Interactive effects of fire and grazing on soil and forage nitrogen dynamics in a tallgrass prairie

by

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Abstract

Fire and large herbivores are dominant regulators of nutrient cycling in tallgrass prairie ecosystems, yet their combined effects on soil nitrogen (N) dynamics and links to forage quality across heterogeneous landscapes remain incompletely resolved. This thesis examined how fire frequency, grazing, and landscape position interact to regulate N cycling, N retention, and plant–soil feedbacks in tallgrass prairie systems. Using long-term experimental watersheds at Konza Prairie Biological Station and additional cross-site comparisons, we quantified soil N pools, inorganic N availability (NH_4^+, NO_3^-), potentially mineralizable N (PMN), autoclavable citrate-extractable (ACE) protein, extracellular enzyme activities, and forage quality (N concentration) across burn intervals, grazing regimes, and topographic positions.

Results show that fire and grazing regulate distinct but interacting components of the N cycle. Infrequent fire increased soil N accumulation and proteinaceous organic N pools, reflected in higher total N and ACE protein. In contrast, grazing enhanced N availability and turnover, increasing nitrate, PMN, and shifting microbial enzyme allocation toward carbon acquisition, indicative of reduced microbial N limitation. Grazing effects were strongest in upland soils, and also caused a notable accumulation of soil total N; shallow soil depth and preferential grazer use may have intensified responses.

Forage quality mirrored soil N dynamics, with grazing and less frequent fire producing higher forage N concentrations and lower C:N ratios, reinforcing feedbacks between plant inputs and microbial processes. However, forage N feedbacks were expressed primarily later in the growing season, and were not detected synchronously across multiple sites, despite consistent grazing effects on soil microbial N availability, reflecting the importance of plant growth dynamics in regulating N availability to grazers. Overall, fire and grazing did not uniformly

increase or decrease N pools; instead, they redistributed N among storage, mineralization, and biological demand pathways.

Together, these findings demonstrate that N cycling in tallgrass prairie is governed by the integration of fire, grazing, and landscape heterogeneity. Rather than a single-direction response, fire frequency and grazing create a spatially structured mosaic of N availability and retention, with implications for grassland resilience, management, and soil health under changing environmental conditions.

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Dedication

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Chapter 1 - Introduction

Tallgrass prairies, productive ecosystems maintained by the coupled effects of fire and large herbivore grazing, once dominated central North America (Fuhlendorf & Engle, 2004; A. K. Knapp et al., 1999). Today, only a small fraction of this system remains (F. B. Samson & Knopf, 1996), and many surviving prairies have experienced altered fire regimes and the loss or modification of native grazing communities (R. C. Anderson, 2006; Hayden, 1998; A. Knapp & Seastedt, 1998; Savage, 2004). These changes have reshaped plant communities and ecosystem processes (S. L. Collins & Calabrese, 2012; Turner et al., 1997), yet their consequences for belowground nutrient cycling—particularly nitrogen (N)—remain incompletely understood.

Nitrogen is a fundamental constraint on productivity in grassland ecosystems because it is required for plant growth, microbial metabolism, and protein synthesis (Schimel & Bennett, 2004; Vitousek et al., 1997). Although atmospheric N is abundant, ecosystem productivity depends on the transformation of N into biologically accessible forms. In soils, N is primarily stored in organic matter and microbial biomass and becomes available through microbial depolymerization and mineralization processes (Booth et al., 2005; Kuzyakov & Xu, 2013; Schimel & Bennett, 2004). These transformations are governed by microbial activity and enzyme production, linking N availability directly to plant–microbe interactions and environmental conditions (Schimel & Weintraub, 2003; R. L. Sinsabaugh et al., 2009).

Fire and grazing influence N cycling through distinct but interconnected mechanisms. Fire removes aboveground biomass and volatilizes N, reducing surface litter inputs and potentially altering long-term accumulation of soil organic matter (R. C. Anderson, 2006; Pellegrini et al., 2022; Seastedt et al., 1991). Under frequent fire, reduced substrate availability and changes in resource quality can increase microbial and plant demand for N, leading to

greater allocation toward N acquisition (Dell et al., 2005; Dell & Rice, 2005; Schimel & Weintraub, 2003). In contrast, infrequent fire allows organic matter and N to accumulate, slowing N uptake and creating the potential for denitrification and/or leaching (Pellegrini et al., 2020; Seastedt & Knapp, 1993).

Grazing modifies these dynamics by redistributing N through herbivory and excretion. Grazers consume plant biomass and return N to soils in dung and urine, creating localized pulses of bioavailable N that can stimulate microbial activity and N transformations (Frank & Evans, 1997; Johnson & Matchett, 2001; Vega Anguiano et al., 2024; Vinton et al., 1993). Grazing also alters plant regrowth and tissue quality, often increasing forage N concentration and lowering C:N ratios, thereby influencing both plant inputs and microbial processing of organic matter (Blair, 1997; Johnson & Matchett, 2001). These mechanisms suggest that grazing can accelerate N cycling and reduce microbial N limitation without necessarily increasing total soil N pools (Frank et al., 1995; Frank & Groffman, 1998; Vega Anguiano et al., 2024).

Importantly, fire and grazing do not operate independently. In tallgrass prairie, their interaction is expressed through pyric herbivory, where grazers preferentially select recently burned patches with high-quality forage (Allred et al., 2011; Fuhlendorf et al., 2009; Raynor et al., 2015). This feedback creates spatial heterogeneity in plant biomass, nutrient distribution, and microbial activity, structuring ecosystem processes across the landscape (Fuhlendorf et al., 2009; Fuhlendorf & Engle, 2004; Kerby et al., 2007). Landscape position further modifies these interactions. Upland soils are typically shallow, well-drained, and more sensitive to surface disturbances, while lowland soils are deeper, more mesic, and capable of greater organic matter accumulation (Briggs et al., 1998; Oviatt, 1998; Ransom et al., 1998). These differences influence water availability, substrate supply, and microbial activity, potentially altering how fire

and grazing effects propagate through the N cycle (Collins & Calabrese, 2012; Robertson et al., 1997; Turner et al., 1997). Despite this, relatively few studies explicitly evaluate how fire–grazing interactions regulate N cycling across topographic gradients.

Understanding these dynamics requires integrating multiple indicators of N cycling. Measures of inorganic N quantify immediate plant-available pools (Booth et al., 2005; Schimel & Bennett, 2004), while potentially mineralizable N reflects microbial capacity to release N from organic matter (Drinkwater et al., 1997; Keeney & Bremner, 1966). Autoclaved citrate-extractable (ACE) protein represents a pool of proteinaceous organic N associated with microbial residues and SOM, often interpreted as a reservoir of potentially available organic N rather than immediately accessible inorganic N (Hurisso et al., 2018; Naasko et al., 2024; Stott, 2019), and extracellular enzyme activities provide insight into microbial resource allocation and nutrient demand (Schimel & Weintraub, 2003; R. L. Sinsabaugh et al., 2009). Together, these metrics allow differentiation among N storage, availability, and microbial demand, offering a more complete understanding of soil N dynamics.

