PATCH-BURN GRAZING IN SOUTHWESTERN NORTH DAKOTA: ASSESSING ABOVE AND BELOWGROUND RANGELAND ECOYSTEM RESPONSES

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Patch-burn grazing in southwestern North Dakota: assessing above and belowground rangeland ecosystem responses	
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ABSTRACT

Rangelands are heterogeneous working landscapes capable of supporting livestock production and biodiversity conservation, and heterogeneity-based rangeland management balances the potentially opposing production and conservation goals in these working landscapes. Within fire-dependent ecosystems, patch-burn grazing aims to create landscape patterns analogous to pre-European rangelands. Little work has tested the efficacy of patch-burn grazing in northern US Great Plains. We investigated patch contrast in above and belowground ecosystem properties and processes during the summer grazing seasons from 2017 – 2020 on three patch-burn pastures stocked with cow-calf pairs and three patch-burn pastures stocked with sheep. We focused on vegetation structure, plant community composition, forage nutritive value, grazer selection, livestock weight gain, soil nutrient pools, soil microbial community composition, and decomposition activity. We used mixed-effect models and ordinations to determine whether differences: along the time since fire intensity gradient, between ecological sites, and between grazer types existed. Despite no significant shifts in the plant community, structural heterogeneity increased over time as the number of time since fire patches increased and was higher than homogeneously managed grasslands. Grazing livestock preferred recently burned patches where the available forage had a higher nutritive value and lower available biomass than surrounding patches at a given point in time. With the exception of 2018, livestock weight gains were consistent. Soil nutrient pools and microbial abundances differed more by ecological site than by the time since fire intensity gradient, and ecological sites exhibited similar nutrient and microbial responses to the time since fire intensity gradient. That belowground response variables were mostly resistant to patch-burn grazing is supportive of further use of this management, especially given the desirable results with aboveground response variables.

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CHAPTER ONE – THE GRASS IS GREENER IN THE BURNED PATCH: SPATIO-TEMPORAL PATTERNS OF RANGELAND FORAGE NUTRITIVE VALUE AND GRAZER SELECTION IN US NORTHERN GREAT PLAINS

Abstract

We measured available forage biomass, forage nutritive value, forage moisture content, and grazer selection at monthly intervals on three patch-burn pastures stocked with cow-calf pairs and three patch-burn pastures stocked with gestating ewes over four summer grazing seasons. We determined crude protein, acid detergent fiber, acid detergent lignin, and neutral detergent fiber from monthly forage clippings using near-infrared spectroscopy. We measured average daily gain of calves, cows, and ewes each year to evaluate livestock performance.

Livestock preferred recently burned patches where available forage had lower available forage biomass, fiber components, and lignin and higher crude protein and moisture content than other patches. Except for 2018, livestock weight gains were consistent. Attraction to recently burned patches helps maintain structural contrast. Higher biomass in patches with increased time since fire serves as a grass bank in the event of a drought, maintains fuel load for prescribed burns, and maintains structural heterogeneity.

Introduction

Rangelands are inherently heterogeneous working landscapes challenged with supporting livestock production and biodiversity conservation amidst threats from land use change, climatic variability, and socio-economic change (Fuhlendorf et al., 2017; Derner et al., 2018; Sayre, 2017). Understanding how dynamic processes like grazer selection respond to imposed management actions and interact with ecosystem properties like vegetation structure and aboveground herbaceous biomass informs tradeoffs between potentially conflicting goals in

rangeland landscapes (Porensky et al., 2021; Raynor et al., 2021a; Senft et al., 1987). The inherent heterogeneity is driven by variation in ecosystem properties like topography, soil moisture, nutrient availability, and plant community composition (Fuhlendorf et al., 2017; Li & Reynolds, 1995). Forage nutritive value and biomass production are ecosystem properties that influence grazer selection and are dynamic over time given climatic fluctuations and disturbance regimes (Archibald et al., 2005; Hoover et al., 2021; McGranahan et al., 2016; Sensenig et al., 2010).

Both discrete disturbance events and interactions between multiple disturbance regimes can alter ecosystem properties. The North American Great Plains encompasses rangeland ecosystems that have a long evolutionary history of disturbance through fire and grazing (Anderson, 2006; Fuhlendorf et al., 2009). The interactive effects of these disturbances can maintain contrast in ecosystem properties between disturbed and undisturbed patches of a landscape to a greater degree than either disturbance individually (Augustine et al., 2019; Fuhlendorf et al., 2012). Together, fire and grazing shape ecosystem properties by redistributing nutrient inputs, reducing litter accumulation, and increasing soil temperature (Augustine et al., 2013; Pausas & Bond, 2020; Sitters & Olde Venterink, 2015).

An individual fire requires sufficient fuel, continuity, and weather conditions to spread through grassland fuelbeds (Bond & Keeley, 2005). Patches of low fuels and/or fuels with excessive moisture content can limit the ability of a fire to pass through an area (McGranahan et al., 2013; Raynor et al., 2021). Considering that biomass regrowth following a fire typically has higher protein and lower fiber content than biomass from unburned areas, spatially-discrete fires create patches of available biomass that contrast in nutritive value and available forage (Sensenig et al., 2010).

Herbivores respond to inherent system properties like topoedaphic features and distance to water and shade, as well as landscape-level heterogeneity in forage nutritive value and available forage, when selecting grazing locations (Allred et al., 2011; Charnov, 1976; Laca et al., 2010; Raynor et al., 2021a; Senft et al., 1987). The influence of topoedaphic features and distance to shade and water are more pronounced in extensive, arid, and rugged rangelands (Ganskopp & Bohnert, 2009; Raynor et al., 2021a). The intensity and duration of grazing can determine how system properties like available forage biomass and nutritive value respond to grazing by slowing or accelerating the accumulation of fiber components and decline in crude protein content (Collins & Newman, 2018; Semmartin et al., 2008). In the absence of grazing or fire, the crude protein in available forage is typically higher earlier in the growing season and then declines as the plant reaches maturity, while fiber components and lignin are lowest earlier in the season and increase over time (Collins & Newman, 2018; Fynn, 2012). Grazed areas tend to have lower fiber content than ungrazed areas (Semmartin et al., 2008). Repeated grazing in an area also maintains a lower amount of available forage with higher nutritive value (Grant et al., 2019; Sensenig et al., 2010).

Herbivores often preferentially graze in areas where the available forage has higher crude protein content and lower biomass and fiber content than other areas (Allred et al., 2011; Ganskopp & Bohnert, 2009; Sensenig et al., 2010). Protein and energy requirements vary with the class, age, activity level, breed, and species of grazing herbivores (NASEMR, 2016; National Research Council, 2007). Failure to meet minimum requirements can slow growth and lead to weight loss and animal welfare issues. Although carbohydrates provide an important source of energy for ruminant herbivores, excessive fiber carbohydrates and lignin decrease the palatability and digestibility of available forage (Collins & Newman, 2018; NASEMR, 2016; National

Research Council, 2007). Compared with dairy and feedlot systems, the protein and energy requirements of grazing livestock are poorly understood (Galyean et al., 2016; NASEMR, 2016; National Research Council, 2007; Schwab & Broderick, 2017).

Conventional grazing management often aims for spatially-homogeneous herbivory through even spatial distribution of grazing pressure to maximize utilization of available forage within a rangeland and minimize overusing a given area (Fuhlendorf et al., 2012; Holechek et al., 2010). Managers either attempt to override grazer preference for fine-scale variation in vegetation through fencing, mineral supplementation, or herding, or seek to homogenize the forage resource through whole-pasture burning (Bohnert & Stephenson, 2016; Fynn, 2012; Smith & Owensby, 1978). The desire for homogeneously-dispersed grazing pressure reduces structural heterogeneity in these landscapes and narrows the suitability for rangeland wildlife (Fuhlendorf & Engle, 2004). Patch-burn grazing is an alternative strategy that uses spatially discrete prescribed fires applied to a portion of a larger pasture to encourage heterogeneous grazer spatial distribution and forage use (Fuhlendorf & Engle, 2001). Grazing herbivores have access to the entire pasture, but typically spend more time in recently burned patches throughout the grazing season (Powell et al., 2018; Spiess et al., 2020). Grazer attraction to recently burned patches is found globally in both wild and domesticated herbivores (Allred et al., 2011; Archibald et al., 2005; Sensenig et al., 2010; Wallace & Crosthwaite, 2005). Through this coupling of disturbances, patch-burn grazing emphasizes increasing patch-level contrast in ecosystem properties and processes through management rather than minimizing them.

Although initially proposed as a way to increase structural heterogeneity in rangelands, patch-burn grazing has also shown promise for reducing inter-annual variability in forage production and livestock performance (Allred et al., 2014; McGranahan et al., 2016; Spiess et al.,

2020). Livestock weight gains are a commonly used assessment of grazing livestock performance that are sensitive to variability in biomass production and climatic conditions like precipitation and temperature (Augustine et al., 2020; Fynn, 2012; Reeves et al., 2013, 2014). Although not a guarantee across all implementations, patch-burn grazing has improved livestock performance during drought conditions compared to homogenously managed rangelands under similar conditions (Allred et al., 2014; Spiess et al., 2020). In previous semi-arid patch-burn grazing experiments, burn patch attraction has persisted and diminished over grazing seasons during droughts (Augustine & Derner, 2014; Spiess et al., 2020).

Despite an understanding that forage nutritive value and quantity patterns influence grazer selection, rangeland forage is rarely studied in a manner sensitive to spatial and temporal variability (Allred et al., 2011; Ganskopp & Bohnert, 2009; Spiess et al., 2020). Rangeland forage sampling typically focuses on crude protein and either compositing samples across spatial and/or temporal scales or spatially intensive sampling at a point in time (Allred et al., 2011; Ganskopp & Bohnert, 2009). We have previously found that cattle maintained a preference for recently burned patches with lower available biomass than other patches despite there not being a significant difference between the crude protein content over the grazing season (Spiess et al., 2020). This result suggests that differences in additional nutritive value parameters could be supporting burn patch attraction in the absence of contrast in crude protein (Ganskopp & Bohnert, 2009; Sensenig et al., 2010; Spiess et al., 2020). Further exploring the associations between patterns in forage nutritive value and grazer selection over grazing seasons is crucial to understanding one of the underlying processes supporting increased structural heterogeneity and stabilized livestock production in rangelands managed with patch-burn grazing (Allred et al., 2014; Spiess et al., 2020).

This study is part of a broader investigation into the efficacy of patch-burn grazing on ecosystem properties and process in the northern Great Plains. The objectives of this study were to determine: 1) differences in forage nutritive value between patches in patch-burn grazing pastures; 2) patch level selection patterns of grazing livestock; 3) how forage nutritive value relates to grazer selection and available biomass over multiple grazing seasons; 4) whether livestock weight gains differ between years; and 5) differences in results between cow-calf and sheep patch-burn pastures. We expected the available forage in recently burned patches to have lower fiber values and available biomass than other patches in addition to having higher crude protein and moisture content (Powell et al., 2018; Sensenig et al., 2010; Spiess et al., 2020). We expected livestock to prefer for recently burned patches across grazing seasons (Spiess et al., 2020). We expected to see dissimilarity between fiber components and crude protein, divergence along the time since fire gradient, and divergence along the intra-season month gradient (Sensenig et al., 2010; Spiess et al., 2020). We did not expect major differences between cattle and sheep patch-burn pastures with similar stocking rates (Spiess et al., 2020).

Methods

Study Location and Experimental Design

We conducted our study in the northern US Great Plains on six, 65 ha rangeland pastures in southwestern North Dakota near the town of Hettinger at the Hettinger Research Extension Center (HREC; 46.003150, -102.644529). Hettinger, North Dakota has a mean annual precipitation of 360 mm and mean temperature range during the May to September grazing season of 12°C (May) to 21°C (July and August). The common ecological sites on the pastures include: clayey, loamy, sandy, saline lowland, and thin claypan (Soil Survey Staff, 2020). Having been initially established on former crop fields under the Conservation Reserve Program

in the late 1980s-early 1990s, the pastures are dominated by species typical of conservation plantings, including intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey), alfalfa (*Medicago sativa* L.), and yellow sweetclover (*Melilotus officinalis* [L.] Lam.) (Soil Conservation Service, 1989, 1992). Other common plants include smooth brome (*Bromus inermis* Leyss), Kentucky bluegrass (*Poa pratensis* L.), and crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.).

Three pastures were stocked with gestating Rambouillet ewes (*Ovis aries* L.), and three pastures were stocked with cow-calf pairs (*Bos taurus* L.). We targeted a similar stocking rate (0.5 ha • AUM⁻¹) and stocking period (late May/early June – mid-September) for both grazer types over the course of the study; grazer type was assigned prior to the implementation of burning treatments. Patch-burning consisted of burning a quarter of each pasture (~15 ha) annually in the dormant season (late summer/early fall or early spring) prior to the grazing season. Since this was the initial implementation of patch-burn grazing on these pastures, we had an increasing number of times since fire over the course of the study beginning with prescribed burns in 2016 (ahead of the 2017 grazing season) until completion of the first burn rotation with prescribed burns prior to the 2020 grazing season (Spiess et al., 2020).

Data Collection

Available Forage Biomass and Nutritive Value

We determined available forage biomass with a nested hierarchical sampling design in which we clipped 25 x 25 cm quadrats monthly throughout each grazing season at sampling points distributed across each patch, within each pasture. Within patches, sample points were stratified by dominant soil characteristics, delineated as ecological sites by the USDA Natural Resource Soil Conservation Service (Soil Survey Staff, 2020). We weighed each sample once to

record an initial post-clipping 'fresh' weight and then a second time following drying in an oven for 48 hours at 50° C. We used the post-drying weight as the measurement of available forage biomass at that sampling point and calculated the moisture content of each sample using the preand post-drying weights.

We determined forage nutritive value parameters of each dried forage sample using near-infrared spectroscopy (NIRS). Following the protocol described by Spiess et al. (2020), we ground forage samples to pass a 1 mm mesh, scanned samples using a XDS-NIRS rapid content analyzer (FOSS Analytical, Hillerød, Denmark), and then determined values from scans with ISI scan software version 4.10.015326 (Infrasoft International L.L.C., State College, Pennsylvania) using a calibration curve based on wet chemistry analysis performed by the North Dakota State University Animal Science laboratory on a representative subsample (Spiess et al., 2020). Here, we report NIRS results for each forage sample from the HREC 2017-2020 grazing seasons including: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL).

Grazer Selection

To evaluate grazer selection, we counted fecal pats within 5 m of forage sampling points when clipping monthly forage samples as a passive measurement of livestock presence in that area. Fecal pat density is a commonly used method for passively tracking distribution of both wild and domestic grazers in rangelands (Archibald et al., 2005; McGranahan et al., 2014; Powell et al., 2018; Spiess et al., 2020). To avoid counting fecal pats in successive months, we only note pats that were still fresh in appearance or not entirely dry throughout. We counted distinct cattle pats, but grouped sheep pats within a roughly 10 cm diameter circle as a single unit (Spiess et al., 2020). We calculated a selection index (SI) to standardize the fecal count data

between grazer type and across sampling events following a previous selection index for visual observations on patch-burn grazing pastures (Powell et al., 2018). We divided the proportion of fecal pats at a sampling point relative to the total count for a pasture in a given month by the proportion a point represents of total points in that pasture. A SI of 1 indicates use even with occurrence in pasture; SI > 1 indicates preference; and SI < 1 indicates a use level lower than occurrence in pasture.

Livestock Weight Gains

We monitored the individual performance of calves, cows, and ewes in each pasture by comparing each animals' weight at the end of each grazing season to their initial weight. We weighed animals on two consecutive days prior to release into pastures and following removal from pastures to determine the average starting and finishing weight of each animal. We distributed animals amongst pastures based on starting weight to achieve a similar head count and average starting weight for the three replicate pastures of each grazer type. We calculated the average daily gain for each animal by subtracting the average starting weight from the average finishing weight and then dividing by the number of days that animal grazed in a pasture. We also include livestock weight gain data from 2016 as a comparison point where these pastures were grazed at the same stocking rate, but without fire.

Data Analysis

Linear Regression

We performed all data manipulation and statistical analysis in the R statistical environment assisted by the 'tidyverse' package (R Core Team, 2019; Wickham et al., 2019) To determine differences among response variables (forage biomass, moisture content, SI, CP, NDF, ADF, and ADL) between patches with varying time since fire (TSF) and differences between

grazer treatments (Grazer), we fit a mixed-effect model for each response variable with TSF, Grazer, and the TSF×Grazer interaction as fixed effects using functions *lmer* for responses with either a normal (ADF, NDF, ADL, moisture) or log-normal distribution (forage biomass, CP) and *glmer* for those with a gamma distribution (SI) in the 'lme4' package (Bates et al., 2015). We determined the distribution that best fit each response variable individually using function *fitdist* in the 'fitdistrplus' package (Delignette-Muller & Dutang, 2015). We evaluated fixed effects with analysis of deviance (reported as '\chi2 statistic, p value') using function *Anova* in the 'car' package (Fox & Weisberg, 2019). We created an intermediate TSF factor level (INT) by combining patches with one year since fire and patches with two years since fire after initial analysis revealed negligible differences between the two and similar relationships relative to other factor levels. Thus, we are comparing responses across the following factor levels for the time since fire intensity gradient: not yet burned (NYB), recently burned (RB), intermediate years since (INT), and three years since fire (3YSF).

After determining significant fixed effects, we used post-hoc pairwise comparisons to compare factor levels using the function *emmeans* in the 'emmeans' package (Lenth, 2021). We report the contrast estimate and 95% confidence interval (CI) for significant factor level contrasts to indicate the directionality and size of the difference as '(Level 1 – Level 2: calculated value(CI lower, CI upper))'. For significant TSF×Grazer interactions, we evaluate TSF contrasts within grazer treatments. To determine differences over grazing seasons and across years, we nested pasture within month within year as a random effect for overall mixed-effect models and account for repeated sampling measures over time (Cheng et al., 2010).

Multivariate Analyses

To explore multivariate relationships between nutritive value parameters, grazer selection, and month, we used unconstrained ordination and post-hoc factor and vector fitting with functions *metaMDS*, *capscale*, and *envfit* from the 'vegan' package (Oksanen et al., 2019) and function *pairwise.factorfit* in the 'RVAideMemoire' package (Hervé, 2021). We averaged nutritive value parameters and selection index to the patch level for each pasture, month, and year. We used CP, NDF, ADF, and ADL as the species variables and forage biomass, moisture content, Grazer, TSF, and month as the environmental variables. We used the Euclidean distance measure for the unconstrained ordination after comparing the stress values and proportion of the data explained of multiple distance measures with the nutritive value data.

Livestock Performance

We compared the average daily gains between years for calves, cows, and ewes using mixed-effect models and post-hoc comparisons between years. We used year as a fixed factor effect and pasture nested within year as a random effect for each model. To determine whether animals lost, maintained, or gained weight each year, we used mixed-effect models with a zero intercept and pasture nested within year as a random effect, Tukey post-hoc comparisons against 0 with package 'multcomp' (Hothorn et al., 2020), and function *confint* in base R.

Results

Forage Biomass and Nutritive Value

Time since fire (TSF) and the TSF×Grazer interaction were significant predictor variables for available biomass (TSF χ^2 : 414.13, p < 0.001; TSF×Grazer χ^2 : 52.98, p < 0.001; Figure 1.1). Recently burned patches had the lowest available forage biomass for both cattle and sheep pastures (RB – INT: -0.60(CI: -0.69,-0.51); RB – 3YSF: -0.76(CI: -0.91,-0.61); RB –

NYB: -0.52(CI: -0.62, -0.43)). There were no additional TSF differences in biomass for cattle pastures. However, in sheep pastures, intermediate and patches with three years since fire had higher available biomass than not yet burned patches (INT – NYB: 0.25(CI: 0.13, 0.39); 3YSF – NYB, 0.48(CI: 0.26, 0.7). Furthermore in sheep pastures, patches with three years since fire had higher available biomass than intermediate patches (Estimate = 0.23(CI: 0.03, 0.43)).

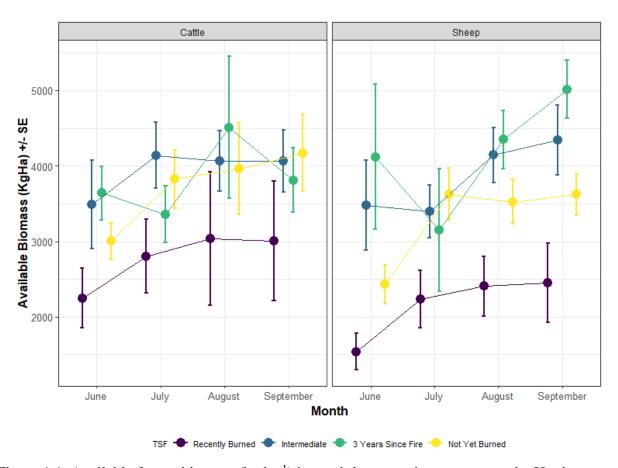


Figure 1.1. Available forage biomass (kg ha⁻¹) in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep).

Recently burned patches had lower available forage biomass than other patches for cattle and sheep pastures. Points represent mean available forage values for patch types \pm standard error.

Time since fire and grazer type were significant predictor variables for neutral detergent fiber (TSF χ^2 : 244.15, p < 0.001; Grazer χ^2 : 7.61, $p \le 0.01$; Figure 1.2). Available forage in recently burned patches had the lowest NDF values for cattle and sheep pastures (RB – INT: -

3.22(CI:-3.79, -2.64), -3.68(CI: -4.66, -2.71), -2.27(CI:-2.86, -1.68)). Intermediate and patches with three years since fire had higher NDF values than not yet burned patches (Estimates: 0.95(CI: 0.33, 1.57), 1.42(CI: 0.37, 2.46)). Although a significant predictor variable, there was not a difference in NDF values between cattle and sheep pastures (Cattle – Sheep: -1.94(CI: -4, 0.12)). Neutral detergent fiber generally increased over time for all patches.

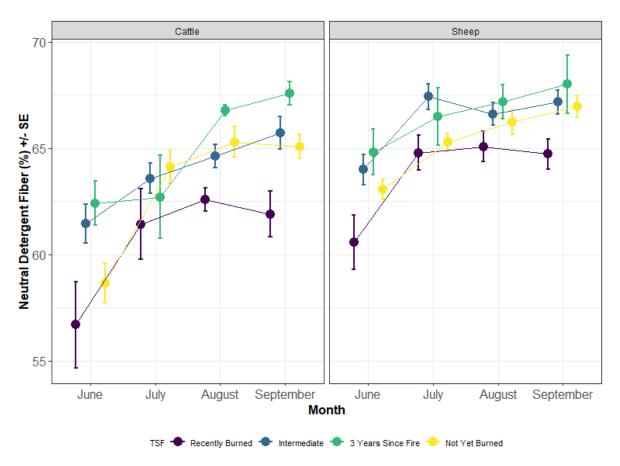


Figure 1.2. Neutral detergent fiber content of available forage biomass in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep). Recently burned patches had lower neutral detergent fiber content than other patches for cattle and sheep pastures. Points represent mean neutral detergent fiber values for patch types \pm standard error.

