Bison as ecosystem engineers: Impacts on tallgrass prairie soil, plant communities, and microbial assemblages

by

Bess Bookout

B.S., Principia College, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
College of Arts and Sciences

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Manhattan, Kansas

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Abstract

The widespread conversion of North American grasslands to agriculture, coupled with the replacement of native megagrazers, primarily bison (*Bison bison*), has limited our understanding of bison's ecological roles. This dissertation investigates how bison, through both keystone herbivory and ecosystem engineering, influence tallgrass prairie in ways that differ from cattle. In Chapter 2, I used a 30-year landscape-scale experiment on fire and grazing to show that grazing increased plant species richness, altered community composition, and increased phylogenetic diversity compared to annually burned, ungrazed prairie. Bison tended to change plant communities more than cattle, though management differences obscure whether these differences are due to the grazer species or aspects of how each species is managed. However, there is one large difference between the two grazers: bison create wallows and cattle do not. In Chapter 3 and 4, I examined the effects of wallows on soil, plant communities, and microbial assemblages compared to non-wallowed bison-grazed and cattle-grazed prairie. I found that wallows had lower nutrient availability, higher salt concentrations, and distinct microbial and plant communities. Some wallows are able to hold water for weeks with consistent rainfall and thus act as ephemeral wetlands, supporting amphibians, aquatic invertebrates, and other organisms. Over time, bison naturally abandon wallows, a process we mimicked by creating exclosures around wallows. We found that unique communities also become established in these transient micro-habitats. Wallows, and their resulting differences in soil physiochemistry and hydrology, increase beta-diversity of soil microbes without decreasing alpha diversity. Bison and their behaviors, especially wallowing, act as important drivers of grassland heterogeneity, underscoring the importance of their reintroduction for restoring ecosystem function and resilience.

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Dedication

To the prairie and your bison – you never cease to inspire me.

Chapter 1 – Introduction

1.1 Background

Temperate grasslands are among the most expansive and ecologically important biomes globally, yet they are also among the most imperiled (Samson and Knopf 1994, Hoekstra et al. 2005). Once covering over 40% of Earth's terrestrial surface (Suttie et al. 2005, Wilsey 2018), grasslands provide essential ecosystem services including carbon storage, support for biodiversity, and food and fiber for human use (Wilsey 2018, Bengtsson et al. 2019). Despite their importance, grasslands have been extensively converted to row-crop agriculture, fragmented by development, and disrupted by loss of historical disturbance regimes (Hoekstra et al. 2005, Lark 2020). In North America, tallgrass prairie is now one of the most altered ecosystems, with estimates suggesting that less than 4% of its original area remains intact (Samson and Knopf 1994, Hoekstra et al. 2005).

Since the last glacial maximum, the tallgrass ecosystem was shaped by a variable climate, frequent fire, and large herbivores (Axelrod 1985, Anderson 2006). Climate variability, including frequent droughts (Axelrod 1985, Anderson 2006), altered when and where fire could occur, either naturally or through Indigenous management (Stambaugh et al. 2013, Roos et al. 2018, Courtwright 2023). Fire kills or knocks back woody vegetation, preventing or slowing woody encroachment and favoring herbaceous vegetation (Collins and Calabrese 2012, Ratajczak et al. 2014). Keystone herbivory, either by large grazers like bison or by mixed-feeders like elk, also likely slowed woody encroachment and increased herbaceous plant richness and diversity (Knapp et al. 1999, Fuhlendorf et al. 2010, O'Connor et al. 2020). Many grazers, native and domesticated, preferentially graze recently burned patches, meaning that fire regimes shape grazing patterns (Fuhlendorf et al. 2009, Raynor et al. 2016). Intensive grazing can also

alter fire frequency and intensity, with grazers and fires effectively "competing" to consume forage (Archibald and Hempson 2016). The interactions of climate, fire, and grazing likely left behind a mosaic of plant communities with high spatial and temporal heterogeneity (Knapp et al. 1999, Adler et al. 2001, Fuhlendorf and Engle 2001).

In the Anthropocene, these historical drivers have been dramatically altered. Precipitation regimes, especially timing and intensity of events, are shifting due to climate change (Kukal and Irmak 2016, Bukovsky et al. 2017), fire is increasingly suppressed or altered (Ratajczak et al. 2012, Twidwell et al. 2013), and native herbivores have been largely replaced by domestic livestock (Anderson 2006, Fuhlendorf et al. 2010). Changes in temporal dynamics of precipitation have been shown to influence plant community traits such as productivity, species evenness, and diversity, which in turn may reduce community resistance and recovery following extreme climatic events (Briggs and Knapp 1995, Nippert et al. 2006, Grant et al. 2017, Shaw et al. 2022). Fire suppression has enabled widespread woody encroachment, which causes reductions in herbaceous productivity and biodiversity (Ratajczak et al. 2012, Anadón et al. 2014, Morford et al. 2022), loss of grassland birds (Silber et al. 2024), and changes in key hydrological processes (Keen et al. 2022).

Loss of keystone herbivores (i.e., bison, elk) and replacement with domestic cattle also may have lasting impacts on grassland biodiversity and functioning (Knapp et al. 1999, Fuhlendorf et al. 2010, O'Connor et al. 2020). Although both bison (*Bison bison*) and cattle *(Bos taurus*) preferentially consume grasses and can increase plant diversity under certain management conditions (Hickman et al. 2004, Towne et al. 2005, Koerner et al. 2018, Ratajczak et al. 2022), they differ in evolutionary history, behavior, and ecosystem effects (Knapp et al. 1999, Fuhlendorf et al. 2010, Kohl et al. 2013). Cattle in the Flint Hills region of the Great Plains

are typically managed in high-density herds, grazed seasonally, and supported by annual burning to maximize forage production (Hoy 2009, Beam 2010). By contrast, bison are often kept in conservation contexts where they graze year-round and are managed under variable fire-return intervals designed to mimic historical heterogeneity (Kohl et al. 2013, USDA 2014). While these management differences obscure species-specific effects, it is often not the species identity alone that causes differences, but differences in functional and behavioral traits (Lundgren et al. 2024). One such difference between cattle and bison is that bison create wallows, and cattle do not (Coppedge and Shaw 1998, McMillan et al. 2011).

Wallowing is not unique to bison. Elk (VerCauteren et al. 2007), fallow deer (Massei and Bowyer 1999), rhinoceros (Wilson et al. 2019), zebra (Wagner et al. 2021), and wild hogs (Eckert et al. 2019; Figure 1.1) wallow. However, the only livestock known to regularly wallow are domestic hogs (Bracke 2011) and water buffalo (Khongdee et al. 2011). By forming wallows, through mudbathing, dustbathing, horning, and trampling, bison create shallow depressions of exposed soil across low-slope areas of the prairie. These wallows are characterized by compacted soils, with elevated clay and salt concentrations, reduced organic matter, and unique hydrological properties (Polley and Collins 1984, Coppedge and Shaw 1998; Bookout et al. *In review*). Plant communities within wallows often differ in composition and structure from the surrounding prairie, with lower overall cover but the presence of wetland species and disturbance-adapted forbs (Collins and Uno 1983, Umbanhowar Jr. 1992, 1992, Trager et al. 2004, McMillan et al. 2011; Bookout et al. *In review*). Some wallows retain water for weeks after rain events, and they can serve as ephemeral wetlands and support many wetland taxa, from anurans (Gerlanc and Kaufman 2003) to invertebrates (Frazier et al. 2024; Figure 1.2). The distinct abiotic and biotic

conditions in wallows likely affect belowground composition and processes (Philippot et al. 2021, West and Whitman 2022, Chen et al. 2024), which is the topic explored in Chapter 4.

Soil microbial communities, including bacteria, archaea, and fungi, are central to nutrient cycling, carbon sequestration, and plant productivity in grassland systems (Fierer and Jackson 2006, Van Der Heijden et al. 2008, Huygens et al. 2011, Aislabie and Deslippe 2013). These communities regulate organic matter decomposition (Grandy and Neff 2008, Goldfarb et al. 2011), nitrogen transformations (Kaye et al. 2005, Philippot et al. 2009), and root-associated feedbacks that can influence plant competition and community composition (Bardgett et al. 1998, Van Der Heijden et al. 2008, Fierer 2017). Microbial community composition, diversity, and activity can be shaped by both abiotic factors (e.g., pH, salinity, temperature) and biotic interactions (e.g., plant root exudates, litter chemistry, microbial antagonism) (Lauber et al. 2008, Tedersoo et al. 2014, Dassen et al. 2017, Fierer 2017, Bahram et al. 2018).

Importantly, microbial and plant communities both respond to disturbance (i.e., fire, grazing intensity, trampling), though the magnitudes and directionality of responses may differ (Dassen et al. 2017, Wang et al. 2019, Reinhart et al. 2022). While plant communities are directly shaped by herbivory and fire (Towne et al. 2005, Collins and Calabrese 2012, Pringle et al. 2023), microbial communities are more sensitive to changes in soil chemistry (i.e., pH, salinity, nutrients), which can be mediated by both abiotic and biotic factors (Fierer 2017, Bahram et al. 2018, Zhou et al. 2020). Moderate grazing can increase plant diversity in some grasslands (Towne et al. 2005, Ratajczak et al. 2022), but grazing also has complex and varying effects on microbial biomass, diversity, and/or activity, and responses of soil microbial communities to grazing, like those of plants, are context and scale dependent (Radl et al. 2007, Eldridge et al. 2017, Xun et al. 2018, Zhang and Fu 2021, Wu et al. 2022, Ma et al. 2022,

Anguiano et al. 2024, Guo et al. n.d.). Bacterial and archaeal communities are generally more sensitive to pH and temperature (Lauber et al. 2008) while fungi respond to changes in plant community structure and nutrient inputs (Tedersoo et al. 2014, Lutz et al. 2025).

Grazing, coupled with soil disturbances can alter microbial activity directly through soil compaction and nutrient redistribution, and indirectly by modifying plant communities and nutrient inputs (Bardgett et al. 1998, Van Der Heijden et al. 2008). In bison-grazed systems, differences in plant composition, root productivity and biomass, and nutrient patchiness may select for different microbial assemblages than those found in cattle systems (Hiltbrunner et al. 2012, Barber et al. 2017, Zaret et al. *In revie*). However, the specific consequences of wallowing for microbial communities have never been tested.

In this dissertation, I investigated how bison affect tallgrass prairie structure across multiple levels of organization, with a focus on plant and microbial communities and the effects of disturbances that create heterogeneity. Specifically, I asked 1) How do grazing by bison and cattle differ in their long-term impacts on plant phylogenetic diversity and drought resilience? 2) How does wallowing affect soil conditions and plant composition compared to surrounding prairie? 3) Do wallows support distinct microbial communities?

In Chapter 2, I used a long-term fire and grazing experiment to evaluate the effects of bison and cattle on plant diversity, community composition, phylogenetic diversity, and drought resilience. In Chapter 3, I compared wallowed and non-wallowed plots to assess how bison wallowing changes soil physiochemistry and plant communities. This chapter also highlights the unique potential of wallows for experimental ecology. In Chapter 4, I used microbial DNA sequencing to characterize prokaryote and fungal communities within and outside of wallows. Together, these chapters allow me to assess how bison influence spatial heterogeneity and

ecosystem function in tallgrass prairie, and whether their effects provide unique conservation and rewilding benefits compared to cattle.

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1.3 Figures

Figure 1.1: A collage of a few examples of animals who wallow and wallows. A) A bison (*Bison bison*) dustbathing at Castle Provincial Park, Alberta, Canada; June 12, 2019; photo taken by Lfhooper1996 (Wikimedia). B) A herd of bison (*Bison bison*) and their wallows at Maxwell Wildlife Refuge, Kansas; June 2019; Google Earth Imagery. C) Two juvenile moose (*Alces alces*) wallowing in a water hole in Kenai National Wildlife Refuge, Alaska; July 11, 2022;

photo taken by Jaimie-Lee Musen with USFWS (Wikimedia). D) A zebra (*Equus sp.*) dustbathing at Allwetterzoo, Munster, Germany; July 25, 2008; photo credits unknown (Wikimedia). E) An adult and juvenile Indian rhinoceros (*Rhinoceros unicornis*) cooling off in a large wallow in Jaldapara National Park, India; April 29, 2008; Photo taken by A.J.T. Johnsingh (Wikimedia). F) A water buffalo (*Bubalus bubalis*) mudbathing in a wallow in Sri Lanka; date and photo credits unknown (Wikimedia). G) An elk (*Cervus canadensis*) wallow; October 1981; place and photo credits unknown (Wikimedia). H) A bull elk (*Cervus canadensis*) in Jasper National Park; September 21, 2019; photo taken by P. Hirsch (Wikimedia). I) A domestic pig (*Sus domesticus*) cooling off in a muddy wallow in Laos; July 20, 2017; photo credits unknown (Wikimedia).

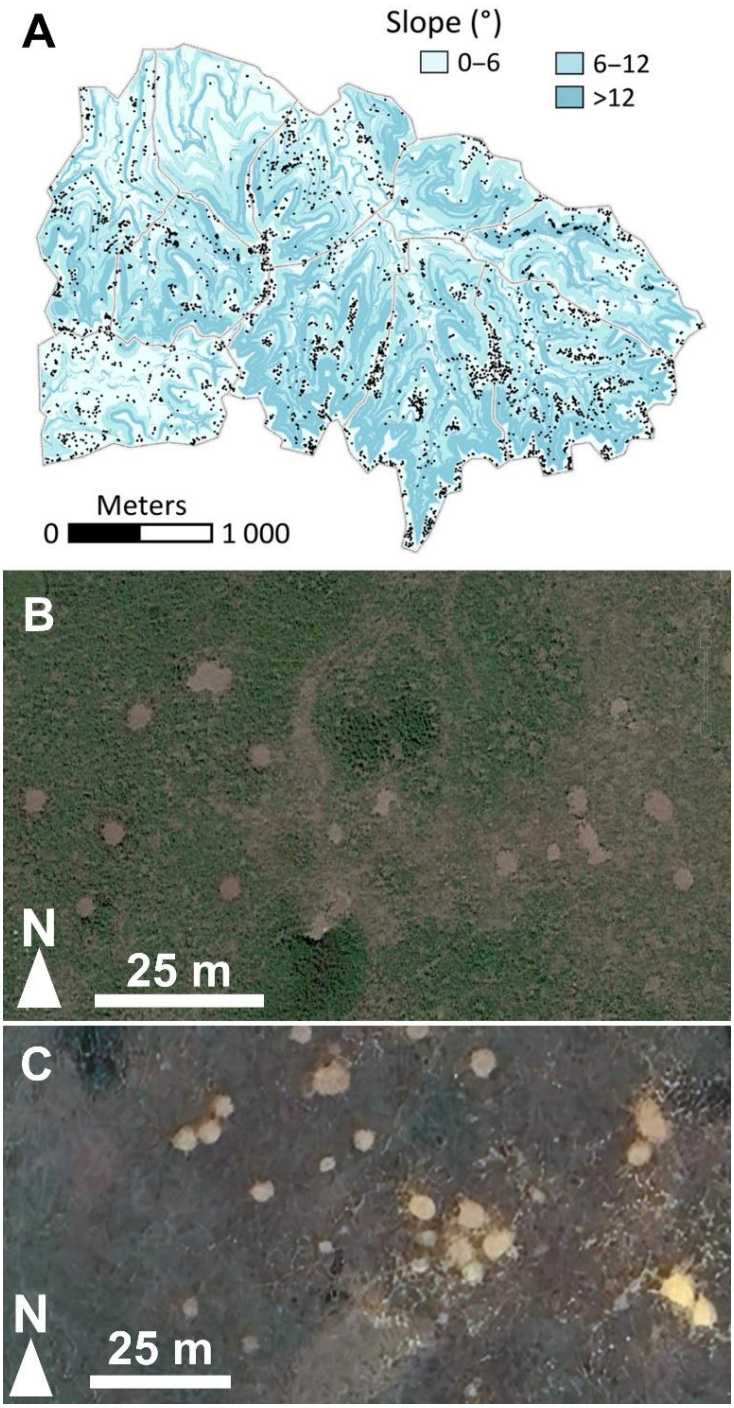

Figure 1.2: Bison wallows and their prevalence and patchy distributions. A) Map of the bison-grazed area at Konza Prairie Biological station with wallows (black dots) overlaid on hillslope. Most wallows are found on flat areas (slope 0-6 degrees). Figure is panel C from Horne et al 2024. B) Aerial image of bison wallows at KBPS (N1A) in spring 2022. Image is from Google Earth Imagery. C) Aerial image of bison wallows at Maxwell Wildlife Refuge, Kansas. Image is from Google Earth Imagery.

Chapter 2 - Resilience and multi-faceted diversity of grazed and ungrazed Great Plains grassland plant communities to severe drought

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2.1 Abstract

The simultaneous agricultural conversion of North American prairies and removal of native megagrazers—primarily bison (*Bison bison*)—has limited our understanding of how bison impact prairie plant communities. Further obscuring the role of native megagrazers is that, in what little prairie remains, either grazers are absent or domestic cattle are dominant. We used a three-decade experiment to assess how fire frequency and reintroducing bison affected upland tallgrass prairie plant communities and their phylogenetic diversity (PD). Grazing and fire shifted plant communities significantly over time. The ungrazed treatment was a largely static community dominated by four grass species, with occasional forbs and subshrubs. In contrast, community composition in the bison treatment shifted dramatically, with increasing abundance of native forbs, subdominant grasses, and small annual plant species. Compared to ungrazed areas, bison treatments had 225% more unique species, 125% more unique plant families, and higher richness within common plant families such as Asteraceae, Fabaceae, and Poaceae. Cattle-grazed communities fell between these two treatments with higher abundance and richness of *Carex spp*. Most treatments had similar, unchanging PD values over time, except annually burned, ungrazed prairie, where PD steadily declined. All treatments were resistant and/or resilient to an extreme drought in 2011 and 2012, with no lasting changes in composition or PD before, during or after the drought. This resistance to change emphasizes the importance of the core grassland plant species to maintain functional group composition. Grazing and fire drove shifts in tallgrass prairie plant communities, which were resilient to extreme drought.

2.2 Introduction

Historically, grasslands were estimated to comprise 42% of the Earth's land cover (Suttie et al. 2005; Wilsey 2018). However, grasslands are in decline worldwide due to land conversion,

removal of large native grazers, and suppression of historical fire regimes (Hoekstra et al. 2005; Anderson 2006). The grasslands of the Great Plains of North America covered approximately 162 million ha before European arrival, with shortgrass prairie in the west, mixed-grass prairie at middle longitudes, and tallgrass prairie to the east. Tallgrass prairie, the most productive Great Plains grassland, now covers less than 5% of its pre-European extent, with approximately 45% of all remaining tallgrass prairie (~1.2 million ha) in the Flint Hills of Kansas (Samson and Knopf 1996). Maintaining biodiversity and ecosystem functions in this remaining landscape is critical to preventing tallgrass prairie from becoming an endangered ecosystem (*sensu* Noss and Scott 1995), along with much of its flora and fauna.

Tallgrass prairie ecosystems are disturbance-dependent, maintained by fire, grazing, and variable climate (Axelrod 1985). Fires in the Great Plains have been controlled by a combination of weather variation, grazing pressure, and, overwhelmingly, human decisions. Many Indigenous groups used prescribed fire in tallgrass prairies to attract game, ease movement across the landscape, and favor certain plants (Stambaugh et al. 2013; Courtwright 2023). In the Flint Hills, white colonizers adopted a culture of setting fires alongside cattle production (Allen and Palmer 2011; Stambaugh et al. 2013). Very frequent fire, such as annual burning, can have a homogenizing effect that promotes dominant grasses at the expense of many of the sub-dominant grasses and forbs that underpin biodiversity in tallgrass prairie (Fuhlendorf et al. 2009; Collins and Calabrese 2012). Long-term fire suppression, with fire return intervals greater than four years, can lead to woody encroachment, where shrubs can establish and shade out herbaceous vegetation. Woody encroachment reduces forage for economically important grazers (Morford et al. 2022), streamflow (Keen et al. 2023), and biodiversity of grassland-adapted plant (Ratajczak et al. 2012) and avian communities (Silber et al. 2024).

Grazing pressure is another important driver of maintaining grasslands in this region and globally (McNaughton 1986; Koerner et al. 2018; Pringle et al. 2023). From the last glacial maximum to early European colonization, large populations of megafauna were widespread, including the megagrazer Plains Bison (*Bison bison bison*) (Axelrod 1985). Most grasslands have coevolved with one or more large grazers (Strömberg 2011). In tallgrass prairie and similar ecosystems, native grazers and domesticated cattle (*Bos taurus*) often can keep dominant grasses in check, allowing forbs and subdominant grasses to compete (Crider 1955; Anderson 2006). While bison and some other native large grazers have escaped near-extinction, they are still relatively uncommon. Instead, they have been replaced by domestic cattle.

Despite domesticated cattle being ubiquitous in grasslands, we have not entirely answered whether domestic livestock are ecologically synonymous with the native grazers that were abundant prior to colonization (Pringle et al. 2023). Several studies emphasize the physiological and behavioral differences between bison and cattle, while others argue that the systems are too complex and the effects too intertwined with differences in management to determine if cattle are markedly different from bison (Coppedge and Shaw 1998; Towne et al. 2005; Kohl et al. 2013; Ranglack and Du Toit 2015). Both species predominantly eat graminoids, an otherwise dominant plant group that reduces forb diversity and abundance (Towne et al. 2005; Collins and Calabrese 2012). However, the few side-by-side studies of diet suggest that cattle eat more forbs and shrubs (10-25% of their diet) than bison (<10%) (Plumb and Dodd 1993; Hartnett et al. 1997; Coppedge and Shaw 1998; Steuter and Hidinger 1999). Both bison and cattle also preferentially choose recently burned patches of grasslands, which can enhance beta-diversity by creating a mosaic of grazing intensities (Coppedge and Shaw 1998; Allred, Fuhlendorf, and Hamilton 2011; Kohl et al. 2013). However, in grasslands, bison tend to

avoid wooded areas and venture farther from water sources than cattle, leading to different patterns of landscape usage (Allred, Fuhlendorf, and Hamilton 2011; Allred et al. 2013; Kohl et al. 2013). These behavioral and physiological differences exhibited by these large grazers could lead to differing plant communities and their response to drought.

In regions where fire and grazing maintain grassland, the third pressure we often consider is the variable climate that predominates in the Great Plains and many other grassland ecosystems (Knapp and Smith 2001). Historically, a major factor shaping grasslands has been periodic extreme droughts (Axelrod 1985), which can have large immediate impacts on ecosystems. However, there are many examples of grasslands being resistant or resilient to such disturbances (Weaver 1954; Hoover et al. 2014; Isbell et al. 2015). Here, we define resistance as the ability of a system to resist change altogether and resilience as the capacity to change but avoid significant ecological shifts and return to pre-disturbance grass-dominated states (e.g., Walker et al. 2004). Resistance and resilience to drought will be critical this century because global circulation models suggest that droughts in the Great Plains will become more common and intense (Cook et al. 2015). Generally, aboveground net primary productivity (ANPP) decreases with decreased precipitation (Nippert et al. 2006; Robinson et al. 2013; Raynor et al. 2024). Beyond these simpler interactions between climate and grassland function, we know far less about how grassland plant communities will fare in the face of more extreme climate change (Ratajczak and Ladwig 2019; Turner et al. 2020), especially those grazed by large herbivores. How large will the initial response be? When we see significant changes in populations and communities, will they recover, and how long will this take? These questions remain largely unanswered in the context of different fire frequencies and the presence of large grazers (Wilcox et al. 2017; Riginos et al. 2018; Ratajczak et al. 2022).

While studies have shown that drought temporarily decreases ANPP and species diversity in mesic grasslands without large grazers, studies also show that grazing can promote plant species diversity. Increased diversity can be important to community resistance and resilience to large disturbances, such as droughts (Díaz et al. 2007; Isbell et al. 2015). Plant communities that are more diverse in form (i.e., species richness, compositional diversity) and function (i.e., functional richness or diversity) are, on average, less susceptible to ecological shifts (Elmqvist et al. 2003; Cadotte et al. 2012; Carpenter et al. 2012; Hoover et al. 2014; Oliver et al. 2015; Loreau et al. 2021). This resilience is often because functional redundancy buffers individual species loss and retains overall ecosystem functioning (Peterson et al. 1998; Oliver et al. 2015; Loreau et al. 2021). Functional diversity and redundancy can be challenging to measure for large plant communities; thus, phylogenetic diversity (PD) has been used as a proxy to estimate functional diversity (Webb et al. 2002; Cadotte et al. 2010). Since closely phylogenetically related species (e.g., two grass species) are likely to fill similar ecological niches than more distantly related species (e.g., a grass and a legume), these species are often assumed to provide similar functions to the community (Prinzing et al. 2001; Wiens et al. 2010; Su et al. 2020). This study uses a long-term experiment to assess the joint effect of grazing and fire on plant community composition and PD to assess how a severe drought affected tallgrass prairie plant communities. In 2012, one of the most severe single-year droughts occurred in the Central Great Plains since the “great drought” associated with the Dust Bowl (Hoerling et al. 2014). This event provided an opportunity to quantify the resistance and resilience of plant communities as a function of grazing and fire. The objectives of this study were to 1) establish a baseline of how cattle and bison alter plant community composition and PD over time; 2) determine if there are differences in resistance and resilience of plant community composition and PD to the extreme

drought based on long-term fire return interval (FRI) and grazing treatment. We hypothesized that community composition and PD would differ between grazing and FRI treatment combinations as recent work showed large differences in alpha diversity metrics (Ratajczak et al. 2022). We also hypothesized that the drought would negatively impact plant communities (i.e., not resistant), especially ungrazed communities, but that they would be resilient to the drought and return to similar communities post-drought.

2.3 Methods

2.3.1 Study system

The study occurred at Konza Prairie Biological Station (KPBS) in the Flint Hills ecoregion of eastern Kansas, USA. KPBS is a native, unplowed tallgrass prairie (Collins and Calabrese 2012). The soils are unglaciated, with all plots included in this study located in thin upland soils (less than 0.5m deep) or indicative of the Florence series of cherty silty clay loam. KPBS has a temperate mid-continental climate with hot summers ranging to 27 C with annual precipitation of 835 mm, most of which occurs during the growing season. Like much of the Flint Hills, KPBS has a hilly topography and rocky soils that preclude plowing. Native prairie grass species, such as big bluestem (*Andropogon gerardii*), yellow prairie grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*), and switchgrass (*Panicum virgatum*), are dominant. However, the community is still diverse owing to many subdominant species.

The site was initially founded to create a factorial experimental design that crossed different fire return intervals (1, 2, 4, and 20 years) with grazing treatments (ungrazed and bison grazed). Historically, fires occurred approximately every three to four years, with spatio-temporal variability (Allen and Palmer 2011; Stambaugh et al. 2013). Today, annual to biannual fire frequencies are common (Ratajczak et al. 2016), with most fires occurring between March and

April before the growing season. The two fire treatments we chose to focus on for this study are annual FRI (the currently dominant fire regime in the region) and three- to four-year FRI, the historically dominant fire regime. This choice also allowed us to make comparisons with cattle, which KPBS added to another portion of the site in areas with an annual and three-year FRI. Therefore, for this study, we had the treatments and corresponding long-term data to factorially compare two FRI (annual and 3-4 years) and three grazing treatments: bison, cattle, and ungrazed.

Bison and cattle on site are managed differently, which reflects how these two grazer species are often deployed in real-world operations. Both in experiments and working ranching operations, we are comparing two land-use systems, not just two different species. At KPBS, bison were reintroduced into a fenced area of 500 ha in 1987 and expanded to 1100 ha in 1991. The bison herd is present year-round, free-ranging among fire treatments, and unmanaged except for an annual round-up to cull excess bison and inoculate them for common bovine diseases. Unlike the bison, all cattle (cow-calf pairs) on site graze between May and October and are moved off-site for winter. The cattle herd in the annually burned catchment used in this data set was established in 1991 and maintained at a density similar to that of local cattle operations in the Flint Hills. In 2008, KPBS implemented a patch-burn-graze (PBG) treatment – a cattle management system that aims to increase biodiversity and maintain cattle production by taking advantage of pyric herbivory (large herbivores preferring to graze in recently burned areas) (Fuhlendorf et al. 2009). The PBG treatment is one pasture containing three catchments in a three-year burning rotation. Prior to the PBG treatment, steers grazed this pasture annually from 1990 to 2008. All grazing treatments maintain grazer densities meant to remove approximately 25% of ANPP (Knapp et al. 2012).

2.3.2 Plant Composition

KPBS established plant composition plots between 1983 and 1994 (for the ungrazed and bison treatments) and 2008 (for the cattle treatment). To achieve a more balanced statistical design, we only included 1994-2021, as 1994 was the first year of plant data where all four bison-grazed and ungrazed catchments included in the study had data collection. The cattle data (2008-2021) covers one catchment for the annually burned treatment and three catchments for the PBG (three-year FRI) treatment.

We had a total of 12 catchments: two annually burned ungrazed catchments, two 3-4 year burn ungrazed catchments, two annually burned bison-grazed catchments, two 3-4 year burn bison-grazed catchments, one annually burned cattle-grazed catchment, and three 3-year burn cattle-grazed catchments. Each catchment (the unit of replication) has 20 plots spread over four 50 m long transects, with five equidistant 10 m^2 plots centered every 10 m across each transect. Each plot was measured in early and late growing seasons to capture both spring ephemerals and late-season plants. Each species cover was assigned to a modified Daubenmire cover class (e.g., 0-1% cover, 1-5% cover, etc.; see Collins and Calabrese 2012), and if a species is recorded in both seasons, only the larger cover class was used in the analysis. Following past studies using this dataset (Collins and Calabrese 2012), we used the midpoint of each cover class.