The overarching objective of this thesis is to determine how fire frequency and grazing jointly regulate N cycling and retention in tallgrass prairie ecosystems, and how these effects are mediated by landscape position and linked to forage quality. I hypothesized that fire and grazing regulate N cycling through complementary pathways, with fire primarily influencing N storage and grazing primarily influencing N availability and turnover. Specifically, I predicted that frequent fire would increase N limitation and promote immobilization of available N (Dell et al., 2005; Seastedt et al., 1991), while grazing would reduce N limitation by increasing the availability and turnover of inorganic N pools (Johnson & Matchett, 2001; Vega Anguiano et al., 2024). I further predicted that these effects would be strongest in upland soils, where shallow soil

profiles and greater grazer use amplify fire impacts (Briggs et al., 1998; Vega Anguiano et al., 2024), and weaker in lowland soils, where greater depth and moisture buffer responses (S. L. Collins & Calabrese, 2012; Oviatt, 1998).

To test these predictions, this thesis integrates soil and forage measurements across experimental fire and grazing treatments, combining indicators of N pools, mineralization, and microbial enzyme activity with measures of forage quality. By linking belowground processes with plant responses, this work provides a mechanistic framework for understanding how disturbance regimes regulate nutrient cycling in grassland ecosystems. Ultimately, this research advances a more nuanced view of prairie N dynamics, demonstrating that fire and grazing do not simply increase or decrease N availability, but reorganize its distribution among storage, microbial processing, and plant uptake pathways across a heterogeneous landscape.

Chapter 2 - Interactive Effects of Fire and Bison Grazing on Soil Nitrogen Dynamics within a Tallgrass Prairie Ecosystem

Abstract

Fire and large herbivores are regulators of nutrient cycling in tallgrass prairie ecosystems, yet their combined effects on soil nitrogen (N) dynamics across heterogeneous landscapes remain incompletely understood. This study examined how fire frequency and bison grazing interact to influence soil N pools, microbial N demand, and N availability in upland and lowland topographical positions at Konza Prairie Biological Station (KPBS), Kansas, USA. We hypothesized that frequent fire would increase N limitation, whereas grazing would reduce N limitation and increase available inorganic N availability and turnover relative to ungrazed prairie, with weaker responses in lowland soils. Surface mineral soils (0-15 cm deep) were collected from replicate watersheds with contrasting historical burn intervals (1-, 2-, 4-, 20-year) with and without long-term bison grazing. To assess N cycling responses, multiple indicators of N dynamics were quantified, including total soil N, inorganic N fractions (NH_4^+ and NO_3^-), potentially mineralizable N over 7 (PMN_{7d}) and 28 days (PMN_{28d}), autoclaved citrate-extractable protein, and extracellular enzyme activities associated with C and N acquisition. Results partially supported predictions: grazing increased soil %N and multiple indicators of N availability in upland soils, while infrequent fire also enhanced N availability across metrics. Fire effects were largely independent of topography, though grazing dampened the fire effect on PMN_{28d}. Notably, in uplands, both infrequent fire and grazing promoted N accumulation to a similar magnitude; upland sensitivity to grazing could be due to differences in soil depth, and/or higher bison usage of upland areas in the landscape. Differential patterns in indices of N availability in short- and long-turnover pools suggest different mechanisms of N partitioning among storage,

mineralization, and microbial demand pathways in infrequently burned prairies. Overall, fire and grazing jointly regulate prairie soil N cycling, with consistent patterns across extractable, mineralizable, and enzymatic indicators of N availability.

Introduction

Grasslands once covered vast portions of the central North American continent; however, of the original expanses that used to dominate from central Texas through south-central Canada, less than 4% of the prairie remains today (Samson & Knopf, 1994). Native tallgrass prairies are characterized by high herbaceous biomass and floral diversity (Freeman, 1998; Samson, 1996), which supported large numbers of ungulate grazers like bison, and were maintained by frequent fire disturbance (Knapp et al. 1999, Fuhlendorf and Engle 2001, Fuhlendorf and Engle 2004). Belowground, grassland soils hold large quantities of organic matter, including the biomass of dense root systems of native grasses, and soil microorganisms that cycle essential nutrients (Bai & Cotrufo, 2022; Ransom et al., 1998; Risser & Parton, 1982). Across much of the historical extent of North American grasslands, soils were tilled and cultivated, and native prairie was replaced by conventional agriculture. In tallgrass prairie remnants such as the Flint Hills of Kansas, while soils remained largely untilled, there has been widespread loss of native grazers, as well as fire suppression (Anderson, 2006; Hayden, 1998; Knapp & Seastedt, 1998; Savage, 2004). While consequent changes in prairie vegetation are well-documented, the results of concurrent shifts in prairie fire and grazing for soil health properties are less well understood.

Soil health is defined by the Natural Resource Conservation Service (NRCS) as a soil's capacity to sustain a variety of lifeforms (Brown & Herrick, 2016; Stott & Moebius-Clune, 2017). Many soil factors support life in the prairie, arguably foremost among them soil nitrogen (N) availability, because N is a necessary and limiting element for activity and growth of all

organisms within prairie ecosystems (Schimel & Bennett, 2004; Vitousek et al., 1997). Although atmospheric N is abundant, terrestrial productivity is constrained by the accessibility of reactive N forms (Blair et al., 1998). The transformation of N from inert N_2 gas to and among its many biologically accessible forms requires reduction, oxidation, and depolymerization process that are fundamentally governed by electron movement through microbial metabolic networks (Blair et al. 1998, Kirk 2023). In soils, N is stored predominantly in organic compounds like proteins and microbial biomass or necromass, and is most accessible for plant growth in its inorganic forms like ammonium (NH_4^+) or nitrate (NO_3^-) (Booth et al., 2005; Kuzyakov & Xu, 2013; Schimel & Bennett, 2004). Frequent fire and ungulate grazing influence N cycling through the tallgrass prairie landscape in both direct and indirect ways.

Fire removes aboveground biomass and dead plant litter, directly volatilizing a portion of the ecosystem N stock (Anderson et al., 2006; Pellegrini et al., 2022; Seastedt et al., 1991). Consequently, frequent burning prevents surface organic matter accumulation (Knapp et al. 1986, Seastedt and Knapp 1993, Pellegrini et al. 2020), and can promote greater fine root production by plants (Johnson and Matchett, 2001). Thus, soil N is scarce relative to biological demand, and the N-limited native prairie plants and soil microbes assimilate soil available N more quickly, and retain N longer within their biomass, in annually burned than infrequently burned prairies (Dell et al., 2005; Dell & Rice, 2005). In response to N limitation, prairie plants increase their root biomass to forage for N (Johnson & Matchett, 2001), and soil microbes increase production of enzymes that scavenge organic N compounds, like amino acids and amino sugars, from SOM (Schimel & Weintraub, 2003; Sinsabaugh et al., 2009). As such, high plant root biomass and high microbial enzymatic N demand are both characteristics of frequently burned, N-limited, native tallgrass prairies (Hobbs et al., 1991b; Johnson & Matchett, 2001).