Time since fire was the only significant predictor variable for acid detergent fiber (TSF χ^2 : 388.67, p < 0.001; Figure 1.3). Available forage in recently burned patches had lower ADF values than all other patches (RB – INT: -2.63(CI: -3.01, -2.24), RB – 3YSF: -3.19(CI: -3.84, -

2.54), RB – NYB:-1.98(CI: -2.37, -1.59)). Intermediate and patches with three years since fire had higher ADF values than not yet burned patches (INT – NYB: 0.65(CI: 0.24, 1.05), 3YSF – NYB: 1.21(CI: 0.52, 1.9)). Time since fire was the only significant predictor variable for acid detergent lignin i.e. ADL (TSF χ^2 : 288.56, p < 0.001; Figure 1.4). Available forage in recently burned patches had the lowest ADL values for cattle and sheep pastures (RB – INT: -0.52(CI: -0.62, -0.43), RB – 3YSF: -0.49(CI: -0.66, -0.32), RB – NYB: -0.56(CI: -0.66, -0.46)). Acid detergent fiber and lignin generally increased over time for most patches.

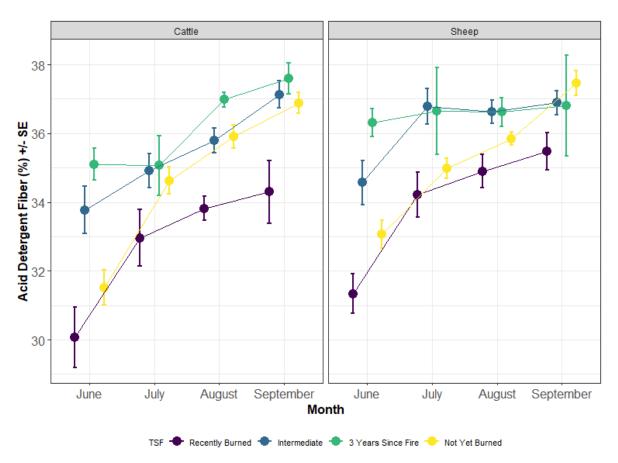


Figure 1.3. Acid detergent fiber content of available forage biomass in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep). Recently burned patches had lower acid detergent fiber content than other patches for cattle and sheep pastures. Points represent mean acid detergent fiber for patch types ± standard error.

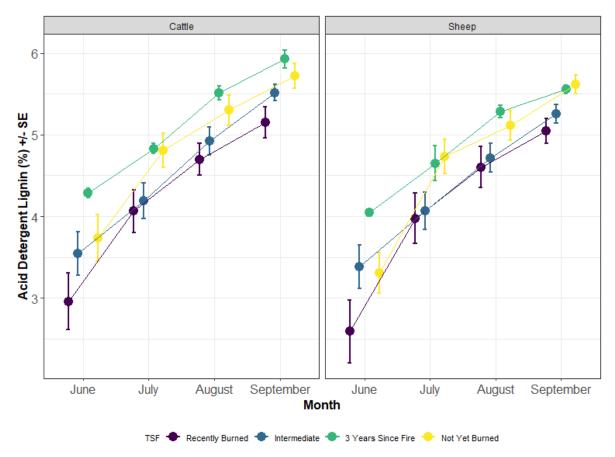


Figure 1.4. Acid detergent lignin (%) in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep).

Recently burned patches had lower acid detergent lignin content than other patches for cattle and sheep pastures. Points represent mean acid detergent lignin for patch types \pm standard error.

Time since fire and the TSF×Grazer interaction were significant predictor variables for crude protein (TSF χ^2 : 436.12, p < 0.001; TSF×Grazer χ^2 : 7.61, p < 0.001; Figure 1.5). In cattle pastures, available forage in recently burned and not yet burned patches had higher CP values than intermediate and patches with three years since fire (RB – INT: 0.22(CI: 0.18, 0.27), RB – 3YSF: 0.39(CI: 0.32, 0.47), NYB – INT: 0.22(CI: 0.17, 0.26), NYB – 3YSF: 0.39(CI: 0.3, 0.47)), and intermediate patches had higher CP values than patches three years since fire (INT – 3YSF: 0.17(CI: 0.10, 0.24)). In sheep pastures, recently burned patches had higher CP values than all other patches (RB – INT: 0.21(CI: 0.17, 0.25), RB – 3YSF: 0.27(CI: 0.19, 0.34), RB –

NYB: 0.15(CI: 0.1, 0.19)), and not yet burned patches had higher CP values than intermediate and patches with three years since fire (NYB – INT: 0.06(CI: 0.02, 0.11), NYB – 3YSF: 0.12(CI: 0.04, 0.20)). Crude protein generally decreased over time for most patches.

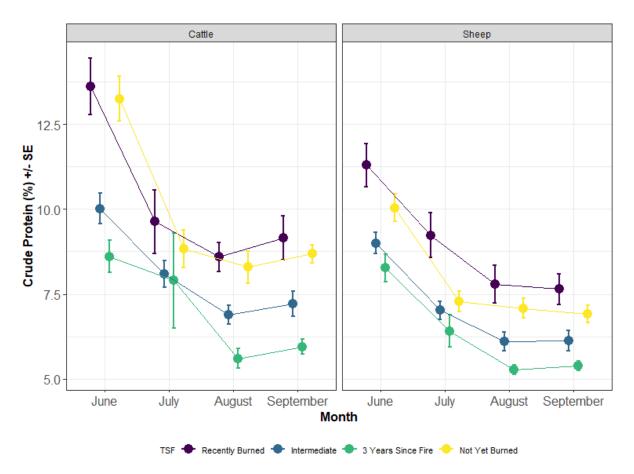


Figure 1.5. Crude protein content of available forage biomass in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep).

Recently burned patches had higher crude protein content than intermediate and patches with three years since fire for cattle pastures. Crude protein content was highest in recently burned patches for sheep pastures followed by not yet burned patches. Points represent mean crude protein content for patch types \pm standard error.

Time since fire was the only significant predictor variable for moisture content (TSF χ^2 : 410.17, p < 0.001; Figure 1.6). The moisture content of available forage biomass was highest in recently burned patches for cattle and sheep pastures (RB – INT: 7.31(CI: 6.18, 8.44), RB – 3YSF: 10.03(CI: 8.08, 11.98), RB – NYB: 6.82(CI: 5.65, 8.0)), and intermediate and not yet

burned patches had higher moisture content then patches with three years since fire (INT – 3YSF: 2.72(CI: 0.88, 4.57), NYB – 3YSF: 3.21(CI: 1.11, 5.31)). Moisture content generally decreased over time for each patch.

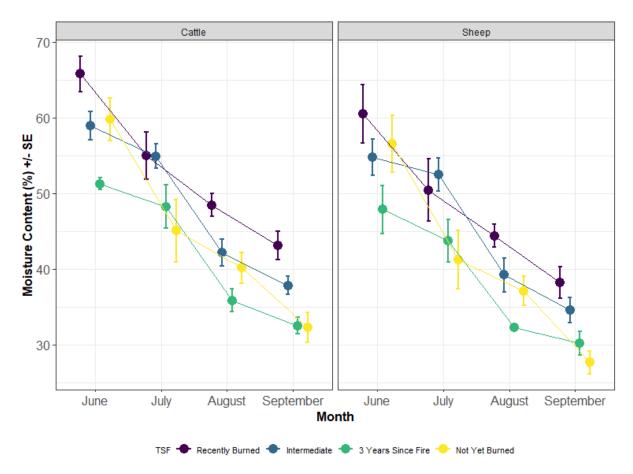


Figure 1.6. Moisture content of available forage biomass in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep).

Recently burned patches had higher moisture content than other patches cattle and sheep

pastures. Points represent mean forage moisture content than other patches cattle and sheep pastures. Points represent mean forage moisture content for patch types ± standard error.

Grazer Selection and Ordinations

Time since fire was the only significant predictor variable for selection index (TSF χ^2 : 130.96, p < 0.001; Figure 1.7). Recently burned patches had the highest SI values for cattle and sheep pastures (RB – INT: 1.19(CI: 0.8, 1.54), RB – 3YSF: 3.34(CI: 1.7, 4.96), RB – NYB: 0.69(CI: 0.4, 0.95)). Not yet burned and intermediate patches had higher SI values than patches

with three years since fire (INT – 3YSF: 2.15(CI: 0.4, 3.8), NYB – 3YSF: 2.65(CI: 1.01, 4.2)), and not yet burned patches had higher SI values than intermediate patches (NYB – INT: 0.5(CI: 0.1, 0.9)).

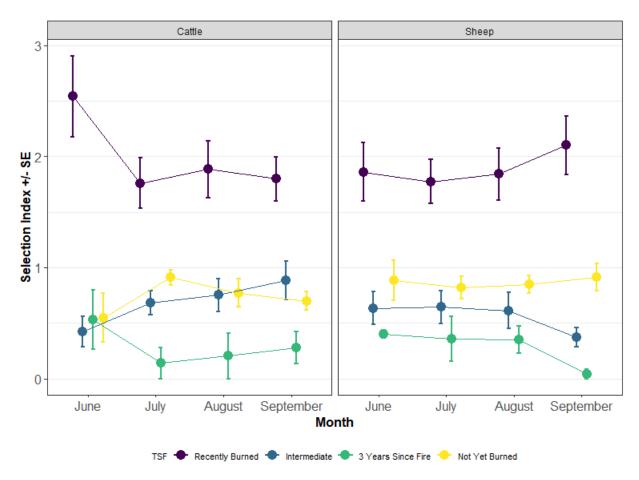


Figure 1.7. Selection indices calculated from fecal count data in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota separated by patch-level time since fire (TSF) and grazer type (cattle or sheep).

Recently burned patches had higher selection indices than other patches cattle and sheep pastures indicating preference. Points represent mean selection index for patch types \pm standard error.

The first two axes of the nutritive value parameter ordination explain 99% of the variation in the data (PCA Axis 1: 71%, PCA Axis 2: 28%). We found dissimilarity along the moisture content ($r^2 = 0.63$, p = 0.002), biomass ($r^2 = 0.2$, p = 0.002), and selection index ($r^2 = 0.12$, p = 0.002) vector gradients (Figure 1.8). We also found dissimilarity with factor variables month ($r^2 = 0.24$, p = 0.002) and time since fire ($r^2 = 0.10$, p = 0.002). All patches were

significantly different from each other ($p \le 0.001$), and recently burned patches were more associated with CP and less associated with ADF, ADL, and NDF than other patches (Figure 1.9). All months were significantly different from each other ($p \le 0.001$) and separated in order of occurrence along the first axis with June more associated with CP and September less associated with CP (Figure 1.10).

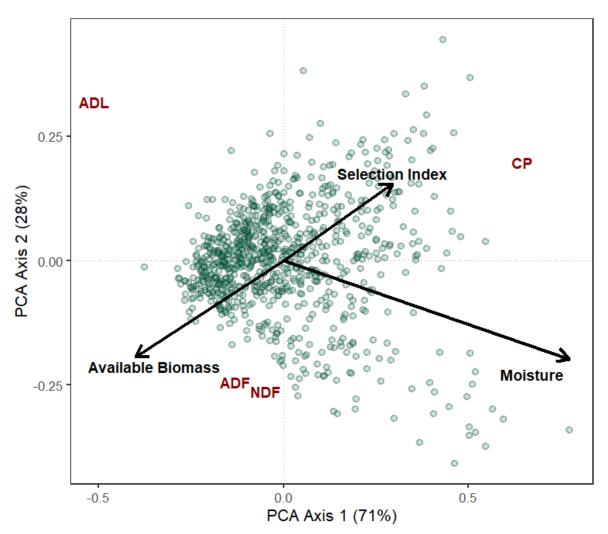


Figure 1.8. Unconstrained ordination of forage nutritive value parameters collected at the Hettinger Research Extension Center in North Dakota showing significant vector gradients. The ordination uses the Euclidean distance measure. PCA Axis 1 (71%) and PCA Axis 2 (28%) explain 99% of the relationship between acid detergent fiber (ADL), acid detergent lignin (ADF), neutral detergent fiber (NDF), and crude protein (CP). Available forage biomass ($r^2 = 0.20$, p = 0.002), grazer selection index ($r^2 = 0.11$, p = 0.002), and forage moisture content ($r^2 = 0.64$, p = 0.002) were significant vector gradients. Points represent calculated patch level site scores.

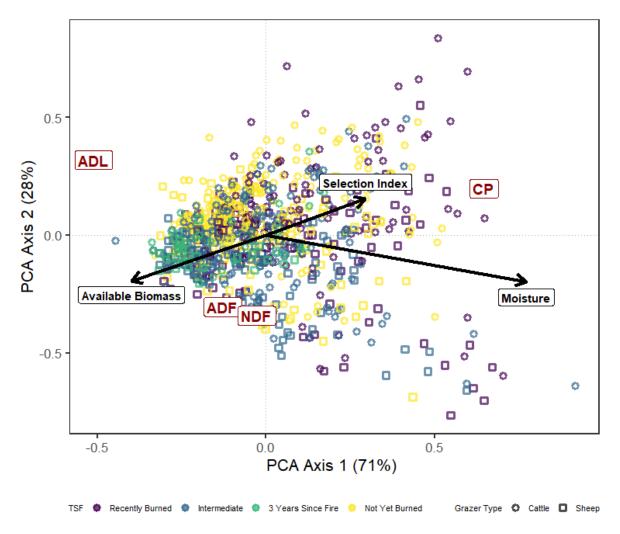


Figure 1.9. Time since fire associations in an unconstrained ordination with forage nutritive value parameters collected at the Hettinger Research Extension Center in North Dakota. The ordination uses the Euclidean distance measure. PCA Axis 1 (71%) and PCA Axis 2 (28%) explain 99% of the relationship between acid detergent fiber (ADL), acid detergent lignin (ADF), neutral detergent fiber (NDF), and crude protein (CP). Points represent calculated patch level site scores. Time since fire was a significant factor variable that explained 10% of the variation in the ordination with all TSF patches being significantly different from each other.

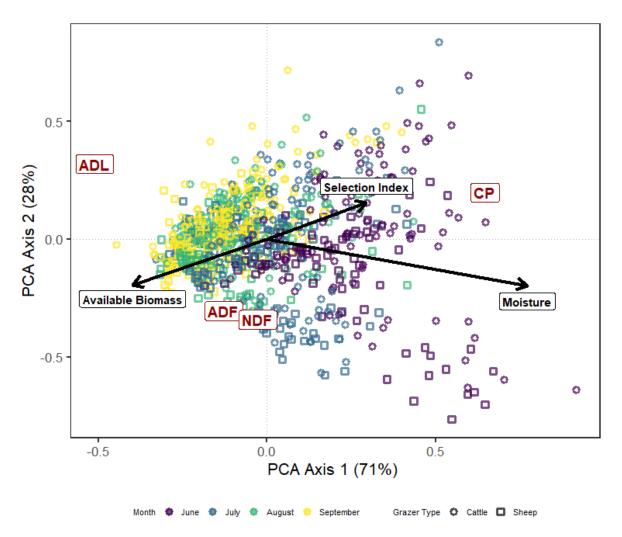


Figure 1.10. Associations in an unconstrained ordination between month and forage nutritive value parameters collected at the Hettinger Research Extension Center in North Dakota. The ordination uses the Euclidean distance measure. PCA Axis 1 (71%) and PCA Axis 2 (28%) explain 99% of the relationship between acid detergent fiber (ADL), acid detergent lignin (ADF), neutral detergent fiber (NDF), and crude protein (CP). Points represent calculated patch level site scores. Month was a significant factor variable that 24% of the variation in the ordination with all months being significantly different from each other.

Livestock Performance

Average daily gains (ADG) were positive for each year and class of livestock (Figure 1.11). Year was a significant predictor variable for each livestock class, indicating potential differences in weight gains between years (Range $\chi 2$: 52.94 – 101.22, p < 0.001). Cow ADG was highest in 2018 (2018 – 2016: 0.64(0.17 – 1.11), 0.83(0.36 – 1.31), 0.66(0.18 – 1.13), 0.67(0.19)

- 1.14)), while calf ADG was lowest in 2018 (Estimates: -0.34(-0.58 – -0.1), -0.45(-0.69 – -0.21), -0.31(-0.56 – -0.08), -0.33(-0.57 – -0.08)). Ewe ADG was highest in 2018 (2018 – 2016: 0.09(CI: 0.04, 0.13), 2018 – 2017: 0.06(CI: 0.01, 0.10), 2018 – 2019: 0.06(CI: 0.02, 0.11), 2018 – 2020: 0.12(CI: 0.07, 0.16)), and ewe ADG in 2020 was also lower than in 2017 and 2019 (2020 – 2017: -0.06(CI:-0.1, -0.01), 2020 – 2019: -0.06(CI: -0.1, -0.01)).

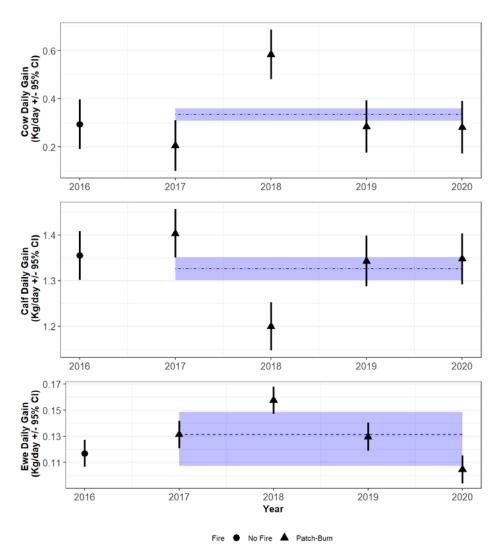


Figure 1.11. Average daily gains (kg • day⁻¹) for calves, cows, and ewes at the Hettinger Research Extension Center in North Dakota from the 2016 – 2020 summer grazing seasons. Points are the parameter estimate with 95% confidence intervals. The dashed line is the overall estimate for the average daily gain with a 95% confidence interval when the pastures were managed with patch-burn grazing (2017-2020). Pastures were managed as continuous, seasonlong grazing without fire in 2016. Average daily gains in 2018 were significantly different from other years for calves, cows, and ewes.

Discussion

Heterogeneity-based management practices offer solutions to balance livestock production and biodiversity conservation in rangeland ecosystems under persistent threats from land use, climate, and socio-economic change (Augustine et al., 2019; Derner et al., 2018; Fuhlendorf et al., 2017; Lark et al., 2020). With a decreasing land base, there is a greater need to find solutions that avoid putting production and conservation at cross-purposes (Porensky, 2021; Wilmer et al., 2019). In this study, we show how disturbance-driven contrast in the nutritive value of available forage among patches within grazed pastures corresponds with patterns in grazer selection. Both sheep and cattle preferred recently burned patches where available forage was not only highest in protein and moisture content, but also lowest in fiber and lignin.

Factors influencing grazer selection in rangeland landscapes are a persistent area of interest for managers and researchers globally (Archibald et al., 2005; Grant et al., 2019; Raynor et al., 2021a; Senft et al., 1987). The low amount of topographic variability and the size of our patch-burn grazing pastures emphasizes the connections between forage nutritive value and grazer selection relative to more rugged and more extensive rangelands (Ganskopp & Bohnert, 2009; Raynor et al., 2021a). Our burn patch attraction results are consistent within the broader context of the fire-grazing interaction and in the northern Plains (Allred et al., 2011; Archibald et al., 2005; Powell et al., 2018; Sensenig et al., 2010). However, a comparable study from semi-arid rangelands in the Great Plains had a similar pasture size, but did not have a persistent and strong attraction to the burned patch due to drought and variable responses by ecological site (Augustine & Derner, 2014). That our study had variable precipitation and consistent results underscores how site context potentially mediates the fire-grazing interaction (McGranahan et al., 2013ab; Raynor et al., 2021b; Scasta et al., 2016a).

Our study adds further evidence to the stabilizing effect of disturbance-driven heterogeneity on ecosystem properties relevant to sustainable livestock production. In the southern Plains, spatial heterogeneity has been shown to reduce annual variability in forage availability (McGranahan et al. 2016); the availability of reserve forage in unburned patches helps stabilize cattle weights during drought (Allred et al. 2014; Spiess et al. 2020). While annual variability in livestock weight gains persisted in this ranch-scale study, weight gains were not as tied to precipitation as in studies without heterogeneously-managed rangeland (Reeves et al., 2013, 2014). Deviations for each livestock class occurred in 2018 when growing season precipitation was relatively normal rather than with drought (2017 & 2020) or above-normal (2019) growing season precipitation (NDAWN, 2021; Appendix B). Elsewhere in the Great Plains, previous research on long-term datasets from continuous, season-long pastures found a positive relationship between growing season precipitation and weight gains and a negative relationship with spring temperature (Reeves et al., 2013, 2014). Likewise, a larger ranch-scale study in semi-arid Colorado rangeland found that steer weight gains differed between years in pastures managed as continuous, season-long or as adaptive rotational grazing (Augustine et al., 2020).

An assumption for stabilized livestock performance with patch-burn grazing is that the imposed heterogeneity in forage nutritive value at the pasture scale will persist throughout the grazing season and benefit grazing livestock. However, studies to date have not examined patterns within grazing seasons. Increasing heterogeneity in forage biomass at the pasture scale with patch-burn grazing reduces inter-annual variability in forage production and livestock weight gain, but this is reliant on persistent homogeneity within patches of pastures to maintain patch contrast (Allred et al., 2014; McGranahan et al., 2016). Incomplete burns, drought, and

stocking rate are known challenges for creating and maintaining patch contrast in forage nutritive value and grazer selection (Augustine & Derner, 2014; McGranahan et al., 2012; Scasta et al., 2016a). In our study, patch contrast persisted over grazing seasons in all measured forage variables and grazer selection. Maintained patch contrast in forage biomass is also an indicator of meeting rangeland structural heterogeneity goals (Hovick et al., 2015).

Differences between cattle and sheep patch-burn grazing pastures in this study were primarily related to additional patch contrasts within grazer treatments. Our previous finding of available forage in cattle pastures having higher crude protein values than sheep pastures did not persist (Spiess et al., 2020). Similarly, we expected the lower forb and legume cover in sheep pastures to translate to significantly higher fiber and lignin components values in sheep pastures as a further example of increased selectivity from sheep. These selection differences have more direct implications for pollinator communities on the pastures with lower floral abundance in sheep pastures (Cutter et al., 2021).

The significant month and forage moisture gradients in the nutritive value ordination show that intra-season variability still existed in the study following the spring to fall growing season despite differentiation along the time since fire gradient. Crude protein content is usually highest at the beginning of the growing season and declines as plants reach maturity and senesce. Conversely, the concentration of fiber components (ADF, NDF) and lignin increase over the growing season (Collins & Newman, 2018). Future analysis can explore how precipitation events prior to sampling influenced forage moisture content and nutritive value.

