Central Grasslands Research Extension Center

2023 Annual Report

Grazing – Forage – Wildlife – Livestock

NDSU | North Dakota Agricultural Experiment Station NDSU | Extension

Summary of the Year

Welcome to the 2023 CGREC Annual Report

The year 2023 grazing season started late with the long winter. The center received over 100 inches of snow during the 2022-2023 winter, with many heavy snow events and a lot of wind. Once the snow finally melted in early May, moisture became very scares in May and June.

Our grazing program focused on two large scale experiments included 1) testing heterogeneity-based grazing treatments studies and 2) integrated livestock-cropping system trials. The focus of the heterogeneity-based treatments was designed to create different levels of structure and plant diversity across the landscape to see which treatments created better wildlife and pollinator habitat while enhancing livestock performance. The integrated livestock-cropping system trials were designed to test the pros and cons of adding livestock grazing on cropland and test if we can enhance soil health while maintaining or improving crop production. See the research reports for our updated reports on these trials.

Our forage program in 2023 was designed to assess different supplemental feeds and silage production. Again, see the research reports for our updated reports on these trials.

The wildlife and pollinator trials focused on impacts of the grazing treatments that were designed to assess heterogeneity-based grazing on habitat. We studied patch-burn grazing, a modified twice-over rest rotation grazing system, and continuous grazing. See reports for latest updates.

Finally, our livestock trials focus on impacts of limit feed vitamins and minerals, or dietary energy on heifer and fetal development. See reports for latest updates.

I would like to end by providing an update on our capital projects. We received special funding from the 2021-2023 state legislative session for a new livestock working facility and research complex. We broke ground in 2023 and hope to have the project completed by 2024. We are hoping to go out on bids for the director’s residence this spring and have a completed project by fall 2024. We received funding to build new pasture working facilities, with these projects projected completed in 2023.

Our 2024 annual field day is scheduled for July 8. We plan to run two tours, one focusing on a new trial that will start in May 2024 looking at a new heterogeneity-based trial using virtual fencing. We think this treatment will allow heterogeneity in structure and plant community composition similar to patch-burn grazing (without fire) without giving up livestock performance that we see with the modified twice-over rest rotation grazing system. Fire does create a flush of flowering plants that grazing alone does not achieve, so I do not expect the grazing with virtual fencing treatment to change that impact. We will also tour the new livestock – cropping system study looking at grazing winter rye prior to planting silage corn to assess soil health impacts of this type of integrated system. All are welcome and come enjoy a good meal and southcentral North Dakota hospitality.

Lastly, we said goodbye to our Sandi Dewald on March 5, 2024. She worked at Central Grasslands REC for 30 years and will be truly missed. We Love You Sandi!

We hope to continue serving you for many years. You are always welcome to stop by anytime and see our research or just visit.

Kevin Sedivec, Interim Director

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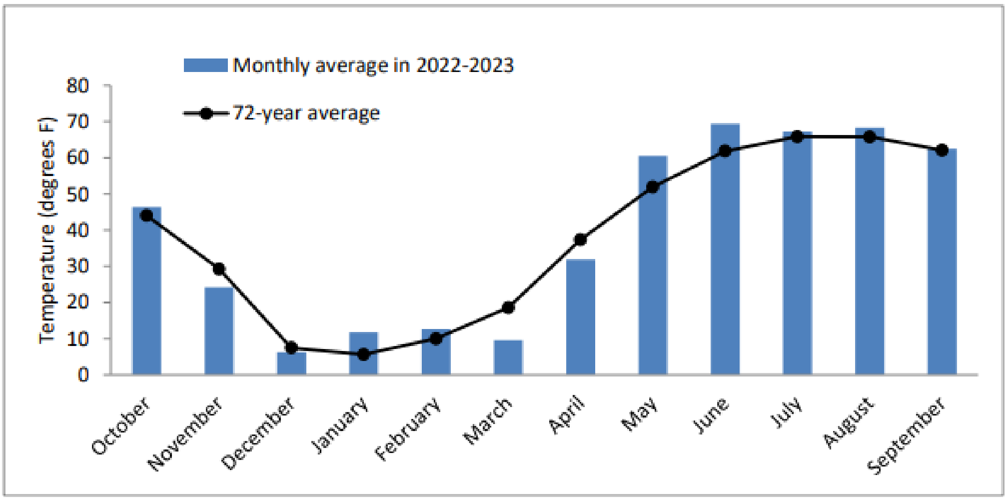
Monthly Temperatures for the 2022-2023 Crop Year



Last spring frost: May 2 (27°F)

First fall frost: Oct. 7 (28°F)

158 frost-free days

Average[1] last spring frost: May 13

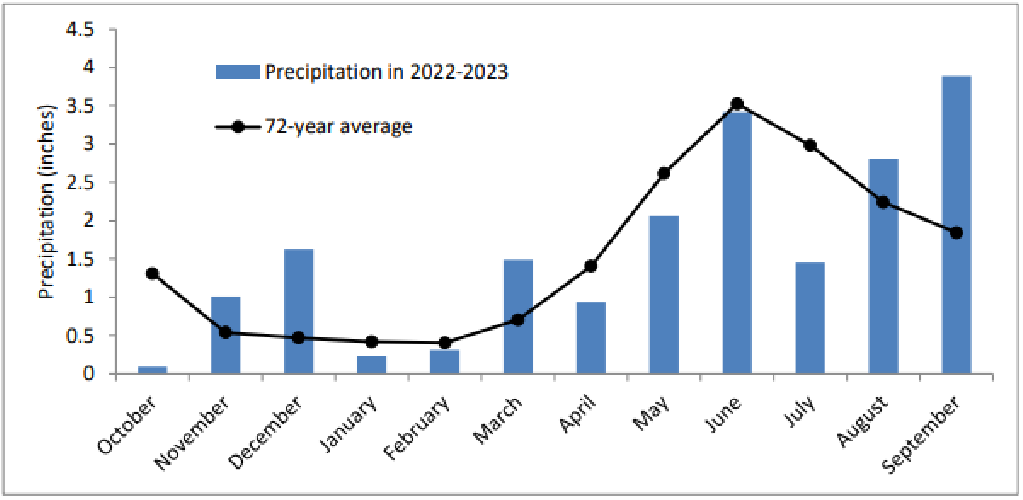
Average first fall frost: Sept. 22

Average: 132 frost-free days

Month	Maximum temperature[2]	Minimum temperature	Average temperature	Long-term[1] average temperature	2022-2023 deviation from long-
October	77	19	46.4	44.0	2.4
November	75	-3	24.2	29.2	-5.0
December	32	-25	6.2	7.4	-1.2
January	36	-24	11.7	5.6	6.1
February	40	-26	12.7	10.0	2.7
March	31	-11	9.5	18.5	-9.0
April	60	0	31.9	37.4	-5.5
May	88	26	60.5	51.9	8.6
June	95	47	69.4	61.8	7.6
July	98	46	67.2	65.9	1.3
August	91	48	68.3	65.8	2.5
September	96	44	62.5	62.1	0.4

[1] 1951-2023; 72 years [2] Degrees F

Monthly Precipitation for the 2022-2023 Crop Year



Month	Precipitation[1]	Long-term[2] average precipitation	Deviation from long-term average	Percent of long-term average	Accumulated precipitation	Accumulated long-term average	Snow[3]
October	0.09	1.31	-1.22	7	0.09	1.31	0
November	1.01	0.54	0.47	188	1.1	1.85	17.5
December	1.63	0.47	1.16	347	2.73	2.32	35.5
January	0.23	0.42	-0.19	55	2.96	2.73	3
February	0.31	0.40	-0.09	77	3.27	3.14	7.5
March	1.49	0.70	0.79	212	4.76	3.84	31.5
April	0.94	1.41	-0.47	67	5.7	5.25	10
May	2.06	2.62	-0.56	79	7.76	7.86	0
June	3.42	3.53	-0.11	97	11.18	11.39	0
July	1.45	2.98	-1.53	49	12.63	14.37	0
August	2.81	2.24	0.57	125	15.44	16.61	0
September	3.89	1.84	2.05	211	19.33	18.46	0
Total	**19.33**	**18.46**	**0.87**	**105**	**19.33**	**18.46**	**105**

[1] Rain and melted snow in inches [2] 1951-2023; 72 years [3] Depth in inches

Using Axillary Buds to Assess Timing of Grazing on Smooth Brome Dominated Range

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Summary

Invasive species can harm grassland ecosystems and degrade biodiversity. Smooth brome is an introduced, perennial, cool season (C_3) grass that has been planted widely and is invasive in the Northern Great Plains. Land that has not been grazed or burned for extended periods is especially prone to invasion by smooth brome. Smooth brome has belowground axillary buds from which new stems and rhizomes develop. Because of that, belowground axillary buds play a large role in the persistence of many perennial grasses and quantifying them can provide information regarding the effects of grazing on a species. Recent studies suggest that grazing has a role in preventing smooth brome invasion, which posed the question of whether grazing could increase diversity of already invaded prairie. To test how timing of grazing affects diversity and smooth brome in an invaded prairie of south-central North Dakota, we quantified plant community canopy cover, and smooth brome tiller and their axillary bud abundance. Compared to non-grazed control, spring+fall and spring only grazing had significantly lower relative cover of smooth brome, while just the spring only treatment had significantly greater relative cover of non-native forbs. Our measures of axillary bud were similar among treatments and the control, except there was a significantly greater number of active buds from fall only grazing compared to spring+fall but neither differed from the control. We suspect that grazing may have led to a greater proportion of tillering and rhizome production by breaking apical dominance, but that survivorship may have been lower in some of the treatments and will test this further.

Introduction

Invasive species displace native species and can harm native ecosystems, often leading to changes in biodiversity, ecosystem services, or ecosystem functioning (Pejchar and Mooney 2009). Lower diversity plant communities are less stable overtime and take longer to recover from drought, compared to more diverse communities (Tilman 1996). Diverse plant communities not only support a greater number of animal species, but also stabilize wildlife communities (Fuhlendorf et al. 2006; Ellis-Felege et al. 2013). Diverse plant communities have also been shown to stabilize livestock production (Allred et al. 2014). Yet, biodiversity of plant communities in the Northern Great Plains (NGP) have declined for a variety of reasons, including the introduction of non-native plants that have become invasive (DeKeyser et al. 2013; Toledo et al. 2014; Grant et al. 2020). For example, the invasive Kentucky bluegrass (*Poa pratensis* L.), makes up at least 50% of the plant community in much of the eastern half of the NGP, especially the Dakotas (Toledo et al. 2014).

Another invasive, smooth brome (*Bromus inermis* Leyss.), is an introduced, perennial, cool season (C_3), rhizomatous grass (Otfinowski et al. 2007). It is a persistent invader of prairie in the more mesic eastern part of the NGP (Palit and DeKeyser 2022). Smooth brome was introduced to North America on multiple occasions, the earliest of which was by the University of California Agricultural Experiment Station around 1880 (Dwinelle 1884; Dunn 1985; Otfinowski et al. 2007). Early distribution of this species in North America was likely motivated by its use as a hay and forage species for livestock. It was distributed to numerous researchers and research stations across the continent by the end of the 19th century, including the Great Plains (Newell and Keim 1943; Dunn 1985). Overtime, smooth brome has been used extensively for hay and forage, soil stabilization of newly constructed roadsides, dense nesting cover for some species of gamebirds, mine tailings, and conservation programs such as the Conservation Reserve Program (CRP) (Otfinowski et al. 2007; Ellis-Felege et al. 2013; Dixon et al. 2019). Smooth brome spread in the NGP from areas where it was cultivated into a vast part of the landscape, including native prairie (Otfinowski et al. 2007; DeKeyser et al. 2013; Grant et al. 2020; Palit and DeKeyser 2022).

Idle managed prairie is especially prone to invasion by smooth brome. Idle managed prairie are lands that has not been burned, grazed, or mowed for an extended period (e.g. decades). Grant et al. (2020) conducted a survey of the approximate 90,000 hectares (222,394 acres) of prairie managed by the U.S. Fish and Wildlife Service, specifically the area north and east of the Missouri River in the Dakotas and north-east Montana. This land was idle managed, and it was estimated that smooth brome made up more of the upland plant community than all native grasses and forbs combined (Grant et al. 2020). When compared to nearby privately grazed land, idle managed land had lower species richness and greater abundance of smooth brome (Murphy and Grant 2005; Coleman et al. 2023). These results suggest that grazing has a role in the prevention of smooth brome invasion (Coleman et al. 2023).

Since grazing seems to have a role in preventing smooth brome invasion (Murphy and Grant 2005; Coleman et al. 2023), it may be important to increasing diversity of already invaded prairie. Herbivory forces plants to use energy and resources to regrow, rather than grow and reproduce (Straus and Agrawal 1999), thus repeated grazing may harm smooth brome populations over time and allow other species to grow. Smooth brome grows in the early spring and fall (Otfinowski et al. 2007), so grazing during the early spring and fall may have the greatest negative effect to this species. Smooth brome also begins growing sooner in the spring than most native species (Otfinowski et al. 2007), and rapidly establishes the canopy thus reducing the amount of sunlight reaching later growing species. We speculate that grazing during the early spring and fall may support these later growing species by opening the canopy and increase the sunlight that reaches them, which might increase diversity and indirectly reduce smooth brome through competition overtime.

The bud bank is the collection of all buds (meristems) that could lead to vegetative regeneration of a plant (Klimešová and Klimeš 2007) (Figure 1). As is typical of many perennial herbaceous plants, successful recruitment of shoots from seeds is rare, in fact most of the annual emergence/renewal of shoots comes from below ground buds (Ott et al. 2019). Further, buds play a major role in the regeneration of individual plants and populations after disturbance, however germination from seeds becomes more important for persistence of populations as the severity of disturbance increases (Klimešová and Klimeš 2007; Ott et al. 2019). For instance, Russell et al. (2019) studied responses of belowground axillary buds in three native grasses to fire and found the number of actively growing buds per tiller was greater after fire for species that tolerate fire, but only when burns occurred in certain seasons and fire return intervals. The greater activity of buds post fire in these species suggests that regeneration of fire adapted species would occur primarily from buds, while regeneration of fire sensitive species would occur primarily from new individuals germinating from seeds. Since outgrowth from axillary buds plays such a large role in the annual persistence of many perennial grasses and their regeneration from disturbance (Ott et al. 2019), evaluating the abundance of axillary buds and their outgrowth can provide valuable information regarding a species response to management.

Figure 1 – Stem with daughter tillers emerging from the belowground portion (left). Belowground axillary buds and daughter tillers (right). Axillary buds can grow out into tillers and rhizomes.

We hypothesize that cattle grazing in the spring to a moderate use, fall to a moderate use, or a combination of the spring and fall to a full use, will increase native species, and decrease the abundance of smooth brome in a smooth brome dominated rangeland of the Missouri Coteau. We speculate that these grazing treatments will promote native species by opening the canopy and decrease competition with smooth brome. To test our hypothesis, the objectives of the study are to: (1) Monitor the plant community composition by estimating aerial plant cover. (2) Monitor the abundance of smooth brome stems/tillers. (3) Evaluate the effects of timing of grazing on bud banks of smooth brome.

Methods

Site Description

To test our hypothesis, research was conducted at NDSU's Central Grasslands Research Extension Center located near Streeter, ND. Specifically, the study took place within the Long Pasture, an approximately 150-acre area that lies within the Missouri Coteau ecoregion. The site is categorized as mixed-grass prairie that was historically dominated by cool-season species such as western wheatgrass (Pascopyrum smithii), green needlegrass (*Nassella viridula*), and needle-and-thread (*Hesperostipa comata*). Warm-season grasses, such as little bluestem (*Schizachyrium scoparium*) and blue grama (*Bouteloua gracilis*), various forb species and sedges (*Carex* spp.) were also common (Barker and Whitman 1988; Limb et al. 2018). The pasture was idle managed from 1981-2007 during which smooth brome (*Bromus inermis* Leyss.) invaded the site. The site has been grazed since 2007; however, grazing was infrequent and never above a moderate rate. It occurred primarily from the spring through the summer, and rarely the fall. Long Pasture was grazed four years prior to the study (2018-2021), and smooth brome still dominated the site at the beginning of the study in 2022.

Study Design

We divided the pasture into four blocks using interior fencing (Figure 2). Within each block, we randomly placed an experimental plot on upland loamy sites (Figure 2A). Each experimental plot consisted of four subplots, one for the control and each of the treatments, which were grazing during the spring only, fall only, and a combination of both spring and fall (spring+fall). Each subplot measured ~9.75 x 9.75 meters (32 x 32 feet). Blocks were grazed by a separate herd of cattle at approximately the same stocking rate at the same time. Cattle had access to their entire block but cattle panels restricted access to specific subplots (Figure 2B/C). During the spring, cattle had access to spring+fall and spring-only subplots while the fall-only and control subplots were enclosed to prevent grazing (Figure 2B). In the fall, the fall-only subplots were opened to allow grazing, while the spring-only subplots were closed to prevent grazing (Figure 2C). Cattle were stocked to achieve a 40-60% degree of disappearance in the spring+fall treatment which was verified by clipping and weighing biomass. However, due to a snowstorm ending the fall 2022 grazing season earlier than planned, degree of disappearance was estimated visually for spring+fall and fall only treatments in 2022 by Dr. Kevin Sedivec. We clipped biomass from five 0.25 m^2 quadrats per subplot and dried them. Degree of disappearance was calculated by dividing biomass in a treatment by biomass of the control and subtracting it from one to get a percentage. Grazing dates and stocking rates for each treatment are shown in Table 2.

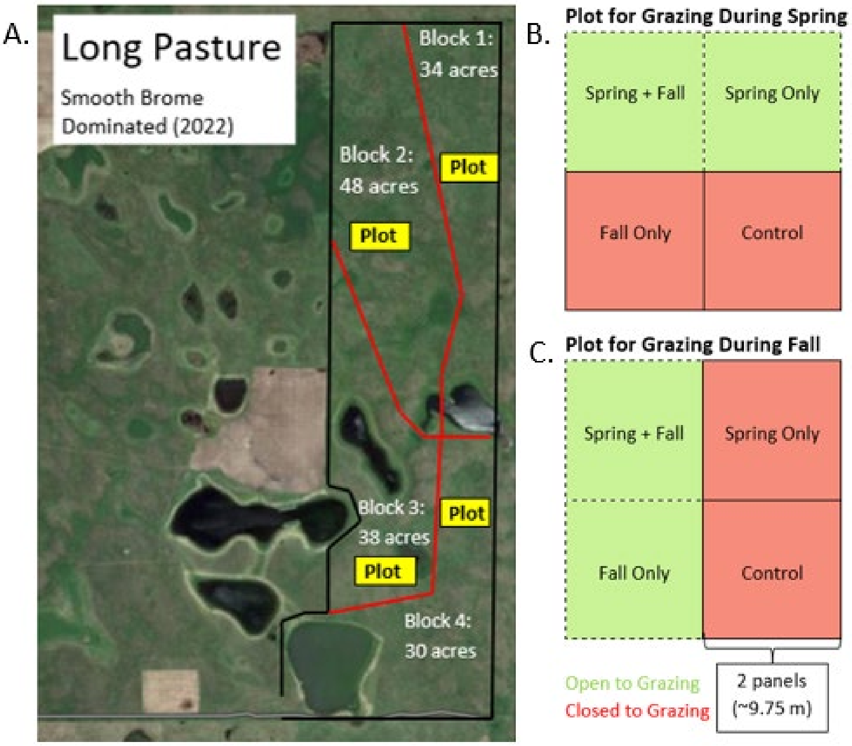

Figure 2 – Experimental design. Long Pasture was divided into four blocks, each grazed by a separate herd of cattle at the same stocking rate (A). Within each block, an experimental plot was established on upload loamy sites (A). Each experimental plot consisted of four subplots, one for the control and each treatment (B/C). Cattle panels were used to restrict access to subplots, spring+fall was always open to allow grazing, spring only was open during the spring, fall only was open during the fall, and control was never open to grazing (B/C).

Table 1 – Grazing dates with start date, end date, total months, AUM/ha, and Degree of Disappearance. Months were calculated by taking the number of days divided by 30.4375. Cattle were removed from the pasture for a 9-day period in the spring of 2023 for breeding purposes (indicated by the except dates). AUM/ha are shown for each treatment.

Grazing Period	Grazing Dates	Months	AUM/ha	Degree of Disappearance
Spring 2022	6/1 - 7/7/2022	1.22	Spring Only: 2.05	Spring Only: 55%
Fall 2022	9/1 – 11/4/2022	2.14	Fall Only: 5.40 Spring+Fall: 7.45	Fall Only: 43% Spring+Fall: 54%
Spring 2023	5/15 – 7/6/2023 (except 6/20 – 6/29)	1.45	Spring Only: 1.27	Spring Only: 33%
Fall 2023	8/30 – 10/30/2023	2.04	Fall Only: 2.1 Spring+Fall: 3.37	Fall Only: 22% Spring+Fall: 46%

Data Collection

To monitor plant community composition, we estimated canopy cover during peak production in 2022 and 2023 (~early-July). We sampled during peak production to account for both cool-season (C_3) and warm-season (C_4) plants. To avoid edge effects, four 1 m^2 quadrats were randomly placed after taking two steps from each corner of subplots (Figure 3). Canopy cover was estimated to the nearest percentage for each species, however species that made up considerably less than 1% of the cover were recorded as 0.1%.

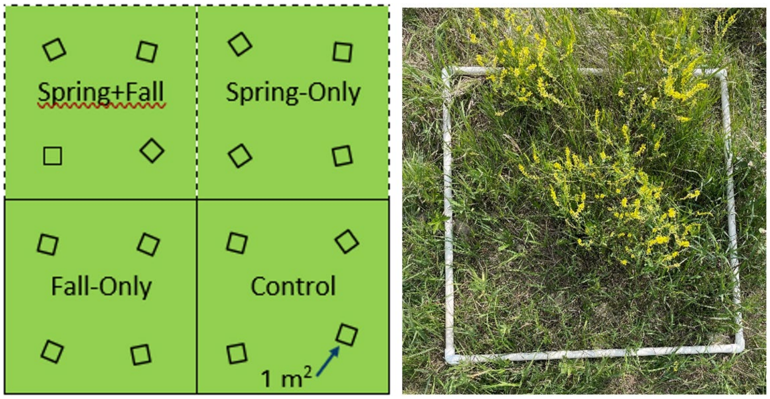

Figure 3 – Study design for canopy cover. Example placement of quadrats to estimate canopy covers for each experimental plot and subplots, each quadrat was randomly placed after taking two steps from a corner of each subplot (left). Four 1 m^2 quadrats (right) were used per subplot. Cover of each species was estimated to the nearest percentage, except species that made up considerably less than 1% were recorded as 0.1% to account for them in the analysis. Canopy estimates for the four quadrats were then averaged for each subplot.

We recorded the abundance and survival of smooth brome tillers during each spring and fall. Spring surveys occurred prior to cattle turnout (Table 1) and fall surveys occurred around mid-September. Four permanent quadrats (15 cm x 15 cm; 0.0225 m^2) were established in each subplot at the beginning of the study by placing two nails and whiskers in opposite corners of the quadrat, which allow the quadrat frames to be removed and replaced each survey (Figure 4A/C).

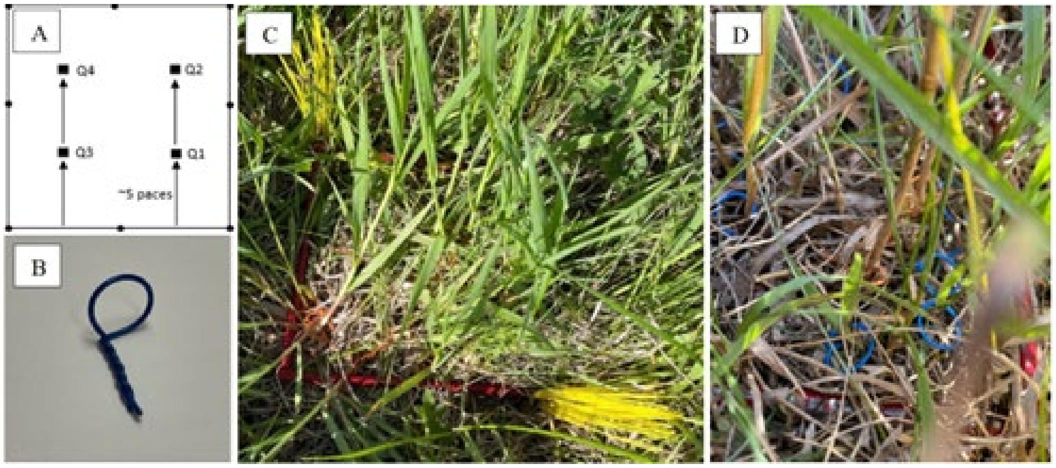

Figure 4 – Study design for smooth brome tiller abundance. Quadrat placement within subplots, four subplots per treatment (A). A tiller ring made from a small piece of wire (B). Quadrat with two nails and whiskers marking opposite corners to allow for frame removal and replacement (C). Rings placed around the base of newly emerged tillers (D). Different colored rings were used for each survey. Tiller data was averaged per subplot for statistical analysis. See Figure 2 for a greater picture of the experimental design.

Tillers that emerged from the soil surface since our last survey (new tillers) were marked using colored rings (Figure 4B/D), and we recorded the number of new tillers (Figure 5). We also recorded the morphological stage of each new tiller (Figure 5). The three morphological stages were vegetative, elongated, and reproductive. Vegetative tillers did not possess a palpable node or a visible inflorescence, elongated tillers had a palpable node but no visible inflorescence, and reproductive tillers had an inflorescence.

Survival of previously marked tillers was then determined, and we recorded the number of previously marked tillers that were still alive, dead, or missing (Figure 5). Alive tillers were recorded when a ring contained a tiller with any visible green tissue. Dead tillers were recorded when a ring either contained a tiller with no visible green tissue or no tiller altogether. Missing tillers were recorded when we could not find a previously placed ring altogether. For example, if we previously marked 10 tillers with rings and we only found 8 rings in the quadrat during the current survey, then 2 of the tillers from the previous survey would be recorded as missing. We removed rings from dead tillers each survey. The number of alive tillers for each quadrat during each survey was calculated by adding the number of new tillers and the number of tillers still alive from previous surveys.

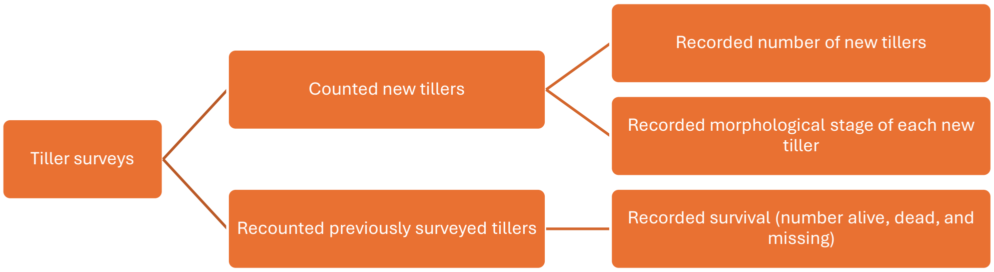

Figure 5 – Data collection during tiller surveys. We recorded the number of new tillers and the morphological stage of tillers for each quadrat. We also recorded the number of previously marked tillers that were alive, dead, and missing. See main text for definitions.

We collected four smooth brome tillers per subplot to assess the abundance of axillary buds, daughter tillers, and rhizomes per tiller. Tillers were collected after surveys in approximately the same morphological stage ratio as we recorded during the survey. For example, if we found that 57% of new tillers were vegetative and 43% were elongated, then we collected 2 vegetative (50%) and 2 elongated tillers (50%) for each subplot. This served as a proxy to achieve a better estimate of axillary buds per tiller because the development of axillary buds in plants can differ with morphological stage (Grbic and Bleecker 2000; Alvarez et al. 2006).

Tillers were dissected with the aid of a dissection scope to expose axillary buds and we recorded the number of axillary buds, daughter tillers, and rhizomes for each tiller (Figure 1). Viability was then determined using a rapid dual staining procedure, see Busso et al. (1989), Hendrickson and Briske (1997), and Russell et al. (2015) for more details. Tillers were placed into the Triphenyl Tetrazolium Chloride (TTC) solution in the dark, at room temperature, for 22-24 hours. TTC stains metabolically active buds pink/red, while the Evan's blue solution enters degraded cell membranes and dyes dead buds a deep blue color (Busso et al. 1989; Hendrickson and Briske 1997; Russell et al. 2015). Buds that stained red/pink were recorded as active. Tillers were then placed in the Evan's Blue solution for ~ 20 minutes at room temperature. Buds that were stained deep blue were recorded as dead, while those that remained white/yellowish in color were recorded as dormant. Stains were also used to aid in the distinction of daughter tillers and rhizomes, though tillers and rhizomes that were obviously alive or dead were recorded as such. Daughter tillers or rhizomes were recorded as alive or dead only, not dormant.

We statistically analyzed plant community and axillary bud data; however, tiller abundance data are still being analyzed. Absolute canopy cover of each species was converted to relative cover, which is the proportion of a species cover divided by the total plant cover of that quadrat. Relative cover of for each species was averaged for each subplot. Relative cover of smooth brome and Kentucky bluegrass (*Poa pratensis* L.) were kept by themselves, while all other species were grouped into either native graminoids, native forbs, other non-native graminoids, non-native forbs, or shrubs. Relative cover data for the groups, smooth brome, and Kentucky bluegrass were analyzed using generalized linear mixed models using the glmmTMB package in R (Brooks et al. 2017; R Core Team 2023). Number of total structures (all buds, daughter tillers, and rhizomes), active buds, and active outgrowth (active daughter tillers + active rhizomes) were analyzed to test for differences between treatment and season of collection (spring or fall) using generalized linear mixed models using the glmmTMB packa in R as well (Brooks et al. 2017; R Core Team 2023).

Preliminary Results

Relative cover of smooth brome and non-native forbs differed significantly between treatments, p-values < 0.001. There was significantly less relative cover of smooth brome in spring+fall and spring only treatments compared to the fall only treatment and control (Figure 6). Relative cover of non-native forbs was significantly greater in the spring fall and spring only treatment compared to the fall only treatment, however only the spring only treatment significantly differed from the control (Figure 6). Relative cover of native graminoids did not significantly differ between treatments but did differ by year, where there was more in 2023 compared to 2022 (p-value < 0.01). However, relative cover was exceptionally low in both years, an average of just 0.1% in 2022 and 0.8% in 2023. Overall, plant community composition appears similar between

2022 and 2023, but we may see greater differences between treatments for some groups between 2022 and 2023 (Figure 7).

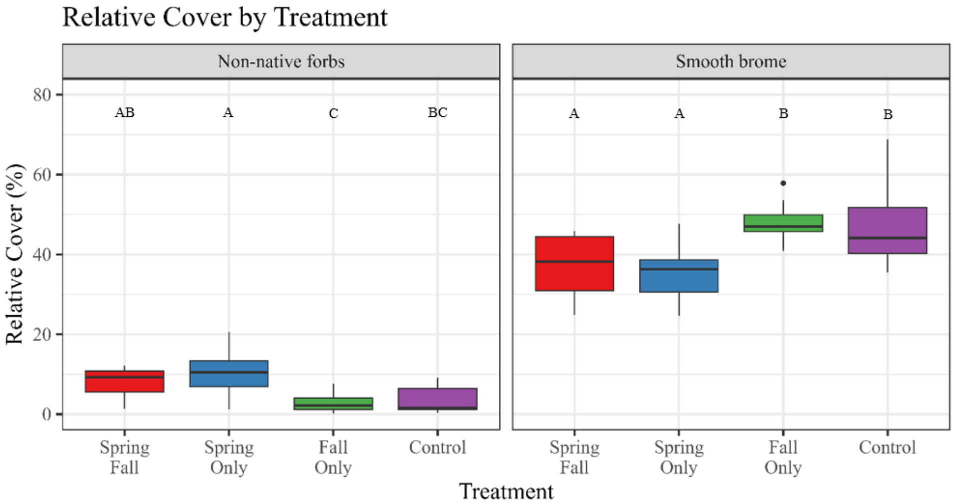

Figure 6 – Relative cover of non-native forbs (left) and smooth brome (right) by treatment. Different letters depict statistical significance between treatments within each pane.

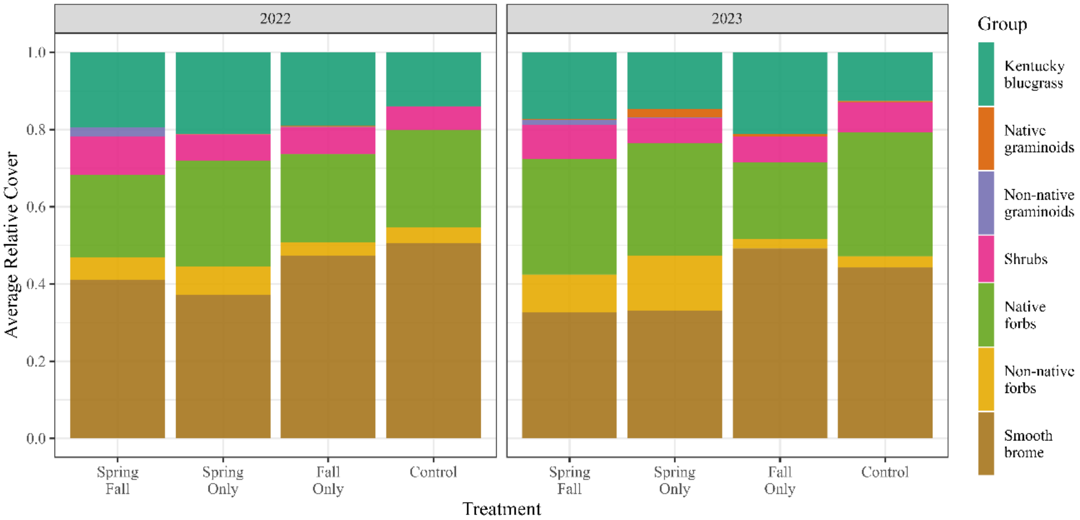

Figure 7 – Average relative cover of groups by treatments during 2022 (left) and 2023 (right). Colors for functional group are shown in the legend at the right.

Statistical analysis of tiller abundance data is still in progress, none of the following results have been tested so we cannot be confident whether the trends are valid. The average difference in tiller abundance from control was positive for the spring+fall and fall only treatment, and negative for the spring only treatment in the spring of 2022. However, in the spring 2023, the average difference for all three treatments were positive, though only by 0.5 tillers for the fall only treatment (Figure 8).

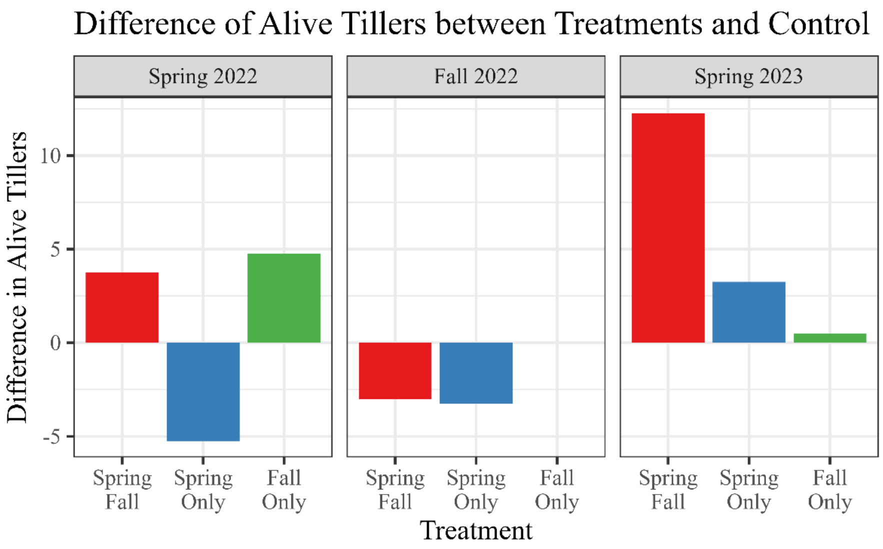

Figure 8 – Average difference between treatment and control in alive smooth brome tillers per quadrat (0.0225 m^2; 15 cm x 15 cm), during the spring of 2022 (Left), fall of 2022 (Middle), and spring of 2023 (Right). We conducted our survey for spring of 2022 prior to any grazing treatments, so the spring 2022 tiller abundance data serves as baseline data. Mean tillers for fall only and control were the same in the Fall of 2022. Statistical analysis is still in progress so we cannot be confident that the trends shown in this figure are valid.

We did not find any significant interaction between treatment and season in any of our axillary bud models ($p > 0.05$), so we removed the interaction term from the models. Total number of axillary buds, daughter tillers, rhizomes, and leaf scars per tiller significantly differed by treatment, ($p = 0.07$; Figure 9A). The number of active axillary buds did differ by treatment ($p = 0.03$). There were fewer active buds in the spring+fall than the fall only treatment ($p = 0.0488$), but neither spring+fall nor fall only differed from the spring only treatment or the control (Figure 9B). Active outgrowth per tiller did not significantly differ by treatment ($p = 0.15$; Figure 9C).

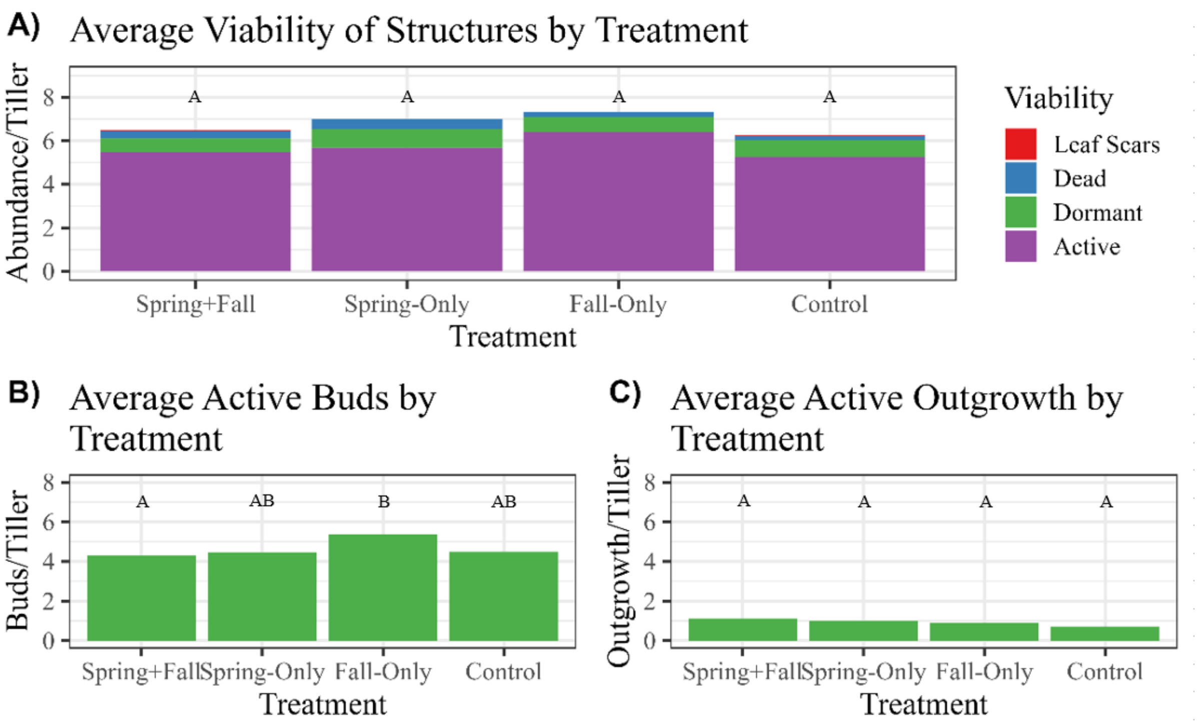

Figure 9 – Average structures (buds, daughter tillers, rhizomes, and leaf scars) per tiller by their viability (A), active buds per tiller (B), and active outgrowth per tiller (C). Different letters signify statistical difference between treatments within each graph. Leaf scars are a rare event in which a bud, tiller, or rhizome is missing from an axil, we only recorded 5 leaf scars in the 256 tillers collected. Outgrowth is the number of daughter tillers and rhizomes combined, graph C only included active daughter tillers and rhizomes. Letters in graph A are for the total structures (leaf scars, dead, dormant, and active) per tiller, does not represent difference in viability of each treatment.

Discussion

We measured canopy cover, tiller abundance, and belowground axillary bud abundance from the spring of 2022 through the fall of 2023. Out preliminary results suggest that relative cover of smooth brome was significantly lower in the spring+fall and spring only treatments than the fall only treatment and control. Non-native forbs were significantly greater in the spring+fall and spring only treatments than fall only, but spring only was the only treatment that had significantly more than the control (Figure 6). Lastly, relative cover of native graminoids was significantly greater in 2023 than 2022, however their prevalence was exceptionally low in both years. Based off the tiller data and observations in the field, we might be seeing more numerous but smaller smooth brome tillers in spring+fall and spring only treatment than the control and will test these claims further. The average number of active buds was significantly lower in the spring+fall treatment than the fall only, but neither treatment differed from the spring only or control.

Although our results seem to suggest that fall only grazing led to no-change or undesirable results, it is still possible that grazing during the fall only could increase diversity and decrease the relative cover of smooth brome in our study. Because of the timing of our cover sampling

(~early July), fall only treatments had received less treatment applications than the other treatments as the spring only received two applications, spring+fall received one and a half applications (two spring and one fall), while fall only grazing received just one application by the time we estimated cover in 2023. There may be a lag effect in plant community composition from grazing that has not been accounted for yet in the fall only treatment that might become apparent during the next year of data collection.

We only found a significant difference in the number of axillary buds per tiller between the spring+fall and fall only treatment, however neither of these treatments differed from the control (Figure 9B). It seems likely we may have gotten this result between the spring+fall and fall only treatments because of differences in the intensity of grazing, where spring+fall tillers were subjected to greater defoliation than the fall only (Table 1). We did not find any difference in the number of structures or active outgrowth between treatments. This may be due to lag effects, where we could see differences after another year of treatments in 2024. However, it seems plausible that the proportion of active outgrowth could be greater per tiller in grazing treatments than the control (Figure 9B/C) and intend on testing this in the future. As a tiller grows, it produces axillary buds (Gurevitch et al. 2021), which can lead to greater capacity for outgrowth. However, the phenomenon of apical dominance and tillering are complex, and we may see proportionally greater outgrowth in the grazing treatments than the control because of increased tillering following the break of apical dominance (Kebrom et al. 2013). Although there may be more tillering with grazing, sufficiently more intense grazing might make it difficult for the new shoots to survive. Alternatively, if the grazing in our study is insufficient in killing new shoots, then it may invigorate smooth brome tiller growth. The proportion of active outgrowth in tillers may not be different for the fall only treatment which could be due to the spring being smooth brome's primary growing season (Otfinowski et al. 2007). If this is the case, grazing during the fall only might induce tillering for the next spring which might have higher survival than spring+fall and spring only grazing because less to no resources are lost at the population level from grazing during its primary growing season in the fall only treatment. Regardless of complexities with apical dominance and tillering, grazing seems to have a role in the long-term prevention of smooth brome invasion and could have a role in improving diversity of invaded sites (Murphy and Grant 2005; Coleman et al. 2023).

