Investigating the impact of bison grazing on native tallgrass prairie plant communities in northeastern South Dakota

Sophie Wong Purcellville, Virginia

A Thesis presented to the Faculty of the University of Virginia in Fulfillment of the Distinguished Majors Program

Department of Environmental Sciences

University of Virginia April 2022

> Howard E. Epstein, Thesis Advisor

Thomas A. Smith, Director of Distinguished Major Program

Abstract

Tallgrass prairies in the central United States have been fragmented and degraded due to conversion to managed rangeland and cropland. Bison, as historical grazers of tallgrass prairie, may serve as a means to restore native plant communities. This study sought to demonstrate the viability of bison grazing as a restoration method for tallgrass prairies through the investigation of plant community diversity and species abundance changes, as well as changes to soil C and N, under different grazing treatments.

By comparing plant communities and soil samples from fields under bison and cattle grazing in Sisseton, South Dakota, along with an unmanaged control field at Waubay National Wildlife Refuge, I investigated the effects of bison and cattle grazing on native plant abundances. I hypothesized that bison grazing would increase the abundance of forbs, increase N in soils and plant biomass, and promote the return of native warmseason tallgrasses. I used Braun-Blanquet community composition data from 2018 to 2021 from experimental plots in South Dakota, as well as bulk vegetative biomass and soil cores for C and N analysis. Bison grazing promoted lower graminoid cover, lower dead graminoid biomass, greater graminoid richness, higher forb biomass than cattlegrazed plots, and a greater proportion of native species in the plant communities. Bison and cattle grazing promoted higher soil C and N, and a lower C:N ratio for the grazed plots than the control plots. Over the three-year study period (2018-2021), there was an increase in total plant biomass, a decrease in forb biomass, an increase in native species area cover and the proportion of native species per plot, as well as a decrease in soil C:N regardless of grazing treatment. This study provides support for the use of bison grazing as a restoration method for degraded native tallgrass prairie plant communities dominated by non-native species and graminoids.

Acknowledgements

This research would not have been possible without the help of all the UVA students who have worked on this project, and specifically Megan Eisenfelder, Lexi Tilot, Maggie Matthews, and Henry Chin. I'd like to thank Howie Epstein for his guidance these four years and his unwavering dedication to this project. Thank you to Elise Heffernan for her help with the statistics and figure creation for this project. I'd also like to thank the organizations that have funded this project over the years: the Jefferson Trust, the UVA Center for Global Health Equity, the Office of Undergraduate Research, the College Science Scholars, and the Hart family. Thank you to Gerald German for welcoming us into his house and allowing us to conduct this experiment in his fields. Thank you to Anna Liang for being a friend and partner throughout this year and the whole DMP process. Finally, thank you to Emily Burnett for her constant support, company, and generous editorial skills throughout this year's work.

I want to acknowledge that this work was conducted on the Lake Traverse Reservation territory of the Sisseton-Wahpeton Oyate tribe as well as the Charlottesville-area territory taken from its original stewards, the Monacan and Manahoac tribes.

Introduction

Bison are a keystone species of tallgrass prairie grasslands. As the largest land mammal in North America, weighing from 0.5 to 1 metric ton (USFWS, 1997), their grazing and non-grazing behavior, such as wallowing and horning, acts as a disturbing force that can maintain higher levels of biodiversity in tallgrass prairies (McMillan et al., 2011). The small-scale disturbance of their grazing in combination with the Miocene-Pliocene transition water stress on shrubs and trees is believed to have supported the evolution of the grassland biome (Axelrod, 1985; Knapp et al., 1999). Before 1830, bison herds were estimated to have been as large as 60 million individuals, which declined drastically as a result of disease and slaughter by colonizers of the Great Plains.

Native tallgrass prairie in North America once stretched from Canada down to Mexico, but due to homesteading beginning in 1830, an estimated 99% of tallgrass prairie has been lost (Samson & Knopf, 1994). Several species of native prairie herbivores and carnivores have gone extinct, and many grassland bird species are considered endangered (Samson & Knopf, 1994). In terms of plant species diversity in grasslands, forbs (herbaceous, non-woody plants) outnumber graminoids (grasses) by an order of magnitude (Damhoureyeh & Hartnett, 1997). Non-native grasses introduced as alternative forage crops have displaced many native species, resulting in a decline of plant diversity (Smith & Knapp, 2001).

Burning has been used as a method for restoring native prairie, and bison grazing may serve as a less-intensive alternative or complement to burning (Fuhlendorf, 2019; D. M. Larson et al., 2013; Starns et al., 2019). Grazing has been shown to cause similar increases in root tissue quality as burning, but conversely increased the rate of N mineralization and decreased the rate of soil respiration relative to burning (Johnson & Matchett, 2001). Since burning can increase water stress in prairies, selecting for drought-tolerant species and understanding how bison and cattle grazing affect soil moisture is also important (Fahnestock & Knapp, 1994). Previous management with grazing resulted in prairies with moderate net primary productivity (NPP), high species diversity, high spatial heterogeneity, and high N availability. Burning management regimes resulted in high NPP, dominance of grasses, low spatial heterogeneity, and low to moderate N availability (Knapp et al., 1999).

Grazing by large herbivores creates patchiness on prairies through defoliation, trampling and wallowing, and waste production (Damhoureyeh & Hartnett, 1997). Wallowing, where the animal rolls in soft dirt while scraping horns and hooves against the ground, creating a circular depression of bare soil, is a non-grazing behavior specific to bison but not cattle (McMillan et al., 2011). This disturbance creates gaps in the plant community that allows some subdominant species to escape competitive exclusion of dominant grasses, leading to greater plant diversity (Trager et al., 2004). Both cattle and bison feed primarily on graminoids, with large overlaps between their diets, but cattle have a lower percentage of graminoids and a higher percentage of forbs in their diet relative to bison (Damhoureyeh & Hartnett, 1997). Bison have been shown to selectively graze key prairie species such as *Andropogon gerardii* (big bluestem) and *Panicum virgatum* (switchgrass), which can promote higher relative growth rates under short-term grazing, but decreased growth under long-term grazing -- an interesting

consideration for the restoration of these vital native species (Vinton & Hartnett, 1992). Bison also graze in two distinct patterns, creating either 20-50 m² patches, or >400 m² lawns (Knapp et al., 1999). Forbs and woody vegetation are usually avoided by bison, leaving these species ungrazed in a lawn of grazed graminoids (Knapp et al., 1999). By initially selecting patches dominated by grasses, bison grazing can convert these sites to communities with a greater abundance of forbs and shrubs, increasing species diversity in these areas (Hartnett et al., 1996; Knapp et al., 1999).