Previous patch-burn grazing research has primarily focused on the implications for rangeland biodiversity (Scasta et al., 2016b). This presents a potential barrier to implementation for private land managers that often have different management priorities than conservationists

and public land managers attempting to incorporate livestock (Becerra et al., 2013; Bendel et al., 2020; Sliwinski et al., 2018). Private land managers are not a monolith, but there are some commonalities between desired outcomes for private rangelands, patch-burn grazing, and current strategies used for achieving these outcomes (Derner et al., 2018; Derner & Augustine, 2016; Sliwinski et al., 2018; Wilmer et al., 2019). Consistent livestock performance, reducing drought effects, and minimizing costs are persistent themes in semi-arid regions of the Great Plains (Sliwinski et al., 2018; Wilmer et al., 2019). The rest and forage banking concepts used in adaptive rotational management to improve drought resiliency are similar to the available forage and grazer selection patterns that occur under patch-burn grazing (Augustine et al., 2020; Derner & Augustine, 2016; McGranahan et al., 2014; Spiess et al., 2020). Our findings here support the expectation that recently burned patches attract grazing livestock and allow for biomass accumulation in adjacent patches regardless of grazing species.

We did not track wool quality or production on the ewes in this study, but the relationship between grazing management strategies, forage nutritive value, and wool production is an interesting avenue for future research that seems to be absent (National Research Council, 2007). More laboratory analysis is required to determine the energy values and protein degradability of collected forage samples which would help move the rangeland forage and livestock knowledge base closer to the granularity in dairy and feedlot systems (NASEMR, 2016; Schwab & Broderick, 2017). The economic viability of implementing patch-burn grazing in the northern Great Plains is still a lingering question. Cost-share programs, potential savings on growing season protein and energy supplementation, potential savings on mineral supplementation, and drought mitigation are some of the factors that would need to be included in a ranch-scale economic analysis.

Conclusion

Understanding how ecosystem properties respond to management actions and influence processes like grazer selection provides a baseline of expectations for practitioners and land managers. We used a spatially intensive and temporally extensive sampling design to evaluate how rangeland forage patterns related to grazer selection in semi-arid North Dakota pastures managed with patch-burning. Grazing livestock utilized recently burned patches where forage was higher in crude protein and moisture content and lower in fiber components, lignin, and biomass than other areas of the pastures. Aside from 2018, livestock weight gains were consistent across years for cows, calves, and ewes. With prescribed fire currently an uncommon management practice in the northern Great Plains, these data work towards filling a knowledge gap for the region and illustrate how grazing livestock can benefit from implementation of patch-burning in addition to maintaining structural heterogeneity.

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CHAPTER TWO – PATCH-BURN GRAZING INCREASED STRUCTURAL HETEROGENEITY IN SOUTHWESTERN NORTH DAKOTA RANGELANDS

Abstract

Persistent land use change and degradation throughout the North American Great Plains increases the need to maintain and improve ecosystem service delivery from remaining rangelands to meet production and conservation goals. In this study, we investigated the efficacy of patch-burn grazing to create and increase structural heterogeneity in semi-arid North Dakota. We surveyed plant community composition and structural characteristics on heterogeneously managed patch-burn grazing pastures during summers 2017 – 2020 and comparison grassland units homogeneously managed through haying or idling during summers 2018 - 2020. Time since fire was significant for all structural characteristics with recently burned patches being different from other patches. Structural heterogeneity on patch-burn grazing pastures increased over the study and was greater than conventional management for conservation grasslands in the region. Increased structural heterogeneity is important for supporting a broad suite of rangeland wildlife and can stabilize forage production.

Introduction

Rangeland livestock production and biodiversity are reliant on the preservation and improvement of rangeland landscapes amidst climatic variability, land use change, and degradation (Sayre 2017; Augustine et al., 2019; Lark et al., 2020). Conversion to other land uses like row-crop agriculture or energy development continually decreases the overall amount of rangelands and intensifies remaining rangelands (Thompson et al., 2015; Trainor et al., 2016; Lark et al., 2020; Spiess et al., 2020). Invasive species can further diminish the production potential and biotic integrity of these working landscapes through increasing the amount of

unpalatable forage, homogenizing the plant community, and reducing the variety of niches available (Hein & Miller 1992; Eddy et al., 2003; Bardgett et al., 2021). Thus, sustaining both the provisioning and conservation components of rangeland landscapes requires efforts that finds balance between the two.

Heterogeneity in ecosystem properties influences ecosystem processes and has implications for the native and domesticated flora and fauna within a landscape (Li & Reynolds 1995; Fuhlendorf et al., 2017). Vegetation structure, for example, is an ecosystem property that is a combination of vegetation height and density that is commonly used to evaluate rangeland suitability for grassland birds and other taxa (Hovick et al., 2015; Ahlering & Merkord 2016; Schultz et al., 2017; Davis et al., 2020). Heterogeneity in vegetation structure is typically associated with the number of niches available within the landscape (Fuhlendorf & Engle 2001; Tews et al., 2004). Reducing structural heterogeneity through management or shifts to homogeneous plant communities threaten the biotic integrity of rangeland ecosystems (Fuhlendorf et al., 2017; Bardgett et al., 2021).

Conventional rangeland management in the North American Great Plains has emphasized spatially-homogenous grazing pressure through an even distribution of herbivory by grazing livestock, which reduces heterogeneity in vegetation structure across rangeland landscapes (Holechek et al., 2010; Fuhlendorf et al., 2012). Although perceived as beneficial for livestock production, homogeneous land use provides resources for a narrow range of rangeland biodiversity (Fuhlendorf et al., 2006). Increased structural heterogeneity also benefits livestock production through a stabilized forage base with less inter-annual variability (Allred et al., 2014; McGranahan et al., 2016). In conservation-focused grasslands like those enrolled in the Conservation Reserve Program and some state and federal properties, management has indirectly

emphasized homogeneity within grassland landscapes through the removal of disturbances altogether, which has favored homogeneous plant communities in the northern US Great Plains (DeKeyser et al., 2013; Dixon et al., 2019; Grant et al., 2020). Management that promotes heterogeneity has been proposed as a way of balancing livestock production and conservation needs on rangelands (Toombs et al., 2010; Fuhlendorf et al., 2017).

Unfortunately, implementing heterogeneous management strategies does not guarantee achieving all management objectives. Recent research has found mixed success with vegetation structure and bird communities when comparing proposed heterogeneous management strategies from season-long, continuous grazing (Sliwinski et al., 2019; Vold et al., 2019). Stocking rate or grazing intensity, rather than grazing system, is often a better predictor of vegetation structure and bird community assemblage (Ahlering & Merkord 2016; Raynor et al., 2021; Sliwinski et al., 2019; Vold et al., 2019).

Patch-burn grazing is an alternative land management strategy that differs from conventional rangeland management in the implicit use of prescribed fire but also in the degree of trust and control over meeting management objectives placed in grazer selection (Toombs et al., 2010; Fuhlendorf et al., 2012; Freese et al., 2014). In theory, applying a spatially discrete prescribed fire to a portion of a pasture or management unit creates initial structural contrasts between the burned patch and adjacent patches. Grazing herbivores then preferentially graze recently burned patches over the grazing season which maintains structural contrast and allows for biomass accumulation in other patches (Fuhlendorf & Engle 2004). This creates a shifting mosaic of vegetation structure along the time since fire gradient within the pasture that encourages differences between patches within a rangeland landscape and produces a consistent variety of structural combinations through space and time (Fuhlendorf & Engle 2001; Fuhlendorf

& Engle 2004). But in practice, the strength of grazer attraction and contrast in structural characteristics is variable with climate, plant community, and stocking rate driving some of the variability in results (McGranahan et al., 2013; Augustine & Derner 2014; Scasta et al., 2016a; Arterburn et al., 2019). When quantified as patch contrast, the degree of difference between areas in a given landscape is used to evaluate the heterogeneity of rangeland landscapes (Kotliar & Wiens 1990; McGranahan et al., 2012).

Field-based evaluations of rangeland wildlife habitat typically focus on methods for quantifying vegetation structure, but vegetation structure is not the only structural characteristic that wildlife respond to or that we would expect to differ within patch-burn grazing pastures (McGranahan et al., 2013; Ahlering & Merkord 2016; Sliwinski et al., 2019; Davis et al., 2020). Plant height contributes to overall structure, is a predictor of space use by grazing herbivores, and is a common covariate for rangeland game bird site selection (Archibald et al., 2005; Geaumont et al., 2017; Geaumont & Graham 2020). Litter depth typically does not contribute to an overall structure measurement, but it does have implications for nesting and foraging behavior of grassland birds and soil exposure (McGranahan et al., 2013; Ahlering & Merkord 2016). Deep litter is generally preferred in rangeland health evaluations in native plant communities (Printz et al., 2014), but an invasive species in the northern Great Plains, Kentucky bluegrass (*Poa* pratensis L.), produces a thick horizontal litter layer that is likely detrimental for species selecting for bare ground (Gasch et al., 2020; Grant et al., 2020). The balance between the arrangement of herbaceous, vertical litter, ground litter, and bare ground cover are also useful for characterizing selected and avoided areas by various wildlife taxa (McGranahan et al., 2013; Geaumont et al., 2017; Schultz et al., 2017; Geaumont & Graham 2020).

Patch-burn grazing is predominantly practiced in the more humid regions of the Great Plains and uses either cattle or bison for the primary grazing component (Scasta et al., 2016b). In semi-arid regions of the Great Plains, patch contrast in structure characteristics is partially contingent on the capacity of the plant community to exhibit a range of structures following disturbance (Augustine & Derner 2015). While patch-burn grazing research has been conducted in the semi-arid northern US Great Plains (Powell et al., 2018; Spiess et al., 2020), patch contrast has not yet been evaluated within patch-burn grazing pastures and relative to conventional management. There is a prevalence of grasslands dominated by introduced cool grasses in this region of the Great Plains through revegetation efforts and invasions (DeKeyser et al., 2013; Scasta et al., 2016b; Dixon et al., 2019; Grant et al., 2020). The Conservation Reserve Program and wildlife refuges helped increase the amount of perennial grassland acres in this region using introduced forage species like intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey) and alfalfa (Medicago sativa L.; Soil Conservation Service 1992; Dixon et al., 2019). Similarly, smooth brome (Bromus inermis Leyss), Kentucky bluegrass, and crested wheatgrass (Agropyron cristatum [L.] Gaertn.) are prevalent across public and private rangelands in the region (DeKeyser et al., 2013; Toledo et al., 2014; Grant et al., 2020). While these species offer increased forage production potential for livestock producers, they threaten native flora and homogenize the vegetation structure within these landscapes (Gasch et al., 2020; Grant et al., 2020).

With a dwindling rangeland base, there is increased attention on incorporating seminatural and novel ecosystems into conservation planning and efforts (Miller & Bestelmeyer 2016; Augustine et al., 2019). The Conservation Reserve Program is one of few pathways for increasing hectares of private grasslands in the US. Under enrollment, management activity is typically restricted to haying and grazing as part of mid-contract management or during an emergency declaration during drought. As lands exit enrollment, they can transition into hayfields and pastures, but generally revert to row-crop agriculture (Skaggs et al., 1994; Johnson et al., 1997; Geaumont et al., 2017; Morefield et al., 2016). In North Dakota, for example, over 500,000 ha of perennial vegetation is set to exit CRP by 2030 (Farm Service Agency 2018). Thus, we are interested in the efficacy of patch-burn grazing as an alternative management strategy that use fire and livestock to increase the suitability of currently homogenized grasslands in the northern Great Plains for a broader suite of wildlife through increased structural heterogeneity. If successful, this would provide support for more disturbance-inclusive management of invaded rangelands, actively enrolled CRP land, and a potential pathway for maintaining lands exiting CRP enrollment in a grassland state.

This study is part of a larger project investigating the efficacy of patch-burn grazing on ecosystem properties and services in northern Great Plains rangelands including post-Conservation Reserve Program grasslands. The objectives of this study were to determine: 1) if differences in plant community composition and structural characteristics exists between patches in patch-burn grazing pastures; 2) if differences in plant communities and structural characteristics exist between cow-calf and sheep patch-burn pastures; 3) if structural patch contrasts increased over time on patch-burn pastures; and 4) whether structural patch contrast was higher on patch-burn pastures relative to conventional management for active and post-CRP grasslands in the area.

Methods

Study Location and Tract Selection

We conducted our study in the northern US Great Plains on privately owned rangeland pastures near the town of Hettinger in southwestern North Dakota. The patch-burn grazing pastures are leased and heterogeneously managed by the Hettinger Research Extension Center (HREC), and comparison units are homogeneously managed through haying or idling by HREC and cooperating landowners. The area has a mean annual precipitation of 360-mm and mean temperature range during the May to September grazing season of 12 °C (May) to 21 °C (July and August). Ecological sites, a land classification system based on topographic, soil, and plant data, on HREC pastures include: clayey, loamy, sandy, saline lowland, and thin claypan (Soil Survey Staff 2020)

Following enrollment in the Conservation Reserve Program in the late 1980's and early 1990's, the patch-burn grazing pastures were planted with a CP1 seed mix including: 60% intermediate wheatgrass, 30% alfalfa, and 10% yellow sweetclover (*Melilotus officinalis*; Soil Conservation Service 1989; Soil Conservation Service 1992). Common plants currently include: intermediate wheatgrass, smooth brome, Kentucky bluegrass, alfalfa, and crested wheatgrass.

To compare pasture-level heterogeneity outcomes on our experimentally managed pastures against conventionally-managed tracts, we selected comparison units that had similar introduced cool-season plant community, and had a mixed management history of being idled, hayed, and/or periodically grazed. The conventional management comparison units were not experimentally manipulated, ranged from 5 – 45 ha in size, and were either idled or hayed during the summers when surveyed. In 2018 and 2019, we surveyed eleven comparison units (Hay: 2, Idle: 9), and we surveyed seven comparison units in 2020 (Hay: 2, Idle: 5).

Experimental Design

At HREC, we managed six, 65 ha pastures with patch-burn grazing in an effort to increase structural heterogeneity within the pastures. Three pastures were stocked with gestating Rambouillet ewes (*Ovis aries* L.), and three pastures were stocked with cow-calf pairs (*Bos taurus* L.). The pastures are arranged in three blocks that create three paired sheep and cattle blocks. We had a similar stocking rate (0.5 ha AUM-1 and stocking period from late May/early June – mid-September.

We conducted all prescribed burns in the dormant season prior to the 2017 – 2020 summer grazing seasons and burned a quarter of each pasture (~15 ha) annually to establish a four-year fire return interval. Because this was the initial implementation of patch-burn grazing on these pastures, we have an increasing number of times since fire over the course of the study beginning with prescribed burns in 2016 prior to the 2017 grazing season until completion of the first four-year burn rotation with prescribed burns prior to the 2020 grazing season (Spiess et al. 2020).

Data Collection

We measured vegetation structural characteristics and determined community composition in HREC pastures and comparison units along 100 m long transects in mid-June – late July when herbaceous biomass is roughly peaking. Transects on patch-burn pastures were previously established for grassland bird and butterfly surveys in a nested hierarchical sampling structure with three transects per patch of each pasture for a total of twelve transects per patch-burn pasture. We used a similar nested hierarchical sampling structure to establish transects on comparison units by subdividing units into four pseudo-patches and then distributing 2-3 transects per patch depending on overall size of each unit. We measured structural characteristics

and determined species composition within 0.5 m2 (0.5 m x 0.5 m) quadrats along both sides of each transect at 10-m intervals starting at 0-m and ending with 100-m for 22 quadrats per transect in HREC pastures. We measured 11 quadrats per transect in comparison units spaced at 10-m intervals along each transect due to the decreased emphasis on documenting species present in comparison units and logistical constraints of doubling the amount of transects.

At each quadrat, we measured vegetation structural density using visual obstruction readings (VOR) with a modified Robel pole (Robel et al., 1970) that has markings at every 0.25 dm. We placed the Robel pole in the center of the quadrat, stood 4 m away from the center of the quadrat at a height of 1 m, and recorded the lowest interval on the pole not obscured by vegetation from the north, east, south, and west sides of the quadrat. A higher VOR is indicative of increased vegetative height and density. We also recorded the tallest standing live and dead plant material (Max Live and Max Dead, respectively) in decimeters using the Robel pole. We expected Max Dead to be lowest in recently burned patches. After removing the Robel pole from the quadrat, we measured litter depth in centimeters using a ruler.

We then measured the canopy cover of species present in each quadrat as well as bare ground, ground litter, and vertical litter using the Daubenmire (1959) cover class method, with species not present receiving a separate 0 cover class rather than receiving a 1. We recorded cover classes (0 – 6) in the field and then analyzed results using the midpoints for each cover class (class 0: 0, class 1: 2.5, class 2: 15, class 3: 37.5, class 4: 62.5, class 5: 85, and class 6: 97.5). We calculated functional group composition from species level cover data using native status for North Dakota and growth habit (native C3 grass, introduced C3 grasses, native C4 grass, native forb, introduced forb, native legume, introduced legume, and native shrub).

Data Analysis

We performed all statistical analysis in the R statistical environment assisted by the 'tidyverse' package (R Core Team 2019; Wickham et al., 2019). To determine contrast in measured structural characteristics and broad functional groups (grass, forb, and legume cover) between patches with varying time since fire (TSF) and differences between grazer treatments (Grazer), we fit a mixed-effect model for each response variable that included TSF, Grazer, and the TSF×Grazer interaction as fixed effects using function *lmer* in the 'lme4' package (Bates et al., 2015). Then, we evaluated the significance of fixed effects using analysis of deviance (reported as ' χ^2 ' statistic, *p* value') using *Anova* in the 'car' package (Fox and Wesiberg 2019). We created an intermediate time since fire factor level by combining patches with one year since fire and patches with two years since fire after finding negligible differences between factor levels and similar relationships when compared with either recently burned, not yet burned, and patches with three years since fire. Thus, we are comparing responses across the following factor levels for the time since fire intensity gradient: not yet burned (NYB), recently burned (RB), intermediate years since (INT), and three years since fire (3YSF).

After determining significant fixed effects for a given response variable, we then used post-hoc comparisons to compare factor levels for significant terms using the function *emmeans* with the pairwise distinction in the 'emmeans' package (Lenth 2021). We report the contrast estimate and 95% confidence interval (CI) for significant factor level contrasts to indicate the directionality and size of the difference as '(Level 1 – Level 2: calculated value(CI lower, CI upper))'. When TSF or Grazer were significant, the estimated marginal means for the specified factor are averaged across the other factor. When the TSF×Grazer interaction was significant, we evaluated TSF contrasts within Grazer. To determine differences across years and account for

repeated sampling measures, we used the transect nested within pasture within year as a random effect for mixed-effect models with continuous structural characteristics and used pasture nested within year as a random effect for cover data averaged to the transect level for analysis (Cheng et al., 2010).

We used a variance partitioning framework to evaluate patch contrast over time on patch-burn pastures and to compare structural patch contrast between patch-burn pastures and conventional management for currently enrolled and post-CRP grasslands (McGranahan et al., 2012; McGranahan et al., 2018; Raynor et al., 2021). For patch contrast over time, we fit random-effect models for each structural variable and broad functional group (grass, forb, and legume) with Grazer and Patch as random effects. We calculated the variation attributed to the separate random effects for each patch-burn block (1 cattle pasture, 1 sheep pasture) each year using function *VarCorr* in the 'lme4' package, and then compared values from 2020 and 2017 to determine if the Patch or Grazer attributed variation increased. A higher value for the patch term indicates higher contrast amongst patches within pastures, and a higher value for the grazer term indicates higher contrast between sheep and cattle pastures. We tested for significant differences between years using analysis of variance in base R with function *aov* and directionality of significant differences between years using a Tukey post-hoc comparison.

For comparing structural patch contrast between patch-burn grazing pastures and conventional management units, we fit a random-effect model for VOR with Patch as the random effect. We calculated the variation attributed to the patch term for each pasture and comparison unit each year to determine if patch-burn pastures had higher patch contrast than conventional homogeneous management. We tested for significant differences between management types with analysis of deviance using a mixed-effect model with management as

the fixed effect and year as a random effect. We then determined directionality of difference between heterogeneous and homogeneous management using a Tukey post-hoc comparison.

To determine whether TSF and/or Grazer were associated with composition and associations between structural characteristics and community composition at the species or functional group level, we used unconstrained ordination and post-hoc factor and vector fitting with functions *metaMDS*, *capscale*, and *envfit* from the 'vegan' package (Oksanen et al., 2016) and function *parwise.factorfit* in the 'RVAideMemoire' package (Hervé 2021). We averaged the species level quadrat data to the patch level for each patch-burn pasture and then calculated the relative abundance for each species across all patch-burn pastures and years.

For the species level ordination, we used patch level data for each year and then removed species occurring in <10% of patches to improve the fit of the ordination. For the functional group ordination, we averaged the quadrat level functional group data calculated from the species data to the patch level for each year. We averaged structural variables to the patch level for inclusion as environmental variables at the same scale as the species and functional group data. We also used the patch level structural variables to test associations between structural variables, time since fire, and grazer type in a separate unconstrained ordination. We compared the metaMDS stress values and proportion of the explained through the first three axes using different distance measures for all three ordinations. We set our thresholds for an ideal unconstrained ordination at a metaMDS stress value < 0.13 and capscale proportion explained value \geq 0.70. The Euclidean distance had the lowest stress values and highest proportion explained for all ordinations. We tested TSF, Grazer, and environmental variables for ordinations using an envfit with a significance level of < 0.05, 499 permutations, and a combination of pasture and year as the strata.

Results

We documented 86 species on transects in patch-burn grazing pastures over the course of the study (Appendix A). Intermediate wheatgrass, smooth brome, Kentucky bluegrass, alfalfa, and crested wheatgrass accounted for 82% of the overall plant community cover (Figure 2.1). The most common native C3 grasses were slender wheatgrass (*Elymus trachycaulus* [Link] Gould ex Shiners, 2%), foxtail barley (*Hordeum jubatum* L., 1%), and western wheatgrass (Pascopyrum smithii [Rydb.] Á. Löve, 1%), and the most common introduced C3 grasses were intermediate wheatgrass (23%), Kentucky bluegrass (21%), smooth brome (21%), and crested wheatgrass (3%). The most common native C4 (warm season) grasses were inland saltgrass (Distichilis spicata [L.] Greene, 2%) and prairie cordgrass (Spartina pectinata Bosc ex Link, 1%). The most common native forbs were common yarrow (Achillea millefolium L., 0.79%) and white heath aster (Symphyotrichum ericoides [L.] G.L. Nesom, 1%), and the most common introduced forbs were field bindweed (Convolvulus arvensis L., 1%) and dandelion (Taraxacum officinale F.H. Wigg., 1%). The most common native legume was American licorice (Glycyrrhiza lepidota Pursh, 0.3%), and the most common introduced legumes were alfalfa (14%) and yellow sweetclover (1%).