Another year of data and statistical analyses of the tiller and axillary bud data will help us draw better conclusions from this research. Based off the current data, we expect plant community composition will consist of less smooth brome in the spring+fall and spring only treatments than the control in 2024. We also expect to see a reduction in smooth brome tillers for the spring+fall and spring only treatments, because we expect other plants to begin to compete with smooth brome in 2024. However, we do expect more tillering from belowground axillary buds in the spring+fall and spring only treatments but that survivorship of those tillers would be lower than that of the control. We plan to answer how tiller survivorship differs between treatments using tiller survivorship data we collected during this experiment (not shown in this report). If we find such results, it will seem likely that grazing during the spring+fall or spring only could be appropriate management, however, fall only grazing could still increase in plant diversity, relative to the control, by the final year of the study (2024). If we do not see a change in plant community composition of fall only treatments and results remain consistent for spring+fall and spring only treatments, then we may conclude that grazing that achieves a full use during both the spring and fall or moderate use during the spring only may increase plant community diversity in loamy sites of smooth brome dominated rangeland within the Missouri Coteau ecoregion. However, those grazing treatments may not increase native plant diversity.

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Enhancing profitability of soybean production and soil health through livestock integration

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Summary

Cover crop management within the Northern Great Plains can be difficult due to short growing seasons and climatic factors. Livestock integration can offer enhancements on cover crop soil health benefits, as well as be utilized as a forage source to extend the grazing season and provide a return on the investment of the cover crop. This study aims to determine the impacts of livestock integration and cover crop management on soil health, crop production, and livestock performance.

Introduction

While cover crop adoption has risen throughout much of the United States, North Dakota and much of the Northern Great Plains (NGP) have been slow to adopt the practice (Wallander et al., 2021). Winter cover crops within the NGP are planted following a cash crop harvest for soil health benefits such as erosion control, nutrient retention, soil aggregation, and promotion of soil biological activity (Weyers et al., 2019; Bawa et al., 2021). Cover cropping can also reduce weed pressure by providing competition during otherwise fallow periods of a crop (Beres et al., 2010; Pinnamaneni et al., 2022). Even with these soil benefits, the financial investment of cover cropping may be a limiting factor for cover crop adoption.

Livestock integration offers both a potential return on cover cropping through forage use, and the enhancement of soil health benefits of cover crop strategies. Livestock integration enhances nutrient cycling by freeing assimilated nitrogen within cover crop biomass, and can increase soil aggregate stability and soil organic carbon (Assmann et al., 2014; Bansal et al., 2022). Within other regions of the United States, such as the Corn Belt, both fall or winter and spring grazing may be utilized as a part of cover crop management (Sulc and Tracy, 2007). However, due to short growing seasons and climatic factors within the NGP, it can be challenging to integrate livestock into a typical crop rotation.

A two-year study was established to determine the effects of livestock integration through dual season (fall and spring) grazing of cover crops on soil properties, crop production, livestock performance, and economic outcomes.

Methods

In fall of 2022, a two-year project was established at the Central Grasslands Research Extension Center (CGREC), located 7 miles northwest of Streeter, North Dakota. A field of approximately 39-acre was divided into 9 equal plots of 4.3 acres and randomly assigned one of 3 treatments: dual graze (DG), spring graze (SG) or no graze (NG). The no graze plots were split to create an additional no rye (NR) treatment which was left unseeded.

Following a hay crop of German millet (*Setaria italica*), winter rye (*Secale cereale*) was no-till seeded on 9/18/2022, at a rate of 60 lbs/ac. Seeding depth was set to 1.25 inches and row spacing was 7.5 inches. Prior to grazing, high tension wire electric fencing was constructed around each plot assigned a grazing treatment and a water source was established. Following the spring grazing treatment, winter rye was chemically terminated with glyphosate and soybean (*Glycine max*) was no-till seeded at a rate of 58 lbs/ac with a row spacing of 15 inches.

Forage samples were collected prior to and following each grazing period. Forage yield was estimated by clipping six 0.25m^2 quadrats. Samples were oven dried at 60℃ for 48 hours and dry matter content was determined from the dried weight. Post grazing samples were used to estimate grazing use. Stocking rates were calculated from the estimated forage yield. Dried samples were sent to the NDSU Nutrition lab to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and in vitro dry matter digestibility (IVDMD). Absolute ground cover was classified during pre- and post-grazing clipping by classifying ground cover within each quadrat into percent bare ground, live rye, residue, or annual weed.

Stocking rates were determined from estimated forage yield. In the fall grazing period, four bred heifers averaging 988 pounds grazed each paddock. In the spring grazing period, nine open yearling heifers averaging 689 pounds grazed each paddock. Livestock performance was evaluated through average daily gain (ADG) and body condition scoring (BCS). Body condition scores were omitted during the fall due to the short grazing period.

Soil samples were collected following the spring grazing period. All soils samples were collected within the same soil unit according to the USDA-NRCS Soil Web Survey to reduce variability. Nutrient analyses were collected at depths of 0-6 and 6-12 inches using a 0.75-inch step-probe. Eight cores were collected within each plot and composited into a single sample. Nutrient analysis include soil nitrate (NO_3), phosphorous (P), potassium (K), total nitrogen (TN), organic matter (OM), inorganic carbon (SIC), and organic carbon (SOC) and total carbon (TC) Samples were sent to AgVise laboratories (Northwood, N.D.) for analysis as according to Recommended Chemical Soil Testing Procedures for the North Central Region (Nathan and Gelderman, 2015).

Soil biological health was evaluated by soil fungi hyphae length, Samples were collected at a depth of 0-6 inches using a 15-inch step-probe. Eight samples were collected within each plot and composited into a single subsample. Samples were immediately placed on ice and frozen before hyphae extraction adapted from Jakobsen et al. (1992).

Soil physical properties were evaluated through bulk density and aggregate stability. Bulk density samples were collected at depths of 0-1.2 in and 2-3.2 in using a slide hammer corer.

Samples were oven dried at 140°F for 24 hours and bulk density was calculated by dividing the dried sample weight by the volume of the core. Aggregate stability samples were collected at a depth of 0-6 inches by removing an undisturbed slice of soil. Samples were sent to AgVise Laboratories for analysis by automated slake wet sieving method derived from Six et al (1998) and utilized sand correction (Mikha and Rice, 2004).

Results and Discussion

Fall forage yield in 2022 was limited due to drought conditions, resulting in a short grazing period. Fall forage yields did not differ ($P > 0.05$) pre-grazing (figure 1). Fall forage yields pre-grazing were 156 lb/ac for DG, 119 lbs/ac for SG, and 101 lbs/ac for NG. Post-grazing yields did not differ ($P > 0.05$), likely due to drought conditions slowing growth and livestock consumption of previous crop residues reducing forage utilization. Spring forage yield was not significantly affected ($P > 0.05$) by grazing the previous fall at 371 lbs/ac for DG, 534 lbs/ac for SG, and 406 lbs/ac for NG. Spring forage yields post-grazing within DG and SG did not differ from each other ($P > 0.05$) but were expectedly significantly ($P < 0.05$) lower than NG due to utilization by livestock.

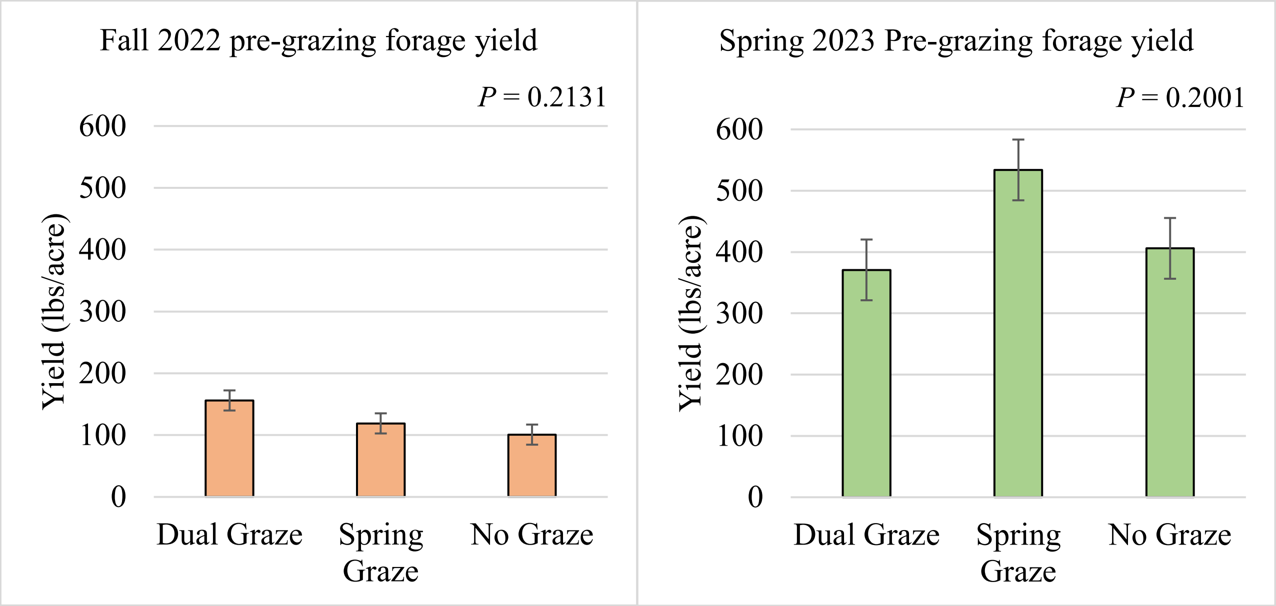
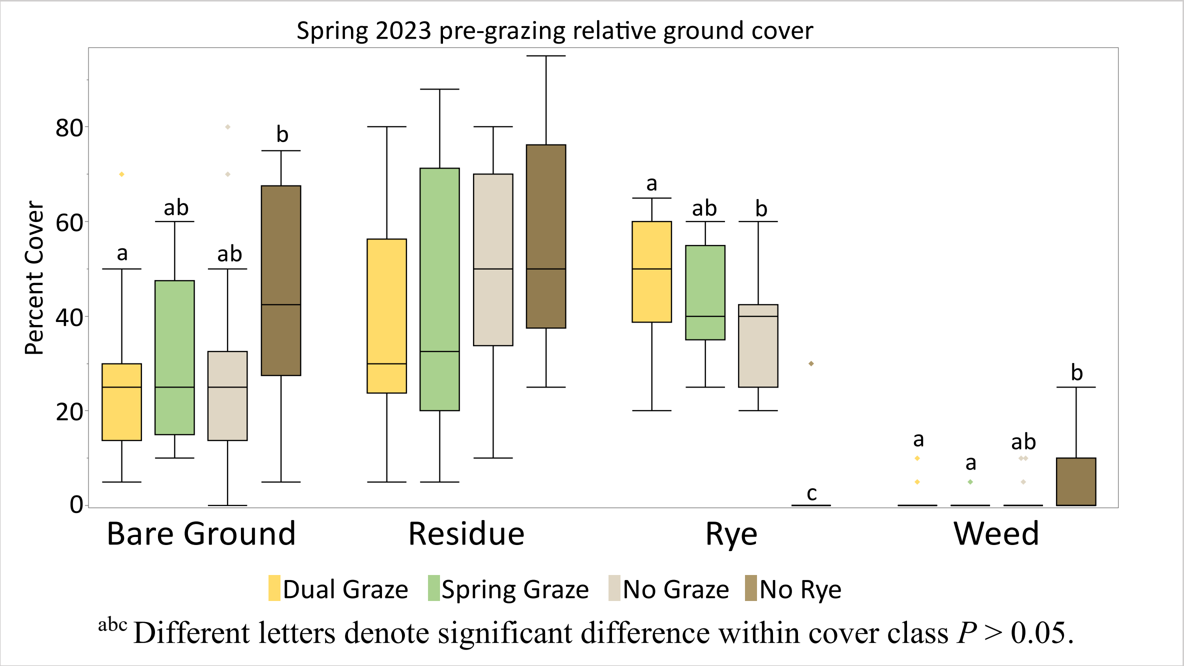


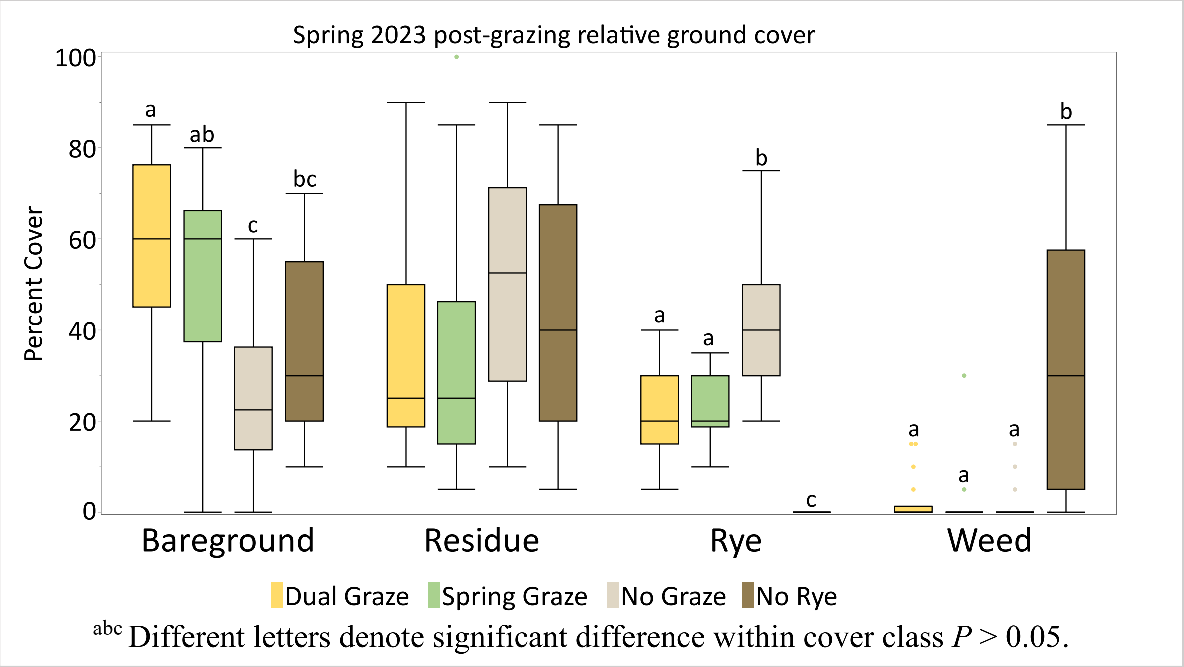
Figure 1. Pre-grazing forage yields in fall 2022 (left) and spring 2023 (right) at Central Grasslands Research Extension Center.

Pre-grazing absolute ground cover was significantly different ($P < 0.05$) between NR and NG, DG, and SG in bare ground, live rye, and annual weed cover classes (figure 2). Pre-grazing live rye cover was significantly higher in DG compared to NG, however no difference was observed in SG. These differences in pre-grazing rye cover were likely due to drought conditions creating variation within rye stands. Post-grazing absolute ground cover experienced significant differences ($P < 0.05$) in bare ground, live rye, and annual weed (figure 3). Bare ground was reduced within NR during the grazing period as lack of plant cover allowed weed species such as pigeon grass (*Setaria pumila*) and kochia (*Bassia scoparia*) to readily establish, significantly increasing weed cover. Live rye cover was significantly higher in NG with no difference between DG or SG post-grazing Residue cover was not significantly different pre- or post-grazing.



^{abc} Different letters denote significant difference within cover class $P > 0.05$.

Figure 2. Spring 2023 pre-grazing relative ground cover. Cover was classified into four categories: bare ground, residues, living rye, and annual weeds.



^{abc} Different letters denote significant difference within cover class $P > 0.05$.

Figure 3. Spring 2023 post-grazing relative ground cover. Cover was classified into four categories: bare ground, residues, living rye, and annual weeds.

Livestock performance varied by season. During the fall grazing period, four bred heifers averaging 987 pounds grazed each DG replicate for five days, totaling 0.6 animal use months (AUMs) per paddock. Cattle saw an average loss of daily gain of -2.75 lbs/day. The spring grazing period grazed nine open heifers averaging approximately 680 pounds grazed each DG and SG replicate for 16 days, totaling 3.36 AUM per paddock. Average daily gain was 0.47 lbs/day in DG, 0.61 lbs/day in SG, and 1.28 lbs/day in the dry lot control group. Only DG and the dry lot control group were significantly different ($P < 0.05$) in rate of gain. The reduced or loss of gain experienced during grazing was likely due to the short grazing periods not providing adequate time for cattle to adjust and reducing gains, as forage quality and availability was adequate.

No effects were observed to soil chemical, physical, or biological properties ($P > 0.05$). While often a concern for producers, there was no effect on soil bulk density by grazing animals (table 1). Literature has shown an increase in soil aggregate stability through cover crop and livestock integration practices; however, these changes are often slow to develop, and require a longer period to be observed (Dhaliwal and Kumar, 2021).

Table 1. Post-grazing soil chemical properties and bulk density at Central Grasslands Research Extension Center spring 2023

Treatment	**Total Nitrogen (%)**	**Nitrate-N (lbs/ac)**	**Olsen-P (lbs/ac)**	**Potassium (lbs/ac)**	**Organic matter (%)**	**Bulk Density (g/cm^2)**
Dual Graze	0.175	10	20	208	3.63	1.03
Spring Graze	0.142	8	24	198	2.65	1.14
No Graze	0.178	6	10	214	3.68	1.05
No Rye	0.147	10	8	204	3.21	1.07

The cover crop did affect soybean plant size throughout the cropping season. Soybeans within NR were significantly ($P < 0.05$) larger staging at V4 compared to V2 within DG, SG, and NG. This difference in maturity was likely due to water usage of the winter rye cover crop prior to soybean seeding and drought conditions delaying the emergence of soybeans within treatments planted with winter rye. These larger plants did not equate to higher yield, as no differences in yield observed ($P > 0.05$). However, soybean yield in NR plots may have been reduced due to increased weed pressure. Average soybean seed yield for each treatment were 14.7 bu/ac for DG, 12.5 bu/ac for SG, 12.5 bu/ac for NG, and 12.4 bu/ac for NR.

Year one of this study demonstrated no negative impacts to soybean production following a grazed or non-grazed cover crop. Fall grazing had no impact on spring forage yield and ground cover which is an important aspect due to its influence on erosion control provided by a cover crop. Grazing also did not affect weed establishment compared to non-grazed cover crops. Grazing did not affect soil physical properties or crop production within the short-term. Fall grazing provided an additional 0.6 AUM per plot, demonstrating the ability to further extend the grazing season without harming forage yield the following spring. The spring grazing period provided 3.3 AUM per plot prior to cover crop termination and soybean planting. Year-two of the project will aid in determining further impacts to soil health as well as management strategies to maximize cover crop benefits and increase economic return on the investment of a cover crop.

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Impacts of Snowpack Reduction on Kentucky bluegrass in an invaded mixed grass prairie

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Summary

Global temperatures are expected to rise between 1.7°C and 4.8°C by the end of the 21st century. The rise temperatures will reduce the impacts of, the winter season, and extent and duration of snowpack in the Northern Plains. Since snowpack acts as an insulator, reductions in snowpack could expose plants to extreme temperature conditions. Which could increase the possibility of overwinter plant survival, leading to changes in plant community composition. Kentucky bluegrass (Poa pratensis L.) has invaded the Northern Plains and much of the United States, resulting in the decline of plant and animal diversity, ultimately lowering biotic integrity of the resource; but it is unknown how the expected temperature variation will impact its survival.

To better understand these impacts on Kentucky bluegrass, we monitored the effect of snowpack reduction on Kentucky bluegrass and the overall plant community abundance in North Dakota. We established 10 plots (10 x 19m) each containing three treatments including: (1) snow reduction all winter, (2) partial season snow reduction (snow reduction 2 weeks following first snowfall and after March 30th), and (3) no snow removal as the control.

In the first year after treatments, we found overall plant communities within the treatments had not changed. However, the percent of Kentucky bluegrass cover declined ($P<0.05$) in the snow reduction season treatment while the snow reduction (all winter) and control treatments did not change ($p>0.05$). Our preliminary data suggests that reducing the snowpack by multiple weeks could potentially lower Kentucky bluegrass cover. If a reduced snowpack lowers Kentucky bluegrass cover other plant species could fill in the niche created. This study will take place for a second year to see if this trend continues and if plant composition changes.

Introduction

Climate change and invasive species both pose a significant threat to ecosystems and biodiversity around the world (Mainka and Howard 2010). Climate change is predicted to alter climate patterns through rising temperatures, changes in precipitation, and increase severe weather events (Rummukainen 2012). These changes in climate are expected to alter biodiversity through changes in species ranges and interactions, community composition, and phenology (Gilman et al. 2010, Walther 2010). Changes in climate could potently favor invasive species over natives due to their faster growth rates and higher phenotypic plasticity (Davidson et al. 2011), possibly enabling them to expand their range. This is problematic considering invasive species pose risks for human health, impact economics, and cause significant ecological changes worldwide through loss of biodiversity, and alterations in ecosystem function and species composition (Mazza et al. 2014, Fantle-Lepczyk et al. 2022, Mollot et al 2017).

One consequence of rising temperatures in the Northern Hemisphere is the reduction of the extent and duration of the snowpack (Brown and Mote. 2009). Alterations in the natural snowpack have potential to alter biodiversity. Reductions in snowpack can impact the population dynamics of native and invasive species and in order to maintain biodiversity it's important to investigate these effects (Concilio et al. 2013, Gornish et al. 2015).

The projected global average surface temperature is expected to rise between 1.7°C and 4.8°C by the end of the 21st century (IPCC 2014). However, the severity of these changes will vary by region. For example, temperatures within the Great Plains are projected to increase between 3.7 and 6.1°C by 2090 (Ojima et al. 2002). Temperature increases associated with global warming are expected to be greatest during the winter months across northern regions and snowpack is expected to decrease (IPCC 2014). Reductions in snowfall have already been recorded in the Northern hemisphere (Brown and Mote. 2009, Knowles et al. 2006) and as global mean surface temperatures increase heat waves are expected to occur more frequently (IPCC 2014). This will result in reduced snowfall amounts and winter season lengths, with the occasional cold extreme winters still occurring (IPCC 2014). The reduction of snowpack weakens insulative properties and exposes plants and soils to colder wintertime temperatures, increasing the chances of frost damage to above-ground tissues and roots of plants (Sanders-DeMott and Templer 2017, Sherwood et al. 2017). Exposure to harsh wintertime conditions can decrease the possibility of overwinter survival of plant species leading to changes in the overall plant community (Kreyling and Laudon 2012).

Kentucky bluegrass is an invasive species that is found in all fifty states and is thought to have been introduced to the United States during the 1600s through hay, bedding, and seed mixtures (Huff 2003, Carrier and Bort 1916). Kentucky bluegrass is a perennial, cool season (C3), sod-forming grass which forms extensive rhizomes (Stubbendieck et al. 2017). Kentucky bluegrass is prevalent among the Great Plains, covering as much as 50% of rangelands in North Dakota (Toledo et al. 2014). The expansion of Kentucky bluegrass in the Great Plains has been associated with declines in plant and animal diversity along with changes in community function and structure (Cully and Hiebert 2003, DeKeyser et al. 2013, Toledo et al. 2014). As Kentucky bluegrass invades an area it lowers species diversity pushing once native areas towards monocultures (DeKeyser et al. 2013). This leaves native wildlife who are dependent on habitat diversity in a venerable position and supports more generalist species (Toledo et al. 2014). Considering Kentucky bluegrass is already photosynthetically active in early spring and late fall, increases in the growing season associated with climate change could provide an opportunity for growth and expansion for Kentucky bluegrass. It is integral to investigate how climate change will impact the spread of Kentucky bluegrass to maintain biodiversity throughout the Great Plains. To better address this topic, we are investigating how alterations in snowpack influence Kentucky bluegrass to promote effective management in a changing climate.

Methods

Study Site

This study took place at the North Dakota State University Central Grassland Research Extension Center (CGREC) for two growing seasons (2022-2023) and will continue for a third year (2024). Located within the Missouri Coteau ecoregion, the study site has characteristic rolling hills with a high concentration of scattered wetlands. Average winter temperatures range from -7.2 °C in December to -1.9 °C in March, with January, on average, experiencing the coldest temperatures during the winter months (NDAWN 2023; Figure 1.1). The CGREC receives, on average, a cumulative total of 289 cm of snowfall each year which often falls from October through May, with December receiving the highest monthly total (NOAA 1981-2023; Figure 1). This location has a yearly average precipitation of 504 mm with over 70 percent occurring between May and September (NDAWN 2023).

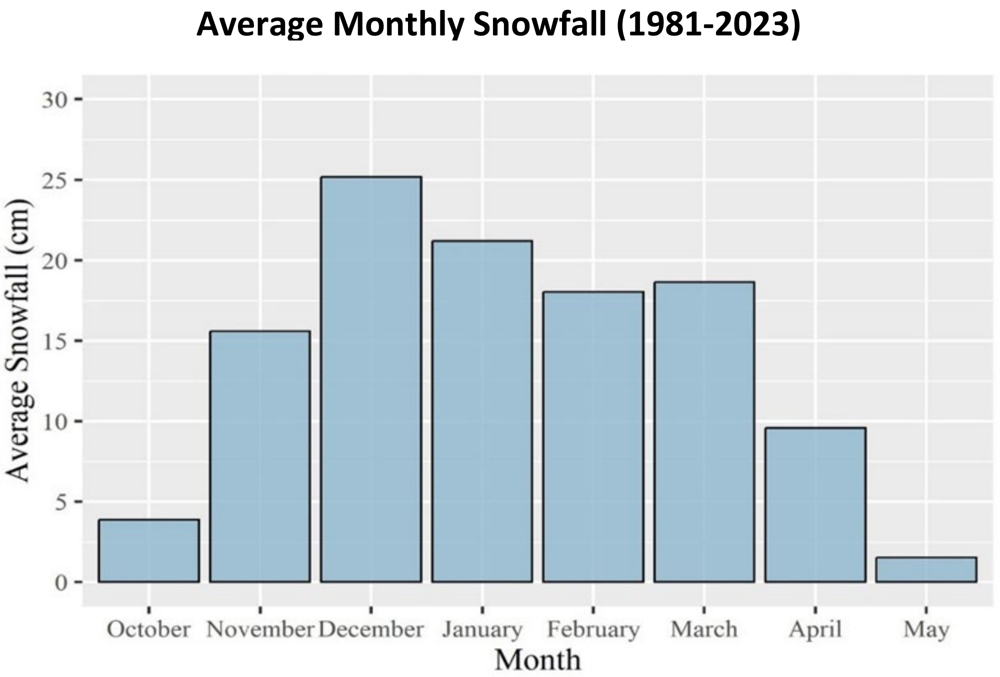

Figure 1. Average monthly snowfall amounts at CGREC from 1981-2023 (NOAA 2023).

Historically, the CGREC contained a mixed grass prairie primarily comprised of cool-season grasses, including western wheatgrass (*Pascopyrum smithii,* [Rydb.] À. Löve.) and green needlegrass (*Nassella viridula,* [Trin.] Barkworth) (Limb et al. 2018, Duquette et al. 2022). Within the landscape warm-season grasses such as little bluestem (*Schizachyrium scoparium*, [Michx.] Nash) and blue grama (Bouteloua gracilis, [Willd. Ex Kunth.] Lag. Ex Griffiths), and various forb species (*Solidago* spp., *Artemisia* spp., *Cirsium* spp.) are commonly found (Limb et al. 2018).

Currently, much of the CGREC, like most of the Northern Great Plains, has become heavily invaded by Kentucky bluegrass (*Poa pratensis* L.), smooth brome (*Bromus inermis* Leyss.), and western snowberry (*Symphoricarpos occidentalis* Hook.) due to alterations in disturbance regimes, like fire suppression and changes in grazing practices (Limb et al. 2018), and climate change (DeKeyser et al. 2015).

Experimental Design

In June of 2022, we established 10 plots (10 x 19m) each containing three treatment areas (10 x 5m) with a 2m buffer between each treatment in 2022 (Figure 1). Each treatment area was divided into 15 sub-plots that are all 1m apart (Fig. 1). The three treatment areas were randomly assigned to include: (1) snow reduction all winter (SR/AS,) (2) partial season snow reduction (SR: snow reduction 2 weeks following first snowfall and after March 30th), and (3) no snow removal as the control (C). In order to simulate a more extreme representation of reduced snowfall caused by climate change one treatment will have snow reduced throughout the winter months. To represent a less extreme representation of reduced snow another treatment will be to reduce any snowfall for two weeks following the first snowfall event to represent delayed snowfall in the fall and after March 30th to represent advanced snowmelt in the spring. On average, the last day of snowfall for the CGREC is April 1st (NOAA 2023) so, in order to advance spring melt, we found on average when there were five consecutive days after the last day of snowfall where minimum temperatures were above freezing. These five days (April 13th-17th) were used to represent springtime thaw so to advance snowmelt by two weeks snowpack will be removed from March 30th onward. These dates were chosen because North Dakota has seen on average a 1.2-day increase in the growing season per decade, with the first day of fall frost occurring later in time and the last day of spring frost occurring earlier in time (Badh et al. 2009). In conjunction with growing season changes, North America has had the highest declines in snow cover area in March (Brown 2000). The final treatment will be a control plot which will have no manipulations.

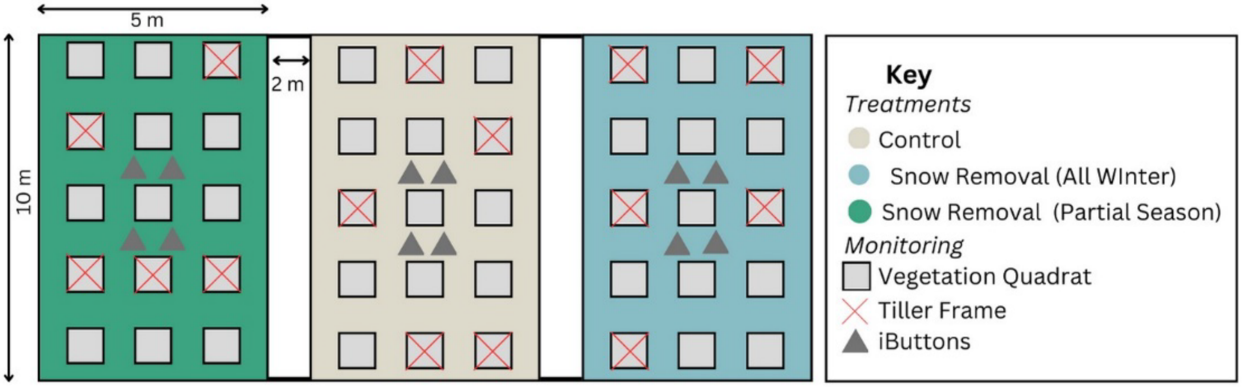

Figure 1. Control (C): Unaltered snow pack, Snow Reduction (SR/AS): Snow reduced after each snow event, Growing Season (SR): Snow reduced 2 weeks following first snowfall event and past March 30th.

Snowpack Reductions

Throughout the winter months (November-April) snow will be removed on a weekly basis through the use of a skid steer and shovels as long as 10 cm of snow has accumulated either through precipitation or drift (Figure 2). Snow will first be removed using a skid steer raised approximately 5 cm from the ground surface to avoid damages, then shovels will be used to remove any remaining snow.

Temperature Monitoring

We will monitor how snowpack reductions impact the soil microclimate by recording soil temperature. Soil and ground surface temperature will be monitored every 1 h throughout the winter till complete snowmelt (November-April) using Thermocron iButtons (DS1925L-F5#; Maxim Integrated Products, 125 Sunnyvale, CA, USA). Each treatment area will have 4 iButtons installed towards the center of the treatment area: two will record soil temperature at a depth of 5 cm, and two will be installed at the soil surface to record surface temperatures (Figure 2).

Figure 2. Top Left: Snow removal with skid-steer. Top Right: Plot after snow removal. Bottom: iButton instillation

Vegetation Sampling

Plant species composition and abundance surveys were conducted in mid-July during the peak of the growing season. Each treatment area has 15 evenly placed 1m^2 quadrats throughout the treatment area (10 plots per treatment x 15 1m^2 quadrats = 150 sampled quadrats per treatment which equal 450 total sampled quadrats per yea; Figure 1) for sampling canopy cover. The canopy cover of all plant species present within each 1m^2 quadrat will be recorded using a modified Daubenmire cover class system (Table 1, Daubenmire 1959).

Table 1. Cover classes are used to measure the canopy cover of all vegetation present within 1x1 quadrats.

Cover Class	Midpoint Value	% Range	Cover Class	Midpoint Value	Percent Range
1	.05	Trace-1%	**9**	55	50-60%
2	1.5	1-2%	**10**	65	60-70%
3	3.5	2-5%	**11**	75	70-80%
4	7.5	5-10%	**12**	85	80-90%
5	15	10-20%	**13**	92.5	90-95%
6	25	20-30%	**14**	96.5	95-98%
7	35	30-40%	**15**	98.5	98-99%
8	45	40-50%	**16**	99.5	99-100%

Analysis

We used R version 4.3.2 to test for treatment effects on Kentucky bluegrass abundance, native and non-native forb, grass, legume, and woody abundance using a generalized linear mixed effect model (GLMM) (Brooks et al. 2017; R Core Team 2023). Generalized linear mixed effect models were used because the data has a non-normal distribution and it enables us to test for random and fixed effects. For each GLMM we used the change in abundance from 2022 to 2023 as our response variable, treatments were used as our fixed effect, and plot number as a random effect. We used a Gamma distribution with an inverse link function followed by the 'emmeans' function within the *emmeans* package to see how each group varied within treatments.

Preliminary Results

In the first year after treatments, we found the percent of Kentucky bluegrass cover declined (p = 0.0433; Figure 4) in the snow reduction partial season treatment while the snow reduction all winter and control treatments did not change (p>0.05). Functional groups for native and non-native forb, grass, legume, and woody abundance within the treatments did not change (p>0.05). Temperatures were lowered by in both SR and SR/AS treatments (Figure 5).

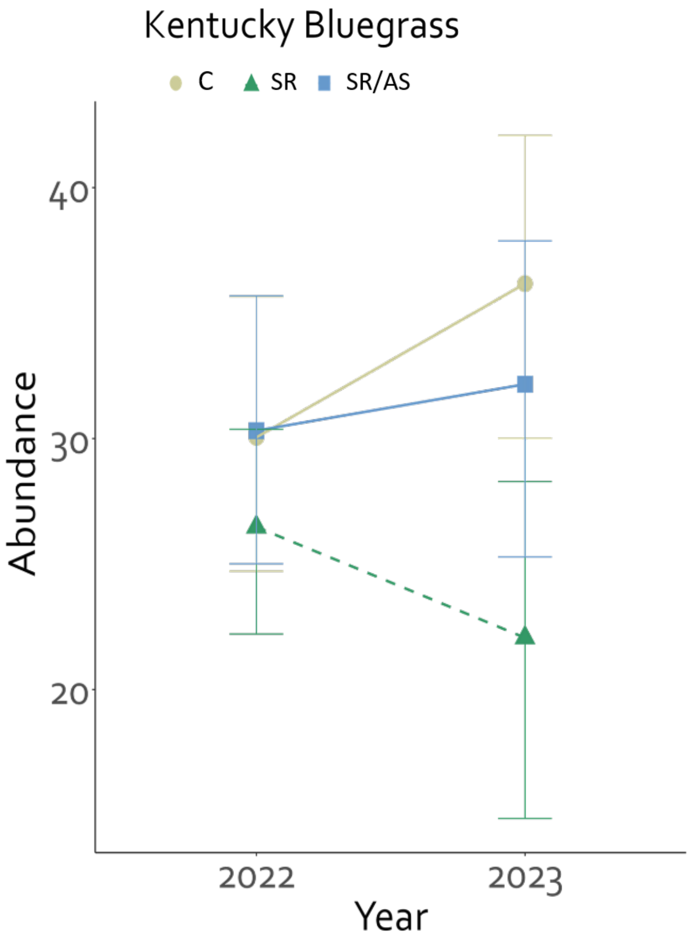

Figure 3. Line graphs showing the overall abundance of Kentucky bluegrass between treatments. Dashed lines indicate significance (p < .05).

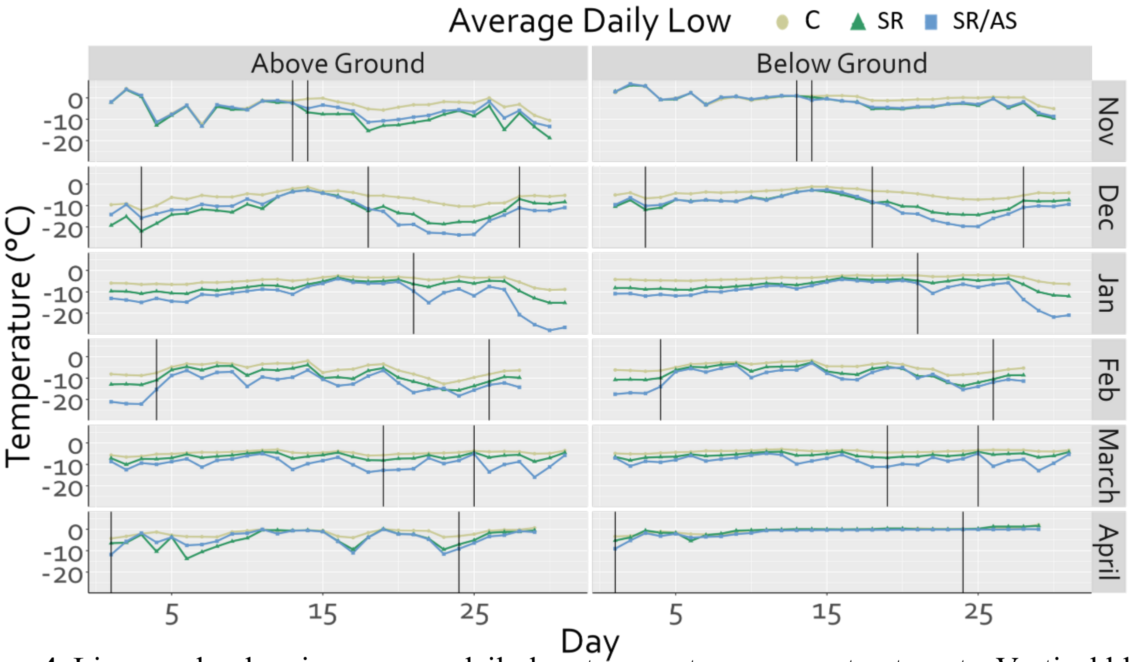

Figure 4. Line graphs showing average daily low temperatures across treatments. Vertical black lines indicate days where snowpack was reduced. C = Control, SR = Growing Season, SR/AS = Snow Reduction All Season.

Discussion

Within our snowpack manipulations we did not see any changes in abundance within our functional groups, native and non-native forb, grass, legume, and woody. However, there was significant decline of Kentucky bluegrass abundance in our snowpack reduction partial season treatment. As we continue to see changes in snowpack associated with climate change it's important to understand how these changes will impact our plant communities. Our preliminary data suggests that reducing snow pack by multiple weeks could potentially lower bluegrass cover. If bluegrass cover continues to decline other plant species within the plant community will fill in the niche created. This information will help improve our understanding of how climate change might impact bluegrass and the overall plant community enabling us to better prepare and create more effective management in a changing climate.

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Effects of Time since Prescribed Fire with Grazing on Rangeland Plant Community Characteristics

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Summary

Current rangeland management practices prioritize cattle production through common techniques such as uniform grazing and fire suppression. Methods such as these lead to pastures of homogenous plant communities and a lack of floral resources necessary for native pollinators and wildlife survival. Prescribed fire use can influence grazing selection and quality while encouraging the healthy production of native plant species and their floral resources. We designed a study in south-central North Dakota to observe mixed-grass prairie plant community response throughout different seasonal and annual timings of prescribed fires, with the addition of moderate grazing. Canopy cover class measurements were taken of vegetation ground cover, and VOR was recorded as well as litter and thatch depth to determine the initial community traits. The first prescribed fires were implemented in late August 2023 and sampling will continue into 2024 with the addition of floral observations (ramet counts) and biomass clippings. We expect our study will find the greatest shifts in plant community composition coming from sites most recently disturbed by fire and grazing, with sites left idle (no fire, no grazing) showing little relative change. We also anticipate plant biomass to remain consistent throughout all treatments, while species richness and floral abundance improve in areas with more disturbance compared to rested plots. Our study is intended to provide insight into the outcomes of varying rangeland management strategy timings by understanding the local vegetation's response. Results from this study may help land managers improve the timing and implementation of techniques deemed important for healthy rangeland ecosystems.

Introduction

The Great Plains ecoregion of the United States evolved through a disturbance regime that included large mammal herbivory from the American plains bison (*Bison bison* Linnaeus) and other herbivores, as well as frequent wildfires that disrupted plant communities across the landscape (Gleason, 1922; Anderson, 2006). Mixed-grass prairies possess a typical fire return interval of 5 to 10 years, which is characteristic of a highly resilient, well-adapted plant community in terms of large-scale disturbance (Wright and Bailey, 1982; Kral-O'Brien et al., 2020). Prescribed fire is a land manipulation tool used for rangeland management that has been around for ages, becoming more widely used by landowners and agencies in recent decades. While the uses of fire vary depending on the management goal, the results of burns have shown trends in plant community resilience throughout the northern Great Plains region of the U.S., meaning many native plant species have evolved through rangeland fire events (Vermeire et al., 2014; Gates et al., 2017; Kral-O'Brien et al., 2020). There are a fair number of papers that have studied plant responses to fire on rangelands, though many studies (64%) researching prescribed burns did not extend past 3 years in duration (Fuhlendorf et al., 2011). It is known that rangeland disturbance events will commonly alter the distribution of plants across mixed-grass prairies.

However, further research is needed to understand how disturbance regimes inclusive of fire and grazing will affect growth and composition at a community level over various lengths of time. In North Dakota, less than 1,000 acres were burned during the 2018 fire season, with over 8.5 million acres being burned throughout the rest of the United States (2020 National Prescribed Fire Annual Report). Prescribed fire use in North Dakota grasslands falls far behind grazing in terms of total annual acres affected, making cattle herbivory the most prominent technique of rangeland management. Reasons for such a small amount of burned acres may include social perceptions of prescribed fires and the absence of a prescribed fire council in the state or prescribed burn associations within the counties or regions (Clark et al., 2022; Great Plains Fire Science Exchange 2022). In comparison to the southern portion of the Great Plains, the northern rangelands have higher annual mean precipitation and a shorter annual burning window (fewer days deemed suitable to burn), contributing to relatively less use of fire as a management technique (Yurkonis et al., 2019).

Prescribed fires may be used to control woody encroachment or invasive species like Kentucky bluegrass (*Poa pratensis*) from spreading and manipulating grazing within pastures (Kral et al., 2018; Vermiere et al., 2004). Prescribed fires are also commonly used to eliminate the build-up of dead plant biomass on the soil surface and promote the growth of new vegetation, leading to the grazing of more nutritious plants with higher crude protein content (McGranahan et al., 2014; Powell et al., 2018). Once paired with grazing, fire is proven to improve species richness while promoting floral abundance and resources available for pollinator utilization (Duquette et al., 2022).

The amount of time since burn completion and the season in which the burn occurs continue to be major aspects in prescribed fire studies looking at effects on rangeland plant communities. It is understood that these factors, once paired with climate and other disturbances, play an important role in determining how a rangeland will recover following a fire event (Fuhlendorf et al., 2009). This study will further our knowledge of fire timing, providing land managers with additional detail on how disturbance practices interact with plant community characteristics over time.