Prairie fire also may have indirect effects on grassland soil N cycling. Frequent fire prevents the expansion of shrubs and trees into grass-dominated systems, also known as woody encroachment, and promotes the growth of warm-season C_4 grasses by increasing light availability and removing surface-level litter and shading (Bond et al., 2005; Ratajczak et al., 2014; Turner et al., 1997). Woody encroachment could change soil N cycling dynamics if different plant-microbe relationships follow the shift in vegetation; however, evidence for turnover in N-cycling soil microbial communities following woody encroachment is weak (Connell et al., 2020; Nieland et al., 2024). In addition, the immediate post-fire environment is characterized by pulses of mineral nutrients in ash (Sánchez-García et al., 2023). Nitrogen is volatilized to the atmosphere during combustion; however, ash contains phosphorus and base cations (Hulbert, 1988; Z. Li et al., 2025; Roshan & Biswas, 2023; Sánchez-García et al., 2023; Wan et al., 2001), which may stimulate microbial processes by supplying readily soluble micronutrients (Kang et al., 2025; Z. Li et al., 2025). However, N is less limiting to tallgrass prairie plant productivity under intermediate fire frequency, when fire increases light availability after some mineralizeable N has accumulated from litter decomposition (Blair 1997).

Grazing affects tallgrass prairie N cycling by reducing grass biomass and redistributing N across the landscape. Grazing animals consume N-containing plant biomass and subsequently deposit dung and urine that return organic and bioavailable N to the soil (Frank & Evans, 1997; Johnson & Matchett, 2001; Vega Anguiano et al., 2024; Vinton et al., 1993). Thus, grazer egestion creates localized pulses of available N that can alleviate microbial N demand and increase nitrification potential (Vega Anguiano et al., 2024). Urine and dung deposition can also increase soil pH, which increases nitrification potential (Dell et al., 2005; Dell & Rice, 2005). In this way, grazing can increase N cycling activity and availability, without necessarily increasing

the total amount of N in the soil (Frank et al., 1995; Vega Anguiano et al., 2024). Therefore, N-limitation is lessened, and plant root foraging and soil microbial enzymatic N demand are also lower, in grazed relative to ungrazed prairies.

Large grazers, like *Bison bison*, have historically interacted with fire in a coupled disturbance regime, as new N-rich growth within the burnt patches attracts herbivory (Coppedge & Shaw, 1998; Fritch & Us, 2024; Martin et al., 2023; Olson et al., 2022; Whitney, 1994). Pyric herbivory, the natural feedback between fire and grazing, may structure N cycling and redistribution across the prairie (Allred et al., 2011; Fuhlendorf et al., 2009; Raynor et al., 2015). Grazing also discourages woody encroachment, as large animals may wallow, trample, and browse on saplings if their preferred forage is in low supply (Bookout, 2025; Coppedge & Shaw, 1997; Fuhlendorf et al., 2009; Vinton et al., 1993). Woody plants can change the soil microbial community, as well as available water and light at the soil surfaces (Ratajczak et al., 2014; Sha et al., 2025); these factors can change grass and soil microbial nutrient cycling capabilities. Finally, grazing reduces the amount of plant biomass that accumulates as aboveground litter, which in turn reduces return of N to the soil through surface litter decomposition.

Landscape topography further affects N cycling dynamics through its influence on soil development and physiochemical soil properties (Philipp et al., 2025). In the untilled prairie of the Flint Hills in Kansas, upland soils tend to be shallower, rocky, and more prone to water limitation (Briggs et al., 1998; Oviatt, 1998; Ransom et al., 1998). Accordingly, root systems and accompanying microbial communities are shallower and more easily impacted by surface disturbances in uplands. Lowland soils are deeper, finer-textured, and more mesic, capable of sustaining greater organic matter accumulation and deeper root profiles (Briggs et al., 1998; Collins & Calabrese, 2012; Oviatt, 1998). Fire can influence N cycling differently in lowlands,

because deeper soils may retain nutrients and support faster post-fire recovery of N pools through their relatively higher stores of organic matter (Cheng et al., 2025; Cheng et al., 2025). Upland soils may also be more available for restoration of N pools through dung and urine deposition, as bison preferentially graze upland sites, concentrating excreta inputs in the more shallow soils (Koirala et al., 2026; Vega Anguiano et al., 2024). However, fewer investigations have explicitly evaluated how interacting fire and grazing regimes affect nutrient cycling across heterogeneous landscapes. This study examines how fire frequency and bison grazing interact to regulate N cycling and retention in tallgrass prairie soils across upland and lowland positions.

I hypothesized that fire and grazing interactions influence ecosystem N retention by altering N cycling through plant and microbial pathways. Specifically, I predicted that more frequent fire would increase N limitation and prevent total N accumulation, while grazing would reduce N limitation and stabilize or elevate bioavailable N quantities relative to ungrazed prairies. Also, I expected that topography would mediate these responses, with lowland soils' deeper soil horizons acting as a buffer against surface impacts of fire and grazing, resulting in greater access to bioavailable NH_4^+, NO_3^-, and potentially mineralizeable N which would reduce microbial N limitation relative to upland soils. To evaluate how these factors interact to influence soil N pools, microbial N demand, and N mineralization potential, this study integrates multiple complementary indicators of N dynamics. Extracellular enzyme activities estimate microbial allocation toward nutrient acquisition, autoclaved citrate-extractable protein reflects operationally defined pool of proteinaceous organic N, often interpreted as a reservoir of potentially available organic N, and potentially mineralizable nitrogen quantifies labile N pools that are readily mineralized. Together with bulk soil N measurements, these methods allow

differentiation between N storage, accessibility, and microbial demand among fire and grazing regimes across the topographically varied landscape.

Methods

Study site and experimental design

This study was conducted at the Konza Prairie Biological Station (KPBS), a 3,487-ha tallgrass prairie research site in the Flint Hills of northeastern Kansas, USA (39 05'N, 96 35'W). KPBS land is co-owned by The Nature Conservancy and Kansas State University (KSU), managed as a field station by KSU, and hosts an NSF Long-Term Ecological Research (LTER) project. The site is in the Flint Hills ecoregion, the largest remaining tract of native tallgrass prairie in North America and has been the location of research on experimentally controlled fire and grazing regimes for more than four decades. Climate at KPBS is continental, with mean annual precipitation (MAP) of approximately 850 mm and mean annual temperature (MAT) of 12.6°C (National Ecological Observatory Network (NEON), 2026b). Soils are derived from shale and limestone residuum. Uplands are characterized by cherty, silty clay loams over limestone bedrock (National Cooperative Soil Survey, 2020), whereas lowland soils are deeper and alluvial and colluvial (Oviatt, 1998).

