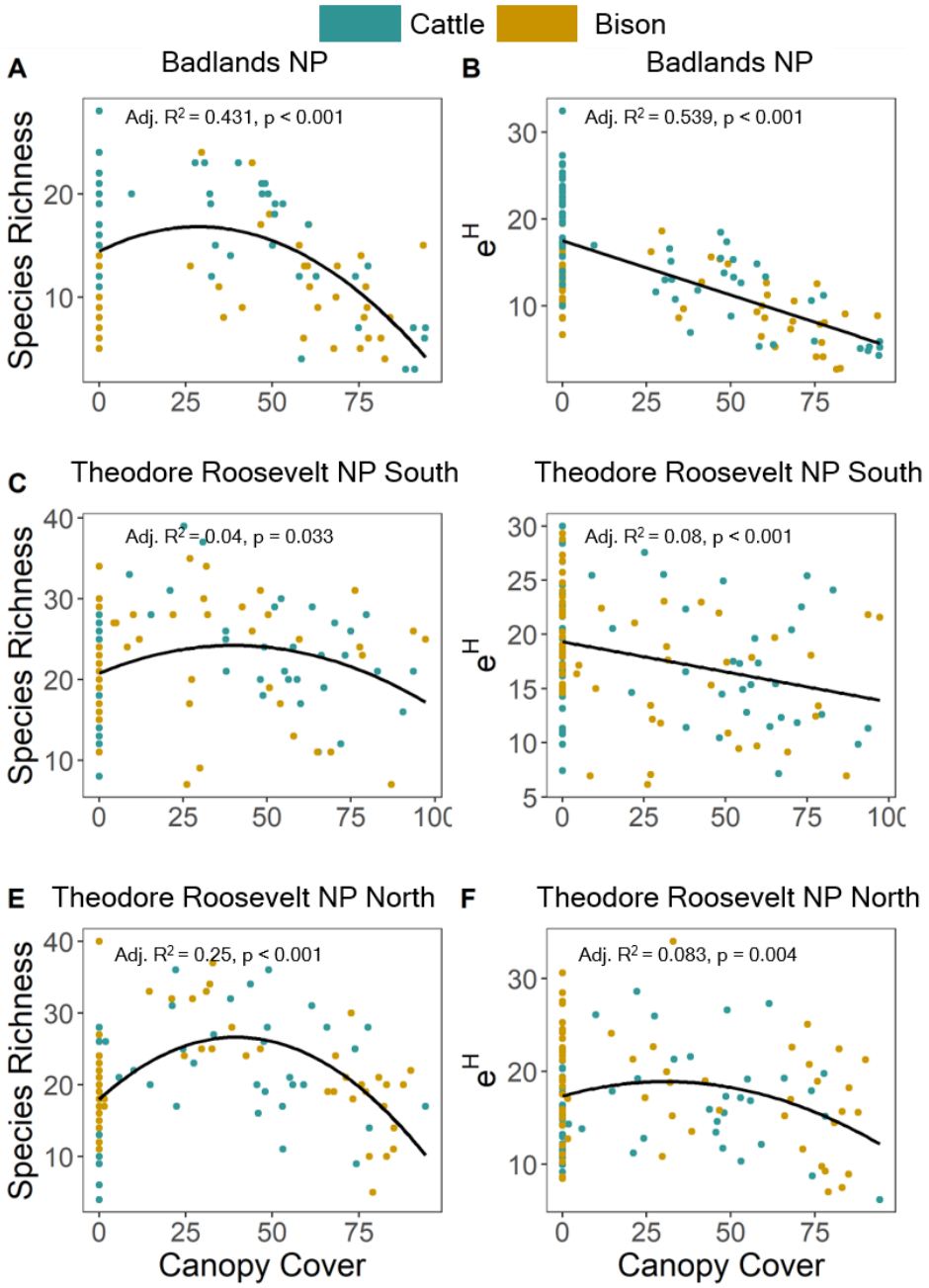

Figure 4.9: Scatterplots examining the relationship between canopy cover and species richness and a diversity index (e^H) across sites. Each plot differentiates data points based on bison's presence (orange) or absence (teal). The lines in each plot represent the fitted regression models, and the plots display the adjusted R^2 values and p-values, indicating the strength and significance of the relationships.