Our study objective in this study is to comprehend the short-term legacy effects of spring and late-summer prescribed fires on northern Great Plains rangelands throughout 6 growing seasons. This project is designed to 1) understand how burning, paired with the season and amount of time since fire, plays a role in the response of the plant community in terms of vegetational production, structure, and cover while we also 2) determine if cattle grazing post-fire has any effect on the composition of plants found on site. Additionally, we are 3) monitoring the amount of floral resource availability to better understand the role disturbances have on local vegetation used by pollinator species.

Methods

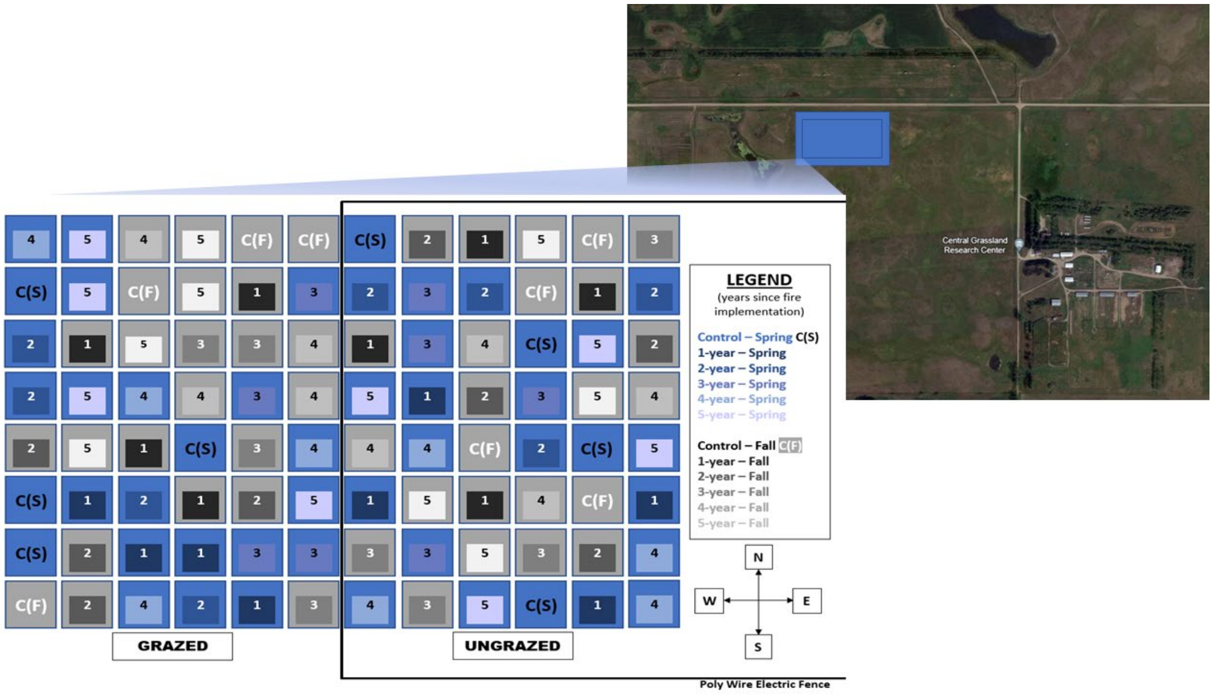
Site Description

Research for this project is taking place near Streeter, North Dakota at the Central Grasslands Research Extension Center (46.715739°, -99.446745°; 596 m above sea level), which is located within the Missouri Coteau ecoregion in the south-central part of the state. The average length of the growing season (considered between the last spring frost and the first fall frost) for this mixed-grass prairie region is between 110 and 140 days (NRCS, 2023). The 30-year average annual precipitation is 397 mm of rainfall, with temperatures in this region typically averaging 20.5 ºC in July and -12.1 ºC in January (NDAWN, 2023). The ecological site description for each plot within the study is a sandy loam on a 0 to 6 percent slope (NRCS, 2023). Common grass species currently found on-site are Kentucky bluegrass (*Poa Pratensis)* and smooth brome (*Bromus inermis*), both invasive cool-season species. Native grasses that are prevalent on site include western wheatgrass (*Pascopyrum smithii*), and green needlegrass (*Nassella viridula*). Common species of forbs within the research area include yellow and white sweet clover (*Melilotus officinales* and *Melilotus albus*). Historically, other species of forbs found within the blocks include goldenrods (*Solidago* spp.) and sages (*Artemisia* Spp.) (Limb et al. 2018). Woody shrubs that were often observed include western snowberry (*Symphoricarpos occidentalis*), wild rose (both *Rosa woodsii* and *Rosa arkansana*), and leadplant (*Amorpha canescens*).

Project Design

Disturbance effects on rangeland plants are frequently researched by looking at various aspects of growth concerning the season in which fire takes place, often disregarding effects after an extended amount of time (i.e. 3+ years) has surpassed (Fuhlendorf et al. 2011). We wanted to ensure that this study would take two main causes of controlled disturbance in the forms of fire and grazing, and place them into a setting where they could be observed through elapsed time and seasonality of fire occurrence to better understand their relationship in terms of the plant communities.

In the early summer of 2023, we created a section of 96 plots (**Figure 1**), positioned in a single 8-by-12 block, each with an area of $10m^2$. To determine relationships between timing and disturbance, we implemented a factorial design on our treatments that allowed for plots to be assigned differing burn timings (**Figure 2**). This experiment is a randomized complete block design and has two blocks, each with 48 plots, with one block being grazed while the other remains ungrazed for the duration of the study. Within each block, there are 4 replicates of 6 different fire return intervals (0 - control, 1, 2, 3, 4, or 5 years). Using R 4.2.3 (R Core Team, 2023), individual plots were randomly assigned a treatment that included designations of spring fire, fall fire, or neither, cattle grazing or absence of cattle grazing, and fire intervals of 0-5 years (**Figure 2**). Approximately 1-meter-wide alleyways were installed to create vegetative fire breaks among plots and to allow for the movement of larger fire equipment between burns. We used a temporary poly wire fence to manipulate cattle movement and restrict access across the blocks. Corners for each of the plots were marked with bright-colored metal disks atop a stake that was then placed in the ground for ease of relocation.



4	5	4	5	C(F)	C(F)	C(S)	2	1	5	C(F)	3
C(S)	5	C(F)	5	1	3	2	3	2	C(F)	1	2
2	1	5	3	3	4	1	3	4	C(S)	5	2
2	5	4	4	3	4	5	1	2	3	5	4
2	5	1	C(S)	3	4	4	4	C(F)	2	C(S)	5
C(S)	1	2	1	2	5	1	5	1	4	C(F)	1
C(S)	2	1	1	3	3	3	3	5	3	2	4
C(F)	2	4	2	1	3	4	3	5	C(S)	1	4

Figure 1. Layout of all 96 plots and their treatment designations. Blue squares indicate spring fire plots while grey squares indicate fall fire plots. Numbers within each box represent the number of years since a prescribed burn (ex. all "1's" will be burned in 2027, "2's" will be burned in 2026 & 2027, "3's" in 2025, 2026, and 2027, and so on). Geographic location of plots on Central Grassland Research Extension Center property. Image Credit: Google Earth.

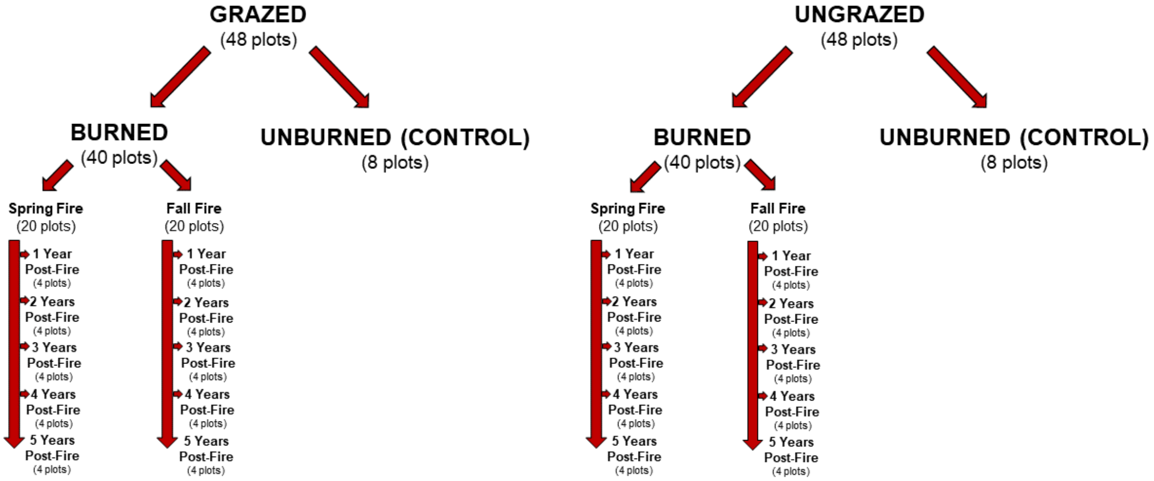


Figure 2. Factorial design used for the assignment of treatments within this project.

Livestock were allowed access to half of the plots following the first round of prescribed fire treatment. Commercial crossbred Angus cattle were placed on pasture from late August until late November and left to graze at a moderate utilization rate (40-60% degree of use). Yearling heifers were used at a stocking rate of approximately 0.89 AUMs/ha in 2023.

Initial Data Collection

Pretreatment plant composition data was collected in July 2023 using various plant community measurements (**Table 1**), with the first prescribed burns occurring later that summer (August 25th and 28th). The composition and abundance of species were obtained using a modified Daubenmire cover class (**Table 2**) while observing canopy closure (Daubenmire, 1959). To gather canopy cover data, two transects were placed running longitudinally, 3 meters in from both the east and west boundaries of a plot. Along each of the transects were 5 evenly-spaced 0.25 m^2 frame quadrats used to obtain cover percentages of each plant species present (**Figure 3**). Cover values for litter, fecal pats, rocks, and bare ground followed the same modified Daubenmire cover class guidelines as vegetation. Additionally, measurements of litter depth, thatch depth (defined as the mat of dead organic material left behind by bluegrass atop the soil horizons), and vegetation obstruction readings (VOR) were recorded. Thatch depth and litter depth were measured to the nearest millimeter using a ruler by probing 5 locations in the corners and center of each plot (**Figure 3**). Thatch samples were gathered using a soil sampling probe and VOR was obtained using a Robel pole (Robel et al. 1970).

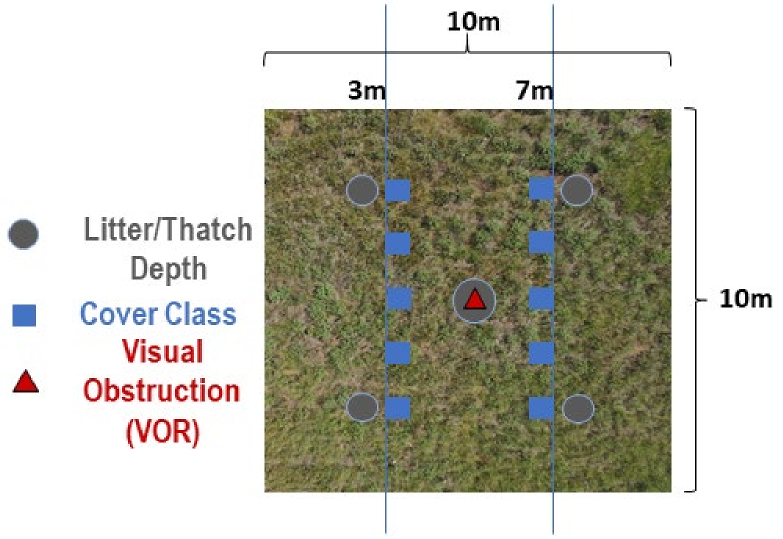
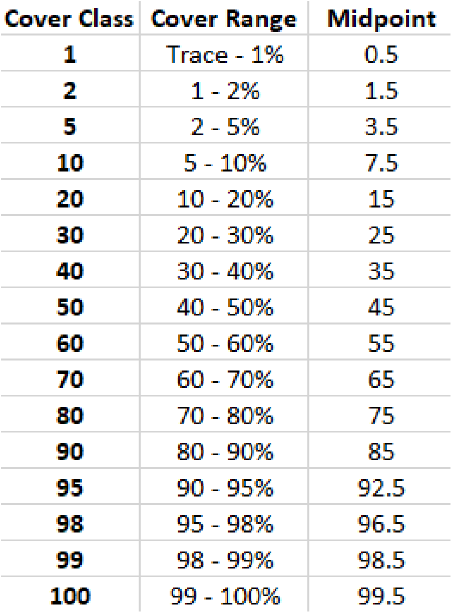


Figure 3. Image example of where each measurement is taken inside of an individual plot.

Table 1. List of each type of measurement being taken and how they will be recorded.

Measurement	**Unit**	**Tools & Method**
Canopy Cover Class (vegetation by species, basal litter, standing litter, bare ground, rock, and fecal pat)	% Canopy Closure	Modified Daubenmire cover class system (**Table 1**) with a 0.25 m frame placed along two transects within the plot (10 times per plot)
VOR (Visual Obstruction Reading)	Decimeter	A Robel pole placed in the direct center of the plot and measured from all 4 sides
Thatch Depth	Millimeter	Ruler to measure the "mat" of organic material atop a core from a soil sampling probe
Litter Depth	Millimeter	Ruler to measure the height of dead vegetation from the surface of the ground
Biomass (Vegetative Production) **sampling beginning 2024*	Kilogram	Clippers to cut a 0.25 m quadrat, separate plants by woody, forb, grass, dry to a consistent weight, weigh using a scale
Floristic Abundance (Total Ramets) **sampling beginning 2024*	Tally Count	Tape measures and a meter stick to measure 1 meter off of each transect, visual observation of ramets within the 1 meter (either side) of the transect will be counted and totaled



Cover Class	Cover Range	Midpoint
1	Trace - 1%	0.5
2	1 - 2%	1.5
5	2 - 5%	3.5
10	5 - 10%	7.5
20	10 - 20%	15
30	20 - 30%	25
40	30 - 40%	35
50	40 - 50%	45
60	50 - 60%	55
70	60 - 70%	65
80	70 - 80%	75
90	80 - 90%	85
95	90 - 95%	92.5
98	95 - 98%	96.5
99	98 - 99%	98.5
100	99 - 100%	99.5

Table 2. Modified Daubenmire cover class system to measure plant canopy cover.

Figure 4. Ring fire technique being utilized on an individual plot.

Fires were implemented using a ring fire technique (**Figure 4**) where the perimeter vegetation is burned first to create a blackline. Wind then moves flames toward the remaining fuel near the center of the plot. Spring fires will be implemented on plots requiring a spring burn once the snowpack has melted and vegetation has become exposed enough to dry, typically around mid to late April. Fall burns are being conducted following the data of historical records, which show fires predominately occur during late summer from late July to August (Higgins, 1984). Relative humidity, wind direction, and wind speed are all accounted for while selecting the best window to burn as they play key roles in determining burn behavior and safety.

Preliminary Results

Data collected during July of 2023 give us a good idea of the preliminary plant composition distributed amongst our plots before the implementation of disturbance treatments. Through our initial data collection (**Figures 5**), we found the average abundances of Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*) to be 43.1% and 20.6% respectively. Both white and yellow sweet clover (*Melilotus albus, Melilotus officinalis*) were at 25.8% and 22.6% relative abundances for a total abundance of 48.5% among plots. Thatch measured out to an average of 1.29 cm within the plots. Initial vegetation structure measurements show an average VOR reading of 5.67 decimeters between both blocks, with the largest recordings appearing in plots invaded by sweet clover. The average basal litter cover was valued at 11.5% while litter depth came out to be approximately 2.23 cm among all plots.

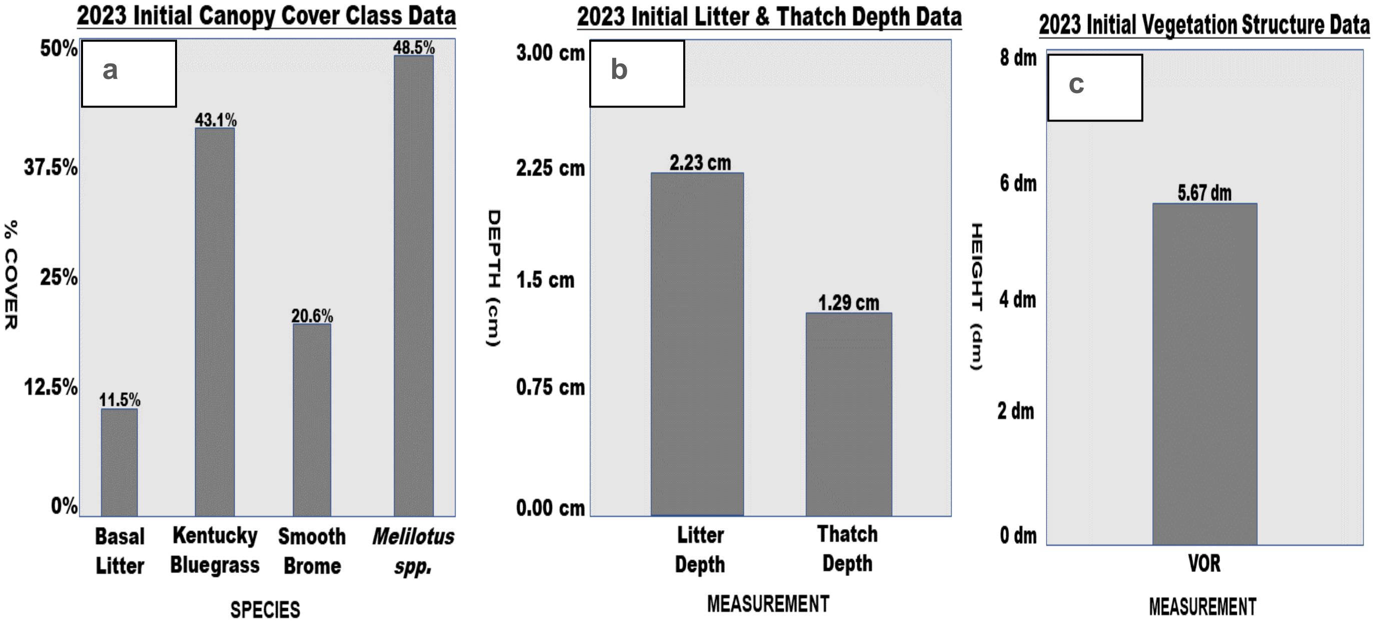


Figure 5. Average canopy cover value (%) for various species across all 96 plots, pretreatment (a), average depth (cm) of basal litter and thatch across all 96 plots, pretreatment (b), and average height (dm) of vegetation structure across all 96 plots, pretreatment (c).

We anticipate the outcome of this study to show a shift of plant functional group composition for plots with the most recent (1-year since fire) and heavily applied disturbance (plots that are both burned and grazed) when compared to control plots that were rested and left unburned. We expect that plots that have been more disturbed by fire and grazing will show less abundance in cool-season invasive species and a greater dominance of native grasses and forbs (Kral et al., 2018; Vermeire and Russell, 2018). We believe the difference between current-year biomass in our rested control and treatment plots will be negligible (Dornbusch et al., 2020). Further, we believe that the amount of years since a fire took place will have a relatively greater impact on the plant community composition than the seasonality in which a fire takes place.

Future Work

Biomass samples will be collected throughout each plot beginning in 2024 by using 0.25m^2 frames placed in a consistent location. Each frame will have its vegetation clipped near the base to simulate the effects of grazing. Plants will then be separated by their functional group into three categories: graminoids, forbs, or woody shrubs before being dried and weighed.

Floral resource sampling will begin in May of 2024 with ramet counts being obtained weekly until fall fires are implemented. Individual plot counts will be completed by visually observing all flowering species within 1 m of both transects and gathering a tally count of available ramets. Following the results of Duquette et al. (2022), we anticipate species richness and floral abundance values to rebound in plots that were burned, reaching or surpassing plots that were left unburned. Sites that have been grazed are expected to have greater amounts of floral resources than sites remaining idle from cattle use, due to less cool-season grass invasion (Murphy and Grant, 2005).

Fires within the plots will continue into 2024, with prescribed burns anticipated for this spring to accomplish the first of this project’s early-season fires. A total of 40 plots will undergo fire treatment in (or close to) April once snowmelt has been completed and vegetation is exposed. Late summer fires will be implemented for the second consecutive year this August, with an additional 32 plots set to be burned on the season.

Discussion

Difficulties within our study may be associated with climate and its unpredictable variability. One major factor of climate is the amount of precipitation since fire behavior often corresponds to values associated with moisture (i.e. recent rainfall totals, relative humidity). Drought often makes burning conditions difficult (patchy, dispersed fuel loads) to impossible (red flag conditions) with an increased risk of danger or escape. In the presence of a drought, we will still attempt to burn when the conditions allow but shall use the results from that year or season with additional caution. If there is no burn availability for the duration of the season, this will be acknowledged in our data and our results will likely reflect a plant community differing from that of a typical treatment inclusive of fire and cattle grazing. An additional pitfall found within the design of our experiment is the randomization aspect of the grazing disturbance. Fire disturbance was able to be assigned fully at random among all plots but the presence of grazing wasn’t as simple since temporary electric fencing was required to exclude cattle from the plots. This could mean geographic variability may have to be accounted for within the grazing portion of the study when compared to the fire.

Our study is designed so that the results will help show the impact of disturbance regimes on rangeland plants that make up a majority of pasturelands found in the northern Great Plains. Through our research, we hope to better understand what will happen to a plant community's presence, structure, and composition regarding the timing of disturbance. Rangeland managers should use data from this study to help determine the best management practices utilized to achieve plant community goals. We expect our results will suggest that returning historic fire intervals to the northern Great Plains region of the United States is important for maximizing the production of grasses and forbs, controlling the spread of cool-season invasive species, and improving the presence and abundance of native plant communities.

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Fleeting Effects of Management Actions in Kentucky Bluegrass (*Poa pratensis*) Invaded Rangelands

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Summary

Rangelands in the northern Great Plains are being converted into novel ecosystems by invasive grasses, especially Kentucky bluegrass (*Poa pratensis*; hereafter 'bluegrass'). Bluegrass invades rangelands and forms a dense litter layer, which leads to the formation of thatch, a unique pseudo-soil layer. Thatch and litter accumulation alter water and nutrient cycling, suppress native plants, and alter soil microbial communities. These factors transform once native rangelands to novel ecosystems, warranting bluegrass management and mitigation. Management of bluegrass using some combination of fire, grazing, and herbicide is effective, but these benefits do not persist. To better understand bluegrass dynamics in different management practices, we monitored how bluegrass and the surrounding native plant communities changed with increasing time since fire and grazing intensity in patch-burn grazing and heterogeneity-based rotational grazing over six years. We found that both management strategies have at least some level of disturbance (either fire followed by grazing or grazing by itself), that halted the spread of bluegrass. For example, bluegrass abundance in patch-burg grazing pastures decreased the year of a fire. However, in the same pastures we also found that bluegrass increased in abundance every year following a burn, with the greatest increases occurring three years post fire. Indicating that a shorter fire return interval may prove more effective in managing or reducing bluegrass abundance. Additionally, all grazed paddocks in the heterogeneity-based rotation pastures maintained bluegrass abundance while rested paddocks allowed bluegrass to increase in abundance. These results provide insight into how bluegrass abundance varies within different management practices and helps us understand how certain management actions impact bluegrass and when those actions might best be applied.

Introduction

The high levels of heterogeneity and biodiversity, once endemic to the Great Plains of North America, has been lost over time due to changes in fire and grazing regimes that have allowed invasive plants to invade, suppressing native species and homogenizing the ecosystem (DiTomaso, 2000; Barnes *et al.*, 2013; Dekeyser *et al.*, 2013; Toledo *et al.*, 2014; DeKeyser *et al.*, 2015). Kentucky bluegrass (*Poa pratensis* L.), one of the most influential exotic grasses in the northern Great Plains, invades native communities and suppresses native species (Toledo *et al.*, 2014). This process is achieved mostly through the build-up of a novel thatch layer (Figure 1) and the accumulation of low lignin litter, but other mechanisms have been described that aid Kentucky bluegrass (Hendrickson et al., 2021; Nouwakpo et al., 2019; Palit et al., 2021; Printz and Hendrickson, 2015). Currently management of Kentucky bluegrass typically involves some combination of fire, herbicide, and grazing (Bahm *et al.*, 2011; Adkins and Barnes, 2013; Dornbusch *et al.*, 2020; Hendrickson *et al.*, 2020; Duquette *et al.*, 2022b). Herbicide, fire, and grazing in combination or alone have been shown to temporarily halt Kentucky bluegrass expansion and promote native plants (Bahm *et al.*, 2011; Dornbusch *et al.*, 2020; Duquette *et al.*, 2022b). However, if management actions are not consistently applied year-after-year, these benefits begin to disappear (Adkins and Barnes, 2013; Dornbusch *et al.*, 2020), though how long these benefits take to disappear and the implications for how often management needs to occur remain unclear.

Preserving or increasing biodiversity while also increasing environmental heterogeneity can benefit not only the surrounding ecosystem itself, but also those that use the ecosystem (Fuhlendorf *et al.*, 2009). Increased diversity resulting from increased environmental heterogeneity can provide increased floral resources for pollinators (Duquette *et al.*, 2022a), increase cattle production (Limb *et al.*, 2011), mitigate the negative effects of variable climate on cattle production (Spiess *et al.*, 2020), and help prevent invasive species from invading native ecosystems (Melbourne *et al.*, 2007; Gao *et al.*, 2021). Conversely, decreasing environmental heterogeneity can allow for already established invasive species to drastically increase in abundance (Melbourne *et al.*, 2007; Tognetti and Chaneton, 2015).

Historically, landscape-level heterogeneity in the Great Plains of North America (hereafter; the Great Plains), was maintained by the interactive effects of fire and grazing (Fuhlendorf *et al.*, 2009). In the Great Plains, plant litter would accumulate over time and eventually fire, either natural or human-induced, would burn through an area removing dead plant material and promoting the growth or regrowth of easily accessible, highly nutritious plant material (Fuhlendorf and Engle, 2001, 2004; Fuhlendorf *et al.*, 2009). This new, nutritious growth attracted large ungulates (historically American bison, *Bison bison* L.) to the recently burned area where they would preferentially graze, causing shifts in plant community composition and structure that affected wildlife occupying the ecosystem (i.e., pyric herbivory; Fuhlendorf *et al.*, 2009; Allred *et al.*, 2011). Sometime after a fire event, another area would be burned, attracting bison to that recently burned area, allowing the previously burned and grazed area a chance to regrow (Fuhlendorf and Engle 2004). This process of fire followed by intense grazing, and then another fire that would promote grazing elsewhere would create a shifting mosaic of heterogeneous vegetation structure that supported high levels of native diversity (Fuhlendorf and Engle, 2004). However, following European colonization of the Great Plains, fire suppression and homogenous grazing management (or "management-toward-the-middle") have decoupled the fire-grazing interaction, reducing landscape-level heterogeneity and allowing for an increase in invasive species (Melbourne *et al.*, 2007; Fuhlendorf *et al.*, 2009; DeKeyser *et al.*, 2015; Palit *et al.*, 2021).

There are numerous invasive species in the Great Plains, one of the most widespread and troublesome is Kentucky bluegrass (hereafter, "bluegrass"; (DiTomaso, 2000; Barnes *et al.*, 2013; Dekeyser *et al.*, 2013; DeKeyser *et al.*, 2015; Hendrickson *et al.*, 2020). Currently, the success of bluegrass in the Great Plains is owed primarily to the build-up of a novel that layer (Figure 1) and accumulation of dense litter, though other mechanisms that aid bluegrass invasion have been described (Samson and Knopf, 1994; Toledo *et al.*, 2014; Printz and Hendrickson, 2015; Nouwakpo *et al.*, 2019; Hendrickson *et al.*, 2021; Palit *et al.*, 2021). Over time, litter accumulation compresses older litter into loosely intermingled layer comprised of decaying litter, live and dead buds, and roots called "thatch" (Hurto *et al.*, 1980; Gaussoin *et al.*, 2013). Thatch is similar to an O-horizon in a soil profile with unique biological and chemical properties (Millar *et al.*, 1966; Gaussoin *et al.*, 2013). However, many soil profiles in the northern Great Plains do not historically contain O horizons, causing issues for native plants (Millar *et al.*, 1966; Toledo *et al.*, 2014; DeKeyser *et al.*, 2015) Both bluegrass thatch and litter alter water and nutrient cycling (primarily total N and soil organic C), shade out competing species, and suppress native seed recruitment (Printz and Hendrickson, 2015; Sanderson *et al.*, 2017; Nouwakpo *et al.*, 2019; Hendrickson *et al.*, 2021).

Currently, management of bluegrass invasion uses a variety of approaches (specifically, some combination of fire, herbicide and grazing) to reduce or control bluegrass abundance while simultaneously promote native diversity (Bahm *et al.*, 2011; Adkins and Barnes, 2013; Dornbusch *et al.*, 2020; Hendrickson *et al.*, 2020; Duquette *et al.*, 2022b). Early spring (prior to or at the start of the growing season), targeted grazing can promote native grass abundance while controlling or reducing bluegrass abundance, depending on the year (Hendrickson *et al.*, 2020). In other scenarios, grazing during the growing season appears to increase bluegrass abundance (Murphy and Grant, 2005). Fall herbicide application, and the combination of fire and herbicide can decrease bluegrass abundance, releasing native species from competition, allowing them to flourish (Bahm *et al.*, 2011; Adkins and Barnes, 2013). Additionally, management not explicitly designed to combat bluegrass invasion, but rather promote landscape-level heterogeneity, can also halt bluegrass expansion, while simultaneously promoting native species (Dornbusch *et al.*, 2020; Duquette *et al.*, 2022b; a). However, all these benefits from management begin to disappear after one or more years (Bahm *et al.*, 2011; Dornbusch *et al.*, 2020), meaning that repeated management actions over many years are likely needed to even halt bluegrass expansion, let alone reduce its abundance on the landscape. In order to properly time bluegrass management, a deeper understanding of exactly when in the management cycle benefits from bluegrass management begin to disappear is needed. This work is meant to expand upon research already conducted by Duquette et al. (2022b), providing a deeper understanding of how bluegrass and native plant abundances fluctuate in accordance with increasing time since fire and grazing intensities.

We present six years of data from pastures managed to create landscape-level heterogeneity using either pyric herbivory (the interaction of fire and grazing) or through varied grazing intensity. We will use this data to examine how bluegrass abundance and native forb, legume, and graminoid abundance change with the number of years post fire or grazing intensity. Specifically, we hypothesize that (1) bluegrass abundance will decrease immediately after a fire, and will increase as time since fire increases, (2) and conversely that native plant abundance will increase immediately after a fire and decrease as time since fire increases. Additionally, (3) we predict that following grazing modest rates (40-75% degree of disappearance) during the growing season will result in no change of bluegrass abundance, but increase native abundance. (4) Conversely, we predict that less grazing pressure (between 0 and 40% degree of disappearance), during the growing season will result in an increase in bluegrass abundance and a decrease in native plant abundance.

Methods

Study Site

This study occurred at North Dakota State University Central Grasslands Research Extension Center (CGREC) in Stutsman and Kidder Counties near Streeter, ND USA (46°45’N, 99°28’W) from 2017 to 2022. Historically this site consisted of mixed-grass prairie dominated primarily by cool-season grasses, such as western wheatgrass (*Pascopyrum smithii*, [Rydb.] À. Löve.) and green needlegrass (*Nasella viridula*, [Trin.] Barkworth) (Barker and Whitman, 1988). However, warm-season grasses such as little bluestem (*Schizachyrium scoparium*, [Michx.] Nash) and blue grama (*Bouteloua gracilis* [Willd. Ex Kunth] Lag.ex Griffiths), and various forbs (*Artemisia* and *Solidago* spp) and sedges (primarily *Carex* spp., rarely *Cyperus* spp.) are also common in this landscape (Barker and Whitman, 1988; Limb *et al.*, 2018). Fire suppression and change in climate and grazing regimes have allowed these prairies to be invaded by western snowberry (*Symphoricarpos occidentalis* Hook.) and Kentucky bluegrass (*Poa pratensis* L.) (DeKeyser *et al.*, 2015; Limb *et al.*, 2018).

Our study utilized four 65-ha replicates (pastures) of two different conservation-based grazing practices, patch-burn grazing, and heterogeneity-based rotational grazing. The patch-burn grazing treatment was designed so a different quarter (approximately 16-ha) of each patch-burn pasture (approximately 64-ha) was burned every spring prior to cattle turn out in mid-May (Table 1), and cattle could graze and move freely, but preferentially grazed the recently burned area due to abundant, nutritious regrowth (see Allred *et al.*, 2011). Prescribed fires were not conducted in 2022 due to a lack of fuel accumulation from an exceptional drought in 2021 (NDAWN, 2023).

As an alternative way of creating landscape-level heterogeneity, we developed a heterogeneity-based rotational grazing system in the form of a modified twice-over rest-rotational grazing system in 2018. Heterogeneity was created by restricting cattle movement to one of four different paddocks (16-ha) within each pasture (65-ha) at a given time. Each paddock was stocked and grazed for different lengths of time each year to create different levels of grazing disturbance. The grazing duration in different paddocks were 0 days for rested paddocks, approx. 21 days for moderate use paddocks (20-40% degree of disappearance), approx. 50 days for full use paddocks (40-60% degree of disappearance), and approx. 77 days for heavy use paddocks (> 60% degree of disappearance). Grazing duration for a given paddock type (i.e., heavy, full, or moderate) varied from year to year to account for differences in vegetative production. Upon cattle turn out (once again mid-May), cattle were placed into the heavy use paddocks and would then be rotated to the full use paddock followed by the moderate use paddocks. After grazing in the moderate paddocks for approximately a week, cattle would be rotated back into heavy use paddocks and complete a second rotation through each paddock. Cattle spent about 40% of the grazing season on the first rotation and 60% of the season on the second rotation. Grazing intensities rotated between paddocks each year so that each paddock would receive a different intensity each year using a four-year cycle. This rotation of paddock grazing intensities also mimics the shifting mosaic of vegetation structure that is commonly seen in patch-burn grazing pastures (Fuhlendorf and Engle, 2004). However, the progression of vegetation structure for any given paddock is the opposite of what would occur in a patch-burn system. I.e., in a patch-burn grazing system, a single patch would progress from being burned (shortest vegetation structure) to being 3 years post-fire (tallest vegetation structure). Whereas a paddock in our heterogeneity-based rotational grazing system would progress from a rested paddock (tallest vegetation structure) to a heavy-use paddock (shortest structure). This reversed progression was intentional because mimicking the progression of patch-burn grazing would have meant repeatedly grazing areas that had been heavily grazed without allowing the paddock to recover, which can permanently shift the plant community (Limb et al. 2018).

Stocking rates were similar among each treatment each year, ranging from 1.59 to 2.48 animal unit months per hectare (AUM/ha). Stocking rates were developed based on a harvest efficiency of 30% for the relative forage production for each hectare of each of ecological site (USDA - NRCS, 2018) found within the treatment area and achieving an average herbage removal of approximately 40-50% of standing crop biomass across the entire treatment. Cattle grazed each pasture annually from mid-May to mid-to-late October (Table 1). Patch-burn patches and the initial location of the different levels of use within the heterogeneity-based rotational pastures were randomized, but season-long treatments were not due to management restrictions that prohibited fire and additional fencing. It should also be noted that neither management practice was implemented with the intention of controlling Kentucky bluegrass, rather they were intended to increase biodiversity at multiple levels by increasing landscape level heterogeneity (Fuhlendorf *et al.*, 2006). For data collection purposes, each pasture was further subdivided into sixteen hypothetical 8-ha sub-patches (or sub-paddocks).

Data Collection

Within any given sub-patch we placed on 60 meter transect on the loamy ecological site to reduce variation in the plant community that might be due to the underlying soil (USDA NRCS, 2021). This meant we had 64 transects in total and 32 transects per treatment. Along each transect we sampled the plant community every other meter for a total of 31 sample points per transect. From 2017 to 2020, we recorded community composition at each sample point by identifying every species in a 0.25 m^2 quadrat and assigning them a cover class using a modified Daubenmire cover system that detect small changes in abundance for both minor and major abundant species (Daubenmire, 1959). Starting in 2021 each sample point was sampled with a 1 m^2 quadrat to both increase the total area sampled in each treatment and to provide greater sampling resolution. Data collection occurred from July to mid-August each year to allow us to capture the greatest variation in the plant community, as this time window corresponds to the portion of the growing season when cool-season plants begin to senesce and warm season plants are beginning to grow and reach peak biomass for the year. All observers were trained in plant identification each year and observers calibrated cover estimations to one another prior to each bout of data collection to minimize observer bias. One graduate student served as the calibration metric each year to further reduce observer bias.

Data analysis

Data were analyzed using R 4.2.3 (R Core Team, 2022). To assess how our different management strategies changed Kentucky bluegrass and native forb, legume, and grass relative abundance in a specific patch or paddock over time, we use the ‘abundance_change’ function from the *codyn* package (Hallett *et al.*, 2020). By setting a reference year, we were able to track how Kentucky bluegrass abundance differed in a given patch or paddock between the initial year a treatment was implemented, and the year data were collected, as patches got further away from a fire event in the patch-burn pastures or as paddocks experienced different grazing intensities in the heterogeneity-based rotational pastures. By tracking changes in abundance in this manner, we are able to assess how the abundances have changed relative to their pre-treatment levels, accounting for differential abundances and production between patches. Relative abundance was used to control for differences in sampling extent between data collected in 2017-2020 and those collected in 2021 and 2022. We then utilized Generalized Linear Mixed Effect Models (GLMM) from the *glmmTMB* package to assess how Kentucky bluegrass, native forb, legume, and grass relative abundance in patch-burn pastures changed as time since fire increased (Brooks *et al.*, 2017). For heterogeneity-based rotational pastures we chose to use GLMM to model how grazing intensity in the previous year influenced Kentucky bluegrass, native forb, legume and grass relative abundance in the current year because Kentucky bluegrass is an early emerging plant making it more likely to respond to changes in conditions prior to the growing season (e.g., the vegetation structure leftover from the previous year's grazing), than it is to respond to changes in conditions in the current year (Dornbusch *et al.*, 2020; Palit *et al.*, 2021). We used GLMM because it allowed us to account for differences in land-use history by treating pasture ID as a random effect, and because some of our data did not follow a normal distribution. Specifically, legume and forb abundance in patch-burn pastures followed a student's T distribution instead of a normal distribution. As such, all changes in plant abundance for this subset of the data were analyzed with a student's T distribution, the assumption of which were assessed using the *DHARMa* package (Hartig, 2022). Pairwise comparisons examining how plant abundances changed between years was conducted using Tukey's-HSD in *emmeans* (Lenth, 2020). Additionally, to assess if the changes in plant relative abundance over time differed from zero at each time since fire or grazing intensity, we used 'wilcox.test' from base R to conduct a non-parametric two-tailed Wilcoxon Rank Sum test.

Results

Changes in plant abundance in patch-burn pastures

Kentucky bluegrass increased as time since fire increased ($p = 0.0013$, Table 2a; Figure 2a). Although Kentucky bluegrass appeared to decrease immediately following a fire (zero years post fire), this decrease was not significantly different from zero, indicating that fire did not reduce Kentucky bluegrass below pre-treatment levels ($p = 0.4067$; Table 3a). However, across all patch-burn pastures, when patches were one, three, or four years post-fire, Kentucky bluegrass saw increases in abundance that were significantly different from zero and patches that were four years post fire saw a marginally significant increase in Kentucky bluegrass abundance ($p_{One} = 0.0133$, $p_{Three} = 0.0001$, $p_{Four} = 0.0663$; Table 3a). Patches that were two years post fire had saw a slight, but non-significant increases in Kentucky bluegrass abundance (Table 3a). Increases in Kentucky bluegrass abundance were greatest in patches that were three years post fire (a 15.1 ± 3.1 percent increase from its abundance at the start of the experiment). Indicating that Kentucky bluegrass abundance continues to increase following a fire, exceeding its initial abundance.

The change in abundance of all native functional groups decreased as time since fire increased ($p_{Forb} < 0.0001$, $p_{Legume} < 0.0001$, $p_{Graminoid} = 0.0193$, Table 2a; Figure 2a). Immediately following a fire, there were no significant changes in native abundance (Table 3a). However, starting one year post fire and continuing for two- and three-years post fire, native graminoids saw decreases in abundance that were significantly less than 0 ($p_{One} = 0.0018$, $p_{Two} = 0.0269$, $p_{Three} = 0.0259$; Table 3a). At four years post fire, the decreases in native graminoid abundance were no longer significantly less than zero (Table 3a). Native forb and legume abundance followed similar trends. One year post fire, forb and legume abundance did not change (Table 3a). After two years post fire, decreases in native legume, but not forb abundance were significant ($p_{Legume}= 0.0011$, $p_{Forb} = 0.2574$; Table 3a). For patches that were three- and four-years post fire, decreases in both native forb and legume abundance were significant ($p_{ThreeForb} < 0.0001$, $p_{FourForb} = 0.0230$, $p_{ThreeLegume} < 0.0001$, $p_{FourLegume} = 0.0004$; Table 3a). In short, native forb, legume, and graminoid abundance do not significantly change following a fire, but as time since fire increases, the abundance of all native functional groups decreases.

Changes in plant abundance in heterogeneity-based rotational pastures.

Resting a paddock allowed Kentucky bluegrass to increase beyond pre-treatment levels but grazing at any intensity brought Kentucky bluegrass expansion back to pre-treatment levels. As grazing intensity in the heterogeneity-based rotational pastures increased, Kentucky bluegrass abundance tended to decrease, but not significantly (*p* = 0.9765, Table 2b; Figure 2b). However, this trend is a bit misleading as only one grazing intensity (or rather, lack of grazing) caused significant changes in bluegrass abundance. As bluegrass abundance significantly increased above pre-treatment levels when paddocks had been rested the year prior (*p* = 0.0099; Figure 2b; Table 3b). Conversely, even though Kentucky bluegrass abundance slightly increased following moderate grazing and slightly decreased following full or heavy grazing, these changes were not significant different from zero, meaning that Kentucky bluegrass abundance in these paddocks were the same as pre-treatment levels (Table 3b).

The magnitude and direction of the changes in native plant abundance did not change as grazing intensity increased (*p* > 0.05 for all, Table 2b; Figure 2b). Similarly, all native functional groups saw significant decreases in abundance following every level of grazing intensity, including rest (Table 3b). Essentially, in heterogeneity-based rotational grazing pastures, native functional group abundance consistently decreased over time, regardless of grazing intensity.