2.3.3 Phylogenetic diversity

To calculate PD, we first updated the KPBS plant species list (all vascular species found at KPBS) to match the nomenclature of The Catalogue of Life (https://www.catalogueoflife.org/) (Banki et al. 2022). We constructed a 'regional' phylogeny based on this updated species list using the V.PhyloMaker version 0.1.0 (Jin and Qian 2019) in R. This package utilizes the combined phylogenetic mega-trees of seed plants (Smith and Brown 2018) and pteridophytes

(Zanne et al. 2014) that encompass all extant vascular plants. PD metrics calculated using synthesis phylogenies, such as those produced by V.PhyloMaker, strongly correlate with PD values calculated using phylogenies created using gene sequence data (Allen et al. 2019; Jantzen et al. 2019; Li, Trotta, et al. 2019).

To build our phylogenetic tree, we then used the default build.nodes.1, which defined a genus as the most recent common ancestor of all the tips in the largest cluster of the genus when species in a genus were spread over multiple clusters, and scenario.3, where if a genus was not already in the mega-tree, the tip for the new genus is bound to the ½ point of the family branch of the phylo.maker function of V.Phylomaker. If a new tip was added to a genus, the tip was added to the basal node of the genus. The resulting tree was scaled to time and contained molecular branch lengths.

With a regional phylogenetic tree created, we then calculated PD metrics, including Faith's PD (PD_{Faith}), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD). PD_{Faith} measured the sum of all phylogenetic branch lengths at a site (Faith, 1992). MPD was the mean pairwise phylogenetic distance between all pairs of taxa (Webb 2000), whereas MNTD was the mean distance between taxa and their closest relative (Webb et al. 2002). Because MPD summarized all phylogenetic distances between species in a tree, this metric captured deep branching patterns in the phylogeny, whereas MNTD captured patterns in the terminal branches. To account for species richness, we calculated z-scores (standardized against a null community) for PD metrics, including PD_{SES} (Faith's PD standardized effect size), NRI (net relatedness index), and NTI (nearest taxon index), for subsequent analyses (Table 1).

To examine how rare species impact PD differences within treatments, we analyzed data from 2019-2021, which allowed us to focus on how rare species contribute to the current plant

communities while ensuring a balanced representation across treatments. Within each treatment, we ranked species by the number of plots they appeared in over the three years, with "common" species having the greatest number of observations and "rarest" species having the fewest. Following Mi et al. (2012), we calculated PD stepwise by sequentially adding species from most to least common within each treatment and recalculating PD after each addition until all species were added. For tied ranks (species had the same number of observations), we randomized the rank order for the tied species, repeated PD calculations 50 times, and calculated the mean value for each rank.

2.3.4 Analytical Methods

To assess how grazing and FRI alter plant community composition over time, we created a species-by-site matrix by averaging species cover within each catchment for each year (e.g., one "site" = 0n4d_2012). This process created a matrix with 233 species over 280 combinations of catchment and years (see Table S1 for more details). The community matrix was then square-root transformed to reduce the mean-variance correlation within the data (Warton and Hui 2017). We visualized the Bray-Curtis dissimilarity of plant communities using non-metric multidimensional scaling (NMDS; metaMDS in *vegan* package v. 2.5-7; Oksanen et al. 2024) and tested the significance of FRI, grazing treatment, and time period, year, and their interactions using a permutational multivariate ANOVA (PERMANOVA; adonis2 in *vegan*). For time period, we selected five two-year periods to represent how plant communities changed over the short- and long-term: early (1994-1995), pre-drought (2009-2010), during-drought (2011-2012), post-drought (2013-2014), and late (2020-2021; see table S2 for more details). We conducted the NMDS and PERMANOVA on the full 30-year-long dataset and a subset of 2009-2014 (two

years pre-drought, two years during, and two years post-drought) to minimize long-term year effects.

To evaluate the effects of grazing and fire on plant PD, we calculated the standardized effect size of Faith's PD (PD_{SES}), net relatedness index (NRI), and nearest taxon index (NTI) on a per-catchment, per-year basis. We used mixed-effects linear models (LMMs; lmer in the lme4 package; Bates et al. 2025) to test the significance of FRI, grazing treatment, and their interaction, with catchment as a random intercept to account for repeated measures. We conducted pairwise comparisons among the groups using the emmeans function followed by the pairs function in the *emmeans* package (Lenth et al. 2024). To assess how PD has changed over time, we applied individual generalized additive models (GAMs; gam in the *mgcv* package v 1.9-1; Wood 2011) to each fire and grazing treatment where the response variable was a PD metric, and the smoothed predictor variable was year.

The effects of drought on temporal changes in plant community composition were quantified by calculating the Euclidean distance between consecutive NMDS coordinates for each site (fire, by grazing, by catchment combination) for each year except for the first year. These distances, representing annual community "movement" or change in NMDS space, were compared among two-year pre-, during-, and post-drought periods (within fire and grazing treatment combinations) using one-way ANOVAs with post hoc Tukey's HSD tests (aov and TukeyHSD in the *stats* package; R Core Team 2024).

To test for drought effects on PD, we used LMMs (lmer function in lme4; Bates et al. 2025) with FRI, grazing treatment, time period (using the same periods as the PERMANOVA) as fixed effects and catchment as a random intercept. We calculated pairwise comparisons

among drought periods within fire and grazing treatment combinations using the emmeans and pairs functions in *emmeans*.

2.4 Results

Over 30 years, 233 plant species were recorded in twelve catchments representing six grazing by fire treatment combinations. Between 2019 and 2021, bison-grazed catchments had, on average, 110.5 species, which was 200% higher than the ungrazed treatment (56.3 species) and 150% higher than the cattle-grazed treatment (75.8 species; Table 2). This pattern held regardless of FRI treatment. The cumulative number of unique species (gamma diversity) recorded in bison-grazed catchments was 225% more than in cattle-grazed catchments and 180% more than in ungrazed areas (Table 2). The bison-grazed catchments also had the most families represented (125-180% more than both cattle and ungrazed areas, respectively) and the highest species richness for the most common families (140-200% more species of Asteraceae, Fabaceae, or Poaceae than both cattle and ungrazed catchments; Table 3). For the most dominant plant family across treatments, Poaceae, we found similar grass species richness in bison-grazed areas regardless of 1- or 4-year FRI (28 and 29 species, respectively) while annually burned ungrazed (13 species) and cattle-grazed (15 species) areas had fewer grass species compared to their 3- or 4-year FRI counterparts (16 and 24 species for ungrazed and cattle-grazed, respectively).

Of the top 25 most abundant and frequently found plant species, *Andropogon gerardii* was the most dominant – found in every catchment each year (ranging from 1-75% cover, overall mean: 38%; see Table S3 for full summary). Thirteen other species were found in every catchment each year but at lower cover, including two warm-season grass species (*Schizachyrium scoparium*: 0.5-43% cover; overall mean: 14% and *Sorghastrum nutans:* 0.5-37%; overall mean: 8%), two perennial asters *(Ambrosia psilostachya:* 0.5-57%; overall mean:

7% and *Symphyotrichum ericoides:* 0.5-32%; overall mean: 4%), and a common flowering sub-shrub from the Fabaceae family *(Amorpha canescens;* 1-37%; overall mean: 7%).

2.4.5 Grazing and fire drives community change over time

The presence of megagrazers and the difference in FRI both had highly significant effects, leading to diverging plant communities over the last 30 years. The NMDS showed distinct clusters primarily based on grazing treatments and secondarily on FRI (Figures 2.1 and S2.1). The PERMANOVA showed that grazing treatment, FRI, year, and the interaction of FRI and grazing all significantly impacted plant community composition (p-values < 0.001) (Tables S4). This pattern was similar using the subset of community data (2009-2014) except for the year effect, which was no longer significant (Table S2.5).

Phylogenetic diversity, in terms of PD_{SES} and NTI, within the catchments per year was low compared to the regional community (overall mean PD_{SES} = -1.92; mean NTI = -1.16) and was highly variable (range PD_{SES} = -3.66 to -0.04; range NTI = -3.48 to 1.04) depending on the year and treatment. Most of the catchments had similar median PD except ungrazed, annually burned communities, which had significantly lower median PD_{SES} (-2.69) and NTI (-0.85) than all other fire and grazing treatments ($p<0.001$) (Tables S2.6-S2.9).

Despite the species composition (Figure 2.1) and richness (Figure 2.2) changing over time, the phylogenetic relatedness of the species present was largely unchanged for most treatments. The only treatment that showed large shifts was the annually burned ungrazed treatment, which had significantly lower NTI compared to other treatments (LMM pairwise comparisons: $p<0.05$) and showed a sharp decline beginning between 2007 and 2012 (Figure 2.3; GAM: r^2 =0.61, $p < 0.001$). Over 27 years (1994 – 2021), these annually burned, ungrazed catchments lost 26 species in 14 families, including four unique families no longer found in those catchments (Table S2.10).

Only ungrazed catchments burned every four years had slight but statistically significant declines in NTI (GAM: $R^2 = 0.12$, p= 0.02); no other treatment showed any significant trends (Figure 2.3; Table S11). PD_{SES} showed similar trends as NTI, with annually burned, ungrazed catchments declining significantly (GAM: $R^2 = 0.43$, $p < 0.001$). Additionally, PD_{SES} in annually burned, bison-grazed areas saw slight changes, although the trend is weak ($R^2 = 0.11$, $p = 0.04$), and the 95% confidence intervals for the first year of data and the last year of data overlap (Figure S2.2). Meanwhile, NRI showed no significant changes over time for any treatment (Figure S2.3).

Several treatment-level patterns stand out when adding species sequentially from most common to most rare. Plant communities in all treatments appear phylogenetically similar for the first ~30 species. Out of the top ten most frequently observed species for each treatment combination (based on the number of observations in 2019-2021; see section 2.3, last paragraph), three families are always present (Poaceae, Asteraceae, Cyperaceae), with Fabaceae making the top ten in a few treatments (Table S2.12). However, rare species do impact PD in some treatments. For example, annually burned, grazed treatments (both cattle and bison) experience large increases in NTI due to their rarest species, as a few of these species come from more distantly related families (Figure 2.4). For the cattle treatments, there are large increases in NTI between the 60-77th species added, which correspond to *Cornus drummondii* (a woody shrub), *Ceanothus herbaceus* (a woody sub-shrub), and *Juncus interior* (a rush), which all had branch lengths that are 2.8-7.5 times greater than the mean branch length. Both of the ungrazed treatments also get a boost in NTI by their rarest species, corresponding to *Rosa arkansana* in the annually burned treatment (a woody sub-shrub; 8.4 times greater than the mean branch length) and, in the 3-4 year FRI treatment, *Delphinium carolinianum* (a perennial forb) and *Gleditsia triacanthos* (a tree; 3.3-4.6 times greater than the mean). Interestingly, NTI in the annually

burned bison-grazed treatment (Figure 2.4) is rescued by several long-branched rare species (*Ophioglossum vulgatum*, *Spiranthes vernalis*, *Evolvulus nutallianus*, *Sanicula canadensis*, and *G. triacanthos*; 3.12 – 10 times greater than the mean), while the 3-4 year FRI treatment was not despite having several long-branched rare species (*J. interior*, *Senna marilandica*, *S. vernalis*, and *Dianthus armeria*; 3-8.3 times greater than the mean). NRI and PD_{SES} behaved similarly, with slightly more muted responses in NRI to the impacts of rare species (Table S2.13).

2.4.6 Community drought resistance

Plant communities showed small but significant shifts from pre-drought composition to during-drought and post-drought composition. The PERMANOVA showed that the time period (early, pre, during, post, late) had a significant effect on community composition ($p<0.01$; Table S2.4-S2.5); however, the interaction between FRI, grazing, and period was not significant, indicating that within FRI and grazing treatment combinations, plant communities did not shift significantly due to the drought. This resistance to drought was confirmed when we calculated the average distance between sequential points to assess if drought affected the magnitude of community shifts, and there was no significant difference before, during, or after the drought (Figure 2.5; Kruskal-Wallis $p>0.05$). Plant PD was also resistant to change during the drought with no effect of drought pre-, during-, or post-drought within treatments (LMM pairwise comparison $p > 0.05$; Table S2.13).

2.5 Discussion

While many studies have looked at the combined impacts of fire and grazing, this study had the advantage of reflecting long-term land-use differences and how these land-use systems are often implemented. Despite similarities between bison and cattle diet and foraging behavior, we found that plant species composition varied depending on the grazing treatment and sometimes

due to FRI. In contrast, measures of PD were similar between most grazed treatments, reflecting how common the Poaceae, Asteraceae, and Fabaceae families are in mesic grasslands. This inference on PD appears robust because we performed the typical analyses for comparing PD and contrasted them with a novel approach that assessed whether PD was robust to realistic species losses (rarest to most common) from each treatment.

2.5.7 Fire return interval and grazing drive community composition change over time

Humans alter fire regimes in many grassland ecosystems, and these management decisions can have critical impacts on the conservation of those grasslands (Fuhlendorf et al. 2009; Courtwright 2023). In ungrazed areas, changes in FRI shaped plant communities (Figure 2.1) with annual fire increasing the dominance of tall grasses at the expense of most other species (Collins and Calabrese 2012; Ratajczak et al. 2022), leading to decreased SR and PD. In contrast, in the four-year ungrazed FRI treatment, dominant grass cover is about 50% lower (Ratajczak et al. 2022), which increased forbs and other typically sub-dominant species and thus SR (Figure 2.2). Our results are another example that in high-productivity grasslands, such as tallgrass prairie, factors that reduce dominant grass cover lead to increases in other functional groups (Koerner et al. 2018) and, sometimes, native plant diversity. However, there are trade-offs – increasing FRI in these mesic prairies means that they can quickly turn into shrublands and closed canopy systems, which are difficult to reverse and have low plant diversity (Ratajczak et al. 2014; Archer et al. 2017). Land managers with ungrazed prairies should balance FRI to increase biodiversity and minimize the chances of woody invasion. However, the impacts of fire on plant communities are moderated by the presence of large grazers, which may change management decisions related to burning.

In the presence of grazers, gamma diversity, annual SR, and family richness all increased. Bison treatments had the highest plant SR, and cattle had moderately higher SR than ungrazed (200% and 135% higher for bison and cattle, respectively), corresponding to previous work (Ratajczak et al. 2022). Moderate grazing generally increases plant SR in tallgrass prairie (Plumb and Dodd 1993; Knapp et al. 1999; Koerner and Collins 2014), provided that grazers reduce the abundance of otherwise dominant species (Koerner et al. 2018), as in typical keystone consumption (Paine 1966). Grazing could also increase the heterogeneity of soil resources (Adler et al. 2001), allowing for greater resource niche partitioning (MacArthur 1960). However, the effects of FRI diminished within the grazing treatments, suggesting that grazing overrides FRI as the primary driver of plant diversity (Fuhlendorf et al. 2009).

This lack of impact of FRI within grazing treatments was unexpected, as more frequent fires in the grazed treatments lead to more frequent grazing as bison and cattle "chase" fire to take advantage of high-nutrition forage (Raynor et al. 2015; Pringle et al. 2023), resulting in compound disturbances that we expected to filter for a very disturbance adapted community in annually burned areas while 3-4 year FRI areas provided refugia for more disturbance-sensitive species. However, the minimal differences between different FRIs in grazed areas suggested that having some form of competitive release from dominant grasses is the important aspect of having grazers present, which superseded the effects of fire (Koerner et al. 2018; Pringle et al. 2023). While both bison and cattle increased plant diversity, their effects were not equal and were likely mediated by differences in management practices, which influence spatiotemporal land use by grazers and their interactions with fire.

Bison and cattle are managed quite differently, in general, and at KPBS. Like most bison herds (USDA 2014), the KPBS bison herd is on site year-round and allowed to move throughout

a large enclosure, which at KPBS includes ten catchments spanning four FRI treatments (1, 2, 4, and long-term unburned). Thus, they can preferentially graze any of the recently burned catchments (Raynor et al. 2017), avoiding long-term nutrient loss found in repeated annually burned grasslands (Soong and Cotrufo 2015) and increased forb presence that deters bison grazing (Collins and Calabrese 2012). In contrast, the cattle are managed in two systems: annual burning of PBG (Hoots 2011; Courtwright 2023). Cattle in the annually burned treatment only have access to annually burned prairie and might graze it more uniformly than if they had access to less frequently burned areas (Ling et al. 2019; Ling et al. 2023). In contrast, the PBG treatment allows cattle to move freely and access recently burned areas that were not burned the previous season, increasing spatial and temporal heterogeneity. While both cattle treatments increased diversity compared to the ungrazed treatment, the PBG treatment had higher gamma diversity than the annually burned treatment (Plumb and Dodd 1993; Towne et al. 2005; Allred, Fuhlendorf, Engle, et al. 2011). Our data adds another study that reinforces the potential conservation benefits of PBG compared to traditional methods.

2.5.8 Grazing and FRI influence phylogenetic diversity

Low PD is typical of tallgrass prairie, where a few families tend to be dominant (i.e., Poaceae, Asteraceae, Fabaceae) (Turner and Knapp 1996; Aust et al. 2015; Li, Miller, et al. 2019; Herzog and Latvis 2022). The dominance of these plant families is likely due to historical species filtering favoring traits that are adapted to frequent disturbance (e.g., fires and grazing), limited precipitation, and high light availability (Grime 1973; Dinnage 2009; Brunbjerg et al. 2012). We found FRI has a larger effect on PD in ungrazed treatments than in grazed treatments, with generally lower NTI in annually burned treatments than in 3-4 year FRI treatments (Figure 2.3).

While bison grazing increased species richness over nearly 30 years (~160% increase; Figure 2.2), we saw no significant change in most PD metrics (Figures 3 and S1-S2) and little change as we added more rare species (Figure 2.4). This pattern suggested that 'rarer' species are closely related to those already present (phylogenetic clustering). For instance, we observe many rare species from the same closely related families as the dominant species, such as grasses (Poaceae) and asters (Asteraceae), rather than species from more distantly related families. Although our analysis suggests phylogenetic saturation, where adding new species led to minimal increases in PD, future studies should explore whether rare species contribute directly to increases in functional diversity. While PD has been used as a proxy for functional diversity (Faith 1992; Cadotte et al. 2012), and conserving high PD can have conservation benefits (Forest et al. 2007; Winter et al. 2013; Tucker et al. 2019), more empirical work needs to be done to explore these relationships and, in general, multiple types of diversity should be considered in conservation decisions (Mazel et al. 2018; Tucker et al. 2019).

2.5.9 Community composition and PD are resilient to drought via resistance

Grasslands worldwide are experiencing biodiversity declines due to global change pressures, including nutrient pollution (Carroll et al. 2022), changes in average climate (Parmesan 2006), invasive species (Mack et al. 2000), and habitat fragmentation (Tilman et al. 1994). These losses are often non-random, with rare species often disappearing first (Suding et al. 2005). The phylogenetic clustering we measured in grazed areas, especially in bison-grazed areas, should build robust family-level functional redundancy. Thus, these communities could lose more species before they experience a decline in measures of PD. This redundancy could buffer against both slower-moving global change pressures and extreme disturbances, including droughts (Oliver et al. 2015; Biggs et al. 2020; Gao et al. 2022).

Extreme droughts have affected most grasslands historically (Axelrod 1985) and will likely become more common in the 21st century (Cook et al. 2015), hence a growing emphasis on climate extremes (Jentsch and Beierkuhnlein 2008; Turner et al. 2020). However, we found that the plant communities were resistant to the extreme drought in 2011 and 2012. Plant communities in neither the species composition nor PD responded, which was surprising considering the temporary decline in species richness during this drought (Ratajczak et al. 2022). This resistance to change was likely due to dominant species and plant families largely remaining throughout the drought (Smith and Knapp 2003). Based on our rare species analysis, the loss of rare species was not likely to decrease PD unless they were in very distantly related families.

Surprisingly, despite their lower gamma diversity and narrower botanical family representation, neither the annually burned ungrazed nor cattle-grazed treatments exhibited significant shifts in community composition or PD during the drought. Generally, increased SR, especially within families, prolongs the ability to lose rare species without losing large amounts of PD or functional diversity (Cadotte et al. 2012; Oliver et al. 2015). In this study, the bison-grazed catchments should be more resistant to loss of function due to the higher SR within families. For example, if we lose 10 Asteraceae species in the bison units, we lose 33% of the species represented; however, if we lose 10 species of asters in the cattle or ungrazed units, we lose 40-77% from this family. However, we saw no difference in the response to drought between grazing treatments. This resistance to change in PD suggests that, so far, the cattle treatment contains those core drought-resistant species that are also in bison-grazed treatments. Thus, while cattle grazing is beneficial for increasing SR and redundancy compared to ungrazed treatments, bison-grazed communities exhibit the highest levels of redundancy and SR, which

may benefit them in future compound disturbances (Oliver et al. 2015; Biggs et al. 2020; Loreau et al. 2021). However, while non-random species loss may not contribute to decreases in ANPP (Smith and Knapp 2003), more empirical work needs to be done to determine if other functions (e.g., pollinator resources, plant-herbivore interactions, nutrient cycling) are lost (Dehling et al. 2022; Wu et al. 2023).

2.6 Conclusion

The presence, type, and management of megagrazers and FRI significantly impacted plant communities, and these communities changed significantly over the last 25+ years. The bison-grazed communities had diverged considerably from the ungrazed treatments of the same fire frequencies and showed significant differences from cattle-grazed prairie at KPBS. Recognizing that these differences between bison-grazed and cattle-grazed communities are deeply intertwined with the management of these megagrazers, our findings indicate that cattle, as managed at KPBS, are not comparable to bison. However, compared to ungrazed areas, cattle can provide beneficial increases in plant species richness and bolster species redundancy, leading to more resilient grasslands. These results could apply more widely because the annually burned cattle grazed treatment is a common land use type in the region, and the patch-burn-grazing treatment is a conservation-oriented approach to grassland management (Fuhlendorf et al. 2009), which not only supports higher plant species diversity, but also supports endemic fauna, such as grassland bird species (Winder et al. 2017; Winder et al. 2018).

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2.8 Tables

Table 2.1. Table of important phylogenetic diversity metrics and definitions used in this paper.

Term	**Definition**	**Interpretation**	**Citation**
Faith's PD (PD_{Faith})	Sum of all branches of a tree representing a community	High PD among communities with the same species richness indicates older divergences of species	Faith et al 1998
Standardized effect size of Faith's PD (PD_{SES})	Standardized effect size of total branch length vs a randomized null community	Negative values indicate more clustering than expected while positive values indicate overdispersion	Webb et al 2008
Mean pairwise distance (MPD)	Average length between any (and all) pairs of species in a tree	Low MPD indicates more recent speciation while higher MPD indicates older speciation events (deep tree relationships)	Webb et al 2000
Net relatedness index (NRI)	MPD of the community compared to null tree	Similar to MPD	Webb et al 2002
Mean nearest taxon distance (MNTD)	Average length between a species and it's closest relative	Low MNTD indicates that most species are highly related (more recent speciation)	Webb et al 2002
Nearest taxon index (NTI)	MNTD of the community compared to null tree	Similar to MNTD	Webb et al 2002

Table 2.2. Summary table of the number of plant families, gamma diversity, and species richness in the grazing and fire treatments from 2019-2021. Grazing denotes the grazing treatment and FRI denotes the fire return interval.

Grazing	*FRI*	*# of families*[1]	*Avg # of families*[2]	*Gamma diversity*[3]	*Avg gamma diversity*[4]	*Avg SR/yr*[5]	*Avg SR/yr/ grazing*[6]
Bison	1	37	36.5	155	153	111	110.5
Bison	4	36		151		110	
Cattle	1	28	29	77	95	71.7	75.8
Cattle	4	30		113		79.8	
Ungrazed	1	14	20.5	57	76	47.2	56.3
Ungrazed	4	27		155		65.3	

[1] Number of families per fire and grazing treatment combinations; [2] Average number of families per grazing treatment regardless of fire; [3] Total number of species recorded per fire and grazing treatment combinations in 2019-2021 (gamma diversity); [4] Average total number of species recorded per grazing treatment regardless of fire in 2019-2021 (gamma diversity); [5] Average number of species recorded per fire and grazing treatment combinations per year (species richness; SR); [6] Average number of species recorded per grazing treatment regardless of fire (species richness; SR).

Table 2.3. Summary table of species richness in each of the top three most common families recorded from 2019-2021. Grazing denotes the grazing treatment and FRI denotes the fire return interval.

Grazing	*FRI*	*Asteraceae*[1]	*Avg*[2]	*Fabaceae*[3]	*Avg*[4]	*Poaceae*[5]	*Avg*[6]
Bison	1	31	30.5	17	16.5	28	28.9
Bison	4	30		16		29	
Cattle	1	20	22	9	12	15	19.5
Cattle	4	24		15		24	
Ungrazed	1	13	17.5	11	11	13	14.5
Ungrazed	4	22		11		16	

[1] Number Asteraceae species recorded in each treatment; [2] Average number of Asteraceae species per grazing treatment regardless of fire; [3] Number Fabaceae species recorded in each treatment; [4] Average number of Fabaceae species per grazing treatment regardless of fire; [5] Number Poaceae species recorded in each treatment; [6] Average number of Poaceae species per grazing treatment regardless of fire.

2.9 Figures

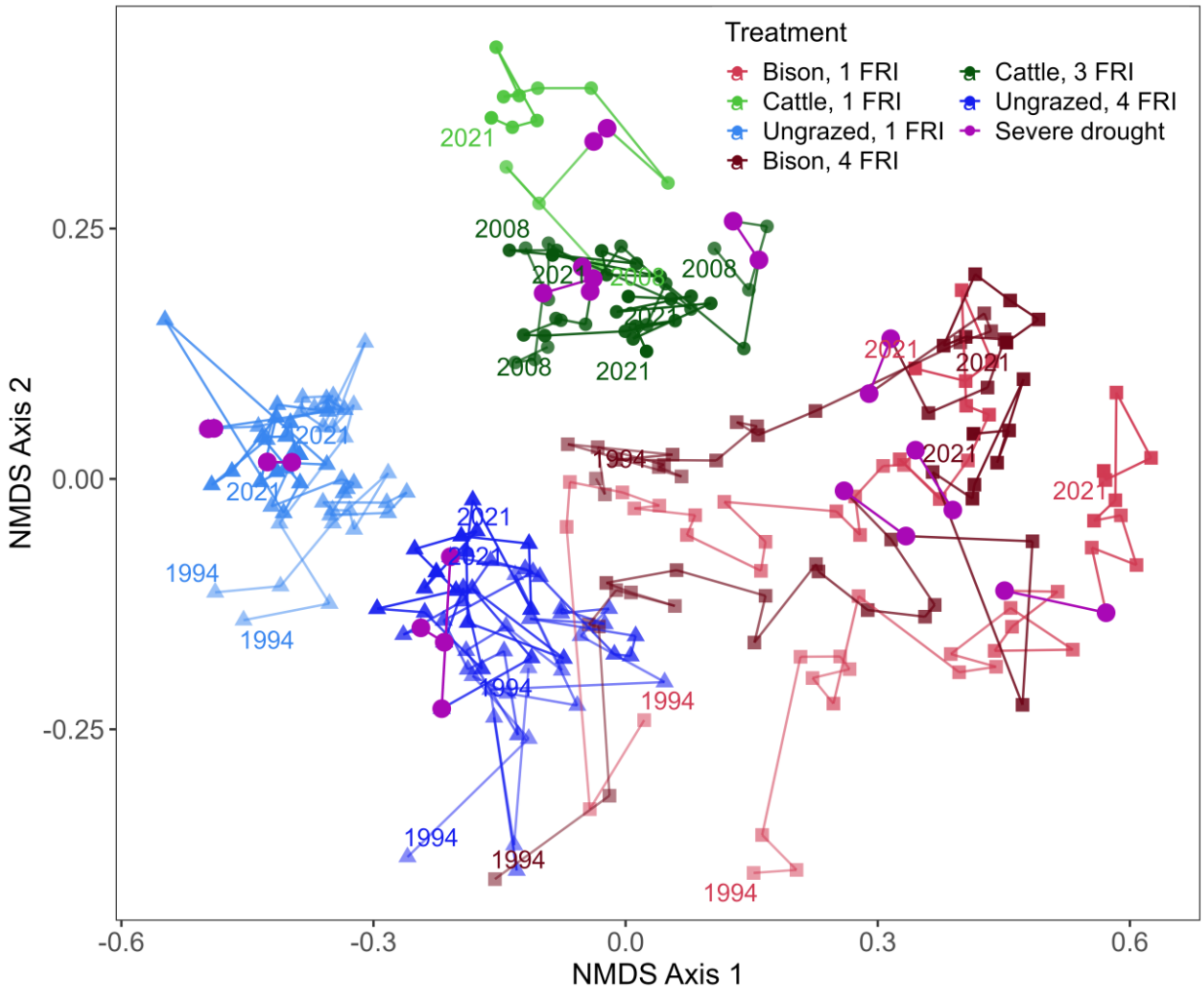

Figure 2.1: NMDS showing community composition dissimilarity (Bray-Curtis) from 1991-2021. Large pink dots denote 2011 and 2012, which were severe drought years. Red/orange squares represent bison-grazed treatments, green circles represent cattle-grazed treatments (only 2008-2021), and blue triangles represent ungrazed-treatments. The years label the beginning and end of each catchment as they move through time.