Bison were reintroduced to KPBS in the late 1980s (Knapp & Seastedt, 1998). The herd size is now around 225 bison that have unrestricted access to all four historical fire treatments within their grazed area; the bison are maintained at a stocking rate of 0.98 acres per bison (0.4 ha per animal unit month (AUM)) and are present throughout the year (Blair, 1997; Vega Anguiano et al., 2024). Cattle are maintained on site from May to October in cow-calf pairs, at a rate of approximately 1 acre per adult or 0.7 ha per AUM (Olson, 2023). Experimental fire treatments on replicate watersheds, with burn intervals of 1-, 2-, 4-, or 20-year fire frequency,

began in the 1980s (Blair & O'Neal, 2026), with year-of-fire varying based on individual watershed management (Table 2.1). Grazing treatments include long-term ungrazed areas and bison-grazed areas, within which are located two replicate watersheds of each burn interval. Within each watershed, composite soil samples were collected parallel to established 50-m LTER vegetation transects at both landscape topographic positions, totaling four upland and four lowland transects. In sum, 128 composite soil samples were collected from eight fire and grazing treatment combinations at each landscape position, at four replicate transects within each of two watersheds per fire and grazing treatment combination.

Soils were collected in June 2023, with three mineral soil cores up to 15 cm in depth from each transect using a 2-cm diameter Oakfield soil corer (Oakfield Apparatus Co., Oakfield, WI, USA), into composite bags and mixed in the field. Corers were cleaned with 95% ethanol between transects, and nitrile gloves were worn during sampling to minimize contamination. Samples were transported to the laboratory, where they were sieved (<4 mm) to remove rocks and root material while preserving aggregate structure, and split into subsamples for different analyses. For available N assays, soils were refrigerated at 4°C and processed with 48 h, and for extracellular enzyme activities, soils were stored at -20°C before analysis.

Potentially mineralizable nitrogen

Potentially mineralizable nitrogen (PMN) was assessed using both a 7-day anaerobic incubation (PMN_{7d}) and a 28-day aerobic incubation (PMN_{28d}), to estimate the short and medium-term capacity of soil microbial communities to convert organic N into plant available N forms (Drinkwater et al., 1997; Keeney & Bremner, 1966). PMN represents realized microbial mineralization potential under controlled laboratory conditions. To measure PMN_{7d}, fresh sieved soils were saturated with water (anaerobic) and incubated at room temperature in the dark for 7

days. To measure PMN_{28d}, field-moist soils were adjusted to approximately 60% water holding capacity and incubated in sealed but regularly aerated containers at room temperature in the dark for 28 days. Inorganic N (NH_4^+-N and NO_3^- -N) was extracted at day 0, and day 7 or day 28 of each incubation using 2M KCl. Soil extracts were analyzed for NH_4^+-N and NO_3^- -N concentrations using microplate assays (Hood-Nowotny et al., 2010; Nieland et al., 2021). Soil extracts were combined with VCl_3 solution and Greiss reagent to measure NO_3^- -N concentrations at an absorbance of 545 nm, and NH_4^+-N was measured using a modified indophenol assay, both using a microplate spectrophotometer (FilterMax F5, Molecular Devices, San Jose, CA, USA). PMN was calculated as the net accumulation of inorganic N over the incubation period:

$$PMN_{7d} = (NH_4^+\text{-}N)_{day7} - (NH_4^+\text{-}N)_{day0}$$

$$PMN_{28d} = (NH_4^+\text{-}N + NO_3^-\text{-}N)_{day28} - (NH_4^+\text{-}N + NO_3^-\text{-}N)_{day0}$$

and results were expressed as μg N kg^{-1} dry soil.

Autoclaved citrate-extractable protein

Autoclaved citrate-extractable (ACE) protein is a pool of proteinaceous organic N. This operationally defined pool represents accumulated microbial biomass turnover products and protein-rich compounds. Air-dried soil was extracted into 50mM sodium citrate buffer (pH 8.0), autoclaved at 121°C for 30 min, cooled, and centrifuged. Protein concentration in the supernatant was determined using the Bradford colorimetric assay (Hurisso et al., 2018; Stott, 2019) using Coomassie Brilliant Blue dye absorbance measured at 595 nm using a microplate spectrophotometer (FilterMax F5, Molecular Devices, San Jose, CA, USA), and a standard curve prepared with bovine serum albumin (BSA) and expressed as mg protein g^{-1} dry soil.

Extracellular enzyme activity

Extracellular enzyme activities (EEA) were measured to assay decomposition potentials and microbial production of enzymes for N acquisition relative to C acquisition (Saiya-Cork et al., 2002; Sinsabaugh, 2000). Three hydrolytic enzymes were assayed: β-glucosidase (βG), which produces C-containing glucose from cellulosic organic matter; N-acetylglucosaminidase (NAG), which produces N- and C-containing amino sugars from bacterial and fungal cell wall compounds, and leucine aminopeptidase (LAP), which produces N-containing amino acids from proteinaceous organic matter. EEAs followed standard fluorometric protocols, in 50mM sodium acetate buffer (pH 5.0), with analytical replicates, substrate blanks, reference standards, and quench controls; βG assays were incubated for 1.5-3 hrs, NAG for 3-4 hrs, and LAP for 16-18 hours. Fluorescence of the substrate reporters methylumbelliferyl (βG and NAG) and methyl coumarin (LAP) was measured at 360 nm excitation and 450 nm emission using a FilterMax F5 microplate reader (Molecular Devices, San Jose, CA, USA), and activities were expressed as nmol substrate hydrolyzed g^{-1} dry soil h^{-1}. To estimate microbial N demand relative to C acquisition, the ratio was calculated as:

$$\ln(\beta G) \,/\, \ln(NAG + LAP)$$

Lower ratios indicate greater microbial investment towards N-acquisition relative to C-acquisition.

Statistical analysis

All statistical analyses were conducted in R (R Core Team, 2025). Three-way analysis of variance (ANOVA) was used to evaluate the effects of burn interval, grazing treatment, and topographic position on all response variables, using the *aov()* function. Model assumptions were evaluated by inspecting residual plots and conducting Shapiro-Wilk tests for normality and

Levene's tests for homogeneity of variance. Log transformations were applied where necessary to improve normality and variance structure prior to analysis. Post hoc comparisons among treatment combinations were conducted using estimated marginal means with Tukey-adjusted pairwise comparisons, using the *TukeyHSD()* function. Data visualization was conducted using the *ggplot2* package.

Results

Soil properties

Soil field gravimetric water content was lower in grazed prairies and in uplands (Table 2.2, Figure 2.1 A). Soil pH was affected by grazing, as grazed soils had a higher pH than ungrazed soils, and was higher in lowlands (Table 2.2, Figure 2.1 B). Total soil C concentration was highest in infrequently burned prairies, in grazed prairies, and in uplands (Table 2.2, Figure 2.2 A). Total N concentration was also highest in infrequently burned prairies, and higher in upland soils than lowland soils, and responded to grazing in the uplands only (Table 2.2, Figure 2.2 B). In upland prairie soils, grazing increased %N across all fire frequencies. Soil C:N molar ratios were higher in prairies burned every 2 years than every 4 or 20 years and were lower in grazed upland soils burned annually and every 4 or 20 years (Table 2.2, Figure 2.2 C).