Supplemental Figures and Tables

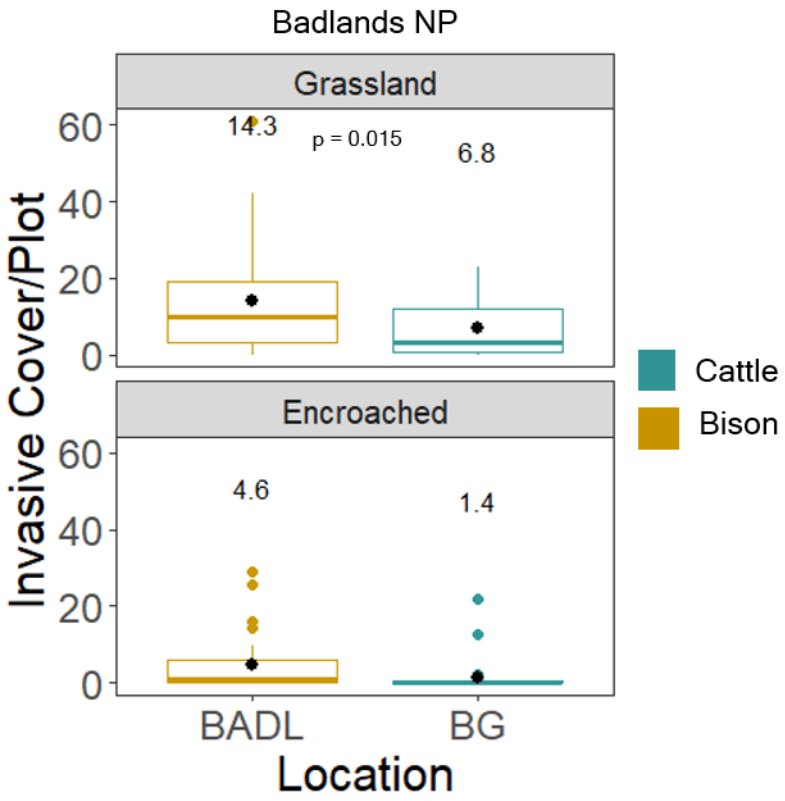

Figure S 4.1: Boxplot of invasive plant cover per plot at Badlands National Park and Buffalo Gap National Grassland. The invasive plant cover within the grasslands was significantly higher with bison presence. The numbers represent the mean invasive plant cover and the black dots represent the mean.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	Adjusted P-Value
Bison	Yes - No	-5.5	-7.3049	-3.6951	<0.00001
Year:Bison	2022:Yes - 2022:No	-6.9	-10.2598	-3.5402	<0.00001
	2023:Yes - 2022:No	-3.8333	-7.1931	-0.4735	0.01854
	2022:Yes - 2023:No	-7.1667	-10.5265	-3.8069	<0.00001
	2023:Yes - 2023:No	-4.1	-7.4598	-0.7402	0.01006
Type:Bison	Grassland:No - Encroached:No	4.0	0.6402	7.3598	0.01271
	Grassland:Yes - Encroached:No	-3.8333	-7.1931	-0.4735	0.01854
	Encroached:Yes - Grassland:No	-7.1667	-10.5265	-3.8069	<0.00001
	Grassland:Yes - Grassland:No	-7.8333	-11.1931	-4.4735	<0.00001
Year:Type:Bison	2022:Grassland:Yes - 2023:Encroached:No	-5.6667	-11.2945	-0.0389	0.04715
	2022:Encroached:Yes - 2022:Grassland:No	-8.9333	-14.5611	-3.3055	0.00009
	2023:Encroached:Yes - 2022:Grassland:No	-5.6667	-11.2945	-0.0389	0.04715
	2022:Grassland:Yes - 2022:Grassland:No	-9.4	-15.0278	-3.7722	0.00003