Grassland soils are one of the largest sinks of organic carbon (C; approximately 20 kg C/m²) (Schlesinger & Bernhardt, 2013). Ungulate grazing redistributes nitrogen (N) sequestered in biomass back into the environment as dung and urine, conserving N cycling in the tallgrass prairie ecosystem (Johnson & Matchett, 2001). Bison grazing was found to have the counterintuitive short-term effect on prairie plants of increased carbon relocation from roots to aboveground biomass, resulting in decreased root C and increased C allocation to shoots (Johnson & Matchett, 2001). A study on the Konza Prairie in eastern Kansas showed that this effect was due to increased rates of N cycling, reducing C allocation to the roots and increasing allocation to shoot growth, decreasing belowground C cycling (Johnson & Matchett, 2001). Cattle grazing, in comparison, increased total soil N and presumably the rate of N cycling due to consumption during grazing, but also increased total soil C on Minnesota prairie (Larson et al., 2020).

The spatial patterns of bison and cattle grazing differ, with bison more widely distributed across an area and cattle clustering near water sources (Damhoureyeh &

Hartnett, 1997; Fuhlendorf & Engle, 2001; Knapp et al., 1999). The preference of bison for open rangeland versus cattle for wooded habitats could impose different intensities of grazing on the prairie plants, resulting in different growth responses and distributions of C and N nutrients across the landscape (Knapp et al., 1999) The comparison of these two types of ungulate grazing on C and N cycling and storage in tallgrass prairies with reference to changes in plant communities is an under-investigated area of tallgrass prairie ecology.

For my study, I was interested in the effects of bison versus cattle grazing on plant community composition and ecosystem properties such as carbon, nitrogen, and soil moisture. I examined changes in overall abundance and diversity of forb, shrub, and graminoid species based on type of grazing and time since the ungulates were introduced to the fields. This study attempted to advance the current understanding of the effects of grazing on nutrient cycling with a focus on changes in plant communities under grazing pressure, as well as determine whether bison and cattle are functional equivalents in terms of maintaining plant species diversity, a question raised by Knapp et al. (1999).

I hypothesized that bison grazing would increase diversity and forb biomass in tallgrass prairies, whereas cattle grazing would increase forb diversity but not promote an increase in biomass due to less selective grazing (Damhoureyeh & Hartnett, 1997). I also hypothesized that bison grazing and cattle grazing would increase N concentrations and reduce C concentrations in soil samples, as well as increase N concentrations in plant tissues. I hypothesized that bison grazing would promote

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increased abundance and diversity of native prairie species (graminoids, forbs, and woody shrubs) relative to cattle grazing through their spatial grazing pattern and avoidance of forbs, resulting in greater abundance of the most diverse type of vegetation in tallgrass prairies (Knapp et al., 1999).

Methods

Study Sites

For this study, I utilized three fields on the Lake Traverse Reservation in Sisseton, South Dakota. Two of these were grazed separately by bison and cattle, owned and managed by our rancher partner (Figure 1). Originally both cattle fields, one of these fields was converted to bison grazing in 2017, the first year of this project. Data collection began in 2018 and continued uninterrupted every summer except 2020 due to complications of COVID-19. A control field at the Waubay National Wildlife Refuge has been unmanaged for the past 11 years (Figure 2), prior to which it was mowed and burned, but it has been unmanaged throughout the duration of this study.



Figure 1. Map of plot distributions in bison (left of the road) and cattle fields (right). Plots are distributed across the environmental gradients in both fields.



Figure 2. Map of plot distributions in Waubay National Wildlife Refuge.

Sample Collection and Analysis

Sampling areas in each field consisted of two 150-meter transects, separated into three five-by-five meter plots, one every 50 meters, for a total of 18 plots, six per field. Data collection consisted of a Braun-Blanquet survey to assess plant species

diversity and composition (Furman et al., 2018), as well as a harvesting of two randomly selected 0.5 m² quadrats of vegetation adjacent to each plot and two soil cores to 10 cm depth from random locations adjacent to the plot. Soil moisture readings were collected within the plot using a Hydrosense soil moisture probe and replicated three times in different locations in the plot.

Plant and soil samples were shipped back to the lab at UVA. Vegetation samples were dried at 60 degrees Celsius for 48 hours prior to being sorted by vegetation type and then weighed (i.e. forbs, dead grass, live grass, shrub wood, and shrub foliage). The soil samples were sieved using a 2 mm sieve to remove litter and rocks, then dried at 115 degrees Celsius for 24 hours. Vegetation and soil samples were ground using a ball mill and were then tinned for elemental carbon and nitrogen analysis. Vegetation samples were coded for nativity as well as functional type (forbs, shrubs, graminoids). *Statistical Analysis*

The data collected during this experiment were analyzed across time and grazing types. With data collected from 2018, 2019, and 2021, there were three years of community composition and diversity, soil and biomass, and C and N data. I ran ANOVAs on vegetation and soil C, N, and C:N ratios; biomass of different plant types; and soil moisture data for differences based on grazing and year. I ran a multivariate analysis on plant community composition and abundance data across treatments and time using a non-parametric multidimensional scaling (NMS) ordination. I used these results to examine relationships among the C, N, and C:N ratios and the Braun-Blanquet plant abundance data. I also included the biomass measurements of the three

functional groups in this NMS ordination, which assisted in determining drivers of dominant biomass under each of these treatments.

All statistics were completed in R. Non-parametric multidimensional scaling (NMS) was used to scale and analyze the Braun-Blanquet plant abundance data, since NMS does not assume linear relationships among variables. Rare species, or species that were in fewer than 5% of plots (2 plots for this experiment), were removed from the data set. The abundance data were log transformed using equation 1. Relativization was determined to be not necessary for this data set using equation 2 below, which was taken from McCune et al. (2002), which resulted in a value of 15.37, below the threshold for necessary relativization.

$$\log(\text{abundance} + 10^{-7}) + 7 \tag{1}$$

$$100 * \sigma / \mu \tag{2}$$

Information about the functional group of each species in the dataset, as well as whether it was non-native or native, was added to the dataset. The percent carbon and nitrogen, the C:N ratio, and biomass were averaged to plot level across the categories of live grass, dead grass, forbs, shrub wood, and shrub foliage. The soil carbon, nitrogen, and C:N ratios were also averaged to plot level.

No moisture samples were collected in 2019 due to probe failure in the field. Since the soil moisture data were only obtained for 2018 and 2021, two NMS ordinations were run: one without all 2019 data, and one with all three years of data but no soil moisture data. There was one plot in the cattle grazing treatment from 2018 that lacked soil element data, so it was removed from the dataset used for these ordinations. The experiment was a randomized complete block design, with year as the block. Several two-way ANOVAs and Tukey tests were performed in order to determine the specific effects of the grazing treatments on our variables of interest. A two-way ANOVA without interactions was determined to be the best fit for the data after using AIC (Akaike information criterion) on two-way and interaction models of a randomly chosen set of variables. Alpha values were set at 0.05.