Discussion

Invasive plants invade rangelands, decreasing both native diversity and the historic environmental heterogeneity necessary to support that diversity (Davies *et al.*, 2005; Melbourne *et al.*, 2007; Davies, 2011). Kentucky bluegrass is an exotic perennial cool-season grass that has invaded much of North America, subsequently decreasing rangeland diversity and structural heterogeneity (Toledo *et al.*, 2014; DeKeyser *et al.*, 2015; Gasch *et al.*, 2020). Management of bluegrass invaded rangelands involving some combination of fire, grazing, and herbicide have been successful in mitigating the spread of bluegrass and promoting native plants (Bahm *et al.*, 2011; Dornbusch *et al.*, 2020; Hendrickson *et al.*, 2020; Duquette *et al.*, 2022b). However, these success are short lived, fading away quickly after the cessation of management (Bahm *et al.*, 2011; Palit *et al.*, 2021). Understanding the rate at which these benefits decay is crucial to creating management plans that can implement effective control measures at a rate that is frequent enough to control bluegrass and promote native plants without causing semi-permanent shifts in rangeland function that are difficult to reverse (Suding *et al.*, 2004; Limb *et al.*, 2018). Our results suggest that both patch-burn grazing and heterogeneity-based rotational grazing, two management strategies designed to create landscape-level heterogeneity and promote biodiversity, have the potential to stop the spread of bluegrass, but not reduce it. Also, only patch-burn grazing had the potential to maintain or promote native plant abundance; native plant abundance in the heterogeneity-based rotational grazing pastures consistently decreased over time.

Patch-burn grazing can halt bluegrass expansion, but effects may vary in accordance with fire return interval.

As any given patch got further away in time from a fire event, bluegrass abundance increased, with those increases becoming larger as time since fire increased. Also, bluegrass abundance tended to be lower immediately following a burn, though this decrease was not significant. This implies that fire can at the very least prevent bluegrass expansion, but the more years that pass following a fire, the more bluegrass will expand. These results add to a growing body of literature that suggest that patch-burn grazing can be an effective tool for controlling or potentially reducing bluegrass abundance when growing season precipitation is near or above the 30-year average (Kral *et al.*, 2018; Dornbusch *et al.*, 2020; Duquette *et al.*, 2022b).

The other additional context our results provide pertains to how bluegrass fluctuates in response to time since fire, with bluegrass expansion continuing after a fire, typically exceeding pre-fire levels. From other work, we know that a four year fire return interval with spring burns can maintain, but not decrease the spread of bluegrass (Dornbusch *et al.*, 2020; Duquette *et al.*, 2022b), our results confirm this. Our results also indicate that a spring burn can offset a roughly 15 percent increase in bluegrass abundance after three years with no fire, returning bluegrass abundance to its initial levels. Because we know that bluegrass abundance steadily increases after a fire, a more frequent fire return interval that burns an area before bluegrass increases by 15 percent, may actually decrease bluegrass abundance, instead of simply maintaining it over many years. Additionally, while we only used spring fires, there is evidence to suggest that varying the seasonality of burns, and fall burns in particular, may be effective at controlling and even reducing bluegrass abundance (Ereth *et al.*, 2017; Kral *et al.*, 2018) However, more work that varies both the fire return interval and season of burn is needed to understand the potential implications of a shorter, more varied fire return interval.

Native plant abundance does not change following a fire, but does decrease as time since fire increases.

Existing literature suggests that fire, especially when applied in discrete patches, should promote native plant abundance and diversity (Hartnett *et al.*, 1996; Baum and Sharber, 2012; Duquette *et al.*, 2022a; b). Our results do not support this conclusion. Instead, our results suggest that fire coupled with grazing maintains native plant abundance and that as time since fire increases, native plant abundance decreases. Suggesting that even though relatively frequent fire coupled with grazing may not increase native plant abundance, the combination of fire and grazing `is critical in maintaining native plant abundance. Even though our results may not have seen an increase in native plant abundance, patch-burn grazing may provide other benefits that we did not measure. For example, the same patch-burn grazing pasture we examined have been shown to increase floral resources for pollinators and mitigate the effects of moderate drought on livestock production (Spiess *et al.*, 2020; Duquette *et al.*, 2022a). That being said, more long-term research that considers trends in precipitation and ecological site is needed to truly understand how patch-burn grazing influences native plant abundance in the northern Great Plains.

Rested paddocks in heterogeneity-based rotational grazing promote bluegrass expansion that is stopped by grazing.

The impacts of grazing without fire on bluegrass are mixed, and likely dependent on both stocking rate and the timing of grazing; however, there are generally two ways in which grazing can influence bluegrass abundance (Murphy and Grant, 2005; Dornbusch *et al.*, 2020; Hendrickson *et al.*, 2020; Palit *et al.*, 2021). Early-season grazing, coupled with a relatively high stocking rate (i.e., early-intensive grazing), has been shown to either decrease or control the spread of bluegrass (Dornbusch *et al.*, 2020; Hendrickson *et al.*, 2020). However, excessively high stocking rates, especially over long periods promotes bluegrass invasion (Murphy and Grant, 2005; Limb *et al.*, 2018). Our results provide evidence that there is a third way in which grazing can affect bluegrass invasion. In paddocks that were grazed the year prior with moderate to heavy grazing (20 to approx. 70 percent degree of disappearance; Table 1 and 3b), bluegrass abundance did not change. However, when paddocks were rested one year, the following year, bluegrass abundance would increase, exceeding its abundance at the start of the experiment. A paddock was always rested after receiving the heavy grazing treatment. Grazing at a heavy intensity (greater than 60 percent degree of disappearance) removes litter from a paddock (Vermeire *et al.*, 2004; Bailey and Brown, 2011). The lack of basal litter then increases light availability early in the growing season, allowing bluegrass, typically the first plant to green up after snowmelt, to take advantage of the increased light availability, increasing its biomass and shading out neighboring plants (Borer *et al.*, 2014; Toledo *et al.*, 2014; Palit *et al.*, 2021). Ultimately, this suggests that grazing at relatively modest rates (i.e. between 20 and 70 percent degree of disappearance) can stop the spread of bluegrass, and that rest following grazing promotes bluegrass expansion.

Increases in bluegrass abundance may also explain the decrease in native plant abundance in the rested pastures, however, it does not explain the decreases in native abundance in grazed pastures. The heterogeneity-based rotation pastures are relatively heavily encroached by a native shrub, Western Snowberry (or buckbrush, *Symphoricarpos occidentalis* Hook, hereafter called, "buckbrush"; Duquette *et al.*, 2022b). Woody shrubs like buckbrush can alter cattle grazing behavior, causing cattle to graze in relatively shrub free areas (Calleja *et al.*, 2019), making buckbrush stands refugia for bluegrass (Pelton, 1953). Additionally, native plants, which do not often occur under a buckbrush canopy (Pelton, 1953), are often of higher nutritive quality than exotic plants (Savage and Heller, 1947), causing cattle to preferentially graze them (Savage and Heller, 1947; Bailey *et al.*, 1996; Prache *et al.*, 1998). The compounding effects of low native diversity under snowberry canopy coupled with the attractiveness of native plants to cattle likely explains why native forb, graminoid, and legume abundance declines regardless of grazing intensity in our heterogeneity-based rotation system. This would also explain the decline of forb abundance seen in Duquette et al. (2022b).

Conclusion

Our study supports a small body of literature that suggest that management not explicitly designed to control Kentucky bluegrass, but rather enhance biodiversity by promoting landscape-level heterogeneity can halt, but not reverse bluegrass invasion (Dornbusch *et al.*, 2020; Duquette *et al.*, 2022b). We found that bluegrass continues increases in abundance every year following a spring burn with a four-year fire return interval, and that a shorter fire return interval may prove more effective in controlling and potentially reducing bluegrass abundance. Additionally, we have shown that while modest grazing intensities enacted during the growing season can halt bluegrass invasion, rest following heavy grazing promotes bluegrass expansion. Ultimately, our results provide insight into how bluegrass abundance varies within different management practices, helping us understand how best to manage bluegrass under different climatic conditions and when to apply certain management actions, such as grazing and prescribed fire.

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Table 1: Stocking rates (AUMs/ha), grazing season, and precipitation (mm) per treatment for each year of the experiment. Stocking rates were calculated under the assumption that cattle would achieve a forage efficiency of 30 percent per hectare (USDA NRCS, 2003) with a targeted overall degree of disappearance between 40 and 50 percent each year. This operates under the assumption that cattle will graze roughly 30 percent of available forage with the remaining 10 to 20 percent lost due to senescence or damage during non-grazing behaviors (walking, resting, defecating, etc.).

Year 0 (2017)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	2.22	May 18 - October 19	293.7
Heterogeneity-Based Rotational Grazing	NA		

Year 1 (2018)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	2.32	May 23 - October 18	478.7
Heterogeneity-Based Rotational Grazing	2.33		

Year 2 (2019)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	2.32	May 22 - October 22	552.3
Heterogeneity-Based Rotational Grazing	2.33		

Year 3 (2020)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	2.37	May 19 - October 22	233.6
Heterogeneity-Based Rotational Grazing	2.48		

Year 4 (2021)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	1.59	May 22 - October 22	332.6
Heterogeneity-Based Rotational Grazing	2.07		

Year 5 (2022)

Treatment	Stocking Rate (AUMs/ha)	Grazing Season	Precipitation (mm)
Patch-burn Grazing	2.37	May 19 - October 22	342.8
Heterogeneity-Based Rotational Grazing	2.48		

Table 2: Analysis of Deviance Results from generalized linear mixed-effect models to determine how (a) time since fire or (b) grazing intensity influences Kentucky bluegrass, native forb, legume, and grass abundance in patch-burn grazing and heterogeneity-based rotational grazing pastures, respectively. Provided a relationship is significant, the estimate column indicates both the direction and relative magnitude of the relationship.

a) Patch-Burn Grazing

Response Variable	Predictor	Estimate	X^2	Df	*p*-value
Kentucky Bluegrass	Time Since Fire	3.3547	10.3788	1	0.0013
Native Forbs		-0.6510	17.0569	1	<0.0001
Native Graminoids		-0.4767	5.4775	1	0.0193
Native Legumes		--1.1369	28.5473	1	<0.0001

b) Heterogeneity-Based Rotational Grazing

Response Variable	Predictor	Estimate	X^2	Df	*p*-value
Kentucky Bluegrass	Grazing Intensity	0.0354	0.0009	1	0.9765
Native Forbs		0.0733	0.0733	1	0.7866
Native Graminoids		-0.0348	0.018	1	0.8932
Native Legumes		0.2087	1.0467	1	0.3063

Table 3: The results of a two-tailed Wilcoxon Rank-Sum Test to determine if each functional group and Kentucky bluegrass experienced increases or decreases in abundance that differed from 0 as (a) time since fire increased in patch-burn pastures, or (b) as grazing intensity increased in heterogeneity-based rotational pastures. The test statistic (V-statistic) and *p*-value do not signify the direction of the relationship, only whether or not it was significantly different from zero. The directionality of the relationship can be seen in Figures 2a and 2b.

a)	**0 YSF**		**1 YSF**		**2 YSF**		**3 YSF**		**4 YSF**	
	V-Statistic	*p*-value	V-Statistic	*p*-value	V-Statistic	*p*-value	V-Statistic	*p*-value	V-Statistic	*p*-value
Kentucky Bluegrass	528	0.4067	1452	0.0133	893	0.1965	681	0.0001	141	0.0663
Native Forbs	673	0.5538	860	0.1659	483	0.2574	52	<0.0001	39	0.0230
Native Graminoids	590	0.8283	591	0.0018	845	0.0269	245	0.0259	47	0.5457
Native Legumes	647	0.7374	850	0.1468	364	0.0011	72	<0.0001	14	0.0004

b)	**Heavy Grazing**		**Full Grazing**		**Moderate Grazing**		**Rested Pastures**	
	V-Statistic	*p*-value	V-Statistic	*p*-value	V-Statistic	*p*-value	V-Statistic	*p*-value
Kentucky Bluegrass	192	0.1838	201	0.2462	316	0.3403	400	0.0099
Native Forbs	152	0.0358	123	0.0073	120	0.0061	103	0.0019
Native Graminoids	81	0.0003	99	0.0014	75	0.0002	31	<0.0001
Native Legumes	83	0.0004	129	0.0105	98	0.0013	13	<0.0001

Figure 1: A profile of Kentucky bluegrass (*Poa pratensis* L.), containing the various aspects of Kentucky bluegrass. a) Aboveground biomass. b) Aboveground litter. c) The thatch layer, which is a mix of loosely compressed and intermingling decaying litter, roots, and both live and dead stems and buds. d) The non-thatch bound root mat. e) The underlying mineral soil.

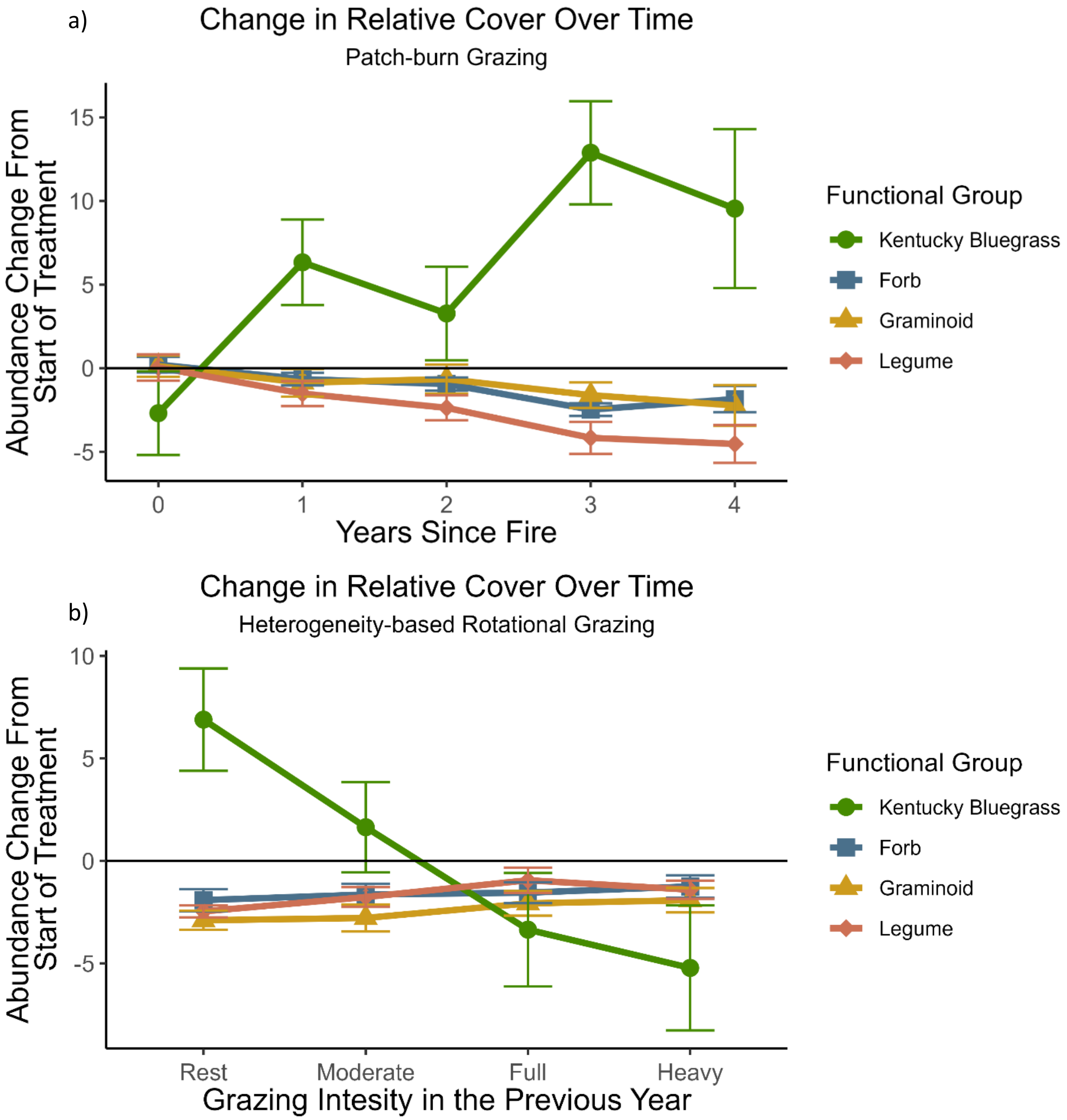

Figure 2: Differences in Kentucky bluegrass (green), native forb (blue), legume (orange), and graminoid (yellow) abundance from the creation of (a) patch-burn pastures and (b) heterogeneity-based rotational pastures. The data displayed here are taken from plant community data collected over several years and represent how much plant abundances have changed since the start of a grazing management practice (2017 for patch-burn pastures, 2018 for heterogeneity-based rotational pastures) when a patch is a certain number of years post-fire (a) or when a paddock experienced a specific grazing intensity the year prior (b). Error bars represent 95% confidence intervals.

Do cows eat wildflowers? Assessing cattle grazing selection of vegetation using three different grazing intensities

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Summary

Our study found cattle grazed wildflowers, with greater use occurring in pastures stocked at a higher grazing intensity. We also found cattle will graze wildflowers at a higher rate during drought years, likely due to less grass and more mature grass, thus less palatable. We couldn't tell if there was a preference for any one species of wildflower as we clipped all wildflowers within the frame without identifying key species.

Introduction

The subject of diet selection by grazing animals is a vast one. While it is widely accepted that there may be a set of generalized principles underlying foraging behavior and diet selection by grazing animals, the current belief is cattle are primarily graminoid eaters, goat's shrub consumers, and sheep prefer grasses earlier in the grazing, but eat flowering plants and shrubs more readily during the second half of the grazing season. In general, the domesticated ruminant animals which have been studied most frequently, i.e. cattle, sheep, deer and goats, have adopted slightly different foraging strategies along the continuum of grazers, mixed grazers/browsers and browsers; with cattle considered as grazers and goats being the nearest species to a browser.

Plumb and Dodd (1993) observed forbs (wildflowers) contributed 15% and shrubs 10% of cattle diet in June and early July, declining by late July. They showed the proportion of the cow's diet was higher than the proportion available in June, but found no difference in August (no positive or negative selection preference).

We started a trial in 2018 to assess impacts of a heterogeneity grazing system on herbage production using a modified twice-over rest rotation grazing system (MTRG). One of the objectives was to determine if cows ate forbs and at what level of degree of use was achieved by grazing cows with calves.

Methods

Study Site

The system was designed to graze cattle with nursing cows in the MTRG for varying lengths of time across four separate paddocks to achieve the desired amount of vegetation biomass removal (targeted grazing intensity).The stocking durations in the MTRG paddocks were 0 day (rested, 0% vegetation removal), 21 days (moderate,20%–40% vegetation removal), 50 days (full, 40%–60%vegetation removal), and 77 days (heavy, 60%–80% vegetation removal), divided into two grazing intervals each year, with 40% of the days grazed during the first rotation and 60% grazed during the second rotation.

The targeted grazing intensity in each paddock rotated from year to year (i.e., heavily stocked paddocks transitioned to rested pad-docks in the subsequent year, etc.) at a 4-year return interval. The stocking rate was 2.26–2.31 Animal Unit Months/ha for all treatments over the 4-year cycle. The stocking rate was determined to achieve, on average across the pasture, a degree of utilization of 40%–50%. We conducted biomass clippings in grazing exclosures using a pair plot technique (non-grazed cage with a matched grazed site) and a 0.25 m^2 frame at the beginning and end of each rotation to calculate the degree of disappearance for the graminoids and forbs. We clipped vegetation from five pair plots (five cages) at four different locations in each grazing intensity pasture. Each clipped frame was sorted by grasses, wildflowers, and standing litter. The study was conducted way four years (2019-2022).

Results

On average, cows grazed wildflowers at a higher level for degree of use with the higher stocking rates. Over the three grazing levels, cows grazed wildflowers at 21.1 percent in the moderate grazing intensity pastures, and increased to 46.5 percent in the full use grazing intensity (**Table 1**). Unlike grasses, degree of use of wildflowers to not increase when we grazed at a high intensity level.

Table 1. Degree of utilization of forbs (wildflowers) and grasses by grazing intensity in a modified twice-over rest rotation grazing system designed to created structural and plant diversity heterogeneity at the Central Grasslands Research Extension Center near Streeter, ND.

Grazing Intensity Treatment	Forbs (Wildflowers)[1]	Graminoids (grasses + grass-like)[1]
Moderate use (20-40%)	21.1	34.3
Full use (40-50%)	46.5	46.8
Heavy use (+60%)	44.5	63.5

[1] Average forb composition on the grazing system was 15.3 percent, graminoid composition 84.7 percent.

The environment played a role each year in wildflower degree of use. Degree of utilization of wildflowers was highest in the drought year 2021, with an average use of 69.7 percent across all three grazing intensities. When moisture was average to above average, such as in 2022, average use was 24.9 percent across all three grazing intensities.

Effect of a Propionic Acid-Based Additive on the Chemical Composition and Fermentation of Corn Silage.

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Summary

Corn is relatively easy to ensile when harvested at the optimum DM content. Chemical additives may be required to enhance fermentation when harvest conditions are not ideal. This study was conducted to investigate the effect of an acid-based additive on silage chemical composition, fermentation and bacterial and fungal communities. Corn silage was harvested at 30 and 40% DM and treated with an acid-based additive, SAVOR® Plus. Silage chemical composition was mostly influenced by DM content at ensiling but the additive reduced the concentration of ADIN (%CP) and increased water-soluble carbohydrate concentration. Acetic acid concentration was lower and ammonia-N concentration greater in additive-treated silage. Treatment of corn silage with an acid-based additive reduced the relative abundance of Saccharomycetales, the predominant order within the Ascomycota phylum. More research is underway to examine higher concentrations of acid-based additives on bacterial and fungal communities.

Introduction

When harvested at optimum DM content of 32 to 35%, corn is relatively easy to ensile due to the large amounts of fermentable water-soluble carbohydrate and low buffering capacity (Muck, 2007). In many cases, due to a variety of reasons, it may not be possible to harvest corn at the optimum DM content. For example, inadequate capacity to harvest large amounts of forage in a timely manner due lack of equipment or missing optimal harvest times due to poor monitoring (Windle et al., 2014). Early frost also can result in either premature harvest of the corn crop or harvest of high DM corn silage. When less than optimum conditions are encountered, chemical additives may be required to enhance the fermentation process. Propionic acid-based preservatives have antimycotic properties that limit spontaneous heating and improve aerobic stability of silage (Coblentz et al., 2021). Studies on the use of organic acids such as propionic have shown that propionic acid may improve silage quality and reduce aerobic deterioration but the effect depends on rate of application of propionic acid (Kung et al., 2004; Kleinschmit et al., 2005). Currently, the effect of acid-preservatives on bacterial and fungal communities in corn silage has not been adequately evaluated. This study was conducted to investigate the effect of an acid-based additive on silage chemical composition, fermentation and bacterial and fungal communities in corn ensiled at two dry matter contents.

Methods

Corn (CP3200SRR, Winfield Solutions LLC, St. Paul, MN) was harvested on two dates, September 16 and 26, 2022, to achieve two different DM contents of 30 and 40% at harvest. At each DM content, approximately 50kg of forage from each DM content was treated with deionized water (Control) or an acid-based additive. The acid-based additive, SAVOR® Plus, (KEMIN Industries, Des Moines, Iowa), was applied at 0.5kg/t of fresh forage. The acid-based additive was sprayed onto forage during mixing. After thorough mixing, four bags of treated forage (approximately 500g/bag) for each treatment were ensiled in 300 x 220 mm vacuum-sealed polyethylene bags (Jarden Consumer Solutions, Boca Raton, FL). Vacuum sealing was accomplished using a commercial sealer (Maxvac 250, LEM Products, West Chester, Ohio, lemproducts.com). All bags were stored at ambient temperature (21°C) for 180 d. After 180 days, bags were opened and subsampled. Silage samples were submitted to Dairyland laboratories (Dairyland Laboratories Inc., St. Cloud, MN) for chemical composition, and silage quality at. Another set of subsamples was submitted to Jonah Ventures laboratory (Jonah Ventures LLC, 5485 Conestoga Ct STE 210 Boulder, CO; https://jonahventures.com) for bacterial and fungal identification.

Results and Discussion

The effect of DM content at ensiling and treatment on the chemical composition of corn silage is shown in Tables 1. Concentration of CP tended to be greater ($P = 0.082$) in corn ensiled at 30% DM relative to corn ensiled at 40% DM (Table 1). As well, CP concentration tended to be greater ($P = 0.059$) in treated corn silage relative to control silage (Table 1). Concentration of ADIN (%CP) was influenced ($P = 0.053$) by DM content at ensiling and treatment (Table 1). Treatment of silage with an additive reduced ADIN concentration at both DM contents at ensiling but the difference was more pronounced in corn ensiled at 40% DM mainly due to a greater ADIN concentration in the control silage (Fig. 1). Acid detergent insoluble N is an indicator of heat damage and is proportional to the degree of heating. Thus, more heating occurred in corn ensiled at 40% DM and this was effectively reduced by addition of the acid-based additive.

Water-soluble carbohydrate concentration was influenced ($P = 0.049$) by DM at ensiling and treatment (Table 1). Concentration of WSC was lower in control silage at both DM contents but the difference between treatments was greater in corn ensiled at 40% DM (Fig. 2). This study supports findings (Hu et al., 2009) which reported greater concentration of WSC concentration in "normal" silage relative to higher DM silage. In this study, treatment of corn silage with a propionic acid-based reduced microbial activity during ensilage.

Concentration of starch was greater ($P < 0.001$) in corn ensiled at 40% DM relative to corn ensiled at 30% DM (Table 1). Increase in starch concentration with increasing DM at ensiling reflects an increase in starch as maturity progressed. Dry matter content at ensiling and treatments did not influence silage NDF and NDF concentration (Table 1). Lignin concentration tended to be influenced ($P = 0.062$) by DM content at ensiling and treatment (Table 1).

Silage pH, organic acids, ethanol and ammonia-N, the most common measurements for evaluating silage fermentation (Kung et al. 2018) are shown in Table 2. Silage pH was lower ($P \leq 0.001$) in corn ensiled at 30% DM relative corn ensiled at 40% DM (Table 2). Treatment of corn with an additive increased ($P < 0.001$) silage pH (Table 2). Silage pH in all treatments was within the normal pH of 3.7 to 4% expected in corn silages (Kung et al. 2018). Lactic acid concentration was greater ($P \leq 0.001$) in corn ensiled at 30% DM relative corn ensiled at 40% DM (Table 2). Acetic acid concentration was influenced ($P = 0.018$) by DM content at ensiling and treatment (Table 2). Treatment of silage with an additive reduced acetic acid concentration at both DM contents but the difference between treatments was more pronounced in corn ensiled at 30% DM. (Fig. 3). Treatment of silage with an acid-based additive containing propionic acid increased ($P = 0.019$) propionic acid concentration of treated silage (Table 2) which was expected since the additive contained propionic acid.

Concentration of total acids was greater ($P \leq 0.001$) in corn ensiled at 30% DM relative corn ensiled at 40% (Table 2). Similarly, lactic acid, as a percent of total acid, was greater in corn ensiled at 30% DM relative corn ensiled at 40% DM (Table 2). Lactic acid made up at least 65 to 73% of the total acids in corn ensiled both DM contents, indicating relatively optimum fermentation. Lactic acid should be at least 65 to 70% of the total silage acids in good silage (Kung and Shaver, 2001). The ratio of lactic acid to acetic acid was greater ($P = 0.001$) in corn ensiled at 30% DM relative to 40% DM (Table 2) probably due to a greater concentration of lactic acid produced in corn ensiled at 30% DM. Also, the ratio of lactic to acetic acid was greater ($P = 0.039$) in treated corn silage relative to control corn silage (Table 2) due to more production of acetic acid in the control silage. A lactic to acetic acid ratio about 2.5 to 3.0 indicates good silage fermentation (Kung et al. 2018).

Ethanol concentration was not influenced by DM content at ensiling or by treatment (Table 2). In general, concentration of ethanol was low in all silage treatments indicating favorable conditions for good-quality silage. High concentrations of ethanol in silages (>3 - 4%) are often associated with high numbers of yeasts, and such silages usually spoil readily when exposed to air because some yeasts can assimilate lactic acid under these conditions (Kung et al. 2018).

Concentration of ammonia-N (% CP) was influenced ($P = 0.001$) by DM content at ensiling and treatment (Table 2). Treatment with additive increased ammonia-N concentration at both DM contents but the difference was greater in corn ensiled at 30% DM (Fig. 4). This finding is supported by a study that evaluated corn silage treated with several additives including a propionic acid-based additive similar to that evaluated in this study (Queiroz et al., 2013). Concentration of ammonia-N in the current study ranged from 7 to 11% (Table 2). Greater NH_3-N concentrations (>12% of CP) indicate excessive protein breakdown caused by a slow drop in pH or clostridial action during ensilage (Kung and Shaver, 2001).

Traditional culturing methods showed that lactic acid bacteria counts were influenced ($P = 0.001$) by DM at ensiling and treatment (Table 2). There was no difference in lactic acid bacteria counts between control silage and treated silage in corn ensiled at 30% DM. Lactic acid bacteria counts were, however, greater in control silage relative to treated silage in corn ensiled at 40% DMNext-generation sequencing techniques identified *Firmicutes* and *Proteobacteria* as the predominant bacterial phyla, making up approximately 76.1% and 21.8% of the 16S sequences, respectively, in corn silage. Three main fungal phyla, *Ascomycota, Basidiomycota,* and *Mucoromycota,* were identified in corn silage. Treatment of corn silage with an acid-based additive reduced ($P = 0.001$) the relative abundance of *Saccharomycetales,* the predominant order within the *Ascomycota* phylum.

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Table 1. Chemical composition of corn silage ensiled at two DM contents and treated with an acid-based additive.

	DM content at ensiling, %			Treatment (T)[2]			P-value		
	30	40	SE	CON	ADD	SE	DM	T	DM x T
DM at ensiling, %	30.4^{b}	40.3^{a}	0.34	35.7	35.0	0.34	<0.001	0.074	0.077
CP, %	7.1	6.8	0.12	6.8	7.2	0.12	0.082	0.059	0.403
ADIN, %CP	3.1^{b}	3.4^{a}	0.06	3.7^{a}	2.9^{b}	0.23	0.006	0.031	0.053
WSC[2], %	5.6^{a}	3.7^{b}	0.17	4.3^{b}	5.0^{a}	0.17	<0.001	0.002	0.049
Starch, %	28.8^{b}	41.3^{a}	0.37	35.3	34.7	0.88	<0.001	0.543	0.791
NDF, %	30.3	31.7	0.91	29.9	32.0	0.91	0.070	0.231	0.998
ADF, %	17.9	19.8	0.74	19.0	18.7	0.74	0.059	0.759	0.184
Lignin, %	2.3	2.2	0.09	1.9^{b}	2.6^{a}	0.13	0.429	0.007	0.062
DM loss, %	2.7^{b}	3.0^{a}	0.11	2.9	2.7	0.19	0.022	0.210	0.006

[1] CON, ADD = control, acid-based additive, respectively.

[2] Water-soluble carbohydrate

[a-c] Means within DM content and within treatment with a different letter differ significantly (*P* ≤ 0.05).

Table 2. Silage pH and concentration of organic acids, ethanol and ammonia of corn silage ensiled at two DM contents and treated with an acid-based additive.

	DM content at ensiling, %			Treatment (T)[2]			P-value		
	30	40	SE	CON	ADD	SE	DM	T	DM x T
pH	3.66^{b}	3.90^{a}	0.017	3.72^{b}	3.84^{a}	0.017	<0.001	<0.001	0.410
Lactic acid, %	7.22^{a}	5.54^{b}	0.052	6.31	6.45	0.304	<0.001	0.667	0.061
Acetic acid, %	2.32^{b}	2.47^{a}	0.062	2.56^{a}	2.23^{b}	0.062	0.048	0.001	0.018
Propionic acid, %	0.39	0.41	0.073	0.24^{b}	0.59^{a}	0.083	0.832	0.019	0.673
Total acids, %	9.93^{a}	8.42^{b}	0.090	9.11	9.24	0.346	<0.001	0.727	0.164
Lactic acid, % total acid	72.7^{a}	65.9^{b}	0.38	69.0	69.6	1.10	<0.001	0.590	0.531
Lactic/acetic acid	3.16^{a}	2.26^{b}	0.089	2.47^{b}	2.94^{a}	0.157	0.001	0.039	0.094
Ethanol, %	0.86	0.50	0.254	0.81	0.55	0.254	0.201	0.329	0.676
Ammonia-N, %CP	9.03^{b}	9.34^{a}	0.112	7.34^{b}	11.03^{a}	0.302	0.050	<0.001	0.001
Lactic acid bacteria[2]	4.40	5.30	0.137	5.39	4.30	0.143	0.003	0.002	0.001

[1] CON, ADD = control, acid-based additive, respectively.

[2] Log cfu/g fresh silage

[a-c] Means within DM content and within treatment with a different letter differ significantly (*P* ≤ 0.05).

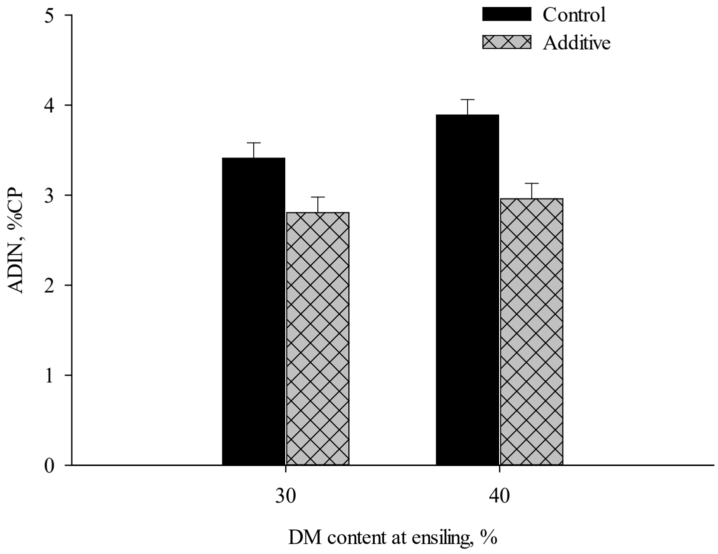

Figure 1. ADIN (%CP) concentration in corn silage ensiled at two DM contents and treated with an acid-based additive.

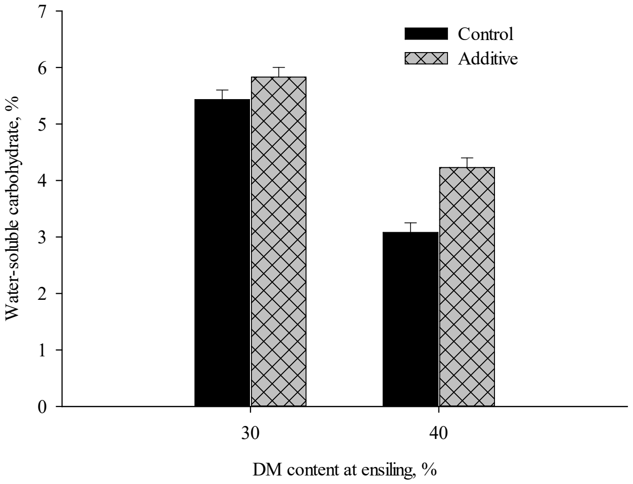

Figure 2. Water-soluble carbohydrate concentration of corn silage ensiled at two DM contents and treated with an acid-based additive.

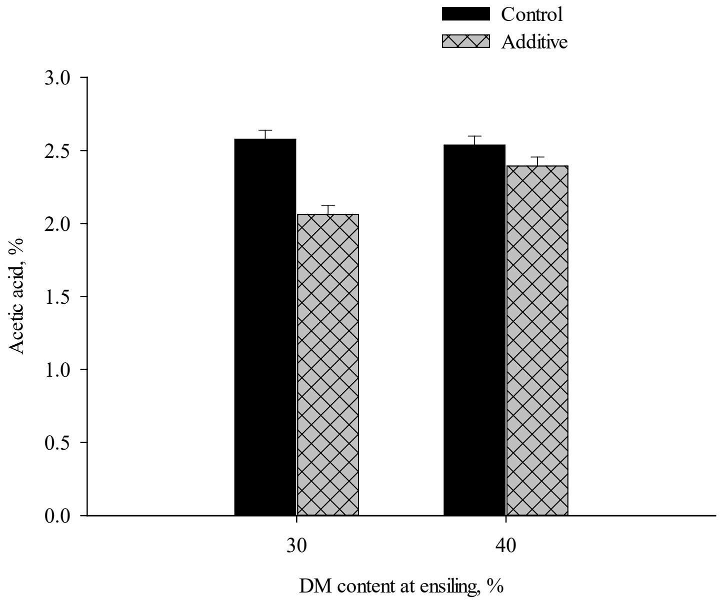

Figure 3. Acetic acid concentration in corn silage ensiled at two DM contents and treated with an acid-based additive.

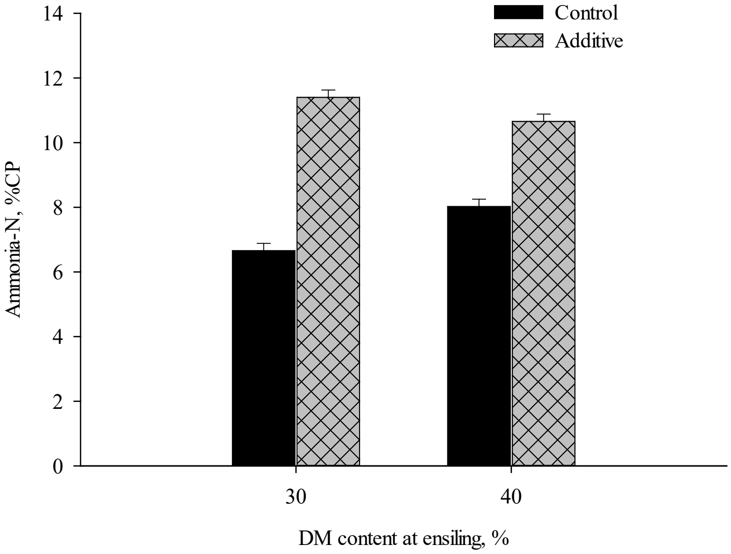

Figure 4. Ammonia-N concentration in corn silage ensiled at two DM contents and treated with an acid-based additive.

Economic Potential of Field Peas as an Alternative to Corn Distillers Dried Grain with Solubles (DDGS) in Beef Heifer Growing Diets

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Field peas can effectively replace corn DDGS in growing heifer diets. However, the high price of field peas normally limits utilization of field peas. This study was conducted to a) determine the economic potential of field peas relative to corn DDGS in diets of growing heifers and, b) identify price points for competitive utilization of field peas as an alternative to corn DDGS in diets of growing heifers. The study was conducted with growing heifers which fed corn DDGS-based and field peas-based diets formulated to produce similar heifer performance. The relative value of field peas as an alternative to corn DDGS in diets for growing heifers was mainly driven by the level of incorporation of field peas into diets and the relative price of field peas to DDGS. When compared to a corn DDGS-based diet, a field peas-based diet that met nutrient requirements of growing heifers required approximately 43% more field peas. At this level of incorporation, field peas would be an economically-feasible replacement for corn DDGS in growing heifer diets when the price of the field peas is less than or equal to 71% of the price of corn DDGS. Results from this study offer the field pea processing industry useful economic information about the range of prices that beef cattle producers can afford to pay for peas relative to DDGS.

Summary

Corn distillers' dry grains with solubles (DDGS) is one of the most common supplements for cattle in North Dakota. Utilization of corn DDGS will likely be affected by availability and pricing, thus, there is continual need to evaluate alternative feed ingredients. Field peas can effectively replace corn DDGS in cattle rations. This study was conducted to determine a price point at which field peas can competitively replace corn DDGS in diets of growing heifers. The study was conducted with growing heifers which fed corn DDGS-based and field peas-based diets which were formulated to produce similar heifer performance. This response allowed comparative ration cost analysis to be conducted without the need to account for differences in animal performance. The relative value of field peas as an alternative to corn DDGS in diets for growing heifers was mainly driven by the level of incorporation of field peas into diets and the relative price of field peas to DDGS. When compared to a corn DDGS-based diet, a field peas-based diet that met nutrient requirements of growing heifers required approximately 43% more field peas. At this level of incorporation, field peas would be an economically-feasible replacement for corn DDGS in growing heifer diets when the price of the field peas is less than or equal to 71% of the price of corn DDGS. Results from this study offer the field pea processing industry useful economic information about the range of prices that beef cattle producers can afford to pay for peas relative to DDGS.

Introduction

Field peas are another source of protein and energy, and desired by livestock, making them a valuable livestock feed (Anderson et al., 2007). The energy content of field peas is similar to cereal grains such as corn and barley when included in high-concentrate finishing diets (Lardy et al., 2009). Field peas have been primarily grown for human consumption and for the pet food industry (Lardy et al., 2009; Troyer et al. 2020). However, the livestock industry is a potential market for field peas in situations where there is excessive field pea production that may saturate the pet food market (Troyer et al. 2020) or field peas which do not meet specifications for human consumption (Lardy et al., 2009; Troyer et al. 2020). The major field pea growing areas include North Dakota and Montana in the US (Lardy et al., 2009), and Manitoba, Saskatchewan and Alberta in western Canada (Chen et al., 2003). In such areas, feeding peas to livestock presents a realistic, on-farm value-adding opportunity for pea growers (Chen et al., 2003).

Field peas have been successfully included in cattle finishing diets (Lardy et al., 2009), lamb finishing diets (Loe et al., 2004), backgrounding beef heifers (Chen et al., 2003), and lactating dairy cow diets (Vander Pol et al., 2009). In such diets, peas have to compete with feeds such as barley, corn, wheat middling, distillers' grains, and oil seed meals (Anderson et al., 2007). Compared to other feedstuffs, the price of field peas is likely to be a major factor in determining utilization of field peas in cattle rations (Anderson et al., 2007). However, identifying a price for field peas as livestock feed presents quite a challenge since field peas for livestock do not have a formal market compared to other feeds as they are normally priced for human food and pet food markets (Lardy et al., 2009; Troyer et al. 2020). Therefore, there is need for data on which to base reliable recommendations on the economic viability of utilizing field peas as a replacement of supplements such as corn DDGS in growing heifer diets.
This study was conducted to a) determine the economic potential of field peas relative to corn DDGS in diets of growing heifers and, b) identify price points for competitive utilization of field peas as an alternative to corn DDGS in diets of growing heifers.

Methods

Animal handling and care procedures were approved by the North Dakota State University Animal Care and Use Committee.
This study extended over 2 years, starting on Nov. 24, 2020 through Feb. 17, 2021 (year 1) and Nov. 8, 2021 through Feb. 24, 2022 (year 2). Starting in the fall of each year, 162 growing Angus heifers (2020/2021, BW = 312 ± 38 kg; 2021/2022, 283 ± 32 kg) were divided into 2 groups of similar average body weight and the groups were randomly assigned to 6 dry lot pens. Dry lot pens were surrounded by 2.5m high wooden windbreaks on 3 sides of the pen. Each pen contained a 16m long feed bunk and a winterized water bowl (Richie Industries Inc., Conrad, IA, USA). Six groups of heifers (27 heifers/pen) were assigned randomly to either a field pea-based or corn DDGS-based total mixed ration (TMR). The diets were formulated to be isocaloric and isonitrogenous and to meet nutrient requirements of growing heifers. Formulation of heifer diets was accomplished using CowBytes® Ration Balancing Program (CowBytes Beef Ration Balancer Program, Version 5.3.1, AAFRD, Edmonton, Alberta). The diets were fed as a total mixed ration using cane molasses to minimize ingredient separation from forages. Field peas were coarse-rolled through a roller mill before incorporation into TMR.