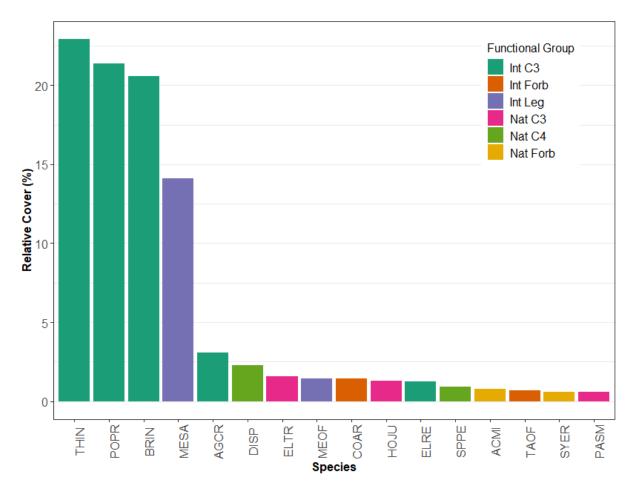


Figure 2.1. Top sixteen species by relative cover found on patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. Introduced C3 grasses made up the largest portion of the plant community followed by introduced legumes. Corresponding common names for codes: THIN = intermediate wheatgrass, POPR = Kentucky bluegrass, BRIN = smooth brome, MESA = alfalfa, AGCR = crested wheatgrass, DISP = inland saltgrass, ELTR = slender wheatgrass, MEOF = yellow sweetclover, COAR = field bindweed, HOJU = field barley, ELRE = quackgrass, SPPE = prairie cordgrass, ACMI = common yarrow, TAOF = common dandelion, SYER = white heath aster, PASM = western wheatgrass. Functional groups: Int C3 = introduced cool season grasses, Int Forb = introduced forbs, Int Leg = introduced legumes, Nat C3 = native cool season grasses, Nat C4 = native warm season grasses, Nat Forb = native forbs.

Structural Characteristics

For vegetation structure, as measured by VOR, time since fire was the only significant predictor variable (TSF χ^2 : 77.83, p < 0.001; Figure 2.2). Recently burned patches had the lowest vegetation structure (RB – INT: -0.41(CI: -0.54, -0.29), RB – 3YSF: -0.5(CI: -0.72, -0.28), RB –

NYB: -0.25(CI:-0.37, -0.12)), and structure was higher in intermediate and patches with three years since fire than in not yet burned patches (INT – NYB: 0.17(CI: 0.03, 0.47), 3YSF – NYB: 0.25 (CI: 0.03, 0.47)).

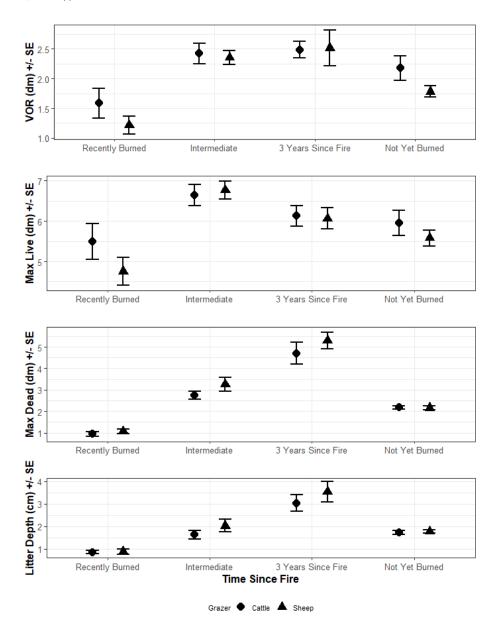


Figure 2.2. Vegetation structure (VOR, dm), maximum live height (Max Live, dm), maximum dead height (Max Dead, dm), and litter depth (cm) in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. With the exception of Max Live, recently burned patches were significantly lower than all other patches. There were no significant differences between cattle and sheep pastures. Points represent means \pm standard error.

Time since fire was the only significant predictor variable for Max Live height (TSF χ^2 : 32.83, p < 0.001; Figure 2.2). Intermediate patches had higher Max Live heights than recently burned and not yet burned patches (INT – RB: 1.01(CI: 0.32, 1.71), INT – NYB: 1.62(CI: 0.88, 2.36)). Time since fire was also the only significant predictor variable for maximum dead height (TSF χ^2 : 266.51, p < 0.001; Figure 2.2). Recently burned patches had the lowest maximum dead heights (RB – INT: -2.02(CI: -2.46, -1.58), RB – 3YSF: -4.22(CI: -4.97, -3.48), RB – NYB: -1.12(CI: -1.54, -0.7)). Patches with three years since fire had the highest maximum dead heights (3YSF – INT: 2.21(CI: 1.48, 2.93), 3YSF – NYB: 3.1(CI: 2.36, 3.84)), and intermediate patches also had higher maximum dead heights than not yet burned patches (INT – NYB: 0.9(CI: 0.48, 1.31)).

Time since fire was the only significant predictor variable for litter depth (TSF χ^2 : 113.62, p < 0.001; Figure 2.2). Recently burned patches had the lowest litter depths (RB – INT: -0.31(CI: -0.45, -0.18), RB – 3YSF: -0.82(CI:-1.05, -0.6), RB – NYB: -0.4(CI:-0.52, -0.27),). Patches with three years since fire also had higher litter depths than intermediate and not yet burned patches (3YSF – INT: 0.51(CI: 0.29, 0.73), 3YSF – NYB: 0.43(CI: 0.21, 0.64)).

Time since fire was the only significant predictor variable for bare ground cover (TSF χ^2 : 117.17, p < 0.001; Figure 2.3). Recently burned patches had the highest bare ground cover (RB – INT: 0.65(CI: 0.35, 0.94), RB – 3YSF: 1.51(CI: 0.99, 2.02), RB – NYB: 1.0(CI: 0.71, 1.29)). Intermediate patches had higher bare ground cover than not yet burned and patches with three years since fire (INT – NYB: 0.35(CI: 0.05, 0.66), INT – 3YSF: 0.86(CI: 0.37, 1.35)).

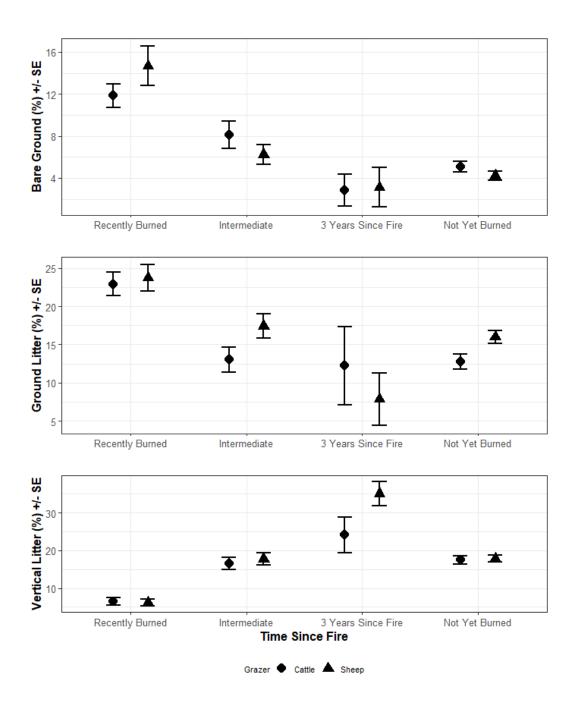


Figure 2.3. Percentages of bare ground, ground litter, and vertical litter cover on patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. Recently burned patches had higher bare ground and ground litter cover and lower cover for vertical litter than other patches. There were no differences between cattle and sheep pastures. Points represent means \pm standard error.

Time since fire was the only significant predictor variable for ground litter cover (TSF χ^2 : 55.36, p < 0.001; Figure 2.3). Recently burned patches had the highest ground litter cover (RB –

INT: 8.03(CI: 4.16, 11.9), RB – 3YSF: 8.84(CI: 5.08, 12.59), RB – NYB: 13.67(CI: 7.05 – 20.29)). Time since fire was also the only significant predictor variable for vertical litter cover (TSF χ^2 : 160.08, p < 0.001; Figure 2.3). Recently burned patches had the lowest vertical litter cover (RB – INT: -10.27(CI: -13.57, -6.98), RB – 3YSF: -22.19(CI: -27.97, -16.42), RB – NYB: -11.55(CI: -14.78, -8.32)), and patches with three years since fire had the highest vertical litter cover (3YSF – INT: 11.92(CI: 6.4, 17.44), 3YSF – NYB: 10.65(CI: 4.64, 16.65)).

The unconstrained ordination with structural characteristics had a stress of 0.05 and explained 92% of the variation through three axes (PCA Axis 1: 58%, PCA Axis 2: 20%, PCA Axis: 14%; Figure 2.4). We found dissimilarity in structural characteristics with time since fire $(r^2 = 0.38, p = 0.002)$ but not with grazer type $(r^2 = 0.01, p > 0.1)$. Recently burned patches were dissimilar from all other patches (p = 0.002). Patches with three years since fire were dissimilar from intermediate and not yet burned patches (p = 0.006), and there was no dissimilarity between intermediate and not yet burned patches (p > 0.2).

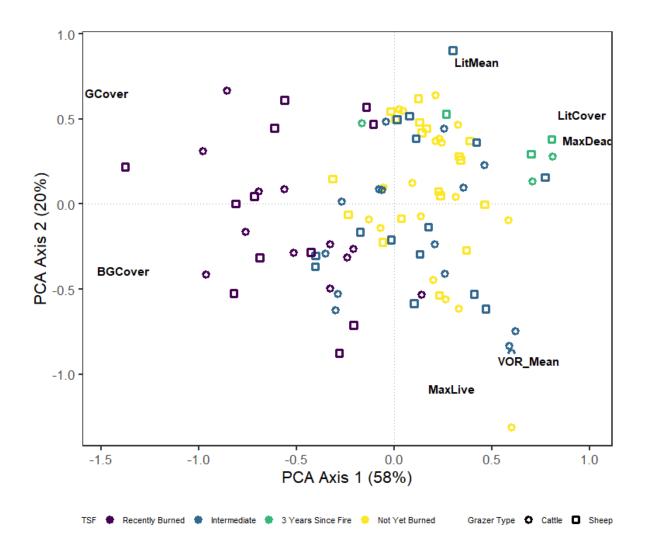


Figure 2.4. The unconstrained ordination of structural characteristics on patch-burn grazing pastures had a stress of 0.05 and explained 92% of the variation through three axes (PCA Axis 1: 58%, PCA Axis 2: 20%, PCA Axis 3: 14%).

We found dissimilarity in structural characteristics with time since fire ($r^2 = 0.38$, p = 0.002) but not with grazer type ($r^2 = 0.01$, p > 0.1). Recently burned patches were dissimilar from all other patches (p = 0.002). Patches with three years since fire were also dissimilar from intermediate and not yet burned patches (p = 0.006), and there was no dissimilarity between intermediate and not yet burned patches (p = 0.006). Structural abbreviations: BGCover = bare ground cover, GCover = ground litter cover, LitCover = vertical litter cover, LitMean = litter depth, MaxDead = maximum dead height, MaxLive = maximum live height.

Community Composition

Grass cover varied by time since fire (TSF χ^2 : 23.46, p < 0.001), but not by grazer type (Grazer χ^2 : 0.25, p = 0.61; Figure 2.5). Recently burned patches had the lowest grass cover (RB –

INT: -4.92(CI: -9.7, -0.14), RB – 3YSF: -11.31(CI: -19.69, -2.92), RB – NYB: -7.10(CI: -11.79, -2.41)). For forb cover, time since fire, grazer type, and the TSF×Grazer interaction were all significant predictor variables (TSF χ^2 : 9.83, $p \le 0.02$; Grazer χ^2 : 6.48 $p \le 0.01$; TSF×Grazer χ^2 : 14.98, p < 0.002; Figure 2.5). Cattle pastures had higher forb cover than sheep pastures (Cattle – Sheep: 0.84(CI: 0.04, 1.64)). Within cattle pastures, recently burned and intermediate patches had higher forb cover than not yet burned patches (RB – NYB: 0.52(CI: 0.17, 0.87), INT – NYB: 0.58(CI: 0.22, 0.95)). There were no significant differences between TSF patches in sheep pastures.

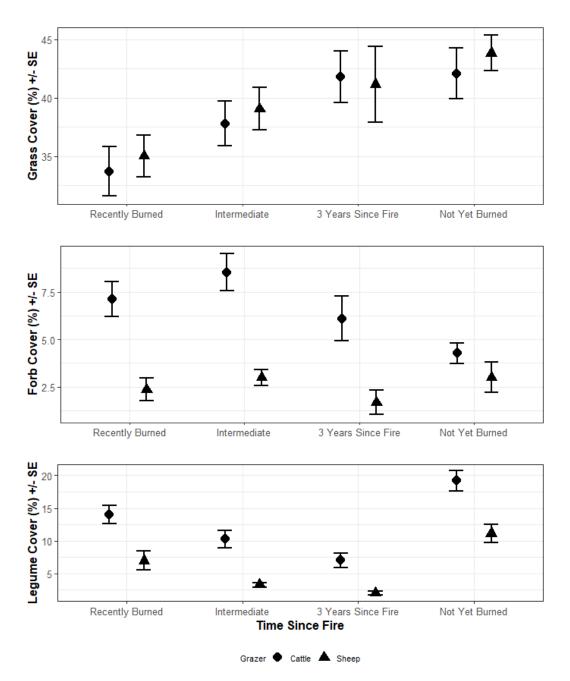


Figure 2.5. Percentages of grass, forb, and legume cover on patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. Recently burned patches had the lowest grass cover. Forb cover in cattle pastures was lowest in not yet burned patches. Sheep pastures had lower forb cover than cattle pastures. Points represent

means \pm standard error.

For legume cover, time since fire, grazer type, and the TSF×Grazer interaction were all significant predictor variables (TSF χ^2 : 15.04, $p \le 0.002$; Grazer χ^2 : 9.67 $p \le 0.002$; TSF×Grazer

 χ^2 : 13.48, $p \le 0.004$; Figure 2.5). Legume cover in cattle pastures was statistically equivalent to sheep pastures (Cattle – Sheep: 0.6(CI: -0.03, 1.23)). Within cattle pastures, recently burned patches had higher legume cover than intermediate patches (RB – INT: 0.55(CI: 0.13, 0.97)), and not yet burned patches had higher legume cover than intermediate and patches with three years since fire (NYB – INT: 0.84(CI: 0.4, 1.28), NYB – 3YSF: 1.01(CI: 0.23, 1.79)). There were no significant differences between TSF patches in sheep pastures.

The unconstrained ordination at the species level with the Euclidean distance had a metaMDS stress of 0.1 and explained 73% of the variation through three axes (PCA Axis 1: 32%, PCA Axis 2: 30%. PCA Axis 3: 11%; Figure 2.6). We did not find dissimilarity with time since fire ($r^2 = 0.08$, p > 0.08) or grazer type ($r^2 = 0.09$, p > 0.1). Similarly, none of the environmental structural variables explained much of the variation at the species level or were significant (r^2 min: 0.002, r^2 max: 0.061, p > 0.2).

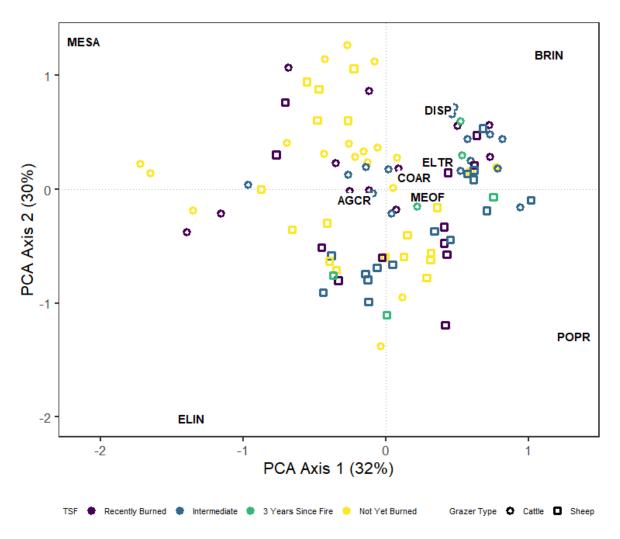


Figure 2.6. The ordination of plant species and potential time since fire association. The ordination with the Euclidean distance had a stress of 0.1 and explained 73% of the variation through three axes (PCA Axis 1: 32%, PCA Axis 2: 30%). We did not find dissimilarity with time since fire ($r^2 = 0.08$, p > 0.08) or grazer type ($r^2 = 0.09$, p > 0.1). Similarly, none of the environmental structural variables explained much of the variation at the species level or were significant (r^2 min: 0.002, r^2 max: 0.061, p > 0.2). Species: AGCR = crested wheatgrass, BRIN = smooth brome, COAR = field bindweed, DISP = inland saltgrass, ELIN = intermediate wheatgrass, ELTR = slender wheatgrass, MESA = alfalfa, MEOF = yellow sweetclover, POPR = Kentucky bluegrass.

The unconstrained ordination at the functional group level with the Euclidean distance had a metaMDS stress of 0.07 and explained 85% of the variation through three axes (PCA Axis 1: 51%, PCA Axis 2: 20%, PCA Axis 3: 14%; Figure 2.7). We did not find dissimilarity with time since fire ($r^2 = 0.06$, p > 0.1) or grazer type ($r^2 = 0.08$, p > 0.1). The environmental

structural variables had a higher range of variation explained than for the species level ordination, but none significantly influenced composition (r^2 min: 0.03, r^2 max: 0.12, p > 0.07).

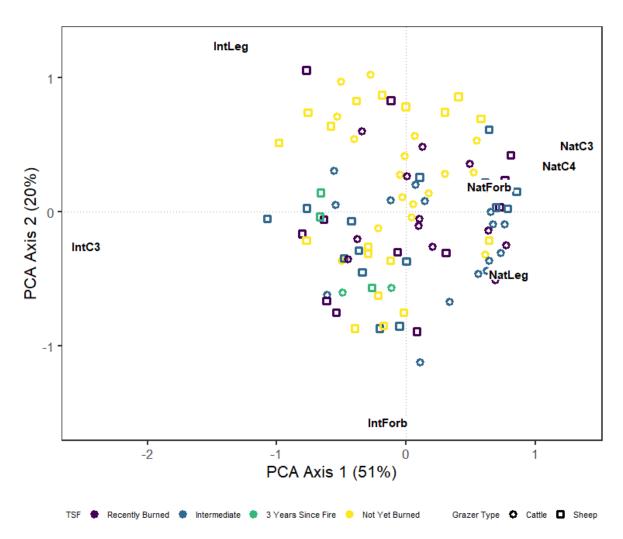


Figure 2.7. The ordination of plant functional groups and potential time since fire association. The unconstrained ordination had a stress of 0.07 and explained 85% of the variation through three axes (PCA Axis 1: 51%, PCA Axis 2: 20%). We did not find dissimilarity with time since fire (r2 = 0.06, p > 0.1) or grazer type (r2 = 0.08, p > 0.1). The environmental structural variables had a higher range of variation explained than for the species level ordination, but none significantly influenced composition (r^2 min: 0.03, r^2 max: 0.12, p > 0.07). Functional groups: IntC3 = introduced cool season grasses, IntForb = introduced forbs, IntLeg = introduced legumes, NatC3 = native cool season grasses, NatC4 = native warm season grasses, NatForb = native forbs.

Patch Contrast

Structural patch contrast was higher in heterogeneously managed patch-burn grazing pastures than in homogeneously managed comparison units (χ^2 : 19.21, p < 0.001; Heterogeneous – Homogeneous: 0.14(CI: 0.07, 0.2); Figure 2.8). Patch-level variation increased over the study for all measured structural characteristics, and there was little variation attributed to grazer type. For vegetation structure, patch contrast increased (F: 10.56, p ≤ 0.03; 2020 – 2017: 0.15(CI: 0.02, 0.29)) and grazer type contrast did not change (F: 1.33, p ≥ 0.31; Figure 2.9). Patch contrast in maximum live height increased (F: 19.63, p ≤ 0.01; 2020 – 2017: 0.98(CI: 0.37, 1.59)), and grazer type contrast did not change (F: 1, p ≥ 0.37; Figure 2.9). Patch contrast in maximum dead height increased (F: 174.5, p < 0.001; 2020 – 2017: 0.22(CI: 0.18, 0.27)), and grazer type contrast did not change (F: 3.49, p ≥ 0.14; Figure 2.9). Patch contrast in litter depth increased (F: 58.8, p ≤ 0.002; 2020 – 2017: 0.36(CI: 0.23, 0.49)), and grazer type contrast did not change (F: 0.2, p = 0.68; Figure 2.9).

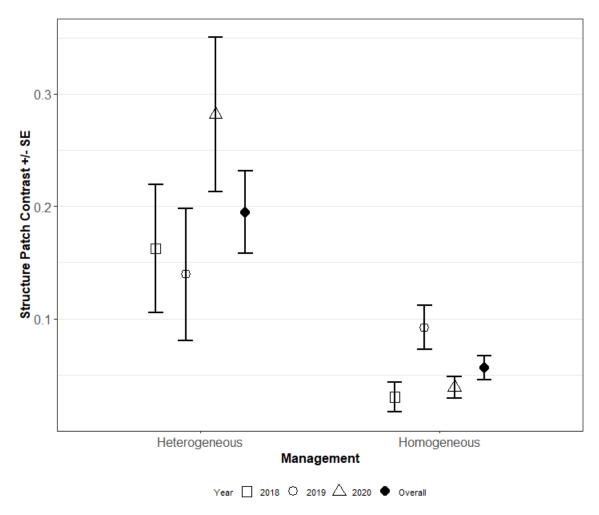


Figure 2.8. Overall comparison of patch-level contrast in vegetation structure between heterogeneous management (patch-burn grazing) and homogeneous management (hay or idle) in southwestern North Dakota.

Heterogeneous management had higher patch-level contrast than homogeneous management in each year of the study and overall. Points represent means \pm standard error.

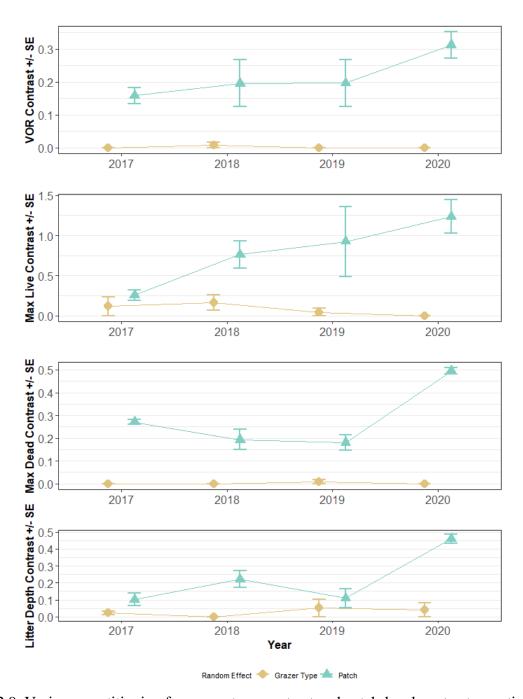


Figure 2.9. Variance partitioning for grazer type contrast and patch-level contrast over time in vegetation structure (VOR), maximum live height, maximum dead height, and litter depth on patch-burn grazing pastures at the Hettinger Research Extension Center. Patch-level contrast increased, and grazer type contrast did not change from 2017 - 2020. Points represent means \pm standard error.