Pseudoreplication is a limitation of this study, due to there being only one bison field available within a reasonable geographic range at the beginning of this study. However, this study could provide the basis for the establishment of more bison rangeland on the Lake Traverse Reservation depending on the results. The bison and cattle fields are also very close geographically, but any animal-distributed seeds or plant propagules are under the same grazing pressure as the rest of the field, so I determined this to not be of concern for my study.

Results

ANOVAs

Percent Functional Group Coverage





Figure 3. Bar plots of percent coverage for forb (a), graminoid (b), and shrub (c) functional groups, averaged by year and treatment. Standard error bars are included. Bars are colored by grazing treatment. Capital letters indicate significance among grazing treatments, while lowercase letters in the boxes at the top of each plot indicate significance among years, based on a two-way ANOVA and post-hoc Tukey HSD test (p<0.05).

The percent coverage was determined for each plant functional type and run against the variables of year and grazing treatment. The forb coverage data (Fig. 3a) failed the Brown-Forsythe test (p <0.001), but scatterplots of the residuals formed a straight diagonal line and a histogram of residuals was normal, so I proceeded with the two-way parametric ANOVA. I did not find a statistically significant difference for year on forb coverage (F(2) = 0.35, p = 0.707), but I did find a statistically significant difference between the control and grazed treatments (F(2) = 41.22, p <0.001). A Tukey post-hoc test showed the control treatment had significantly higher percent forb coverage than the bison treatment (p <0.001) and the cattle treatment (p <0.001).

The graminoid percent cover (Fig. 3b) also failed the Brown-Forsythe test (p <0.001), but the qqplot and histogram of residuals were normal, so I proceeded with the two-way ANOVA. There was not a significant difference for year on percent grass cover (F(2) = 2.631, p = 0.082), but there was a significant difference among the grazing treatments (F(2) = 15.539, p <0.001). A Tukey test showed significantly greater graminoid coverage for the cattle treatment than the bison (p = 0.019), the control than the bison (p = 0.022), and the control over the cattle (p <0.001).

For percent shrub cover (Fig. 3c), the data passed the Brown-Forsythe test (p=0.268) so I proceeded with the two-way ANOVA. There were not significant differences between years or among grazing treatments on percent shrub coverage.



Total biomass (g) was summed from the mass of each functional type sorted from the vegetation samples taken from each plot. The total biomass data (Fig. 4a) passed the Brown-Forsythe test (p=0.092), so I proceeded with a two-way ANOVA testing differences among grazing treatment and year. There was a significant difference in total biomass among years (F(2) = 41.295, p <0.001) as well as among grazing treatments (F(2) = 4.312, p = 0.016). A Tukey post-hoc test for years showed significantly greater total biomass in 2019 than 2018 (p <0.0001), as well as greater biomass in 2021 than 2018 (p <0.001). The Tukey test showed also greater biomass in the control fields than the cattle treatment plots (p = 0.011).

For live graminoid biomass (Fig. 4b), the data passed the Brown-Forsythe test (p = 0.991), so I proceeded with the ANOVA. There was a significant difference in graminoid biomass across years (F(2) = 46.810, p < 0.001), but I did not find significant differences among grazing treatments (F(2)= 0.018, p = 0.982). A Tukey test showed that grass biomass in 2019 was lower than 2018 (p < 0.001) and higher in 2021 than 2019 (p < 0.001).

For dead graminoid biomass (Fig. 4c), the data passed the Brown-Forsythe test (p = 0.056), so I proceeded with a two-way ANOVA. There was a significant difference in dead grass biomass for both year (F(2) = 84.994, p <0.001) and treatment (F(2) = 7.706, p <0.001). A Tukey test for year showed an increase in dead biomass in 2019 from 2018 (p <0.001), in 2021 from 2018 (p <0.001), and in 2021 from 2019 (p <0.001). A Tukey test for grazing treatments showed the bison treatment had less dead biomass than the control (p <0.001) as did the cattle treatment (p = 0.039).

Forb biomass data (Fig. 4d) did not pass the Brown-Forsythe test (p < 0.001), but the qqplot was straight and the histogram of residuals was normal, so I proceeded with the ANOVA. Both year (F(2) = 11.08, p < 0.001) and grazing treatment (F(2) = 17.72, p < 0.001) showed a significant difference in forb biomass. Tukey tests for year showed biomass in 2019 was greater than 2018 (p < 0.001), but overall lower in 2021 than 2018 (p < 0.001). Tukey tests for grazing showed cattle was lower than bison (p = 0.006), the control was greater than bison p = 0.016) and the cattle (p < 0.001).

Last, for shrub biomass (Fig. 4e), which was the sum of both shrub wood and shrub foliage, the Brown-Forsythe test was not statistically significant (p = 0.084), so I conducted the ANOVA. There was a significant difference between years (F(2) = 5.036, p = 0.008), but no significant difference between grazing treatments (F(2) = 2.768, p = 0.067). A Tukey test showed an increase in 2019 from 2018 (p = 0.007), and decrease from 2019 to 2021 (p = 0.076).

b) a) Mean Graminoid Species Richness Mean Total Species Richness AB В В 2018 -- a **2018** -- a 20 2019 -- a 2019 -- a 2021 -- a 2021 -- a 7.5 Mean Species Richness Species Richness Mean С 2.5 0. Year Year 2018 2019 2021 2019 2019 2021 2018 2021 2018 2021 2021 2018 2019 2021 2018 2018 c) Mean Forb Species Richness d) Mean Shrub Species Richness В 15 2.5 **2018** -- a 2018 -- a В 2019 -- a 2019 -- a AB 2021 -- a 2021 -- a 2.0 Mean Species Richness Mean Species Richness Α Grazing Bison Cattle Control 0.5 0.0 2019 2018 Year Year 2021 2018 2019 2018 2021 2018 2021 2021 2018 2021 2018 2019 2021

Figure 5. Bar plots of species richness, total and grouped by functional group, averaged by grazing treatment for each year. Standard error bars are included. Bars are colored by grazing treatment. Capital letters indicate significance among grazing treatments, while lowercase letters in the boxes at the top of each plot indicate significance among years, based on a two-way ANOVA and post-hoc Tukey HSD test (p<0.05).