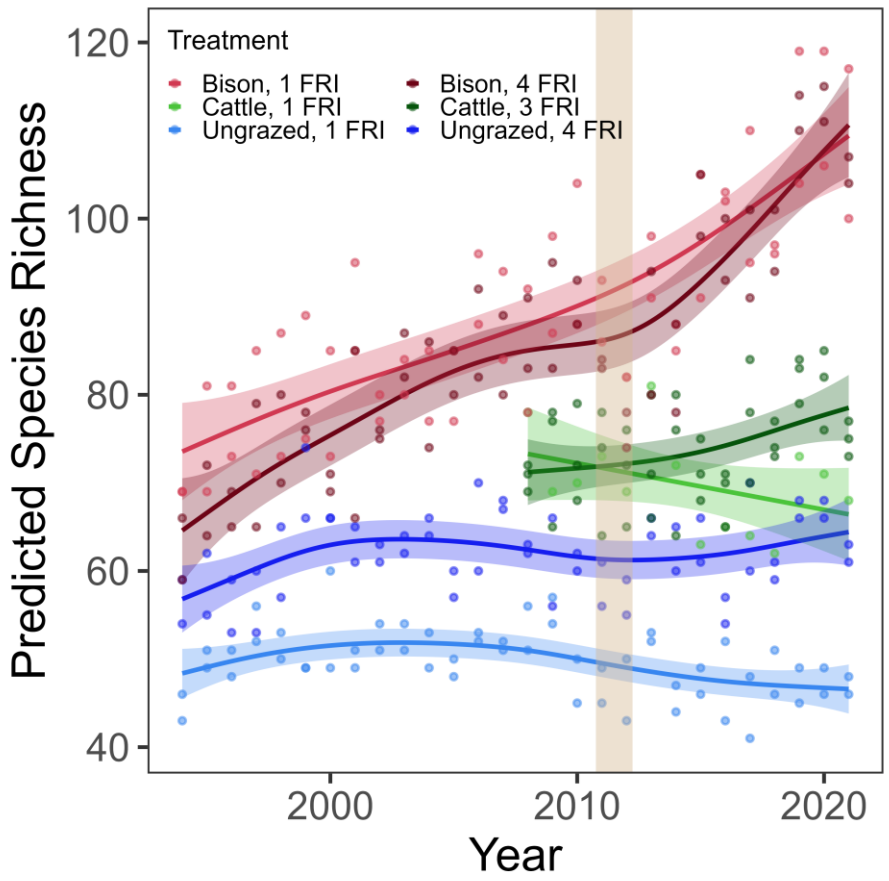

Figure 2.2: General additive models (GAMs) of catchment-scale species richness (SR) as a function of time. The vertical tan bar represent 2011 and 2012, which were major drought years and the shaded areas for each line are the 95% confidence interval. Annual fire is the 1 year FRI; frequent fire is the 3-4 year FRI.

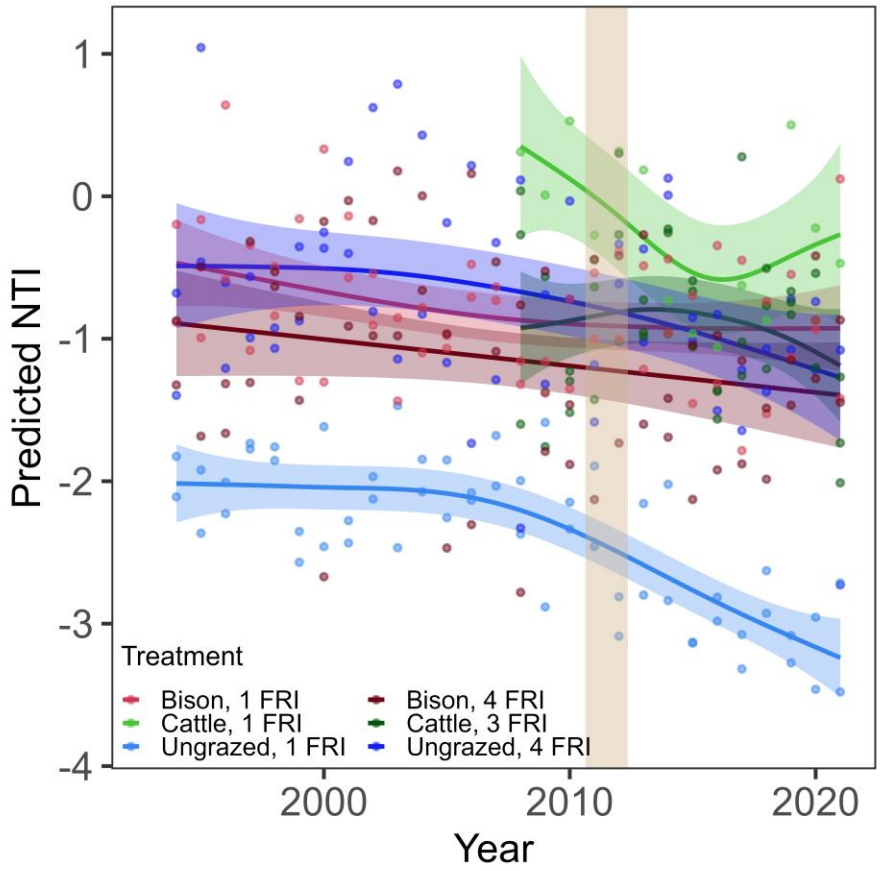

Figure 2.3: General additive models (GAMs) of predicted phylogenetic diversity (NTI, nearest taxon index) as a function of time on a catchment-level. The vertical tan line indicates the major drought years (2011-2012). Points are the observed annual NTI and shaded areas span the 95th confidence interval of GAMs.

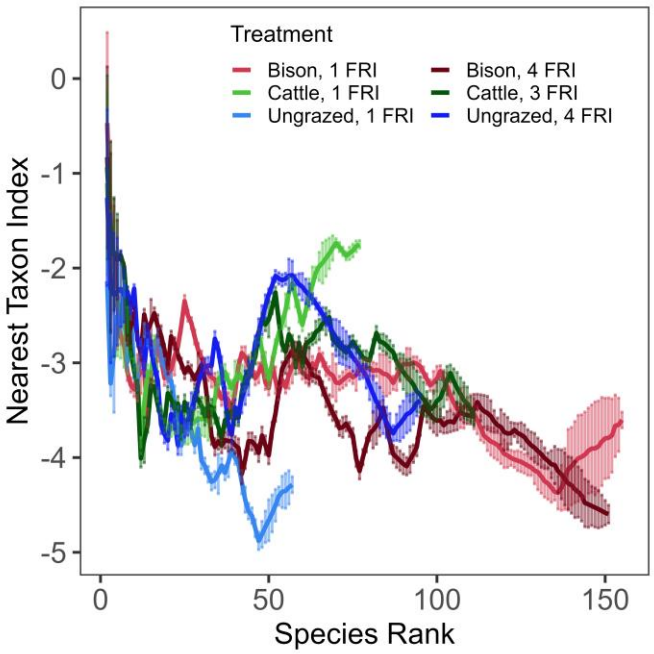

Figure 2.4: The mean nearest taxon index (NTI) per species rank as species are added sequentially from most frequent to least frequent. Error bars are the mean ± the standard deviation at each species rank. Higher NTI values indicate less phylogenetic clustering while lower NTI indicates more phylogenetic clustering.

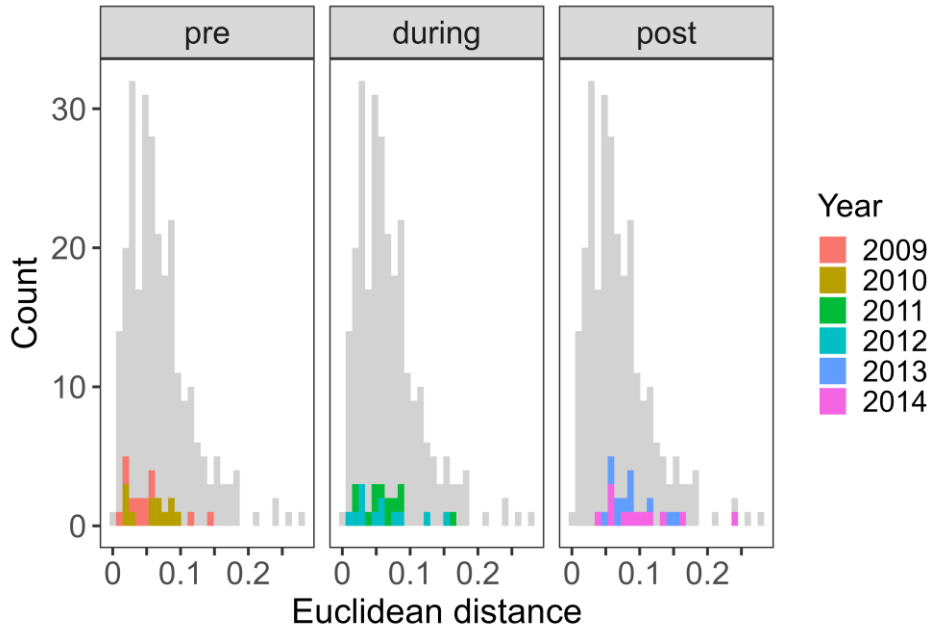

Figure 2.5: Histograms showing the distribution of Euclidean distance between consecutive plant communities in NMDS space. Highlighted areas denote the distance between plant communities either pre-drought (2009-2010), during the drought (2011-2012), or post-drought (2013-2014). There was no significant change in the NMDS distance moved between pre-, during-, or post-drought (Kruskal-Wallis $p>0.05$).

Appendix A – Chapter 2 supplemental materials

Supplementary Tables

Table S2.1. Details for the catchments used for each fire and grazing treatment included in the study.

Fire treatment	Average Fire Return Interval[1]	Grazing	Catchment name	Years of data included	Sample size (catchment/yr)
Annual	1	ungrazed	1d	1994-2021	28
Annual	1	ungrazed	SpB	1994-2021	28
Four-year	3.4	ungrazed	4B	1994-2021	28
Four-year	3.9	ungrazed	4A	1994-2021	28
Annual	1	bison	N1a	1994-2021	28
Annual	1	bison	N1d	1994-2021	28
Four-year	3.9	bison	N4c	1994-2021	28
Four-year	3.9	bison	N4d	1994-2021	28
Annual	1	cattle	C1a	2008-2021	14
PBG (3-year)	3	cattle	C3a	2008-2021	14
PBG (3-year)	3	cattle	C3b	2008-2021	14
PBG (3-year)	3	cattle	C3c	2008-2021	14

[1]Average FRI could be different from the treatment due to accidental wildfires

Table S2.2. Independent variables for the PERMANOVA and linear mixed effects models (LMMs).

Predictor	Levels
FRI	1, 4*
Grazing	ungrazed, bison, cattle
Drought period	early, pre, during, post, late**

*PBG (3-year FRI) was included in the 4-year FRI level; **early = 1994, 1995; pre = 2009, 2010; during = 2011, 2012; post = 2013, 2014; late = 2020, 2021

Table S2.3. Top 25 dominant plant species in terms of both cover and frequency for all treatments combined. Summary statistics on average percent cover (of twenty 5 m^2 plots along four transects) per catchment, per year (1994-2021).

Species	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	Family
Andropogon gerardii	0.75	25.68	38.88	37.75	51.12	74.62	Poaceae
Schizachyrium scoparium	0.5	2.35	10.37	13.86	20.2	43.42	Poaceae
Rhus glabra	0	0	0.88	7.66	8	85	Anacardiaceae
Sorghastrum nutans	0.5	2.325	5.88	7.59	10.78	36.63	Poaceae
Amorpha canescens	0.63	1.79	4.68	7.15	10.15	37.21	Fabaceae
Ambrosia psilostachya	0.5	0.75	3	7.14	8.725	57	Asteraceae
Symphyotrichum oblongifolium	0	0.58	1	5.24	6.81	34	Asteraceae
Bouteloua dactyloides	0	0	0.5	4.75	7	37.5	Poaceae
Symphyotrichum ericoides	0.5	0.68	2.23	3.88	5.78	31.93	Asteraceae
Salvia azurea	0.5	0.77	1.85	3.83	5.38	37.03	Lamiaceae
Ceanothus herbaceus	0	0.88	1	3.73	6.5	17.33	Rhamnaceae
Poa pratensis	0	0.5	0.73	3.72	4.25	40.13	Poaceae
Solidago missouriensis	0	0.7	2	3.06	4.39	15.61	Asteraceae
Bromus arvensis	0	0	0	3.02	0.57	67.39	Poaceae
Artemisia ludoviciana	0	0.61	1.56	2.78	3.76	21.48	Asteraceae
Sporobolus heterolepis	0	0.5	0.8	2.67	3.33	22.75	Poaceae
Rosa arkansana	0	0.5	0.64	2.66	1	37.5	Rosaceae
Panicum virgatum	0.5	0.57	0.7	2.64	2.75	54.08	Poaceae
Dichanthelium oligosanthes	0.5	0.65	0.98	2.54	3.08	20.48	Poaceae
Bouteloua curtipendula	0.5	0.65	1.38	2.5	3.48	24.55	Poaceae
Carex inops	0	0.53	0.75	2.38	3.65	16.95	Cyperaceae
Bouteloua gracilis	0	0.5	0.7	2.32	2.3	26.25	Poaceae
Koeleria macrantha	0	0.5	0.63	2.15	2.21	28.68	Poaceae

Sporobolus compositus	0.5	0.53	0.63	1.99	2.04	18.40	Poaceae
Mimosa quadrivalvis	0	0.6	0.8	1.89	2.69	15	Fabaceae

Table S2.4. PERMANOVA (adonis2) results table of the Bray-Curtis dissimilarity of the community composition matrix ~ burn*graze*period+year. Data are from 1994-2021.

	DF	SUM OF SQS	R2	F	PR(>F)
FRI	1	1.5844	0.06524	42.5349	0.001***
GRAZING	2	7.9807	0.32864	107.1244	0.001***
PERIOD	5	1.3721	0.0565	7.3671	0.001***
YEAR	1	0.7911	0.03258	21.2383	0.001***
FRI:GRAZING	2	2.3096	0.09511	31.0021	0.001***
FRI:PERIOD	5	0.1737	0.00715	0.9324	0.573
GRAZING:PERIOD	9	0.7103	0.02925	2.1188	0.001***
FRI:GRAZING:PERIOD	9	0.2361	0.00972	0.7042	0.988
RESIDUAL	245	9.1262	0.37581		
TOTAL	279	24.2843	1		

Table S2.5. PERMANOVA (adonis2) results table of the Bray-Curtis dissimilarity of the community composition matrix ~ burn*graze*period+year. Data are subsetted to 2009-2014 to limit long-term year effects.

	DF	SUM OF SQS	R2	F	PR(>F)
FRI	1	0.3488	0.05661	11.3889	0.001***
GRAZING	2	2.9489	0.47855	48.1391	0.001***
PERIOD	2	0.2134	0.03464	3.4841	0.001***
YEAR	1	0.0549	0.00891	1.7919	0.095.
FRI:GRAZING	2	0.7511	0.12189	12.2612	0.001***
FRI:PERIOD	2	0.0376	0.0061	0.6137	0.852
GRAZING:PERIOD	4	0.1226	0.0199	1.001	0.453
FRI:GRAZING:PERIOD	4	0.0615	0.00997	0.5017	0.994
RESIDUAL	53	1.6233	0.26344		
TOTAL	71	6.1622	1		

Table S2.6 Median standardized phylogenetic metrics (standardized effect size of Faith's PD [PD_{SES}], nearest taxon index [NTI], and net relatedness index [NRI]) as well as the 95% confidence intervals. Ungrazed, annually-burned catchments (1_ungrazed) had significantly lower NTI and PD_{SES} than all other treatments (LMM: $p<0.05$). Data from 1994-2021.

Treatment	n (Catchments*Years)	Metric	Median	Lower CI	Upper CI
1_bison	56	NTI	-0.846	-0.989	-0.667
1_bison	56	PD_{SES}	-1.7	-1.83	-1.66
1_bison	56	NRI	-1.66	-1.83	-1.58
1_cattle	14	NTI	-0.248	-0.75	0.248
1_cattle	14	PD_{SES}	-1.59	-1.82	-1.47
1_cattle	14	NRI	-1.5	-1.55	-1.43
1_ungrazed	56	NTI	-2.31	-2.5	-2.13
1_ungrazed	56	PD_{SES}	-2.69	-2.8	-2.59
1_ungrazed	56	NRI	-1.48	-1.52	-1.45
4_cattle	42	NTI	-0.959	-1.21	-0.715
4_cattle	42	PD_{SES}	-1.8	-2.01	-1.66
4_cattle	42	NRI	-1.64	-1.78	-1.58
4_bison	55	NTI	-1.09	-1.43	-0.912
4_bison	55	PD_{SES}	-1.92	-2.18	-1.66
4_bison	55	NRI	-1.85	-2.19	-1.62
4_ungrazed	56	NTI	-0.818	-1.04	-0.615
4_ungrazed	56	PD_{SES}	-1.49	-1.72	-1.36
4_ungrazed	56	NRI	-1.44	-1.5	-1.37

Table S2.7. Pairwise comparison of linear mixed model of nearest taxon index (NTI) and fire frequency*grazing treatment on the catchment scale, with catchment as a random intercept to account for repeated measures. The number indicates the fire return interval (1 is annually burned; 3-4 is frequently burned) and text is grazing treatment (bison, cattle, or ungrazed).

	1_bison	1_cattle	1_ungrazed	3_cattle	4_bison
1_bison					
1_cattle	n.s				
1_ungrazed	***	***			
3_cattle	n.s	n.s	***		
4_bison	n.s	n.s	***	n.s	
4_ungrazed	n.s	n.s	***	n.s	n.s

Note: pvalues are less than *** 0.001, ** 0.01, *0.05

Table S2.8. Pairwise comparison of linear mixed model of standardized effect size of Faith's PD (PD_{SES}) and fire frequency*grazing treatment on the catchment scale, with catchment as a random intercept to account for repeated measures. The number indicates the fire return interval (1 is annually burned; 3-4 is frequently burned) and text is grazing treatment (bison, cattle, or ungrazed).

	1_bison	1_cattle	1_ungrazed	3_cattle	4_bison
1_bison					
1_cattle	n.s.				
1_ungrazed	n.s.	n.s.			
3_cattle	n.s.	n.s.	n.s.		
4_bison	n.s.	n.s.	n.s.	n.s.	
4_ungrazed	n.s.	n.s.	n.s.	n.s.	n.s.

Note: pvalues are less than *** 0.001, ** 0.01, *0.05

Table S2.9. Pairwise comparison of linear mixed model of net relatedness index (NRI) and fire frequency*grazing treatment on the catchment scale, with catchment as a random intercept to account for repeated measures. The number indicates the fire return interval (1 is annually burned; 3-4 is frequently burned) and text is grazing treatment (bison, cattle, or ungrazed).

	1_bison	1_cattle	1_ungrazed	3_cattle	4_bison
1_bison					
1_cattle	n.s.				
1_ungrazed	*	#			
3_cattle	n.s.	n.s	*		
4_bison	n.s.	n.s.	*	n.s.	
4_ungrazed	n.s.	n.s.	*	n.s.	n.s.

Note: pvalues are less than *** 0.001, ** 0.01, * 0.05, # 0.1

Table S2.10. Summary of families lost and gained between 1994 and 2021. “Families Lost” are those families that were found in the catchments in the early years (1994 or 1995) but not found in the plots in the later years (2020 or 2021). “Families Gained” are those families that were found in the later years, but not the earlier years. Cattle plots were excluded as data were not available until 2008. All families gained or lost only had one representative except for Anacardiaceae and Polygonaceae in the infrequently burned, bison-grazed catchment(denoted by the *), which had two species representing them.

Treatment	Families Lost	Families Gained
1 year FRI, bison-grazed	Polygonaceae	Cannabaceae
	Scrophulariaceae	Nyctaginaceae
		Ophioglossaceae
		Orchidaceae
		Primulaceae
		Rubiaceae
3-4 year FRI, bison-grazed	Rubiaceae	Anacardiaceae*
		Malvaceae
		Polygonaceae*
		Ranunculaceae
		Cannabaceae
		Orchidaceae
		Juncaceae
		Liliaceae
1 year FRI, ungrazed	Linaceae	None
	Malvaceae	
	Oxalidaceae	
	Violaceae	
3-4 year FRI, ungrazed	Boraginaceae	Cannabaceae
	Brassicaceae	
	Caryophyllaceae	
	Rubiaceae	

Table S2.11 Results from individual general additive models (GAMs) on diversity metrics as a function of year (1994-2021 for bison and ungrazed; 2008-2021 for cattle).

Treatment	Diversity metric[1]	Overall Fit (R^2) [2]	Overall model significance[3]	Smooth term Signficance[4]
1 year FRI, ungrazed	NTI	0.61	p<0.001	ns
	NRI	0.05	p<0.001	0.01
	PD SES	0.43	p<0.001	0.00
	SR	0.25	p<0.001	ns
3-4 year FRI, ungrazed	NTI	0.12	p<0.001	0.02
	NRI	0.01	p<0.001	ns
	PD SES	0.00	p<0.001	ns
	SR	0.16	p<0.001	0.04
1 year FRI, bison-grazed	NTI	0.09	p<0.001	ns
	NRI	0.07	p<0.001	ns
	PD SES	0.11	p<0.001	0.04
	SR	0.67	p<0.001	p<0.001
3-4 year FRI, bison-grazed	NTI	0.03	p<0.001	ns
	NRI	0.00	p<0.001	ns
	PD SES	-0.01	p<0.001	ns
	SR	0.78	p<0.001	p<0.001
1 year FRI, cattle-grazed	NTI	0.32	ns	ns
	NRI	-0.08	p<0.001	ns
	PD SES	0.05	p<0.001	ns
	SR	0.09	p<0.001	ns
3-4 year FRI, cattle-grazed	NTI	0.04	p<0.001	ns
	NRI	-0.02	p<0.001	ns
	PD SES	0.01	p<0.001	ns
	SR	0.17	p<0.001	0.02

[1] Nearest taxon index (NTI), net relatedness index (NRI), standardized Faith's PD (PD SES), species richness (SR); [2] overall model fit; [3] overall model p-value; [4] p-value of the smooth term where $p<0.05$ signifies directional change (i.e., the confidence intervals of the first year and last year of data overlap)

Table S2.12. Table of the top 10 most frequently encountered species, and their families, in each treatment. All treatments are dominated by grasses (Poaceae) and asters (Asteraceae).

Treatment	Species name	Family	# of observations
1 year FRI, bison-grazed	*Ambrosia_psilostachya*	Asteraceae	120
	Andropogon_gerardii	Poaceae	120
	Bouteloua_curtipendula	Poaceae	120
	Carex_inops	Cyperaceae	120
	Sorghastrum_nutans	Poaceae	120
	Symphyotrichum_ericoides	Asteraceae	120
	Dichanthelium_oligosanthes	Poaceae	119
	Solidago_rigida	Asteraceae	116
	Sporobolus_compositus	Poaceae	112
	Artemisia_ludoviciana	Asteraceae	111
3-4 year FRI, bison-grazed	*Ambrosia_psilostachya*	Asteraceae	120
	Andropogon_gerardii	Poaceae	120
	Carex_inops	Cyperaceae	120
	Dichanthelium_oligosanthes	Poaceae	120
	Koeleria_macrantha	Poaceae	120
	Sorghastrum_nutans	Poaceae	120
	Bouteloua_curtipendula	Poaceae	119
	Artemisia_ludoviciana	Asteraceae	118
	Salvia_azurea	Lamiaceae	117
	Solidago_rigida	Asteraceae	117
1 year FRI, cattle-grazed	*Andropogon_gerardii*	Poaceae	60
	Carex_inops	Cyperaceae	60
	Carex_meadii	Cyperaceae	60
	Dichanthelium_oligosanthes	Poaceae	60
	Koeleria_macrantha	Poaceae	60

	Schizachyrium_scoparium	Poaceae	60
	Sorghastrum_nutans	Poaceae	60
	Sporobolus_compositus	Poaceae	60
	Symphyotrichum_ericoides	Asteraceae	60
	Eragrostis_spectabilis	Poaceae	58
3-4 year FRI, cattle-grazed	*Andropogon_gerardii*	Poaceae	180
	Carex_inops	Cyperaceae	180
	Dichanthelium_oligosanthes	Poaceae	180
	Koeleria_macrantha	Poaceae	180
	Sorghastrum_nutans	Poaceae	180
	Symphyotrichum_ericoides	Asteraceae	180
	Sporobolus_compositus	Poaceae	179
	Bouteloua_curtipendula	Poaceae	178
	Carex_meadii	Cyperaceae	178
	Poa_pratensis	Poaceae	175
1 year FRI, ungrazed	*Andropogon_gerardii*	Poaceae	120
	Bouteloua_curtipendula	Poaceae	120
	Sorghastrum_nutans	Poaceae	120
	Ambrosia_psilostachya	Asteraceae	117
	Schizachyrium_scoparium	Poaceae	117
	Dichanthelium_oligosanthes	Poaceae	114
	Carex_inops	Cyperaceae	113
	Symphyotrichum_ericoides	Asteraceae	109
	Amorpha_canescens	Fabacaea	101
	Carex_meadii	Cyperaceae	100
3-4 year FRI, ungrazed	*Andropogon_gerardii*	Poaceae	120
	Bouteloua_curtipendula	Poaceae	120
	Carex_inops	Cyperaceae	120

	Dichanthelium_oligosanthes	Poaceae	120
	Poa_pratensis	Poaceae	120
	Schizachyrium_scoparium	Poaceae	120
	Sorghastrum_nutans	Poaceae	120
	Symphyotrichum_ericoides	Asteraceae	120
	Sporobolus_compositus	Poaceae	119
	Salvia_azurea	Lamiaceae	118

Table S2.13. Pairwise comparisons from linear mixed models on NTI as a function of FRI, grazing, and time period. Both PED_{SES} and NRI were similarly non-significant with the exception of 4-yr FRI, ungrazed post and late being significantly different from one another.