Heifer feeding was accomplished using a clean bunk feeding management. The goal of clean bunk management is for all feed delivered to a pen to be consumed daily, with bunks being empty for a certain period of time prior to next feeding, without restricting feed intake (Erickson et al., 2003). The heifers were fed once daily at approximately 09:00 each day and feed bunks were targeted to be empty of feed by 16:00. Amount of feed delivered to bunks each week was based on bunk clearance from the previous week. Heifers had *ad libitum* access to fresh water. Heifer performance was assessed from average of two-day body weights taken at the start and end of the study.

Economic evaluation of the feed costs for each TMR dietary treatment (DDGS and Peas) was based on the two-year average measures of DMI (kg hd^{-1} day^{-1}), TG (kg hd^{-1}), and DOF. Total mixed rations fed in this study were formulated to be isocaloric and isonitrogenous which hypothesis testing revealed similar performance between the heifers on both treatments. This response allowed for an economic comparative ration cost analysis without the need to account for differences in animal performance. Therefore, enterprise budgeting techniques were used to calculate the two-year average costs of individual ingredients for each diet treatment (AAEA, 2000). On March 1, 2022, prices of corn grain, hay, and DDGS were obtained from a local farm input supplier (Farmers Coop Elevator Company, Streeter, ND) and were priced at \$275, \$88, and \$325 MT^{-1}, respectively. In addition, a price of \$34 MT^{-1} for corn silage was used and based on local production and estimated from corn production (LaPorte, 2019). Identifying a consistent price for field peas as livestock feed is challenging since field peas are normally priced for human food and pet food markets (Lardy et al., 2009; Troyer et al. 2020). Also, in March of 2022, based on conversations with field peas producers, the price of field peas was concluded to be in a range between \$294 and \$404 MT^{-1} (\$8 to \$11 bushel^{-1}). For the analysis, we used the average base-case price of \$366 MT^{-1} for field peas.

Ration costs (\$ hd^{-1}) were calculated as the product of each daily individual ingredient dry matter intake (DMI) and individual ingredient price. Individual ingredient DMI was calculated from feed delivered (kg hd^{-1} day^{-1}) and diet composition (Table 1). Over a two-year period, an average of 4.2% DDGS and 6% field peas were required in the corn DDGS-based and field peas-based diets, respectively. At a feed intake of approximately 8 kg day^{-1} for both diets, 0.30 and 0.43 kg day^{-1} of corn DDGS and field peas, respectively, were included in the respective diets.
In some cases, prices for certain feed ingredients are correlated with prices of other co-product ingredients. For example, because DDGS are a co-product of corn processing (e.g., ethanol, corn oil, corn syrup), the price of DDGS typically increases (decreases) when corn prices increase (decrease). The same is true for soybean meal; that is, soybean meal prices tend to increase (decrease) when the price of soybeans increase (decrease). However, prices for some feed ingredients move independently of other feed ingredients. For instance, the price of field peas as an animal feed is determined in part by the price of field peas for human and pet food consumption; however, the price of field peas as a source of feed for animal production is not likely directly affected by the price of corn, DDGS or soybean meal. Therefore, to understand market situations where dry field peas are more cost effective than DDGS, and vice versa, sensitivity analysis was conducted to calculate relative total cost of feeding peas versus DDGS for a number of combinations of prices of DDGS and field peas. For this analysis, price combinations for DDGS and field peas were used that ranged between -50% and +50% of the base-case prices of \$325 and \$366 MT^{-1} for DDGS and field peas, respectively.

Results and Discussion

Field peas utilized in total mixed rations contained 25.7% CP and 1.41 Mcal/kg which was within expected ranges reported in similar studies (Anderson et al., 2007; Troyer et al., 2020). At 30.5% CP and 1.35 Mcal/kg, the composition of corn DDGS was also comparable to other studies (Troyer et al., 2020). The diets offered to heifers were formulated to be isonitrogenous and isocaloric (Table 1). In 2020, approximately 3.9 and 5.8% of DDGS and field peas, respectively, were required to produce diets containing 12% CP and NEg of 0.7 Mcal kg^{-1}. In 2021, approximately 4.5 and 6.2% of DDGS and field peas, respectively, was required to produce diets containing 13.5% CP and NEg of 0.6 Mcal kg^{-1}.

Animal performance data are shown in Table 2. Dry matter intake was not influenced ($P > 0.05$) by diet but there were seasonal differences, with intake being greater in winter relative to summer. When expressed as a percent of BW, there were no dietary differences in DMI but DMI was greater in the fall relative to winter. Initial BW, final BW, total gain, and ADG were not influenced ($P > 0.05$) by diet but there were seasonal differences. Initial BW was greater ($P < 0.001$) in winter relative to fall, which was expected since the same heifers were utilized in winter. The same trend was observed for final BW. However, total gain and ADG were greater in the fall relative to winter. In general, animal performance was not impacted by diet, which was expected since the diets were formulated to be isocaloric and isonitrogenous.

Quantities of individual feed ingredients fed to heifers by dietary treatment (DDGS and peas) and feeding period (fall and winter) are reported in Table 3. Heifer performance was similar when 0.33 kg d^{-1} and 0.48 kg d^{-1} of DDGS and field peas, respectively, were incorporated into DDGS-based and field peas-based rations, respectively. Corn DDGS-based and field peas-based rations for winter were greater than the TMR for fall, likely due to heifers eating more during the colder winter weather to maintain necessary energy levels. For the DDGS TMR, the total quantity of feed consumed for the winter-feeding period was 72.8 kg more than the feed fed during the fall period. For the Peas TMR, the total quantity consumed by heifers in winter was 51.6 kg more than the fall. Overall, the DDGS and Peas rations average out over the two feeding periods to 8 kg d^{-1}.

The individual feed quantities reported in Table 3 were used to calculate the two-year average cost of feed for each ingredient on a (\$ $head^{-1}$ day^{-1}) and (\$ $head^{-1}$) basis. These costs are reported in Table 4. The cost of hay, silage, corn grain, and supplements equaled \$82.09 $head^{-1}$ (or \$1.84 $head^{-1}$ day^{-1}) over the total feeding period, accounted for 87% and 81% of the total cost of the corn DDGS-based ration and dry field peas-based ration, respectively. The total cost of feed for a representative heifer for the total (fall plus winter) feeding period for the corn DDGS-based ration was \$93.89 $head^{-1}$ (or \$2.10 $head^{1}$ day^{-1}) and \$6.88 $head^{-1}$ (7.3%) less than the dry peas-based ration cost of \$100.77 $head^{-1}$ (or \$2.26 $head^{-1}$ day^{-1}) for base-case prices of \$325 MT^{-1} and \$366 MT^{-1} for corn DDGS and field peas, respectively. For perspective, at the base-case prices, a producer interested in feeding a group of 100 heifers similar to those fed in the study, the cost of feeding field peas instead of DDGS in the TMR would cost an extra \$688 over the total feeding period.

Table 5 reports differences in the total cost of field peas relative to the total cost of DDGS for alternative combinations of prices of field peas and DDGS that range from -50% to +50% of the base-case prices. For reference, price combinations that have a negative total cost indicate market situations where field pea-based rations have an economic advantage over corn DDGS-based rations. For instance, in cases where field peas can be purchased at a price 30% below the base-case price for peas (i.e., peas can be purchased for \$256 MT^{-1} instead of \$366 MT^{-1}) and the price of DDGS is at 30% higher than the base-case price (i.e., \$423 MT^{-1} instead of \$325 MT^{-1}), peas would have a \$2.28 $head^{-1}$ economic advantage over DDGS. For a market scenario where peas can be purchased at a price that is 50% less than the base-case price of peas and the price of DDGS is priced 50% higher than the base-case price, a producer would benefit economically from buying peas and saving \$8.37 $head^{-1}$ of feed cost, holding all other feed ingredient prices constant. Overall, for a base-case average price of \$325 MT^{-1} for DDGS, the breakeven price for field peas was equal to \$231.15 MT^{-1}, which was 36.8% less than the base-case price of \$366 MT^{-1} for peas, and 71% of the base-case price of DDGS. Conversely, for the base-case price of field peas of \$366, the breakeven price of DDGS was equal to \$514.60 MT^{-1}, which was 58.3% more than the base-case price of \$325 MT for DDGS and 140.60% more than the base-case price for field peas. At the respective breakeven prices, producers would be indifferent between using field peas or corn DDGS in their TMR.

When we used the base-case prices of \$325 MT^{-1} and \$366 MT^{-1} for DDGS and field peas, respectively, in the analysis, our results support studies that showed a competitive economic advantage of corn DDGS relative to feeds such as dry-rolled corn (Buckner et al., 2008) or field peas (Troyer et al. 2020). Inclusion of corn DDGS in the diet resulted in higher profits relative to a dry-rolled corn-based diet (Buckner et al., 2008). A recent economic evaluation of field peas and corn DDGS as a supplement for heifers grazing crested wheatgrass showed field peas can be utilized as a supplement when the peas are competitively priced at 90% of the price of corn DDGS (Troyer et al. 2020). Due to lower costs relative to feeds such as wheat, barley, corn, canola meal, and soybean meal, feed co-products such as DDGS will likely continue to be common and cost-effective ingredients in beef and dairy diets (Paz et al., 2013). Our study also agreed with the argument by Chen et al. (2003) that the inclusion of field peas into livestock diets will likely increase total feed costs due to a larger quantity of peas required for a balanced ration compared to DDGS and a relatively high price of field peas.

Situations that result in excess production of field peas, resulting in drastically lower prices of field peas, might offer opportunities for competitively-priced field peas for use in cattle diets. However, Kowk (2022) cautions that cattle producers typically rely on feed supply companies that have built a trusting relationship for their feed ingredients, and these relationships have been developed over time because they are accompanied with a reliable supply and are competitively priced. So, if the situations where excess production of field peas, and corresponding prices, are not reliable each year, then producers will not likely switch away from a feed product that is reliable from a trustworthy supplier.

The relative value of field peas as an alternative to corn DDGS in diets for growing heifers was mainly driven by the level of incorporation of field peas into diets and the relative price of field peas to DDGS. When compared to a corn DDGS-based diet, a field peas-based diet that met nutrient requirements of growing heifers required approximately 43% more field peas. At this level of incorporation, field peas would be an economically-feasible replacement for corn DDGS in growing heifer diets when the price of the field peas is less than or equal to 71% of the price of corn DDGS. Results from this study offer the field pea processing industry useful economic information about the range of prices that beef cattle producers can afford to pay for peas relative to DDGS. This information will help pea processors and feed supply dealers develop a reliable supply chain for a beef cattle quality field pea source of feed. As a result, future research to investigate the production and economics of supply chain reliability of excess field peas is warranted.

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Table 1. Ingredients and chemical composition of total mixed rations fed to growing heifers.

	Year			
	2020/2021		2021/2022	
	Corn DDGS	Field peas	Corn DDGS	Field peas
Ingredients, % as fed				
Hay	37.9	36.7	42.3	43.1
Corn silage	40.7	40.6	41.3	39.6
Corn grain	14.4	13.8	9.1	8.3
Peas	-	5.8	-	6.2
DDGS	3.9	-	4.5	-
CM30[1]	3.1	3.1	2.8	2.8
Chemical composition, %DM				
CP	12.2	12.3	13.4	13.6
NEg, Mcal/kg	0.71	0.67	0.65	0.61
NDF	42.9	42.1	47.7	44.7
ADF	29.4	29.0	32.1	31.3
EE	2.9	2.0	3.2	2.7
Ca	0.82	0.86	1.2	1.1
P	0.27	0.27	0.35	0.29
Mg	0.35	0.21	0.30	0.28
K	1.57	1.43	1.88	1.86

[1] Core Max 30 liquid protein supplement (30% CP, 0.1 % CF, 11.3 to 13.5 % Ca, 0.08 % P, 4.4 to 5.4 % Salt, 2% K, 5.5 ppm Se, 50,000 IU/LB Vit., 8% total Sugars, 33.0 % moisture).

Table 2. Performance of growing heifers consuming field peas-based or corn DDGS-based total mixed rations.

	Diet			Season			P-value		
	DDGS	Peas	SE	Fall	Winter	SE	Diet	Season	Diet x Season
DMI, kg/d	7.9	8.0	0.09	7.6^b	8.2^a	0.03	0.550	<0.001	0.718
DMI, %BW	2.6	2.5	0.06	2.6^a	2.5^b	0.03	0.772	0.001	0.750
Initial BW, kg	297	300	9.6	280^b	316^a	2.9	0.707	<0.001	0.655
Final BW, kg	331	336	8.9	317^b	349^a	3.0	0.602	<0.001	0.685
Total gain, kg	34.7	35.7	1.23	37.4^a	32.9^b	0.66	0.439	<0.001	0.533
ADG, kg/d	0.77	0.75	0.050	0.84^a	0.67^b	0.016	0.696	<0.001	0.657

$^{a\text{-}c}$Means with a different letter within column for diet or season differ significantly ($P \leq 0.05$).

Table 3. Quantities of individual feed ingredients by DDGS-based and field peas-based rations by feeding period.

	Corn DDGS				Peas			
Feed ingredient	Fall	Winter	Average	Total	Fall	Winter	Average	Total
Hay (kg/d)	2.96	3.40	3.18	-	2.99	3.27	3.13	-
Silage (kg/d)	3.07	3.50	3.28	-	3.00	3.46	3.23	-
Corn (kg/d)	0.94	1.02	0.98	-	0.83	1.08	0.95	-
Supplement (kg/d)	0.22	0.25	0.24	-	0.22	0.26	0.24	-
Dry distiller grains solubles (kg/d)	0.31	0.36	**0.33**	-	0.00	0.00	0.00	-
Dry field peas (kg/d)	0.00	0.00	0.00	-	0.45	0.50	**0.48**	-
Total (kd/d)	7.50	8.53	8.01	-	7.49	8.57	8.03	-
Hay (kg/period)	132.14	161.55	146.85	293.70	141.01	152.63	146.82	293.65
Silage (kg/period)	135.73	165.61	150.67	301.34	139.33	161.43	150.38	300.76
Corn (kg/period)	39.76	48.07	43.92	87.83	36.71	50.13	43.42	86.84
Supplement (kg/period)	9.82	11.95	10.89	21.77	10.18	12.12	11.15	22.30
Dry distiller grains solubles (kg/period)	13.82	16.91	15.36	**30.72**	0.00	0.00	0.00	0.00
Dry field peas (kg/period)	0.00	0.00	0.00	0.00	21.02	23.56	22.29	**44.58**
Total (kg/period)	331.27	404.09	367.68	735.36	348.26	399.88	374.07	748.13

Table 4. Two-year average cost of feed on a ($/hd/day) and ($/hd) basis for individual feed ingredients for TMR dietary treatments for a representative heifer being developed for breeding purposes by fall, winter and total grazing periods.

	Fall		Winter		Total	
	$/hd/d	$/hd	$/hd/d	$/hd	$/hd/d	$/hd
Hay	0.32	14.61	0.36	16.80	0.66	29.91
Silage	0.13	5.68	0.14	6.76	0.27	12.07
Corn grain	0.29	12.78	0.35	16.41	0.68	29.92
Supplement	0.10	4.71	0.12	5.68	0.23	10.20
Corn DDGS	0.12	5.46	0.14	6.68	0.26	11.79
Total feed cost when DDGS was in included in the TMR	0.96	43.24	1.11	52.32	2.10	93.89
Field peas	0.20	9.35	0.22	10.48	0.42	18.68
Total feed cost when field peas were included in the TMR	1.04	47.13	1.20	56.12	2.26	100.77
Difference in cost between DDGS- and Peas-based rations	0.08	3.89	0.08	3.80	0.16	6.88

Table 5. Difference in total cost ($/hd) of field peas relative to cost of corn DDGS for alternative price ($/MT) combinations.

	Peas								
	%	**-**	**-50%**	**-30%**	**-10%**	**Base[1]**	**10%**	**30%**	**50%**
Corn DDGS	**-**	**$/MT**	**183**	**256**	**329**	**366**	**403**	**476**	**549**
	-50%	**163**	3.42	7.15	10.88	12.76	14.65	18.38	22.10
	-30%	**228**	1.07	4.79	8.52	10.41	12.29	16.02	19.75
	-10%	**293**	-1.29	2.43	6.16	8.05	9.94	13.66	17.39
	Base[1]	**325**	-2.45	1.27	5.00	6.89	8.77	11.76	16.23
	10%	**358**	-3.64	0.07	3.80	5.69	7.58	11.30	15.03
	30%	**423**	-6.01	-2.28	1.44	3.33	5.22	8.94	12.67
	50%	**488**	-8.37	-4.64	0.53	2.42	2.86	6.59	10.31

[1] Base-case net return assuming a price of \$325 MT^{-1} and \$366 MT^{-1} for corn DDGS and field peas, respectively.

Does a liquid foliar application of a mineral enhanced product improve forage production and quality of a perennial grass hay?

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Introduction

Fertilization is a common practice used to increase forage production of hay. Forage crops remove large amounts of nutrients, affecting both the future productivity of the field and the mineral balance in the animals that consume the forage. Best management practices for forage fertilization will optimize yield and quality, and minimize impacts on the environment. The International Plan Nutrition Institute (IPNI) effectively summarizes forage fertilization into four steps: Apply the right source, at the right rate, at the right time, in the right place. Primary fertilization options in the Northern Plains are manure, and N, P, and K fertilizer, with occasional micronutrient issues (Cherney and Cherney 2015).

Forage plants require essential nutrients for healthy growth. Fertilizers provide these nutrients, including nitrogen (N), phosphorus (P), and potassium (K). Without adequate nutrients, plant growth can be limited. Nitrogen fertilization generally increases both yield and crude protein content in cool and warm-season grasses. Properly fertilized forages tend to produce higher yields, which is beneficial for livestock feed. Fertilization can also reduce stored carbohydrates in plants, resulting in more succulent forage. This can positively impact palatability and digestibility for grazing animals.

Most studies show nitrogen is the most limiting nutrient, impacting forage production potential and forage quality. However, can fertilizing with minerals, specifically trace minerals increase forage production without the addition of nitrogen by enhancing soil microbial activity. Can fertilizing with minerals increase forage quality?

This study was designed to test if fertilizing with a mineral rich product can impact forage production and quality. Will adding a mineral based product enhance the existing soil microbes to convert inaccessible nitrogen to a more accessible nitrogen for plant growth? The objective of the study was to test different rates of Nutri-Graze, a mineral based product, on forage production and quality of a smooth bromegrass hay field.

Methods

Study Area

This trial was conducted at NDSU Central Grasslands Research Extension Center located near Streeter, North Dakota. Treatments were designed using a randomized block design at two locations. Block size was 30 x 120 feet and plot size were 30 x 30 feet with four replicated blocks. Site #1 (high yield producing site) will be hay land that is predominately smooth bromegrass (*Bromus inermus*) with a soils complex of Williams-Bowbells complex, deep loamy soils, and a slope of 1-2 percent. Site #2 (low yield producing site) will be hay land that is predominately meadow bromegrass (*Bromus biebersteinii*) with a soils complex of Wabek-Appam complex, gravelly sandy loam soils and a slope of one percent.

Treatments

- Control
- Nutri-Graze 1 pint per acre one-time application
- Nutri-Graze 2 pint per acre one-time application

- Treatments were foliar applied and mixed with 10 gallons of water on May 16, 2023, at the 2-3 leaf stage.
- Two sampling periods for biomass yield were collected by sub-sampling two $0.25m^2$ frames per clipping period. Both sub-samples were used for forage production, one sub-sample used for nutritional analysis. The collection dates were June 6 when the grass was in the boot stage of plant growth and June 29 when the grass was in the heading stage.
- For both sampling periods, two clipping samples were collected per plot and composited per individual replication.
- All samples were oven dried at 60° C (140° F) in a Grieve oven for 72 hours, weighed to determine forage production.
- Treatment and clipping period were tested for significance using a mixed procedure of SAS. Difference were reported at a p-value 0.05.

Results

Forage Production

Nutri-Graze was most effective at increasing forage production on the loamy, higher production site compared to the shallow loamy, low production site during both clipping periods (Table 1). There was no difference in forage production between the recommended rate (1 pt/acre) and 2x recommended rate (2 pt/acre) for both production sites at both clipping periods.

Only the 2x recommended rate increase forage production compared to the control for the low production treatment during the second clipping (Table 1). The 1 pt/acre treatment was not different ($P>0.05$) from the control at 21 and 45 days following application. The 2 pt/acre was also not different ($P>0.05$) from the control 21 days after application; however, it was different ($P\leq0.05$) from the control 45 days after application, providing 23.1 percent greater production after 45 days after application.

Forage production was higher ($P\leq0.05$) for both application rates compared to the control on the higher production site during both clipping periods (Table 1). The 1 pt/acre rate producing 23.5 and 44.3 percent greater ($P\leq0.05$) forage than the untreated control 21 and 45 days after application; respectively, on the high production site.

Table 1. Forage production of smooth bromegrass hay field by Nutri-Graze treatment at the Central Grasslands Research Extension Center near Streeter, ND in 2023.

Treatment	Low Production Soils[1]		High Production Soils[2]	
	June 6 (21 days after application)[3]	June 29 (45 days after application)[4]	June 6 (21 days after application)	June 29 (45 days after application)
	Lb/acre ± SE[5]		Lb/acre ± SE	
Control[6]	1199 ± 62.3 ^{ax}	1271 ± 30.6 ^{ax}	2623 ± 78.6 ^{ay}	2742 ± 40.2 ^{ay}
1 pt/acre	1451 ± 52.6 ^{ax}	1359 ± 73.3 ^{abx}	3239 ± 93.4 ^{by}	3956 ± 131.5 ^{bz}
2 pt/acre	1387 ± 77.9 ^{ax}	1564 ± 67.8 ^{bx}	3093 ± 67.3 ^{by}	3732 ± 82.3 ^{bz}
[1] Shallow loamy soil.				
[2] Loamy soil				
[3] Smooth brome grass in the vegetative growth stage				
[4] Smooth brome grass in the heading growth stage				
[5] Production values within columns with the same letters (a,b) are not significantly different (P>0.05).				
[5] Production values within rows with the same letters (x,y,z) are not significantly different (P>0.05).				

Economics

The economics of applying Nutri-Graze was determined based on $10 per acre for one pint/acre and $20 per acre for two pints/acre of product plus custom rate costs to apply product. Based on the North Dakota custom rate survey (USDA NASS 2023), the average custom rate to apply a liquid fertilizer is $7.38 per acre. Total cost per acre to apply Nutri-Graze was $17.38 per acre using 1 pt/acre and $27.38 per acre using 2 pt/acre.

The high production site achieved greater benefits from Nutri-Graze at 45 days following application, and was achieved at the recommended rate of 1 pint/acre. When treated with Nutri-Graze, the high production site had an increase of 1,214 lb/acre or 0.61 tons/acre. The input costs of $17.38 per acre to generate an additional 0.61 tons/acre was $28.49 per ton.

The low production site only received a positive increase in forage production 45 days following application with the 2x rate of 2 pints/acre. The increase in production was 293 lb/acre, or 0.15 tons/acre. The input costs of $27.38 per acre to generate an additional 0.15 tons/acre was $182.53 per ton.

Summary

- Forage production did not change by clipping period (plant maturation) using the 1 pt/acre treatment compared to 2 pints/acre treatment for either production levels.

- Nutri-Graze did increase forage production on the high production site by 1,214 lb/acre, or 44 percent using the recommended rate.
- Nutri-Graze applied at the recommended rate was cost-effective, with a cost per ton of added hay at $28.49 per ton.
- There was no benefit to applying 2 pt/acres compared to 1 pt/acre on the high production site; however, the 2 pt/acres increased forage production by 23 percent (292 lb/acre) on the low production during the heading growth stage, but was not cost effective.

Implications

- 1 pt/acre was the most cost-effective foliar rate on the high production site, as there was no difference in forage production between the 1 and 2 pt/acre rates.
- Only the 2 pt/acre increased forage production on the low productive site in the heading growth phase, but it wasn't cost-effective.

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Enhancing Avian Territory Quality with Heterogeneity-based Grazing Practices

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Summary

Rangelands cover almost half of the Great Plains and offer promising opportunities to enhance habitat for imperiled grassland birds. However, ninety-one percent of these rangelands lie in the hands of private landowners, many of whom manage their land for uniform utilization of forage. This reduces habitat quality by altering prey availability, brood parasite abundance, and niche availability for many grassland specialist species. Contemporary grassland bird management advocates for management practices that restore rangeland heterogeneity to improve habitat quality for grassland birds.

We examined the impacts of two heterogeneity-based and one conventional grazing practice on territory size, a proxy for territory quality, of the Western Meadowlark (*Sturnella neglecta*). We delineated territories from mid-May to early June and characterized vegetation structure and composition along 100-meter transects within each territory. We delineated a total of 38 territories across 2022 and 2023 and analyzed the direct and indirect impacts of grazing practice on habitat quality.

Kentucky bluegrass and standing litter were the only vegetation variables that positively influenced territory quality (standardized $\beta = 0.10$ and 0.06, respectively). Territory quality was negatively impacted by vegetation density and the percent cover of litter, smooth brome, and western snowberry (standardized $\beta = -0.05$, -0.09, -0.01, and -0.11, respectively). Grazing practice did not directly influence territory quality, but patch-burn grazing was the only grazing practice to consistently reduce cover of the vegetation variables that negatively impacted territory quality.

Our research suggests that rangeland management decisions have cascading and compounding effects on the wildlife that inhabit these landscapes. These unexpected indirect consequences of management decisions can complication the incorporation of conservation into working lands. Understanding the complex interaction between grazing practices, vegetation structure, and habitat quality will allow land managers to tailor management to avian conservation goals.

Introduction

Rangelands, covering approximately 45% of the Great Plains, present an excellent opportunity to improve habitat for imperiled grassland birds (Rosenberg et al. 2019; Augustine et al. 2021). However, nearly all rangelands (91%) are privately owned and managed for uniform utilization of forage (Becerra et al. 2017; Robinson et al. 2019). This management approach leads to a reduction in vegetation structure and composition variation, contributing to the widespread decline of grassland bird populations by limiting niche diversity (Sauer et al. 2013; Rosenberg et al. 2019). Contemporary rangeland management strategies for grassland birds advocate for heterogeneous vegetation structure and composition, often employing methods such as patch-burn grazing or rest-rotation grazing (Fuhlendorf and Engle 2001; Churchwell et al. 2008; Campomizzi et al. 2019; Verheijen et al. 2019; Duquette and Hovick 2020). These approaches promote vegetation variation more closely aligned with natural rangeland conditions and support a broader range of species' vegetation preferences (Fuhlendorf and Engle 2001). Understanding the direct and indirect impacts of such grazing systems on grassland bird habitat quality is essential for grassland bird conservation in working landscapes.

In the Northern Great Plains, season-long grazing and rotation grazing are common management practices (Whitt and Wallander 2022). Both methods aim to utilize forage uniformly throughout the growing season (Sedivec and Barker 1991; Fuhlendorf and Engle 2001). While season-long grazing utilizes the entire pasture continuously during the growing season, conventional rotation grazing moves cattle between two or more subunits, herein referred to as paddocks, throughout the grazing season (Briske et al. 2008; Whitt and Wallander 2022). Recent research suggests that rest-rotation grazing can be adapted to create heterogeneous pastures by varying the duration each paddock is grazed during the growing season (Duquette and Hovick 2020).

Another method to introduce heterogeneity is patch-burn grazing, which manipulates grazing patterns through prescribed fire (Fuhlendorf and Engle 2001). By strategically burning patches within pastures, cattle are drawn to regrowth in recently burned areas, reducing grazing pressure elsewhere in the pasture (Fuhlendorf et al. 2009). Each year, a new quarter of the pastures is burned until the entire pasture has been burned and the process begins again (Fuhlendorf et al. 2009). Over time, this creates a shifting gradient of vegetation structure and composition across the pasture with varying time-since-fire (Augustine and Derner 2015).

Given that grassland birds evolved in heterogeneous landscapes, restoring heterogeneity is vital for avian conservation on working landscapes (Fuhlendorf et al. 2006; Augustine and Derner 2015). Heterogeneity has been linked to increased avian diversity and species densities, as well as decreased annual turnover in the bird community (Hovick et al. 2015). It also influences prey availability (Engle et al. 2008), brood parasite abundance (Churchwell et al. 2008), and niche availability for specialist grassland species, particularly those that specialize on the extreme ends of the vegetation gradient (Coppedge et al. 2001).

While previous research has explored the impact of grazing practices and vegetation structure on avian diversity, density, and nest survival, understanding the complex, cascading effects across trophic levels requires nuanced investigation (Pillsbury et al. 2011; Hovick et al. 2012; Holcomb et al. 2014; Sandercock et al. 2015). Changes in vegetation, whether from management practices or invasive species, may have compounding impacts on wildlife, potentially stronger than previously reported. We aim to investigate how three management strategies—patch-burn grazing, a modified rotation grazing system, and traditional season-long grazing—affect avian habitat quality. We hypothesize that treatments resulting in greater heterogeneity in vegetation structure and composition will result in smaller territories (Whitaker and Warkentin 2010). Smaller territory sizes lead to adults spending less time away from the nest and more time caring for nestlings which should result in larger nestling body mass and higher nest success (Wiens et al. 1985; Whitaker and Warkentin 2010; Cooper et al. 2014; Diemer and Nocera 2014). Our objective is to quantify the direct and indirect impacts of grazing intensity on territory size, providing a deeper understanding of the relationship between grazing practices and avian habitat quality. Notably, this study will be the first to measure the cascading effects of grazing practices on avian habitat quality, adding significant insight to existing literature on the subject.

Methods

Study Area

Situated within the Missouri Coteau ecoregion and centrally located in North Dakota, the Central Grasslands Research Extension Center (CGREC) spans Kidder and Stutsman counties. The region experiences a temperate, continental climate characterized by a mean growing/grazing season (April to September, May to October, respectively) precipitation of 433 mm and temperatures of 13.6° C (NDAWN 2022).

Rangeland at CGREC is classified as Northern mixed-grass prairie, hosting an herbaceous community primarily composed of perennial, cool season (C_3) grasses such as western wheatgrass (*Pascopyrum smithii)* and green needlegrass (*Nassella viridula*). However, invasive species including Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*) have established dominance across most pastures (Patton et al. 2007; Limb et al. 2018).

The forb community is diverse and includes goldenrods (*Solidago* spp.), sages (*Artemisia* spp.), prairie coneflower (*Ratibida columnifera*), thistles (*Cirsium* spp.), as well as many other herbaceous species. Woody vegetation predominately comprises western snowberry (*Symphoricarpos occidentalis*) with scattered patches of silverberry (*Eleagnus commutata*) and wild rose (*Rosa arkansana*) (Patton et al. 2007; Limb et al. 2018).

Treatment Structure

The experiment design encompasses four 65-ha replicates, each subjected to one of three treatments: 1) season-long grazing (SLG), 2) patch-burn grazing (PBG), 3) and a modified twice over rest-rotation treatment (MTORG). Stocking rates were maintained between 1.59-2.45 Animal Unit Months/ha for all treatments to achieve a 40 to 50% degree of disappearance.

SLG represents a conventional grazing practice in North Dakota, serving as a baseline for comparison with two heterogeneity-based grazing approaches (Whitt and Wallander 2022). PBG manipulates cattle grazing patterns through the discrete application of fire in spring to a designated quarter of the pasture rather than the use of interior fencing (Coppedge and Shaw 1998; Raynor et al. 2022). In this system, one quarter of the pasture is burned during the dormant season on a 4-year fire return interval. This practice was initiated in 2017 and has been burned every year with the exception of 2022 due to weather constraints.

The MTORG system is a unique rotational grazing system which divides a pasture into four quarters, herein referenced to as paddocks, including a rested paddock (0% utilization), moderate paddock (20-40% utilization), full paddock (40-60% utilization), and heavy paddock (60+% utilization). The desired utilization is achieved by varying the number of days that a paddock is grazed throughout the growing season (0, 21, 50, and 77 days, respectively). The grazing days are split into two intervals with 40% of the days occurring during the first rotation and 60% in the second. Cattle movement within the 65-hectare pasture is constrained to each paddock (approximately 16 hectares) using interior fencing (Duquette et al., 2022). The system was established in 2018 and had completed one full cycle in 2021.

Study Species

The Western Meadowlark (*Sturnella neglecta;* WEME), a species of conservation concern in North Dakota, was selected as the focal species for this study (Dyke et al. 2015). An obligate grassland bird, the WEME exclusively relies on grasslands for its life history requirements and is found abundantly across all three treatments (Duquette and Hovick 2020; Duquette 2020).

The WEME builds an inconspicuous, domed nest on the ground with the female constructing the nest and caring for the eggs/nestlings (Baicich and Harrison 2005; Davis and Lanyon 2020). Females lay 3-7 (usually 5) eggs which are incubated for 12-15 days, and nestlings leave the nest at 10-12 days old but remain ground relatively flightless for another week (Baicich and Harrison 2005; Davis and Lanyon 2020). However, strong and nearly full-sized legs allow fledglings to leave the nest (Davis and Lanyon 2020). By studying this species, we aim to establish insights into the response of ground-nesting, obligate grassland bird management techniques while gathering crucial data on the population dynamics of a declining species.

Banding and Territory Mapping

Annual surveys were conducted to locate territorial WEME and previously banded individuals. Specific-target mist-netting with audio lures was used to capture unbanded birds with experience banders removing birds from the net and performing standard morphological measurements (weight, wing chord [length of the folded wing], rectrix length, tarsus length, and furcular cavity fat deposits) (Pyle 1997; Barg et al. 2005). WEME lack a suspended molt and other age indicators typically used post-fledging, so all birds were aged as after hatch year (in at least their second year). We used morphological measurements and behavioral observations to determine sex (Pyle 1997). Birds were banded with the standard US Fish and Wildlife Service band and a combination of 3 color bands (Ralph et al. 1993).

We created territory maps using a combination of flush- and spot-mapping techniques (Wiens 1969; Fletcher et al. 2003; Jones 2011). The initial visit was based on the location of the individual bird that was banded and the area was searched until the target bird was flushed. Upon flushing, we marked all flush and landing sites that day until we reached 10-12 locations. We repeated this over the course of 3-4 days to collect between 30-40 locations per bird (Verheijen et al. 2019). Territory mapping began at sunrise and concluded by approximately 14:00 to avoid disturbing nests during the hottest part of the day (Verheijen et al. 2019). We used the adehabitatHR package in R to generate minimum convex polygons and estimate territory area (ha) (Calenge 2006; Cooper et al. 2014; R Core Team 2023).

Vegetation Surveys

We quantified territory vegetation composition and structure using three 100-meter transects placed perpendicular to the longest axis of each territory (Figure 1). Plant communities were sampled every 10 meters starting at 0 and ending at 100 meters (11 quadrats per transect). At each sampling point, we set up a 1-m^2 quadrat and recorded cover of every plant species identified within the quadrat (Daubenmire 1959). Additionally, we took structural measurements including bare ground cover, litter cover and litter depth, thatch depth, and visual obstruction reading (VOR). Litter depth was measured in the NW corner of each quadrat and VOR was measured in each cardinal direction at a 4-meter distance with the observer 1-meter above the ground (Robel et al. 1970).

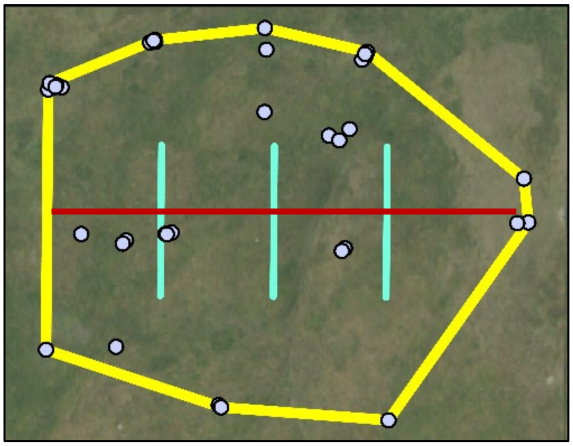

Figure 1. An example of a Western Meadowlark territory. The points represent locations the bird was observed, and the yellow line is the territory boundaries based on a minimum convex polygon. Vegetation survey transects (teal lines) were laid out perpendicular to the longest axis (red line) for each territory and spaced equidistant from each other.

Statistical Analysis

We averaged all vegetation variables for each territory and relativized them by dividing each percent cover by the total cover within that territory. Simpson's diversity index was then calculated for a territory based on the relativized cover of each species for further analysis. We assessed the direct impact of grazing practice on territory quality and vegetation variables using the glmmTMB package (Brooks et al. 2017), specifying a Gamma family with a log link function and band ID as a random effect.

Using the piecewiseSEM package (Lefcheck 2016), we constructed our initial model based on a conceptual framework (Figure 2). We specified a Gamma family with a log link function and incorporated band ID as a random effect to address repeated measures of birds that returned to the same territory between years using the glmmTMB package (Brooks et al. 2017). The goodness-of-fit of the structural equation model (SEM) was assessed using Fisher's C statistic with significant ($p < 0.05$) C values indicating that the model fails in fitting data due to missing links (Shipley 2013). We then removed non-significant pathways until a reduction in Akaike's information Criterion adjusted for (AIC) scores was no longer possible to reach the most parsimonious model (Shipley 2013). We then standardized beta coefficients by dividing them by the standard deviation of the response variable. We used these standardized coefficients to calculate cumulative effect sizes (Lefcheck 2016). Direct effects are represented by the standardized coefficients whereas indirect effects are calculated by multiplying standardized coefficients for each pathway together (Lefcheck 2016).

While a generalized linear model (GLM) can quantify direct relationships between variables, it falls short in accounting for indirect relationships. For instance, while a GLM can quantify the relationship between grazing practice and territory quality or vegetation structure and territory quality separately, it cannot simultaneously consider their combined and cascading effects on territory quality or the indirect impact of grazing practices on territory quality through changes in vegetation structure (Grace and Keeley 2006). In contrast, an SEM enables the quantification of direct and indirect effects between variables, providing a more comprehensive understanding of cascading effects.

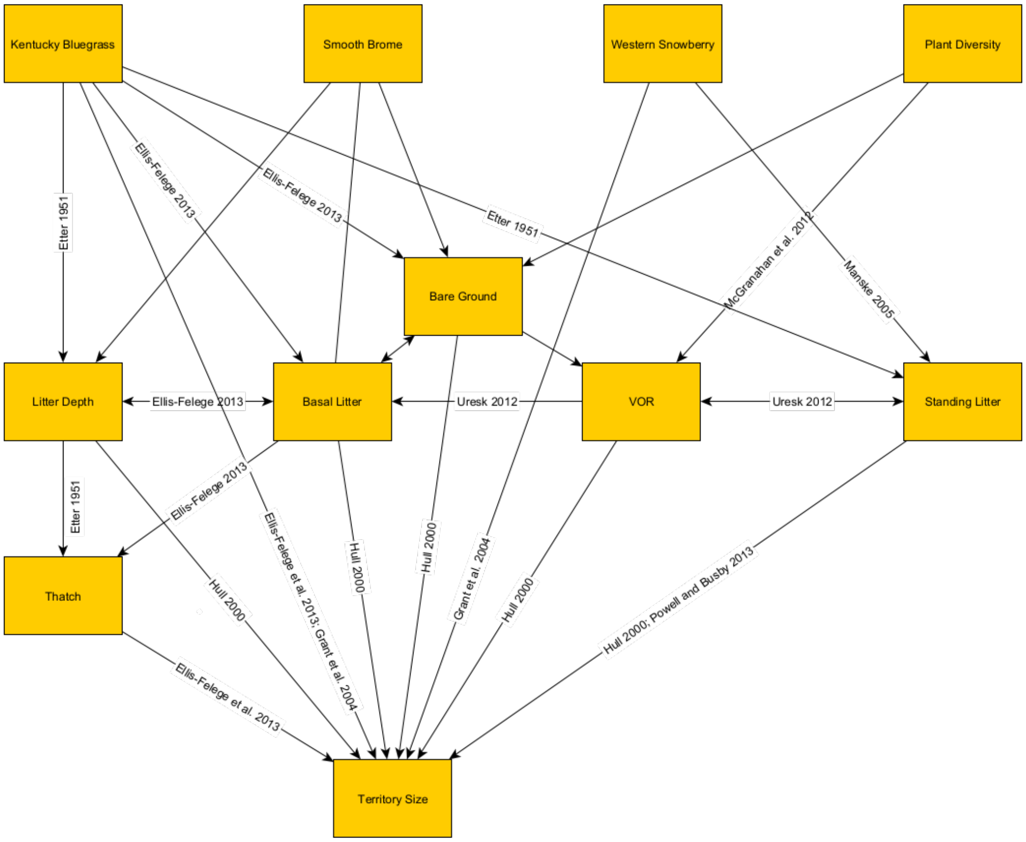

Figure 2. A conceptual model representing the hypothesized relationships between vegetation structure and territory quality. The pathways in this model are based on earlier studies as indicated by the provided citations. The conceptual model was used to guide the initial model building process (Grace et al. 2012).

Results

We delineated a total of 38 territories that ranged in size from 3.78 ha to 13.36 ha (mean = 7.22 ha). The average territory size was not significantly different between grazing practices ($p > 0.05$) however, the SLG treatment had much greater variability in territory size than either heterogeneity-based treatment (Table 1).

Table 1. Western Meadowlark territory size across season-long (SLG), patch-burn (PBG), and modified twice-over rest-rotation (MTORG) grazing across 2022 and 2023 at the Central Grasslands Research Extension Center. There was no significant difference in average territory size among grazing practices, but SLG had more variable territory sizes than either heterogeneity-based grazing practice.

Grazing Practice	Territory Count	Average Size	Standard Deviation
SLG	12	7.37	3.22
PBG	13	7.17	2.10
MTORG	13	7.14	2.53

The final structural equation model included 8 vegetation variables that directly or indirectly impacted territory quality. Vegetation density, measured with VOR, was the only variable to directly impact territory quality with increasing VOR resulting in reduced territory quality. The only two variables to positively impact territory quality were Kentucky bluegrass and standing litter (standardized $\beta = 0.10$, 0.06, respectively; (Figure 4). Territory quality was negatively impacted by vegetation density and the percent /cover of litter, smooth brome, and western snowberry (standardized $\beta = -0.05$, -0.09, -0.01, and -0.11, respectively; (Figure 4).

While grazing regime did not directly impact territory quality, it did alter vegetation variables associated with territory quality. Litter cover was significantly higher in the MTORG than PBG, but SLG was not significantly different from either. Smooth brome cover was significantly higher in SLG than either heterogeneity-based grazing practice. Lastly, western snowberry was significantly higher in MTORG than either SLG or PBG (Figure 4).

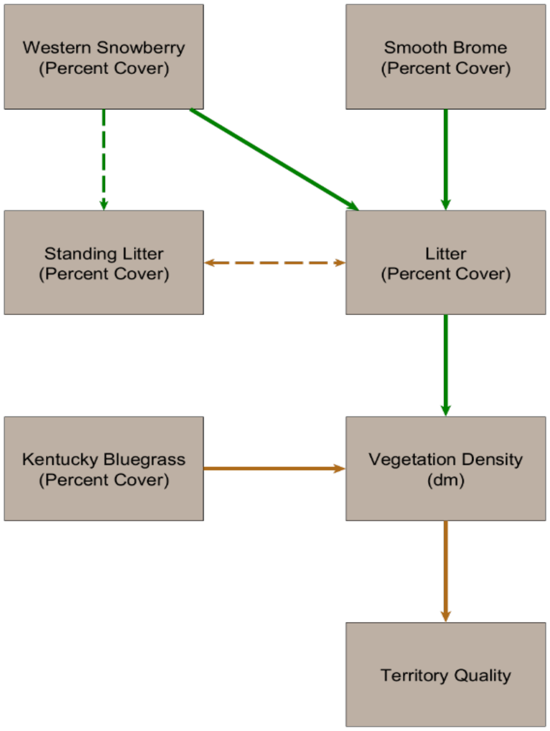

Figure 3. The final structural equation model showing the impact of vegetation variables on territory quality. Brown lines indicate a negative impact on territory quality and green lines indicate a positive impact. Significant relationships ($p < 0.05$) are indicated by a solid line and marginally non-significant relationships ($p < 0.10$) are indicated by dashed lines. Only eight of the original ten variables had significant ($p < 0.05$) or marginally non-significant ($p < 0.10$) impacts on territory quality.