Total species richness (Fig. 5a) is the total number of species per plot. These data failed the Brown-Forsythe test (p = 0.027), but the qqplot and histogram of residuals were normal, so I proceeded with a two-way ANOVA comparing among year

and grazing treatments. The difference between grazing treatments was statistically significant (F(2) = 4.023, p = 0.0241), but there was no statistically significant difference among years. A Tukey test showed that total species richness was greater for the control than the bison (p = 0.024).

Next, I determined the species richness for each functional group: graminoids, forbs, and shrubs. For graminoids (Fig. 5b), the data failed the Brown-Forsythe test (p <0.001), but passed the other tests of normality. There was a statistically significant difference in graminoid species richness across the grazing treatments (F(2) = 30.144, p <0.001), but not for year (F(2) = 0.374, p = 0.690). Tukey tests showed the cattle treatment had greater graminoid richness than bison (p = 0.005), while the control had lower richness than bison (p <0.001) and cattle (p <0.001). For forbs (Fig. 5c), the data also failed the Brown-Forsythe test (p < 0.001) but passed the other tests, so I continued with the ANOVA. There was a statistically significant difference between the control and grazed treatments (F(2) = 12.335, p < 0.001), but no significant difference across years (F(2) = 1.115, p = 0.336). Tukey post-hoc tests showed that the control had greater forb richness than the bison treatment (p < 0.001) and the cattle treatment (p < 0.001). Last, for shrub species richness (Fig. 5d), the data failed the Brown-Forsythe test (p = 0.014), but the rest of the tests were normal so I continued with the ANOVA. I found a statistically significant difference for grazing treatments (F(2) = 4.726, p = 0.013), but not for year (F(2) = 0.734, p = 0.485). Tukey tests showed the control had greater shrub richness than the bison treatment (p = 0.010).

Native Species



Figure 6. Bar plots for percent area of plots covered by native (a) and non-native (b) species. Standard error bars are included. Bars are colored by grazing treatment. Capital letters indicate significance among grazing treatments, while lowercase letters in the boxes at the top of each plot indicate significance among years, based on a two-way ANOVA and post-hoc Tukey HSD test (p<0.05).

Species were grouped as native or non-native, and percent cover for these categories was averaged for treatments and years. The percent area of native species (Fig. 6a) failed the Brown-Forsythe test (p <0.001), but passed the other normality tests, so I proceeded with a two-way ANOVA testing grazing treatment and year. There were significant differences among years for the percent cover of native species (F(2) = 7.71, p = 0.001) as well as treatment (F(2) = 19.13, p < 0.001). Tukey tests showed 2021 had greater native cover than 2018 (p = 0.003), as well as 2019 (p = 0.005). The control plots had greater native cover than the bison treatment (p < 0.001) and the cattle treatment (p < 0.001).

For percent cover of non-native species in each plot (Fig. 6b), the data passed the Brown-Forsythe test (p=0.853), so I used a two-way ANOVA. There was a statistically significant difference among years for non-native cover (F(2) = 14.006, p <0.001), but not for grazing treatments (F(2)=0.241, p = 0.787). Tukey tests showed that non-native cover increased between 2019 and 2018 (p <0.001), and decreased between 2021 and 2019 (p <0.001).



Figure 7. Bar plots of proportion of native (a) and non-native (b) species in a plot out of the total number of species in that plot. Standard error bars are included. Bars are colored by grazing treatment. Capital letters indicate significance among grazing treatments, while lowercase letters in the boxes at the top of each plot indicate significance among years, based on a two-way ANOVA and post-hoc Tukey HSD test (p<0.05).

Then, I was interested in what proportion of the species in each plot were native and non-native, so I grouped the species by nativity, then found the proportion for each plot. For proportion of native species (Fig. 7a), the data failed the Brown-Forsythe test (p = 0.003), but the rest of the tests were normal, so I still used an ANOVA. There were significant differences in proportion of native species across grazing treatment (F(2) = 10.85, p < 0.001) and year (F(2) = 16.97, p < 0.001). Tukey tests showed an increase between 2021 and 2018 (p < 0.001), and 2021 and 2019 (p < 0.001). Tukey tests for treatment groups also showed the cattle treatment had a lower proportion of native species than the bison treatment (p = 0.003) and relative to the control (p < 0.001).

For the proportion of non-native species (Fig. 7b), the data passed the Brown-Forsythe test, so I used an ANOVA. There was a statistically significant difference among years (F(2) = 16.908, p < 0.001), but not among grazing treatments (F(2) =3.154, p = 0.052). Tukey tests on year showed a decrease in the proportion of nonnatives between 2021 and 2018 (p < 0.001) and between 2021 and 2019 (p < 0.001).

Soil Elemental Properties



Figure 8. Bar plots of average soil elements for grazing treatments each year. Standard error bars are included, and bars are colored using grazing treatment. Capital letters indicate significance among grazing treatments, while lowercase letters in the boxes at the top of each plot indicate significance among years, based on a two-way ANOVA and post-hoc Tukey HSD test (p<0.05).

To analyze the percent carbon content of the soil samples (Fig. 8a), I used a twoway ANOVA with grazing treatment and year as variables, even though the data failed the Brown-Forsythe test (p<0.001) but passed the other normality tests. There was a significant difference in soil carbon for grazing treatments (F(2) = 16.531, p <0.001), but not for year (F(2) = 2.782, p = 0.0667). Tukey tests on treatments showed that the cattle treatments had lower carbon content than bison (p <0.001), as did the control fields (p <0.001).

For soil nitrogen content (Fig. 8b), the data failed a Brown-Forsythe test (p <0.001) but passed normality tests of residual qqplots and histograms, so I used a twoway ANOVA. There were significant differences among years (F(2) = 34.76, p <0.001) and among grazing treatments (F(2) = 27.69, p <0.001). Tukey tests for treatment showed the bison treatment had greater soil nitrogen content than the cattle (p <0.001) and control treatments (p <0.001), and the cattle treatment had greater soil nitrogen than the control (p = 0.040). Tukey tests for year showed increases in nitrogen between 2019 and 2018 (p <0.001), and between 2021 and 2018 (p <0.001).

Last, the C:N ratio was calculated by dividing the carbon content for individual soil samples by the nitrogen content (Fig. 8c). The data failed a Brown-Forsythe test (p = 0.007), but passed the other normality tests, so I continued with an ANOVA. There were significant differences for year (F(2) = 98.05, p <0.001) and treatment (F(2) = 13.37, p <0.001). Tukey tests for year showed a decrease in C:N ratio between 2019 and 2018 (p <0.001), and between 2021 and 2018 (p <0.001). Tukey tests for treatment showed a larger C:N ratio for the control than the bison (p <0.001) and the cattle (p <0.001).

NMS Without Soil Moisture

For the run without the soil moisture data, two dimensions and 100 iterations were used, resulting in a stress of 0.21 when the solution was reached on run 20. The abbreviations used in the data are explained in Table S1, and the NMDS factors can be seen in Table S2.