Patch contrast in bare ground cover increased (F: 16.89, $p \le 0.02$; 2020 – 2017: 0.48(CI:

(0.16, 0.8)), and grazer type contrast did not change (F: (2.69, p = 0.18); Figure (2.10)). Patch

contrast in ground litter cover increased (F: 19.17, $p \le 0.01$; 2020 – 2017: 4.16(CI: 1.52, 6.79)), and grazer type contrast did not change (F: 0.5, $p \ge 0.52$; Figure 2.10). Patch contrast in vertical litter cover increased (F: 38.81, $p \le 0.003$; 2020 – 2017: 4.5(CI: 2.49 – 6.5)), and grazer type contrast did not change (F: 1, $p \ge 0.37$; Figure 2.10).

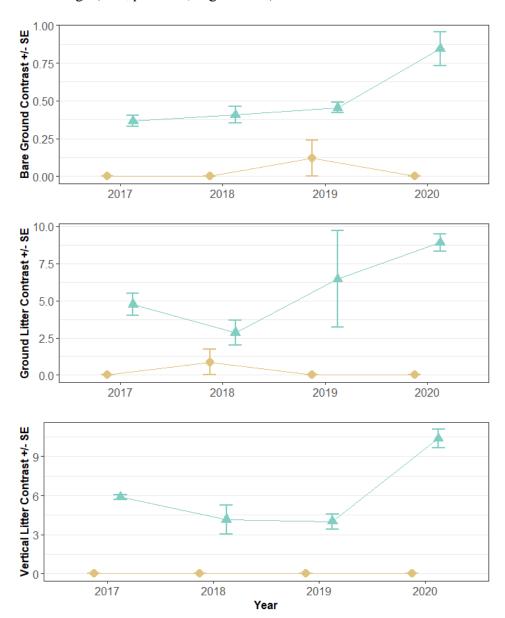


Figure 2.10. Variance partitioning for grazer type contrast and patch-level contrast over time in bare ground, ground litter, and vertical litter cover on patch-burn grazing pastures at the Hettinger Research Extension Center.

Patch-level contrast increased, and grazer type contrast did not change from 2017 - 2020. Points represent means \pm standard error.

For grass, forb, and legume cover, changes in patch and grazer type contrast were less consistent than structural responses with grazer type contrast increasing over the study for legume cover. Patch and grazer contrast in grass cover did not change (Patch F: 0.01, p > 0.9; Grazer F: 1, $p \ge 0.34$; Figure 2.11). Patch contrast and grazer type contrast in forb cover did not change (Patch F: 1.17, $p \ge 0.34$; Grazer F: 1.41, $p \ge 0.2$; Figure 2.11). Patch contrast in legume cover did not change (F: 0.1, F) 0.2; Figure 2.11), but grazer contrast in legume cover increased (F: 11.22, F) 0.03; Estimate = 0.46, CI: 0.08 – 0.85).

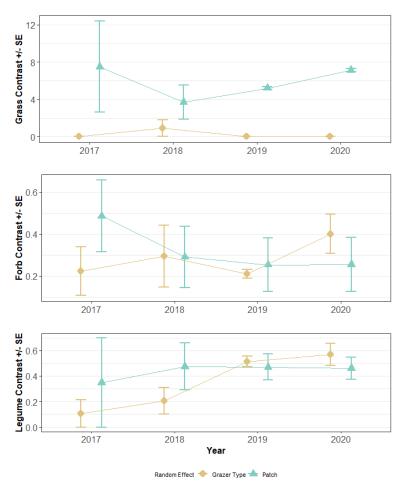


Figure 2.11. Variance partitioning for grazer type contrast and patch-level contrast over time in grass, forb, and litter cover on patch-burn grazing pastures at the Hettinger Research Extension Center.

Patch-level contrast did not change. Grazer type contrast increased from 2017 - 2020 for forb and legume cover indicating divergence between cattle and sheep pastures over time. Points represent means \pm standard error.

Discussion

In this study, we show that patch-burn grazing created and increased structural heterogeneity in semi-arid rangelands in the northern US Great Plains. This study directly addresses questions about the efficacy of patch-burn grazing and heterogeneity focused land management to alter vegetation structure in the northern Great Plains and semi-arid rangelands (Augustine & Derner 2015; Sliwinski et al., 2019; Vold et al., 2019; Epstein et al., 2021). Prior mixed and inconclusive results with patch-burn grazing in the region have been attributed to plant community composition, variable precipitation, and management decisions like stocking rate and the timing of fire (Augustine & Derner 2015; Arterburn et al., 2019; Spiess et al., 2020). These constraints are consistent with those found across the broader range of patch-burn grazing research throughout the Great Plains (McGranahan et al., 2013; Scasta et al., 2016; Scasta et al. 2016b). We maintained a moderate stocking rate, experienced variable precipitation over the course of this study, and met vegetation structure objectives using patch-burning within an introduced cool season grass plant community.

Variability in results of heterogeneity focused management strategies highlights the importance of making management decisions that will satisfy as many objectives as possible for a given rangeland (McGranahan et al., 2013; Porensky 2021). Viewing rangelands as socioecological systems provides additional challenges, but can also help managers prioritize objectives and direct actionable research for stakeholders (Wilmer et al., 2019; Epstein et al., 2021). While private rangeland managers in our study region do not typically prioritize biodiversity conservation, some of the adaptive strategies that they use for drought resiliency also contribute to heterogeneity amongst pastures (Toombs et al., 2010; Sliwinski et al., 2019; Manfredo et al., 2021). Prescribed fire is not a common management tool for private rangeland

managers in this region currently (Bendel et al., 2020), but we hope that some of the connections between patch-burn grazing and stabilized livestock performance will promote discussion of prescribed burning within livestock production focused groups (Allred et al., 2014; McGranahan et al., 2016; Spiess et al., 2020; Chapter 1).

Heterogeneity focused rotational grazing approaches have had mixed success in meeting vegetation structure, grassland biodiversity, and livestock production goals (Sliwinski et al., 2019; Vold et al., 2019; Davis et al., 2020). The increased costs of implementing multi-paddock rotational grazing and negligible differences in livestock production compared with continuous, season-long grazing presents a tradeoff between economic viability of a private ranching operation and achieving structural heterogeneity (Briske et al., 2011; Windh et al., 2019; Davis et al., 2020). However, cost-share programs and prioritization of alternative management objectives like soil and riparian health help promote implementation of various rotational grazing systems (Toombs & Roberts 2009; Roche et al., 2015).

The conservation potential of invaded, semi-natural, and novel rangeland ecosystems has been recognized as rangelands face persistent threats of land use change (Backstrom et al., 2018; Augustine et al., 2019; Lark et al., 2020). While the plant community in our study pastures does not resemble the native plant community, it does provide perennial grassland vegetation with a higher production potential that is beneficial for livestock production and for game birds and waterfowl in the region (Geaumont et al., 2017; Backstrom et al., 2018; Dixon et al., 2019). Furthermore, these species are also prevalent on public and private rangelands in the region (Toledo et al., 2014; Dixon et al., 2019; Grant et al., 2020; Gasch et al., 2020). Our findings provide support for calls to reintroduce fire and grazing where possible and to actively manage idle rangeland landscapes (Fuhlendorf et al., 2017; Augustine et al., 2019). More work is still

needed to improve the diversity of floral resources on these pastures and better serve pollinator communities (Cutter et al., 2021).

Differences between cattle and sheep pastures in this study were primarily related to forb and legume cover and additional differences between TSF patches for some structural characteristics. During the study period, sheep pastures also had lower floral abundance and floral diversity (Cutter et al., 2021). This is indicative of the increased grazing selectivity of sheep preferentially targeting forbs and legumes that typically have higher nutritive value than grasses (Collins & Newman 2018). Practitioners might be able to utilize this preference with a combined targeted grazing and prescribed burning approach to invasive legumes like yellow sweet clover and sericea lespedeza (*Lesepedeza cuneata* [Dum. Cours.] G. Don).

Our finding of no visual obstruction distinction between intermediate time since fire patches and three years since fire patches is similar to previous semi-arid patch-burn grazing research (Augustine & Derner 2015). However, some measured structural characteristics, like maximum dead height, followed the typified time since fire gradient and had higher values in the three years since fire patches than what previously existed in the not yet burned patches.

Continued data collection on HREC patch-burn pastures will be able to evaluate if these results persist and whether any species composition trends emerge from successive burn rotations.

Conclusion

Patch-burn grazing increased structural heterogeneity in low diversity grasslands in the northern Great Plains over time and compared with conventional homogeneous management.

Time since fire had a significant effect for all measured structural characteristics. Differences between sheep and cattle pastures were primarily found in forb and legume cover, but the grazer treatments were structurally similar. Despite achieving increased structural heterogeneity, there

were no significant shifts in plant community composition at the species or functional group level on this first cycle through the burn rotation. Increasing structural heterogeneity is associated with benefiting grassland wildlife through a more diverse offering of potential niches and more consistent forage for grazing livestock. Practitioners interested in increasing rangeland structural heterogeneity need to know what to expect when implementing heterogeneity focused land management strategies. Here, patch-burn grazing with moderate stocking rates successfully imposed and increased heterogeneity in the measured aboveground ecosystem properties regardless of grazer type. Mixed results across previous research illustrate the importance of considering how ecosystem properties like plant community and management decisions like stocking rate can mitigate attempts at imposing heterogeneity.

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CHAPTER 3 – SOIL NUTRIENT POOLS AND MICROBIAL COMMUNITIES RESISTANT TO PATCH-BURN GRAZING IN NORTHERN GREAT PLAINS

Abstract

Understanding how management that targets aboveground heterogeneity translates to soil properties and processes provides an important whole-system context for practitioners. We investigated how patch-burn grazing associated with soil nutrients, decomposition activity, and microbial abundance and composition on semi-arid post-Conservation Reserve Program rangelands in southwestern North Dakota. We collected soil samples during 2018 – 2020 summer grazing seasons in three cow-calf patch-burn pastures and three sheep patch-burn pastures. We tested for differences between grazer type, ecological sites, and patches with varying time since fire using mixed-effect models and ordination. There were no differences between cattle and sheep pastures. Ecological sites explained more variation in response variables than time since fire. Recently burned patches primarily had equivalent nutrient pools and microbial abundances to patches with increased time since fire. Hesitancy regarding reintroducing fire and grazing into conservation grasslands and fire into private rangelands is likely unsupported from a soil perspective in this region.

Introduction

Grasslands are among the most threatened biomes, globally, by land use change and conversion (Hoekstra et al. 2005, Bardgett et al. 2021). This loss in land base and the degradation of remaining grasslands coincides with a reduction in the ecosystem services grasslands provide (Augustine et al. 2019, Lark et al. 2020, Bardgett et al. 2021). With climate change expected to compound conflicts between grassland biodiversity conservation and provisioning services, it is important to identify and implement strategies for retaining and improving ecosystem service

delivery in these imperiled socio-ecological systems (Augustine et al. 2019, Ahlering et al. 2020, Epstein et al. 2021, Bardgett et al. 2021).

Heterogeneity-based management is one proposed solution for improving ecosystem service delivery in working grassland landscapes (Toombs et al. 2010, Fuhlendorf et al. 2017). Previous research into heterogeneous management has exhaustively covered aboveground properties and processes like avian responses, vegetation structure, and grazer selection (Scasta et al. 2016b). With an increased interest in rangeland soil health and calls to reintroduce disturbances like fire and grazing into conservation-focused grasslands, there is a need to understand how these disturbances affect belowground ecosystem properties and processes (Byrnes et al. 2018, Derner et al. 2018, Augustine et al. 2019, Sanderson et al. 2020). Conventional management of production and conservation-focused grasslands has prioritized creating and encouraging homogeneous levels of utilization or disturbance within a landscape, which supports a narrow suite of wildlife taxa (Fuhlendorf and Engle 2004, Holechek et al. 2010, Fuhlendorf et al. 2017, Dixon et al. 2019). Aboveground ecosystem processes like grazer selection and grassland bird nest-site selection are related to the heterogeneity of ecosystem properties like forage nutritive value and vegetation structure (Ganskopp and Bohnert 2009, Hovick et al. 2015, Ahlering and Merkord 2016). The inherent, or initial, heterogeneity of aboveground ecosystem properties is driven by variation in topo-edaphic features and properties like soil moisture and nutrient availability (Williamson et al. 2016, Fuhlendorf et al. 2017). Whether occurring naturally or imposed through management, disturbances influence aboveground processes through the alteration of ecosystem properties (Fuhlendorf et al. 2017).

Ecological sites are a useful framework for classifying the inherent heterogeneity of a landscape by grouping soil and landscape position with climate and plant community

information (Williamson et al. 2016, Natural Resource Conservation Service 2018). Ecological responses to management and disturbances can vary by ecological site (Wonkka et al. 2016, Williamson et al. 2016, Davis et al. 2020). Thus, identifying the degree to which ecological sites differ under similar disturbance regimes informs future adaptive management decisions and when inherent heterogeneity might override imposed heterogeneity (Vermeire et al. 2005, Augustine and Derner 2014, Wonkka et al. 2016).

Patch-burn grazing is a heterogeneity-focused land management strategy that uses prescribed burning and grazing to impose and encourage contrasts in ecosystem properties and processes (Fuhlendorf and Engle 2004). Livestock are not commonly used across conservation-focused grasslands, and prescribed fire is typically applied to an entire pasture when used in production-focused grasslands (Smith and Owensby 1978, Matthews et al. 2012, DeKeyser et al. 2013, Dixon et al. 2019).

With the typical implementation of patch-burn grazing, a prescribed fire is applied to a spatially discrete portion of a pasture to create an initial contrast between the recently burned patch and adjacent patches that were burned in previous years (Fuhlendorf and Engle 2004). At a landscape or pasture level, the intention is to create an intensity gradient that follows the time since fire sequence in a shifting mosaic as different patches are burned each year (Fuhlendorf and Engle 2004). Grazing animals that have access to the entire pasture prefer recently burned patches which then maintains contrasts in aboveground biomass over the grazing season (Allred et al. 2011, Powell et al. 2018, Spiess et al. 2020). The preference to graze plants in recently burned patches is consistent across domestic and wild herbivores and is often attributed to the available forage in recently burned patches having higher protein content and lower fiber content (Archibald et al. 2005, Sensenig et al. 2010, Allred et al. 2011). Creating and maintaining patch

contrast in aboveground biomass results in increased structural heterogeneity benefiting a broader suite of wildlife taxa than homogeneity-focused land management strategies (Fuhlendorf et al. 2017).

For plants in recently burned patches to maintain grazer attraction throughout a grazing season, there needs to be sufficient soil moisture and nutrients otherwise aboveground patch contrast diminishes (Anderson et al. 2006, Augustine et al. 2010, Augustine and Derner 2014, McGranahan et al. 2014). Drought can diminish grazer attraction within a grazing season, but is not a guarantee (Augustine and Derner 2014, McGranahan et al. 2014, Spiess et al. 2020). This indicates that climate conditions and the inherent heterogeneity in the soil properties of a site can mitigate imposed heterogeneity in addition to known constraints including plant community composition, stocking rate, and timing of fire (McGranahan et al. 2013, Scasta et al. 2016a, Arterburn et al. 2019, Raynor et al. 2021).

We previously found that cattle and sheep on patch-burn grazing pastures in North

Dakota, USA maintained a preference for recently burned patches and patch contrast in

aboveground biomass during a drought (Spiess et al. 2020). This prompted a question whether

managing for aboveground patch contrast comes at the expense of belowground properties and

processes. For aboveground contrasts in patch-burn grazing pastures, some measured properties,

like vegetation structure and available forage biomass, should be lower in recently burned

patches than patches with increased time since fire. Other properties, like forage crude protein

content and bare ground cover, should be higher in recently burned patches (Fuhlendorf and

Engle 2004). The expected relationship between time since fire and belowground properties is

less clear. However, recently burned patches with reduced nutrient concentrations and microbial

abundance are not a desirable management objective under soil health principles. If measured

responses are lower in recently burned patches, if and when they recover informs the long-term efficacy of this management strategy.

The combination of fire and grazing presents a confluence of the disturbance specific effects on soil nutrient cycling and availability. Due to the dearth of direct patch-burn grazing research focused on soil responses, we instead relied on prior works focusing on combinations of fire and grazing to formulate our expectations and specific research questions (Anderson et al. 2006, Scasta et al. 2016b). Possible pathways for fire to influence belowground properties and processes include: altering nutrient inputs, volatilization of nutrients in aboveground biomass, increased soil temperature, changing evapotranspiration rates, altering pH, and potentially reducing microbial abundance (Hobbs et al. 1991, Neary et al. 1999, Dooley and Treseder 2012, McLauchlan et al. 2020). Possible pathways for grazing to influence belowground properties and processes include: altering the quantity and quality of litter inputs and biomass, altering soil physical properties, and redistributing nutrients through waste deposition (Sitters and Olde Venterink 2015, Derner et al. 2018, Sitters et al. 2020).

Nitrogen is a limiting nutrient in most temperate grasslands, but is not the only possible nutrient pool affected by fire (Blair 1997, LeBauer and Treseder 2008, Kirkman et al. 2014, Reinhart et al. 2016). Grasslands in the North American Great Plains, where patch-burn grazing is predominantly practiced (Scasta et al. 2016b), are generally limited in nitrogen and soil moisture along a precipitation gradient (high to low) from East to West and South to North (LeBauer and Treseder 2008, Kunkel et al. 2013, Kirkman et al. 2014). Plant available forms of nitrogen, ammonium and nitrate, are often higher following a fire for a period of time (Wan et al. 2001, Augustine et al. 2010). Ash produced from forest wildfires can contain carbon compounds, nitrogen compounds, calcium, magnesium, sodium, potassium, phosphorous, and sulfur (Blank

and Zamudio 1998, Pereira et al. 2012). In other rangeland studies, prescribed fire has resulted in an increase in the availability of soil nitrogen, potassium, phosphorous, and zinc (Blank et al. 2007, Reinhart et al. 2016).

The degree to which a fire can influence nutrient availability and microbial abundance depends on the individual fire and overall fire regime that an ecosystem experiences. The fire regime refers to a collection of parameters that can be used to describe a fire and usually includes: intensity (the amount of energy released), season (when fire occurs), type (where fire occurs), extent (how much is burned), and frequency (how often burning occurs (Krebs et al. 2010, McGranahan and Wonkka 2021). The fire return interval is important to consider when evaluating the relationship between fire and soil nutrient availability. Repeated annual burning and an increased fire return interval are often associated with decreasing plant available nitrogen and net mineralization rates for nitrogen through continuous removal of aboveground biomass and litter (Ojima et al. 1994, Blair 1997, Pellegrini et al. 2018). While aboveground and belowground biomass production in a tallgrass prairie experiment were unhindered by annual burning, the greatest aboveground biomass production occurred when burned at infrequent intervals (Blair 1997). This is attributed to the transient maxima hypothesis which describes a transition between resource limitations in the post-fire environment where grasslands go from primarily space and light limited in the pre-fire environment to primarily water and nutrient limited in the post-fire environment (Seastedt and Knapp 1993).

Soil microbial communities mediate soil processes like decomposition, nitrification, and mineralization and have a mixed relationship with fire (Van der Heijden et al. 2007, Dooley and Treseder 2012, Cotrufo et al. 2013, Reinhart et al. 2016, Pausas and Bond 2020). The post-fire environment is thought to improve the conditions for microbial mineralization, but fires

generally reduce both microbial abundance and diversity (Hobbs and Schimel 1984, Anderson et al. 2006, Pressler et al. 2019, Dove et al. 2021). Across biomes, soil bacteria are more resilient to disturbances than fungi (Van der Heijden et al. 2007, Pressler et al. 2019, Dove et al. 2021). Soil temperature and moisture content are important regulators of nitrogen mineralization; mineralization increases as soil temperature increases and as soil moisture decreases from saturation (Sierra 1997, Wang et al. 2006). On the other end of the moisture spectrum, net nitrification and mineralization increased in an incubation experiment as soil moisture increased from 15% to 35% (Wang et al. 2006). In high intensity fires, a hydrophobic coating can form on the soil from the volatilization of organic matter that reduces infiltration and increases repellency which could reduce microbial activity (DeBano 1981, Zavala et al. 2009). Fortunately, the intensity of prescribed fires in grasslands are typically lower than in forests due to the lower amount and smaller size classes of available fuel and shorter residence time (Zavala et al. 2009, Shakesby et al. 2015), so grassland fires are presumably less likely to severely impact soil properties and processes. In a long-term experiment of fire return interval and nitrogen, there were no differences in nitrogen mineralization between annually burned, triennially burned, and unburned plots (Coetsee et al. 2008).

Grazing can influence the nutrient ratios of aboveground vegetation positively and negatively for microbial decomposition (Georgiadis and McNaughton 1990, Tanguy and Michel 2001, Semmartin et al. 2008, Sitters and Olde Venterink 2015). The chemical composition (C:N:P ratios) of available substrates like plant litter and livestock waste influence microbial community composition and decomposition (Cleveland and Liptzin 2007, Cotrufo et al. 2013, Sitters and Olde Venterink 2015). Similar to digestibility for livestock, litter lignin content and lignin:nitrogen ratios are indicators of degradability by the microbial community (Fortunel Claire

et al. 2009, Austin et al. 2010). Conceptually, grazing while grasses are still in the vegetative state prolongs and potentially skips the transition into the reproductive state prior to dormancy. Compared to an ungrazed grassland, grazing reduced the lignin:nitrogen ratio of aboveground biomass (Semmartin et al. 2008). Grazing at a low intensity can increase the buildup of fibrous compounds and hasten the decline in crude protein (Georgiadis and McNaughton 1990). If grazer attraction is maintained throughout the growing and grazing season in patch-burn grazing pastures, the remaining plant biomass should still have lower fiber content than plant biomass from other patches that receive less grazing pressure (Sensenig et al. 2010).

Grazing animals also influence nutrient cycling in grasslands by reallocating nutrients and increasing the turnover rate from plant uptake to deposition and decomposition. Plant nitrogen and phosphorous remain relatively constant while herbivore nitrogen and phosphorus cycling are variable depending on forage concentrations and the animal's requirements with excess nutrients deposited through waste (Sterner 1990, Tanguy and Michel 2001). Additionally, any nutrients acquired in less frequented parts of a landscape could be deposited in frequented areas (Augustine et al. 2013, Sitters and Olde Venterink 2015). We would then expect the majority of nutrient redistribution through waste to favor recently burned patches (Archibald et al. 2005, Sensenig et al. 2010, Powell et al. 2018, Spiess et al. 2020).

Erosion and soil moisture issues are potentially exacerbated by grazing following a fire. Soils are already prone to increased runoff and erosion after a fire from increased surface exposure and potential development of a hydrophobic coating (DeBano 1981, Johansen et al. 2001). Compaction following intensive grazing could limit soil moisture by decreasing water infiltration and constrain microbial activity (Warren et al. 1986, Schrama et al. 2013). In a tallgrass prairie patch-burn grazing study, however, soil moisture did not differ during the

growing season between annually burned, patch-burn, and unburned treatments (Anderson et al. 2006). In another tallgrass prairie study, fire and intensive grazing increased temperature and erosion on sandy soils, but did not affect soil moisture (Vermeire et al. 2005).