FRI	Grazing	contrast	estimate	SE	df	t.ratio	p.value
1	**Bison**	1_early-2_pre	0.594182	0.346	78	1.717	0.4294
1	**Bison**	1_early-3_during	0.172235	0.346	78	0.498	0.9873
1	**Bison**	1_early-4_post	0.217404	0.346	78	0.628	0.97
1	**Bison**	1_early-5_late	0.216541	0.346	78	0.626	0.9705
1	**Bison**	2_pre-3_during	-0.42195	0.346	78	-1.22	0.7401
1	**Bison**	2_pre-4_post	-0.37678	0.346	78	-1.089	0.8117
1	**Bison**	2_pre-5_late	-0.37764	0.346	78	-1.092	0.8104
1	**Bison**	3_during-4_post	0.045169	0.346	78	0.131	0.9999
1	**Bison**	3_during-5_late	0.044306	0.346	78	0.128	0.9999
1	**Bison**	4_post-5_late	-0.00086	0.346	78	-0.002	1
1	**Cattle**	2_pre-3_during	0.246261	0.489	78	0.503	0.9581
1	**Cattle**	2_pre-4_post	0.540439	0.489	78	1.105	0.6878
1	**Cattle**	2_pre-5_late	0.615551	0.489	78	1.258	0.5922
1	**Cattle**	3_during-4_post	0.294178	0.489	78	0.601	0.9314
1	**Cattle**	3_during-5_late	0.36929	0.489	78	0.755	0.8744
1	**Cattle**	4_post-5_late	0.075112	0.489	78	0.154	0.9987
1	**Ungrazed**	1_early-2_pre	0.182917	0.346	78	0.529	0.9841
1	**Ungrazed**	1_early-3_during	0.506558	0.346	78	1.464	0.5886
1	**Ungrazed**	1_early-4_post	0.398309	0.346	78	1.151	0.7787
1	**Ungrazed**	1_early-5_late	1.096812	0.346	78	3.17	0.018
1	**Ungrazed**	2_pre-3_during	0.323641	0.346	78	0.935	0.8822
1	**Ungrazed**	2_pre-4_post	0.215392	0.346	78	0.623	0.971
1	**Ungrazed**	2_pre-5_late	0.913896	0.346	78	2.641	0.0727
1	**Ungrazed**	3_during-4_post	-0.10825	0.346	78	-0.313	0.9979
1	**Ungrazed**	3_during-5_late	0.590255	0.346	78	1.706	0.4363

1	**Ungrazed**	4_post-5_late	0.698504	0.346	78	2.019	0.2667
4	**Bison**	1_early-2_pre	0.321327	0.346	78	0.929	0.8849
4	**Bison**	1_early-3_during	0.085275	0.346	78	0.246	0.9992
4	**Bison**	1_early-4_post	0.150568	0.346	78	0.435	0.9924
4	**Bison**	1_early-5_late	-0.091	0.346	78	-0.263	0.9989
4	**Bison**	2_pre-3_during	-0.23605	0.346	78	-0.682	0.9597
4	**Bison**	2_pre-4_post	-0.17076	0.346	78	-0.494	0.9877
4	**Bison**	2_pre-5_late	-0.41233	0.346	78	-1.192	0.756
4	**Bison**	3_during-4_post	0.065293	0.346	78	0.189	0.9997
4	**Bison**	3_during-5_late	-0.17628	0.346	78	-0.51	0.9862
4	**Bison**	4_post-5_late	-0.24157	0.346	78	-0.698	0.9563
3	**Cattle**	2_pre-3_during	-0.70703	0.282	78	-2.503	0.0673
3	**Cattle**	2_pre-4_post	-0.60408	0.282	78	-2.138	0.1501
3	**Cattle**	2_pre-5_late	-0.01929	0.282	78	-0.068	0.9999
3	**Cattle**	3_during-4_post	0.102957	0.282	78	0.364	0.9833
3	**Cattle**	3_during-5_late	0.687748	0.282	78	2.435	0.0789
3	**Cattle**	4_post-5_late	0.58479	0.282	78	2.07	0.172
4	**Ungrazed**	1_early-2_pre	0.318264	0.346	78	0.92	0.8884
4	**Ungrazed**	1_early-3_during	0.556202	0.346	78	1.608	0.4971
4	**Ungrazed**	1_early-4_post	-0.05906	0.346	78	-0.171	0.9998
4	**Ungrazed**	1_early-5_late	1.067701	0.346	78	3.086	0.0228
4	**Ungrazed**	2_pre-3_during	0.237938	0.346	78	0.688	0.9586
4	**Ungrazed**	2_pre-4_post	-0.37732	0.346	78	-1.091	0.8109
4	**Ungrazed**	2_pre-5_late	0.749437	0.346	78	2.166	0.2035
4	**Ungrazed**	3_during-4_post	-0.61526	0.346	78	-1.778	0.3935
4	**Ungrazed**	3_during-5_late	0.511499	0.346	78	1.478	0.5794
4	**Ungrazed**	4_post-5_late	1.126761	0.346	78	3.257	0.014

Supplementary Figures

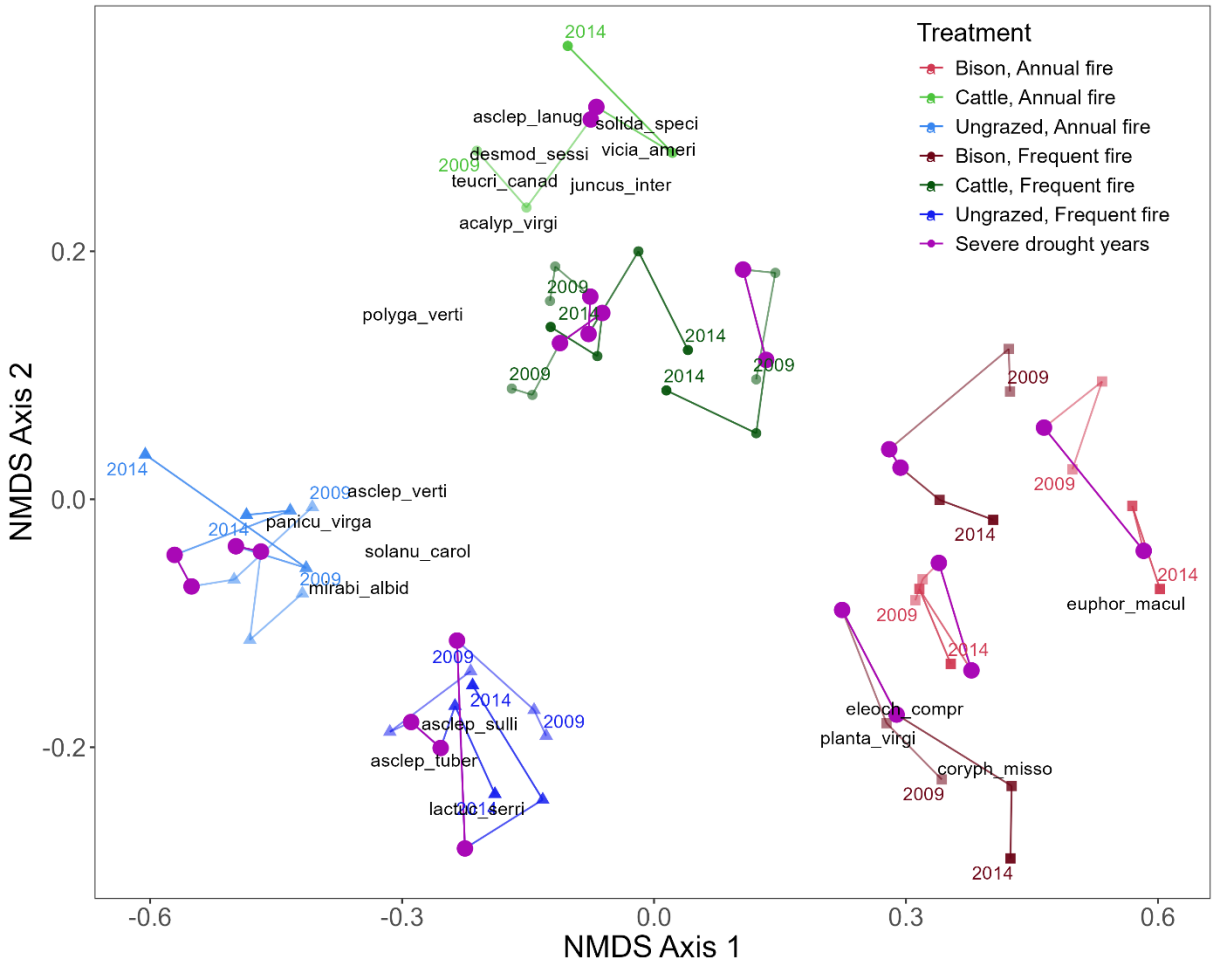

Figure S2.1. NMDS of the Bray Curtis dissimilarity of a subset of the community composition matrix (2009-2014) to highlight the years surrounding the 2011-2012 drought. Pink dots show 2011 and 2012. Annual fire is the 1 year FRI; frequent fire is the 3-4 year FRI.

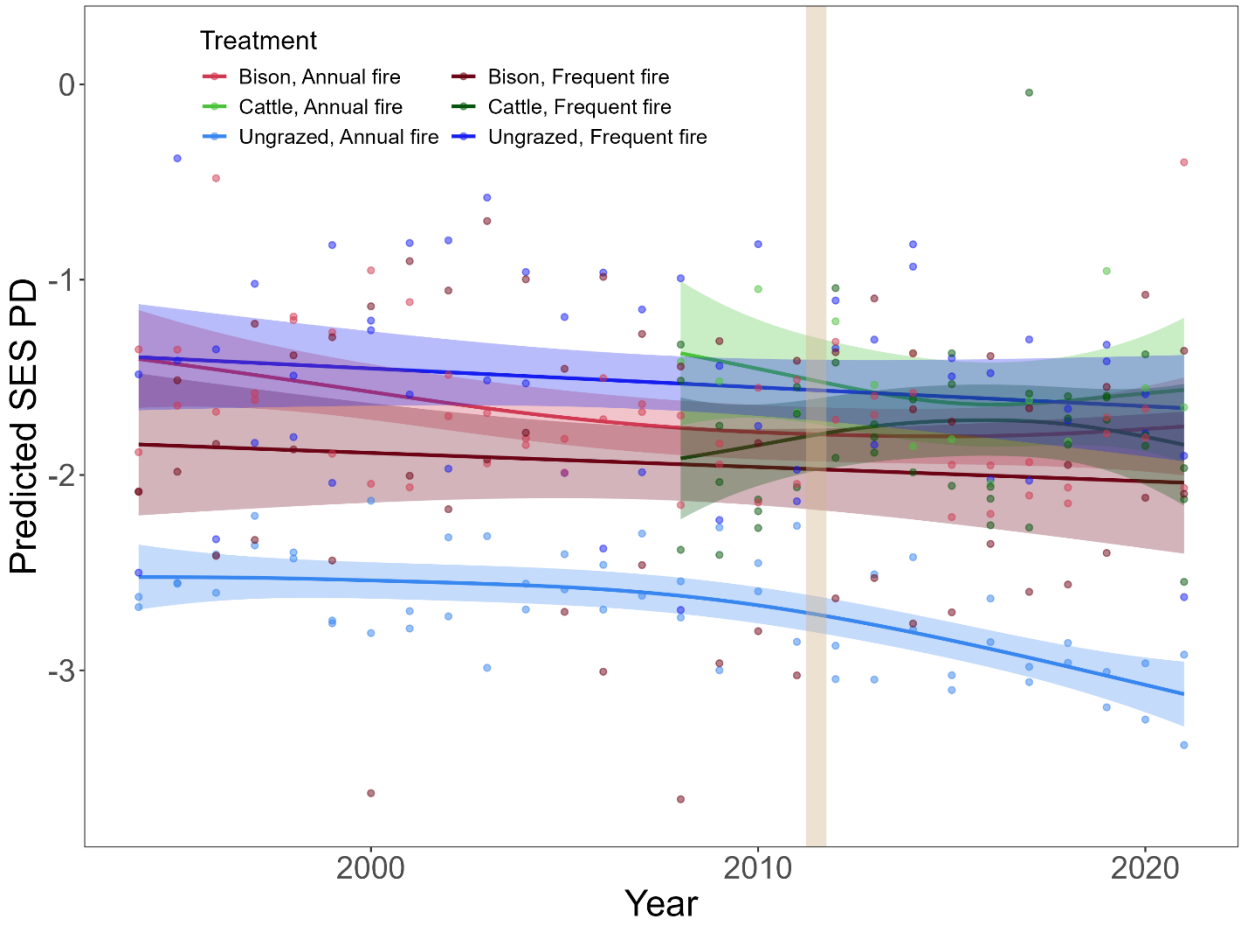

Figure S2.2. General additive models (GAMs) of catchment-scale phylogenetic diversity (SES Faith's PD) and time. The vertical tan bar represent 2011 and 2012, which were major drought years and the shaded areas for each line are the 95% confidence interval. Annual fire is the 1 year FRI; frequent fire is the 3-4 year FRI.

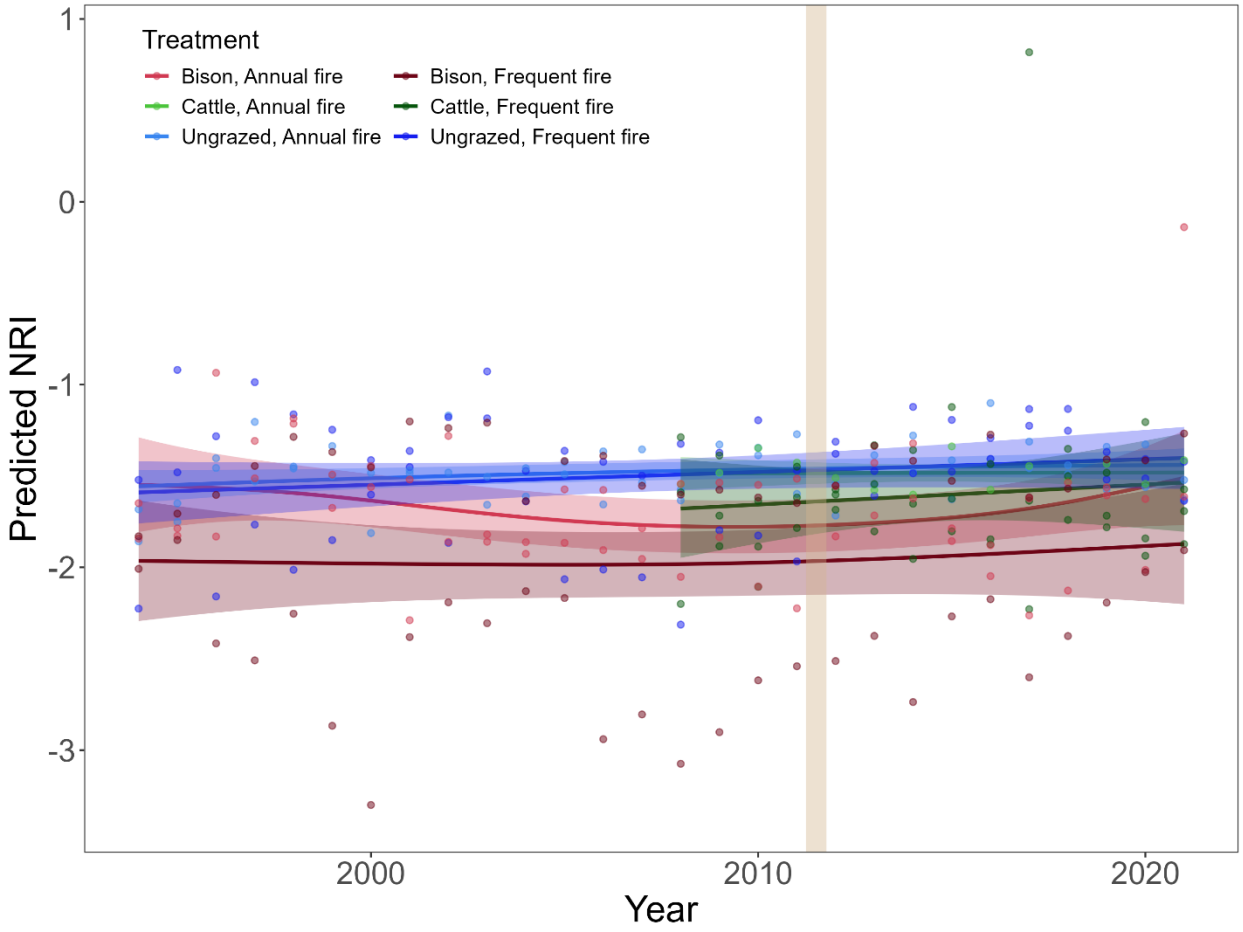

Figure S2.3. General additive models (GAMs) of catchment-scale phylogenetic diversity (NRI, net relatedness index) and time. The vertical tan bar represent 2011 and 2012, which were major drought years and the shaded areas for each line are the 95% confidence interval. Annual fire is the 1 year FRI; frequent fire is the 3-4 year FRI.

Chapter 3 - Reintroducing bison restores landscape diversity, widespread salty ephemeral wetlands, and heterogeneity

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One of humans' most widespread impacts on the natural world is the reduction or extinction of megafauna, including whales, mammoths, and bison (Smith et al. 2023). With their high abundance and biomass, megafauna likely exerted strong effects on the environment, such as supporting complex interaction webs with parasites, detritivores, and plants. Megafauna are often ecosystem engineers, which is our focus here. Like several other native herbivores globally (e.g., cape buffalo, elephants, warthogs), Plains bison (*Bison bison*) form wallows—bare earth depressions left behind from bison dustbathing, mudbathing, and trampling. New data allow us to estimate how abundant wallows were before colonization of North America and whether wallows provided sharply different microsites compared to the surrounding prairie.

We offer that wallows present a range of research opportunities, spanning classical natural history and experimental ecology. **Natural history:** in many areas, the loss of bison had the surprising effect of removing a vast network of wetland meta-communities, including distinct communities that are rare or absent in native grasslands (Figure 1). Some wallows are salty, which affects salt-limited herbivores and food webs. **Concepts and theory:** the size and density of wallows make them an elegant observational and experimental study system for microbiomes, plant populations, and consumer meta-populations (Appendix S1: Figure S1).

After the last glacial maximum, North America was home to three mega-herbivores, bison, elk, and moose. Among them, bison were the largest and most widespread. Once numbering in the tens of millions, bison now represent less than 5% of their pre-colonization abundance and geographic range (Sanderson et al. 2008). In many areas where bison have returned, the reappearance of wallows is a conspicuous change on the landscape. Bison do not create lasting wallows in all ecosystems, but where they do, the numbers can be staggering, especially in tallgrass prairie. The largest remaining landscape of tallgrass prairie is the Flint

Hills ecoregion of North America (~25,700 km^2). Since being reintroduced in 1992, bison have created ~300 wallows per km^2 (Horne et al. 2024) at Konza Prairie Biological Station (KPBS) which is located within the Flint Hills. Using a conservative extrapolation (150 wallows km^{-2}), the Flint Hills alone could have contained ~4 million wallows. Assuming an order of magnitude lower wallow density (15 wallows per km^2), the entire tallgrass prairie biome could have once conservatively contained at least 10 million wallows. Although many wallows appear to be barren depressions, or worse, weedy thickets, recent data suggests the opposite.

We took measurements at KPBS, an experimental research station featuring a 35 km^2 (13.5 mi^2) area with large-scale manipulations of fire frequency and the presence/absence of large grazers (bison or cattle). We established 72 permanent plots across wallows (n = 24), adjacent bison-grazed non-wallowed prairie (n = 24), and cattle-grazed prairie (n = 24). We conducted vegetation surveys twice per year, in the mid and late growing season, to capture seasonal dynamics. We identified all plants to species, except for some *Carex* species, and recorded species abundance using a cover class scale. We collected soil samples at the end of the growing season each year, which we analyzed for nutrients, organic matter, cations, pH, and soil texture. We also monitored standing water in wallows during the growing seasons of 2022 (n = 48) and 2023 (n = 172) following rainfall events. We analyzed differences in plant community composition due to plot type (wallow, bison non-wallow, cattle) using a PERMANOVA and differences in species richness, diversity, bare ground, and soil properties among plot types using linear mixed-effects models, with a random effect for sample sites. Pairwise comparisons for each response variable were conducted with Tukey honest squared difference tests (see Appendix S1: Section 1 for more details on methods).

Over two growing seasons (2022-2023), we found that after a substantial rainfall event,

~38% of wallows held standing water for at 1-7 days and 13% held water for >7 days (Figure 2f). With consistent rainfall, wallows can hold water for 30 consecutive days, supporting amphibian reproduction (Gerlanc and Kaufman 2003) and ephemeral pool specialists like *Triops* (Figure 1f-g; Frazier et al. 2024). Larger vertebrates, including bison, use these wallows as primary watering holes (Nippert et al. 2013).

Wallows support contrasting plant communities, spread across two zones: the inner depression of the wallow and the wallow edge, which form a miniature ecotone with open grasslands (Figure 3d). On average, wallows have lower plant species diversity than non-wallows (Figure 3a-b). Yet wallows feature distinct communities, especially within their interior (Figure 3g). These plant communities often favor ruderal forbs that may increase pollinator resources. Wetland vegetation sometimes emerges in wallows that hold water, including two species rarely found outside of ephemeral pools, *Bacopa rotundifolia* and *Cyperus acuminatus* (Figure 3e-f). Studies on wallows are surprisingly few, but data from Oklahoma prairies also found *B. rotundifolia* and *C. acuminatus* to be confined to bison wallows (Polley and Wallace 1986). These wetland plants rely on low-competition ephemeral bodies of water that might once have been more abundant with bison.

Wallows soils were distinct, with elevated clay content (150% higher than non-wallows), reduced total nitrogen (70% higher), and altered potassium (140% more than cattle-grazed areas and 80% less than ungrazed areas, Figure 2a-e). Altered nutrients reflect bioturbation and lower organic matter inputs from plants (Mallen-Cooper et al. 2019). These biogeochemical changes increase fine-scale heterogeneity, likely contributing to the increased beta diversity found in bison-grazed grasslands (Knapp et al. 1999).

One of the most surprising aspects of wallows was their salinity. The median wallow had

similar salinity as nearby areas without wallows, but the 30% of wallow had greater sodium concentration than the maximum recorded sodium in all non-wallow plots (bison and cattle combined; Figure 2d). Elevated sodium likely stems from urine inputs and evaporative concentration. Bison might also seek out naturally sodic locations to form wallows, as recent experiments suggest herbivores can detect salt in forage (Welti and Kaspari 2021). Therefore, elevated sodium in wallows may have important implications for prairie food webs. Sodium is an essential but often limiting nutrient for terrestrial herbivores, especially insects, and sodium addition can increase insect abundance by 45% (Welti and Kaspari 2021). As patches of concentrated sodium, wallows may altering patterns of foraging, movement, and even population dynamics.

Despite how widespread wallows likely were historically, many questions remain. For instance, why do some wallows retain water while others do not? How does wallow hydrology vary within and across regions? To determine where bison increased ephemeral wetlands historically will require further research, probably with a biogeographic and critical zone approach. For instance, wallow research has occurred more in the lower Great Plains in tallgrass prairie where annual precipitation is >300 mm (Polley and Collins 1984; McMillan et al. 2011). In more water-limited grasslands (e.g., shortgrass prairie) and areas with sandier soils, wallows will be less likely to serve as ephemeral wetlands. Like many impacts of bison, there are undoubtedly meaningful regional differences.

The relatively small size, discrete boundaries, and high density of wallows make them a particularly tractable experimental study system. The size of wallows also means that rain-out shelters can entirely cover them to determine the effects of drought on community assembly or biodiversity. As natural mesocosms embedded in nature, wallows offer a rare opportunity to

experimentally test dispersal limitation, environmental filtering, biotic interactions, and community assembly in a field setting – linking theory to real-world processes. A difficulty in community ecology is knowing when one community ends and another begins. Empirical approaches can answer this question but are data-hungry. With wallows, the boundary of communities is clear, allowing strong inference of within vs between community interactions.

Bison wallows are a chance to address long-standing questions on succession and ecosystem development in grasslands – an ecosystem where work on these topics has revolved around agricultural disturbances. Researchers can easily exclude bison to study community assembly through passive recolonization or active restoration via seeding. Wallows show that natural processes also cause major soil disturbances and raise questions about how succession in these patches compares to anthropogenic disturbances. For instance, does succession in wallows follow the typical successional pattern of an increase in species diversity, followed by a peak or plateau, or something else? These questions are especially relevant in wallows as bison continue to visit some wallows and abandon others, creating a mosaic of different successional stages.

Wallows also provide a mosaic of patch habitats ideal for studying spatial dynamics in metacommunities on how dispersal limitation, local extinctions, and recolonization shape genetic and community structure, especially for taxa restricted to ephemeral pools. Bison may increase connectivity among these isolated populations, acting as vectors for seeds, microbial spores, and small invertebrates. Wallow formation strongly tracks topography, with more wallows formed in flat areas (Horne et al. 2024). Erosion creates variation in the size and arrangement of flat areas, resulting in wallow networks with different characteristics (e.g., size, modularity), which should theoretically affect each network's capacity to support viable meta-populations.

The disappearance of bison and their wallows from North American grasslands

represents a deeper loss of ecological complexity. We find that wallows are important hotspots of activity and biological diversity. They alter hydrology, soil chemistry, plant assemblages, and the availability of key nutrients like sodium. Their loss has meant shifts in ephemeral wetland networks and metacommunities, the decline of specialized habitats, and reduced sodium availability across the Great Plains. In an era of accelerating biodiversity loss, we need to protect grasslands and native grazers. In reintroducing bison, we are not just bringing back keystone herbivory – a role that cattle can potentially mimic. We are rewilding important processes, restoring forgotten ecological dynamics, and rebuilding a mosaic of disturbance-driven habitats that amplify diversity. Finally, the Great Plains was once a shallow inland sea ~200 million years ago. It is a whimsical thought that returning native megafauna also results in the creation of these wallows that resemble miniature seas in some respects, with salty water and microbiota.

Acknowledgements:

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3.2 Figures

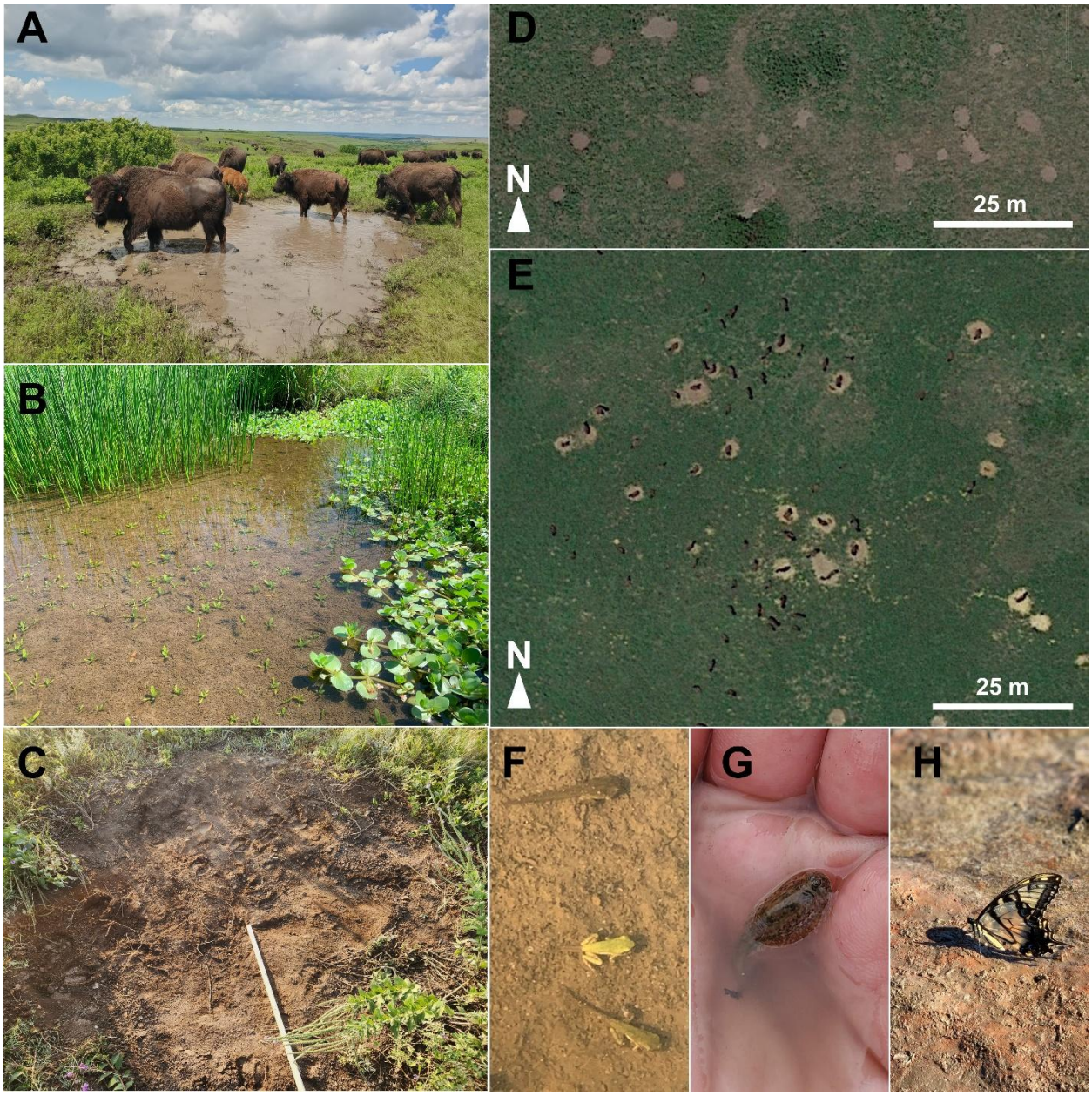

Figure 3.1: A) Bison using rain-filled wallow for water and thermoregulation; photo by B. Bookout (BB), June 2, 2021. B) Rain-filled wallow with wetland plants and tadpoles; photo by K. Stevermer (KS), July 9, 2022. C) Recently used wallow with measuring pole (~1 m) for scale; photo by BB, August 7, 2023. D) Aerial image from Google Earth showing the density of wallows in some areas on KPBS in spring 2022. E) Aerial image of bison and wallows in June 2019 at Maxwell Wildlife Refuge, McPherson County, Kansas. Image from Google Earth. F) Tadpoles and froglets in rain-filled wallow; photo by KS, July 8, 2022. G) *Triops longicaudatus* from a rain-filled wallow; photo by BB May 24, 2021. H) *Papilio glaucus* puddling in a drying wallow; photo by KS, July 9, 2022.