Figure 4. The cumulative effect sizes for each vegetation variable on Western Meadowlark territory quality in the final SEM. Brown boxplots represent a net negative effect and green boxplots represent a net positive effect. Vegetation density was measured with a visual obstruction reading while all other measurements were relativized percent cover. Kentucky bluegrass and standing litter cover were the only two variables that positively impacted territory quality.

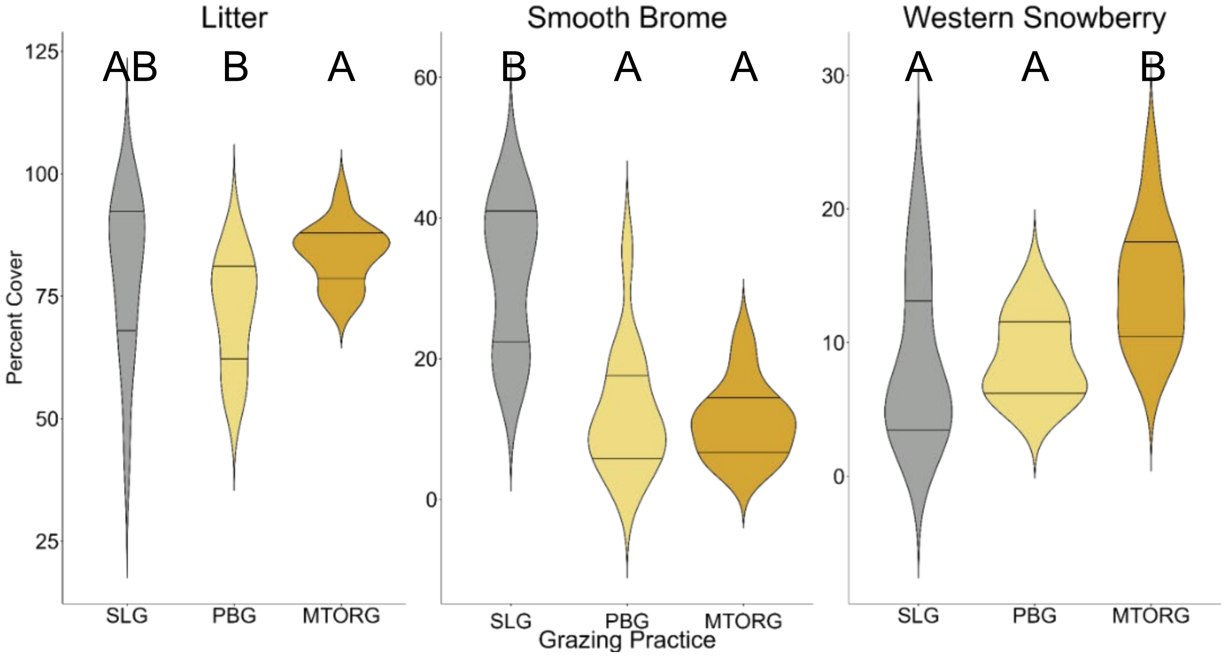

Figure 5. The impact of grazing practice on variables that impacted Western Meadowlark territory quality. Only variables with a significant difference between grazing practices are shown. While grazing practice did not significantly impact territory size, it did alter vegetation variables impacting territory quality. Patch-burn grazing consistently reduced cover of vegetation variables that negatively impacted territory quality.

Discussion

We found that grazing practice did not directly impact territory quality, rather it has an indirect impact through changes in vegetation composition and structure. Notably, season-long grazing showed greater variability in territory quality than either heterogeneity-based grazing practice. This may indicate small patches of high-quality habitat exist within traditionally grazed pastures. Prolonged season-long grazing can promote the development of static "grazing lawns" where cattle repeatedly graze the same areas year-after-year, resulting in patches of short-stature vegetation (McNaughton 1984). These grazing lawns may provide fine-scale heterogeneity which may only benefit a small portion of the breeding population, resulting in greater variation in territory sizes.

Changes in vegetation structure played a significant role in determining territory quality. Kentucky bluegrass and standing litter were the only vegetation variables positively correlated with territory quality, likely because of the structure they provide. The thick mat of litter produced by Kentucky bluegrass offers abundant resources for female WEME to weave their nests from (Toledo et al. 2014; Davis and Lanyon 2020). Moreover, standing litter may serve dual purposes as it often functions as song perches for WEME, facilitating mate attraction and aiding in territorial defense against rival birds (Davis and Lanyon 2020).

In contrast, the density of vegetation and the cover of western snowberry, litter, and smooth brome were negatively correlated with territory quality. Previous studies have shown that WEME are sensitive to woody vegetation (Grant et al. 2004), possibly due to heightened nest predator activity (Klug et al. 2010). This may work in tandem with increased vegetation density and litter cover which can restrict the mobility of recently fledged young that primarily rely on strong legs to escape predators (Doxon and Carroll 2010; Davis and Lanyon 2020). Smooth brome impacted territory quality through changes in litter cover, both of which increase without adequate disturbance (Grant et al. 2004). In fact, all four vegetation variables that negatively impacted territory quality increase as the frequency and intensity of disturbance decreases (Grant et al. 2004).

Although grazing practice did not have a direct effect on territory quality, it exerted an indirect influence by modifying vegetation structure and composition. Specifically, all grazing practices led to a reduction in at least one of several vegetation variables that adversely affected territory quality. These findings underscore the importance of adequate disturbance in enhancing grassland bird habitat as all of the vegetation variables that negatively impacted territory quality typically increase with rest (Grant et al. 2004). However, not all grazing practices yielded the same level of habitat improvement. For instance, PBG consistently showed reduced cover of negative vegetation variables whereas MTORG and SLG only exhibited decreased cover of some of these variables. These results highlight the importance of considering the indirect and cascading effects that grazing practices can have on the wildlife that inhabit these landscapes. Understanding the complex interaction between grazing practices, vegetation structure, and habitat quality will allow land managers to more effectively tailor management to avian conservation goals.

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Avian Nesting Survival in a Heterogeneity-based Rotation Grazing System

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Summary

Traditional grazing management focuses on maximizing cattle production through uniform forage utilization, resulting in reduced variation in vegetation structure and composition. This homogenization decreases avian niche diversity, contributing to ongoing declines in grassland bird communities. Recoupling fire and grazing can restore rangeland heterogeneity but limited regional knowledge and resources for prescribed fire prevent widespread application across the Great Plains. This suggests a need for innovative grazing practices that utilize existing infrastructure to restore heterogeneity.

In 2018, we established a modified rotation grazing system that varies grazing intensity to create heterogeneity across paddocks. Our treatment structure includes four replicates, each split into four paddocks based on the degree of disappearance (DoD): heavy (60+%), full (40-60%), moderate (20-40%), and rest (0%). We assessed the efficacy of this system in creating heterogeneity and the subsequent impacts on grassland birds by quantifying grazing intensity impacts on 1) vegetation structure, 2) avian community composition, and 3) nest survival. We conducted vegetation sampling to quantify vegetation structure and rope dragging to locate nests within paddocks. Nests were subsequently monitored to determine fate. We incorporated vegetation structure and composition measurements taken at each nest into a hierarchical modeling scheme using RMark to assess nest survival.

We found that modifying grazing intensity within a pasture creates heterogeneity in litter depth across years, but changes in vegetation density were less consistent. Changes in the nesting community between grazing intensity were also only seen in 2021 ($p < 0.05$). However, we found that nest survival of four of the eight species was impacted either directly by grazing (Red-winged Blackbird) or indirectly by changes in vegetation structure associated with grazing (Northern Pintail, Blue-winged Teal, Brewer's Blackbird). Additionally, nesting density of the Gadwall was significantly higher in the moderately grazed paddock than the heavily grazed paddock and densities of both the Gadwall and Clay-colored Sparrow were indirectly impacted through changes in litter depth. Responses to grazing and vegetation variables were species-specific, reflecting the importance of heterogeneity when managing for diverse grassland birds.

Our results demonstrate the potential for an alternative grazing practice to restore heterogeneity and improve grassland bird conservation. We recommend land managers incorporate grazing strategies that promote heterogeneity to benefit grassland birds.

Introduction

Historically, fire and grazing shaped North American grasslands and avian communities by creating a shifting mosaic of vegetation structure and composition (Fuhlendorf & Engle, 2001). In contrast, current rangeland management focuses on uniform forage utilization to maximize cattle production, resulting in reduced variation in vegetation structure and composition (Becerra et al., 2017; Fuhlendorf et al., 2009). Homogenization of rangelands decreases avian niche diversity, contributing to ongoing grassland bird declines (Rosenberg et al., 2019; Sauer et al., 2013). Since grassland birds evolved in heterogeneous landscapes shaped by the interaction of large herbivores and fire, restoring vegetation structural variability in rangelands is a crucial part of avian conservation on working landscapes (Christensen, 1997; Fuhlendorf & Engle, 2001; Ostfeld et al., 1997; Wiens, 1997).

Heterogeneity-based management practices can blend the needs of conservation while simultaneously promoting cattle production (Fuhlendorf et al., 2006). Management focused on creating heterogeneous vegetation structure can increase avian prey availability, decrease brood parasite abundance, and increase habitat for specialist grassland bird species (Churchwell et al., 2008; Coppedge et al., 2001; Engle et al., 2008). Diversity in vegetation structure is especially beneficial for grassland birds that rely on the far ends of the vegetation structural gradient. For example, the upland sandpiper relies on dense vegetation for nesting but forages in open patches (Sandercock et al., 2015). Additionally, species such as the Chestnut-collared Longspur and Northern Pintail rely on the sparser end of the vegetation spectrum whereas Le Conte's Sparrows can be found at the denser end (Beauchamp et al., 1996; Davis et al., 1999; Hovick et al., 2014).

Patch-burn grazing has emerged as an effective method for achieving heterogeneity in rangelands (Duchardt et al., 2016; Fuhlendorf & Engle, 2001; McNew et al., 2015). This management practice relies on the patchy application of fire within a pasture to manipulate the grazing patterns of livestock (Fuhlendorf et al., 2009). However, many land managers in the northern Great Plains are hesitant to implement fire for reasons ranging from a lack of tools to a cultural aversion (Clark et al., 2022a, 2022b; Sliwinski et al., 2018). Reluctance to use prescribed fire creates a need for innovative methods to promote grassland heterogeneity for the conservation of declining grassland birds.

A preliminary study has demonstrated that twice-over rest-rotation grazing, a widespread practice in north Dakota, can be modified to create heterogeneity in vegetation structure (Duquette et al., 2022; Whitt & Wallander, 2022). The modification creates heterogeneity by altering the number of days that a paddock is grazed to achieve different grazing intensities and maximize conservation potential (Duquette et al., 2022). Varying grazing intensities creates a vegetation structural gradient that will alter grassland bird species composition, specifically benefiting specialist grassland species at the extreme ends of the structural gradient (Coppedge et al., 2008; Holcomb et al., 2014; Pillsbury et al., 2011). Moreover, utilizing grazing systems common in the Northern Great Plains may expedite adoption by landowners using twice-over rotational grazing because it requires minimal changes to the current grazing practice.

We hypothesize that avian nesting communities, nest survival, and nest densities will vary across paddocks, with species exhibiting preferences for different types of vegetation structure. Nesting density should be lowest at the extreme ends of this structural gradient because relatively few specialized species, including species of special concern like the chestnut-collared longspur, rely on short vegetation structure (Churchwell et al., 2008). Our objectives are to assess the efficacy of this system in achieving heterogeneity and the subsequent impacts on grassland birds by quantifying grazing intensity impacts on 1) vegetation structure, 2) avian nesting community composition, and 3) nest survival and density.

Methods

Study Area

The Central Grasslands Research Extension Center (CGREC) is located in the Missouri Coteau ecoregion and the central part of North Dakota along the border of Kidder and Stutsman counties. The study area has a temperate, continental climate with an average growing/grazing season (April to September, May to October, respectively) precipitation of 433 mm and average growing season temperatures of 13.6° C (NDAWN, 2022). However, 2021 was a drought year with above-average temperatures (15.4° C) and below-average precipitation (333 mm). Temperatures were high but within the normal range in 2022 and high early in the season in 2023 but dropped closer to normal temperatures later in the season (13.8 and 14.3° C, respectively). Precipitation was lower than normal in both 2022 and 2023 (343 and385 mm, respectively; Figure 6).

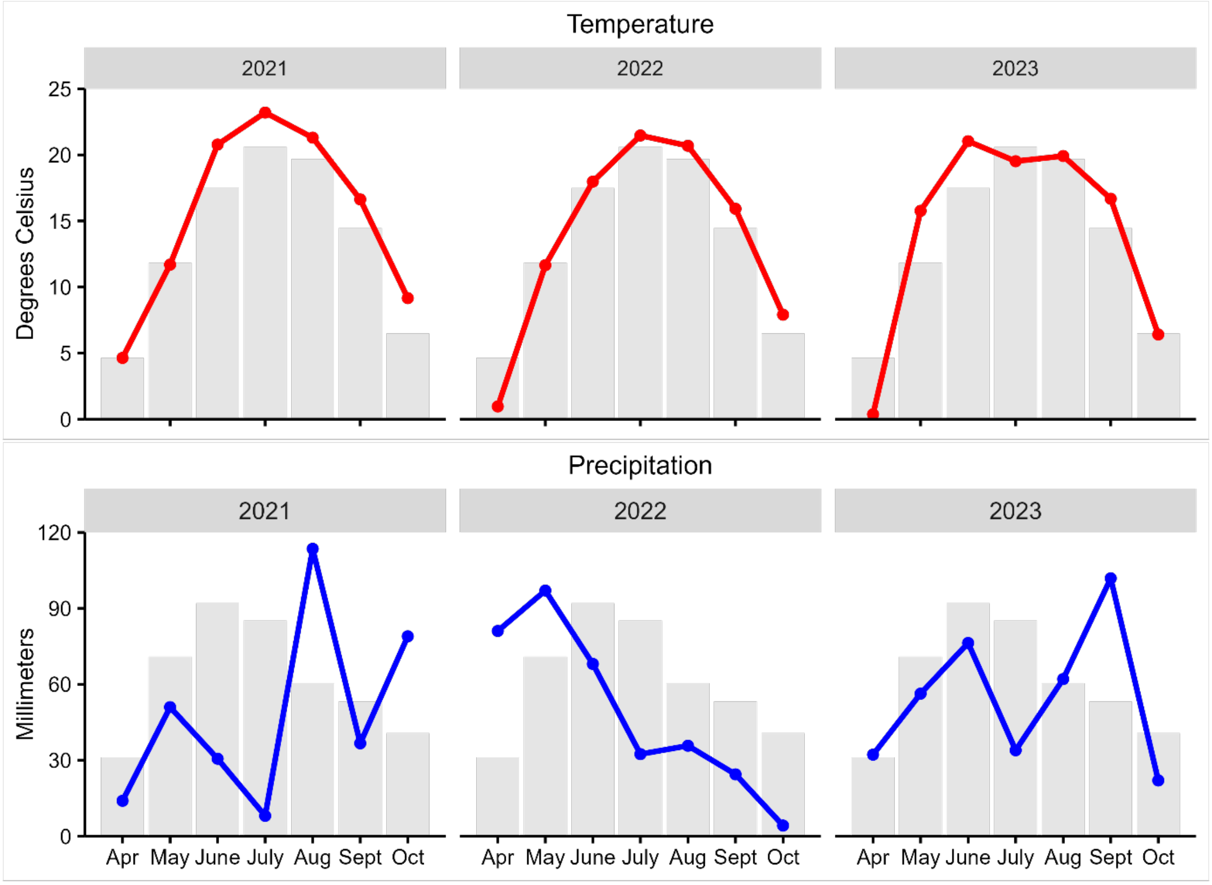

Figure 6. Growing season temperature (top) and precipitation (bottom) from the Streeter North Dakota Agricultural Weather Network (NDAWN) accessed on February 4, 2023. The colored lines represent the conditions within each year and the gray bars are the 30-year average.

Rangeland at CGREC is classified as Northern mixed-grass prairie, with an herbaceous community comprised of perennial, cool season (C_3) grasses including western wheatgrass (*Pascopyrum smithii)* and green needlegrass (*Nassella viridula*), although invasive plants including Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*) have become dominant in most of the paddocks (Limb et al., 2018; Patton et al., 2007).

The forb community is diverse and includes goldenrods (*Solidago* spp.), sages (*Artemisia* spp.), prairie coneflower (*Ratibida columnifera*), thistles (*Cirsium* spp.), as well as many other herbaceous species. The woody vegetation is predominately western snowberry (*Symphoricarpos occidentalis*) with patches of silverberry (*Eleagnus commutata*) and wild rose (*Rosa arkansana*) (Limb et al., 2018; Patton et al., 2007).

Treatment Structure

The modified twice-over rest-rotation grazing (MTORG) system is designed to create heterogeneity in vegetation structure by varying grazing intensities within a pasture. The study system includes four replicates with each replicate split into quarters, herein referenced to as paddocks, based on degree of disappearance. The four paddocks within each of the four experimental replicates and their degree of disappearance are as follows: rested (0%), moderate (20-40%), full (40-60%), and heavy (60+%). Throughout the treatment, a moderate stocking rate (5 year average = 2.34 AUM/ha) has been used with varied lengths of grazing periods to achieve the desired degree of disappearance (Duquette et al., 2022).

Grazing intensity rotates each year, with the rested paddock becoming the moderate paddock, the moderate to full, the full to heavy, and the heavy to rest. Cattle movement within the 65-hectare pasture is constrained to each paddock (approximately 16 hectares) using interior fencing (Duquette et al., 2022). The system was established in 2018 and had completed one full cycle in 2021.

Vegetation Surveys

We measured vegetation structure within each grazing intensity using three 25-m transects in each paddock. Vegetation measurements were taken at 0, 12.5, and 25 m along each transect. Structural measurements included visual obstruction readings (VOR) and litter depth.

We determined VOR using the height at which a Robel pole was 50% obscured at a 4-m distance with the viewer's eye-level 1 m above the ground (Robel et al., 1970). This measurement was taken in each cardinal direction and averaged together for each location along the transect. Litter depth was measured in the northwestern corner of a 0.5-m x 1-m quadrat centered at each transect location (Daubenmire, 1959; Duquette et al., 2019). Vegetation measurements were taken at the end of the breeding season during the first two weeks of August.

Nest Searching/Monitoring

We searched for nests by dragging a 30-m rope with aluminum can bundles attached every 3 m (Winter et al., 2003). As we surveyed each paddock, we placed pin flags marking one end of the rope at approximately 50-m intervals and used global position system (GPS) tracks to ensure complete coverage of the paddocks (Hovick et al., 2012). We repeated this process from 20 May to mid-July between 0530 to 1100 in the morning with each paddock searched four times in 2021, and three times in 2022 and 2023.

When a bird flushed from the rope, we identified the species and began searching for the nest. We recorded nest locations with a GPS and placed flags approximately 5 m to the north and south of the nest and low in the vegetation to prevent trampling and avoid attracting predators

(Winter et al., 2003). If we were unable to locate the nest but observed secondary indicators (chipping, broken wing display, adults nearby), we marked the approximate location on a GPS and searched again within three days (Hovick et al., 2012; Shew et al., 2019).

We candled two representative eggs from each nest to determine the age and subsequently monitored nests every 2-4 days (Johnson & Temple, 1990; Lokemoen & Koford, 1996). We recorded the nesting stage (laying, incubating, nestling), number of host eggs, and number of Brown-headed Cowbird (*Molothrus ater*) eggs (a common brood parasite) during each monitoring event (Johnson & Temple, 1990).

We continued monitoring until the nest was fledged, depredated, or abandoned (Hovick et al., 2012; Winter et al., 2005). We considered nests successful if they fledged at least one conspecific individual (Hovick et al., 2012; Shew et al., 2019). We confirmed fledging by resighting a fledgling or using adult behavioral indicators such as nearby adults appearing agitated/chipping or adults carrying food to a nearby area (Duquette et al., 2019; Hovick et al., 2012; Shew et al., 2019). Nests that lacked these indicators or that were clearly disturbed, such as being ripped from the vegetation or trampled, were considered failures.

We calculated expected fledge dates using known incubation and nestling periods as well as the current age of the nest, which was determined upon discovery using the candling method described above. Collecting vegetation data on actual or expected fledging dates standardizes vegetation data collection and prevents bias by collecting data, regardless of the outcome, at a standardized time relative to nest completion for each nest (McConnell et al., 2017).

We measured vegetation using a 0.5-m^2 quadrat centered on the nest bowl. Vegetation was assessed based on functional groups using the midpoint of the following cover classes: 0%, 1-4%, 5-24%, 25-49%, 50-74%, 75-95%, and 95-100% (Duquette, 2020). We split Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*) from the other grasses due to their unique structure and invasiveness (Duquette, 2020). Structural measurements included VOR, vegetation height, and litter depth.

Analysis

All statistical analyses were conducted within R version 4.3.2 (R Core Team, 2023). The glmmTMB package was used to create generalized linear mixed-effects models comparing vegetation structure in each grazing intensity (Brooks et al., 2017). We controlled for non-independence while maximizing sampling points by incorporating transect ID nested within pasture as a random effect.

Avian species were divided into functional groups based on habitat preferences. Obligate grassland birds rely exclusively on grasslands for their life history, whereas facultative grassland birds rely on grasslands in conjunction with other habitat types (Vickery et al., 1999). Wetland species are those that rely on wetlands for much of their life history. Nonmetric multidimensional scaling with Bray-Curtis dissimilarity was used with the vegan package to visualize differences in avian community composition across each of the grazing intensities within the MTORG system (Oksanen et al., 2022). PerMANOVA was used to determine any statistical difference in the nesting communities (Oksanen et al., 2022).

Nest survival was analyzed using the RMark interface, which utilizes a maximum-likelihood estimator and logit function to calculate daily survival rates (DSR) (Dinsmore et al., 2002;

Laake, 2013). We only included species with more than 30 nests in our analysis (C. Duquette & Hovick, 2020). We assessed grazing intensity impacts on DSR using a hierarchical modeling scheme with the top models from each model set included in the subsequent model set (Burnham & Anderson, 2004; Dinsmore & Dinsmore, 2007; Hovick et al., 2012; Winter et al., 2006). We assessed management and vegetation impacts on nest survival in four steps: 1) temporal impacts, 2) biological impacts, 3) treatment impacts and 4) nest site vegetation (Table 2). Top models were based on Akaike's information criterion with the MuMIn package, adjusted for a small sample size, and carried over into the next model set (AIC_c; Bartoń, 2023; Burnham & Anderson, 2004).

We calculated nest densities in each paddock using the following formula:

$$Density = \frac{N}{DSR^{a} * A}$$

Table 2. We used a four-step hierarchical modeling scheme to assess nest survival for all birds with more than 30 nests. The steps included temporal, biological, grazing, and nest site vegetation covariates. Brown-headed Cowbird (BHCO) parasitism covariates were only included in passerine models. At each step, models were assessed AIC_c where competing models were within 2Δ AIC. The top model in each step was carried forward into the next model stage.

Step	Covariates
1. Temporal	Null
	Year
	Julian Day (linear
2. Biological	Number of BHCO eggs/nestlings
	Presence of BHCO eggs/nestlings
	Nest Age
	Nest Stage
3. Grazing	Days a nest experienced grazing
	Grazing presence/absence
	Previous years degree of disappearance
4. Nest Site Vegetation	Kentucky Bluegrass
	Smooth Brome
	Litter Cover
	Bare Ground Cover
	Forb Cover
	Grasslike Cover
	Woody Cover
	Litter Depth (mm)
	Vegetation Height (mm)
	Visual Obstruction Reading (dm)

Where *N* represents the number of nests found, *DSR* is the constant daily survival rate calculated within RMark, *a* is the average age (days) of nests when first found, and *A* is the area of each paddock in hectares (Arnold et al. 2007; Duquette 2020). This formula corrects for apparent nesting densities by accounting for nests that failed before they were able to be discovered. Nesting densities were then compared between treatments using a Wilcoxon rank sum test with a Bonferroni correction for multiple comparisons (Hollander & Wolfe, 1973; Saville, 1990). We tested the impact of vegetation structure at the paddock scale on nesting densities using the glmmTMB package replicate nested within year as a random effect (Brooks et al., 2017). Given the nature of our response variable and the potential for excess zeros, we utilized a zero-inflated gamma distribution to account for both over dispersion and excess zeros in the data. Zero-inflated models include both a conditional model and zero-inflated model. The conditional model quantifies the relationship between the response variable and the explanatory variable while accounting for random effects. The zero-inflated model quantifies the probability of observing more zero counts in the data than would be expected under a standard distribution (Brooks et al., 2017).

Results

Objective 1: Vegetation Structure Changes

We did not see a consistent trend in vegetation density across years. In 2021, a drought year, the heavily grazed paddock was significantly ($p = 0.007$) lower than all other paddocks with no other significant differences. In 2022, full and heavily grazed paddocks were significantly ($p < 0.0001$) lower than rested and moderate paddocks. However, in 2023 we did not see a significant difference between any paddocks ($p > 0.05$; Figure 7).

Litter depth followed a lag effect with the moderate paddock (rested in the previous year) having higher litter depth than the heavily grazed ($p < 0.0001$) and rested paddock ($p = 0.0006$) in all years except 2023 where moderate was only significantly higher than heavy ($p = 0.02$). The full intensity paddock (moderate in previous years) was only significantly ($p = 0.047$) higher than the heavy in 2021.

Objective 2: Avian Nesting Community Composition

We found a total of 1,229 nests across the three years of the study. This includes a total of 8 obligate and 17 facultative grassland birds as well as 7 wetland-associated birds (Table 3). Avian breeding communities were significantly different between full and heavy grazing intensities in 2021 (PERMANVOA, $p = 0.03$) but no other years showed a significant difference between communities (Figure 8).

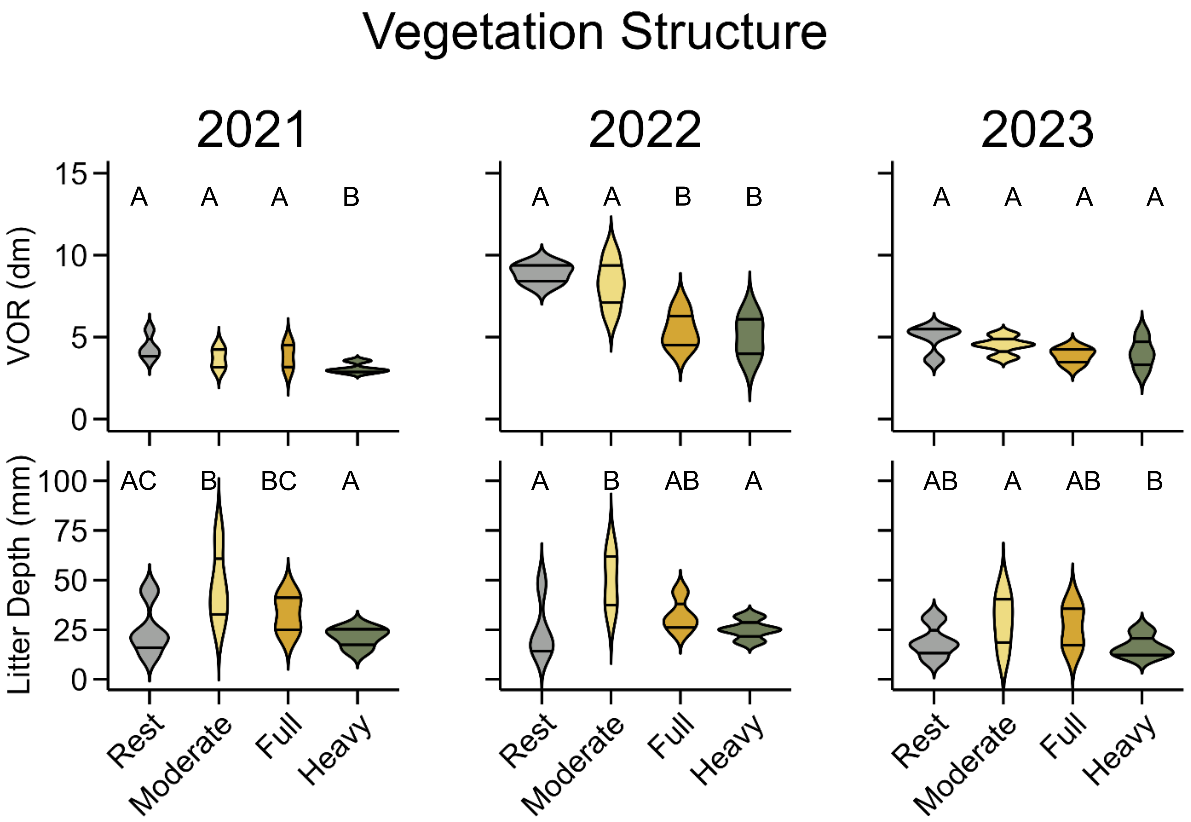

Figure 7. Violin plots showing two vegetation structural measurements taken in a modified twice-over rest-rotation grazing system at the Central Grasslands Research Extension Center in North Dakota. Measurements included litter depth and visual obstruction reading (VOR), a measure of vegetation density, and were taken in August 2021, 2022, and 2023 within four replicates of each grazing intensity. Colors represent each grazing intensity, and the shape indicates the distribution of values where wider sections represent the accumulation of data points. Same letters indicate no statistical difference (P>0.05) among grazing intensities within a year.

Table 3. Summary of nest data with American Birding Association (ABA) codes collected at the Central Grasslands Research Extension Center from mid-May to mid-July 2021, 2022, and 2023. Rest, moderate, full, and heavy correspond to grazing intensities within a modified twice-over rest-rotation grazing system. Obligate (OBL) grassland species rely exclusively on grassland for their life history. Facultative (FAC) grassland species may use grassland habitats in conjunction with other habitats. Wetland (WET) species are those that nest within wetlands.

Species	ABA	Group	Rest	Moderate	Full	Heavy	Totals
Pied-billed Grebe	PBGR	WET	1	0	2	2	5
American Bittern	AMBI	FAC	0	4	0	0	4
Mallard	MALL	FAC	1	19	7	4	31
Gadwall	GADW	FAC	18	30	21	9	78
Northern Pintail	NOPI	FAC	14	27	11	7	59
American Wigeon	AMWI	FAC	0	2	2	1	5

Northern Shoveler	NSHO	FAC	3	12	10	1	26
Blue-winged Teal	BWTE	FAC	26	30	33	23	112
Green-winged Teal	GWTE	FAC	1	3	0	2	6
Lesser Scaup	LESC	FAC	0	0	1	0	1
Sharp-tailed Grouse	STGR	OBL	0	3	4	1	8
American Coot	AMCO	WET	1	2	4	11	18
Sora	SORA	WET	1	1	2	2	6
Killdeer	KILL	FAC	2	0	0	1	3
Willet	WILL	FAC	3	1	0	0	4
Upland Sandpiper	UPSA	OBL	2	2	0	0	4
Wilson's Snipe	WISN	WET	1	0	1	1	3
Wilson's Phalarope	WIPH	WET	1	0	3	1	5
Mourning Dove	MODO	FAC	11	17	10	12	50
Eastern Kingbird	EAKI	FAC	6	4	4	0	14
Marsh Wren	MAWR	WET	0	0	1	1	2
Dickcissel	DICK	OBL	4	4	3	0	11
Clay-colored Sparrow	CCSP	FAC	85	152	150	96	483
Grasshopper Sparrow	GRSP	OBL	2	2	2	6	12
Savannah Sparrow	SAVS	OBL	1	0	1	1	3
Chestnut-collared Longspur	CCLO	OBL	4	4	1	3	12
Western Meadowlark	WEME	OBL	26	17	21	12	76
Bobolink	BOBO	OBL	3	6	3	0	12
Yellow-headed Blackbird	YHBL	WET	5	1	0	12	18
Red-winged Blackbird	RWBL	FAC	30	32	35	22	119
Brewer's Blackbird	BRBL	FAC	11	17	7	3	38
Common Grackle	COGR	FAC	0	1	0	0	1
Totals			263	393	339	234	1229

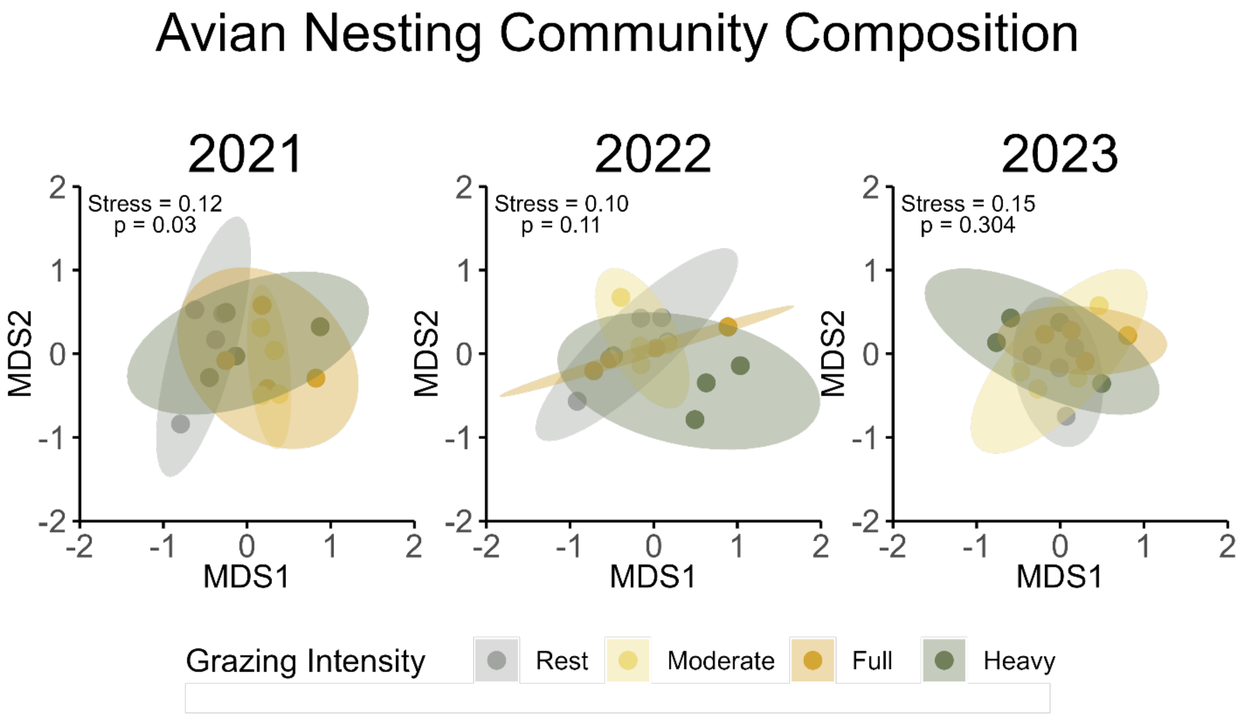

Figure 8. Non-metric multidimensional ordinations comparing avian nesting communities within a modified twice-over rest-rotation grazing system at the Central Grasslands Research Extension Center. Avian communities were assessed from mid-May to mid-July in 2021 and 2022. Points represent avian communities within each replicate where closer points are more similar. Ellipses show 95% confidence intervals. Nesting communities were significantly different between heavy and full in 2021 but no significant differences were seen in 2022 or 2023.

Objective 3: Avian Nest Survival

We were able to assess daily nest survival metrics on every species with more than 30 total nests (eight species, Table 4). Species that included year or stage in the top model have daily survival rates for each grouping variable. The average daily survival rate across the breeding season is reported for models that included a temporal covariate (Table 4). For all species, nest survival was consistently highest in 2022, and passerines consistently had higher nest survival in the nestling stage than the incubation stage (Table 4).

Gadwall (*Mareca strepera*) nest survival varied across years with higher nest survival in 2021 and 2022 than 2023 (0.97, 0.98, 0.93, respectively). This corresponds with a 0.44, 0.57, and 0.14 period survival rate, respectively. Daily survival rate increased with forb cover but decreased with increasing nest age. Gadwall nesting densities were significantly higher in the moderate paddock than the heavy paddock which closely mirrored the pattern seen in litter cover between grazing intensities (Figure 9). Additionally, while litter depth did not impact variability in density (*p* = 0.78), it did significantly impact the probability of occurrence (*p* = 0.02). This suggests that as litter depth increased, the likelihood of observing excess zero values in nesting density decreased significantly (Figure 9). The impact of visual obstruction reading was only marginally non-significant (*p* = 0.09).

Table 4. Daily survival rates (DSRs) and final hierarchical model coefficients for avian nesting data collected at the Central Grasslands Research Extension Center from mid-May to mid-July in 2021 and 2022. DSRs represent the probability that a nest survives from one day to the next. Yearly DSRs are included for all species. Stage specific DSR is only included for passerines. Coefficient directionality is represented by a + or - with parentheses indicating 85% confidence limits that overlap 0. Only species with greater than 30 nests between the two years were included in the nest survival analysis.

Species	Sample Size	Constant Daily Survival Rate	Year Daily Survival Rate	Stage Daily Survival Rate	Top Model Covariates
Gadwall	76	0.96	2021: 0.97 2022: 0.98 2023: 0.93	N/A	Year Nest Age - Forb Cover +
Northern Pintail	57	0.93	2021: 0.92 2022: 0.94 2023: 0.94	N/A	VOR +
Blue-winged Teal	103	0.96	2021: 0.97 2022: 0.97 2023: 0.94	N/A	(Year) Nest Age - Veg Height +
Mourning Dove	49	0.93	2021: 0.94 2022: 0.95 2023: 0.81	Incubating: 0.81 Nestling: 0.97	Julian Day Quadratic - Stage + Kentucky Bluegrass +
Clay-colored Sparrow	467	0.89	2021: 0.87 2022: 0.91 2023: 0.91	Incubating: 0.80 Nestling: 0.94	Year Stage +
Western Meadowlark	71	0.92	2021: 0.91 2022: 0.93 2023: 0.90	Incubating: 0.83 Nestling: 0.95	Stage +
Red-winged Blackbird	112	0.95	2021: 0.92 2022: 0.96 2023: 0.94	Incubating: 0.85 Nestling: 0.97	Julian Day Quadratic - Stage + Days Grazed +
Brewer's Blackbird	37	0.94	2021: 0.92 2022: 0.99 2023: 0.95	Incubating: 0.82 Nestling: 0.97	(Year) Stage + Litter Cover -

Northern Pintail (*Anas acuta*) did not include year in the top model and constant nest survival was 0.93. This corresponds with a 0.16 period survival rate. The nest daily survival rate decreased with increasing litter cover at the nest site. There was no significant difference in nesting densities between grazing intensities although densities were slightly higher in the moderate paddock (Figure 9). Nesting density was not significantly impacted by vegetation structure, but visual obstruction reading was only marginally non-significant (p = 0.058).

Blue-winged Teal (*Anas discors*) nest survival varied across years with survival decreasing from 2021 to 2023 (0.97, 0.97, 0.94, respectively). This corresponds with a 0.48, 0.48, and 0.23 period survival rate, respectively. Blue-winged teal nest daily survival increased with vegetation height but decreased with increasing nest age. There was no significant difference in nesting densities between grazing intensities although densities were slightly higher in the full paddock (Figure 9). Nesting density was not significantly impacted by vegetation structure, but visual obstruction reading was only marginally non-significant (p = 0.052).

Mourning Dove (*Zenaida macroura*) nest survival did not include year but did include nest stage. Survival was higher in the nestling stage (0.97) than the incubation stage (0.81). This corresponds with a period survival rate of 0.04 for incubation and 0.63 for nestlings. Nest survival was highest at the beginning and end of the season for both stages. Increasing Kentucky bluegrass cover at the nest site improved nest survival. Mourning Dove nest survival varied across time with higher daily survival rates at the beginning and end of the breeding season. There was no significant difference in nest densities although densities showed an increase with increasing grazing intensity (Figure 9). Nesting density was not significantly impacted by vegetation structure.

Clay-colored Sparrow (*Spizella pallida*) nest survival varied with both year and stage. Nest survival improved across years (0.87, 0.91, 0.91 respectively). This corresponds with a period survival of 0.04, 0.11, and 0.11, respectively. Nest survival was higher in the nestling stage (0.94) than the incubation (0.80). These correspond to a period survival rate of 0.04 for incubation and 0.57 for nestlings. There was no significant difference in nesting densities between grazing intensities although densities were slightly higher in the moderate and full paddocks (Figure 9). However, nesting densities were positively correlated with litter depth (p = 0.01) with both occurring at their highest in the moderate paddock and the lowest in the rested paddock.

Western Meadowlark (*Sturnella neglecta*) did not include year in the top model but did include nest stage. Nest survival was higher in the nestling (0.95) than the incubation stage (0.83). This corresponds with a period survival of 0.06 for incubation and 0.54 for nestlings. There was no significant difference in nesting densities between grazing intensities although densities showed a decrease with increasing grazing intensity (Figure 9). Nesting density was not significantly impacted by vegetation structure.

Red-winged Blackbird (*Agelaius phoeniceus*) did not include year in the top model but did include nest stage. Nest survival was higher in the nestling (0.97) than the incubation stage (0.85). This corresponds with a period survival rate of 0.12 for incubation and 0.72 for nestlings. Daily survival rate increased with the number of days that grazing occurred while the nest was active. Additionally, nest survival varied across time with higher daily survival rates at the beginning and end of the breeding season. There was no significant difference in nesting densities between grazing intensities although densities decreased as grazing intensities increased (Figure 9). Nesting density was not significantly impacted by vegetation structure.

Brewer's Blackbird (*Euphagus cyanocephalus*) nest survival varied with year and stage. Nest survival was highest in 2022 and lowest in 2021 (2021: 0.92, 2022: 0.99, 2023: 0.95). Period survival rates for each year were 0.11, 0.77, and 0.26, respectively. Nest survival was higher in the nestling stage (0.97) than the incubation period (0.82). This corresponds with a period survival rate of 0.08 for incubation and 0.67 for nestlings. Nest survival was negatively impacted by increasing litter depth. There was no significant difference in nesting densities between grazing intensities although densities were slightly higher in the moderate and full paddocks (Figure 9). Additionally, vegetation did not significantly impact nesting densities although the zero-inflated litter depth model was only marginally non-significant ($p = 0.069$).