This study presents an opportunity to evaluate soil nutrient pools and microbial communities under a disturbance regime that adapts the historic disturbance regime of the North American Great Plains to meet modern logistical constraints. There is a perception that the use of fire with or without grazing is detrimental to soil nutrient availability, and inorganic nitrogen availability in particular, in grasslands despite some evidence to the contrary (Blair 1997, Anderson et al. 2006, Coetsee et al. 2008, Augustine et al. 2010). The relationship between fire and soil properties and processes has been studied more frequently in forests than in grasslands (Wan et al. 2001, Pressler et al. 2019). Similarly, soil microbial communities and processes have been studied less frequently in grasslands compared to other ecosystems and lack the combined use of prescribed fire and grazing (Dooley and Treseder 2012, Burns et al. 2013, Risch et al. 2019). The plant community and inclusion of a sheep grazing treatment are also novel contributions to the realm of patch-burn grazing research that will expand the baseline of expectations beyond using cattle or bison (Scasta et al. 2016b). One of the differences between sheep and cattle is that sheep have a higher preference for forbs and legumes than cattle (Chang et al. 2018, Cuchillo-Hilario et al. 2018, Jordon 2020). We have observed this on our study pastures, but whether this corresponds with belowground differences was an initial point of interest (Spiess et al. 2020, Cutter et al. 2021). Differences in the spatial dispersion of livestock and chemical composition of waste products are additional pathways for grazer type to influence the soil environment (Sitters et al. 2017, McGranahan et al. 2018).

This study is part of a broader investigation into the efficacy of patch-burn grazing on ecosystem properties and processes in the northern Great Plains. The primary objectives of this study were to determine: 1) whether soil nutrient pools, microbial abundance, soil moisture, and decomposition activity differ between patches with varying time since fire; 2) whether inherent heterogeneity mitigated imposed heterogeneity; 3) the microbial community composition in post-Conservation Reserve Program rangelands managed with patch-burn grazing; and 4) multivariate associations between microbial community composition, soil nutrient pools, ecological sites, and time since fire. We expected recently burned patches to have equal to or higher measured nutrient concentrations and soil moisture than patches with increased time since fire (Blair 1997, Anderson et al. 2006, Coetsee et al. 2008). We expected decomposition activity to be highest in recently burned patches due to the increased deposition of labile fecal material and reallocation of nutrients from other patches (Augustine et al. 2013, Sitters and Olde Venterink 2015). We expected sandy sites to have lower nutrient concentrations regardless of time since fire (Vermeire et al. 2005, Paul 2014). We expected soil bacteria to be more associated with recently burned patches and soil fungi to be more associated with patches with increased time since fire that also have less grazing pressure (Van der Heijden et al. 2007, Dooley and Treseder 2012, Dove et al. 2021).

Methods

Study Site

We conducted our study in the northern North American Great Plains on privately owned experimental rangeland pastures previously enrolled in the Conservation Reserve Program in southwestern North Dakota near the town of Hettinger and managed by the Hettinger Research Extension Center (HREC; 46.003150, -102.644529). Hettinger, North Dakota has a mean annual

precipitation of 360-mm and mean temperature range during the May to September grazing season of 12°C (May) to 21°C (July and August (NDAWN 2021)).

The common ecological sites on the pastures include: clayey, loamy, sandy, saline lowland, and thin claypan (Soil Survey Staff 2020). Following enrollment in the Conservation Reserve Program in the late 1980's and early 1990's, these pastures were planted with a CP1 seed mix including: 60% intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey), 30% alfalfa (*Medicago sativa* L.), and 10% yellow sweetclover (*Melilotus officinalis* [L.] Lam.) (Soil Conservation Service 1989, 1992). While some native plants are present in the pastures, five introduced species account for 82% of the current plant community by cover: intermediate wheatgrass (23%), Kentucky bluegrass (*Poa pratensis* L., 21%), smooth brome (*Bromus inermis* Leyss, 21%), alfalfa (14%), and crested wheatgrass (*Agropyron cristatum* [L.] Gaertn, 3%; Chapter 2).

Experimental Design

At HREC, we managed six, 65 ha pastures with patch-burn grazing in an effort to increase structural heterogeneity within the pastures. Three pastures were stocked with gestating Rambouillet ewes (*Ovis aries* L.), and three were stocked with cow-calf pairs (*Bos taurus* L.). We assigned the species of grazer for each pasture prior to the implementation of patch-burning. We burned a quarter of each pasture (~15 ha) annually in the dormant season (either late summer/early fall or early spring) prior to livestock release into the pastures in late May/early June. We targeted a similar stocking rate (0.5 ha AUM-1) and stocking period (late May/early June – mid-September) over the course of the study (Spiess et al. 2020).

Since this was the initial implementation of patch-burn grazing on these pastures, we had an increasing number of times since fire over the course of the study beginning with prescribed

burns in 2016 prior to the 2017 grazing season until completion of the first burn rotation with prescribed burns prior to the 2020 grazing season (Spiess et al. 2020). In this study, we report results from the 2018, 2019, and 2020 grazing seasons. In 2018, one recently burned patch, one patch with one year since fire, and two not yet burned patches existed in each pasture. In 2019, one recently burned patch, one patch with one year since fire, one patch with two years since fire, and one not yet burned patch existed in each pasture. In 2020, one recently burned patch, one patch with one year since fire, one patch with two years since fire, and one patch with three years since fire existed in each pasture.

Data Collection

We collected monthly soil samples during the 2018, 2019, and 2020 grazing seasons from sampling points distributed across each patch within each pasture to create a nested hierarchical sampling design. We assigned four sampling points per patch split between the two most common ecological sites to get a total of 16 points per pasture and 96 total sampling points that we returned to each month and year. At each point, we took samples to a depth of 10 cm to create an aggregate sample of ~100 g from the immediate area surrounding each sampling point. We placed samples in sealed, labeled plastic bags and stored them in a cooler while sampling. We sent subsamples to the North Dakota State University Soil Testing Lab (Fargo, North Dakota USA) to quantify soil nutrient concentrations and gravimetric soil moisture. We sent subsamples to Microbial ID (Newark, Delaware USA) for phospholipid fatty acid analysis (PLFA).

We determined concentrations of inorganic nitrogen (ammonium and nitrate) from all monthly soil samples in the study and gravimetric soil moisture from monthly samples collected during the 2019 and 2020 grazing seasons. We determined the pH and electrical conductivity (EC) of samples from the 2020 grazing season in the lab using an Oakton pH 150 meter

(OAKTON Instruments, Vernon Hills, Illinois USA) for pH and an Orion Star A112 Benchtop Conductivity Meter (Thermo Fisher Scientific, Waltham, Massachusetts USA) for EC following a 1:1 slurry method (Rhoades 1996, Thomas 1996). We determined concentrations of calcium, magnesium, available phosphorous, potassium, total carbon, and total nitrogen during the July sampling round each study year. We surveyed the abundance and composition of broad taxonomic microbial groups (actinomycetes, arbuscular mycorrhiza fungi, eukaryotes, fungi, Gram-negative bacteria, and Gram-positive bacteria) using PLFA during the June sampling rounds in 2019 and 2020.

We measured decomposition as an indicator of microbial activity at each sampling point throughout the 2019 and 2020 grazing seasons (June – September) using litter bags buried at 10 cm. We built the bags out of 2 mm fiberglass screen mesh following guidelines for excluding macrofauna (Harmon et al. 1999, Karberg et al. 2008). We created two pouches to allow for eventually comparing decomposition of early season smooth brome and Kentucky bluegrass herbaceous material. We kept subsamples of each species to determine carbon, nitrogen, and phosphorous content of substrates. We weighed 10 g of each species for each bag and placed them in separate pouches. We included a numbered cattle tag in the bags, placed a metal washer attached to the bags on top of the soil, and marked the area surrounding with spray paint to relocate and identify buried bags. We compared the weight of each species before and after burial to determine mass lost over the season. For this study, we are looking across species for decomposition.

Data Analysis

We performed all statistical analysis in the R statistical environment assisted by the 'tidyverse' package (R Core Team 2019, Wickham et al. 2019). To determine differences in

response variables between patches with varying time since fire (TSF), ecological sites (ES), and grazer types (Grazer), we fit a mixed-effect model for each response variable that included TSF, ES, Grazer, and the TSF×ES interaction as fixed effects using functions *lmer* for response variables with normal (total nitrogen, pH, decomposition, fungi:bacteria, and AM fungi) and lognormal (ammonium, nitrate, soil moisture, total carbon, phosphorus, potassium, calcium, magnesium, EC, actinomycetes, fungi, eukaryotes, Gram-negative bacteria, and Gram-positive bacteria) distributions and *glmer* for those with a gamma distribution (total microbial abundance) in the 'lme4' package (Bates et al. 2015). We determined the distribution that best fit each response variable individually using function *fitdist* in the 'fitdistrplus' package (Delignette-Muller and Dutang, 2015). The mixed-effect model for decomposition did not include the TSF×ES interaction due to missing factor combinations. We evaluated the significance of fixed effects with analysis of deviance (reported as ' χ^2 statistic, *p* value') using function *Anova* in the 'car' package (Fox and Weisberg 2019).

After exploratory analysis revealed indiscernible differences between grazer types, we did not include any interaction terms that included Grazer in the overall test model for each response variable. Similarly, we created an intermediate time since fire factor level by combining patches with one year since fire and patches with two years since fire after finding negligible differences between the factor levels and similar relationships when compared to either recently burned, not yet burned, and patches with three years since fire. Thus, we are comparing responses across the following factor levels for the time since fire intensity gradient: not yet burned (NYB), recently burned (RB), intermediate years since (INT, 1YSF – 2YSF), and three years since fire (3YSF).

After determining significant fixed effects for a given response variable, we then used post-hoc pairwise comparisons to compare factor levels for significant terms using the function *emmeans* with the pairwise distinction in the 'emmeans' package (Lenth 2021). We report the contrast estimate and 95% confidence interval (CI) for significant factor levels contrasts to indicate the directionality and size of the difference as '(Level 1 – Level 2: calculated value(CI lower, CI upper))'. When TSF, ES, or Grazer were significant, the estimated marginal means for the specified factor are averaged across the other two factors. When the TSF×ES interaction was significant, we evaluated TSF contrasts within ecological sites, and the estimated marginal means are averaged across Grazer. To determine differences over grazing seasons and across years, we nested pasture within month within year as a random effect for ammonium, nitrate, and soil moisture. We used pasture nested within month for pH and EC since those were only collected in 2020. We used pasture nested within year as a random effect for remaining nutrient response variables and microbial abundances. Due to low litter bag recovery rates in some pastures, we only used year as a random effect for decomposition activity (Cheng et al. 2010).

To determine multivariate relationships between soil microbial community composition, soil nutrients, ecological sites, and time since fire, we used unconstrained ordination and post-hoc factor and vector fitting with functions metaMDS, capscale, and envfit from the 'vegan' package (Oksanen et al. 2019) and function pairwise.factorfit in the 'RVAideMemoire' package (Hervé 2021). We compared the metaMDS stress values and proportion of the data explained through the first three ordination axes using different distance measures with the abundance values for the microbial taxonomic groups before selecting the Euclidean distance measure. We set our threshold for an ideal unconstrained ordination at a metaMDS stress value < 0.13 and capscale proportion explained \geq 0.70. We used the June measurements for ammonium, nitrate,

and moisture and the July measurements for remaining nutrients as potential environmental vector gradients. We included ES, TSF, and Grazer as potential environmental factor variables.

We tested factor and vector environmental variables using an envfit with a significance level of < 0.05, 499 permutations, and a combination of pasture and year as the strata.

Results

Ecological site was significant for more measured response variables (18/20, 90%) than time since fire (11/20, 55%), the TSF×ES interaction (4/20, 20%), and grazer type (0/20). When ecological site was significant, values in sandy sites were typically lower than other sites. There were no significant instances of measured nutrient pools in recently burned patches having lower concentrations relative to the less disturbed ends of the TSF intensity gradient (not yet burned and patches with three years since fire). For significant interaction terms, divergent TSF responses were found in saline lowland and clayey sites.

Soil Nutrient Pools, Moisture, and Chemical Properties

Nutrient Pools and Moisture

The overall mean ammonium concentration across the 2018-2020 grazing seasons was 9.09 ppm (± 0.44 SE). Time since fire was the only significant predictor variable for ammonium (TSF χ^2 : 11.35, $p \le 0.01$), but there were no significant differences between TSF patches (Figure 3.1). The overall mean nitrate concentration across the 2018-2020 grazing seasons was 4.54 ppm (± 0.16 SE). Time since fire and ecological site were the significant predictor variables for nitrate (TSF χ^2 : 9.29, $p \le 0.03$; ES χ^2 : 9.43, $p \le 0.02$). There were no significant differences between TSF patches or between ecological sites (Figure 3.2).

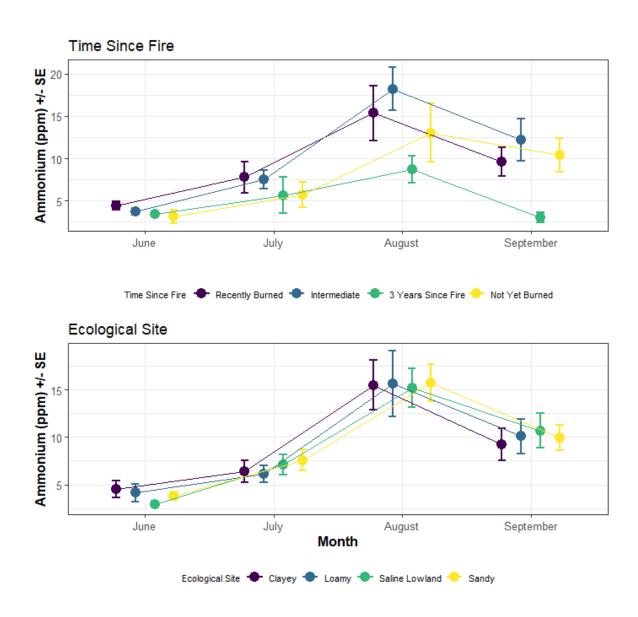


Figure 3.1. Soil ammonium (NH4 ppm) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by patch-level time since fire and ecological site. Neither time since fire or ecological site significantly influenced ammonium concentrations. Ammonium peaked in August for all patches. Points represent mean values \pm standard error.

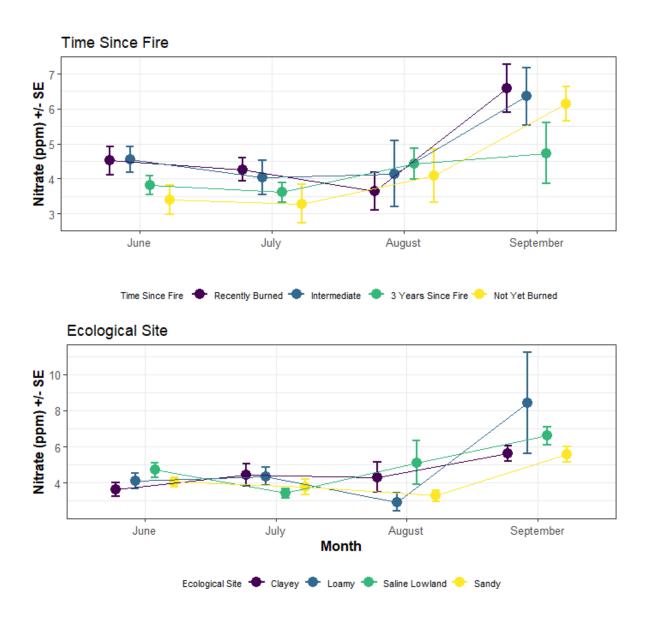


Figure 3.2. Soil nitrate (NO3 ppm) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by patch-level time since fire and ecological sites. Neither time since fire or ecological site significantly influenced ammonium concentrations. Nitrate peaked in September for most patches and ecological sites. Points represent mean values \pm standard error.

The overall mean gravimetric soil moisture content across the 2019 and 2020 grazing seasons was 12.39% (\pm 0.24 SE). Time since fire and ecological site were significant predictor variables for soil moisture (TSF χ^2 : 29.4, p < 0.001; ES χ^2 : 146.89, p < 0.001). Not yet burned patches had the lowest soil moisture (NYB – RB: -0.09(CI: -0.18, -0.01), NYB – INT: -0.12-(CI:

-0.18, -0.04), NYB – 3YSF: -0.13(CI: -0.24, -0.02); Figure 3.3). All ecological sites had higher moisture content than sandy sites (Clayey - Sandy: 0.14(CI: 0.07, 0.21), Loamy – Sandy: 0.15(CI: 0.05, 0.24), SaLo – Sandy: 0.23(CI: 0.16, 0.29)), and clayey sites had lower moisture content than saline lowland sites (Clayey – SaLo: -0.09(CI: -0.16, -0.01)). Within seasons, most patches and sites exhibited variable moisture content between months with peaks generally in July and September.

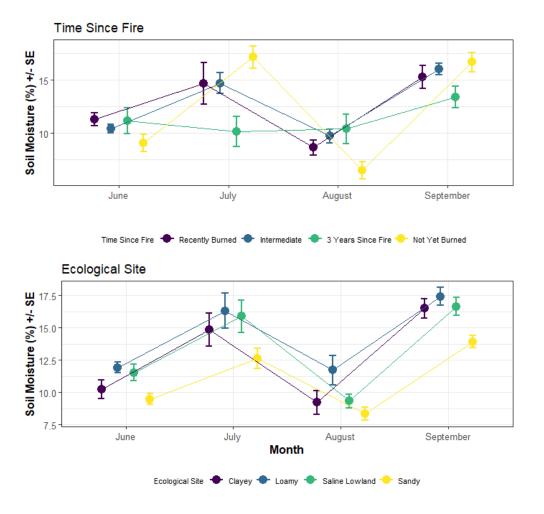


Figure 3.3. Gravimetric soil moisture content (%) in the top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by time since fire and ecological sites. Recently burned, intermediate, and patches with three years since fire had higher gravimetric soil moisture content than not yet burned patches. Sandy sites had the lowest gravimetric moisture content, and saline lowland sites had higher moisture content than clayey sites. Month to month variability was lowest in patches with three years since fire. Points represent mean values \pm standard error.

The overall mean percent total carbon across the 2018-2020 grazing seasons was 1.93% (± 0.05 SE). Time since fire and ecological site were significant predictor variables for total carbon (TSF χ^2 : 9.87, $p \le 0.02$; ES χ^2 : 89.78, p < 0.001). There were no significant differences between TSF patches (Figure 3.4). Total carbon was lowest in sandy sites (Clayey – Sandy: -0.12(CI: -0.21, -0.03), Loamy – Sandy: -0.19(CI: -0.32, -0.06), SaLo – Sandy: -0.27(CI: -0.36, -0.18)). Clayey sites had lower total carbon than saline lowland sites (SaLo – Clayey: -0.15(CI: -0.04, -0.25)).

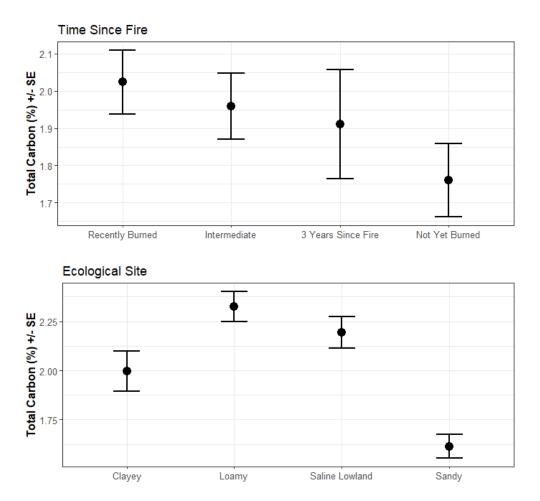


Figure 3.4. Total soil carbon (%) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by time since fire and ecological site.

There were no significant differences between TSF patches. Sandy sites had the lowest total carbon, and saline lowland sites were significantly higher than clayey sites. Points represent mean values \pm standard error.

The overall mean percent total nitrogen across the 2018 – 2020 grazing seasons was 0.20% (\pm 0.004 SE). Time since fire and ecological site were the significant predictor variables for total nitrogen (TSF χ^2 : 7.93, p \leq 0.05; ES χ^2 : 79.24, p < 0.001). There were no significant differences between TSF patches (Figure 3.5). Total nitrogen was lowest in sandy sites (Sandy – Clayey: -0.03(CI:-0.06, -0.01), Sandy – Loamy: -0.04(CI: -0.32, -0.004), Sandy – SaLo: -0.06(CI: -0.09, -0.04)). Clayey sites had lower total nitrogen than saline lowland sites (Clayey – SaLo: -0.03(CI: -0.06, -0.001)).

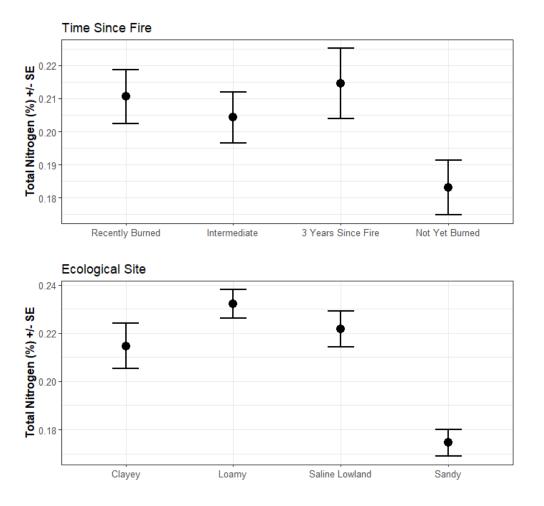


Figure 3.5. Total soil nitrogen (%) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by time since fire and ecological site.

There were no significant differences between TSF patches. Sandy sites had the lowest total nitrogen, and saline lowland sites were higher than clayey sites. Points represent mean values \pm standard error.

The overall mean calcium concentration across the 2018 – 2020 grazing seasons was 2272.25 ppm (\pm 116.90 SE). Ecological site was the only significant predictor for calcium (ES χ^2 : 14.05, $p \le 0.003$). Clayey sites had lower calcium concentrations than sandy sites (Clayey – Sandy: -0.25(CI: -0.48, -0.01); Figure 3.6).

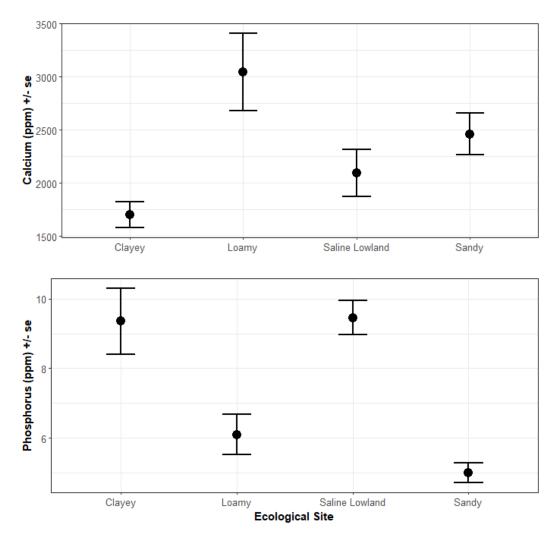


Figure 3.6. Soil calcium and available phosphorus (ppm) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by ecological site. Loamy and sandy sites had higher calcium concentrations than clayey sites. Sandy sites had the lowest available phosphorus concentrations. There no significant differences between TSF patches for calcium or phosphorus. Points represent mean values \pm standard error.