Soil inorganic N pools

Soil extractable NH_4^+-N and total inorganic N were both higher in ungrazed than grazed prairies, and higher in prairies burned every 20 years than every 2 years, in both uplands and lowlands (Table 2.2, Figure 2.3 A, C). Soil extractable NO_3^--N and the ratio of NH_4^+-N:NO_3^--N showed a three-way fire, grazing, and topography interaction, such that there was more NO_3^--N, and more NO_3^--N relative to NH_4^+-N, in grazed uplands burned every 2y and 20y (Table 2.2, Figure 2.3 B, D).

Extracellular enzyme activities

Soil βG activity was higher in uplands than lowlands but did not respond to fire or gazing (burn interval post-hoc results were $P > 0.1$) (Table 2.2, Figure 2.5 A). Soil NAG activity was lower in grazed prairies across all fire treatments and landscape positions and was higher in annually burned than infrequently burned lowlands (Table 2.2, Figure 2.5 B). Soil LAP activity was higher in grazed prairies burned every 4 years, and was higher in lowlands (Table 2.2, Figure 2.5 C). Ratios of ln(βG)/(ln[NAG+LAP]) were consistently lower in upland relative to lowland soils, and higher in grazed soils across all fire frequencies and landscape positions (Table 2.2, Figure 2.5 D).

Discussion

This study evaluated how fire frequency and bison grazing interact to regulate prairie soil N cycling, with topography mediating these responses. I predicted that frequent fire would increase N limitation and limit total N accumulation, and that grazing would reduce N limitation and stabilize relative quantities of available soil N pools, with weaker effects in lowland soils. Overall, the results partially fit predictions, in that prairie soil %N responded more strongly to grazing in uplands; however, topography did not influence fire effects on soil %N. N cycling was generally enhanced by grazing, where NO_3^--N, PMN_{7d}, and ln(βG):ln(NAG+LAP) all indicated higher N availability, and total %N was higher, in grazed upland soils (Figures 2.2 B, 2.3 B, 2.4 A, 2.5 D; Table 2.2). Fire suppression also enhanced N cycling, with NH_4^+-N, PMN_{7d}, PMN_{28d}, and ACE protein all indicating higher N availability, and total %N being higher, in soils with infrequent fire (Figures 2.2 B, 2.3 A, 2.4 A-C; Table 2.2). Fire effects were generally independent of topography but grazing dampened fire effects on PMN_{28d} (Figure 2.4 B). Results confirm our previous understanding of the direct effects of fire on N cycling and advance our

understanding of how grazing effects on N cycling interact with fire and landscape topography. Finally, differential patterns among extractable, mineralizeable, and enzymatic indices of N availability make these data useful for assessing the utility of different N-cycling indices for interpretation of prairie soil health.

Soil total %N was lower in prairies under annual, 2y, and 4y fire return intervals than in infrequently burned prairies by ~20% (Figure 2.2 B), reinforcing that soil %N declines with frequent fire due to combustion of plant litter-N (Pellegrini et al., 2022; Seastedt et al., 1991). In upland soils, mean total N was 0.44% (SD ± 0.08%) in grazed prairies and 0.38% (SD ± 0.08%) in ungrazed prairies, a 15% increase (Figure 2.2 B). In a related study of N cycling in annually burned upland prairie soils, Vega Anguiano (2024) found that bison grazing increased plant-available N and reduced microbial N limitation relative to ungrazed soils, suggesting accelerating N cycling under grazing (Frank & Groffman, 1998; Towne, 2000; Vega Anguiano et al., 2024). Vega Anguiano (2024) also quantified N uptake into grasses and estimated N egestion through bison dung and noted that egestion rates were much too low to account for the increase in soil N, but N uptake through plant biomass was in the necessary range. Notably, this study shows that total N accumulation through grazing-promoted elevated plant available N and subsequent uptake and decomposition is in the same range as N accumulation through fire suppression in upland prairie soils. Our results align with long-term patterns reported by Connell et al. (2020) who also found that fire suppression increased soil C and N pools, while frequent fire promoted N limitation. While Connell et al. (2020) attributed these patterns primarily to shifts in plant community composition over decades, our data reinforce that these outcomes are underpinned by shorter-term microbial processes, including enzyme allocation and N mineralization. On the other hand, that study concluded that grazing reduced the accumulation of

soil N, in contrast to this study's findings that soil N concentration was higher in grazed uplands. Connell et al. (2020) only analyzed soils collected in lowlands and therefore did not detect the stronger effects of grazing shown here in the uplands of the tallgrass prairie.

Fire and grazing affect different aspects of soil N availability

Predictions that grazing would increase plant-available N were partially supported, in that some but not all indices of N availability were higher in all grazed prairie soils. Soil PMN_{7d} was consistently higher, and ln(βG)/(ln[NAG+LAP]) was consistently lower, in grazed soils regardless of landscape position or fire frequency, both indicating the predicted higher N availability (Figures 2.4A, 2.5D; Table 2.2). However, only extractable NO_3^--N was higher in grazed soils, not extractable NH_4^+-N, and only in upland soils, suggesting that grazing stimulated microbial N processing pathways such as nitrification, more strongly in upland soils (Figures 2.3A-B; Table 2.2). Grazing alters plant-microbe stoichiometry by stimulating plant regrowth, which produces aboveground tissues with relatively higher N concentrations and relatively lower C:N ratios that mature or senesced plant material (Allen & Gillooly, 2009; Blair, 1997; Cui et al., 2025; Johnson & Matchett, 2001). These higher-quality substrates may contribute to the observed shifts in the NH_4^+-N:NO_3^--N ratios across fire and grazing treatments. If this is true, and if the grazing feedbacks on plant and litter quality are stronger in uplands, this could explain the observed pattern in extractable NO_3^--N. Notably, grazing animals redistribute N through egestion, providing localized inputs of NH_4^+-N that can stimulate microbial nitrification activity (Li et al., 2024; Vega Anguiano et al., 2024). Also, changes in plant input quality or N redistribution by grazers may produce stronger shifts in microbial growth and N cycling within these shallow soils (Briggs et al., 1998; Oviatt, 1998; Ransom et al., 1998). This pattern may be further reinforced by bison foraging behavior, as grazers preferentially concentrate on high-

quality forage patches created by fire, maintaining these areas through repeated grazing (Raynor et al., 2015). Because upland soils are shallower and drier, and exhibit greater spatial heterogeneity, this selective grazing likely intensifies localized nutrient inputs and cycling, leading to stronger increases in extractable NO_3^--N compared to lowlands. The deeper lowland soils contain larger organic matter reserves and greater moisture availability (Bian et al., 2022), which may buffer microbial communities against short-term changes in substrate stoichiometry and nutrient inputs.