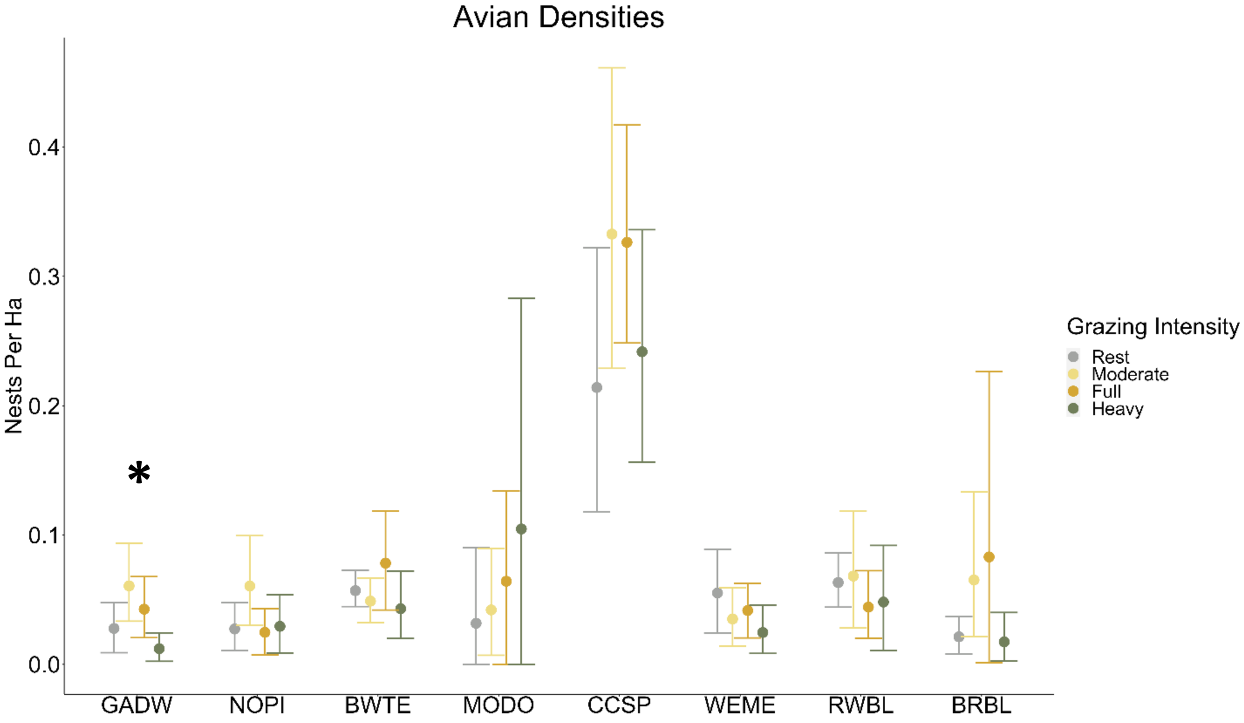

Figure 9. Nesting densities of grassland birds in a modified twice-over rest-rotation grazing (MTORG) system at the Central Grasslands Research Extension Center from 2021 to 2023. Nesting densities corrected for imperfect detection using daily survival rate and error bars represent bootstrapped 95% confidence intervals. Asterisks indicate species with a significant difference in densities among grazing intensities. Gadwall were the only species to show a significant difference with higher densities in the moderate paddock than the heavy paddock.

Discussion

Lower-than-average precipitation early in the 2021 and 2023 field seasons may have impacted the vegetation structural gradient observed between grazing intensities (Derner & Hart, 2007; NDAWN, 2022; Scasta et al., 2016). Despite these climactic challenges, we were able to identify a slight gradient in 2021 with significantly shorter vegetation in the heavy paddock than any other paddocks and a full gradient from tall dense vegetation in the rested and moderate paddocks to short and sparse vegetation in the full and heavy paddocks in 2022. The reduced structural gradient observed in our VOR measurements may result in reduced available niche space which is correlated with lower avian diversity (Coppedge et al., 2008). In contrast, litter depth consistently showed variation attributed to grazing intensity. These changes seemed to follow a lag effect with the previous year's grazing intensity impacting litter more than the current year.

While changes in vegetation structure were evident, particularly for litter depth, nesting communities were only significantly different in 2021. This may be due to a limited species assemblage from the drought and reduced structural gradient, which further suggests that below-average precipitation may have impacted nesting diversity within our grazing system.

Our analysis of nest survival revealed species-specific responses to grazing intensity and changes in vegetation structure. Four out of eight species included in the nest survival analysis were either directly or indirectly impacted by grazing, highlighting the importance of considering species-specific responses in habitat management practices. In the nest density analysis, two more of the eight species showed changes in density either directly or indirectly associated with grazing intensity. The Gadwall was both directly and indirectly impacted by grazing intensity with the highest densities in the moderate paddock and the lowest in the rested, following the same trend as litter depth. The Clay-colored Sparrow nesting densities were also positively impacted by increasing litter depth. While not significant, Western Meadowlark densities decreased with increasing grazing intensities while Mourning Dove nesting densities increased. This further suggests that maintaining structural variability within a pasture is critical to creating high-quality habitat for diverse species assemblages.

The results of this study can inform grassland bird management in the northern Great Plains and will benefit obligate grassland nesting species that are currently listed as species of concern, including Grasshopper Sparrow (*Ammodramus savannarum*), Chestnut-collared Longspur (*Calcarius ornatus*), Northern Pintail (*Anas acuta*), Upland Sandpiper (*Bartramia longicauda*), and Bobolink (*Dolichonyx oryzivorus*) (Duquette, 2020; Dyke et al., 2015).

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Decaying Cattle Carcasses as a Resource for Rangeland Pollinators

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Summary

Pollinators are needed in grazed rangelands to pollinate flowering plants for cattle grazing and diverse plant communities, and, in turn, rangelands provide food and shelter that support pollinator community survival and reproduction. However, as pollinator numbers decline over time, it is important that we explore additional methods to attract and feed these insects. One resource that can be common in grazed rangelands, but it currently not used for ecological purposes, is decaying animal carcasses. Carcasses are well studied for forensic and criminal purposes, but recently there has been more attention to their additional potential benefits for ecosystems, including how they can add nutrients to soil and support wildlife groups. This study focuses on the effect carcasses can have on insect pollinators, both in terms of attracting them to nearby flowers in an area and to act as a direct source of nutrition. We conducted insect floral visitor surveys around five points centered on a decaying calf carcass and five control points in a rangeland during the summers of 2022 and 2023. Our preliminary results indicate that more insect visitors were detected in 2022 than in 2023, but that more pollinators were found directly on calf carcasses in 2023. For both years, the most abundant visitors to surrounding flower species were bumble bees, honey bees, and hover flies. There were also trends in timing of insect visitation to flowers around our points and with pollinators found directly on calves. These observations could be due to the surrounding flowering community composition and/or the stage of carcass decomposition. We plan to further analyze interactions with the flowering community around each point as well as to quantify potential competition on carcasses. In doing so, this research will help better understand the role of decaying matter in supporting pollinator communities and help support future potential applications in a practical and beneficial format for landowners.

Introduction

Pollinators are essential for many plants and their reproduction in natural ecosystems and agricultural landscapes (Aizen et al. 2009, Winfree et al. 2009, Ollerton 2017). Although these insects are greatly needed, they are declining in many areas due to factors such as habitat fragmentation, diseases, and climate change (Potts et al. 2010, Ollerton 2017). Therefore, it is necessary to better understand how we can support insect pollinator communities, especially in areas that provide lots of resources, such as grazed rangelands. Grazed rangelands cover a large expanse of land and have many flower species that pollinators visit to collect nectar and pollen from (Black et al. 2011, Harmon et al. 2011). Besides directly managing rangelands to promote flowering species that pollinators visit, there are potentially other methods to attract and feed pollinators (as well as other ecologically important insects like beetles). One such way is the use of decaying organic matter which is an underrepresented research topic in insect ecology even though it could potentially contribute to biodiversity conservation (Barton et al. 2013). Our study specifically places decaying matter (deceased calves) at an experimental site to understand how this resource may attract and feed pollinators in a rangeland.

Most research involving decaying matter and insects is geared towards forensic entomology, or the study of using insects to determine timing, location, and other specific details to help solve crimes or legal issues (Amendt et al. 2011). Therefore, there are many studies that examine insect life cycles and colonization on carcasses (Goff 2009, Anderson 2011, Zeariya and Kabadaia 2019, Maisonhaute and Forbes 2021). From an ecological standpoint rather than a forensic one, there is little research outside of studying the importance of decaying matter for recycling nutrients into soil and feeding insects that depend on carrion for their life cycle, such as burying beetles and blowflies (Barton et al. 2013). However, we are interested in how decaying carcasses may attract and potentially feed pollinators either by increasing pollinator visits to flowers surrounding carcasses or by directly feeding on the carcass. We hypothesize that both of these instances will occur in our experiment because firstly, there are studies that observe stingless bee species feeding on pig carcasses (Santos et al. 2014, Somavilla et al. 2019), indicating that carcasses are potential direct nutrient sources, and secondly, there is at least one study that supports the idea that decaying odors can attract honey bees to an area (Morice et al. 2019).

Thus far, there is limited research on how decaying matter may attract other insects that are not typically associated with using carcasses in their life cycle, such as bees and butterflies. Only one study that we know of supports that pollinator abundance and pollination services to flowers increase in urban settings when carrion is present (Cusser et al. 2021). Our study will extend this idea to a grazed rangeland system for three main reasons: 1) rangelands are becoming increasingly depended on for supporting pollinators and other ecologically important insects to contribute to sustainable ecosystems that support both wildlife and livestock, 2) the impact of carcasses on insect biodiversity and ecology has not been studied in a rangeland (Barton et al. 2013), and 3) cattle die of natural causes each year in rangelands and are typically disposed of in a designated pit or dumping area rather than being utilized as a nutrient-rich resource. This third reason is of particular importance to our study because it is not a typical idea or practice to utilize carcasses on or near pastures. Besides the fact that carcasses are unpleasant to be near because of their odor and the current societal association with carcasses having unsanitary conditions, they are also an attractant for flies that carry bacteria and mammal predators that could affect livestock, such as coyotes. If our study finds benefits of carcasses for pollinators, it could generate future discussions with landowners to not only relay our findings, but to also come up with carcass utilization methods that would be feasible and beneficial to ranchers. To begin quantifying potential benefits of decaying carcasses for rangeland pollinators, we have two main objectives for this study:

Objectives

1. Quantify abundance and composition of insect visitors to flowering plants surrounding decaying calf carcasses.
2. Examine pollinator abundance around carcasses and feeding behavior (both on floral species and the decaying matter itself).

Methods

Study Site and Experimental Design

We collected data at North Dakota State University's Central Grassland Research Extension Center (CGREC) located near Streeter, North Dakota, (46°45′N, 99°28′W). For both 2022 and 2023, we set up our experiment in the calving pasture which is approximately 28 hectares. Each spring, five newborn calves that passed away due to natural causes were immediately placed in individual trash bags and put into a freezer to prevent decay. The calves were then set out at predetermined spots in the calving pasture each summer (July 6th in 2022 and July 12th in 2023) and immediately caged to prevent natural predators (birds, coyotes, etc.) from accessing the calves (Figure 1). Five control points were also established at predetermined locations and marked with a t-post. All ten points (five calves and five control) were in the southern half of the calving pasture within 5.5 hectares to keep similar soil type, flowering plant community, and elevation. Each point was placed at least 35 meters from one another to minimize overlap in insect activity. These points were then switched in 2023, e.g., calf points in 2022 became control points in 2023 (Figure 2).

Figure 1. A picture of a deceased calf immediately after placing a cage around it in 2023. Photo credit: Rebecca Valentine.

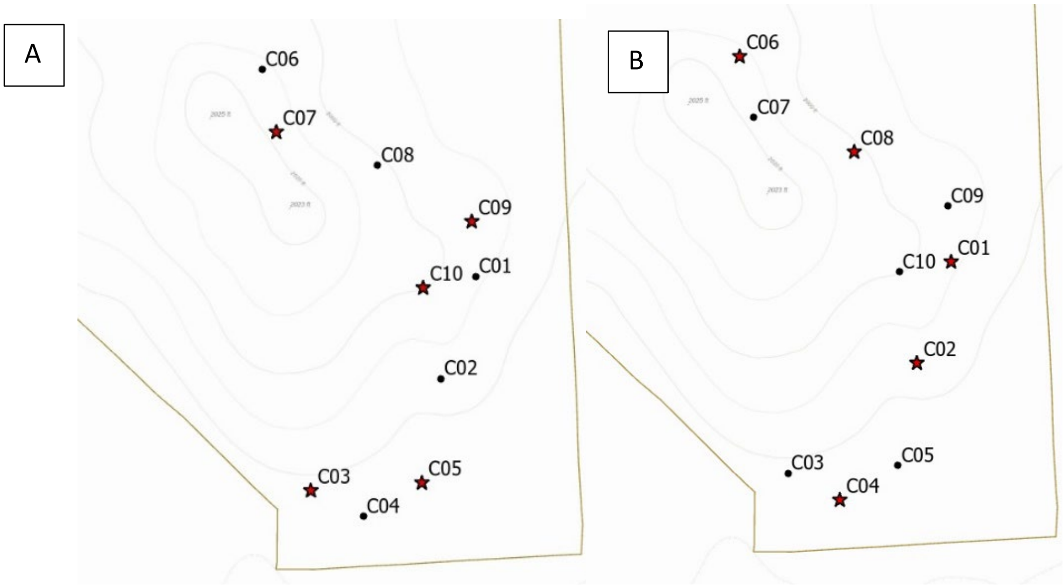

Figure 2. The layout of experimental sites in the 5.5 hectares of the calving pasture at CGREC. Stars indicate the points with a deceased calf and the dots are the control points for A) 2022 and B) 2023.

Floral Visitor Surveys

Each point (for both calves and controls) was surveyed for insect visitors at nearby flowering plants. We surveyed two, 10-meter transects on each side of the point (Figure 3) and recorded all insects visiting flowers as well as the flower height and species, and the flower distance to the center point of the survey (the calf and the control t-posts were considered the center points). Additionally, any insects that were found exhibiting feeding behavior on the calf (outside of flies and beetles) were recorded. We considered an insect to be exhibiting feeding behavior when they were walking on the carcass with their head in the fur and were occasionally pausing to place their respective tongue (glossa in bees, proboscis in butterflies and flies) on the carcass itself. This behavior is often referred to as "puddling" in which an insect soaks up, or drinks, liquid material from sources such as mud, tree sap, or carrion (Molleman 2010). Other insects that were not recorded include grasshoppers and crickets because of their common presence at all points and their behavioral habit of jumping onto a carcass and then immediately off again without exhibiting feeding behavior. The first floral visitor survey was conducted on the same day that calves were placed outside and covered with cages and were repeated at least once a week for four weeks (7 survey rounds in 2022 and 12 survey rounds in 2023).

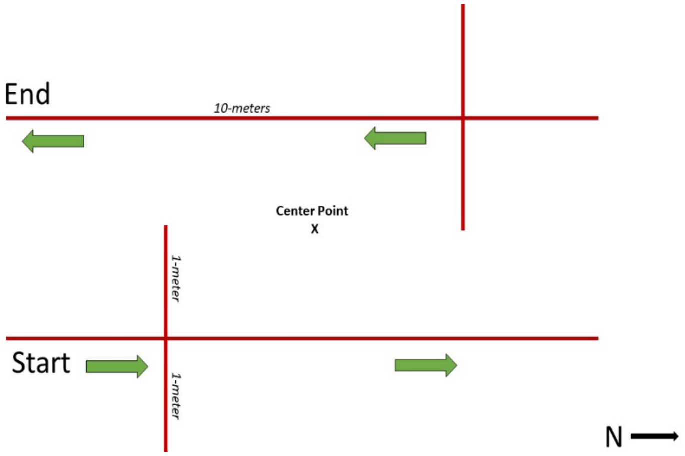

Figure 3. The transect setup for floral visitor surveys around the center point (either the calf or the control t-post). Two, 10-meter transects were walked by the surveyor and all floral plants were examined within 1-m on each side.

Results

Objective 1. Floral visitor abundance and composition.

For our preliminary results, we grouped insect visitors caught either on flowers or on the calf by general insect group: ambush bug, ant, bee (this includes all bees except bumble bees and honey bees), beetles, bumble bees, butterflies, damselflies, dragonflies, flies (this includes all flies except for hover flies), honey bees, and hover flies. We graphed the average number of total bees, butterfly, beetle, and fly individuals by year (2022 and 2023) and treatment (calf and control) (Figure 4). In 2022, we surveyed on seven different dates including the first day that calves were set out. We had more survey dates in 2023, so to keep our results comparable, we only used the data from 2023 on the same (or one day difference) survey dates as 2022.

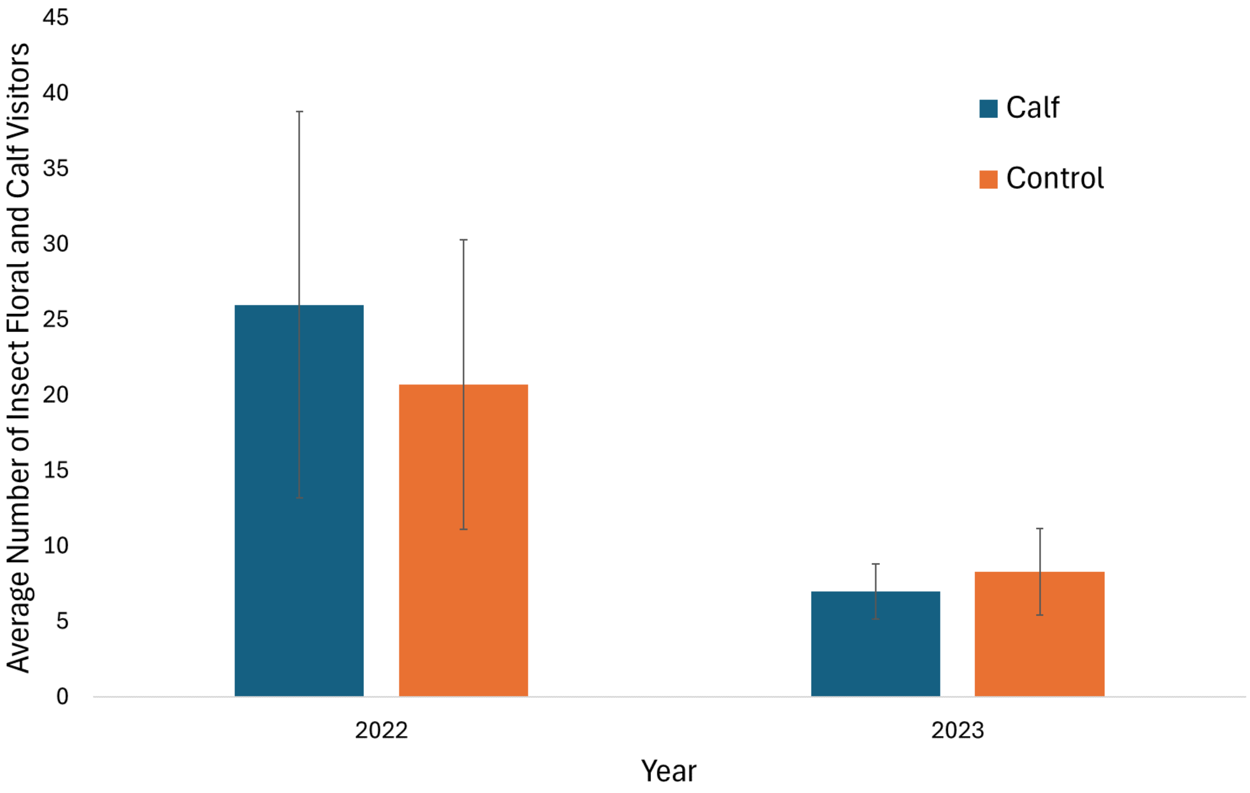

Figure 4. Average number of individual insects visiting flowers and calves in 2022 and 2023 for calf and control treatments with standard error. Insects that were included are bees, butterflies, beetles, and flies on seven survey dates in July.

Objective 2. Pollinator proximity and feeding behavior

We selected the three most abundant pollinator groups from the pollinator surveys to further examine proximity to the center point and feeding behavior directly on decaying matter: bumble bees (genus Bombus), honey bees (genus Apis), and hover flies (family Syrphidae). We visualized the average abundance of each of these groups across years and treatment (Figure 5).

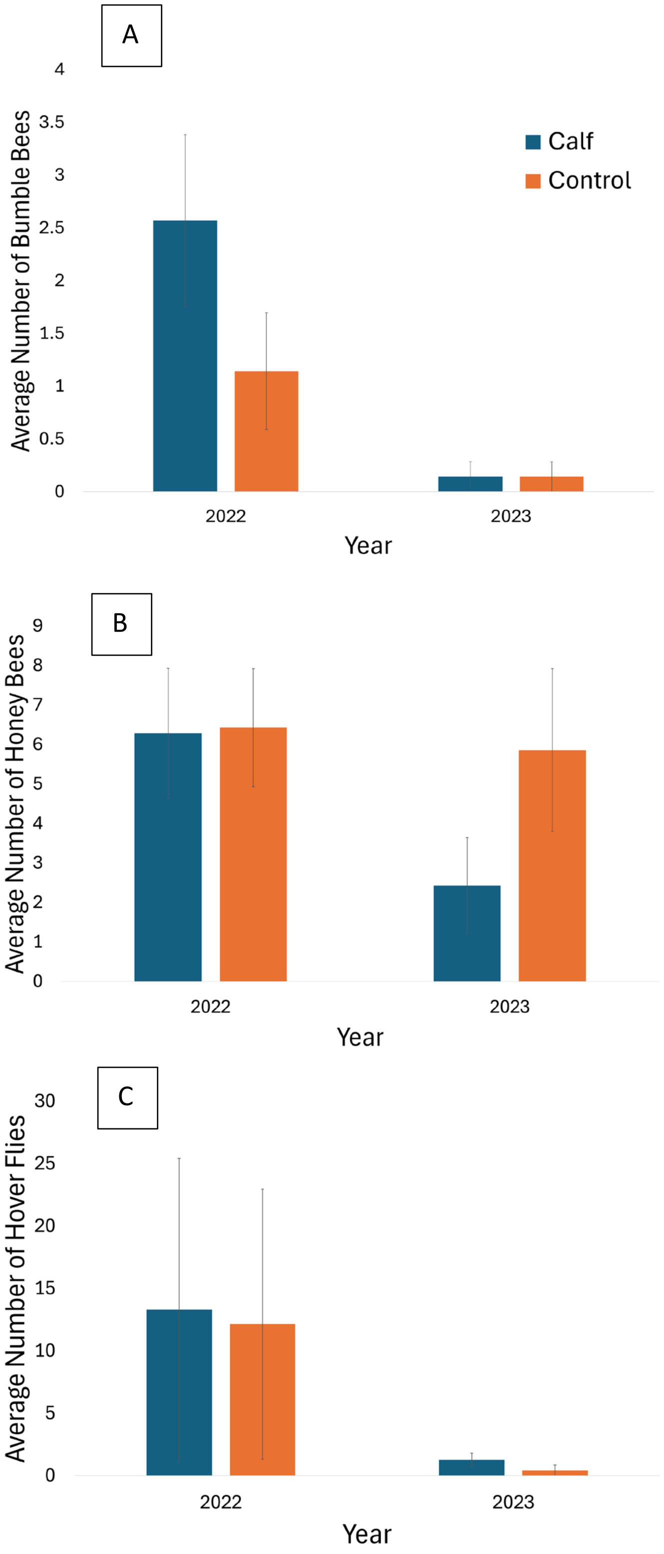

Figure 5. The average number of A) bumble bees, B) honey bees, and C) hover flies with standard error across year and treatment.

We also attempted to record feeding behavior on the calf carcass for each of these three groups (as well as other general pollinator groups that were present such as butterflies) and the calf decay stage during the observation (Table 1). We included all survey dates for both years. In 2023, there were difficulties with counting individuals feeding on calves due to high numbers of individuals feeding alongside carrion fly and beetle species present at the same time, and our inability to catch insects to count captured individuals because the cage prohibits capture with a net. We therefore photographed insects through the cage for identification (Figure 6) and documented them as "too many to count," if needed, indicating a count greater than 10 during that specific survey.

Table 1. The number of pollinating insects recorded under 1-meter from the calf, number of those that were observed directly feeding on the calf, the number of surveys these observations were recorded across, and the decay stage of the calf during the time of the observation.

Year	**Insect Type**	**Under 1-m Distance From Calf**	**Observed Feeding on Calf**	**Number of Surveys**	**Calf Decay Stage**
2022	Bumble Bee	4	0	1	Advanced Decay
	Butterfly	1	1	1	Active Decay
	Honey Bee	2	0	2	Advanced Decay
2023	Sweat Bee	Too many to count	All	1	Advanced Decay
	Bumble Bee	4	4	3	Active to Advanced Decay
	Butterfly (Not Counted)	Too many to count	All	2	Advanced Decay
	Butterfly (Counted)	4	4	3	Advanced Decay
	Honey Bee	Too many to count	All	1	Advanced Decay

Figure 6. A photograph of a female Yellow Bumble Bee (*Bombus fervidus*), a declining species, with collected pollen loads on her legs feeding directly on a calf carcass in 2023. Photo credit: Bethany Roberton

Discussion and Future Work

Our preliminary data suggests more insect visitors to flowers overall in 2022 than in 2023. However, we observed more insects (honey bees, bumble bees, butterflies, and sweat bees) directly on the carcass in 2023 that appeared to be exhibiting feeding behavior. The most common visitors both years were bumble bees, honey bees, and hover flies. Although these three groups did not have any visible trends in preference for calf or control sites across our compared seven survey dates each year, it is possible that they were still attracted to the general area because of the scent from five decaying calves all within a 5.5-ha plot. We will explore this idea further by examining control points that we placed further away in the calving pasture in 2023.

Another aspect we plan to explore is the flowering community at each calf and control point. Each year, we took flowering species surveys following the same floral visitor survey route to analyze the influence of flowering abundance and diversity on floral visitors at the calf and control points. As one example, in 2022, we observed that the high abundances of hover flies and bees did not overlap in terms of timing. Hover flies were caught earlier on in the floral visitor surveys and visited different flower species than honey bees and bumble bees which were detected a couple of weeks later and were visiting more late-season flowering species. In this case, the flowering composition may have been a greater attractant to these insect groups than the calves. Any changes in floral community from 2022 to 2023 may have also influenced the increase in pollinators feeding directly on the carcasses in 2023.

One interesting observation was that we saw more pollinator species on the carcasses in 2023. This indicates that decaying calves could be a potential nutrient resource as well as an attractant to the surrounding flower species in an area. This is especially important because many bee species are in decline, and any nutritious resources utilized by bees are worth further study (especially when we observe workers of identified declining species exhibiting feeding, or puddling, behavior on a carcass (Figure 6)).

One final trend worth examining further is the stage of decomposition that pollinators were caught during, both in proximity to and feeding on the carcass. Most of the insects in 2023 feeding on the carcass were documented from July 20th to 23rd (approximately 14-17 days following placement of the calf outside) during the stages of more advanced decay. This is the typical time that most maggots leave the carcass to pupate in the surrounding soil which decreases the number of predatory beetles present on the carcass. We hypothesize that the decrease in resource competition from flies and beetles during advanced decay may allow more flying pollinator insects to take advantage of the food resource. Additionally, this decay stage has more fluid loss which may be more attractive to pollinators exhibiting puddling behavior (Santos et al. 2014). There therefore may be an essential timing factor that would need to be considered if carcasses are ever strategically placed to attract pollinator insect species to an area. Altogether, there are several ideas that we can further explore to better understand the ecological interactions of essential insect species around decaying carcasses and potentially implement ideas on using carcasses to support pollinators.

Significance

Decaying carcasses as a supporting resource for insect pollinators is an underrepresented topic in the current literature. It is not often regarded as a resource because of societal views on decay and the potential for carcasses to attract flies carrying bacteria and mammal predators, but the ecological importance of this resource should be further explored and considered when it comes to conservation. Due to the declining number of pollinator species and the increase in need for their services in large areas like rangelands, researchers need to be open to the possibility of unconventional avenues. Our preliminary study shows that carcasses can potentially attract different groups of pollinators and possibly change how they utilize surrounding flower species and/or to serve as a direct source of nutrition. As we continue to explore these trends in terms of distances, timing, and floral communities, we hope to bring more attention to the possible use of decay in rangeland ecosystems.

Acknowledgements

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Native bee abundance and floral visitation across three grazing regimes in the summer of 2023

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Summary

Grazed rangelands are important not only for livestock production, but also for supporting wildlife including native bee species. We examined the abundance of native bees and their visits to flowers across three months (June, July, and August) in three different grazing treatments (modified twice-over rest rotational grazing, patch-burn grazing, and season-long grazing). Our preliminary results show that both bee and floral abundance did not differ across our grazing treatments, but were variable based on the month. Many bees were active during August which was evident in both their high abundances during that month as well as more late-season flower visits than expected given their availability. In summary, we detected 13 bee genera that visited a total of 31 flower species during the three months of surveys, most of which were native flower species. Some flower species were only visited in specific months while others, such as western snowberry, were visited throughout the entire season. The visitation of bees compared to the percentage of available flower species indicated preferences for different flower species during each survey round which is likely due to differences in floral composition and bee species across the growing season. Although we have more analyses to conduct to better understand the impacts of timing and grazing treatments on bee diversity, our preliminary results support that floral diversity across land management regimes helps support multiple bee genera throughout the growing season.

Introduction

Bees are important pollinators that provide essential services to natural and agricultural plant communities (Kremen et al. 2002, Klein et al. 2007, Park et al. 2010). Both honey bees and native bees are vital to native and agroecosystems, but their populations have undergone global declines due to habitat loss, agricultural intensification, and climate change (Brown and Paxton 2009, Potts et al. 2010). Native bees especially require support due to their contribution to long-term crop yield and landowner profit (Garibaldi et al. 2014). Rangelands are a crucial source of pollinator food sources and nesting sites (Black et al. 2011), making these areas critical for pollinator conservation (Cole et al. 2017). Yet, rangeland management is critical for determining the success of both rangeland plants and animals (ref). Therefore, grazing management techniques are essential to study for both bees and livestock production.

One of the contributing factors to bee decline is habitat loss, including the conversion of more land to agricultural landscapes (Kline and Joshi 2020). As native, solitary bees are needed to pollinate agricultural fields and, in some cases, can be extremely efficient crop pollinators (Garibaldi et al. 2014), actions must be done to help support these insects (Kline and Joshi 2020). As with crop production, livestock grazing also takes up a large area of land. In the United States, about 33% of land is taken up by production grazing (Theobald 2014). If we are to use this land to efficiently support bee communities, we must explore grazed rangeland management techniques that can create sustainable habitat for bees while also maximizing livestock production.

Grazed rangelands can be managed in different ways including rotational grazing, continuous grazing, and patch-burn grazing (Fuhlendorf and Engle 2001). Grazing management can impact bees in many ways depending on the level of grazing intensity, timing of grazing, and landscape (Sjödin et al. 2008, Lázaro et al. 2016, Smith et al. 2016, Buckles and Harmon-Threatt 2019). For example, patch-burn grazing may negatively or positively impact nesting sites for bees depending on the parameters of specific studies (Buckles and Harmon-Threatt 2019, Bruninga-Socolar et al. 2021). Research specifically carried out in North Dakota has found that grazing can shift bee-plant network structure and composition as well as floral availability (Bendel et al. 2019). Bees depend on floral resources to fill nutritional needs and select flowers based on food source quality (Cnaani et al. 2006, Somme et al. 2015), so it is important to continue studies on bee communities and the floral resources they use across different rangeland management techniques.

To contribute to the study of bees across rangeland management methods, we are examining the effects of three grazing regimes on native bee communities and their floral visitation. All three grazing regimes have rarely been investigated for their effects on bees and there is little research linking bee abundance and diversity along with floral use in the Northern Great Plains. Our two objectives are to examine 1) native bee and floral abundance and 2) floral species availability and bee visitation across three grazing management practices.

Procedures

Study Site

We collected data at North Dakota State University's Central Grassland Research Extension Center (CGREC) located near Streeter, North Dakota, (46°45′N, 99°28′W). The CGREC is characterized as a mixed-grass prairie and is dominated by western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve], green needlegrass [*Nassella viridula* (Trin.) Barkworth], and blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] (Limb et al. 2018). It also contains the non-native grass, Kentucky bluegrass (*Poa pratensis* L.) and the native shrub, western snowberry (*Symphoricarpos occidentalis* Hook.) (Limb et al. 2018). The forb community includes many species such as milkweeds (*Asclepias* spp.), goldenrods (*Solidago* spp.), coneflowers (*Echinacea* spp.) and thistles (*Cirsium* spp.).

Treatment Structure

We used three treatments of approximately 260 hectares each (Figure 1): modified twice-over rest rotational grazing (MTORG), patch-burn grazing (PBG), and season-long grazing (SLG) (Fuhlendorf and Engle 2001). Each treatment has four replicates of equal pasture size (65 ha) which are further designated into eight subplots of 8-ha each. Within the modified twice-over rest rotational treatment, there are different intensities of grazing including heavy, full, moderate, and rested paddocks. The patch-burn grazing treatment is burned on a four-year rotation with a quarter of each pasture being burned every spring. The season-long grazing acts as a control to reflect common regional management.

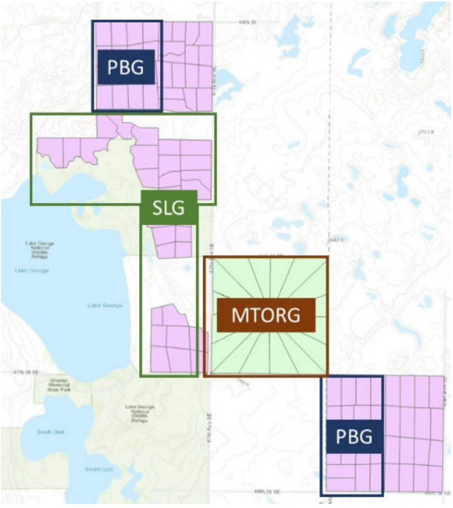

Figure 1. The three grazing treatments used at the CGREC: modified twice-over rest rotational grazing (MTORG, outlined in red), patch-burn grazing (PBG, outlined in blue), and season-long grazing (SLG, outlined in green).

Surveys

Transect Bee Surveys

We surveyed a 100-m transect in every 8-ha subplot to measure bee abundance. In 2023, we surveyed bees on these transects in three rounds: June 6th to July 1st, July 6th to August 1st, and August 2nd to August 27th. We caught all bees within reach (approximately 1 meter on each side of the transect) using a sweep net. All bees except for honey bees were immediately placed in a plastic jar and photographed for identification with a minimum of 5 photos and one, 10-second video. Bumble bees were swabbed for a pollen sample after being photographed and released live. All other native bees were swabbed for a pollen sample after being photographed and then lethally collected to identify them to species and to be swabbed a second time for pollen samples in the lab. For each observed or caught bee in both transect and plot surveys (described below), we recorded the flowering species that the bee was visiting. Bee behavior was also recorded so that we could separate out bees based on whether they were actively collecting nectar or pollen from the flowers at the time of the survey.

Plot Bee Surveys

After each transect bee survey, we immediately searched a 50-m by 50-m plot for 10 minutes to survey bees. We walked slowly throughout the plot without set transects but made sure not to retrace our steps. We also spent more time at floral patches (an extra 30 seconds or so) where we knew bees were more than likely would be. Bees were processed in the same way (photographed, swabbed for pollen, and then bumble bees released while all other native bees were collected) when caught. The timer was stopped while bees were processed and then resumed once more when searching began again.

Transect Floral Surveys

After finishing each transect bee survey and before starting the plot bee survey in each 8-ha subplot, we followed the same 100-m transect for the plant survey. We counted and identified each individual flowering plant (individuals counted as a stem(s) originating from the same location in the soil) within 2.5 meters on either side of the transect.

Results

Objective 1: Native bee and floral abundance

We used a two-way repeated measures ANOVA in R4.3.2 to analyze variation in the number of native bees and then for the number of nectaring native bees within each round (June, July, and August) and between treatments (MTORG, PBG, SLG). No interactions were detected between independent variables (round and treatment), so we conducted post-hoc analyses with paired t-tests with a Bonferroni correction.

For both the number of native bees and number of native bees found feeding on flowers (nectaring), we did not find a statistical difference across treatment. However, we did see a difference between the timing of our three survey rounds for native bees ($P<0.0001$) and for nectaring native bees ($P<0.001$) with the third round being significantly higher than the first and second survey rounds in both cases (Figure 2).

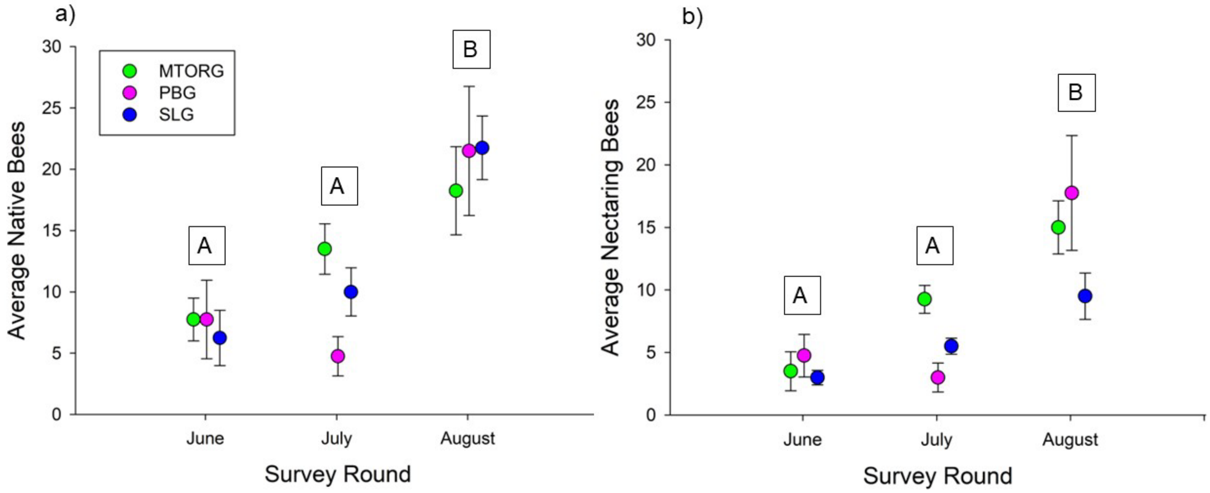

Figure 2. Average bee abundance with standard error of a) total native bees and b) native bees caught nectaring on flowers across survey round (June, July, and August) and treatment (MTORG, PBG, and SLG).

We also used a two-way repeated measures ANOVA in R4.3.2 to analyze variation in the number of flowering plants within each round (June, July, and August) and between treatments (MTORG, PBG, SLG). No interactions were detected between independent variables, so we conducted post-hoc analyses with paired t-tests with a Bonferroni correction. There were no differences between treatments but survey round had a significant difference ($p<0.0001$). The second round of surveys had significantly higher numbers of flowers than both the first and third rounds.

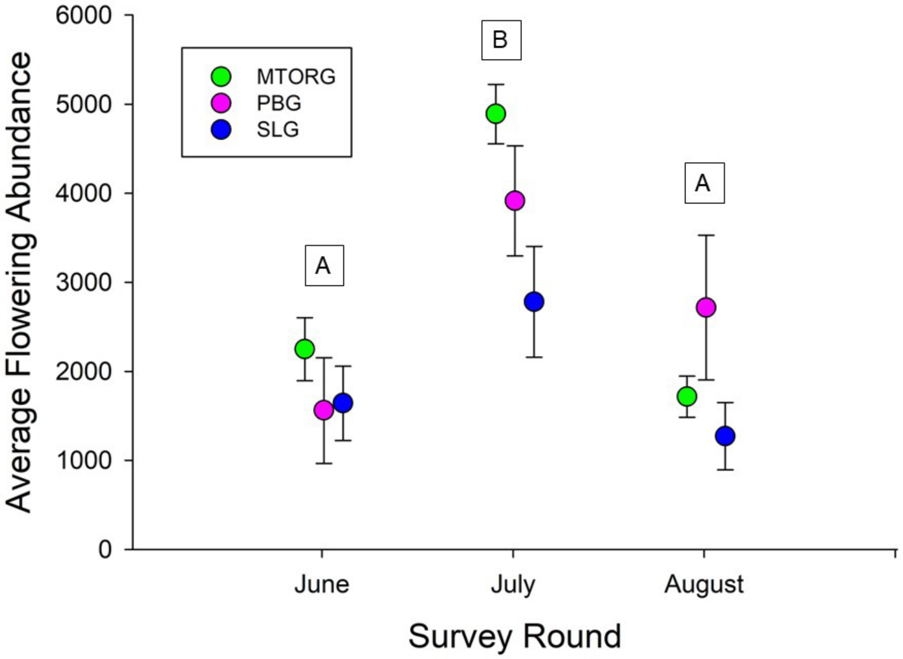

Figure 3. The average abundance of flowering plants with standard error across survey round (June, July, and August) and treatment (MTORG, PBG, and SLG).

Objective 2. Floral species availability and visitation

For the second objective, we visualized how the relative availability of flower species related to visitations by native bees that were found visiting, and presumably nectaring, flowers from both transect and plot surveys (Figure 4). This allowed us to observe the flowers species that are visited disproportionately to their availability. We then compiled a list of flower species that bees were caught on throughout the growing season to examine trends in bee visitation regarding flower status, number of bee visits, and number of bee genera (Table 1). We detected 13 different bee genera visiting 31 flower species throughout the growing season.

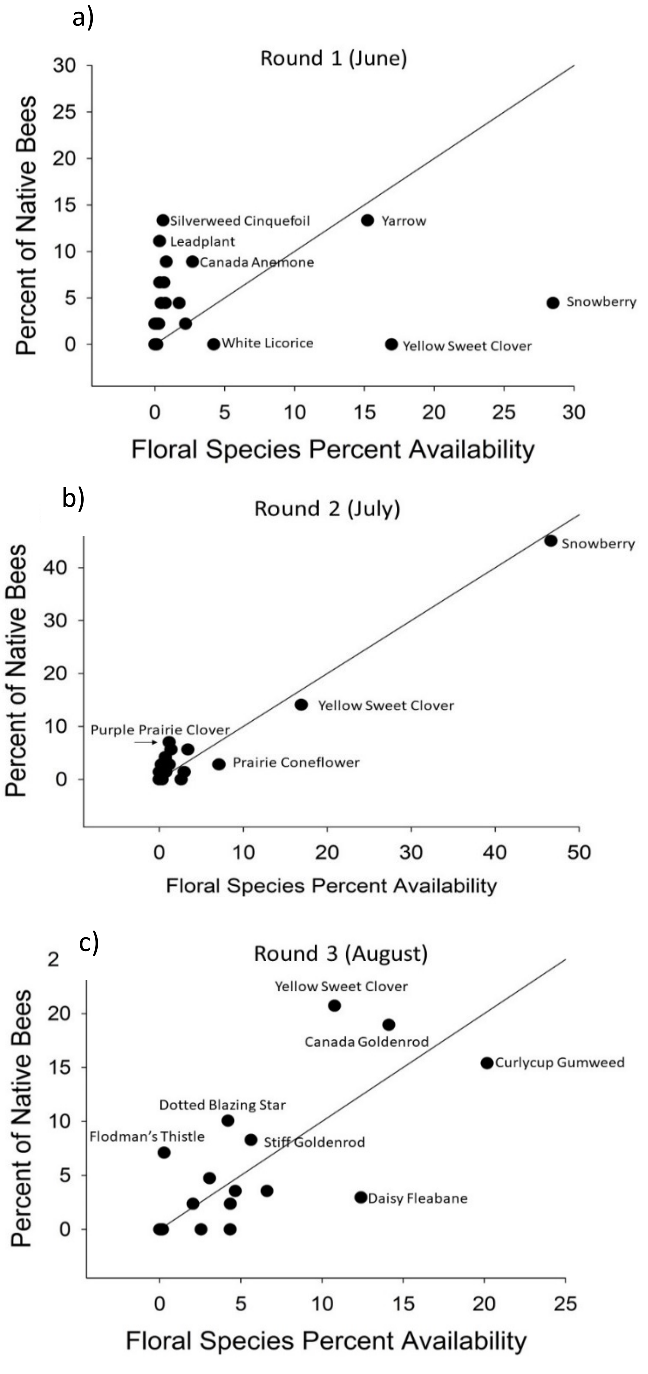

Figure 4. The percent of total nectaring native bees versus the percent floral availability (number of individuals of flower species divided by total number of flowering plants from the growing season) of each flower species those bees were caught visiting for the entire growing season across a) survey 1, b) survey 2, and c) survey 3. The line in each figure represents the expectation that the percentage of bees would match the percentage of floral availability in a one-to-one relationship. Floral species above the line imply more preference (visited more often than expected based on species availability) while floral species below the line imply less preference (visited less often than expected based on species availability).