Functional Group • Forb • Graminoid • Shrub Grazing Treatment • Bison • Cattle • Control

Figure 9. NMDS plot of NMS ordination data, with plant species as circles color coded by functional group, and plots colored by grazing treatment. Overlays are environmental variables with p < 0.001, showing associations between environmental variables and the grazing treatments.

The NMDS plot (Fig. 9) uses species abundance data as dots, color-coded by plant type, and plot information as the squares. It yields an r² value of 0.56 for the treatment variables and gives a p-value of 0.001 for treatment, indicating that there is a moderate but significant amount of the variation in species abundances accounted for by grazing treatment in the NMS. There is separation between the control (Waubay) plots and the cattle and bison plots, which also have a moderate clustering that overlap each other. There does not seem to be clustering of the plant species functional groups across the grazing treatments. The arrows displaying associations of variables with a significance value of p<0.001 are plotted. These vectors include the percent cover of native species per plot (pctNative), the percent cover of forbs per plot (pctF), the cattle grazing treatment (Cattle), the control grazing treatment (Control), time (designated as Year), the percent nitrogen content of live graminoids (G Npct), the percent nitrogen content of forbs (F Npct), the C:N ratio of live graminoids (G CN), the biomass of dead grass (DG biomass), and the biomass of forbs (F biomass). Arrow length corresponds to the strength of the relationship. Along Composite 1, there appears to be a moderate association between forb biomass and the control treatment, as well as the percent cover of native species. Cattle also seems to trend with increasing nitrogen content of grass and forbs. The control plots have greater percent of native species, forb biomass, and percent cover of forbs. To a lesser degree, along Composite 2, the amount of dead grass biomass, percent cover of forbs, and percent cover of native species appear to be increasing over time.

Upon inspection of the NMDS vector significance values, there were a number of vectors with p values of 0.002, so I plotted the NMS data with new overlays including

vectors with p<0.002 (Fig. 10). These new overlays include the bison grazing treatment, the 2021 year of data, and the percent nitrogen of dead grass. Along component 1, there is not a large separation of the three grazing treatments, but along component 2, year and bison grazing treatment are separated, indicating a significant difference in the bison fields with time, specifically for the plant communities observed in 2021. The nitrogen content of dead grass and live grass both appear to be trending with the cattle treatment along component 1.



Functional Group • Forb • Graminoid • Shrub Grazing Treatment • Bison • Cattle • Control

Figure 10. NMDS plot of NMS ordination data, with plant species as circles color coded by functional group, and plots colored by grazing treatment. Overlays are environmental variables with $p \le 0.002$, showing associations between environmental variables and the grazing treatments.

Since there appeared to be similarities between the response variables and the independent variables, I conducted an ANOSIM (Analysis of Similarity) test, which tests the significance of difference among groups. I ran this ANOSIM test using the same data matrices used for the NMDS, and compared the results when the species abundances were grouped by grazing treatment and year using a Bray-Curtis dissimilarity measure and 999 permutations. For data grouped by treatment, p = 0.001 and the R value was 0.62, meaning that there is a moderate, significant difference among the plant communities for the three treatments (cattle, bison, control) (*ANOSIM Test in R*, 2019). For species abundances grouped by year, the ANOSIM yielded a p = 0.001 and an R statistic of 0.27, indicating that there is a weak, significant difference among the plant communities under the grazing treatments from 2018 to 2021.

I ran a Mantel test to determine whether the differences in community composition co-varied with soil C:N ratio. I ran the species abundance data with a Bray-Curtis dissimilarity test and the soil C:N data with a euclidean dissimilarity test comparing the differences in soil C:N among samples. The Mantel test used the Spearman's rank correlation and 999 permutations, resulting in a Mantel statistic (r) of 0.18 and a p-value = 0.003, indicating that there was a significant difference, but the relationship between the community composition and the soil C:N data was very weak. Last, to identify any indicator species for the treatments, I ran an indicator species analysis, the results of which are in Table S3. The bison grazing treatment was defined by three species, *Artemisia frigida* (fringed sagebrush, p = 0.004), *Monarda fistulosa* (wild bergamot, p = 0.006), and *Tragopogon dubius* (goatsbeard, p = 0.047), all three of which are forbs and the first two are native. The cattle treatment had nine indicator

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species, five of which are non-native, and five which are graminoids. The control field had sixteen indicator species, only three of which were non-native and twelve of which are forbs.



NMS With Soil Moisture

Functional Group • Forb • Graminoid • Shrub Grazing Treatment • Bison • Cattle • Control

Figure 11. NMS ordination plot for data including soil moisture (data from 2018 and 2021). Plant species are points colored based on functional group, and plots are squares colored by grazing treatment. Overlays are environmental variables with p<=0.001.

I re-ran the NMS ordination using the 2018 and 2021 data, so that I could include the soil moisture variable (Table S2). A stress of 0.18 was achieved on run 20. The goodness of fit for treatments had an r^2 value of 0.50, and a p-value = 0.001, indicating

a moderate but significant fit of the model to the species abundance and environmental variables data. After plotting the data, it was clear that many more variables were included on the overlay (Fig. 11). There is a strong positive trend between soil moisture (Moisture), soil C:N (SoilC_N), and shrub wood C:N (S_CN) along component 2. Live grass C:N (G_CN) appears to increase with soil moisture and no grazing. Cattle grazing appears to have a strong association with the percent nitrogen of forbs (F_Npct) along component 1. Percent nitrogen of live grass (G_Npct), dead grass (DG_Npct), and soil (SoilNpct) appear to trend positively with increases in time (Year) and cattle grazing (Cattle). Dead grass biomass (DG_biomass), total biomass of sample (Total_Biomass), the percent coverage of native species per plot (pctNative), the percent coverage of forbs (pctF), and live grass biomass (G_biomass) appear to increase with time and no grazing.

There were many interrelated variables revealed in this NMS plot. To test for significance, I used an ANOSIM test with Bray-Curtis dissimilarity and 999 permutations to assess the effects of treatment and year on community composition. For groups of treatment, the ANOSIM R statistic was 0.60, and yielded a p-value = 0.001, indicating a moderate, significant difference in plant communities across treatments. For groups of years, using species abundance and soil moisture, the R statistic was 0.39, and p = 0.001, showing a weak, significant difference in plant communities across the years. For the Mantel test for this data, the r statistic was 0.24 and p = 0.001, showing that soil moisture weakly but significantly selects for certain plant communities. For the same years, using species abundance and soil C:N ratio, the Mantel statistic was 0.15 and p = 0.009, meaning there is a weak but significant

relationship between soil C:N and plant community composition. Last, an indicator species analysis identified three species for the bison treatment, *Artemisia frigida* (fringed sagebrush, p = 0.009), *Poa pratensis* (Kentucky bluegrass, p = 0.005), and *Monarda fistulosa* (wild bergamot, p = 0.015) (Table S3). Two of these species are native forbs, but Kentucky bluegrass is a non-native graminoid. Five species were identified for the cattle treatment, three native and two non-native, and fourteen species were identified for the control fields, thirteen of which were native.