Factor Group	**Comparison**	**Difference**	**Lower Bound**	**Upper Bound**	**Adjusted P-Value**
	2023:Grassland:Yes - 2022:Grassland:No	-6.5333	-12.1611	-0.9055	0.01136
	2022:Encroached:Yes - 2023:Grassland:No	-8.6667	-14.2945	-3.0389	0.00016
	2022:Grassland:Yes - 2023:Grassland:No	-9.1333	-14.7611	-3.5055	0.00005
	2023:Grassland:Yes - 2023:Grassland:No	-6.2667	-11.8945	-0.6389	0.01800

Table S 4.1: Table of all significant ($p<0.05$) Tukey HSD comparisons for species richness for Badlands National Park and Buffalo Gap National Grassland.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	**Adjusted P-Value**
Type	Grassland - Encroached	7.276	5.674	8.879	9.45e-14
Bison	Yes - No	-4.033	-5.636	-2.431	2.26e-06
Year:Type	2022:Grassland - 2022:Encroached	8.209	5.226	11.192	4.95e-10
	2023:Grassland - 2022:Encroached	6.867	3.884	9.850	1.45e-07
	2022:Grassland - 2023:Encroached	7.686	4.703	10.669	4.76e-09
	2023:Grassland - 2023:Encroached	6.344	3.361	9.327	1.17e-06
Year:Bison	2022:Yes - 2022:No	-5.353	-8.336	-2.370	4.75e-05
	2023:Yes - 2022:No	-4.443	-7.426	-1.460	9.89e-04
	2022:Yes - 2023:No	-3.623	-6.606	-0.640	0.01052
Type:Bison	Grassland:No - Encroached:No	10.036	7.053	13.019	2.38e-13
	Grassland:Yes - Encroached:No	3.243	0.260	6.226	0.02744
	Encroached:Yes - Grassland:No	-11.309	-14.292	-8.326	1.06e-13
	Grassland:Yes - Grassland:No	-6.793	-9.776	-3.810	1.96e-07
	Grassland:Yes - Encroached:Yes	4.517	1.533	7.500	7.85e-04
Year:Type:Bison	2022:Grassland:No - 2022:Encroached:No	11.225	6.229	16.222	7.50e-09
	2023:Grassland:No - 2022:Encroached:No	8.307	3.310	13.304	3.23e-05
	2022:Grassland:No - 2023:Encroached:No	11.765	6.769	16.762	1.42e-09
	2023:Grassland:No - 2023:Encroached:No	8.847	3.850	13.844	7.58e-06
	2022:Encroached:Yes - 2022:Grassland:No	-13.561	-18.558	-8.565	4.90e-12
	2023:Encroached:Yes - 2022:Grassland:No	-11.976	-16.972	-6.979	7.38e-10
	2022:Grassland:Yes - 2022:Grassland:No	-8.369	-13.366	-3.372	2.74e-05
	2023:Grassland:Yes - 2022:Grassland:No	-8.135	-13.132	-3.138	5.07e-05
	2022:Encroached:Yes - 2023:Grassland:No	-10.643	-15.640	-5.647	4.36e-08
	2023:Encroached:Yes - 2023:Grassland:No	-9.057	-14.054	-4.061	4.25e-06
	2022:Grassland:Yes - 2023:Grassland:No	-5.451	-10.448	-0.454	0.02228
	2023:Grassland:Yes - 2023:Grassland:No	-5.217	-10.213	-0.220	0.03413
	2022:Grassland:Yes - 2022:Encroached:Yes	5.192	0.196	10.189	0.03564
	2023:Grassland:Yes - 2022:Encroached:Yes	5.427	0.430	10.423	0.02331

Table S 4.2: Table of all significant ($p<0.05$) Tukey HSD comparisons for e^H for Badlands National Park and Buffalo Gap National Grassland.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	Adjusted P-Value
Type	Grassland - Encroached	-3.3	-5.598	-1.002	0.00528
Year:Type	2023:Grassland - 2023:Encroached	-5.3	-9.578	-1.022	0.00867