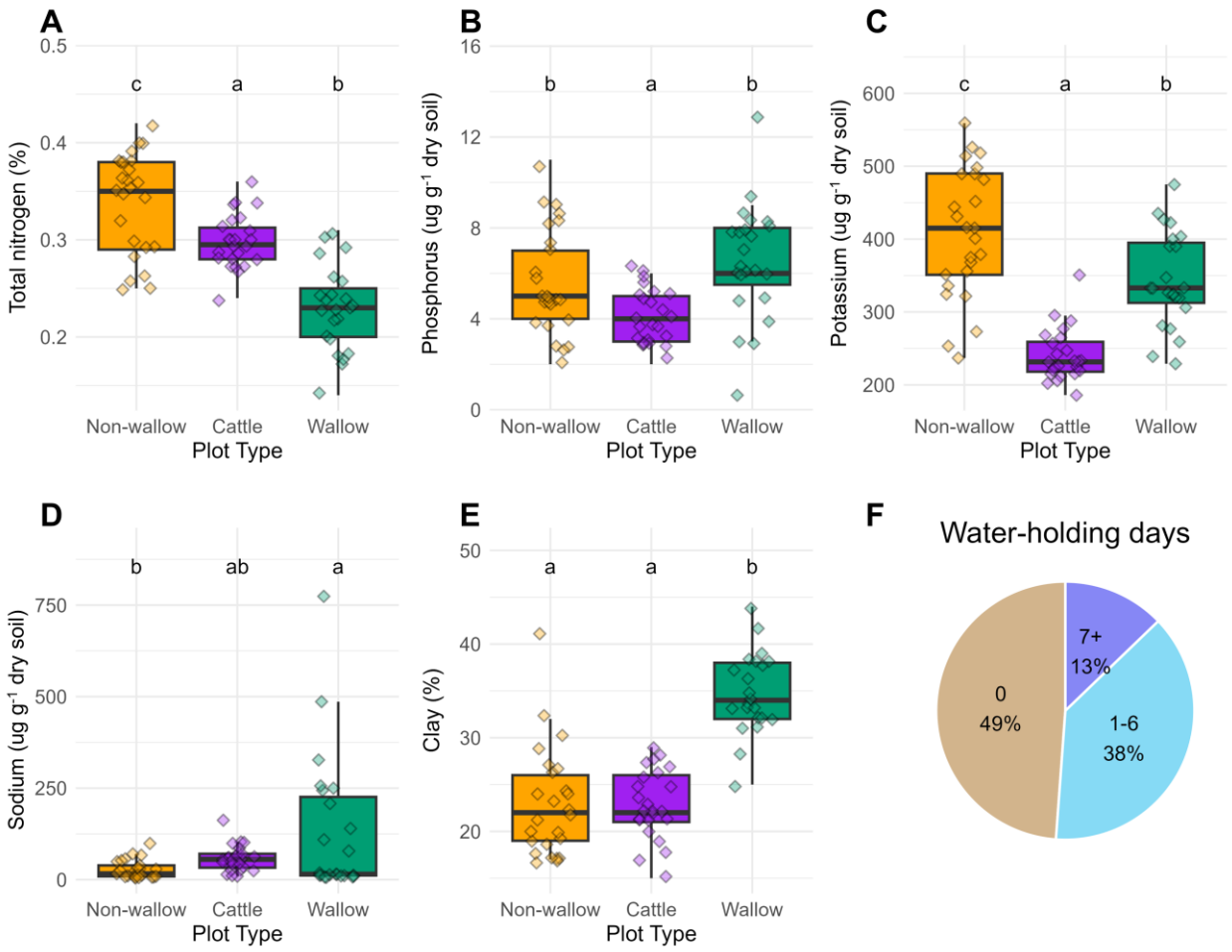

Figure 3.2: Soil characteristics (A-E) and hydroperiods (F). Soil characteristics include A) total nitrogen, B) phosphorus, C) potassium, D) sodium, and E) clay content. Letters above boxplots designate significant differences between the means of groups using a Tukey post-hoc test.

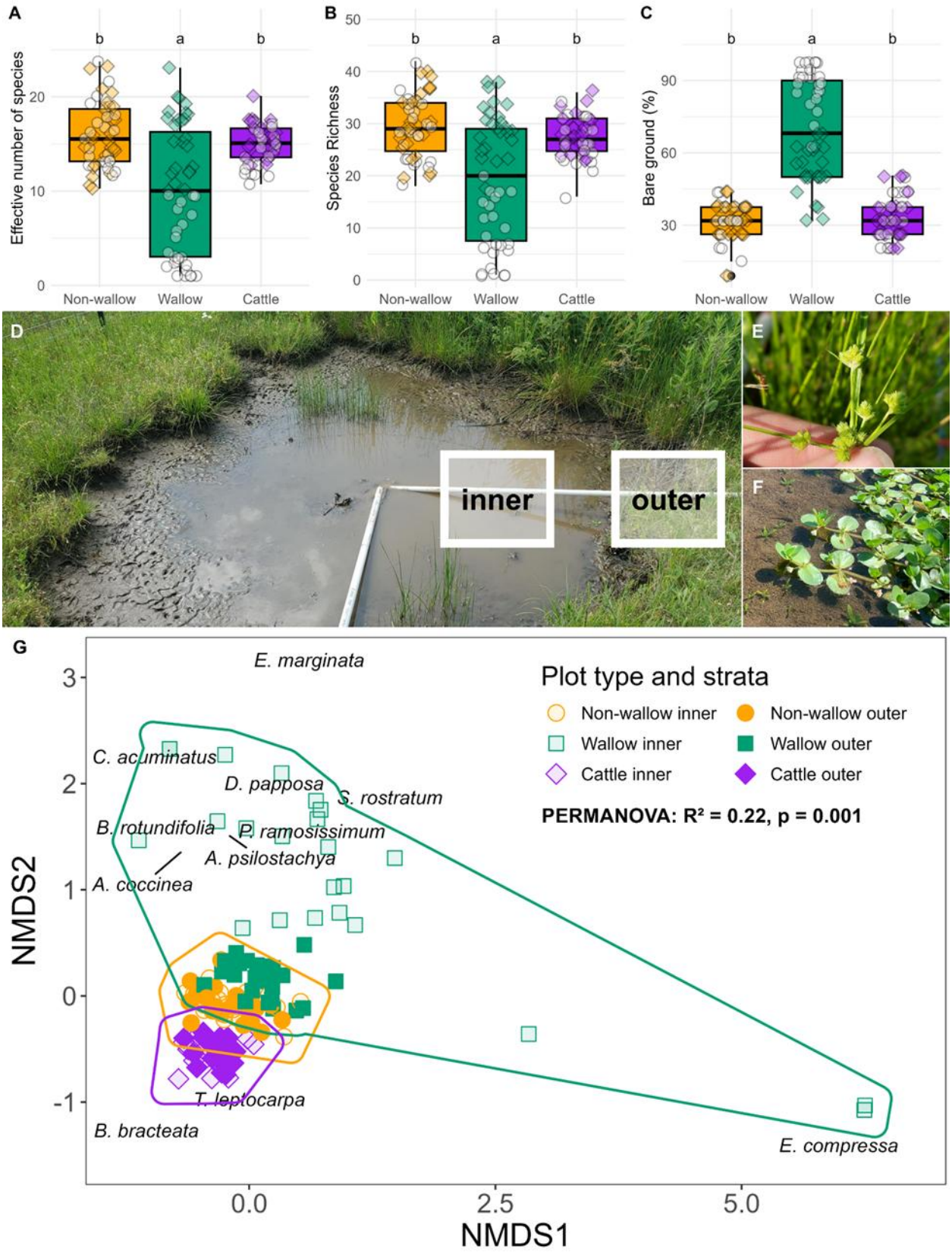
A
Effective number of species
b
a
b
Non-wallow
Wallow
Cattle
B
Species Richness
C
Bare ground (%)
D
inner
outer
E
F
G
E. marginata
C. acuminatus
D. papposa
S. rostratum
B. rotundifolia
P. ramosissimum
A. psilostachya
A. coccinea
T. leptocarpa
B. bracteata
E. compressa
Plot type and strata
Non-wallow inner
Non-wallow outer
Wallow inner
Wallow outer
Cattle inner
Cattle outer
PERMANOVA: R² = 0.22, p = 0.001
NMDS1
NMDS2

Figure 3.3: Plant diversity (A, B, G) and bare ground cover (C) from 24 wallows and 24 adjacent non-wallows. A) Effective number of species (Hill-Shannon diversity) per stratum per plot. B) Plant species richness per stratum per plot. C) Average percent cover of bare ground within strata. White-filled dots indicate the "inner" stratum, while color-filled diamonds indicate the "outer" stratum. Letters designate significant differences between the means of groups using a Tukey post-hoc test. D) Plot design showing the placement of inner and outer subplots in wallows (photo by BB). E) *Cyperus acuminatus* (photo by BB). F) *Bacopa rotundifolia* (photo by KS). G) Non-metric multidimensional scaling plot of Bray-Curtis dissimilarity matrix of 2022 plant communities (stress = 0.13). Black italicized text on the plot indicates plant species that are associated with plots they are near (Appendix S3.2: Table S3.1).

Appendix A - Chapter 3 supplemental materials

Methods

Site Description:

All data was collected at Konza Prairie Biological Station (KPBS) in eastern Kansas, U.S.A (39.1069° N, 96.6091° W). KPBS is an experimentally-managed remnant tallgrass prairie located in the northern Flint Hills ecoregion, the ecoregion with the largest remaining (nearly) continuous tracts of tallgrass prairie in North America (Knapp et al. 1998). KPBS features a factorially crossed grazing and fire return interval (FRI) experiment with large areas divided into ungrazed, bison-grazed, and cattle-grazed sections (Figure S3.2). Within these larger areas, the units are divided into catchment-level treatments with different long-term prescribed fire return intervals (FRIs)—1, 2, 3-4, and 20-year FRIs. We refer to these as "catchments" from here on. Note that many but not all of these factorial combinations of fire and grazer type exist. The data collected for this study were collected in 2022 in 1 and 3-4-year FRI catchments in bison- and cattle-grazed pastures (see catchments marked with an asterisk in Figure S3.2).

Plot selection:

Actively used wallows were chosen if they had a clear boundary, were approximately 8 m^2 (±1 m^2) in area (the average size of recorded wallows; Horne et al. 2024), and contained less than 10% live plant cover in the interior, indicating their active use. To control for topographic effects, all wallows were located on one of two mid-elevation benches in FRI 1 or 4 catchments (with centroids of these locations marked as stars in Figure S3.2). Benches are a common landform in the region, where large flat topographies punctuate hillslopes. Along with flat uplands, most bison wallows are formed on benches and other areas with flat topographies

(Horne et al. 2024). Wallows within 10 m of roads or mown fire breaks were excluded to minimize edge effects. Non-wallow plots (8 m^2) were randomly placed ~5 m from their paired wallow while ensuring a minimum distance of ~5 m from other wallows or other disturbances (e.g., erosive gullies). Each plot was permanently marked with a piece of metal conduit, placed at the center for non-wallows or at the edge for wallows to minimize loss of markers due to wallowing. In each catchment, we identified two distinct benches, and within each bench, we established six permanently marked plots. This yielded 12 wallows, 12 paired bison-grazed non-wallow plots, and 12 cattle-grazed non-wallow plots for each combination of grazer type (bison vs. cattle) and each fire return interval (1 vs. 3-4 years; 72 total plots).

Vegetation Surveys:

Each plot contained eight subplots for plant monitoring. These subplots were stratified to capture both inner (within the interior of the wallow) and edge (the boundary between disturbed and undisturbed area) microhabitats (called strata from here on; Figure S3.3). Each subplot was 0.25 m^2 and was systematically arranged along four evenly spaced transects radiating outward from the plot center at 90-degree intervals. Non-wallow plots followed the same layout to maintain consistency across plot types. Vegetation surveys were conducted both in early June and August to capture seasonal variation in plant communities, capturing both spring ephemerals and late-season species (Collins and Calabrese 2012; Ratajczak et al. 2022). Plant species were identified to species, except for sedges and a few other species that were identified to genus. Their cover was then estimated using a modified Daubenmire scale (1: 0-1%, 2: 1-5%, 3: 5-25%, 4: 25-50%, 5: 50-75%, 6: 75-95%, 7: 95-100%). When a species was recorded in both sampling periods, only the larger of the two cover values was used in subsequent analyses.

Soil Sampling and Analysis:

Soil samples were collected in early September. In each plot, we took four soil cores (6 to 10 cm depth, 1.27 cm diameter) using a soil probe, positioned between the inner and edge vegetation subplots to minimize disturbance. This depth captures the zone of highest root biomass, although many species (especially forbs) often have deeper roots (e.g., Nippert and Knapp 2007; Nippert et al. 2012; O'Keefe and Nippert 2017). The four cores were mixed together, sieved to remove large roots and rocks (to 4 mm mesh), and refrigerated at 4 C to minimize microbial transformations prior to analysis. Samples were sent to the Kansas State University Soil Testing Laboratory (https://www.agronomy.k-state.edu/outreach-and-services/soil-testing-lab/), where they were analyzed for nutrient content, cation concentrations, pH, and soil texture. Nutrient content included phosphorus (Bray P test using HCl-ammonium fluoride extraction), organic matter (loss-on-ignition at 400 C for 3 hours after drying at 150 C for 2 hours), and total nitrogen and carbon (LECO TruSpec CN combustion analyzer). Cations included Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} and were extracted with 1 M ammonium acetate and analyzed by ICP Spectrometry. Soil texture was determined using the hydrometer method after using sodium hexametaphosphate as a dispersing agent.

Water-holding in wallows:

From late June 2022 to September 2022 and early June to mid-July 2023, we monitored the 24 wallows approximately every other day and estimated the percent cover of standing water in each wallow until there was no more standing water. Then, if no rainfall occurred, wallows were checked weekly or bi-weekly. Based on our observations, all wallows were rainfed; none were spring- or stream-fed. When rainfall occurred, monitoring resumed as soon as roads were drivable and continued every other day until wallows re-dried. The visual estimates, paired with rainfall data, were used to estimate hydroperiods (the number of consecutive days that wallows

had at least 5% cover of standing water). Hydroperiods were subsequently binned into three categories: 0 days (consistently no standing water within 48 hours of rainfall), 1-7 days (held less than 20% water within 7 days), and 7+ days (held at least 20% water for more than 7 days). Binning was necessary because we could not always reach each wallow every day after a rainfall event. From June to July 2023, we monitored an additional 148 wallows from KPBS to increase the sample size for hydroperiod data (Table S3.2).

Data analysis

To assess differences in plant community composition, we constructed a site-by-species matrix by averaging percent cover across subplots within each plot and stratum (inner or outer), resulting in 143 observations (site = plot and stratum). Midpoints of Daubenmire cover classes were used to calculate cover. One inner plot was excluded from the multivariate analysis due to complete absence of vegetation across all inner subplots. Bray–Curtis dissimilarities were calculated from that site-by-species matrix and visualized using non-metric multidimensional scaling (NMDS; metaMDS function in the *vegan* package; Oksanen et al. 2024). All species were included in the matrix. To statistically test for differences in community composition among plot types (wallow, bison non-wallow, cattle non-wallow), we performed a PERMANOVA using the adonis2 function.

To determine the effects of plot type (wallow, bison non-wallow, cattle non-wallow) on plant alpha diversity, we calculated species richness (the number of species in each stratum in each plot; specnumber in *vegan*) and Hill-Shannon diversity (effective number of species in each stratum in each plot; Roswell, Dushoff, and Winfree 2021). To calculate Hill-Shannon diversity, we first calculated Shannon diversity index (Equation 1) using the diversity function in *vegan* with the “shannon” option and then exponentiating the result. Shannon diversity is calculated as

$H = -\sum_{i=1}^{S} p_i \, log_b \, p_i$, here p_i is the proportion of species *i*, and *S* is the number of species so that $\sum_{i=1}^{S} p_i = 1$, and *b* is the base of the logarithm. We calculated bare ground as the average percent cover (midpoint of Daubenmire cover class) of the four subplots within each stratum. We then analyzed univariate measures of community diversity and bare ground for the effects of plot type using linear mixed-effects models (lmer in *lme4* package; Bates et al. 2025) with "bench" as a random effect. We then used Tukey HSD post hoc tests (emmeans, followed by pairs in *emmeans* package; Lenth et al. 2024) to calculate pairwise differences between plot types.

Soil variables, which only represent the inner stratum on the plot level (samples homogenized per plot), were analyzed separately for effects of plot type (wallow, bison non-wallow, cattle non-wallow) using linear mix-effects models with "bench" as a random effect (lmer in *lme4* package). We then conducted Tukey's HSD post hoc tests to identify pairwise differences (emmeans, followed by pairs in *emmeans*) .

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Supplementary Tables

Table S 3.1: Plant species labels in the NMDS.

Species	Functional group	Wetland Status[1]
Euphorbia marginata	Forb	FACU
Cyperus acuminatus	Graminoid	OBL
Dyssodia papposa	Forb	UPL
Solanum rostratum	Forb	FACU
Bacopa rotundifolia	Forb	OBL
Polygonum ramosissimum	Forb	FAC
Ambrosia psilostachya	Forb	FACU
Ammannia coccinea	Forb	OBL
Triodanis leptocarpa	Forb	UPL
Baptisia bracteata	Forb	UPL
Eleocharis compressa	Graminoid	FACW

[1]From USDA Plant Database. OBL = wetland obligate, FACW = facultative wetland, FAC = facultative, FACU = facultative upland, UPL = upland

Table S 3.2: Locations and sample size of additional wallows monitored for hydroperiods

Catchment	Number of wallows
N1A	23
N1B	71
N20A	1
N20B	1
N2A	7
N2B	12
N4A	5
N4B	4
N4C	9
N4D	15

Table S 3.3: PERMANOVA results.

	DF	Sum of Squares	R^2	F	Fr(>F)
Plot type	2	8.6	0.22	19.69	0.001
Residual	140	30.54	0.78		
Total	142	39.13	1		

Supplementary Figures

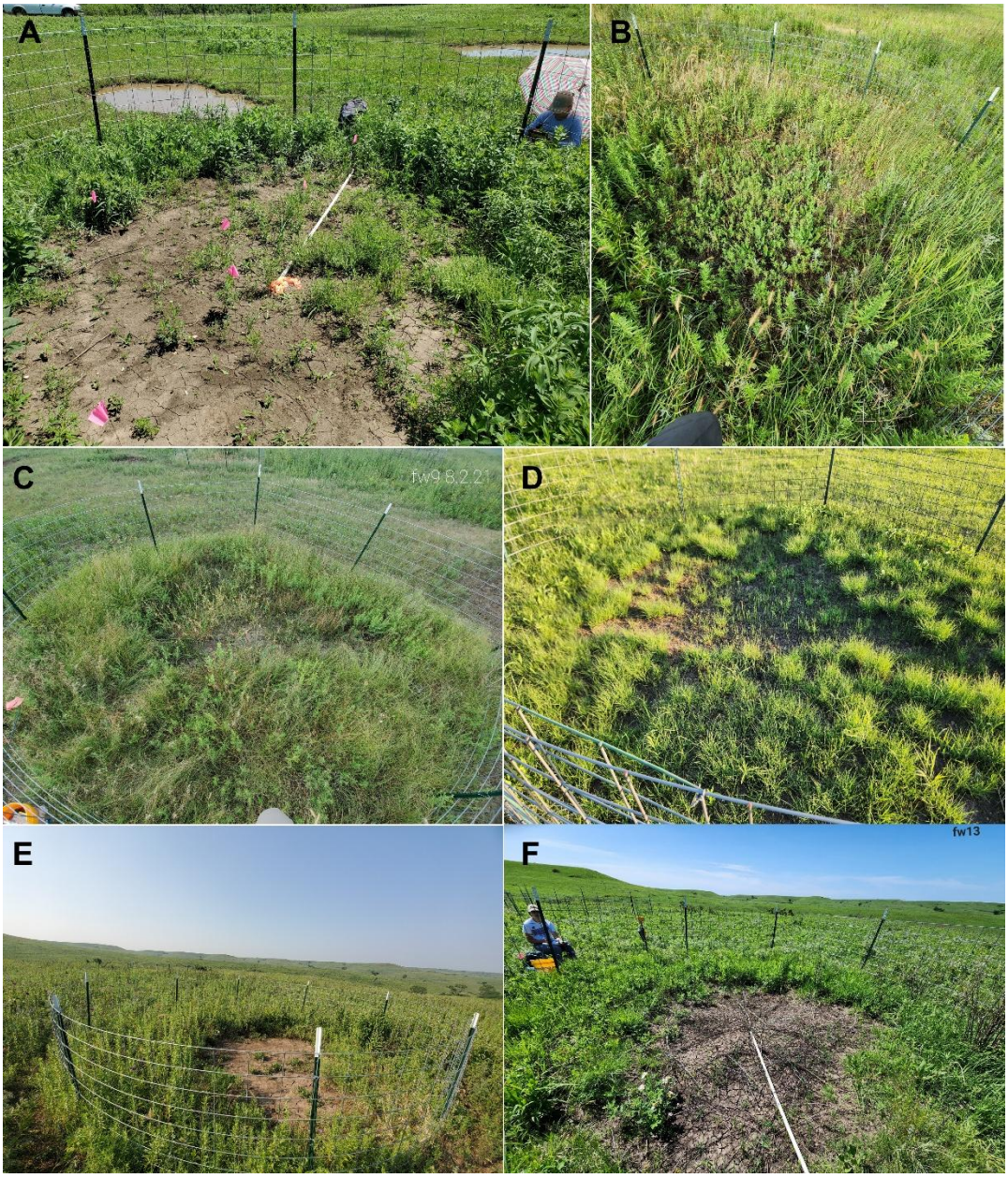

Figure S 3.1: Examples of fenced wallows to allow re-colonization of plants. A-B) Photos of a fenced wallow the spring after it was fenced and ~2 years later (A: May 2021; B: August 2023). C-D) A fenced wallow the spring after it was fenced and ~4 years later (C: August 2021; D: May 2025). E-F) A fenced wallow the spring after it was fenced and ~2 years later (E: August 2021; F: May 2023). All photos taken by B. Bookout.

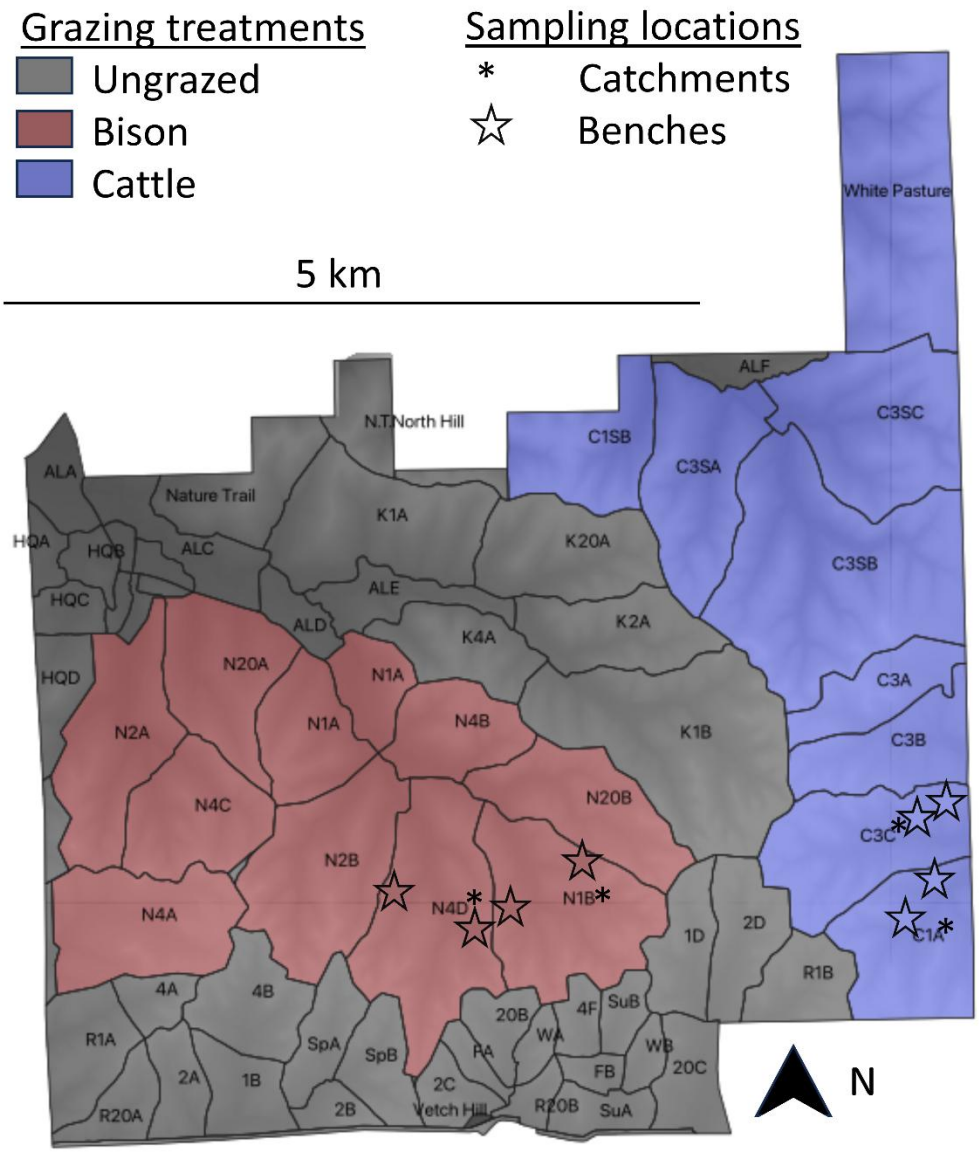

Figure S 3.2: Map of fire and grazing treatments at Konza Prairie Biological Station with catchments (asterisks) and sampling locations marked (stars). Numbers in the catchment names (i.e., N1B) indicate fire return interval. All bison-grazed catchments are in one pasture without cross-fencing. C3A, C3B, and C3C are one pasture split into three fire treatments without cross-fencing. C1A is one pasture with one fire treatment.

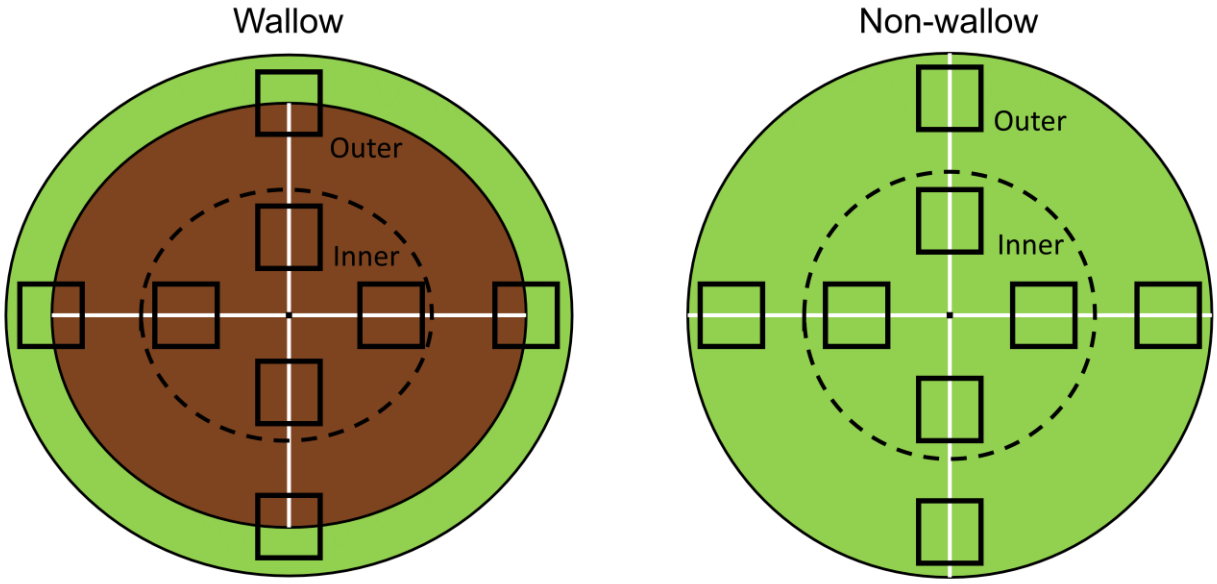

Figure S 3.3: Diagram of plot design for wallow and non-wallow plots. Green indicates non-wallowed grassland and brown indicates a wallow. Subplots (black squares) are stratified between inner (within dashed circle) and outer microsites with inner subplots within the interior of the wallow and outer subplots straddling the boundary between wallow and non-wallow. The size and shape of wallows vary, so the size of wallow plots and spacing of subplots also vary. Non-wallow plots are all the same size and shape and do not vary.

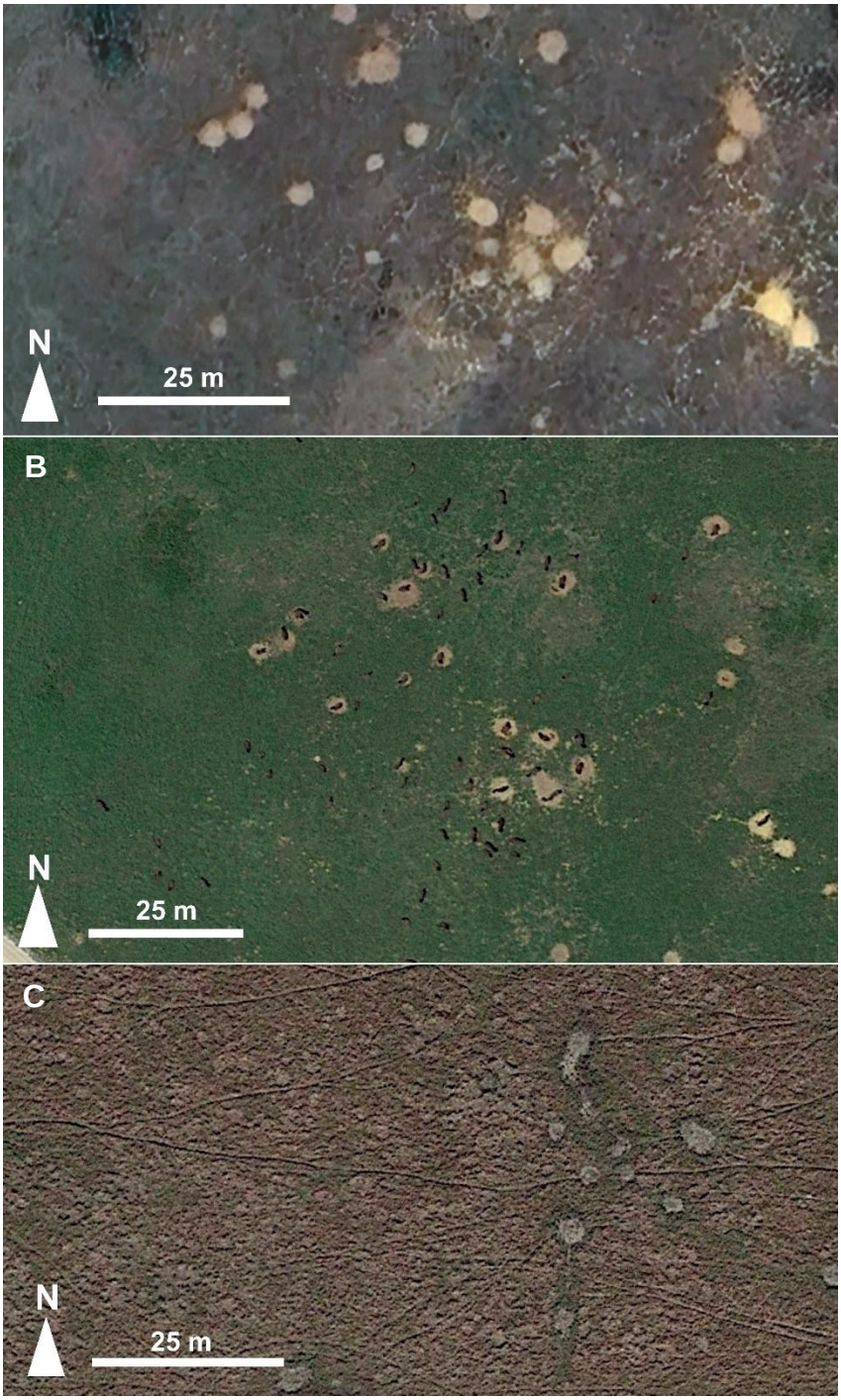

Figure S 3.4: Additional aerial images from Google Earth showing the density and relative size of wallows at different grassland sites. A) Maxwell Wildlife Refuge, McPherson County, KS. B) Maxwell Wildlife Refuge, June 2019. C) Tallgrass Prairie National Preserve, September 2019.