Discussion

To assess the effects of bison grazing versus cattle grazing on plant abundance by species and functional group, soil nutrients, plant nutrient content, and biomass, the NMS ordinations combined the variables of treatment and year with multiple dependent variables, showing relationships between grazing treatments and nutrient content as well as nativity of species. The bison treatment was significant at a p-value of 0.002, the distribution of the plots across all three of the NMS graphs shows distinct clustering of the cattle plots, the bison plots, and the control plots – indicating that there is a degree of separation across them for the environmental variables and species abundances. The bison plots fall between the cattle and control plots on Component 1, indicating that the environmental variables and the species abundances for the bison treatments are intermediate between those of cattle and those of the unmanaged control tallgrass prairie plots, but along Component 2, bison and year separate out quite clearly while cattle and control treatments do not, indicating the effects of the bison grazing treatment are changing over time unlike the other treatments. Another year of data may be enough to provide the temporal separation that would be necessary to see nonoverlapping clustering on the NMDS plots.

While I did not see the increase in species richness under bison grazing versus cattle that I expected, species richness among the functional groups showed some movement of bison rangeland towards historical prairie characteristics. The cattle and bison fields did not have significantly different species richness, but the grazed plots both had higher graminoid species richness than the control plot, although the bison was closer to the control levels, and cattle had higher graminoid richness than bison. Since many of the non-native species are graminoids, this is an interesting indicator that bison grazing may control graminoid species better than cattle. I found that cattlegrazed plots had higher graminoid cover than bison, but bison and cattle had lower graminoid cover than the control at Waubay. This indicates that although bison grazing appears to drive the plant community to a lower number of graminoid species, the decrease in graminoid cover is not what we see in the control plots. The indicator species for the bison treatment fields indicated the dominance of native forb species, but also, a non-native grass that is a historically planted forage grass (Hillenbrand et al., 2019). This indicates that these previously-dominant species are still present in significant proportions on these bison-grazed fields, which may drive the non-graminoid coverage difference among the grazed fields and the control.

One trend I observed was an increase in dead graminoid biomass from 2018 to 2021. The control plot at Waubay had higher dead biomass than bison and cattle, which can increase the fire risk in those areas. The grazing treatments did not have significantly different dead graminoid biomass, indicating that ungulate grazing could

decrease the frequency of wildfires on prairies, although this is to be expected of grazing treatments in general (Starns et al., 2019). A study of burning and grazing interactions showed that grazing reduced fuel load and reducing the frequency and impact of severe wildfires, promoting greater biodiversity and reducing fire spread (Starns et al., 2019). This may indicate that future management strategies for tallgrass prairie should combine grazing and burning regimes.

I was interested in changes in forb abundances and richness, since most traditional medicines used by the Dakota tribe, with whom I collaborated with during this project, are forbs. I did not see a significant difference in forb cover between bison and cattle plots, with both grazed plots having lower forb cover than the control, and the species richness of forbs was also not significantly different between the two grazing treatments but was significantly lower than the richness of the control plots. While there was a decrease in total forb biomass from 2018 to 2021 -- potentially due to the drought the Midwest has been experiencing for the last two years which can be seen in the NMS plot with soil moisture trending opposite year -- bison had higher forb biomass than cattle, putting the bison grazed plots closer to the higher control field level of forb biomass. So, while bison grazing may not drive greater forb cover or richness, it could drive higher biomass of the species already present, increasing the availability of those species for the traditional medicines of the Dakota people. This increase in herbaceous biomass was also seen by Hillenbrand et al. (2019) on shortgrass prairies under bison grazing treatments.

I did observe greater total species richness for control plots than cattle and bison plots, and the proportion of native species was also higher for the control than cattle.

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Although the difference between the bison and cattle fields was not significant for the proportion of native species, bison fell between the cattle fields and the control, potentially indicating that the bison-grazed plots are slowly moving towards the control level of native species, since there was also an increase in the proportion of native species from 2018 to 2021. There was also an increase in the area covered by native species from 2018 to 2021, with bison falling between the higher control average and the lower cattle average. There was an observed decrease in the proportion of nonnative species over time (2018 to 2021), but grazing treatments did not have an effect on this, indicating that this change may be due to different distributions or dominance of native species over non-native as a result of environmental fluctuations. The majority of indicator species for the three treatments across both NMS ordinations were native, and showed that these fields were characterized by different species of native plants. The effects of bison grazing on species richness have been observed to be positively correlated with increasing sample area (Hartnett et al., 1996), so I may not have observed this relationship in my plots due to the limitation of number of bison fields and size of the plots I was able to establish.

Previous work on the Konza Prairie highlighting abundances of two main native graminoids between bison and cattle-grazed plots showed decreases under bison and increases under cattle (Towne et al., 2005), one of which was an indicator species for my control field (*Andropogon gerardii*; Table S3), and the other was an indicator for my cattle field (*Schizachyrium scoparium*; Table S3). Given the abundance of the *A. gerardii* on the reserve fields, with another year of data, it may be possible to observe this species on the bison fields as well if the trend of increased native coverage

continues for this treatment, although Vinton et al. found that bison preferentially grazed areas with lower forb cover and high abundance of *A. gerardii* (1993), which may be the cause of the low measured abundance of this species on my bison-grazed field. *A. gerardii* on the Konza Prairie showed decreased regrowth rate under burning and ungulate grazing, but the burned communities were more homogeneous and dominated by graminoids, so although there may not be dominant communities of *A. gerardii* in the grazed plots of my study, the grazing treatments should drive more heterogeneous plant communities, as seen by Vinton & Hartnett (1992).