Table 1. A list of all the flower species that native bees were caught visiting along with the flower's scientific name and native or non-native status in North Dakota. Flowers highlighted in green bloom from early- to mid-season, and flowers highlighted in yellow bloom from mid- to late-season. For each species, we listed number of bee visits and number of bee genera visiting for each round (June, July, and August).

			Round 1 (June)		**Round 2 (July)**		**Round 3 (August)**	
Flower Common Name	**Flower Species**	**Status**	*# Bee Visits*	*# Bee Genera*	*# Bee Visits*	*# Bee Genera*	*# Bee Visits*	*# Bee Genera*
Western Snowberry	*Symphoricarpos occidentalis*	Native	2	1	31	3	8	1
Prairie Rose	*Rosa arkansana*	Native	3	3	0	0	0	0
Yellow Salsify	*Tragopogon dubius*	Non-Native	1	1	0	0	0	0
Leadplant	*Amorpha canescens*	Native	3	3	0	0	0	0
Lambert's Locoweed	*Oxytropis lambertii*	Native	2	2	0	0	0	0
Narrow-leaved Purple Coneflower	*Echinacea angustifolia*	Native	2	2	0	0	0	0
Canada Anemone	*Anemone canadensis*	Native	2	2	0	0	0	0
Blanketflower	*Gaillardia aristata*	Native	4	3	0	0	0	0
Yarrow	*Achillea millefolium*	Native	6	3	0	0	0	0
Scarlet globemallow	*Sphaeralcea coccinea*	Native	1	1	0	0	0	0
Field Chickweed	*Cerastium arvense*	Native	4	2	0	0	0	0
American Vetch	*Vicia americana*	Native	1	1	0	0	0	0
Purple Prairie Clover	*Dalea purpurea*	Native	2	1	5	4	0	0
Bastard Toadflax	*Comandra umbellata*	Native	1	1	0	0	0	0
Silverweed Cinquefoil	*Potentilla anserina*	Native	6	2	0	0	0	0
Maximilian Sunflower	*Helianthus maximiliani*	Native	1	1	1	1	4	2
Stiff-leaved Sunflower	*Helianthus pauciflorus*	Native	0	0	2	1	4	1
Showy Blue Lettuce	*Lactuca tatarica*	Native	0	0	2	2	0	0
Flodman's Thistle	*Cirsium flodmanii*	Native	0	0	2	2	12	2
Wavy Leaf Thistle	*Cirsium undulatum*	Native	0	0	1	1	0	0
Canada Thistle	*Cirsium arvense*	Non-Native	0	0	4	3	6	3
Yellow Sweet Clover	*Melilotus officinalis*	Non-Native	0	0	10	2	35	3
Wild Licorice	*Glycyrrhiza lepidota*	Native	0	0	1	1	0	0
Rigid Goldenrod	*Solidago rigida*	Native	0	0	1	1	14	4
Curlycup Gumweed	*Grindelia squarrosa*	Native	0	0	4	1	26	7
Dotted Blazing Star	*Liatris punctata*	Native	0	0	1	1	17	1
Hairy False Goldenaster	*Heterotheca villosa*	Native	0	0	1	1	0	0
Prairie Coneflower	*Ratibida columnifera*	Native	1	1	2	2	0	0
Heath Aster	*Symphyotrichum ericoides*	Native	0	0	0	0	5	4
Canada Goldenrod	*Solidago canadensis*	Native	0	0	0	0	32	5
Velvety Goldenrod	*Solidago mollis*	Native	0	0	0	0	6	1

Discussion

We observed that the number of native bees and nectaring native bees were not different across our three grazing treatments. However, there were differences across survey rounds with the third round in August being higher in average abundance of bees than the first survey round in June and the second survey round in July. This is counterintuitive to our floral abundance which was highest during the second round of surveys in July. This suggests that the exact composition of flowers and activity of different bee genera or species will be essential to examine. For example, three goldenrod species, which are late-season flower species, were visited by several bees, supporting that flowering community composition is critical for studying bee visitation.

Additionally, bee communities change throughout the growing season based on life history traits. Bumble bees, for example, have an emergence of new queens and males during August to mate before the end of the growing season. This is the main reason that we separated total native bees from nectaring native bees in our analysis because we observed distinct differences in bumble bee abundance and behavior in the last round of surveys (look at the total abundance of native bees for August in SLG in Figure 2a compared to the nectaring native bees for August in SLG in Figure 2b to see an example of how drastic this difference can be).

The similarities in bee abundance across treatment are not very surprising because our treatments are all very close to each other spatially, so it is possible that bees, especially bees with larger bodies like bumble bees and mining bees, travel freely between treatments. Furthermore, when examining results from a single growing season, we cannot make strong conclusions on which treatments may benefit bees over time. It is important, therefore, that we look at the trends in bee and floral abundance and diversity over several growing seasons and climate conditions.

When we examined the availability of the flowering species with the number of bee visits for 2023, we found several species that were visited in higher proportion to their availability, but the exact species changed over each round. This is expected because flowers bloom at various times throughout the season and because bee species have different activity periods. As one example of how bee composition alters community visitation as a whole, yellow sweet clover was available at a percentage of about 10-20% throughout the entire season but was only visited at higher rates than expected during the third round. This could potentially indicate that bee species that are more active during the late-season may visit yellow sweet clover more often than bee species that are active during the early-season, or that the increased abundance of bees in general at the end of the season increased visitation to yellow sweet clover. Our next steps with this will be to make similar graphs that are broken down by bee genera and treatment to better understand bee floral selection.

We found a trend that most native bees were caught on native flower species. The flower species that were visited by the most genera each round (three or more genera) were prairie rose, blanket flower, leadplant, and yarrow for the June surveys, western snowberry, purple prairie clover, and Canada thistle for the July surveys, and curlycup gumweed, heath aster, Canada thistle, yellow sweet clover, and goldenrod spp. for the August surveys. Some flowering plants had visits from bees on all three rounds including western snowberry and Maximillian sunflower. Altogether, this qualitative chart alongside our other findings support how essential it is to have a variety of flowering species throughout the season to support different abundances and diversity of bees.

Future Work

There are many avenues for us to explore with this data in the future. Firstly, it is important that we separate our data for analysis by bee genera or species within a single genus, different bee species can have varying behavior, floral preferences, and times of activity. If we analyze our data at this detailed level, we may start to see stronger indications that specific grazing treatments support certain types of bees and that bee species have trends of potential floral preference. We also plan to examine the availability of each floral species visited across survey round and within grazing regime. This may help us make connections to which regimes can provide higher availability of important floral species visited by bees.

Significance

This research is necessary because there is an increasing need for more study on bee responses across different management regimes and across multiple bee taxa (Brown and Paxton 2009). Researchers can use this information to help manage rangelands for multiple uses, including promoting bees and their needed resources. So far, data from this single field season suggests that grazing management regimes may not have drastic impacts on bee abundances, but it is essential regardless that a diversity of floral resources is needed for supporting bee diversity throughout the growing season. Bees are essential for multiple ecosystems services (Klein et al. 2007, Lautenbach et al. 2012, Patel et al. 2021), so it is more important than ever to conserve these pollinators. Because of the large areas utilized as rangelands (Foley et al. 2005) and the increasing global need for livestock products (Thornton 2010), findings from studies like this can contribute to a larger goal of benefiting both production and habitat for a variety of wildlife.

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Vitamin/mineral supplementation to beef heifers during pregnancy on immunoglobulin concentrations in colostrum and immune responses in the offspring

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Summary

Two experiments were conducted to evaluate the impacts of feeding a vitamin/mineral supplement to beef heifers throughout gestation on concentrations of immunoglobulin (Ig) in colostrum and in calf serum 24 hours (h) after consumption of maternal colostrum (Exp. 1) or a colostrum replacement product (Exp. 2). Angus-based heifers were provided with a basal diet during gestation (CON) or were provided with the basal diet plus the addition of a vitamin/mineral supplement during gestation (VTM). Colostrum was collected from heifers in both experiments at calving, and blood was collected from calves at birth (pre-suckling) and 24 h after suckling to evaluate passive transfer of Ig through colostrum. Calves in Experiment (Exp.) 1 were evaluated postnatally to determine immune responses to vaccination at birth, pasture turn out, and at weaning. Blood was collected from calves on the day of vaccination and 14 days (d) after to examine antibody responses to Bovine Viral Diarrhea Virus (BVDV) Type 1 and Type 2, Bovine Respiratory Syncytial Virus (BRSV), Infectious Bovine Rhinotracheitis (IBR), and Parainfluenza 3 (PI3). In both Exp. 1 and 2, maternal dietary treatment (CON or VTM) did not affect ($P \geq 0.21$) concentrations of IgG, IgM, or IgA in colostrum at calving or in calf serum at 24 h. However, concentrations of IgG, IgM, IgA, and total Ig in calf serum at 24 h were greater ($P \leq 0.01$) in calves receiving maternal colostrum (Exp. 1) compared with those receiving a colostrum replacement product (Exp. 2). No effect of treatment (CON vs VTM) or the interaction of treatment and day were observed ($P \geq 0.93$) for antibody responses at birth, pasture turn out, or weaning for CON or VTM calves in Exp. 1. Taken together, maternal dietary treatment during pregnancy did not impact colostrum Ig, passive transfer of Ig, or postnatal titer response to vaccination for CON or VTM calves, but consumption of maternal colostrum appeared to be a more effective method of delivery of Ig compared with the colostrum replacement product.

Introduction

The immune system of neonatal beef calves established early in life is integral for the long-term health, productivity, and economics of cow-calf enterprises. In the immediate postnatal period for a newborn calf, it is imperative that calves receive successful passive transfer of immunity via colostrum ingestion and intestinal absorption of immunoglobulins in colostrum either from the dam or a colostrum replacement product (Chase et al., 2008). During fetal development, maternal nutrition may have the potential to affect the physiological, metabolic and immune functions in the gestating calf (Wu et al., 2004; Price et al., 2017), a concept known as fetal/developmental programming (Barker, 2004). Research on vitamin/mineral nutrition, specifically during pregnancy and colostrogenesis, on concentrations of immunoglobulins in colostrum, passive transfer of immunity to the neonatal calf, and postnatal health and immune function of the offspring require further investigation. Therefore, our objectives were to determine the impacts of maternal vitamin/mineral supplementation during gestation in beef heifers on concentrations of immunoglobulins (Ig) in colostrum, passive transfer of immunity in naturally and artificially reared offspring, and postnatal antibody responses to vaccination.

Procedures

Two experiments were conducted to evaluate the impacts of feeding a vitamin/mineral supplement to beef heifers throughout gestation on concentrations of immunoglobulin (Ig) in colostrum and in calf serum 24 h after consumption of maternal colostrum (Exp. 1) or a colostrum replacement product (Exp. 2). Angus-based heifers (n = 72, 14 to 15 months of age, initial body weight [BW] = 838.6 ± 111.47 pounds [lbs]) were managed in an individual feeding system (American Calan; Northwood, NH) at the NDSU Animal Nutrition and Physiology Center (ANPC; Fargo, ND). Heifers were randomly assigned to receive either the basal diet targeting gain of 1 lb/heifer/day (CON; n = 36) or the basal diet plus a loose product vitamin/mineral supplement (Table 1; Purina Wind and Rain Storm All-Season 7.5 Complete, Land O'Lakes, Inc., Arden Hills, MN) top-dressed on the total mixed ration (TMR) at a rate of 4 oz/heifer/day (VTM; n = 36). All heifers were subjected to a 7-day Select-Synch + CIDR estrus synchronization protocol and bred via artificial insemination (AI) to female-sexed semen from a single sire. Transrectal ultrasonography was conducted to determine pregnancies at day 35 post-insemination, and fetal sex was determined at day 65 after AI to confirm pregnancies with female fetuses.

For Exp. 1, diet treatments began at the time of AI. Heifers becoming pregnant with female fetuses after first service AI (CON, n = 14; VTM, n = 17) were transported to the NDSU Beef Cattle Research Complex (BCRC; Fargo, ND), adapted to the Insentec Roughage Intake Control Feeding System (Hokofarm B.V., Marknesse, The Netherlands), and diet treatments were continued throughout pregnancy. The basal diet for heifers on Exp. 1 consisted of corn silage; alfalfa, millet, or prairie hay; and dried corn distillers grains plus solubles. During late-gestation, feed deliveries were adapted to provide *ad libitum* feed intakes through calving. At calving, heifer calves were allowed to nurse their dams and remained alongside their dams until weaning.

For Exp. 2, heifers that did not become pregnant to first-service AI (CON, n = 19; VTM, n = 18) were synchronized for estrus and rebred via AI 60 days after initial dietary treatments began and continued treatments throughout pregnancy at the ANPC. The basal diet consisted of grass hay, corn silage, and dried corn distillers grains plus solubles. During late-gestation, pregnant heifers were transported to the BCRC, and feed deliveries of the basal diet were adapted to provide ad libitum intakes through calving. The basal diet consisted of corn silage, alfalfa hay, dried corn distillers grains plus solubles, and a corn-based premix. Heifer calves born (CON, n = 7; VTM, n = 7) were removed from their dams at birth, relocated to individual pens, and fed 1.5 liters of colostrum replacer containing 150 g globulin protein (Lifeline Rescue High-Level Colostrum Replacer; APC, Inc; Ankeny, IA) via an esophageal feeder within 2 h of birth. Every 12 h, calves were fed 2 liters of milk replacer (Duralife Optimal Non-Medicated Calf Milk Replacer; Duralife; Fort Worth, TX) via an esophageal feeder.

In both experiments, samples of colostrum from the dam at calving were obtained by completely milking the rear-right quarter of the udder using a portable milk machine (InterPuls, Albinea, IT). Blood samples were collected from calves pre-suckling (within 2 h of birth) and 24 h after colostrum consumption via jugular venipuncture. Concentrations of immunoglobulin G (IgG), M (IgM), and A (IgA) were quantified in colostrum and serum (pre-suckling and 24 h post-suckling) using bovine radial immunodiffusion plate kits (Triple J Farms; Bellingham, WA).

For Exp. 1, blood was collected at numerous time points relative to vaccination at 24 h of age, pasture turn out, and at weaning to assess antibody titer response to vaccination. Blood samples were collected on the day of vaccination (24 h of age, pasture turnout, and 7 d pre-weaning) and 14 days following vaccination, totaling six blood collection time points. At 24 h of age, vaccinations administered included protection against respiratory viruses (IBR, PI3, and BRSV) and clostridial diseases. At the time of pasture turn out on native range pasture, calves were administered vaccinations to protect against respiratory viruses (IBR, PI3, BRSV, BVDV-1, BCDV-2, and Mannheimia haemolytica), clostridial diseases, pinkeye, and an anthelmintic was administered. At 7 d prior to weaning, calves received vaccinations to protect against respiratory viruses (IBR, PI3, BRSV, BVDV-1, BVDV-2, and Mannheimia haemolytica), clostridial diseases, and pinkeye.

Blood samples were allowed to clot after collection and placed on ice until centrifugation. Samples were centrifuged at 1,500 × g at 4°C for 20 minutes, aliquoted into 2-mL plastic microtubes, and stored at -20°C until analysis. Serum was analyzed at Oklahoma State University Animal Disease Diagnostic Laboratory (Stillwater, OK) via serum neutralization (SN) for detection of antibodies for BVDV Type 1 and Type 2, BRSV, IBR, and PI3. Data for both experiments were analyzed for the effect of treatment using the MIXED procedure in SAS. Significance was considered at $P \leq 0.05$.

Results and Discussion

Immunoglobulin Concentrations in Colostrum and Serum

Maternal dietary treatment (CON or VTM) did not affect ($P \geq 0.21$) concentrations of IgG, IgM, or IgA in colostrum at calving or in calf serum at 24 h in either experiment (Figures 1 and 2; Table 2). All calves from both experiments had undetectable concentrations of these respective immunoglobulins at birth, which was expected as calves are born with a naïve immune system. In calves that received maternal colostrum, serum concentrations of IgG were greater ($P = 0.01$; average concentration: 2596 ± 535 mg/dL) than that of calves receiving colostrum replacer (average concentration: 1611 ± 335 mg/dL) at 24 h after suckling. Concentrations of IgA, IgM, and total Ig in serum at 24 h were also greater ($P \leq 0.002$) in calves fed maternal colostrum in Exp. 1 compared with artificially reared calves in Exp. 2. Interestingly, we were able to determine Ig concentrations in the colostrum replacement source and found that the product only contained 9494 ± 77.8 mg/dL of IgG, 609 ± 16.6 mg/dL of IgM, and IgA was undetectable. Given the differences in Ig content in the colostrum replacement product, this likely explains the lower serum Ig values reported for calves in Exp. 2.

Our results suggest that maternal vitamin/mineral supplementation throughout gestation did not impact Ig concentrations in colostrum or the resultant serum Ig concentrations in calves either 24 h after suckling or after delivery of a commercial colostrum replacer. Investigating the intestinal morphological characteristics, blood metabolite and endocrine profiles, and other postnatal physiological responses of calves born to CON and VTM dams may support the lack of treatment differences in terms of passive transfer of immunity observed here. However, our results suggest that maternal colostrum is a more effective delivery of immunoglobulins compared with a commercial colostrum replacement product.

Antibody Titer Response to Vaccination

No treatment or treatment by day interactions were observed ($P \geq 0.93$) for antibody responses to vaccinations administered at birth, pasture turnout, or weaning (Table 3). At weaning, calves elicited an immune response as suggested by a day effect ($P \leq 0.02$) between 184.4 ± 3.73 days of age (pre-weaning/day of vaccination) and 198.4 ± 3.73 days of age (post-weaning) to BVD-2, IBR, and PI3. Interestingly, no effects of day ($P \geq 0.87$) were observed at pasture turn out, suggesting that antibody titer levels were similar on the day of vaccination and 14 d following vaccination at pasture turn out. Our results suggest that maternal vitamin/mineral supplementation during pregnancy was not critical in altering postnatal immune responses in naturally reared calves.

Taken together, supplementing vitamins and minerals during pregnancy in beef heifers did not influence passive transfer of immunity or titer response to vaccination in calves from birth to weaning. Investigating additional characteristics of calves exposed to *in utero* vitamin/mineral supplementation may shed light on other potential programming outcomes of the offspring, such as influences on growth performance, efficiency, and future reproductive success.

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Table 1. Composition of VTM supplement[1] provided to beef heifers at breeding until calving[2]; company guaranteed analysis

Item	**Assurance levels**	
Minerals	**Min**	**Max**
Ca, g/kg of DM	135.0	162.0
P, g/kg of DM	75.0	-
NaCl, g/kg of DM	180.0	216.0
Mg, g/kg of DM	10.0	-
K, g/kg of DM	10.0	-
Mn, mg/kg of DM	3,600.0	-
Co, mg/kg of DM	12.0	-
Cu, mg/kg of DM	1200.0	-
I, mg/kg of DM	60.0	-
Se, mg/kg of DM	27.0	-
Zn, mg/kg of DM	3,600.0	-
Vitamins, IU/kg of DM		
A	661,500.0	
D	66,150.0	
E	661.5	

[1] Purina Wind and Rain Storm All Season 7.5 Complete Mineral (Land O' Lakes, Inc., Arden Hills, MN); ingredients: dicalcium phosphate, monocalcium phosphate, processed grain by-products, plant protein products, calcium carbonate, molasses products, salt, mineral oil, potassium chloride, magnesium oxide, ferric oxide, vitamin E supplement, vitamin A supplement, lignin sulfonate, cobalt carbonate, manganese sulfate, ethylenediamine dihydroiodide, zinc sulfate, copper chloride, vitamin D3 supplement, natural and artificial flavors, and sodium selenite.

[2] VTM supplement provided at a rate of 4 oz/heifer/day.

Table 2. Concentrations of immunoglobulin (Ig) G, M and A in colostrum and total colostrum Ig concentrations in beef heifers assigned to receive a basal diet (CON) or a basal diet with the addition of a vitamin/mineral supplement[1] (VTM) during gestation

	Treatment			
Concentration, mg/dL	CON	VTM	SE	*P*-value
Experiment 1[2]				
IgG	12769	13003	1150.8	0.88
IgM	376.03	359.72	25.201	0.64
IgA	293.81	300.95	29.367	0.86
Total Ig	13439	13664	1173.6	0.89
Experiment 2[3]				
IgG	10109	7482	1704.34	0.30
IgM	296	302	48.9	0.93
IgA	198	227	35.7	0.58
Total Ig	10603	8011	1764.5	0.32

[1] Purina Wind and Rain Storm All Season 7.5 Complete Mineral (Land O' Lakes, Inc., Arden Hills, MN). VTM supplement provided at a rate of 4 oz/heifer/day to gestating heifers on respective VTM treatment.

[2] Heifers in Experiment 1 were assigned to dietary treatments of CON or VTM at breeding and remained on respective treatments throughout gestation. Calves born to dams in Exp. 1 were naturally reared and allowed to suckle from their dam.

[3] Heifers in Experiment 2 were assigned to dietary treatments of CON or VTM 60 days prior to AI breeding and remained on respective treatments throughout gestation. Calves born to dams in Exp. 2 were artificially reared; thus, calves were separated from their dams at birth and provided with a colostrum replacement product and milk replacer.

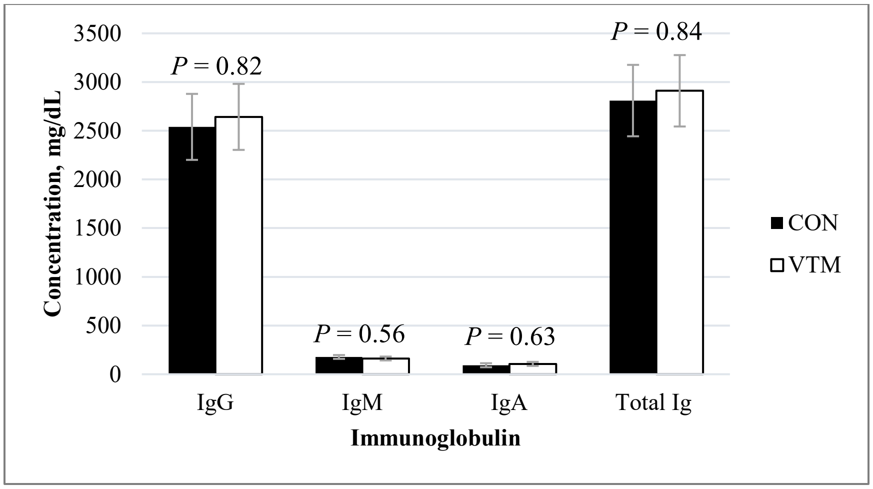

Figure 1. Concentrations of immunoglobulin (Ig) G, M and A in neonatal calf serum 24 h after consumption of dam's colostrum (Experiment 1) and total serum Ig concentrations in female calves born to beef heifers assigned to receive a basal diet (CON) or a basal diet with the addition of a vitamin/mineral supplement (VTM) during gestation. Significance considered at $P \leq 0.05$.

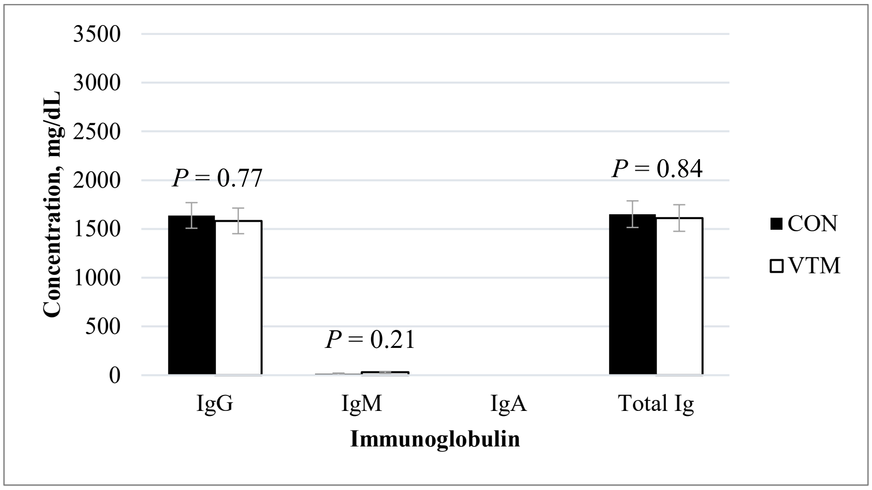

Figure 2. Concentrations of immunoglobulin (Ig) G, M and A in neonatal calf serum 24 h after consumption of colostrum replacement product (Experiment 2) and total serum Ig concentrations in female calves born to beef heifers assigned to receive a basal diet (CON) or a basal diet with the addition of a vitamin/mineral supplement (VTM) during gestation. Significance considered at $P \leq 0.05$

Table 3. Antibody titer responses to vaccination in postnatal beef calves with evaluation at 24 h of age, pasture turn out, and weaning. Dams of the calves born were exposed to the basal diet from breeding to calving (CON) or received the basal diet plus the addition of a vitamin/mineral supplement from breeding to calving (VTM)[1]

Calf Age	**24 h**		**15 d**				*P*-values[4]	
Birth[2]	**CON**	**VTM**	**CON**	**VTM**	SE	TRT	Day	TRTxDay
BVD-1[3]	1082.7	1040.5	249	618.35	420.36	0.70	0.14	0.63
BVD-2	2560	2021.7	1750.7	766.12	802.23	0.35	0.20	0.78
IBR	25	35.53	12.33	14.59	7.01	0.37	0.02	0.56
BRSV	142	178.12	82	79.29	46.40	0.72	0.09	0.68
PI3	341.3	401.9	101.33	198.12	116.66	0.50	0.06	0.88
Calf Age	**40.4 ± 3.73 d**		**54.4 ± 3.73 d**				*P*-values	
Pasture turn out	**CON**	**VTM**	**CON**	**VTM**	SE	TRT	Day	TRTxDay
BVD-1	90	304.24	82	149.65	89.06	0.12	0.37	0.41
BVD-2	151.67	364.24	106.33	255.53	96.65	0.07	0.43	0.74
IBR	5	5.88	4.67	6.59	1.124	0.22	0.87	0.65
BRSV	25.33	32.24	41.33	46.12	12.52	0.64	0.24	0.93
PI3	62.33	77.88	22.33	43.53	23.64	0.44	0.12	0.91
Calf Age	**184.4 ± 3.73 d**		**198.4 ± 3.73 d**				*P*-values	
Weaning	**CON**	**VTM**	**CON**	**VTM**	SE	TRT	Day	TRTxDay
BVD-1	6.33	8.47	20.36	21.65	5.73	0.77	0.02	0.94
BVD-2	21	63.53	312	1130.82	402.06	0.29	0.10	0.34
IBR	4	4	200	113.18	43.13	0.32	0.0008	0.32
BRSV	4	20.25	6.91	61.18	22.10	0.12	0.33	0.39
PI3	5.33	4	431.64	316	123.39	0.64	0.004	0.65

[1] Treatments of the dams were: VTM (n = 17): heifers received the basal diet plus the addition of a vitamin and mineral supplement (Purina Wind and Rain Storm All Season 7.5 Complete Mineral (Land O' Lakes, Inc., Arden Hills, MN) from breeding through parturition; or CON (n = 14): heifers received the basal diet from breeding through parturition.

[2] Collection periods for blood samples occurred at 3 different time points with 2 blood samples per time point – the first sample on the day of vaccination and the subsequent sample on d 14 following vaccination. Calf ages at for birth collections were: 24 h of age and 15 d of age; pasture turn out: 40.4 ± 3.73 d of age and 54.4 ± 3.73 d of age; and weaning: 184.4 ± 3.73 d of age and 198.4 ± 3.73 d of age.

[3] Serum neutralization (SN) analyses were conducted to determine antibody titer response to vaccination at Oklahoma State University Animal Disease Diagnostic Laboratory (Stillwater, OK) for detection of antibodies for Bovine Viral Diarrhea Virus (BVDV) Type 1 and Type 2, Bovine Respiratory Syncytial Virus (BRSV), Infectious Bovine Rhinotracheitis (IBR), and Parainfluenza 3 (PI3).

[4] Significance considered at $P \leq 0.05$.

Effects of replacing dried distillers grains with solubles with heat-treated soybean meal in forage-based growing cattle diets

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Summary

Seventy Angus-based steers were utilized in an 85-day growing study evaluating the partial replacement of 16% dried distillers grains plus solubles (DDGS) with heat-treated soybean meal by substituting 0, 4, 8, and 12% of DDGS (dry matter basis) with AminoPlus® (Ag Processing Inc., Omaha, NE). Diets were formulated to increase metabolizable protein and lysine as the inclusion of treated soybean meal increased. Body weights and blood samples were collected every 28 days. Individual daily feed intake was measured using an automated feed system (Insentec Roughage Intake Control, Hokofarm B. V., Marknesse, The Netherlands). There were no differences ($P \geq 0.27$) in ending body weight, average daily gain, dry matter intake, and feed-to-gain ratio as heat-treated soybean meal replaced DDGS at increased levels. Additionally, residual carcass characteristics from the growing study resulted in no differences ($P \geq 0.13$) in hot-carcass weight, ribeye area, backfat thickness, marbling score, and calculated yield grade. The increasing inclusion rate of heat-treated soybean meal resulting in theoretical increases in metabolizable protein and lysine concentrations, did not affect steer growth performance. Therefore, the inclusion of heat-treated soybean meal in growing cattle diets will likely be decided based on availability and cost.

Introduction

As the biodiesel industry expands, soybean-crushing plants are being built in North Dakota and surrounding states. These processing facilities extract soybean oil and produce feed coproducts such as soybean meal and soy hulls. The North Dakota Soybean Council estimates yearly crushing capacity at 136 million bushels and 940,000 tons of soybean meal. The increase in soybean meal production could allow producers to take advantage of a local feedstuff. Soybean meal (SBM) has a high protein content that offers a balanced amino acid profile, particularly lysine, for beef cattle diets. This could benefit producers in the Midwest as corn-based diets are typically limiting in the essential amino acid, lysine. However, SBM is highly degradable in the rumen at 70% rumen degradable protein (RDP; NASEM, 2016) so most of the lysine originally from SBM does not flow to the small intestine (Borucki Castro et al., 2007).

Increasing the rumen undegradable protein content in SBM through additional processes with non-enzymatic browning increases ruminal bypass that could improve cattle growth performance if protein and lysine requirements are not met (Coetzer et al., 1999). Heat-treated soybean meal (TSBM) supplies cattle with greater amounts of available amino acids, particularly lysine, and metabolizable protein (MP) by increasing the rumen undegradable protein (RUP) content to approximately 70%.

This study evaluated the effects of increasing concentrations of lysine and metabolizable protein by feeding heat-treated soybean meal in forage-based growing cattle diets. We hypothesize that as TSBM replaces dried distillers grains plus solubles (DDGS), growth performance will improve due to increased metabolizable protein and intestinal supply of essential amino acids.

Materials and Methods

The North Dakota State University Institutional Animal Care and Use Committee approved all animal procedures. Seventy Angus-based steers (initial BW = 656 ± 36 lb) were utilized in an 85-day growing study at the NDSU Beef Cattle Research Complex in Fargo, North Dakota. Steers originated from the NDSU Central Grasslands Research Extension Center. Steers were provided ad libitum access to feed and water in a monoslope barn with drylot access. Based on 16% inclusion of DDGS, dietary treatments using TSBM were formulated to replace DDGS at increasing inclusion levels of 0 (TSBM0), 4 (TSBM4), 8 (TSBM8), and 12% (TSBM12) dietary DM on a dry matter basis (Table 1). Diets were formulated using the empirical solutions model of the Beef Cattle Nutrient Requirements Model 2016 (version 1.0.37.15; NASEM, 2016) to increase metabolizable protein and lysine as the inclusion of TSBM increased in the diet. Lysine requirements in the formulated diets were predicted to be deficient for TSBM0, TSBM4, and TSBM8 whereas TSBM12 was modeled to be in excess. In contrast, the metabolizable protein requirement was considered to be in excess for all treatments (Table 2).

Prior to initiation of the study, steers were limit-fed a common diet containing 40% corn silage, 57% oat hay, and 3% dry meal supplement at 1.8% BW for five days followed by three days of weighing to minimize gut fill variation (Watson et al., 2013). The average of the 3-day weights served as the initial BW. Steers were blocked by weight into light (initial BW = 617 ± 14 lb), medium (initial BW = 653 ± 10 lb), and heavy (initial BW = 698 ± 18 lb) blocks and assigned randomly to treatments. The 3-day weight process was repeated at the end of the study to measure the ending BW. On day 0, steers were implanted with 80 mg of trenbolone acetate and 16 mg of estradiol (Revalor®-IS, Merck Animal Health, Summit, NJ). Body weights and blood were collected every 28 days, however feed intake was individually measured daily using an automated feed system (Insentec Roughage Intake Control, Hokofarm B. V., Marknesse, The Netherlands). Blood was collected via jugular venipuncture, processed into plasma and serum samples, and stored at -4˚C. Plasma samples were utilized to analyze amino acids, glucose, blood urea nitrogen (BUN), and non-esterified fatty acids (NEFA).

After completion of the study, steers returned to the NDSU Central Grasslands Research Extension Center to be finished. All steers received a common diet of corn, DDGS, liquid supplement, and corn silage for the first 121 d. Hay replaced corn silage for the remainder of the feeding period. After 150 days, steers were shipped to a commercial abattoir to collect hot-carcass weight (HCW), ribeye area (REA), backfat thickness (BF), marbling score (MARB), and calculated yield grade (CYG).

Dietary dry matter DM was determined weekly by sampling ingredients and oven-drying at 60° C for 48 hours. Weekly ingredient samples were collected and ground through a 1 mm screen using a Wiley Mill grinder (Thomas Scientific, Swedesboro, NJ). Ground samples were composited into 4-week intervals. Composited ingredient samples were analyzed for laboratory DM, crude protein (CP), organic matter (OM), acid detergent fiber (ADF), neutral detergent fiber (NDF), starch, fat, calcium (C), and phosphorus (P).

Growth performance, blood parameters, and carcass characteristics were analyzed as a generalized randomized block design utilizing the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment (n = 4) and block (n = 3) as fixed effects. Treatment effect, DDGS vs. TSBM, linear, and quadratic orthogonal contrasts were evaluated and adjusted using the Tukey-Kramer methods. Orthogonal contrasts were considered if treatment effect is $P \leq 0.05$. Model residual plots were evaluated to ensure mixed procedure assumptions were met, and necessary outliers were removed.

Results and Discussion

While exploring statistical models on growth performance traits collected, two steers were identified as having data on all traits that were outliers compared to other steers (one from TSBM4 and one from TSBM8). Therefore, these two steers were removed from the analysis dataset, leaving sixty-eight steers with data available.

There were no differences ($P \leq 0.27$) for ending body weight, average daily gain (ADG), dry matter intake (DMI), and feed:gain (F:G; Table 3). As TSBM replaced DDGS, the energy of the diet was likely less due to less digestible fiber and oil in TSBM than in DDGS. However, F:G ratio was not different among treatments, and while not statistically different, TSBM12 improved F:G ratio by 8.9% compared to TSBM0 (7.37 and 8.09, respectively). The formulated metabolizable lysine content for TSBM0, TSBM4, and TSBM8 were all deficient (-6.2, -3.1, and -0.2 grams/day). Thus, metabolizable lysine was possibly limiting in the TSBM0 and TSBM4 diets but was met when TSBM replaced 8% of the DDGS. However, because MP was provided above the requirement in all diets, it is possible that as TSBM replaced DDGS, the additional MP provided by TSBM was utilized as energy. Our results agree with previous research when partially replacing wet corn distillers grains included at 20% of the diet with TSBM at 0, 6, and 12% dietary DM did not affect the growth performance of steers fed smooth brome hay-based diets (Spore et al., 2021).

Replacing DDGS with TSBM did not result in differences ($P \geq 0.33$) in glucose and NEFA concentrations in blood plasma on day 85 of the growing experiment, however BUN concentrations on day 85 were different ($P = 0.01$) across treatments (Table 4). Evaluating treatment diets based on with or without TSBM (DDGS vs. TSBM) detected ($P = 0.01$) an increase in BUN with TSBM inclusion. Additionally, BUN linearly ($P = 0.01$) increased with TSBM inclusion. TSBM0 and TSBM4 contained lower concentrations compared to TSBM8 and TSBM12. The differences in BUN concentrations can be attributed to the increase in dietary CP when increasing the inclusion rate of TSBM.

There were no differences ($P \geq 0.13$) in residual carcass characteristics from treatments during the growing period (Table 5). Although no difference was detected for HCW, ending BW for each treatment in the growing period resulted in a similar trend. This suggests that none of the treatment diets were limited in growth potential during the growing period and subsequently did not experience compensatory gain in the finishing phase. Similarly, Heiderscheit and Hansen (2020) did not detect differences in feeding DDGS or replacing DDGS with TSBM in growing and finishing steer diets.

The results of the current study suggest that increasing concentrations of metabolizable protein and lysine through heat-treated soybean meal supplementation does not affect growing cattle performance with the partial replacement of DDGS when included at 16% of the diet. Therefore, replacing DDGS with TSBM will be based on availability and economics.

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Table 1. Experimental diets

Ingredient, % DM	TSBM0	TSBM4	TSBM8	TSBM12
Corn Silage	44	44	44	44
Oat Hay	37	37	37	37
DDGS[1]	16	12	8	4
TSBM[2]	0	4	8	12
Supplement[3]	3	3	3	3

[1] Dried Distillers Grains with Solubles.

[2] Heat-treated Soybean Meal (AminoPlus®, Ag Processing Inc., Omaha, NE).

[3] Supplement formulated to provide 22.9 g/ton monensin (Rumensin 90, Elanco Animal Health). Supplement contained 1.62% fine ground corn, 1.00% limestone, 0.30% salt, 0.05% beef trace mineral, 0.0126% Vitamin A, 0.002% Vitamin D, 0.0003% Vitamin E on a dry matter basis.

Table 2. Nutrient composition of the experiment diets

Item	TSBM0	TSBM4	TSBM8	TSBM12
DM, % As-fed	46.90	46.90	46.90	46.90
CP, % DM	12.57	13.07	13.58	14.08
Fat, % DM	2.61	2.44	2.26	2.08
NDF, % DM	52.15	51.30	50.45	49.60
ADF, % DM	29.70	29.41	29.13	28.85
Starch, % DM	16.95	16.88	16.81	16.74
Metabolizable protein balance[1], g/d	40.5	68.9	89.8	117.5
Lysine balance[1], g/d	-6.23	-3.09	-0.24	2.80

[1] Calculated utilizing the empirical solutions model of the Beef Cattle Nutrient Requirements Model 2016 (version 1.0.37.15; NASEM, 2016).

Table 3. Overall performance of growing cattle on forage-based diets with heat-treated soybean meal

	Treatments[1]					*P* - value			
Item	TSBM0	TSBM4	TSBM8	TSBM12	SEM	TRT	DDGS vs. TSBM	Linear	Quadratic
Steers, n	16	18	17	17	--	--	--	--	--
Initial BW, lb	656	656	656	656	3.8	0.99	0.95	0.95	0.95
Ending BW, lb	834	847	837	846	9.5	0.68	0.39	0.52	0.84
ADG, lb/day	2.09	2.25	2.13	2.24	0.100	0.57	0.31	0.45	0.80
DMI, lb/day	16.9	16.8	16.7	16.5	0.36	0.90	0.59	0.46	0.92
Feed:Gain	8.09	7.47	7.84	7.37	--	0.27	0.14	0.19	0.84

[1] Dietary percent of heat-treated soybean meal (TSBM) replacing a proportion of 16% dried distillers grains plus solubles in the diet; TSBM0: 0% heat-treated soybean meal, TSBM4: 4% heat-treated soybean meal, TSBM8: 8% heat-treated soybean meal, TSBM12: 12% heat-treated soybean meal.

[ab] Different superscripts in the same row differ $P \leq 0.05$.

Table 4. Blood characteristics of growing cattle on forage-based diets with heat-treated soybean meal on day 85

	Treatments[1]					*P* - value			
Item	TSBM0	TSBM4	TSBM8	TSBM12	SEM	TRT	DDGS vs. TSBM	Linear	Quadratic
Steers, n	16	18	17	17	--	--	--	--	
Glucose, µmol/L	4.71	4.60	4.81	4.70	0.080	0.33	0.91	0.65	0.99
BUN, µmol/L	7.60^{a}	8.41^{a}	9.77^{b}	10.68^{b}	0.370	0.01	0.01	0.01	0.89
NEFA, µmol/L	537.81	534.70	559.85	473.85	44.25	0.53	0.77	0.39	0.34

[1] Dietary percent of heat-treated soybean meal (TSBM) replacing a proportion of 16% dried distillers grains plus solubles in the diet; TSBM0: 0% heat-treated soybean meal, TSBM4: 4% heat-treated soybean meal, TSBM8: 8% heat-treated soybean meal, TSBM12: 12% heat-treated soybean meal.

[ab] Different superscripts in the same row differ $P \leq 0.05$.

Table 5. Carcass characteristics of growing cattle on forage-based diets with heat-treated soybean meal

	Treatments[1]					*P* - value			
Item	TSBM0	TSBM4	TSBM8	TSBM12	SEM	TRT	DDGS vs. TSBM	Linear	Quadratic
Steers, n	16	18	17	17	--	--	--	--	
HCW, lb	799	812	798	819	13.8	0.61	0.49	0.44	0.76
REA, lb^2	12.7	12.6	13.0	13.1	0.26	0.50	0.57	0.21	0.69
Backfat, in	0.51	0.53	0.46	0.49	0.03	0.47	0.73	0.46	0.82
Marbling	414	413	388	414	14.7	0.49	0.59	0.25	0.99
Calculated YG	3.35	3.52	3.10	3.27	0.132	0.13	0.71	0.25	0.99

[1] Dietary percent of heat-treated soybean meal (TSBM) replacing a proportion of 16% dried distillers grains plus solubles in the diet; TSBM0: 0% heat-treated soybean meal, TSBM4: 4% heat-treated soybean meal, TSBM8: 8% heat-treated soybean meal, TSBM12: 12% heat-treated soybean meal.

[ab] Different superscripts in the same row differ $P \leq 0.05$.

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The CGREC Annual Field Day will be held on July 8, 2024 and open to the public

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