The overall mean available phosphorus concentration across the 2018-2020 grazing seasons was 7.23 ppm (\pm 0.32 SE). Ecological site was the only significant predictor for

phosphorus (ES χ^2 : 92.36, p < 0.001). Available phosphorous was lowest in sandy sites (Sandy – Clayey: -0.44(CI: -0.63, -0.26), Sandy – Loamy: -0.35(CI: -0.62, -0.09), SaLo – Sandy: -0.45(CI: -0.64, -0.26); Figure 3.6).

The overall mean magnesium concentration across the 2018 – 2020 grazing seasons was 349.07 ppm (\pm 11.61 SE). Ecological site and the TSF×ES interaction were significant predictor variables for magnesium (ES χ^2 : 18.76, p < 0.001; TSF×ES χ^2 : 17.13, $p \le 0.05$). Saline lowland sites had higher magnesium concentrations than sandy sites across TSF patches (SaLO – Sandy: 0.17(CI: 0.01, 0.34); Figure 3.7). Within saline lowland sites, intermediate patches had higher magnesium concentrations than not yet burned patches (INT – NYB: 0.3(CI: 0.5, 0.54)). There were no additional significant TSF differences in magnesium concentrations within ecological sites.

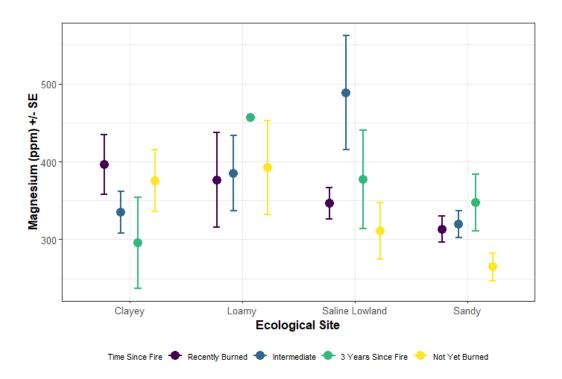


Figure 3.7. Soil magnesium (ppm) in top 10-cm of patch-burn grazing pastures in southwestern North Dakota separated by time since fire within ecological sites. Within saline lowland sites, intermediate patches had higher concentrations of magnesium than not yet burned patches. Points represent mean values \pm standard error.

The overall mean potassium concentration across the 2018 - 2020 grazing seasons was 334.08 ppm (\pm 8.69 SE). Time since fire and ecological site were the significant predictor variables for potassium (TSF χ^2 : 17.49, p < 0.001; ES χ^2 : 76.53, p < 0.001). Not yet burned patches had the lowest potassium concentrations (RB – NYB: 0.19(CI: 0.04, 0.34), INT – NYB: 0.19(CI: 0.05, 0.33), 3YSF – NYB: 0.26(CI: 0.02, 0.5); Figure 3.8). Sandy sites had the lowest potassium concentrations (Clayey – Sandy: 0.27(CI: 0.12, 0.42), Loamy – Sandy: 0.32(0.11 – 0.53), SaLo – Sandy: 0.33(CI: 0.18, 0.48)).

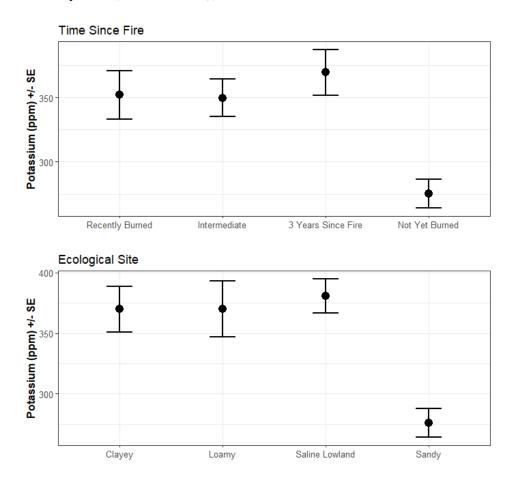


Figure 3.8. Soil potassium (ppm) in top 10-cm of patch-burn grazing pastures at the in southwestern North Dakota separated by time since fire and ecological site. Potassium concentrations were lowest in not yet burned patches. Sandy sites had the lowest potassium concentrations. Points represent mean values \pm standard error.

Chemical Properties: pH and EC

The overall mean pH for the 2020 grazing season was 6.77 (\pm 0.05 SE). Time since fire, ecological site, and the TSF×ES interaction were significant predictor variables for pH (TSF χ^2 : 23.48, p < 0.001; ES χ^2 : 21.22, p < 0.001; TSF×ES χ^2 : 25.69, p < 0.001). Across ecological sites, recently burned patches had lower pH values than intermediate and patches with three years since fire (RB – INT: -0.29(CI: -0.48, -0.1), RB – 3YSF: -0.3(CI: -0.52, -0.07)). Across TSF patches, clayey sites had the lowest pH (Clayey – Loamy: -0.37(CI: -0.71, -0.02), Clayey – Sandy: -0.25(CI: -0.49, -0.06), Clayey – SaLo: -0.27(CI: -0.51, -0.004)). Within clayey sites, patches with three years since fire had higher pH values than recently burned and intermediate patches (3YSF – RB: -0.48(CI: -0.89, -0.07), 3YSF – INT: -0.51(CI: -0.87, -0.15)). Within saline lowland sites, recently burned patches had lower pH values than intermediate patches (RB – INT: -0.62(CI: -0.9, -0.34); Figure 3.9). There were no significant differences between TSF patches within loamy or sandy sites.

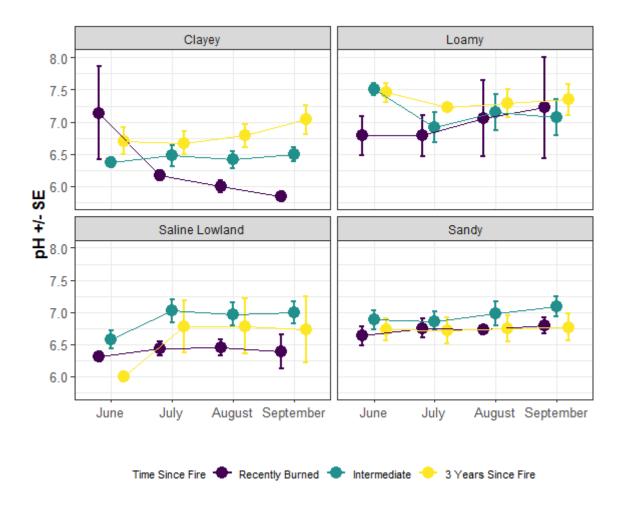


Figure 3.9. Soil pH in top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota.

There was a significant interaction between time since fire and ecological site, indicating varying differences between time since fire patches by ecological site. For sandy and loamy sites, pH did not differ between time since fire patches. In clayey sites, pH in recently burned patches decreased over the season and was lower than patches with three years since fire. In saline lowland sites, pH was higher in intermediate patches than recently burned patches. Points represent mean values \pm standard error.

The overall mean EC for the 2020 grazing season was 250 μ S • cm-1 (\pm 16.86 SE). Time since fire and ecological site were the significant predictor variables for EC (TSF χ^2 : 15.62, p < 0.001; ES χ^2 : 20.79, p < 0.001). Recently burned patches had lower EC values than intermediate patches (RB - INT = -0.28(CI: -0.52, -0.04); Figure 3.10). Although a significant fixed effect for EC, there were no significant differences between ecological sites.

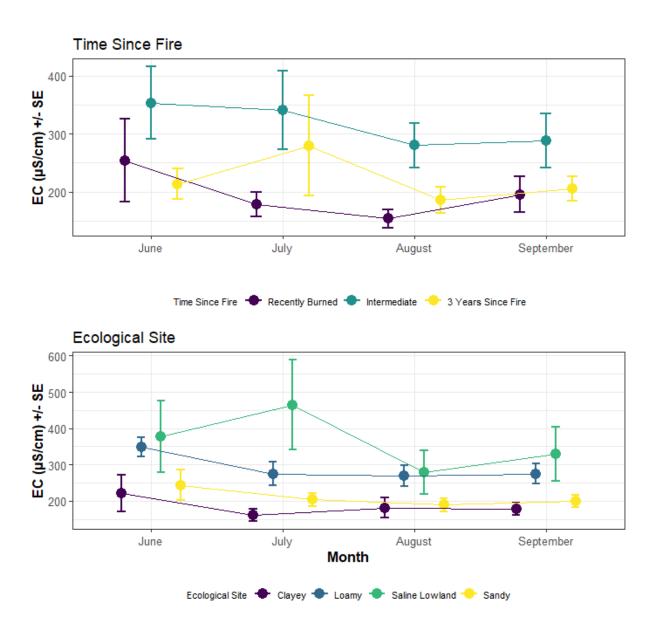


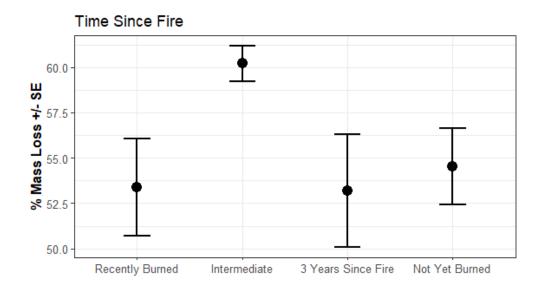
Figure 3.10. Electrical conductivity (EC, micro Siemens • cm $^{-1}$) in top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. Intermediate patches had higher EC values than recently burned patches across ecological sites. Although ecological site was a significant fixed effect for EC, there were no significant differences between ecological sites. Points represent mean values \pm standard error.

Microbial Activity, Abundance, and Composition

Litter Bag Decomposition

We recovered 70% of deployed litter bags in 2019 and 58% in 2020. Sheep pastures had higher recovery rates than cattle pastures. We recovered 83% of deployed litter bags in sheep

pastures in 2019 and 71% in 2020. In cattle pastures, we recovered 56% of litter bags in 2019 and 46% in 2020. For this analysis, we looked at decomposition across both litter substrate species rather than separately. Low recovery rates precluded the full evaluation of the TSF×ES interaction due to some missing factor combinations. Time since fire and ecological site were significant predictor variables for decomposition (TSF χ^2 : 16.96, p < 0.001; ES χ^2 : 10.07, $p \le 0.002$). Intermediate patches had higher decomposition rates than recently burned and not yet burned patches (INT – NYB: 9.28(CI: 2.12, 16.43), INT – RB: 6.24(CI: 0.89, 11.60); Figure 3.11). Loamy sites had higher decomposition rates than sandy sites (Loamy – Sandy: 7.92(CI: 0.93, 14.91)).



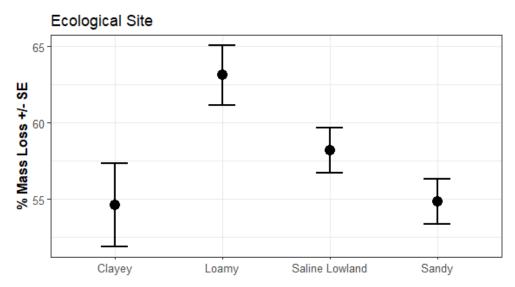


Figure 3.11. Soil decomposition activity (% mass lost) in patch-burn grazing pastures in southwestern North Dakota measured with litter bags buried at 10-cm. Intermediate patches had higher decomposition activity than recently burned and not yet burned patches. Loamy sites had higher decomposition activity than sandy sites. Points represent mean values \pm standard error.

Microbial Abundance and Composition

Gram-negative (37.52% \pm 0.16 SE) and Gram-positive (35.93% \pm 0.17 SE) bacteria accounted for the largest percentages of the microbial community across patches and sites (Figure 3.12). The overall fungi:bacteria ratio of 0.12 (\pm 0.002 SE) was less than 1 across patches

and sites further indicating a community mostly comprised of bacteria. Patches with three years since fire had the highest fungi:bacteria ratio (3YSF – RB: 0.03(CI: 0.01, 0.05), 3YSF – INT: 0.03(CI: 0.01, 0.04), 3YSF – NYB: 0.03(CI: 0.003, 0.05)). Loamy and sandy sites had higher fungi:bacteria ratios than saline lowland sites (Loamy – SaLo: 0.03(CI: 0.01, 0.05), Sandy – SaLo: 0.02(CI: 0.01, 0.03); Figure 3.13).

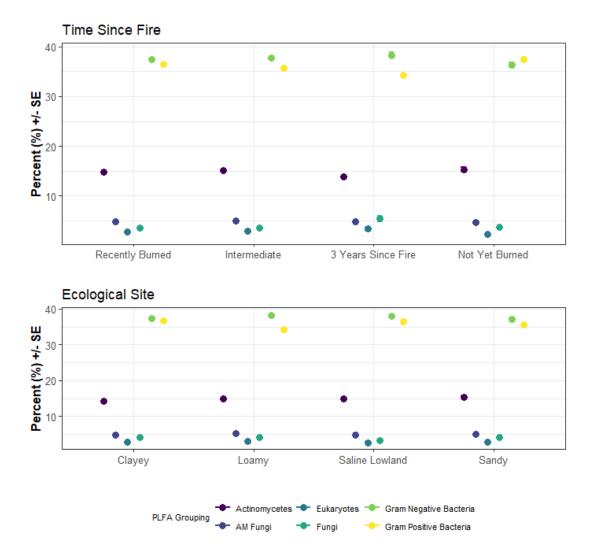


Figure 3.12. Percentages of total abundance of microbial community separated by time since fire patches and ecological sites from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis.

Gram-positive and Gram-negative bacteria made up the largest proportion of the community in each patch type and ecological site.

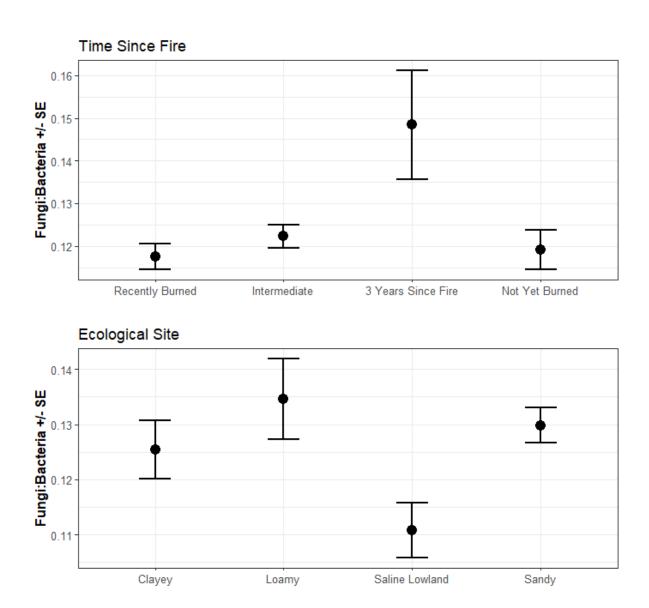


Figure 3.13. Fungi:bacteria ratio of soils separated by time since fire and ecological site from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis. Patches with three years since fire had the highest ratio. Loamy and sandy ecological sites had higher ratios than saline lowland sites. Points represent mean values \pm standard error.

The overall mean total microbial abundance across the 2019 and 2020 grazing seasons was 129.69 nmol • g-1 (\pm 4.38 SE). Ecological site was the only significant predictor variable for total microbial abundance (ES χ^2 : 31.3, p <0.001). Sandy sites had lower total microbial

abundances than loamy and saline lowland sites (Loamy – Sandy: 0.002(CI: 0.0001, 0.003), SaLo – Sandy: 0.002(CI: 0.0001, 0.003); Figure 3.14).

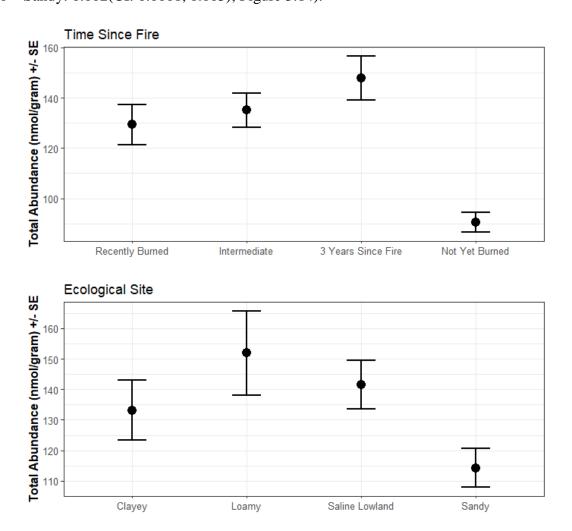


Figure 3.14. Total soil microbial abundance (nanomole • g⁻¹) separated by time since fire and ecological site from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis.

Sandy sites had lower microbial abundances than loamy and saline lowland sites. Time since fire was not a significant predictor variable, and there were no significant differences between time since fire patches. Points represent mean values \pm standard error.

The overall mean abundance of Gram-negative bacteria across the 2019 and 2020 grazing seasons was 48.96 nmol • g-1 (\pm 1.72 SE). Ecological site was the only significant predictor variable for total gram-negative abundance (ES χ^2 : 41.3, p <0.001). Sandy sites had the lowest

abundance of Gram-negative bacteria (SaLo – Sandy: -0.27(CI: -0.42, -0.11), Sandy – Loamy: -0.25(CI: -0.49, -0.02), Sandey – Clayey: -0.18(CI: -0.34, -0.01); Figure 3.15). The overall mean abundance of Gram-positive bacteria across the 2019 and 2020 grazing seasons was 46.23 nmol • g-1 (\pm 1.49 SE). Ecological site was the only significant predictor variable for Gram-positive abundance (ES χ^2 : 40.72, p <0.001). Clayey and saline lowland sites had higher abundances than sandy sites (Clayey – Sandy: 0.2(CI: 0.05, 0.36), SaLo – Sandy: 0.24(CI: 0.9, 0.38); Figure 3.15). There were no significant differences between TSF patches for Gram-positive or Gram-negative bacteria.

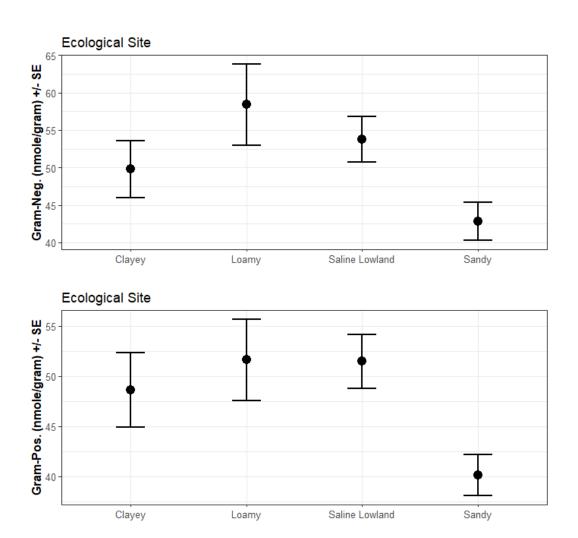


Figure 3.15. Abundance (nanomole \bullet g⁻¹) of Gram-negative and Gram-positive bacteria separated by ecological site from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis.

For Gram-negative bacteria, clayey, loamy, and saline lowland sites had higher abundances than sandy sites. For Gram-positive bacteria, clayey and saline lowland sites had higher abundances than sandy sites. Points represent mean values \pm standard error.

The overall mean abundance of arbuscular mycorrhiza fungi across the 2019 and 2020 grazing seasons was 6.45 nmol • g-1 (\pm 0.24 SE). Time since fire, ecological site, and the TSF×ESD interaction were significant predictor variables for AM fungi (TSF χ^2 : 10.59, $p \le 0.01$; ES χ^2 : 16.55, p < 0.001; TSF×ES χ^2 : 19.09, $p \le 0.02$). Across TSF patches, the abundance was higher in loamy sites than sandy sites (Loamy – Sandy: 2.11(CI: 0.35, 3.88)). Within ecological

sites, the only significant TSF differences were in saline lowland sites where recently burned and intermediate patches had higher abundances of AM fungi than patches with three years since fire (RB – 3YSF: 3.23(CI: 0.21, 6.24), INT – 3YSF: 4.02(CI: 1.15, 6.9); Figure 3.16). The overall mean abundance of fungi across the 2019 and 2020 grazing seasons was 5.2 nmol • g-1 (\pm 0.32 SE). Although the TSF×ESD interaction was the only significant fixed effect for fungi abundance (TSF×ES χ^2 : 22.86, $p \le 0.007$), there were no significant differences in abundances between ecological sites or significant TSF differences within individual ecological sites (Figure 3.16).

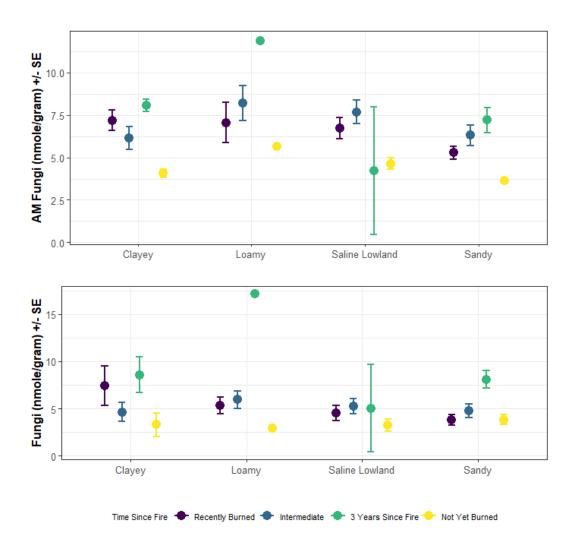


Figure 3.16. Abundance (nanomole • g⁻¹) of arbuscular mycorrhizal fungi and fungi shown as the interaction between time since fire and ecological sites from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis.

For AM Fungi in saline lowland sites, abundances in recently burned and intermediate patches were higher than patches with three years since fire. Although ecological site and the $TSF \times ES$ interaction were significant for fungi, there were no significant differences between sites or within TSF patches for individual sites. Points represent mean values \pm standard error.

The overall mean abundance of eukaryotes across the 2019 and 2020 grazing seasons was 3.85 nmol • g-1 (\pm 0.21 SE). Ecological site was the only significant predictor variable for eukaryote abundance (ES χ^2 : 11.20, $p \le 0.01$). Eukaryote abundance was higher in loamy sites than sandy sites (Loamy – Sandy: 0.3(Ci: 0.01, 0.59); Figure 3.17). The overall mean abundance

of actinomycetes across the 2019 and 2020 grazing seasons was 18.93 nmol • g-1 (\pm 0.61 SE). Time since fire and ecological site were significant predictor variables for actinomycete abundance (TSF χ^2 : 8.36, $p \le 0.04$; ES χ^2 : 42.82, $p \le 0.001$) Saline lowland sites had higher abundances than sandy sites (SaLo – Sandy: 0.22(CI: 0.8, 0.35); Figure 3.17). There were no significant differences between TSF patches for abundances of eukaryotes or actinomycetes.