PMN_{28day} showed an intriguing interaction, with higher PMN_{28day} in biannually burned grazed prairies but lower PMN_{28day} in infrequently burned grazed prairies relative to ungrazed treatments (Figure 2.4 B; Table 2.2). These results suggest that grazing enhances mineralizeable N under frequent fire but can reduce mineralization potential where fire is infrequent. These findings suggest that fire and grazing influence N cycling through interacting mechanisms, with grazing stimulating N turnover under frequent fire while fire frequency and landscape position regulate longer-term N storage in soils. Unlike inorganic N and short-term PMN_{7day} measurements, PMN_{28day} integrates enzymatic activity, substrate quality, microbial biomass, and environmental constraints to estimate net N release over time (Barrett & Burke, 2000; Drinkwater et al., 1997), providing insight into longer-term N storage and stabilization processes, rather than immediate plant-available N supply. Under frequent fire, aboveground litter inputs are limited, and grazing may stimulate N mineralization through several mechanisms (Hobbs et al., 1991a). Grazing can increase root turnover and stimulate new plant regrowth, leading to greater inputs of labile root exudates and fine-root detritus that fuel microbial activity (Johnson & Matchett, 2001; Knapp & Seastedt, 1986). In addition, dung and urine deposition provide localized pulses of N and C, which can also stimulate microbial growth and enzyme production

(Vega Anguiano et al., 2024). These inputs may enhance microbial processing of SOM, resulting in greater PMN despite the reduction of aboveground litter inputs by annual fire.

ACE protein pools were also highest in ungrazed and infrequently burned prairies and were consistently higher in ungrazed relative to grazed soils, contrary to most other data on soil N availability (Figure 2.4 C; Table 2.2). Like PMN_{28day}, ACE protein may be an index of longer-term N storage and stabilization processes, specifically by measuring N retained in proteinaceous organic forms (Graham et al., 2021; Hurisso et al., 2018; Naasko et al., 2024). Grazing may reduce the accumulation of proteinaceous soil N in infrequently burned prairies because herbivory redirects N from slowly decomposing plant litter into faster cycling pathways (Frank et al., 1995; Frank & Groffman, 1998). In ungrazed systems, large amounts of standing dead biomass and litter accumulate, allowing organic N compounds to be incorporated into SOM pools over time (Milchunas et al., 1988; Raven et al., 2005; Seastedt et al., 1991). Grazing reduces this litter accumulation and instead redistributes N through dung and urine deposition, which enter the soil as relatively labile substrates that are rapidly mineralized by microbes (Vega Anguiano et al., 2024). As a result, N may cycle more quickly through microbial biomass and inorganic pools, rather than accumulating as proteinaceous organic matter (Noll et al., 2019). This explanation is partially supported by contrasting patterns in LAP and NAG activities, in which NAG activity reflects the originally predicted increase in more N-limiting conditions, while LAP (the peptidase) activity is higher in infrequently burned prairie soils, where there is also higher ACE protein (Figures 2.4 C, 2.5 B, C).

Topography affects soil N availability

Landscape topography strongly influenced many of the soil attributes measured in this study, underscoring its importance in regulating N cycling across tallgrass prairie ecosystems.

Topography generally affects soil depth, moisture availability, organic matter accumulation, oxygen availability, and hydraulic redistribution of nutrients, all of which influence microbial activity and subsequent N transformations (Briggs et al., 1998; Collins & Calabrese, 2012; Kerby et al., 2007; Robertson et al., 1997; Turner et al., 1997). Lowland soils receive downslope movement of water and dissolved nutrients and support greater plant productivity, which promotes greater organic matter accumulation and can increase total N storage within the deeper soil profile (Kirk, 2023; Knapp & Seastedt, 1998; Oviatt, 1998). As a result, although surface mineral soils in uplands contain higher N concentrations, deeper lowland soils may store larger total N stocks because of greater soil depth and volume (Collins & Calabrese, 2012; Kerby et al., 2007; Oviatt, 1998). These patterns highlight an important distinction between N concentration and total N stocks within soils. The results presented here reflect N concentrations in surface mineral soils and therefore do not directly quantify total ecosystem N storage. Because lowland soils are typically deeper than upland soils, their overall N stocks may exceed those of uplands despite lower surface concentrations. Consequently, the higher %N observed in grazed upland soils should not be interpreted as evidence of greater total N storage.

Topographic variation in soil depth, moisture, and organic matter accumulation also influence microbial nutrient demand and enzyme production, helping to explain variation in indicators such as NAG activity and ln(ßG)/(ln[NAG+LAP]) ratios across upland and lowland soils (Figures 2.5 B, D) (Allison & Vitousek, 2005; Bian et al., 2022). These microbial indicators reflect how microorganisms allocate resources to acquire C and N and provide further insight into how landscape position shapes microbial strategies for nutrient acquisition and N cycling.

Heterogeneity and other influences

Time since last burn may have contributed to variability within burn intervals (Table 2.1). While the fire treatment categories refer to decadal prescribed fire regimes, replicate watersheds are often burned in different years; also, within the bison-grazed prairies, fire was applied annually in the three years preceding this study (Blair & O'Neal, 2026). Recently burned watersheds may experience short-term N pulses through grazing or other effects, while longer burned intervals accumulate organic substrates (e.g., surface litter and detritus) that contribute to elevated ACE protein concentrations (Figure 2.4 C). The consistently higher ACE values and total N concentrations in infrequently burned prairies, particularly within grazed watersheds, suggest greater N accumulation under extended fire return intervals, that was not removed with recent individual fire events.

Weather and precipitation patterns may also influence these outcomes. Soil moisture regulates oxygen diffusion, redox potential, and microbial metabolism rates. Because uplands are generally shallower and more prone to moisture limitation, interannual precipitation variability may disproportionately influence N cycling in those positions. By way of contrast, lowlands may buffer climatic variability due to greater soil depth and moisture retention. The single season-sampling design limits inference about temporal variability; consequently, disturbance effects should be interpreted as condition-specific rather than fixed ecosystem states. Although this study captures long-term fire and grazing treatments, soil sampling represents a single seasonal time point. Nitrogen cycling processes are dynamic and may vary across seasons and years due to changes in soil moisture, temperature, and plant phenology which can influence soil microbial activity and rates of mineralization and immobilization (Groffman et al, 2009). Consequently, measurements from a single sampling period may not capture the full range of in situ N cycling

dynamics. Additionally, PMN assays reflect potential mineralization under controlled laboratory conditions and may not fully represent in situ field rates. These limitations should be considered when interpreting disturbance effects on N retention.