Table S 4.3: Table of all significant (p<0.05) Tukey HSD comparisons for species richness for Theodore Roosevelt National Park South Unit and Little Missouri National Grassland South Unit.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	Adjusted P-Value
Type	Grassland - Encroached	3.514	1.624	5.404	0.000355
Year:Type	2022:Grassland - 2022:Encroached	5.980	2.462	9.498	0.000127
	2022:Grassland - 2023:Encroached	4.182	0.664	7.700	0.01289
Type:Bison	Grassland:Yes - Encroached:No	3.655	0.137	7.173	0.03848
	Grassland:Yes - Encroached:Yes	5.497	1.979	9.015	0.000494
Year:Type:Bison	2022:Grassland:Yes - 2022:Encroached:No	6.237	0.345	12.130	0.02997
	2022:Grassland:Yes - 2022:Encroached:Yes	7.303	1.411	13.196	0.00508
	2022:Grassland:Yes - 2023:Encroached:Yes	6.282	0.389	12.175	0.02799

Table S 4.4: Table of all significant (p<0.05) Tukey HSD comparisons for e^H for Theodore Roosevelt National Park South Unit and Little Missouri National Grassland South Unit.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	Adjusted P-Value
Type	Grassland - Encroached	-5.35	-7.798	-2.902	0.0000325
Year:Type	2023:Grassland - 2022:Encroached	-7.733	-12.290	-3.177	0.0001301
	2023:Grassland - 2023:Encroached	-6.8	-11.356	-2.244	0.0009604
Type:Bison	Grassland:No - Encroached:No	-6.633	-11.190	-2.077	0.001346
	Grassland:Yes - Encroached:No	-5.0	-9.556	-0.444	0.02552
	Encroached:Yes - Grassland:No	5.7	1.144	10.256	0.007871
Year:Type:Bison	2023:Grassland:No - 2022:Encroached:No	-8.667	-16.299	-1.035	0.01456
	2023:Grassland:No - 2023:Encroached:No	-8.0	-15.632	-0.368	0.03288
	2022:Encroached:Yes - 2023:Grassland:No	8.0	0.368	15.632	0.03288

Table S 4.5: Table of all significant (p<0.05) Tukey HSD comparisons for species richness for Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit.

Factor Group	Comparison	Difference	Lower Bound	Upper Bound	Adjusted P-Value
Year	2023 - 2022	-2.391	-4.372	-0.410	0.01845
Year:Bison	2022:Yes - 2023:No	4.347	0.660	8.035	0.01389
Type:Bison	Grassland:Yes - Grassland:No	4.049	0.361	7.736	0.02541
Year:Type:Bison	2022:Grassland:Yes - 2023:Grassland:No	7.396	1.220	13.573	0.00785

Table S 4.6: Table of all significant (p<0.05) Tukey HSD comparisons for e^H for Theodore Roosevelt National Park North Unit and Little Missouri National Grassland North Unit.

Chapter 5 - Conclusion

This dissertation aims to contribute to our understanding of the way bison effect plant communities in the context of woody encroachment and also the effect of bison on plant communities outside of the tallgrass prairie. One chapter focused on the impact of bison on woody plant expansion in an infrequently burned tallgrass prairie and the effect on the plant community. Another chapter investigated the influence of bison on reversing woody plant expansion. Finally, one chapter focused on the effects of bison on plant communities in mixed-grass prairie in the Northern Great Plains while also in the context of woody plant encroachment.

Within tallgrass prairie I have found that bison may inhibit tree expansion, especially *Juniperus virginiana*. Taking advantage of a 20-year fire return interval experiment, with and without bison, there was lower tree cover after 25 years in the presence of bison. Additionally, I tested this experimentally by planting *J. virginiana* seedlings with and without bison. Remarkably, after 3 months, there was 45% mortality with and 5% without. While I did not directly observe bison disturbing the seedlings, there was visual evidence that bison browsed, trampled, and removed the seedlings. These results support recent work in Yellowstone National Park, USA, where bison may inhibit aspen recruitment in the Lamar Valley (Painter et al. 2023).