Chapter 4 - Bison wallows as biogeomorphic disturbances: Impacts on soil, microbes, and vegetation

This chapter is formatted for: ***Microbial Ecology***.
The citation for this chapter is: Bookout, B., Zeglin, L., Ratajczak, Z. Bison wallows as biogeomorphic disturbances: Impacts on soil, microbes, and vegetation. In prep. *Microbial Ecology*.

4.1 Abstract

Large grazers shape grassland biodiversity through both herbivory and ecosystem engineering. In the North American tallgrass prairie, bison wallowing creates bare-soil depressions with altered soil structure and chemistry, but the consequences for belowground microbial communities remain untested. We used a factorial field experiment at Konza Prairie Biological Station, Kansas, USA, to evaluate how patch type (wallow vs. surrounding matrix grassland) and grazer exclusion influence soil physicochemistry, plant composition, and soil bacterial, archaeal, and fungal diversity. Across 96 paired plots, we measured soil properties, plant communities, and microbial amplicon sequence variants (16S rRNA, ITS) over two years. Patch type was the dominant driver of variation in soils and communities. Wallows had higher clay, sodium, and extractable phosphorus, but lower organic matter, carbon, and nitrogen than grasslands. Plant alpha diversity was ~50% lower in wallows, whereas prokaryotic diversity was higher; fungal alpha diversity showed weaker and more variable patch-type effects. Beta-diversity responses diverged by domain: prokaryotes were more compositionally variable in wallows, while fungal dispersion was similar across patch types. Most microbial taxa were unique to a single patch type, with prokaryotes showing fewer shared ASVs but greater ordination overlap, and fungi showing more shared ASVs but stronger compositional separation. Fencing produced rapid, divergent responses: plant and fungal diversity increased in wallows but declined in grasslands, while prokaryotic diversity increased in both. Microbial composition correlated more strongly with soil properties than plant composition, though fungi were more plant-associated than prokaryotes. These results demonstrate that bison wallows restructure soil, plant, and microbial communities, increasing spatial heterogeneity and driving distinct assembly processes across microbial domains. Our study provides the first evidence that wallowing, a historically

widespread megafaunal behavior, shapes belowground microbial diversity and composition in mesic grasslands.

4.2 Introduction

Soil microbes, including bacteria, archaea, and fungi, play key roles in supporting ecosystem functions globally (Fierer and Jackson 2006, Huygens et al. 2011, Aislabie and Deslippe 2013). Microorganisms are critical for cycling of nutrients, including carbon, nitrogen, and phosphorus (Aislabie and Deslippe 2013, Nielsen et al. 2015). Despite advances in sequencing resolution and computational efficiency in microbial ecology, much remains unknown about which abiotic conditions and/or biotic interactions determine microbial diversity, composition, function, and the scale-dependency of such relationships (Fierer 2017). However, pH, temperature, nutrient availability, and oxygen levels affect most aspects of microbial communities, although perhaps to varying degrees (Fierer and Jackson 2006, Bates et al. 2011, Aislabie and Deslippe 2013, Tedersoo et al. 2014). Some ecosystems also show strong correlations between the composition of microbial and plant communities, which is not surprising given that plants are the primary source of carbon for microbes, and microbes are a major source of nutrients for many plants (Van Der Heijden et al. 2008, Prober et al. 2015).

Changes in soil physiochemistry can alter microbial alpha and beta-diversity. For example, at larger scales, pH is often one of the best predictors of variability in community composition, with higher diversity occurring in near-neutral soils (Fierer and Jackson 2006, Lauber et al. 2009). Soil moisture gradients, in space (e.g., ephemeral pool vs upland soils) and time (e.g., drought and re-wetting events), also shape microbial communities, often with lower diversity, biomass, and activity under low moisture availability (Evans et al. 2011, Zeglin et al. 2013, Heděnec et al. 2018). However, prokaryotes and fungi often have contrasting responses to environmental gradients. Prokaryotes are typically more sensitive to pH and temperature (Rousk

et al. 2010, Bates et al. 2011, Zhou et al. 2016), while fungi are more sensitive to changes in nutrient availability and plant communities (Rousk et al. 2010, Tedersoo et al. 2014). Although these effects are often complex and taxa-, scale- and context-dependent (Fierer 2017).

Biotic controls, such as species interactions, can directly affect soil microbial diversity, and indirectly by altering soil attributes that affect microbial communities. For instance, plant communities often alter nutrient availability through carbon and nitrogen inputs via litter and root shedding, and can alter nutrient pools and microbial behavior via root exudates (Bardgett et al. 1998, Hughes et al. 2006, Seaton et al. 2024). The quality of nutrient inputs can determine the microbial composition, with bacteria dominating in soils with higher concentrations of labile carbon sources and low organic matter, while fungi tend to dominate in soils with higher organic matter and recalcitrant plant materials (Six et al. 2006, Van Der Heijden et al. 2008). Plant-microbial associations might also vary due to the relative balance of nitrogen versus phosphorus limitation, and thereby, the strength of plant-microbe mutualisms (Bonneau et al. 2013, Nouri et al. 2014, Zhang et al. 2023).

In grasslands, grazing by large warm-bodied herbivores has affected microbes and plants since the expansion of grassland biomes ~20 million years ago (Strömberg 2011). Large grazers transform and redistribute nutrients via urine and dung to increase spatial heterogeneity in microbial activity and nutrient availability (Bakker et al. 2003, Eldridge et al. 2017, Hawkins and Zeglin 2022, Anguiano et al. 2024). For example, bacterial taxa adapted to nutrient-rich microsites may flourish near dung patches, while fungal decomposers dominate under dense vegetation (San Emeterio et al. 2023). Dung can also act as a vector for microbes to disperse across longer distances, thus homogenizing microbial communities (Hawkins and Zeglin 2022). Grazing also changes plant communities, increasing plant alpha and beta-diversity (Towne et al.

2005, Ratajczak et al. 2022, Bookout et al. 2025) as well as decreasing root biomass (Johnson and Matchett 2001). For instance, one study in tallgrass prairie found that grass root biomass was ~22% higher in areas with no large herbivore (Nippert et al. 2012).

Understandably, work on interactions between large herbivores and ecosystems has focused on their role as consumers (Adler et al. 2001, Koerner et al. 2014, du Toit and Olff 2014, Pringle et al. 2023), with a greater emphasis on carbon and nutrient exchanges in microbial communities (McSherry and Ritchie 2013). However, many large herbivores, especially those that qualify as megafauna (~100 kg or larger), have impacts that qualify as ecosystem engineering (Pringle et al. 2023). A widespread example is wallowing, which is the formation of bare earth depression from rubbing and dustbathing by herbivores (Wilson et al. 2019, Wagner et al. 2021, Eckhoff et al. 2025). Many large native herbivores create or use wallows, including elk, zebra, rhinoceros, wild hogs, and bison—the focus of this study (Geist 1982, Eckert et al. 2019, Wilson et al. 2019, Wagner et al. 2021). In contrast, the only widespread domesticated livestock known to wallow are domestic pigs (Bracke 2011) and water buffalo (Khongdee et al. 2011). Therefore, wallows represent a habitat and source of heterogeneity that was formerly widespread, but now missing from many post-colonial grasslands and savannas (Bookout et al. *In review*). Bison wallows enhance spatial heterogeneity in grasslands, housing unique plant communities (Polley and Collins 1984, Trager et al. 2004, McMillan et al. 2011, Bookout et al. *In review*). As some wallows can hold water for short periods of time (i.e., weeks), they also serve as habitats for herpetofauna (Gerlanc and Kaufman 2003), invertebrates (Nickell et al. 2018, Frazier et al. 2024), and wetland vegetation (Polley and Collins 1984, Umbanhowar Jr. 1992, Horne et al. 2024). Data on wallows is sparse, but the few studies on this topic have found that physically, wallows have distinct and often more variable soil conditions than surrounding

grasslands, including higher salinity and clay content and reduced plant cover (Polley and Wallace 1986, Coppedge and Shaw 2000; Bookout et al. *In review*).

Wallows represent an acute disturbance. Unlike most other disturbances, which many herbaceous grassland plants are adapted to, such as fire and grazing, wallowing removes nearly all plant life for some time, even roots (McMillan et al. 2000, Coppedge and Shaw 2000). This disturbance is recurring in many wallows, unless bison choose to abandon wallows for seasons to years, leaving behind a legacy of reduced soil organic matter after years of reduced plant material (Bookout et al., *In review*). Considering that soil conditions often affect microbial community composition and function, wallowing should directly alter soil communities. For example, seasonal drought or pulse nutrient additions can cause transient shifts in microbial composition or metabolic function (Collins et al. 2008, Shade et al. 2012). Tilling and mixing of soils can disrupt microbial spatial structure, reduce community resistance to disturbance, and homogenize communities by removing microbial niches as soil aggregates are broken down (Shade et al. 2012, Vos et al. 2013, Fierer 2017). Soil compaction, through trampling or large grazers, reduces pore space within soils, reducing gas exchange and soil moisture, which can reduce microbial activity and diversity (Hamza and Anderson 2005, Shade et al. 2012, Chen et al. 2024). Wallows share many characteristics with these well-studied examples of external soil changes affecting soil communities.

Despite the likely ecological significance of wallows for belowground communities, we are unaware of any studies that have investigated the microbial communities associated with bison wallows. Given their distinctiveness, wallows likely support microbial communities that differ from surrounding soils. By studying a natural, behaviorally-mediated disturbance embedded in a long-term experimental grassland landscape, we evaluate how physical

disturbance, soil variation, and biotic communities interact to structure microbial communities. This work contributes to broader questions in community ecology about how disturbance, environmental context, and co-limitation of multiple resources shape microbial diversity and composition.

This study asked: (1) Do wallows alter soil microbial community composition and diversity? With high selective pressures of low nutrients, high salinity, and repeated disturbance, we hypothesized that wallows have reduced microbial diversity due to environmental filtering (West and Whitman 2022, Chen et al. 2024). We hypothesized that physical disturbance of wallows and altered soil microhabitats would shift microbial communities away from soil microbial communities of the surrounding grassland. We expected that these communities would be more similar to one another due to strong filtering effects of disturbance. Alternatively, wallow communities might have higher beta-diversity, reflecting high variability in soil conditions across wallows. (2) Second, does the removal of bison grazing and disturbance (via fenced exclosures) alter microbial communities in two years? We hypothesized that fencing, by removing disturbances and large grazers, would also lead to different microbial communities if they respond quickly to changes in nutrient availability and/or plant communities. However, we also expected that there might be little response to fencing because microbial communities, especially as detected through rRNA sequencing, are slow to respond to environmental shifts.

4.3 Methods

4.3.10 Site Description:

This study took place at Konza Prairie Biological Station (KPBS) in eastern Kansas, USA (39.1069° N, 96.6091° W). KPBS is an experimentally-managed remnant tallgrass prairie located in the northern Flint Hills ecoregion, which contains the largest remaining (nearly)

continuous tracts of tallgrass prairie in North America (Knapp et al. 1998). The soils at KPBS were not glaciated. Topography includes lowlands with deeper soils, clayey loams typified by the Tully series, and shallow clayey rocky uplands with a shallow profile typified by the Florence-Benfield complex. These two topographic positions are connected by slopes with deeper but heterogeneous soils, and flat benches with intermediate soil characteristics. The hilly topography and rocky soils preclude plowing at Konza and much of the Flint Hills. KPBS has a temperate mid-continental climate with hot summers ranging to 27 °C with mean annual precipitation of 899 mm, most of which occurs during the growing season from April to September. Native prairie grass species, such as big bluestem (*Andropogon gerardii*), yellow prairie grass (*Sorghastrum nutans*), little bluestem (*Schizachyrium scoparium*), and switchgrass (*Panicum virgatum*), are dominant. However, the community includes many subdominant species such as perennial asters (*Ambrosia psilostachya* and *Symphyotrichum ericoides*), and a common flowering sub-shrub from the Fabaceae family (*Amorpha canescens*). Bison and cattle grazed communities sometimes have much higher abundance of short grass species (e.g., *Bouteloua spp.*).

KPBS features a factorially crossed grazing and fire return interval (FRI) experiment with ungrazed, bison-grazed, and cattle-grazed experimental units (Figure 4.1). These experimental units are further divided into catchment-level treatments with differing long-term prescribed fire return intervals—1, 2, 3-4, and 20-year FRIs ("catchments" from here on). We collected the data for this study in 2021 and 2022 in 1 and 3-4-year FRI catchments in bison-grazed pastures (see catchments marked with an asterisk in Figure 4.1). Bison-grazed treatments have higher richness and lower graminoid cover than frequently burned areas without large grazers (Ratajczak et al. 2022, Bookout et al. 2025). Less frequently burned (3-4 FRI) catchments have more woody

cover than annually burned catchments (Ratajczak et al. 2014), though all plots in this study were placed in herbaceous locations.

At KPBS, bison were reintroduced into a fenced area of 500 ha in 1987 and expanded to 1100 ha in 1992. The bison herd is present year-round, free-ranging among fire treatments, and unmanaged except for an annual round-up to cull excess bison and inoculate them for common bovine diseases. Burning annually is also common in the Flint Hills as it increases graminoid production (Collins and Calabrese 2012) and decreases woody encroachment (Ratajczak et al. 2012), which translates to consistent cattle weight gain for ranchers (Owensby et al. 2008). All grazing treatments maintain grazer densities meant to remove approximately 25% of ANPP (Knapp et al. 2012).

4.3.11 Plot selection:

In total, our study included 96 plots spanning combinations of fire return interval, patch type (wallow vs matrix grassland), and fencing treatment. In late 2020, we identified two distinct benches in each catchment. Within each bench, we established six plots of each factorial combination of vegetation type (wallow and matrix grassland) and fencing treatment (fenced and unfenced). Across all bison-grazed plots, we fenced a total of 48 plots, 24 wallows and 24 matrix grassland plots (12 per fire regime), to mimic aspects of historical abandonment and allow plant recolonization.

For sampling in bison-grazed areas, we started by identifying actively used wallows and selected wallows with a clear boundary that were approximately 8 m^2 (±1 m^2) in area, which is the average size of recorded wallows on site (Horne et al. 2024). We only used wallows that contained less than 10% live plant cover in the interior, indicating their active use. To control for topographic effects, all wallows were located on one of two mid-elevation benches in catchments

with FRIs 1 year or 3 to 4 years (with centroids of these benches marked as stars in Figure 4.1). Along with flat uplands, most bison wallows are formed on benches and other areas with flat topographies (Horne et al. 2024). Wallows within 10 m of roads or mown fire breaks were excluded to minimize edge effects. Matrix grassland plots (8 m^2) were randomly placed ~5 m from their paired wallow while ensuring a minimum distance of ~5 m from other wallows or other disturbances (e.g., erosive gullies). We marked each plot with a piece of metal conduit, placed at the center for matrix grassland plots and all fenced plots or at the edge for unfenced wallows to minimize loss of markers due to wallowing.

4.3.12 Vegetation Data:

Each of the permanently marked plots contained eight subplots for plant monitoring beginning in June 2021. These subplots were stratified to capture both inner (within the interior of the wallow) and edge (the boundary between disturbed and undisturbed area) microhabitats (Figure 4.1), though for this study we only used plant data from the inner subplots. Each subplot was 0.25 m^2 and was systematically arranged along four evenly spaced transects radiating outward from the plot center at 90-degree intervals. Matrix grassland plots followed the same layout to maintain consistency. We conducted vegetation measurements in early June and August to capture seasonal variation in plant communities, capturing both spring ephemerals and species that peak in cover during the late-season (Collins and Calabrese 2012, Ratajczak et al. 2022). We identified plants to the taxonomic level of species, except for sedges and a few other species that were identified to genus. We assigned each species a cover class using a modified Daubenmire scale (1: 0-1%, 2: 1-5%, 3: 5-25%, 4: 25-50%, 5: 50-75%, 6: 75-95%, 7: 95-100%). When a species was recorded in both sampling periods, we used the larger of the two cover values for

subsequent analyses. Mid-points of cover classes were used for any measurements that require species cover.

4.3.13 Soil Sampling and Analysis:

We collected soil samples in early September 2021 and 2022. In each plot, we took four mineral soil cores (6 to 10 cm depth, 1.27 cm diameter), positioned between the inner and edge vegetation subplots to minimize disturbance. This depth captures zone with highest root biomass of many dominant plant species, although many species (especially forbs) often have deeper roots (e.g., Nippert and Knapp 2007; Nippert et al. 2012; O'Keefe and Nippert 2017), as well as the zone where most microbial activity and biomass occurs (Lavahun et al. 1996). Soil moisture was measured *in situ* at each of the four sampling locations using a HydraGO soil moisture probe (Stevens Water Monitoring Systems). We averaged these values to obtain a single moisture estimate per plot.

We aggregated soil cores at the plot level, sieved them to remove large roots and rocks (to 4 mm mesh), and refrigerated them at 4 °C to minimize microbial transformations prior to analysis. The Kansas State University Soil Testing Laboratory analyzed all samples for nutrient content, soil organic matter, cation concentrations, pH, and soil texture. Nutrient content included phosphorus (Bray P test using HCl-ammonium fluoride extraction), and total nitrogen and carbon (LECO TruSpec CN combustion analyzer), ammonium-N, and nitrate-N. Organic matter was measured using loss-on-ignition at 400 °C for 3 hours after drying at 150 °C for 2 hours. Cations included Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, which were extracted with 1 M ammonium acetate and analyzed by ICP Spectrometry (Robertson 1999). The hydrometer method was used to measure soil texture after using sodium hexametaphosphate as a dispersing agent. In four plots, we did not collect enough soil for texture analyses. Rather than exclude these, we

interpolated values by calculating the mean texture values of plots within the same patch type and fencing treatment.

4.3.14 DNA Extraction and Sequencing

Using approximately 0.5 g of homogenized soil per sample, we extracted total genomic DNA (gDNA) using the Qiagen DNeasy PowerSoil kit (Qiagen Sciences, Germantown, MD, USA). We followed the manufacturer's instructions with the exception of these modifications: PowerBead Tubes were shaken in a MP biomedicals sample disruptor for 20 s set at 4 m/s velocity, the supernatant was transferred using the recommended minimum volume (~400 µL) and for the final DNA elution step 50 µL of solution C6 was added and incubated for 5 minutes at room temperature before spinning down. These modifications followed practices designed for this ecoregion (Hawkins and Zeglin 2022). gDNA was stored at -20°C prior to library prep and sequencing at Kansas State University's Integrated Genomics Facility. The facility prepared separate amplicon libraries following the Illumina recommended protocols. We targeted the V4 region of the 16S rRNA gene using universal archaeal/bacterial primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT), and the internal transcribed spacer (ITS) region of the fungal rRNA operon using standard fungal ITS primers (fITS7: 5'-GTGARTCATCGAATCTTTG-3'; ITS4: 5'-TCCTCCGCTTATTGATATGC-3'). These libraries were sequenced on an Illumina MiSeq platform using 2x250 cycles for 16S and 2x300 cycles for ITS. All sequence data were received demultiplexed and with primer sequences removed.

We processed raw Illumina sequence data using QIIME2 v2022.8 (Bolyen et al. 2019). We inferred amplicon sequence variants (ASVs) using the DADA2 pipeline (Callahan et al. 2016) within QIIME2, including quality filtering, denoising, chimera removal, and paired-end

read joining. We performed taxonomic assignment using the SILVA 138 reference database (Quast et al. 2013) for 16S rRNA gene sequences and the UNITE database (Abarenkov et al. 2021) for ITS sequences. We imported ASV tables and taxonomic assignments into R for downstream filtering and analyses. Low-read samples (<10,000 for 16s rRNA sequences and <5,000 for ITS sequences) were filtered out prior to downstream analyses. No rarefaction or normalization was applied prior to beta-diversity analyses because rarefaction can remove information, add artificial uncertainty, and does not improve statistical performance (McMurdie and Holmes 2014). These unrarefied datasets included 187 samples with 12,489-47,534 reads per sample, for a total of 5,167,093 reads and 74,747 ASVs for 16s and 186 samples with 5,608-125,736 reads per sample, for a total of 5,583,585 reads and 16,795 ASVs for ITS. However, for alpha diversity metrics, we rarefied samples to the minimum library size using rarefy_even_depth in *phyloseq* (McMurdie and Holmes 2013) to minimize effects of sampling depth (Schloss 2023). These final rarefied datasets included 187 samples with 12,489 reads per sample, for a total of 2,335,443 reads and 70435 ASVs for 16s and 186 samples with 5,608 reads per sample, for a total of 1,048,696 reads and 14,857 ASVs for ITS.

4.3.15 Data Analysis

For each dataset (soil, plant, prokaryote, and fungal), we constructed a site-by-variable matrix for downstream analyses, where each row represented an individual plot (n = 187-194 depending on the dataset). Columns represented were either individual soil characteristics (e.g., pH, organic matter), plant species, or microbial ASVs, depending on the dataset. This approach yielded a single, composite value per plot for each soil property, plant species cover, or microbial ASV reads abundance, ensuring consistent sample size across all patch types.

Effect of habitat type and fencing treatment on univariate variables: To assess how soil physiochemistry and plant, prokaryotic, and fungal communities varied across patch types and fencing treatments, we tested for differences in soil characteristics, alpha diversity (richness and Hill-Shannon diversity), and plant functional group metrics. We included 15 soil characteristics: organic matter (%), total carbon (%), total nitrogen (%), nitrate-N (ug g^{-1} dry soil), ammonium-N (ug g^{-1} dry soil), phosphorus (ug g^{-1} dry soil), potassium (ug g^{-1} dry soil), magnesium (ug g^{-1} dry soil), sodium (ug g^{-1} dry soil), calcium (ug g^{-1} dry soil), clay (%), silt (%), sand (%), and soil moisture (%). Alpha diversity included species richness, which we calculated as either the number of plant species or the number of observed bacterial/archaeal or fungal ASVs per plot (using specnumber in *vegan*; Oksanen et al. 2024). We also calculated Hill-Shannon diversity, which decreases the influence of rare species, by exponentiating Shannon diversity index (using diversity in *vegan*) as defined as $H = -\sum_{i=1}^{s} p_i \, log_b \, p_i$, where p_i is the proportion of species i and S is the number of species so that the $\sum_{i=1}^{s} p_i = 1$, and b is the base of the logarithm.

For each response variable defined above, we used two-way ANOVAs (aov in *stats* package; R Core Team 2024) followed by Tukey's HSD post hoc comparisons (TukeyHSD in *stats* package) to test for the effects of patch type and fencing treatment. The model included patch type (wallow or matrix grassland), fencing, and their interaction.

Multivariate community composition*:* To visualize differences in community composition and structure among patch types, we conducted principal coordinates analyses (PCoA; pcoa in *ape*; Paradis et al. 2024) on Bray-Curtis dissimilarities for plant, prokaryote, and fungi communities. Differences in communities by patch type (matrix grassland, wallow), fencing, and their interaction, were tested using PERMANOVA (adonis2 in *vegan* package). To evaluate for differences in community dispersion (beta diversity) among patch types and fencing treatments,

we calculated multivariate dispersion using the betadisper function (*vegan* package) on the same dissimilarity matrices as the PERMANOVA. We then tested for significant differences in the distance to group centroid using one-way ANOVAs, followed by Tukey's HSD for pairwise comparison. We did this twice – once for patch type differences and once for patch type and fencing interactive differences to parse out the effects of fencing within habitat types.

To explore among-group associations among plant and microbial communities as well as with soil characteristics, we conducted pairwise Mantel's tests (mantel in *vegan* package) using the Bray Curtis dissimilarity matrices for plant, prokaryotic, and fungal communities and Euclidean distance matrix on scaled soil data (scale in base R). To test how the soil environment, plant communities, patch type, or their interactions explained variation in prokaryotic or fungal communities, we used variance partitioning after removing collinear variables. To assess which soil properties were most influential in shaping microbial communities, we used canonical correspondence analyses (CCA; cca in *vegan*) with soil variables included as environmental constraints after using stepwise selection (forward and backward; CCA) to reduce the soil properties used.

4.4 Results

4.4.16 Soil physiochemistry by habitat type and fencing

Most soil physiochemical properties differed by patch type ($p < 0.05$; Figure 4.2; Table S4.1): Soil organic matter, % C, % N, molar C:N, nitrate-N, ammonium-N, K^+, % sand, and % silt were higher in the surrounding grassland than in wallows. Extractable P, Mg^+, Na^+, and % clay were higher in wallows than in matrix grassland. The three variables that were not significantly different due to patch type were calcium, ammonium, and pH. Fencing treatment affected fewer soil variables ($p < 0.05$; Figure 4.2; Table S4.1): Fencing decreased nitrate-N but increased

ammonium-N, and increased % clay relative to % silt and % sand. Only soil moisture showed a significant interactive effect between fencing and patch type, with lower moisture in unfenced matrix grassland plots compared to both fenced and unfenced wallows ($p < 0.05$).

4.4.17 Plant and soil microbial alpha diversity

Plant species richness and Hill-Shannon diversity were both approximately 50% lower in wallows than matrix grassland plots, on average ($p < 0.05$; Figure 4.3). Fenced plots had higher alpha diversity than unfenced plots ($p < 0.05$). However, fencing had opposite effects on wallows and grassland plots (interaction: $p < 0.05$): fencing increased plant richness and diversity in wallows, but decreased them in matrix grassland plots.

Soil prokaryote ASV richness and Hill-Shannon diversity were both higher in wallows compared to surrounding grassland, and were higher in fenced than unfenced treatments, with no interaction ($p < 0.05$, Figure 4.3). In contrast, soil fungal alpha diversity showed interactions between patch and fencing ($p < 0.01$), and only in observed richness was there an effect of patch type alone ($p < 0.05$, Figure 4.3). In wallows, fencing increased fungal ASV richness and Hill-Shannon diversity, while in the matrix grassland plots, fencing either did not affect or decreased fungal ASV richness and Hill-Shannon diversity.

4.4.18 Plant and soil microbial beta diversity

More plant community variation was explained by soil variables than microbial community variation, which is typical due to the inherent high dimensionality, variability, and sparseness of large microbial datasets (Bardenhorst et al. 2021). Plant communities differed significantly between wallows and surrounding grasslands ($R^2 = 0.23 > 0.10$; Table 4.1), and had higher dispersion across wallows compared to surrounding grassland plots (58.9% farther from

centroid; $p < 0.05$; Figure 4.4). Fencing had a weaker direct and interactive influence on plant communities ($R^2 = 0.02, 0.03$, Table 4.4). Soil prokaryote communities also differed more between wallows and the surrounding grassland than in response to fencing or the interaction between patch type and fencing ($R^2 = 0.03, 0.01, 0.01$, respectively; Table 4.4). Soil fungal communities followed a similar pattern, with an even stronger effect of grassland versus wallow ($R^2 = 0.08, 0.01, 0.01$, respectively; Table 4.4). Soil bacterial and archaeal communities were significantly more variable (4.2%; $p<0.01$) in wallows compared to matrix grassland plots, but fungal community dispersion was similar in wallows and matrix grassland plots ($p>0.05$).

Plant community variance was independently explained by $_{\text{patch type}}$ and soil (4% and 6%, respectively), and also by their interaction (9%) (VPA; Figure 4.4). In contrast, soil prokaryote community variance was not explained by patch type or plant community variance, but was weakly explained by soil (1%), interactions between soil and plant variance (1%), and a three-way interaction with patch type (1%) (Figure 4.4). Fungal community variance was best explained by plant community variance (7%), followed by soil physiochemistry (3%), the interaction between soil and plant composition (3%), the interaction between plant composition and patch type (1%), and the three-way interaction (3%) (Figure 4.4). Both soil prokaryote and fungal community distances were more strongly correlated with soil distances than plant community distances (Mantel test, Table 4.2). The variation in bacterial and archaeal communities was most explained by changes in magnesium, sodium, pH, and organic matter (CCA; Figure S4.1). For fungal communities, organic matter, magnesium, sodium, potassium, and calcium were the strongest predictors of community change (Figure S4.2).