N cycling and soil health

When considered holistically, total soil N, ACE protein, PMN, and inorganic N fractions illustrate that N retention and N availability are not synonymous, reinforcing that fire frequency and grazing reorganize and redistribute N and its accessibility rather than uniformly increasing or decreasing N stocks. At its core, N cycling in the tallgrass prairie reflects the movement and redistribution of the element through plant-microbe-soil pathways. Across burn intervals and landscape positions, the measured shifts in extracellular enzyme allocation, inorganic N fraction, PMN, and ACE protein illustrate how microbial communities respond to changes in substrate quality, water and pH, and surface inputs and losses. From a soil health perspective, these findings underscore the importance of considering both N storage and accessibility. Tallgrass prairies are one of North America's most endangered ecosystems, and they are also globally significant reservoirs of belowground C and N. Understanding how disturbance regimes regulate nutrient dynamics is essential for sustaining prairie resilience under changing climate allocation and land-use pressures. By integrating disturbance ecology with microbial allocation and disturbance heterogeneity, this study contributes to a more nuanced understanding of how prairies store, mobilize, and redistribute N through time.

Conclusion

Prior research at KPBS and other tallgrass prairie systems has demonstrated that fire and grazing regulate belowground processes and N cycling (Ojima et al. 1994, Johnson and Matchett, 2001; Anguiano et al, 2024). This study extends that body of knowledge by explicitly integrating

extracellular enzyme allocation, ACE protein concentration, and PMN across multiple burn intervals, topographic positions, and grazing regimes. By evaluating both storage pools and microbial allocation strategies, the results provide a more mechanistic understanding of how disturbances impact N accessibility. Importantly, the role of topography and the prevalence of interactive effects highlight that N-cycling ecology in grasslands cannot be interpreted solely through the direct effects of single drivers. Landscape heterogeneity shapes how fire and grazing translate into biogeochemical outcomes. In this way, the present work enlarges the conceptual framework for prairie N cycling by demonstrating that N-cycling regimes are spatially structured and mediated by microsite conditions. This study demonstrated that N partitioning and availability in tallgrass prairie soils are governed not by fire or grazing alone, but by their integration across the heterogeneous landscapes. Burn interval structured total N, inorganic N, ACE protein, and PMN, yet grazing frequently modified those patterns at specific fire frequencies. Topography emerged as a consistent mediator, shaping both the magnitude and the direction of grazing and fire responses. Rather than confirming a uniform decline in N under frequent fire, the results reveal a mosaic of responses driven by interactions between fire, grazing, and landscape position.

Table 2.1. Summary of KPBS soil sample locations collected in June 2023 including watersheds, grazing regimes (ungrazed or Bison-grazed), burn intervals (years), and time from last burn (years (y) and months (mos)). Eight (8) total composite soil samples were collected within each watershed (4 upland, 4 lowland.

KPBS Watershed	Grazing Regime	Burn Interval (years)	Time from Last Burn
1D	ungrazed	1	2 mos
SpA	ungrazed	1	2 mos
N1A	Bison	1	3 mos
N1B	Bison	1	2 mos
2B	ungrazed	2	1 y +3 mos
2D	ungrazed	2	1 y + 4 mos
N2A	Bison	2	1 y + 4 mos
N2B	Bison	2	4 mos
4B	ungrazed	4	2 y + 3 mos
4F	ungrazed	4	3 mos
N4B	Bison	4	4 mos
N4D	Bison	4	2 y + 3 mos
20B	ungrazed	20	6 y + 3 mos
20C	ungrazed	20	2 y + 7 mos
N20A	Bison	20	4 mos
N20B	Bison	20	4 mos

Table 2.2. Summary of ANOVA results evaluating effects of burn interval (01Y, 02Y, 04Y, 20Y), grazing treatment (ungrazed, bison-grazed), and landscape topography (upland, lowland) on all response variables. Some variables were log transformed to approach model assumptions of normality and noted with superscript "l" if so. Reported values are F-statistics with associated numerator and denominator degrees of freedom, and p-value ranges denoted as follows: *** p < 0.001; ** p < 0.01; * p < 0.05; . p < 0.1; NA, p > 0.1.

Response variable	Burn interval	Grazing	Topography	Burn × Grazing	Burn × Topography	Grazing × Topography	Burn × Grazing × Topography
Soil properties							
Soil water content	NA	$F_{1, 112}$ = 15.2***	$F_{1, 111}$ = 5.1**	NA	NA	NA	NA
Soil pH	NA	$F_{1, 112}$ = 52.5***	$F_{1, 111}$ = 6.9**	$F_{3, 111}$ = 2.3 .	NA	NA	NA
[l]Total soil C	$F_{3, 111}$ = 6.2***	$F_{1, 112}$ = 4.9*	$F_{1, 111}$ = 65.8***	NA	NA	NA	NA
[l]Total soil N	$F_{3, 112}$ = 18.4***	$F_{1, 112}$ = 11.6**	$F_{1, 112}$ = 99.2***	$F_{3, 111}$ = 2.5 .	NA	$F_{1, 112}$ = 4.0*	NA
[l]Soil C:N ratio	$F_{3, 112}$ = 9.9***	NA	NA	$F_{3, 111}$ = 5.5 **	NA	$F_{1, 112}$ = 4.7*	NA
Inorganic nitrogen pools							
[l]Extractable NH_4^+-N	$F_{3, 111}$ = 15.1***	$F_{1, 112}$ = 17.5**	NA	$F_{3, 111}$ = 2.5 .	$F_{3, 111}$ = 2.3 .	NA	$F_{3, 111}$ = 2.3 .
[l]Extractable NO_3^--N	$F_{3, 111}$ = 5.7**	$F_{1, 112}$ = 11.4*	$F_{1, 111}$ = 3.0 .	$F_{3, 111}$ = 4.7**	NA	$F_{3, 111}$ = 18.1***	$F_{3, 111}$ = 4.1**
[l]Total inorganic N (TIN)	$F_{3, 111}$ = 15.7***	$F_{1, 112}$ = 9.8**	NA	NA	NA	NA	NA
NH_4^+-N: NO_3^-N ratio	NA	$F_{1, 112}$ = 23.7***	NA	$F_{3, 111}$ = 5.6**	$F_{3, 111}$ = 3.8*	$F_{3, 111}$ = 7.2**	$F_{3, 111}$ = 2.8*
Potentially mineralizable N (PMN) and ACE protein							
[l]PMN-7 day	$F_{3, 107}$ = 5.0**	$F_{1, 107}$ = 30.0***	$F_{1, 107}$ = 43.6***	NA	NA	NA	NA
[l]PMN-28 day	$F_{3, 112}$ = 19.0***	NA	NA	$F_{3, 112}$ = 5.9***	NA	NA	NA
[l]ACE protein	$F_{3, 112}$ = 23.0***	$F_{1, 112}$ = 26.1**	$F_{1, 112}$ = 102.0**	NA	NA	NA	$F_{3, 112}$ = 2.3 .
Extracellular enzyme activities							
[l]BG activity	$F_{3, 112}$ = 2.8*	NA	$F_{1, 112}$ = 36.8***	NA	NA	NA	NA
[l]NAG activity	NA	$F_{1, 112}$ = 22.8**	NA	NA	$F_{3, 112}$ = 5.8**	NA	NA
[l]LAP activity	$F_{3, 112}$ = 3.5*	NA	$F_{1, 112}$ = 4.4*	$F_{3, 112}$ = 5.0**	NA	NA	NA
BG/(NAG+LAP)	NA	$F_{1, 112}$ = 4.8*	$F_{1, 112}$ = 18.4***	NA	$F_{3, 112}$ = 2.3 .	NA	NA