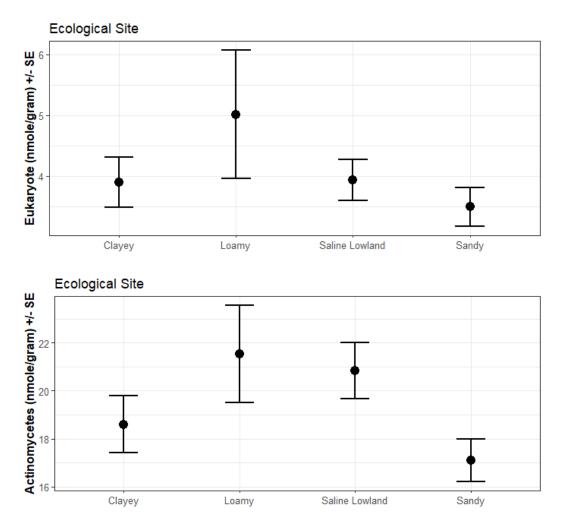


Figure 3.17. Abundance (nanomole • g⁻¹) of eukaryotes and actinomycetes separated by ecological site from top 10-cm of patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota determined using phospholipid fatty acid analysis.

Eukaryote abundance was higher in loamy than sandy sites. Actinomycete abundance was higher in saline lowland sites than sandy sites. There were no significant differences between TSF patches were either group. Points represent mean values \pm standard error.

The unconstrained ordination with the PLFA soil microbial community data had a stress of 0.04 and explained 92% of the variation through three axes (PCA Axis 1: 51%, PCA Axis 2: 29%, PCA Axis 12%; Figure 3.18). We found initial dissimilarity in abundance of PLFA groupings with factor variables time since fire ($r^2 = 0.07$, p = 0.004) and ecological site ($r^2 = 0.04$, p = 0.04). Patches with three years since fire were dissimilar from all other patches (p = 0.002). With ecological site, however, there were no significant differences between ecological sites ($p \ge 0.07$). We found dissimilarity along multiple vector gradients: gravimetric moisture content ($r^2 = 0.06$, p = 0.006), phosphorus ($r^2 = 0.06$, $p \le 0.04$), potassium ($r^2 = 0.07$, $p \le 0.02$), calcium ($r^2 = 0.15$, $p \le 0.002$), total carbon ($r^2 = 0.08$, $p \le 0.004$), and total carbon:nitrogen ratio ($r^2 = 0.13$, $p \le 0.02$).

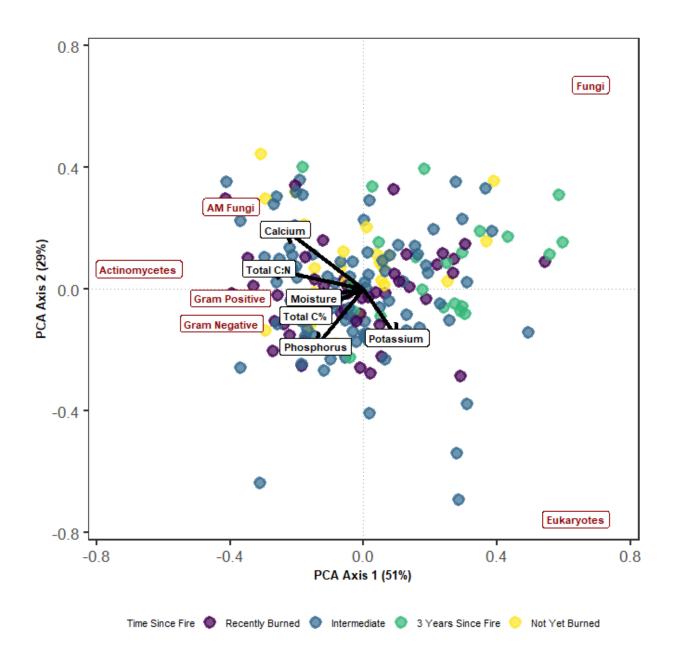


Figure 3.18. Unconstrained ordination of the soil microbial community in patch-burn grazing pastures at the Hettinger Research Extension Center in southwestern North Dakota. Time since fire $(r^2=0.07, p=0.004)$ and ecological site $(r^2=0.04, p=0.04)$ were significantly associated with composition of phospholipid fatty acid analysis microbial groupings. Patches with three years since fire were dissimilar from all other patches, but there were no differences between ecological sites. Multiple vector gradients influenced community composition: moisture content $(r^2=0.06, p=0.006)$, phosphorus $(r^2=0.06, p\leq0.04)$, potassium $(r^2=0.07, p\leq0.02)$, calcium $(r^2=0.15, p\leq0.002)$, total carbon $(r^2=0.08, p\leq0.004)$, and total carbon:nitrogen ratio $(r^2=0.13, p\leq0.02)$.

Discussion

Understanding how above and belowground rangeland system properties respond to intensive disturbance regimes is crucial for evaluating the efficacy of management strategies attempting to improve ecosystem service delivery in these working landscapes. This study presents a seemingly counterintuitive response in soil nutrient pools and microbial communities under rangelands managed with patch-burn grazing. One might expect that the intensive grazer attraction following a prescribed fire would result in a reduction of the soil nutrient pools and microbial communities located in recently burned patches relative to patches with increased time since fire and less consistent grazing pressure. Instead, the measured responses were largely resistant to these combined disturbances, and the variation in measured responses was consistently attributed to the inherent heterogeneity of these rangelands when incorporated as ecological sites.

The lack of negative responses along the implicit time since fire intensity gradient suggests that managing for aboveground heterogeneity did not come at the expense of belowground properties and processes under these conditions. The lack of conflicting interactive effects between the disturbance gradient, analyzed as time since fire, and ecological sites is also promising for wider evaluation of this management strategy in the region. This supports previous findings that aboveground management in rangelands is not always tied to an equivalent response in soil properties and processes (Anderson et al. 2006, Pyle et al. 2019, Sanderson et al. 2020).

Since patch-burn grazing is discussed as a way of balancing livestock production and biodiversity conservation needs in rangelands, it is important to develop a baseline of expectations for ecosystem properties and processes across variations and possible environments

it could be implemented in (Toombs et al. 2010, McGranahan et al. 2013). Patch-burn grazing has typically used either cattle or bison for the grazing component and is predominantly conducted in more humid regions of the Great Plains (Scasta et al. 2016b). The inclusion of a treatment comparing responses between pastures grazed by cattle and pastures grazed by sheep was relevant for the region given the prevalence of sheep ranching operations and novel in the realm of patch-burn grazing research. That we did not observe significant differences in soil nutrients or microbial communities between grazer types when stocked similarly provides useful context for practitioners when making stocking decisions. Other work on these pastures during the study period found that sheep pastures had lower forb cover and floral resources than cattle pastures which has more direct implications for pollinator communities (Cutter et al. 2021, Chapter 2). We are unable to directly compare the degree to which our observed results can be attributed to the use of fire or grazing individually rather than their interactive effects due to the study design prioritizing investigating potential differences along the implicit intensity gradient found in patch-burn grazing systems. Future work could include sampling within grazing exclosures and fire exclusions to isolate contributions of either disturbance across patches (Augustine et al. 2010).

We can infer from the neutral effects on inorganic nitrogen and soil moisture that plants in recently burned patches were not limited by nitrogen or moisture any more than in other patches (Anderson et al. 2006, Augustine et al. 2010). Similarly, no other measured nutrient pools were significantly lower in recently burned patches. These results support previous findings that infrequent rangeland fires do not incur negative responses in measured nutrients (Blair 1997, Anderson et al. 2006, Coetsee et al. 2008). This does, however, raise questions about where the nutrients are coming from. Given the differences in aboveground biomass and

grazer selection, a reasonable assumption is that there is a difference in the type and quantity of aboveground inputs between recently burned and patches with increased time since fire (Chapter 2). The litter bag approach that we used provides a broad estimation of microbial activity over time, but does not directly inform whether the nutrients were mineralized into the soil or immobilized by the microbial community (Harmon et al. 1999, Karberg et al. 2008).

Extracellular enzyme assays and resin bags are two complimentary methods that would be able to quantify which substrates in the soil environment microbes are utilizing and whether mineralization or immobilization is occurring in future work (Binkley et al. 1986, Friedel et al. 2000, Burns et al. 2013, Banerjee et al. 2016).

The inherent heterogeneity, as categorized by ecological sites, did not mediate time since fire effects as might be expected. Responses to imposed disturbances often vary by ecological site indicating research and monitoring should incorporate inherent heterogeneity into design (Vermeire et al. 2005, Augustine and Derner 2014, Wonkka et al. 2016, Davis et al. 2020). Here, the lower diversity plant community shifts the focus of ecological site distinctions to soil type and landscape position. Ecological site was a significant predictor variable for all but two measured response variables. The TSF×ES interaction, however, was only significant for magnesium, pH, AM fungi, and fungi. Within significant interactions, contradictory TSF differences between ecological sites were only found with pH. Thus, we can deduce that ecological sites responded similarly to the time since fire intensity gradient despite inherent variability in response variables between ecological sites. The lower pH values in recently burned patches on clayey sites over the 2020 grazing season is a point of concern, but the values are still within the realm of acceptability for macronutrient availability (Paul 2014).

While we did expect to see a higher proportion of soil bacteria in these pastures, we did not expect soil fungi to be as abundant in recently burned patches. Fungal communities typically have a negative response to fire, especially high intensity fires (Pressler et al. 2019, Dove et al. 2021). Data from prescribed fires on these patch-burn grazing pastures and from a neighboring prescribed fire soil project show that the immediate soil surfaces received a brief period of heating that did not extend into the soil profile past 3 cm (Zopfi 2020, McGranahan et al. currently unpublished). This suggests that the dormant season prescribed fires did not negatively affect the fungal community under the fire conditions experienced. Sampling before and after conducting future prescribed fires would provide more detail on this interaction, and sampling additional times within the grazing season would provide insight into whether composition shifts as grazer attraction increases or decreases.

The soil microbial community in these former CRP rangelands are likely more responsive to plant community turnover than aboveground disturbances. In idle grasslands, changes in the plant community typically result in a shift in the microbial community composition (Bardgett et al. 1999, Van der Heijden et al. 2007). Disturbances, like tillage, that physically disrupt the soil profile result in reduced microbial abundance and diversity (Balota et al. 2004, Van der Heijden et al. 2007, Paul 2014, Coleman et al. 2017). A study of CRP rangelands in semi-arid West Texas found that the microbial community in the top 10 cm of older CRP rangelands were more similar to native rangelands than younger CRP rangelands were, indicating composition shifts over time (Li et al. 2018). We did not observe significant shifts in plant composition on this first cycle through the burn rotation despite altering vegetation structure, but it is something to follow-up on after additional cycles through the burn rotation (Chapter 2).

Rangeland soil health initiatives and the increased interest in rangeland carbon sequestration underscore the importance of understanding how disturbances influence the regulating and supporting ecosystem services that soils provide (Dominati et al. 2010, Sanderson et al. 2020, Bork et al. 2020, Roy and Bagchi 2021). The deliberate increase in bare ground cover and removal of accumulated litter are counter to recommendations for rangeland health indicators and soil health principles (Printz et al. 2014, Derner et al. 2018). Our findings suggest that patch-burn grazing did not have negative effects on common nutrient and microbial indicators used to evaluate rangeland soil health and sequestration potential (Printz et al. 2014, Pyle et al. 2019, Sanderson et al. 2020). We did not find significant differences in total carbon between TSF patches, but recent studies have found that grazing at moderate and even higher intensities can increase soil carbon pools (Bork et al. 2020, Roy and Bagchi 2021). Variability in rangeland soil carbon pools is more often associated with variable weather conditions in shortterm evaluations and climate patterns at long-term evaluations (Sanderson et al. 2020). It is important to note that we focused on identifying potential nutrient pools affected by this management at the onset of the study and thus did not measure some of the variables used to fully account for carbon pools and fluxes like inorganic carbon, soil organic material, soil respiration, root exudate production, and belowground herbaceous production.

Active and intensive management of conservation focused grasslands and the use of prescribed fire in grazed rangelands are currently uncommon in the northern Great Plains, but our findings support efforts of reintroducing fire and grazing into grasslands across the region (Sliwinski et al. 2018, Dixon et al. 2019, Augustine et al. 2019, Bendel et al. 2020). The plant community in our research pastures is representative of enrolled and formerly enrolled Conservation Reserve Program grasslands and revegetated conservation grasslands throughout

the region (Dixon et al. 2019). The predominantly introduced cool season grass communities have been invaded by additional introduced cool season grasses like Kentucky bluegrass and smooth brome, which are also prevalent on private rangelands (DeKeyser et al. 2013, Toledo et al. 2014, Grant et al. 2020, Gasch et al. 2020). While these introduced species provide ample forage for livestock and dense nesting cover, homogeneous plant communities combined with homogeneity-based management limit the potential to meet biodiversity conservation goals beyond preservation of a grassland state (Fuhlendorf et al. 2017, Geaumont et al. 2017, Dixon et al. 2019). What our previous work on these pastures illustrates is that patch-burn grazing increased structural heterogeneity for wildlife and consistently provided forage with a higher nutritive value for grazing animals (Chapters 1 and 2). Here, we are able to show how hesitation regarding the use of prescribed fire from a soil perspective is unsupported in this context (Bendel et al. 2020).

We encourage a more disturbance inclusive perspective of enrolled CRP grasslands and mid-contract management practices. Currently, limited haying and grazing occurs on CRP as a required mid-contract management action or during times of drought and additional management actions are restricted to outside of the primary nesting season (Farm Service Agency 2018). For mid-contract management, enrollees are able to use a variety of disturbances to potentially increase structural and plant community diversity including herbicide, tillage, interseeding, and prescribed burning (Natural Resource Conservation Service 2012, ND State FSA Office 2016). In North Dakota, it is unclear how much prescribed burning is actually conducted as mid-contract CRP management, but it is likely that very little is cost-shared as part of broader USDA conservation efforts on private lands (Toombs and Roberts 2009, Natural Resource Conservation Service 2020).

Conclusion

Here we are able to illustrate patterns in soil nutrient pools and microbial communities useful for evaluating the efficacy of patch-burn grazing beyond the ability to create aboveground structural heterogeneity. Despite experiencing a dormant season prescribed fire and focalized grazing, soil nutrient concentrations and microbial abundances in recently burned patches were equivalent to or higher than patches on the opposite of the intensity gradient. Ecological sites accounted for more variability in measured response variables, and ecological sites did not exhibit differential responses to the time since fire intensity gradient for most measured variables. This suggests that these rangeland soils are resistant to more active management, and more work is needed to directly investigate the underlying processes driving these patterns. Hesitancy regarding reintroducing fire and grazing into conservation-focused grasslands and fire into private rangelands is likely unsupported from a soil perspective in this region.

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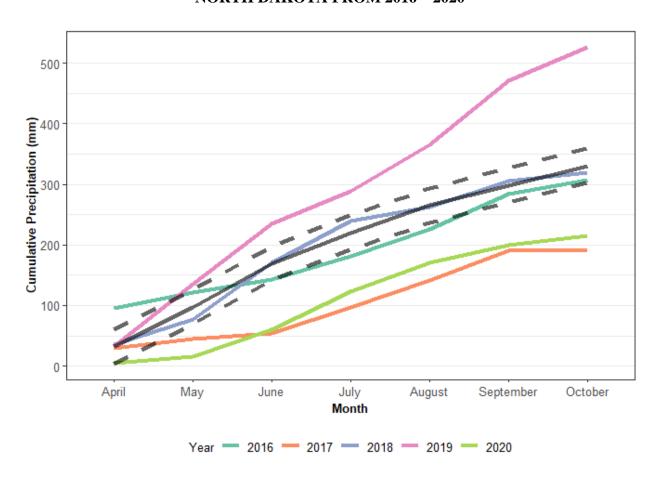
APPENDIX A. LIST OF SPECIES FOUND ON PATCH-BURN GRAZING VEGETATION TRANSECTS FROM 2017 – 2020

Percent	Code	Species	Common	Fine FG
22.919	ELIN	Elymus intermedium	Intermediate wheatgrass	Int C3
21.388	POPR	Poa pratensis	Kentucky bluegrass	Int C3
20.592	BRIN	Bromus inermis	Smooth brome	Int C3
14.109	MESA	Medicago sativa	Alfalfa	Int Legume
3.061	AGCR	Agropyron cristatum	Crested wheatgrass	Int C3
2.281	DISP	Distichlis spicata	Inland saltgrass	Nat C4
1.598	ELTR	Elymus trachycaulus	Slender wheatgrass	Nat C3
1.453	MEOF	Melilotus officinalis	Yellow sweetclover	Int Legume
1.426	COAR	Convolvulus arvensis	Field bindweed	Int Forb
1.313	HOJU	Hordeum jubatum	Foxtail Barley	Nat C3
1.251	ELRE	Elymus repens	Quackgrass	Int C3
0.898	SPPE	Spartina pectinata	Prairie cordgrass	Nat C4
0.792	ACMI	Achillea millefolium	Yarrow	Nat Forb
0.694	TAOF	Taraxacum officinale	Dandelion	Int Forb
0.575	SYER	Symphyotrichum ericoides	White heath aster	Nat Forb
0.566	PASM	Pascopyron smithii	Western wheatgrass	Nat C3
0.456	ERAS	Erysimum asperum	Western wallflower	Nat Forb
0.434	CIUN	Cirsium undulatum	Wavyleaf thistle	Nat Forb
0.324	ARLU	Artemisia ludoviciana	Cudweed Sagewort	Nat Forb
0.294	GLLE	Glycyrrhiza lepidota	American licorice	Nat Legume
0.293	CIAR	Cirsium arvense	Canada thistle	Int Forb

Percent	Code	Species	Common	Fine FG
0.282	SOAR	Sonchus arvensis	Perennial sow thistle	Int Forb
0.28	SEDGE	Carex sp	Sedge	Sedge
0.238	ARFR	Artemisia frigida	Fringed sagewort	Nat Forb
0.234	SPHE	Sporobolus heterolepis	Prairie dropseed	Nat C4
0.217	HEMA	Helianthus maximiliani	Maximillian Sunflower	Nat Forb
0.186	BOGR	Bouteloua gracilis	Blue grama	Nat C4
0.151	GRSQ	Grindelia squarrosa	Curlycup gumweed	Nat Forb
0.142	BUNCH	Unknown Elymus	Unknown Elymus	Nat C3
0.098	RUOC	Rumex occidentalis	Western Dock	Nat Forb
0.09	LOUN	Lotus unifoliolatus	Deer vetch	Nat Legume
0.09	VIAM	Vicia americana	American vetch	Nat Legume
0.083	ASSY	Asclepias syriaca	Common milkweed	Nat Forb
0.079	PSAR	Psoralea argophylla	Silverleaf scurfpea	Nat Legume
0.068	AMPS	Ambrosia psilostachya	Western ragweed	Nat Forb
0.066	DEPI	Descurainia pinnata	Tansymustard	Nat Forb
0.065	IVAX	Iva axillaris	Poverty weed	Nat Forb
0.058	LEDE	Lepidium densiflorum	Common pepperweed	Nat Forb
0.057	NAVI	Nassella viridula	Green needlegrass	Nat C3
0.054	HEPA	Helianthus pauciflorus	Stiff sunflower	Nat Forb
0.054	TYLA	Typha latifolia	Cattail	Cattail
0.05	THAR	Thlaspi arvense	Field pennycress	Int Forb
0.036	CALO	Calamovilfa longifolia	Prairie sandreed	Nat C3
0.031	LATA	Lactuca tatarica	Blue lettuce	Nat Forb
0.03	HEPU	Hedeoma pulegioides	False pennyroyal	Nat Forb

Percent	Code	Species	Common	Fine FG
0.03	SOML	Solidago mollis	Soft goldenrod	Nat Forb
0.03	SOMO	Solidago missouriensis	Missouri goldenrod	Nat Forb
0.03	SPCO	Sphaeralcea coccinea	Scarlet globemallow	Nat Forb
0.03	TRDU	Tragopogon dubius	Goatsbeard	Int Forb
0.029	CASA	Camelina sativa	False flax	Int Forb
0.028	CHAL	Chenopodium album	Lambsquarters	Int Forb
0.027	EUES	Euphorbia esula	Leafy spurge	Int Forb
0.026	COTI	Coreopsis tinctoria	Plains coreopsis	Nat Forb
0.025	HECO	Hesperostipa comata	Needle and thread	Nat C3
0.023	ANMI	Antennaria microphylla	Littleleaf pussytoes	Nat Forb
0.023	SYOC	Symphoricarpos occidentalis	Snowberry	Nat Shrub
0.019	ECAN	Echinacea angustifolia	Purple prairie coneflower	Nat Forb
0.019	PHPR	Phleum pratense	Timothy	Int C3
0.018	LIPU	Liatris punctata	Dotted gayfeather	Nat Forb
0.018	POAN	Poa annua	Annual bluegrass	Int C3
0.018	ROAR	Rosa arkansana	Prairie rose	Nat Shrub
0.018	THRH	Thermopsis rhombifolia	Prairie thermopsis	Nat Legume
0.017	RACO	Ratibida columnifera	Prairie coneflower	Nat Forb
0.015	BSPR	Bassia prostrata	Kochia	Int Forb
0.015	BUDA	Buchloe dactyloides	Buffalograss	Nat C4
0.014	PSES	Psoralea esculenta	Breadroot scrufpea	Nat Legume
0.013	KOMA	Koeleria macrantha	Prairie junegrass	Nat C3
0.01	BASC	Bassia scoparia	Kochia	Int Forb
0.006	MEAL	Melilotus albus	White sweetclover	Int Legume

Percent	Code	Species	Common	Fine FG
0.005	PLPA	Plantago patagonica	woolly plantain	Nat Forb
0.005	SAKA	Salsola kali	Russian thistle	Int Forb
0.004	ANEM	Anemone	Geranium	Nat Forb
0.004	LYJU	Lygodesmia juncea	Rush skeleton plant	Nat Forb
0.004	VEPE	Veronica peregrina	Purslane Speedwell	Nat Forb
0.003	GAAR	Gaillardia aristata	Blanketflower	Nat Forb
0.002	BEIN	Berteroa incana	Hoary false alyssum	Int Forb
0.002	BRJA	Bromus japonicus	Japanese brome	Int C3
0.002	GERI	Geranium richardsonii	Richardson's geranium	Nat Forb
0.002	HEVI	Heterotheca villosa	Hairy goldenaster	Nat Forb
0.002	OPFR	Opuntia fragilis	Fragile prickly pear	Nat Shrub
0.001	ANPA	Antennaria parvifolia	Small-leaf pussytoes	Nat Forb
0.001	ARPY	Arabis pycnocarpa	hairy rockcress	Nat Forb
0.001	BOCU	Bouteloua curtipendula	Sideoats Grama	Nat C4
0.001	DAPU	Dalea purpurea	Purple prairie clover	Nat Legume
0.001	OWL	Orthocarpus luteus	Owl Clover	Nat Forb
0.001	POCO	Polygonum coccineum	Smartweed	Nat Forb



The black solid line is the 25-year average calculated from the NDAWN weather station at the Hettinger Research Extension Center. The dashed black lines represent the 95% confidence intervals for the 25-year average.