4.4.19 Plant and microbial gamma diversity

Most plant species were shared between wallows and surrounding grassland (62.2%), with only 12.6% and 25.3% of species unique to either wallows or matrix grassland, respectively (Table 4.3). The plants unique to wallows tended to be wetland species, and species adapted to especially high disturbance, including *Bacopa rotundifolia*, *Cyperus acuminatus*, and *Euphorbia marginata*. However, a majority of microbial ASVs were unique to either wallows or matrix grassland, with only 10.9% of all prokaryotic ASVs and 30.0% of all fungal ASVs shared between the two (Table 4.3). Compared to the grassland matrix, wallows had a higher proportion of unique prokaryotic ASVs (48.9 versus 40.2%) and a lower proportion of unique fungal ASVs (31.7% versus 38.3%; Table 4.3).

4.5 Discussion

Patch type—wallow versus matrix grassland—emerged as the strongest driver of plant and microbial community composition. Wallow soils differed markedly from surrounding grassland soils, having lower organic matter and nutrients but higher clay content and salinity. Plant communities in wallows were less alpha diverse but were more variable compared to the surrounding prairie. Wallow plant community composition was also distinct from grasslands. Microbial communities also varied by patch type, with bacterial and archaeal beta diversity (beta-dispersion) highest in wallows, but similar fungal beta diversity across both patch types. Bison exclusion, by fencing, had the largest impact on plant communities, simultaneously increasing alpha diversity in wallows and decreasing alpha diversity in matrix grassland plots. Fencing slightly decreased prokaryote alpha diversity, but had less consistent effects on fungal alpha diversity. Our results suggest that changes in soil characteristics significantly impact soil

microbial communities, and to some extent, the altered plant communities found in bison wallows when compared to surrounding grassland plots.

4.5.20 Effects of patch type

In this study, patch type emerged as a primary driver of heterogeneity across soil, plant, and microbial communities. One of the biggest differences between wallows and matrix grassland were in their soil characteristics. Wallows had higher clay content, likely due to bison removing topsoil, exposing subsoil layers, and compacting soil (Polley and Wallace 1986, Coppedge and Shaw 2000). Wallows also had higher salt concentrations than surrounding soils, which is likely the result of evaporative concentration of salts (Liu et al. 2021), inputs by bison urine and dung (Michelini et al. 1999), and higher clay content, allowing wallow soils to hold more cations than soils with less clay. There is also evidence that large herbivores in Africa sometimes seek out more sodic sites to form grazing lawns and wallows (Hempson et al. 2015), which we suspect bison do as well (Tracy and McNaughton 1995; see Hornaday 1889 for an account from the 1800s).

Continuous physical perturbations by bison in wallows led to decreased plant alpha diversity and limited plant inputs, evidenced by low organic matter, total carbon, and total nitrogen compared to surrounding grassland soils. These findings are consistent with previous wallow studies that also found low nutrient pools within wallows (McMillan 1999; Bookout et al *In review*). Plant communities were most variable among wallow plots, indicating that disturbance did not affect these communities uniformly. This increase in heterogeneity disagrees with some general concepts in disturbance ecology, where very frequent disturbance is generally thought to support a narrow set of species (Grime 1988, Turner and Gardner 2015). For instance, frequent fire in grasslands often results in low-diversity plant communities in tallgrass prairies,

unless there are mitigating factors such as high soil heterogeneity or ungulate grazing (Towne et al. 2005, Collins and Calabrese 2012, Baer et al. 2020). We speculate that this large dispersion in wallows reflects priority effects, where the weedy species that are first able to reach wallows become dominant (Fukami 2015). For instance, several species that commonly occupy wallows are annual forbs, such as ragweed (*Ambrosia artemisiifolia*), buffalo bur (*Solanum rostratum*), and snow on the mountain (*Euphorbia marginata*).

Niche differentiation may also drive some of the dispersion in wallow plant communities. Across wallows, we found wide variation in soil nutrients, soil texture, and most notably, soil moisture (Figure 4.2, Bookout et al. *In review*). Wallows span a range from very xeric plots where soil moisture is exceptionally low, to plots that hold standing water for days to weeks at a time (Figure 4.1c; Bookout et al, *In review*). Standing water in this ecosystem is rare outside of riparian corridors and ephemeral streams, except for occasional seeps, human-made livestock troughs or ponds, and some naturally occurring ponds (Lehner and Döll 2004, Downing et al. 2006, Lehner et al. 2011, Grill et al. 2019). Therefore, wallows represent an important water feature on the landscape, where we found wetland plants in the 13% of wallows that often held water (Bookout et al. *In review*). Together, the presence of disturbed patches and increased heterogeneity on the landscape seems to offset much of the local homogenizing effects of frequent wallowing disturbances, resulting in a wide range of plant communities.

For bacteria and archaea, most taxa were unique to either wallows or matrix grassland plots, with relatively few shared ASVs across both patch types. However, the large degree of overlap observed in the PCoA suggests that the shared ASVs were the dominant taxa shaping overall community structure, while rare taxa contribute more to gamma diversity. This pattern is partly an artifact of using DNA sequences that capture both dormant and active taxa (Carini et al.

2016). However, this pattern also aligns with metacommunity theory, where dominant microbial taxa often display broad environmental tolerance and dispersal, while rare taxa are more likely to be shaped by local environmental filters or stochasticity (Riddley et al. 2025). Similar patterns have been observed in prairie and agricultural soils, where dominant bacteria were often more cosmopolitan, but subdominant and rare taxa show greater patch type specificity (Kaminsky and Morales 2018, Jiao et al. 2019, Wu et al. 2021).

Fungal communities followed a contrasting pattern: though more ASVs were shared across patch types, fungal communities were more clearly separated in ordination space. The relatively high ordination separation despite shared ASVs suggests that these shared taxa may respond differently in abundance across patch types, potentially reflecting functional differentiation rather than wholesale taxonomic turnover, which we also see in the plant communities. This difference in microbial groups may reflect the stronger dependence of fungi on plant community composition and plant-derived organic matter inputs compared to bacteria and archaea (Lauber et al. 2008, LeBlanc et al. 2015). Fungal community composition was more strongly influenced by plant community composition (Figure 4.4) and soil organic matter (Figure S4.1-4.2), which is consistent with previous studies showing that fungi are more responsive to plant-derived inputs, litter chemistry, and host specificity than prokaryotes (Tedersoo et al. 2014, Bahram et al. 2018, Lutz et al. 2025).

4.5.21 Removal of grazing and wallowing disturbance

Fencing resulted in strong effects on plant communities, simultaneously increasing alpha diversity in wallows and decreasing it in surrounding grassland plots. When bison were prevented from using wallows, plants recolonized the bare ground, taking advantage of open niche space (Trager et al. 2004, McMillan et al. 2011). However, when bison were prevented

from grazing the surrounding grassland, plant alpha diversity decreased, likely due to the removal of keystone herbivory, as bison preferentially consume the dominant grasses (Raynor et al. 2015), which would otherwise shade out and out-compete subdominant forbs and grasses. This decline was a much faster response than we expected. For instance, it took decades for many of these species to accumulate (Ratajczak et al. 2022, Bookout et al. 2025), but many were lost in a single year (at least aboveground). However, a previous study on-site also found a rapid decline in plant species richness after building similar bison exclosures (Koerner et al. 2014), suggesting that the ability to lose species rapidly might be common in this ecosystem. However, we cannot say yet whether plant species have yet to lose the ability to bounce back if grazing returns, because some belowground meristems and seeds are numerous and long-lived in tallgrass prairie (Dalgleish and Hartnett 2006).

Fencing increased prokaryote alpha diversity, which aligns with findings in other grassland systems, though the response in temperate grasslands appears more muted than in drier grasslands, where excluding grazing had strong positive effects (Shu et al. 2024). However, our results are similar to other studies in grazed and ungrazed tallgrass prairie (Zaret et al, *In review*), where long-term ungrazed areas had higher bacterial and archaeal diversity compared to areas bison had grazed for 30 years (Zaret et al, *In review*). The speed of this response, within two years of fencing, suggests rapid shifts in microbial communities, possibly driven by changes in bison-mediated nutrient inputs and shifts in plant communities. Thus, we might expect the differences between fenced and unfenced plots to increase with time, though wallows and the surrounding grassland might converge over time if grassland plant communities begin to return and soil organic matter starts to accumulate in fenced wallows.

Fencing had more complicated implications for fungal communities. In wallows, fungal alpha diversity increased with fencing, paralleling the assembly of the plant communities, which are often key players in changes in fungal richness through the provision of litter, root exudates, and mycorrhizal hosts (Tedersoo et al. 2014, Prober et al. 2015, LeBlanc et al. 2015). However, in the surrounding grassland plots, fencing decreased or had little effect on fungal alpha diversity. This divergence may reflect the dual influence of plant recolonization and changes in organic matter on fungal community dynamics, as fungal guilds differ in their resource requirements and dispersal abilities (Glassman et al. 2017, Egidi et al. 2019). For example, saprotrophic and mycorrhizal fungi are more sensitive to changes in plant diversity and host identity (Heinemeyer et al. 2004, LeBlanc et al. 2015, Dassen et al. 2017). Mycorrhizal fungi may initially decline if early-successional plant species with low mycorrhizal dependence dominate recolonizing plots (Kokkoris et al. 2020). However, these relationships are context dependent, where disturbance may modulate the relative influence of biotic vs abiotic factors on fungal community assembly (Bowd et al. 2021, Schlatter et al. 2023).

4.6 Conclusion

Our results support the role of patch-scale disturbances in enhancing plant and microbial heterogeneity in mesic grasslands. Bison wallows created persistent and spatially variable disturbance regimes, altering both the abiotic and biotic environment in ways that favored distinct plant, bacterial and archaeal, and fungal communities, which increased gamma diversity. For plant communities, the most surprising results were the high degree of compositional dispersion between wallows and how rapidly the plant community changed after fencing. Bacterial communities responded much like we expected, with shifts that follow large differences in soil physiochemical changes. However, we did not expect the sheer number of

taxa that would be unique to wallows (48.9%), compared to the larger communities in which they occur. The same held for fungi—31.7% of taxa in wallows were never encountered in samples in the grassland matrix soils. We found that fungal communities were more idiosyncratic than bacteria, suggesting that their composition might be associated with patchy plant communities. Together, our results showed that despite plants, bacteria, and fungi experiencing the same set of disturbances and altered soil characteristics posed by wallows, the response of each community followed different paths. Our results reemphasize that reintroducing native megafauna often leads to a multi-faceted increase in heterogeneity in grassland ecology, which extends through soils, bacteria, fungi, and plants. With this being the first study on microbial communities in bison wallows, to our knowledge, we remain excited to see if these results hold across the extensive range of environmental conditions that bison formerly occupied.

4.7 References

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4.8 Tables

Table 4.1. Effects of patch type (wallow vs surrounding grassland) and fencing (fenced vs unfenced) on multivariate plant and microbial communities (Bray-Curtis) using a PERMANOVA.

PERMANOVA **(df, F, R^2, p)**	**Patch type**	**Fencing**	**Patch type *** **Fencing**
Plant	1, 59.4, 0.23, < 0.001	1, 4.3, 0.02, < 0.001	1, 6.9, 0.03, < 0.001
Bacteria & archaea	1, 3.9, 0.02, < 0.001	1, 1.2, 0.01, < 0.01	1, 1.2, 0.01, < 0.02
Fungi	1, 11.8, 0.06 < 0.001	1, 2.6, 0.01, < 0.001	1, 1.8, 0.01, < 0.003

Table 4.2. Multivariate correlations between plants, bacteria and archaea, fungi, and soil data using a Mantel test.

Comparison	**Mantel R**	**p-value**
Plant vs Prokaryote	0.556	0.001
Plant vs Fungi	0.483	0.001
Plant vs Soil	0.574	0.001
Prokaryote vs Fungi	0.567	0.001
Prokaryote vs Soil	0.637	0.001
Fungal vs Soil	0.549	0.001

Table 4.3. Unique taxa and gamma diversity for plants and microbes within wallows and the surrounding grassland.

	Bacteria and archaea		Fungi		Plants	
Patch type	# of ASVs[1]	% of total ASVs[2]	# of ASVs[1]	% of total ASVs[2]	# of species[1]	% of total species[2]
Wallow	44,719 (36,583)	59.8% (48.9%)	10,337 (5,331)	61.5% (31.7%)	95 (16)	74.8% (12.6%)
Matrix grassland	38,164 (30,028)	51.1% (40.2%)	11,444 (6,438)	68.1% (38.3%)	111 (32)	87.4% (25.2%)
TOTAL	74,747	100%	16,795	100%	127	100%

[1]Total number of taxa within each patch type with the number of unique taxa to that patch type in parentheses; [2]Percent of total ASVs found in each patch type with the percent of unique taxa to that patch type in parentheses.

4.9 Figures

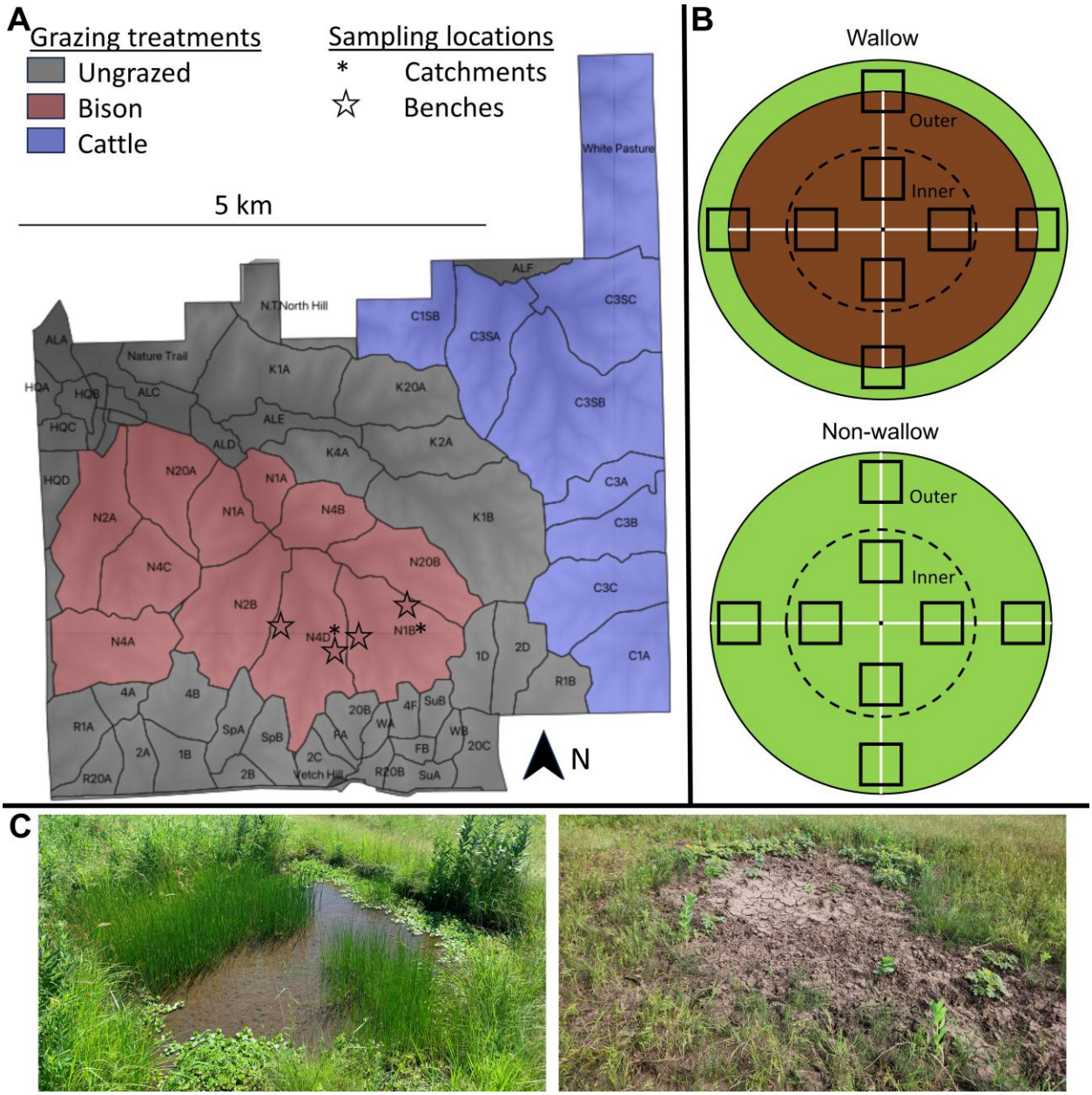

Figure 4.1. A) Map of grazing treatments at Konza Prairie with general sampling locations. B) Plot design for vegetation and soil data collection. Only plant composition in the four inner subplots were used in this study. Soil samples were taken just outside the inner subplots. C) examples of bison wallows that can hold water (left) or remain dry (right). Photos taken by B. Bookout.

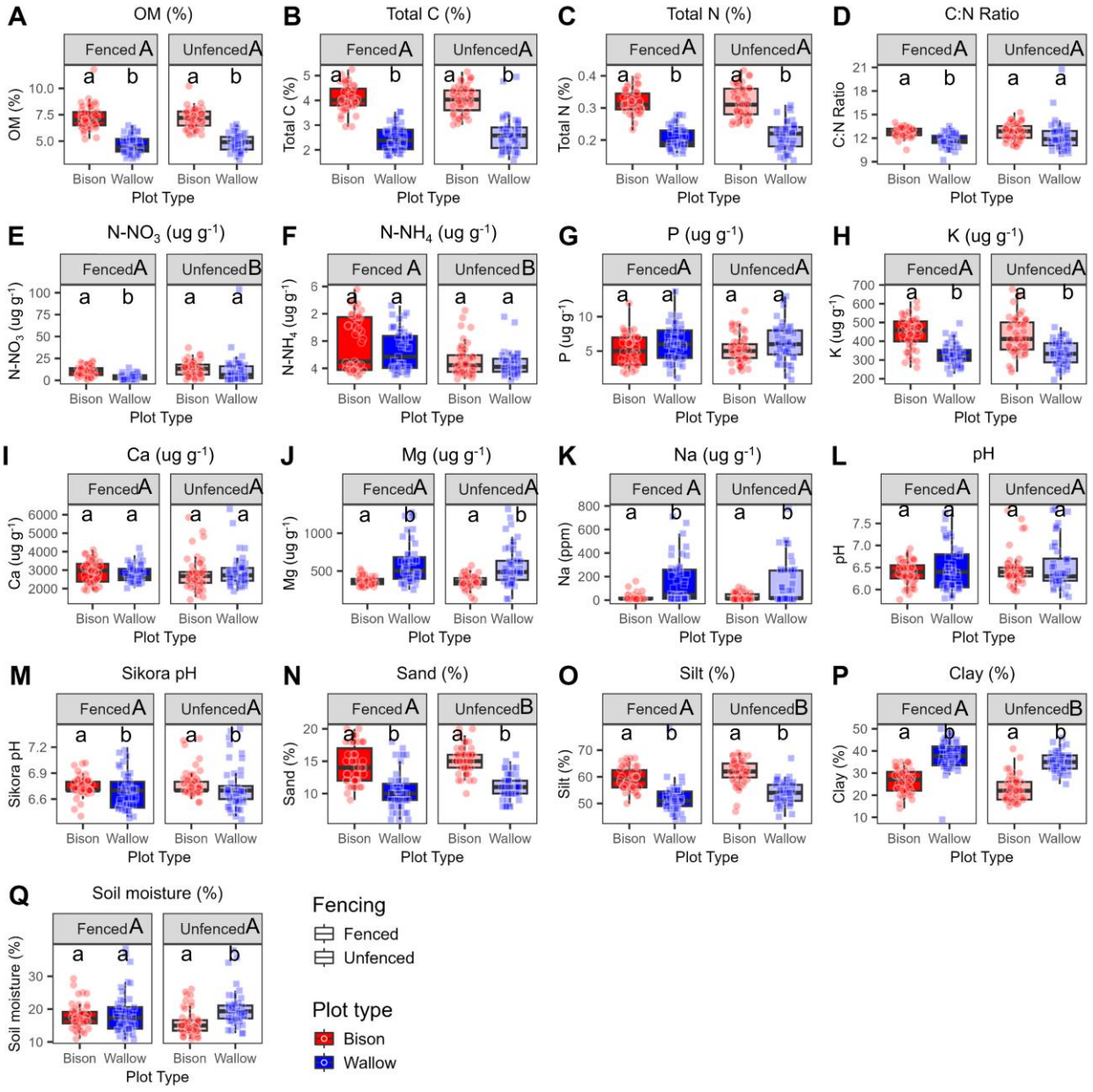

Figure 4.2: Patch type and fencing effects on soil physiochemistry. A) Soil organic matter content (%) by loss-on-ignition. B) Total carbon (%) by combustion analyzer. C) Total nitrogen (%) by combustion analyzer. D) Nitrate-N (ug g^{-1} dry soil). E) Ammonium-N (ug g^{-1} dry soil). F) molar C:N. G) Phosphorus (ug g^{-1} dry soil). H) Potassium (ug g^{-1} dry soil). I) Calcium (ug g^{-1} dry soil). J) Magnesium (ug g^{-1} dry soil). K) Sodium (ug g^{-1} dry soil). L-M) pH and adjusted pH (Sikora pH). M-N) Soil texture (% of each component). O) Soil moisture (%) taken in the field at the time of sampling. Capital letters indicate significant differences between fencing treatments and lowercase letters above boxplots indicate significant differences between habitat types within a certain soil variable (two-way ANOVA). Only soil moisture had a significant interactive effect of habitat and fencing ($p<0.05$)

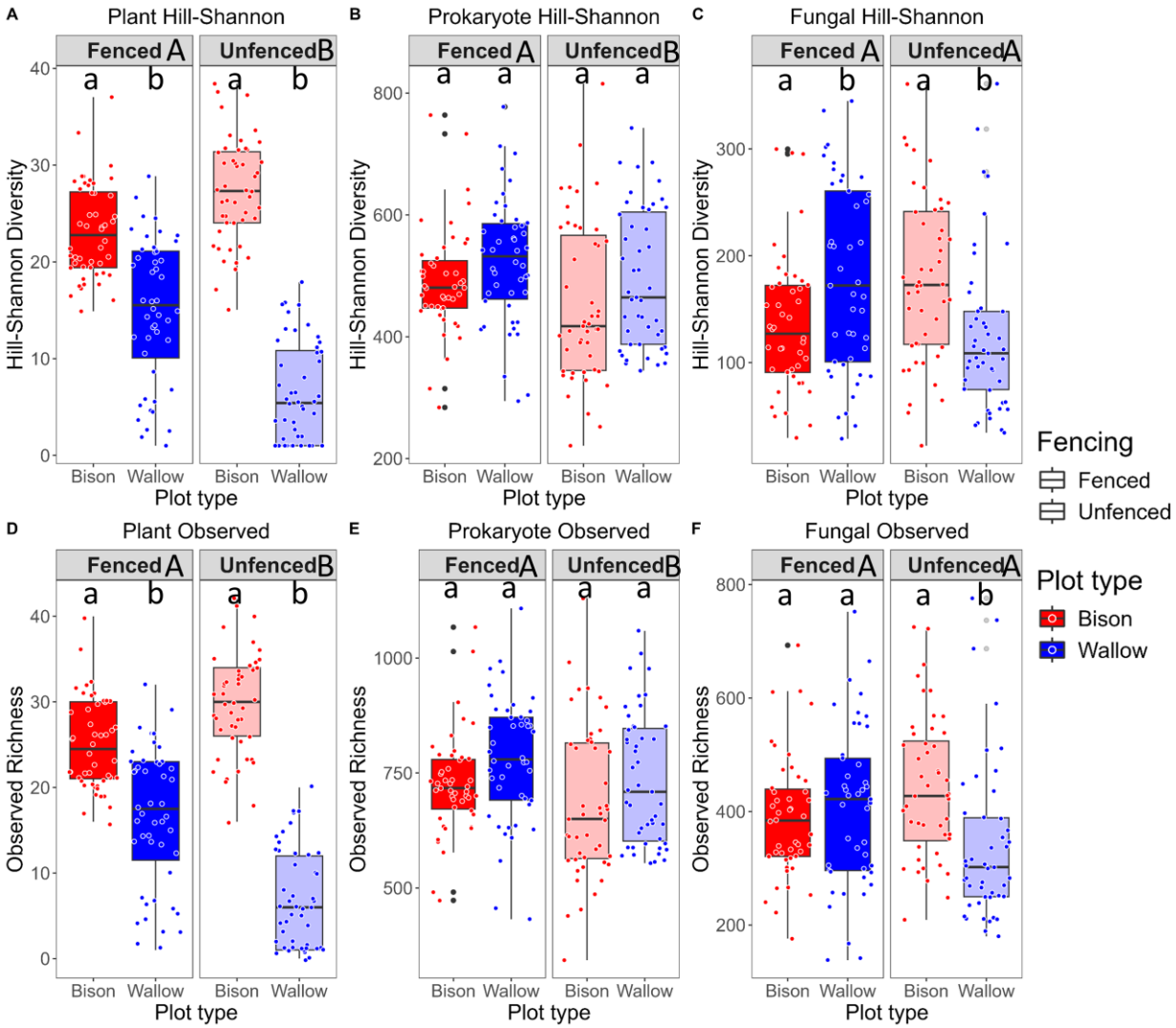

Figure 4.3: Alpha diversity of plants (A,D), bacteria/archaea (B,E) and fungi (C,F). Richness is the number of unique species (for plants) or ASV's (for microbes) within each plot. Hill-Shannon is the exponentiated Shannon diversity index. Capital letters indicate significant differences between fencing treatments and lowercase letters above boxplots indicate significant differences between habitat types within a alpha diversity metric (two-way ANOVA). "Bison" refers to the surrounding grassland plots.

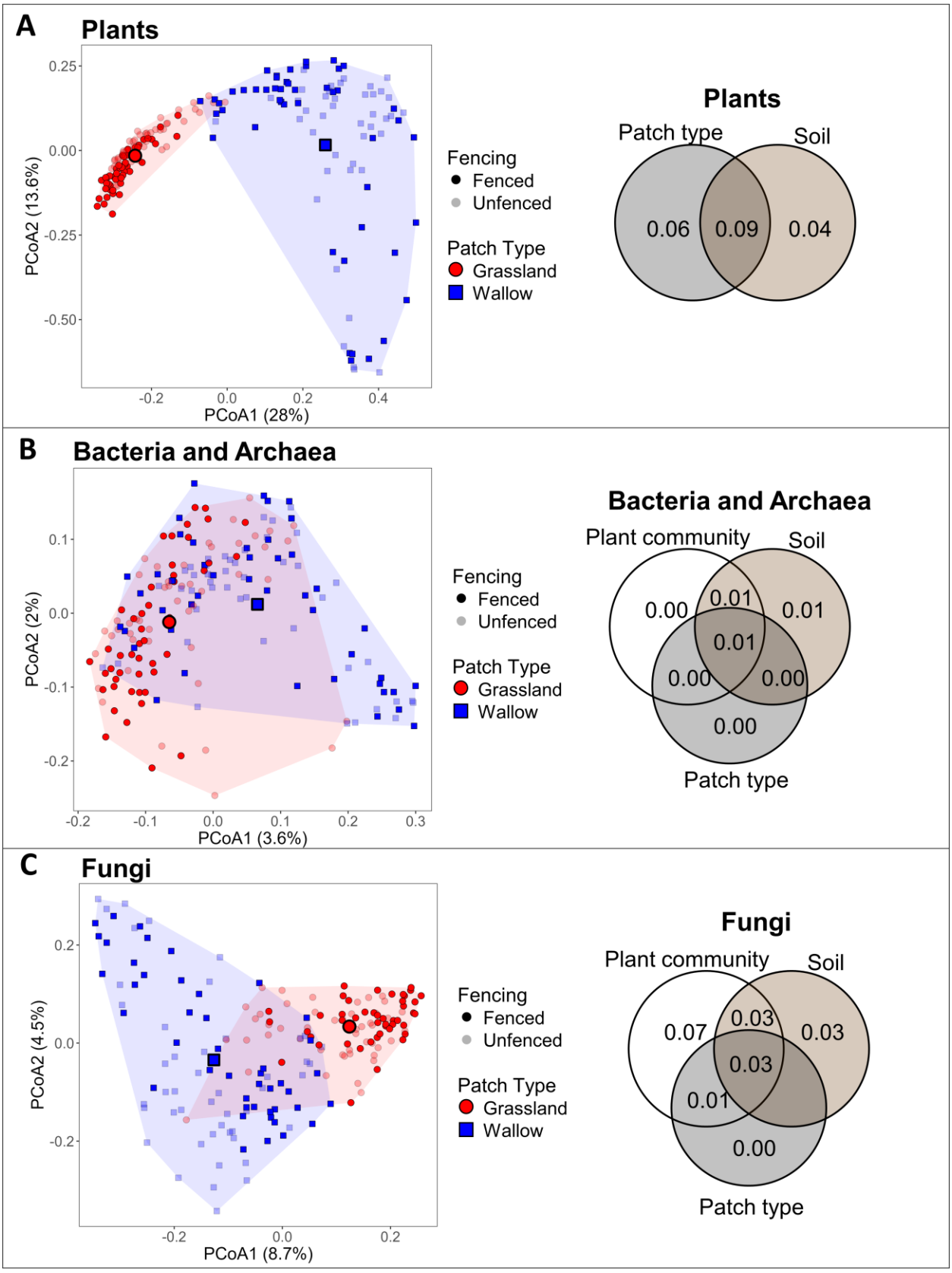
A
Plants
PCoA2 (13.6%)
PCoA1 (28%)
Fencing
Fenced
Unfenced
Patch Type
Grassland
Wallow
Plants
Patch type
Soil
0.06
0.09
0.04
B
Bacteria and Archaea
PCoA2 (2%)
PCoA1 (3.6%)
Fencing
Fenced
Unfenced
Patch Type
Grassland
Wallow
Bacteria and Archaea
Plant community
Soil
0.00
0.01
0.01
0.01
0.00
0.00
0.00
Patch type
C
Fungi
PCoA2 (4.5%)
PCoA1 (8.7%)
Fencing
Fenced
Unfenced
Patch Type
Grassland
Wallow
Fungi
Plant community
Soil
0.07
0.03
0.03
0.03
0.01
0.00
Patch type

Figure 4.4: Principal coordinates analyses (PCoA) and variance partitioning for plant (A), bacterial and archaeal (B), and fungal (C) communities. Large symbols in PCoAs represent the centroid of the patch types. Numbers less than 0 are not shown in Venn diagrams.