Last, I observed that bison grazing contributed to higher soil carbon levels than cattle or the control, and also had the highest soil nitrogen level compared to the other two treatments. Soil nitrogen levels increased from 2018 to 2021, but the control had the lowest soil nitrogen out of the three treatments. This indicates that the animal activity, and potentially, waste products, are significantly changing the soil properties in these fields. 80% to 90% of organic carbon on prairies is in the soil, forming a pool of long-term carbon storage (Sanderson et al., 2020). Increasing soil carbon under bison grazing could be a potential mechanism of compensating for anthropogenic carbon emissions. The C:N ratio of the soil decreased from 2018 to 2021, but the control at Waubay now had the highest ratio in 2021, greater than bison, and then cattle. The grazing treatments, and specifically the bison, may be driving a lower C:N ratio for tallgrass prairie sites, increasing the guality of the soil, which could promote more productive plant communities (Johnson & Matchett, 2001). The litter inputs are of greater N concentration as well, which can increase N immobilization and mineralization by soil microbes (Johnson & Matchett, 2001). Soil moisture was also strongly

associated with the C:N ratios of shrub wood, grasses, and soil, all four of which appeared to be decreasing over time. The observed decrease in soil carbon is not what I had expected, according to the results of Damhoureyeh & Hartnett (1997), but could be due to the cessation of the short-term effects observed in that study in our four-yearlong study, or the decrease in root C input under grazing as found by Johnson & Matchett (2001). Cattle grazing was associated with greater nitrogen contents in grass, dead grass, and forbs, which further research should investigate for a mechanism.

Conclusion

These results have important implications for bison as a tallgrass prairie restoration method. There is evidence that bison grazing can drive graminoid species towards a level seen on unmanaged prairies and promote native species diversity and cover in bison-grazed plots. Bison can also promote higher forb biomass, which would increase the availability of the species in this functional group as traditional medicines. Understanding how these changes in plant communities are affecting ecosystem function and services of tallgrass prairie should be investigated further, although research on shortgrass prairies indicate improvements in ecosystem services under managed bison grazing (Hillenbrand et al., 2019). Future work is necessary to see the long-term effects of bison grazing, and whether these trends continue or the species compositions shift, but this early research shows that bison can serve as a viable restoration method for tallgrass prairie when targeting the specific issues of non-native species and graminoid cover.

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Supplementary Materials

Table S1. Explanation of variable

abbreviations used in NMS

ordination.

Variable Code	
pctInvasive	Percent coverage of non-native species
pctNative	Percent coverage of native species
pctF	Percent coverage of forbs
pctG	Percent coverage of graminoids
pctS	Percent coverage of shrubs
Bison	Bison grazing treatment
Cattle	Cattle grazing treatment
Control	Control grazing treatment, field at Waubay
Transect	Value 1-2, transect per field
Transect_1	Coded as 1 for plot in transect 1, 0 for not
Transect_2	Coded as 1 for plot in transect 2, 0 for not
Time_2018	Coded as 1 for samples from year 2018, 0 for not
Time_2019	Coded as 1 for samples from year 2019, 0 for not
Time_2021	Coded as 1 for samples from year 2021, 0 for not
Year	Year data was collected, i.e. 2018 - 2021
SoilNpct	Percent nitrogen for soil samples
SoilCpct	Percent carbon for soil samples
SoilC_N	C:N ratio for soil samples
G_Npct	Percent nitrogen for live grass samples
DG_Npct	Percent nitrogen for dead grass samples
F_Npct	Percent nitrogen for forb samples
S_Npct	Percent nitrogen for shrub wood samples
SF_Npct	Percent nitrogen for shrub foliage samples
G_Cpct	Percent carbon for live grass samples
DG_Cpct	Percent carbon for dead grass samples
F_Cpct	Percent carbon for forb samples
S_Cpct	Percent carbon for shrub wood samples
SF_Cpct	Percent carbon for shrub foliage samples
G_CN	C:N ratio for live grass samples
DG_CN	C:N ratio for dead grass samples
F_CN	C:N ratio for forb samples
S_CN	C:N ratio for shrub wood samples
SF_CN	C:N ratio for shrub foliage samples
G_biomass	Biomass per sample of live grass
DG_biomass	Biomass per sample of dead grass
F_biomass	Biomass per sample of forb
S_biomass	Biomass per sample of shrub wood
SF_biomass	Biomass per sample of shrub foliage
Total_Biomass	Total biomass of sample

Table S2. Results of NMS ordination on species abundance data with and without the

soil moisture variable. Permutations were free, and 999 permutations were used.