Additionally, long-term plant community data showed that species richness and diversity have continued to increase even with low fire frequency, especially in tallgrass prairie uplands. While, in the ungrazed 20-year fire return interval treatment species richness and diversity has remained stable or declined with the increasing presence of woody plants. My results add to the potential ‘keystone’ effects that bison may have in the tallgrass prairie ecosystem. Furthermore, bison may have prevented woody plant expansion before European colonization when their herds numbered in the millions.

My third chapter further explored the potential for bison to reverse woody encroachment in the context of alternative state theory. There is strong evidence within the Central Great Plains that alternative states or attractors exist based on fire frequency within mesic grasslands (Ratajczak et al. 2014a, Collins et al. 2021). Mesic grasslands rely on frequent fire while decreasing fire frequency can transition grasslands to shrublands and shrublands to woodlands. In the absence of grazers returning frequent fire to the woody-dominated state fails to revert it to grassland, indicating the presence of hysteresis, and thus an alternative state (Collins et al. 2021). At Konza Prairie Biological Station, 16 exclosures were established in an infrequently burned woody-dominated state grazed by bison. Fire was reintroduced annually to the system starting in 2021. After three years of data collection, shrub area has declined but with no difference between exclosures and the presence of bison. Additionally, tree topkill appears higher without bison, probably due to more intense fires. It seems unlikely that bison will affect hysteresis; however, further monitoring is warranted.

My fourth chapter explores the effects of bison on plant communities in the context of woody encroachment within the mixed-grass prairie. Outside of the tallgrass prairie, there is very little evidence and few studies that explore the keystone effect that bison have on tallgrass prairie plant communities. Within tallgrass prairie, at least at Konza Prairie Biological Station, bison have had an enormous impact on tallgrass prairie plant communities, increasing species richness by over 100% since their reintroduction in the 1990s (Ratajczak et al. 2022). However, this appears to be only confined to tallgrass prairie (McMillian et al. 2019) and with mixed results in tallgrass prairie restorations (Wilsey and Martin 2015, Blackburn et al. 2020). While only observational, my results appear to dampen the keystone effect of bison on plant communities outside of tallgrass prairie. At two of my sites I found no difference in plant species richness or

diversity with bison present on the landscape for ~65 years. At one of my sites, Badlands National Park, I found lower plant species richness and diversity in the presence of bison, possibly due to increased invasive plants possibly from disturbance. Furthermore, I found little effect of bison on encroached plant communities, probably due to bison avoiding these areas based on dung counts.

My results from Chapter 4 potentially indicate that the impact of bison as a keystone species on plant communties is not uniform but rather varies with the ecological context. Therefore, for the continued support of large mammal translocation and reintroduction efforts aimed at biodiversity restoration, it is critical to reassess and verify that such keystone processes are applicable in different environments. The ecological relevance of Konza Prairie Biological Station for studying bison dynamics may not fully reflect the broader ecological impacts of bison across larger and more varied landscapes. For example, at Konza Prairie, approximately 200 bison are managed on a relatively small area of 961 hectares, removing about 25% of the annual net primary productivity (ANPP). This intensive management regime contrasts sharply with settings like Badlands National Park, where about 1,200 bison roam over 32,300 hectares, consuming only 12% of the ANPP, and Theodore Roosevelt National Park North Unit, where 300-500 bison access 18,600 hectares and remove about 7% of the ANPP.

Additionally, research from Grasslands National Park in Canada shows bison utilizing foraging patches approximately 11,690 hectares in size, suggesting that bison in larger, mixed-grass prairies may exhibit different foraging behaviors and impacts on plant communities than those observed in the more confined, intensively grazed settings like Konza Prairie (Kohl et al. 2013). Thus, the scale and management practices at Konza may not adequately represent the ecological role of bison in larger and less managed ecosystems, potentially skewing

understanding of their natural behaviors and effects on ecosystem structure and function. When it comes to the reintroduction of bison, numerous ecological, cultural, and aesthetic motivations exist for such initiatives (Freese et al. 2007, Shamon et al. 2022). However, should the reintroduction throughout the Great Plains hinge on anticipated keystone effects, it becomes essential for managers to employ comprehensive and ongoing monitoring strategies to effectively measure the impact of bison on the plant communities of these rangelands.

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