Appendix A

Supplemental Tables

Table S 4.1: Mean differences and Tukey HSD differences between groups, as effects of well as patch type, fencing, and their interactions for two-way ANOVAs on all soil variables.

Soil Variable	Comparison	Mean difference[1]	Tukey HSD p-value[2]	Patch type p-value[3]	Fencing p-value[4]	Interaction p-value[5]
pH	Grassland:Unfenced-Grassland:Fenced	0.09	0.77	0.24	0.21	0.91
	Grassland:Unfenced-Wallow:Fenced	0.01	1.00	0.24	0.21	0.91
	Wallow:Fenced-Grassland:Fenced	0.08	0.80	0.24	0.21	0.91
	Wallow:Unfenced-Grassland:Fenced	0.15	0.31	0.24	0.21	0.91
	Wallow:Unfenced-Grassland:Unfenced	0.07	0.87	0.24	0.21	0.91
	Wallow:Unfenced-Wallow:Fenced	0.07	0.85	0.24	0.21	0.91
C:N Ratio	Grassland:Unfenced-Grassland:Fenced	-0.01	1.00	**0.00**	0.18	0.17
	Grassland:Unfenced-Wallow:Fenced	1.04	**0.00**	**0.00**	0.18	0.17
	Wallow:Fenced-Grassland:Fenced	-1.05	**0.00**	**0.00**	0.18	0.17
	Wallow:Unfenced-Grassland:Fenced	-0.59	0.07	**0.00**	0.18	0.17
	Wallow:Unfenced-Grassland:Unfenced	-0.58	0.07	**0.00**	0.18	0.17
	Wallow:Unfenced-Wallow:Fenced	0.46	0.22	**0.00**	0.18	0.17
Ca (ppm)	Grassland:Unfenced-Grassland:Fenced	-116.85	0.86	0.96	1.00	0.26

	Grassland:Unfenced-Wallow:Fenced	-3.19	1.00	0.96	1.00	0.26
	Wallow:Fenced-Grassland:Fenced	-113.66	0.87	0.96	1.00	0.26
	Wallow:Unfenced-Grassland:Fenced	5.45	1.00	0.96	1.00	0.26
	Wallow:Unfenced-Grassland:Unfenced	122.30	0.84	0.96	1.00	0.26
	Wallow:Unfenced-Wallow:Fenced	119.11	0.85	0.96	1.00	0.26
Clay (%)	Grassland:Unfenced-Grassland:Fenced	-3.55	0.01	**0.00**	**0.00**	0.64
	Grassland:Unfenced-Wallow:Fenced	-15.01	0.00	**0.00**	**0.00**	0.64
	Wallow:Fenced-Grassland:Fenced	11.46	0.00	**0.00**	**0.00**	0.64
	Wallow:Unfenced-Grassland:Fenced	8.66	0.00	**0.00**	**0.00**	0.64
	Wallow:Unfenced-Grassland:Unfenced	12.21	0.00	**0.00**	**0.00**	0.64
	Wallow:Unfenced-Wallow:Fenced	-2.80	0.07	**0.00**	**0.00**	0.64
K (ppm)	Grassland:Unfenced-Grassland:Fenced	-17.09	0.70	**0.00**	0.71	0.24
	Grassland:Unfenced-Wallow:Fenced	101.31	0.00	**0.00**	0.71	0.24
	Wallow:Fenced-Grassland:Fenced	-118.40	0.00	**0.00**	0.71	0.24
	Wallow:Unfenced-Grassland:Fenced	-109.43	0.00	**0.00**	0.71	0.24
	Wallow:Unfenced-Grassland:Unfenced	-92.34	0.00	**0.00**	0.71	0.24
	Wallow:Unfenced-Wallow:Fenced	8.98	0.94	**0.00**	0.71	0.24
Mg (ppm)	Grassland:Unfenced-Grassland:Fenced	-7.50	1.00	**0.00**	0.41	0.57

	Grassland:Unfenced-Wallow:Fenced	-238.86	0.00	**0.00**	0.41	0.57
	Wallow:Fenced-Grassland:Fenced	231.36	0.00	**0.00**	0.41	0.57
	Wallow:Unfenced-Grassland:Fenced	190.83	0.00	**0.00**	0.41	0.57
	Wallow:Unfenced-Grassland:Unfenced	198.33	0.00	**0.00**	0.41	0.57
	Wallow:Unfenced-Wallow:Fenced	-40.53	0.75	**0.00**	0.41	0.57
Na (ppm)	Grassland:Unfenced-Grassland:Fenced	4.92	1.00	**0.00**	0.78	0.59
	Grassland:Unfenced-Wallow:Fenced	-122.48	**0.00**	**0.00**	0.78	0.59
	Wallow:Fenced-Grassland:Fenced	127.40	**0.00**	**0.00**	0.78	0.59
	Wallow:Unfenced-Grassland:Fenced	111.43	**0.00**	**0.00**	0.78	0.59
	Wallow:Unfenced-Grassland:Unfenced	106.50	**0.00**	**0.00**	0.78	0.59
	Wallow:Unfenced-Wallow:Fenced	-15.98	0.94	**0.00**	0.78	0.59
NH4 (ppm)	Grassland:Unfenced-Grassland:Fenced	-2.37	**0.00**	0.10	**0.00**	0.61
	Grassland:Unfenced-Wallow:Fenced	-1.46	0.07	0.10	**0.00**	0.61
	Wallow:Fenced-Grassland:Fenced	-0.91	0.41	0.10	**0.00**	0.61
	Wallow:Unfenced-Grassland:Fenced	-2.85	**0.00**	0.10	**0.00**	0.61
	Wallow:Unfenced-Grassland:Unfenced	-0.48	0.84	0.10	**0.00**	0.61
	Wallow:Unfenced-Wallow:Fenced	-1.94	**0.01**	0.10	**0.00**	0.61
NO3 (ppm)	Grassland:Unfenced-Grassland:Fenced	2.57	0.56	**0.00**	**0.00**	0.09

	Grassland:Unfenced-Wallow:Fenced	9.19	0.00	**0.00**	**0.00**	0.09
	Wallow:Fenced-Grassland:Fenced	-6.62	0.01	**0.00**	**0.00**	0.09
	Wallow:Unfenced-Grassland:Fenced	0.77	0.98	**0.00**	**0.00**	0.09
	Wallow:Unfenced-Grassland:Unfenced	-1.80	0.80	**0.00**	**0.00**	0.09
	Wallow:Unfenced-Wallow:Fenced	7.39	**0.00**	**0.00**	**0.00**	0.09
OM (%)	Grassland:Unfenced-Grassland:Fenced	-0.06	0.99	**0.00**	0.57	0.31
	Grassland:Unfenced-Wallow:Fenced	2.55	**0.00**	**0.00**	0.57	0.31
	Wallow:Fenced-Grassland:Fenced	-2.61	**0.00**	**0.00**	0.57	0.31
	Wallow:Unfenced-Grassland:Fenced	-2.40	**0.00**	**0.00**	0.57	0.31
	Wallow:Unfenced-Grassland:Unfenced	-2.33	**0.00**	**0.00**	0.57	0.31
	Wallow:Unfenced-Wallow:Fenced	0.22	0.67	**0.00**	0.57	0.31
P (ppm)	Grassland:Unfenced-Grassland:Fenced	0.16	0.99	**0.02**	0.74	0.89
	Grassland:Unfenced-Wallow:Fenced	-0.71	0.48	**0.02**	0.74	0.89
	Wallow:Fenced-Grassland:Fenced	0.87	0.29	**0.02**	0.74	0.89
	Wallow:Unfenced-Grassland:Fenced	0.94	0.23	**0.02**	0.74	0.89
	Wallow:Unfenced-Grassland:Unfenced	0.77	0.40	**0.02**	0.74	0.89
	Wallow:Unfenced-Wallow:Fenced	0.06	1.00	**0.02**	0.74	0.89
Sand (%)	Grassland:Unfenced-Grassland:Fenced	0.93	0.29	**0.00**	**0.05**	0.61

	Grassland:Unfenced-Wallow:Fenced	4.76	**0.00**	**0.00**	**0.05**	0.61
	Wallow:Fenced-Grassland:Fenced	-3.83	**0.00**	**0.00**	**0.05**	0.61
	Wallow:Unfenced-Grassland:Fenced	-3.28	**0.00**	**0.00**	**0.05**	0.61
	Wallow:Unfenced-Grassland:Unfenced	-4.21	**0.00**	**0.00**	**0.05**	0.61
	Wallow:Unfenced-Wallow:Fenced	0.56	0.72	**0.00**	**0.05**	0.61
Sikora pH	Grassland:Unfenced-Grassland:Fenced	0.04	0.65	**0.03**	0.09	0.97
	Grassland:Unfenced-Wallow:Fenced	0.11	0.03	**0.03**	0.09	0.97
	Wallow:Fenced-Grassland:Fenced	-0.06	0.38	**0.03**	0.09	0.97
	Wallow:Unfenced-Grassland:Fenced	-0.01	0.98	**0.03**	0.09	0.97
	Wallow:Unfenced-Grassland:Unfenced	-0.06	0.41	**0.03**	0.09	0.97
	Wallow:Unfenced-Wallow:Fenced	0.05	0.62	**0.03**	0.09	0.97
Silt (%)	Grassland:Unfenced-Grassland:Fenced	2.62	**0.03**	**0.00**	**0.00**	0.78
	Grassland:Unfenced-Wallow:Fenced	10.25	**0.00**	**0.00**	**0.00**	0.78
	Wallow:Fenced-Grassland:Fenced	-7.63	**0.00**	**0.00**	**0.00**	0.78
	Wallow:Unfenced-Grassland:Fenced	-5.38	**0.00**	**0.00**	**0.00**	0.78
	Wallow:Unfenced-Grassland:Unfenced	-8.00	**0.00**	**0.00**	**0.00**	0.78
	Wallow:Unfenced-Wallow:Fenced	2.24	0.09	**0.00**	**0.00**	0.78
Soil moisture (%)	Grassland:Unfenced-Grassland:Fenced	-1.89	0.25	**0.00**	0.69	0.03

	Grassland:Unfenced-Wallow:Fenced	-2.80	**0.03**	**0.00**	0.69	0.03
	Wallow:Fenced-Grassland:Fenced	0.90	0.81	**0.00**	0.69	0.03
	Wallow:Unfenced-Grassland:Fenced	2.23	0.13	**0.00**	0.69	0.03
	Wallow:Unfenced-Grassland:Unfenced	4.12	**0.00**	**0.00**	0.69	0.03
	Wallow:Unfenced-Wallow:Fenced	1.33	0.57	**0.00**	0.69	0.03
Total C (%)	Grassland:Unfenced-Grassland:Fenced	-0.06	0.96	**0.00**	0.54	0.19
	Grassland:Unfenced-Wallow:Fenced	1.58	**0.00**	**0.00**	0.54	0.19
	Wallow:Fenced-Grassland:Fenced	-1.63	**0.00**	**0.00**	0.54	0.19
	Wallow:Unfenced-Grassland:Fenced	-1.47	**0.00**	**0.00**	0.54	0.19
	Wallow:Unfenced-Grassland:Unfenced	-1.42	**0.00**	**0.00**	0.54	0.19
	Wallow:Unfenced-Wallow:Fenced	0.16	0.53	**0.00**	0.54	0.19
Total N (%)	Grassland:Unfenced-Grassland:Fenced	0.00	0.99	**0.00**	0.77	0.50
	Grassland:Unfenced-Wallow:Fenced	0.11	**0.00**	**0.00**	0.77	0.50
	Wallow:Fenced-Grassland:Fenced	-0.11	**0.00**	**0.00**	0.77	0.50
	Wallow:Unfenced-Grassland:Fenced	-0.11	**0.00**	**0.00**	0.77	0.50
	Wallow:Unfenced-Grassland:Unfenced	-0.10	**0.00**	**0.00**	0.77	0.50
	Wallow:Unfenced-Wallow:Fenced	0.01	0.90	**0.00**	0.77	0.50

[1] Mean differences between patch type*fencing groups

[2] Pair-wise differences between patch type*fencing groups

[3] P-values for patch type main effects based on two-way ANOVA

[4] P-values for fencing main effects based on two-way ANOVA

[5] P-values for patch type*fencing interactive effects based on two-way ANOVA

Supplemental figures

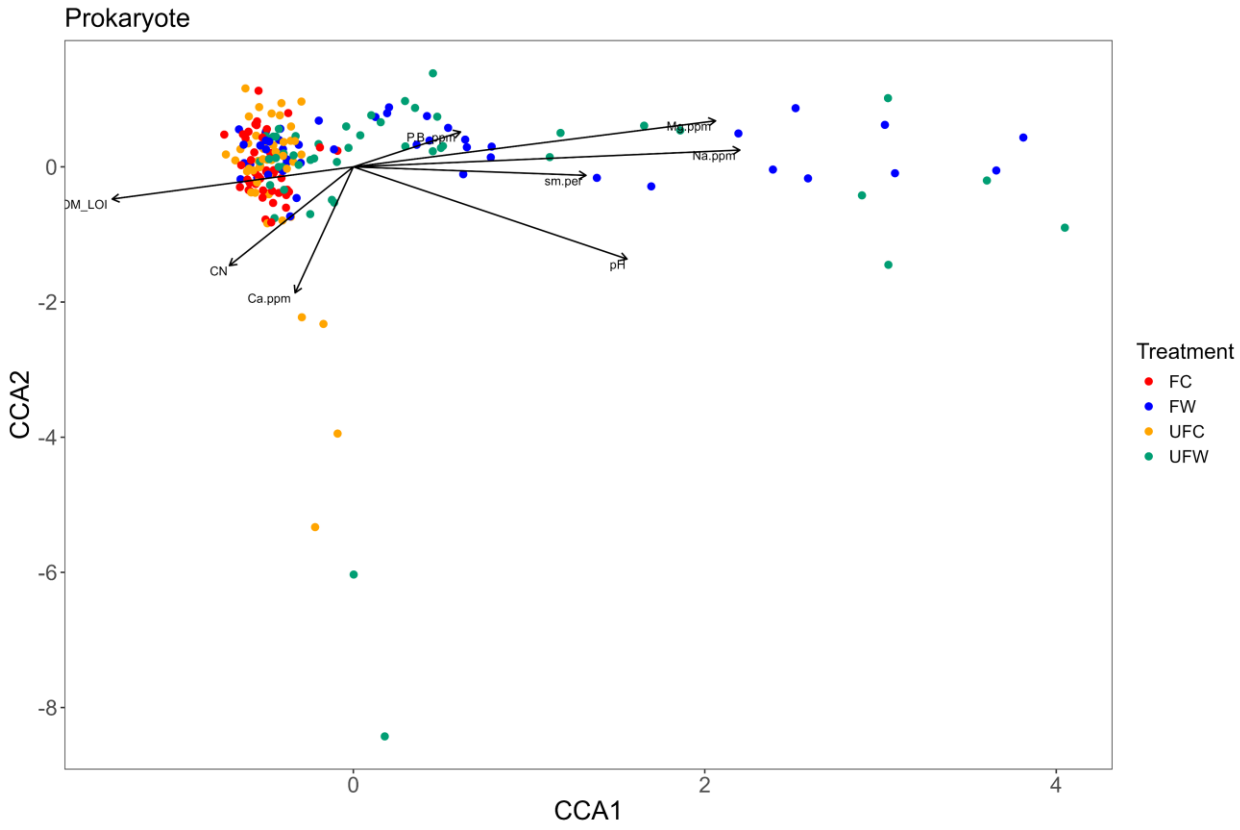

Figure S4.1: Canonical correspondence analysis (CCA) of bacterial and archaeal communities with top explanatory soil variables (stepwise selected). Treatment included fenced grassland (FC), fenced wallow (FW), unfenced grassland (UFC), and unfenced wallow (UFW).

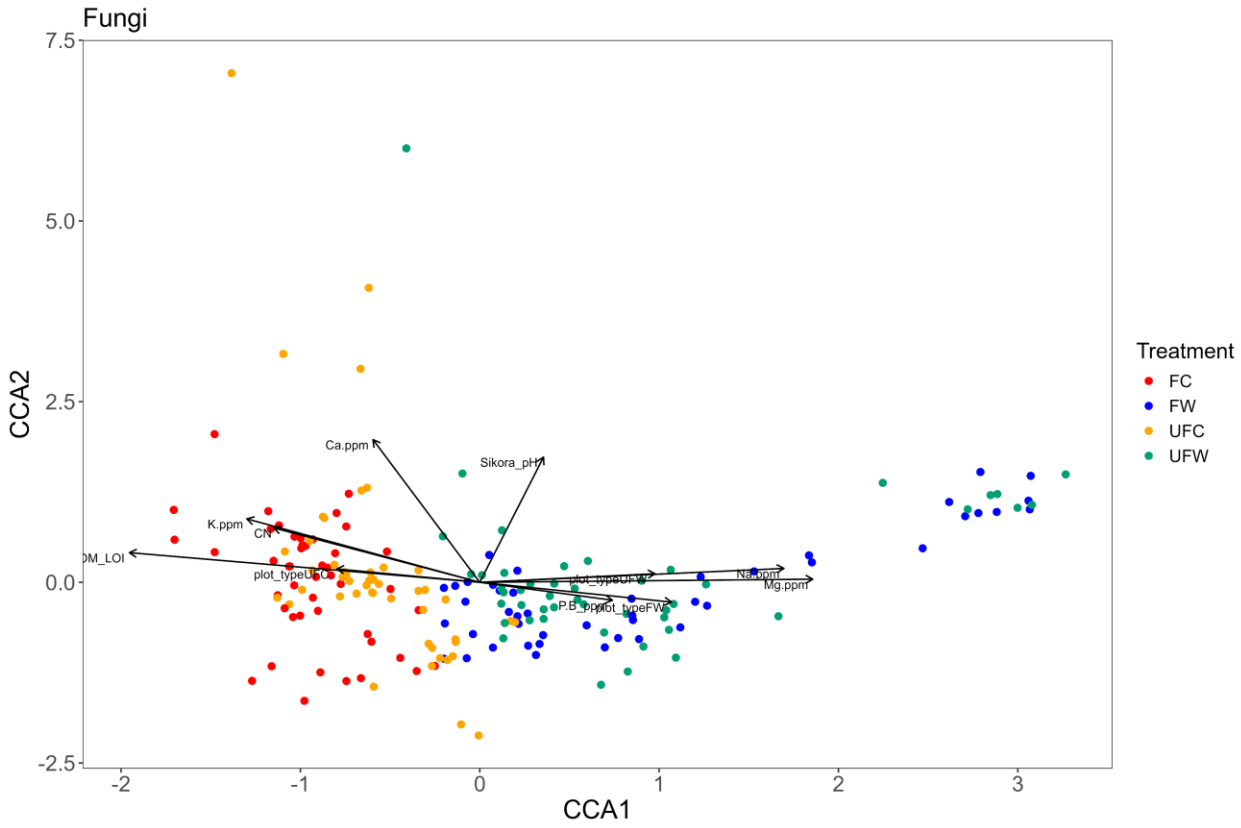

Figure S4.2: Canonical correspondence analysis (CCA) of fungal communities with top explanatory soil variables (stepwise selected). Treatment included fenced grassland (FC), fenced wallow (FW), unfenced grassland (UFC), and unfenced wallow (UFW).

Chapter 5 – Conclusion

5.1 Conclusion

North American grasslands have undergone significant transformation due to widespread agricultural conversion, fire suppression, species invasion, and the removal of native megafauna (Samson and Knopf 1994, Hoekstra et al. 2005, Yu and Lu 2018). These losses extend beyond biodiversity and include essential the de-coupling of ecological processes such as pyric herbivory and the removal of patch-scale soil disturbances, once created by large herbivores like bison (Knapp et al. 1999, Fuhlendorf et al. 2010a, Bråthen et al. 2021). This dissertation examines how fire, climate, and especially megaherbivores, impact tallgrass prairie soil, plant, and microbial diversity and variability.

In Chapter 2, leveraging a 30-year landscape-scale experiment at Konza Prairie Biological Station, we found that bison grazing significantly increased plant species richness and family-level redundancy (i.e., more plant species per family represented) compared to cattle-grazed systems, and both greater species richness and higher phylogenetic diversity compared to annually-burned, ungrazed prairie. Less frequently burned (3-4 year fire return interval) ungrazed prairie also had increased species richness and phylogenetic diversity compared to annually burned ungrazed prairie, but the risk of woody encroachment outweighs the increase in plant diversity. Phylogenetic metrics indicated that bison not only increased species number, but the evolutionary breadth of prairie plant communities, suggesting more functionally diverse assemblages (Cadotte et al. 2009). Additionally, despite lower richness and phylogenetic diversity in some treatments, all plant communities were resistant to an extreme drought.

In Chapter 3, we collected plant, soil, and hydroperiod data from 72 plots, including 24 bison wallows, 24 bison non-wallows, and 24 cattle non-wallows. Bison wallows acted as ephemeral wetlands and disturbance-generate habitats that increased heterogeneity across the landscape. Compared to bison and cattle non-wallows, wallows had less plant richness and diversity and more exposed bare ground. However, they had distinct plant communities, including obligate wetland species that would not be found in upland grassland without wallows present. Wallows are uniquely situated as candidates for both observational and experimental ecological studies as they are small, numerous, and have distinct boundaries. They could be used as natural, in situ microcosms for wetland taxa or processes, as part of metapopulation and dispersal studies, as case studies for community assembly and succession, and more. Wallows are also an intriguing and accessible connection to tallgrass prairie history and ecological research and can be included in K-12 education and outreach for the general public.

In Chapter 4, we examined soil, plant, and microbial communities from 96 plots in a fully factorial design crossing patch type (wallow vs. matrix grassland) and fencing status (fenced vs. unfenced) to assess how bison wallows influence belowground biodiversity. Soil microbial communities in wallows reflected strong shifts in physicochemistry driven by repeated disturbance. Compared to matrix grasslands, wallows had lower organic matter, total nitrogen, and carbon, but higher clay content, sodium, and extractable phosphorus—soil properties known to influence microbial activity and composition (Lauber et al. 2008, Bates et al. 2011, Tedersoo et al. 2014, Fierer 2017). Microbial community composition differed significantly between patch types, but alpha diversity responses varied: prokaryotic diversity was generally higher in wallows than matrix grasslands, whereas fungal diversity responses were weaker and more variable. Across all habitat types, fungal communities were more strongly associated with plant

community composition and nutrient pools than with soil texture or chemistry, consistent with previous work (Tedersoo et al. 2014, Dassen et al. 2017, Lutz et al. 2025). Prokaryotic communities, by contrast, correlated more strongly with soil texture and pH than with plant composition, in line with patterns observed elsewhere (Lauber et al. 2008, Rousk et al. 2010, Dassen et al. 2017).

Together, these chapters demonstrate that bison impact prairie ecosystems across multiple ecological scales, from altering plant evolutionary diversity and redundancy to reshaping microbial composition and plant-microbe-soil relationships. These findings offer evidence that reintroducing bison restores ecological processes that are not fully replicated by cattle grazing or fire management alone (Figure 5.1). Modern grassland management practices, including annual burning and growing season-cattle grazing (Owensby et al. 2008), seem to fall short of replicating the historical disturbance regimes that fostered fine-scale heterogeneity and resilience (Knapp et al. 1999, Towne et al. 2005). While patch-burn-grazing re-couples the interaction of fire and grazing (Fuhlendorf et al. 2009), and increases heterogeneity that is beneficial for some taxa (Fuhlendorf et al. 2006, 2010b, Winder et al. 2017), it still does not replicate the ecosystem engineering of bison wallowing.

In grassland conservation efforts, restoring process, not just presence of megagrazers, should be a priority (Fuhlendorf et al. 2009, Cromsigt et al. 2018). We recognize that cattle will remain the dominant herbivore, as they fuel regional economies and are critical to the cultural identity of many communities (Hoy 2009, Maher et al. 2021). We also see that, compared to ungrazed prairie, they can lead to positive conservation outcomes by facilitating heterogeneity, especially when re-coupled with fire effects (Fuhlendorf et al. 2009, Winder et al. 2018, San Emeterio et al. 2023) or when actively managed for altered movement across the landscape (i.e.,

virtual fencing, rotational grazing; Allred et al. 2011, Lund et al. 2024, Hoag et al. 2024). However, if we aim to reestablish ephemeral wetland networks (Bookout et al *In review*), maintain high plant and microbial diversity (Towne et al. 2005, Bookout et al. 2025; Chapter 4), increase soil and habitat heterogeneity (Knapp et al. 1999, Anguiano et al. 2024; Chapter 4), and meet the cultural needs of many Indigenous groups (Shamon et al. 2022), then rewilding more bison herds is essential. This dissertation reinforces the position that bison reintroduction is a restoration of ecosystem processes, not just restoring a charismatic megafauna.

5.2 References

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5.3 Figures

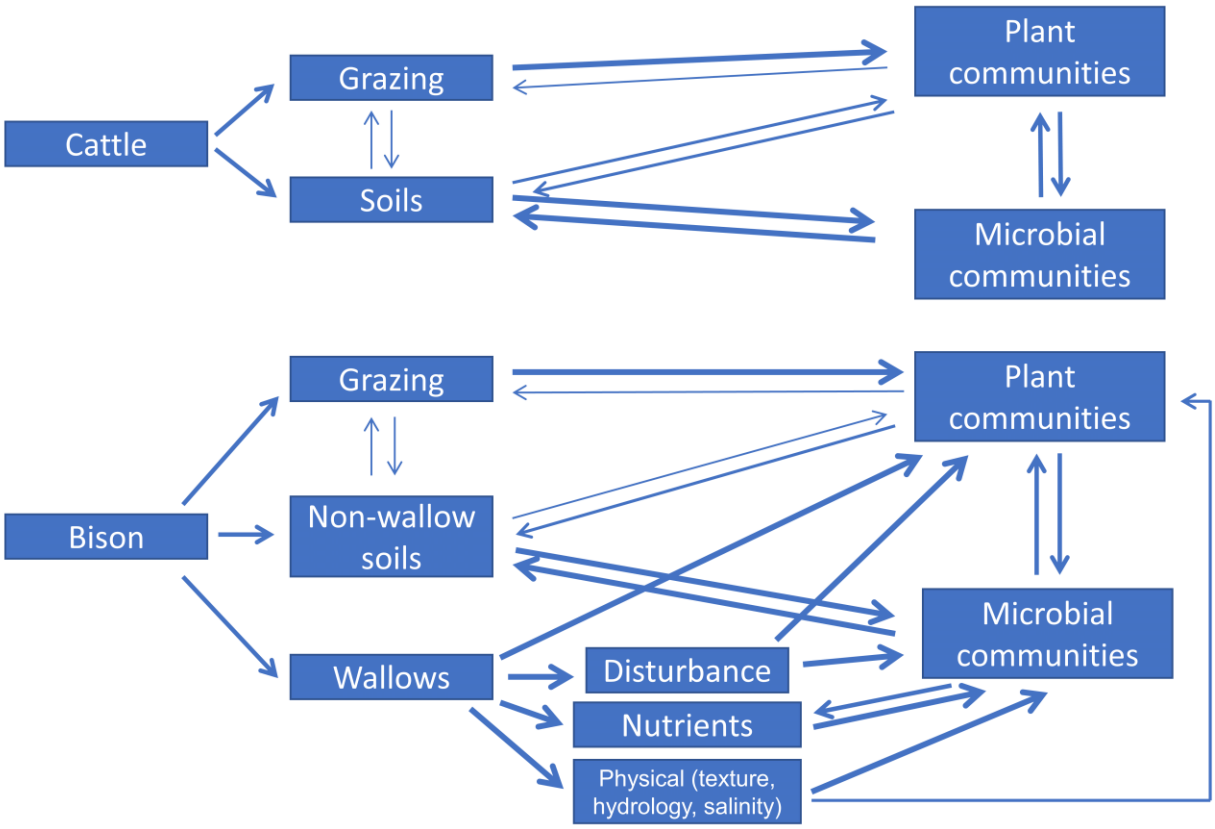

Figure 5.1: A conceptual diagram illustrating the complex dynamics of grazed grasslands, and the increased complexity in bison-grazed grasslands. Width of arrows indicate stronger relationships between boxes. This does not include indirect affects, which are many.