	Runs with Soil Moisture						Runs without Soil Moisture							
	Species Environment						Vectors Environment							
	NMDS1	NMDS2	r2	Vectors	Pr(>r)	Vectors P	?r(>r)	NMDS1	NMDS2	r2	Pr(>	•r)	Vectors (Pr(>r)
pctInvasive	0.37535	-0.92688	0.0612	0.389		0.373		-0.58115	-0.8138	0.0577	0.229		0.247	
pctNative	0.82166	0.56998	0.5905	0.001	***	0.001	***	0.88766	0.46051	0.4288	0.001	•••	0.001	***
pctF	0.95155	0.3075	0.4084	0.001	***	0.001	•••	0.8401	0.54243	0.4559	0.001	•••	0.001	•••
pctG	-0.66508	0.74678	0.154	0.066		0.07		-0.98011	0.19848	0.1727	0.01	••	0.009	**
pctS	0.9417	0.33645	0.029	0.603		0.61		0.51573	-0.85675	0.066	0.18		0.187	
Bison	-0.73934	-0.67334	0.0559	0.428		0.394		-0.38849	-0.92145	0.2264	0.002	••	0.003	**
Cattle	-0.98368	0.17995	0.4687	0.001	•••	0.001	•••	-0.90325	0.42911	0.4772	0.001	•••	0.001	•••
Control	0.99817	0.06055	0.7277	0.001	•••	0.001	•••	0.96685	0.25533	0.7731	0.001	•••	0.001	•••
Transect	0.99833	-0.05782	0.0426	0.498		0.488		0.36192	-0.93221	0.1277	0.045	*	0.035	*
Moisture	-0.12214	-0.99251	0.6317	0.001	***	0.001	***							
Transect_1	-0.99833	0.05782	0.0426	0.498		0.488		-0.36192	0.93221	0.1277	0.045		0.035	
Transect_2	0.99833	-0.05782	0.0426	0.498		0.488		0.36192	-0.93221	0.1277	0.045		0.035	*
Time_2018	0	0	0	1		1		0	0	0	1		1	
Time_2019	0	0	0	1		1		0	0	0	1		1	
Time_2021	0.05934	0.99824	0.7797	0.001	***	0.001	•••	0.24089	0.97055	0.2461	0.002	••	0.001	***
Year	0.05934	0.99824	0.7797	0.001	***	0.001	***	0.19369	0.98106	0.3189	0.001	***	0.001	***
SoilNpct	-0.33391	0.9426	0.4425	0.001	***	0.001	***	-0.88251	0.4703	0.0627	0.214		0.199	
SoilCpct	-0.59987	0.8001	0.1426	0.1		0.101		-0.61696	-0.78699	0.0426	0.347		0.345	I
SoilC N	0.20922	-0.97787	0.4882	0.001	•••	0.001	•••	0.38721	-0.92199	0.1885	0.009	••	0.009	••
AG Npct	-0.82545	0.56447	0.4855	0.001	•••	0.001	•••	-0.89781	0.44038	0.2284	0.001	•••	0.005	••
DG_Npct	-0.41891	0.90803	0.4621	0.001	•••	0.001	•••	-0.56003	0.82847	0.2303	0.002	••	0.001	***
F Npct	-0.99894	-0.04593	0.4462	0.001	***	0.001	•••	-0.9632	0.26879	0.2535	0.001	•••	0.001	***
S Npct	-0.53038	0.84776	0.0443	0.478		0.462		-0.93693	-0.34951	0.0068	0.866		0.836	l
SF Npct	0.37943	-0.92522	0.0362	0.576		0.565		0.28302	-0.95911	0.0304	0.443		0.444	l
AG Cpct	-0.00995	0.99995	0.103	0.145		0.172		0.74187	0.67054	0.0221	0.58		0.575	l
DG Cpct	-0.78739	0.61646	0.1113	0.118		0.142		-0.40241	0.91546	0.0053	0.882		0.855	
F Cpct	0.41177	0.91129	0.0141	0.789		0.786		0.08664	-0.99624	0.0451	0.318		0.324	
S Cpct	0.21041	-0.97761	0.0875	0.211		0.232		0.46366	-0.88602	0.0922	0.084		0.092	
SF Cpct	0.47809	-0.87831	0.115	0.134		0.163		0.41092	-0.91167	0.0999	0.086		0.08	
AG CN	0.90663	-0.42192	0.4855	0.001	***	0.001	***	0.97714	-0.21261	0.2893	0.001		0.002	••
DG CN	0.73263	-0.68063	0.3013	0.003	**	0.005	••	0.84488	-0.53496	0.1704	0.015		0.011	
F CN	0.86444	0.50273	0.222	0.013		0.009	**	0.99612	0.08806	0.139	0.025		0.024	
S CN	0.37429	-0.92731	0.3234	0.001	***	0.002	••	0.56188	-0.82722	0.1419	0.04		0.018	
SF CN	0.58948	-0.80778	0.1858	0.032		0.048		0.52706	-0.84983	0.1548	0.012		0.016	
AG biomass	0.97143	0.23732	0.4147	0.001	***	0.001	***	-0.18861	-0.98205	0.0182	0.635		0.655	
DG biomass	0.30652	0.95186	0.5849	0.001	***	0.001	***	0.53161	0.84699	0.2821	0.001	***	0.001	***
F biomass	0.83913	0.54393	0.3395	0.002	**	0.002	••	0.99927	0.03826	0.2791	0.001	•••	0.002	**
S biomass	-0.1481	0.98897	0.0007	0.989		0.99		-0.14975	-0.98872	0.0931	0.08		0.084	
SF biomass	0.2546	-0.96705	0.0495	0.456		0.476		-0.05804	-0.99831	0.0627	0.193		0.203	
Total Biomass	0.47824	0.87823	0.4669	0.001	•••	0.002	••	0.91111	0.41217	0.104	0.068		0.07	
Signif. codes: 0 (**** 0.001 (** 0.01 (** 0.01 (* 1.001 (** 0.01 (

Table S3. Results of indicator species analysis using data matrices from NMS

 ordination. Left column is data from the NMS with the soil moisture included, and

 right column is data from the NMS without the soil moisture included.

	Run	is with Soil M	isture		Runs without Soil Moisture					
	Tota	al number of spec	cies: 78		Total number of species: 78					
	Select	ted number of sp	ecies: 28		Selected number of species: 35					
	Number of species associate	d to 1 group: 22		Number of species associated to 1 group: 28						
	Number of species associate	d to 2 groups: 6		Number of species associated to 2 groups: 7						
Grazing Treatment	Species name	r Statistic	p-value		Species name	r Statistic	p-value			
	Artemisia_frigida	0.557	0.009	**	Artemisia_frigida	0.493	0.004	**		
Bison	Poa_pratensis	0.511	0.005	••	Monarda_fistulosa	0.414	0.006	••		
	Monarda_fistulosa	0.456	0.015	•	Tragopogon_dubius	0.35	0.047	•		
	Scolochloa_festucacea	0.768	0.001	***	Agrostis_gigantea	0.74	0.001	•••		
	Agrostis_gigantea	0.601	0.003	••	Scolochloa_festucacea	0.572	0.001	•••		
	Trifolium_dubium	0.506	0.005	••	Phleum_pratense	0.467	0.004	••		
	Erigeron_strigosus	0.452	0.011	•	Schizachyrium_scoparium	0.464	0.005	••		
Cattle	Verbena_stricta	0.407	0.037	•	Erigeron_strigosus	0.448	0.006	••		
					Verbena_stricta	0.412	0.01	••		
					Trifolium_dubium	0.39	0.013	•		
					Trifolium_repens	0.332	0.035	•		
					Dichantheium_oligosnthes	0.297	0.048	•		
	Pediomelum_argophyllum	0.808	0.001	•••	Pediomelum_argophyllum	0.79	0.001	•••		
	Rosa_arkansana	0.782	0.001	•••	Galium_boreale	0.751	0.001	•••		
	Galium_boreale	0.75	0.001	•••	Heliopsis_helianthoides	0.705	0.001	•••		
Control	Heliopsis_helianthoides	0.618	0.004	••	Rosa_arkansana	0.694	0.001	•••		
	Dalea_purpurea	0.57	0.003	••	Melilotus_alba	0.629	0.001	•••		
	Helianthrus_pauciflorus	0.557	0.002	••	Solidago_rigida	0.62	0.001	•••		
	Echinaceae_purpurea	0.556	0.006	••	Brickellia_eupatorioides	0.577	0.001	•••		
	Brickellia_eupatorioides	0.55	0.008	••	Echinaceae_purpurea	0.57	0.001	•••		
	Melilotus_alba	0.511	0.007	••	Dalea_purpurea	0.561	0.001	•••		
	Achnatherum_hymnoides	0.497	0.038	•	Helianthrus_pauciflorus	0.556	0.001	•••		
	Solidago_mollis	0.495	0.029	•	Helianthus_maximiliani	0.496	0.002	••		
	Helianthus_maximiliani	0.495	0.03	•	Melilotus_oficinalis	0.459	0.004	••		
	Solidago_rigida	0.481	0.015	•	Andropogon_gerardii	0.44	0.004	••		
	Andropogon_gerardii	0.48	0.015	•	Achnatherum_hymnoides	0.398	0.025	•		
					Solidago_mollis	0.397	0.021	•		
					Liatris_aspera	0.394	0.029	•		