Figure 2.1. A) Soil field water content (g g^{-1}) and B) soil pH across burn intervals (01Y, 02Y, 04Y, 20Y), grazing treatments (ungrazed, grazed), and landscape topography (uplands, lowlands). Box plots show medians and interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

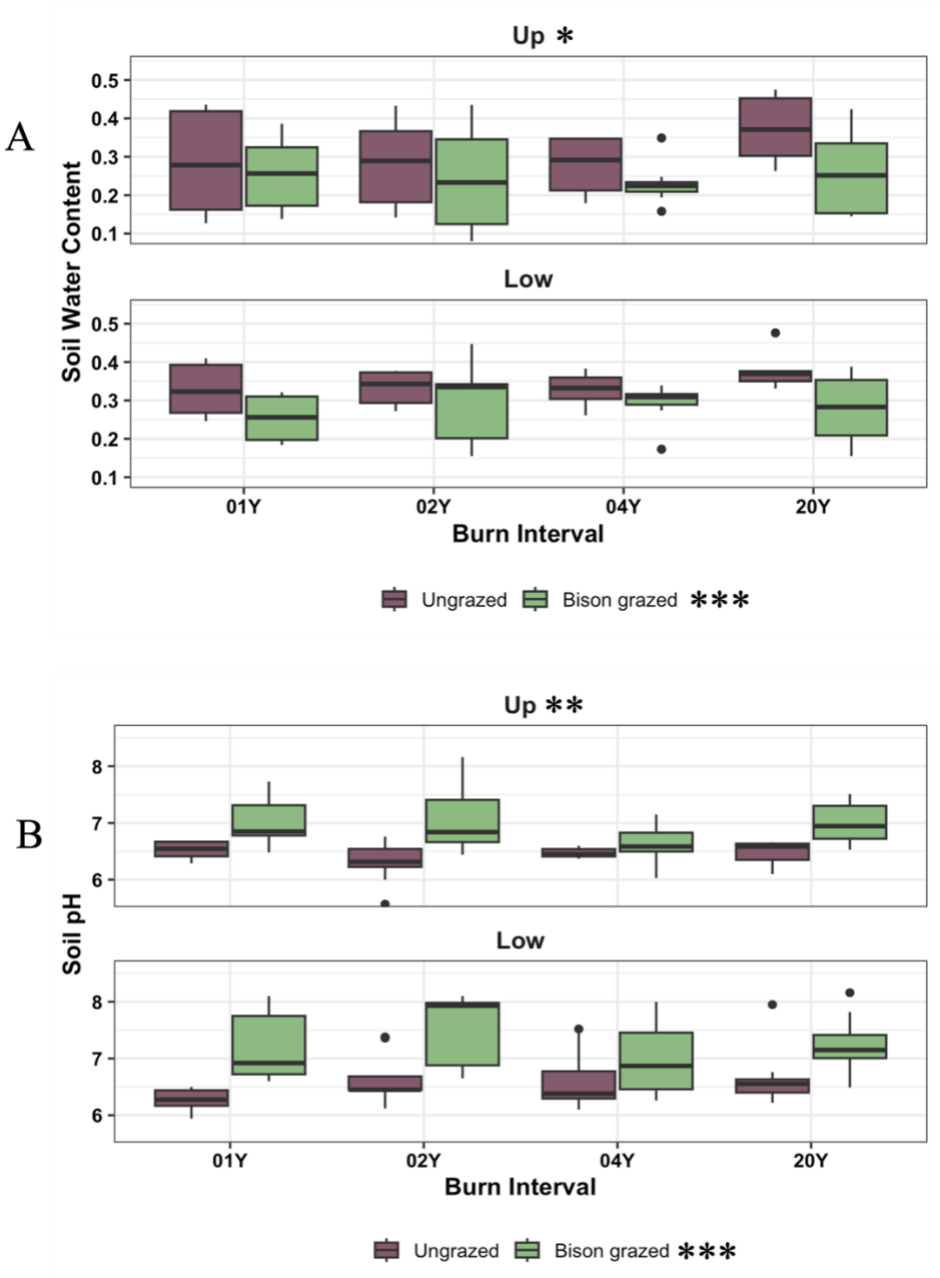


Figure 2.2. A) Total soil %C, B) total soil %N, and C) soil molar C:N across burn intervals (01Y, 02Y, 04Y, 20Y), grazing treatments (ungrazed, bison-grazed), and landscape topography (uplands, lowlands). Box plots show medians and interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

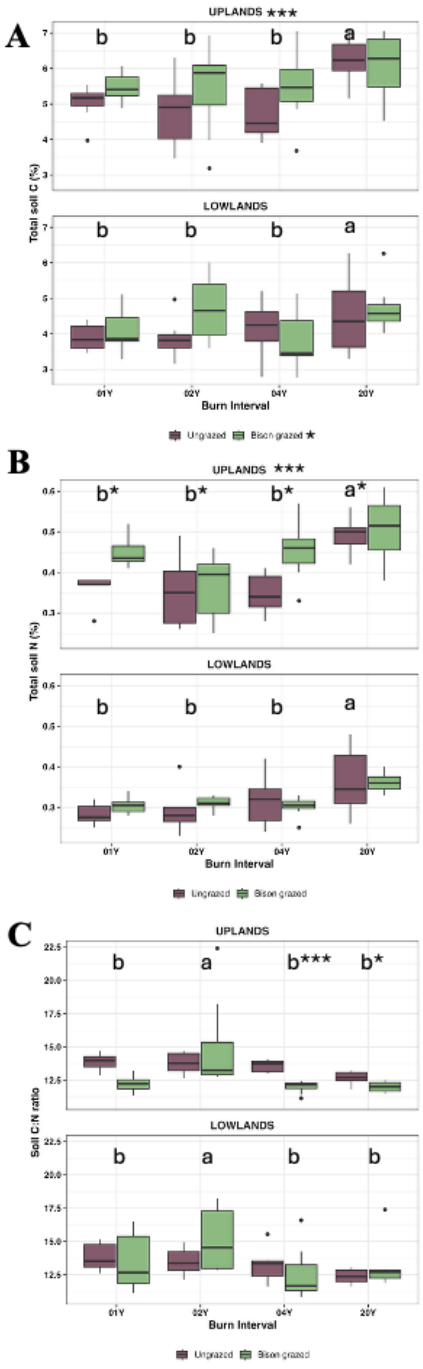


Figure 2.3. Soil extractable A) ammonium-N (NH_4^+-N), B) nitrate (NO_3^--N), C) total inorganic N (TIN), and D) NH_4^+-N : NO_3^--N across burn intervals (01Y, 02Y, 04Y, 20Y), grazing treatments (ungrazed, grazed), and landscape topography (uplands, lowlands). Box plots show medians and interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

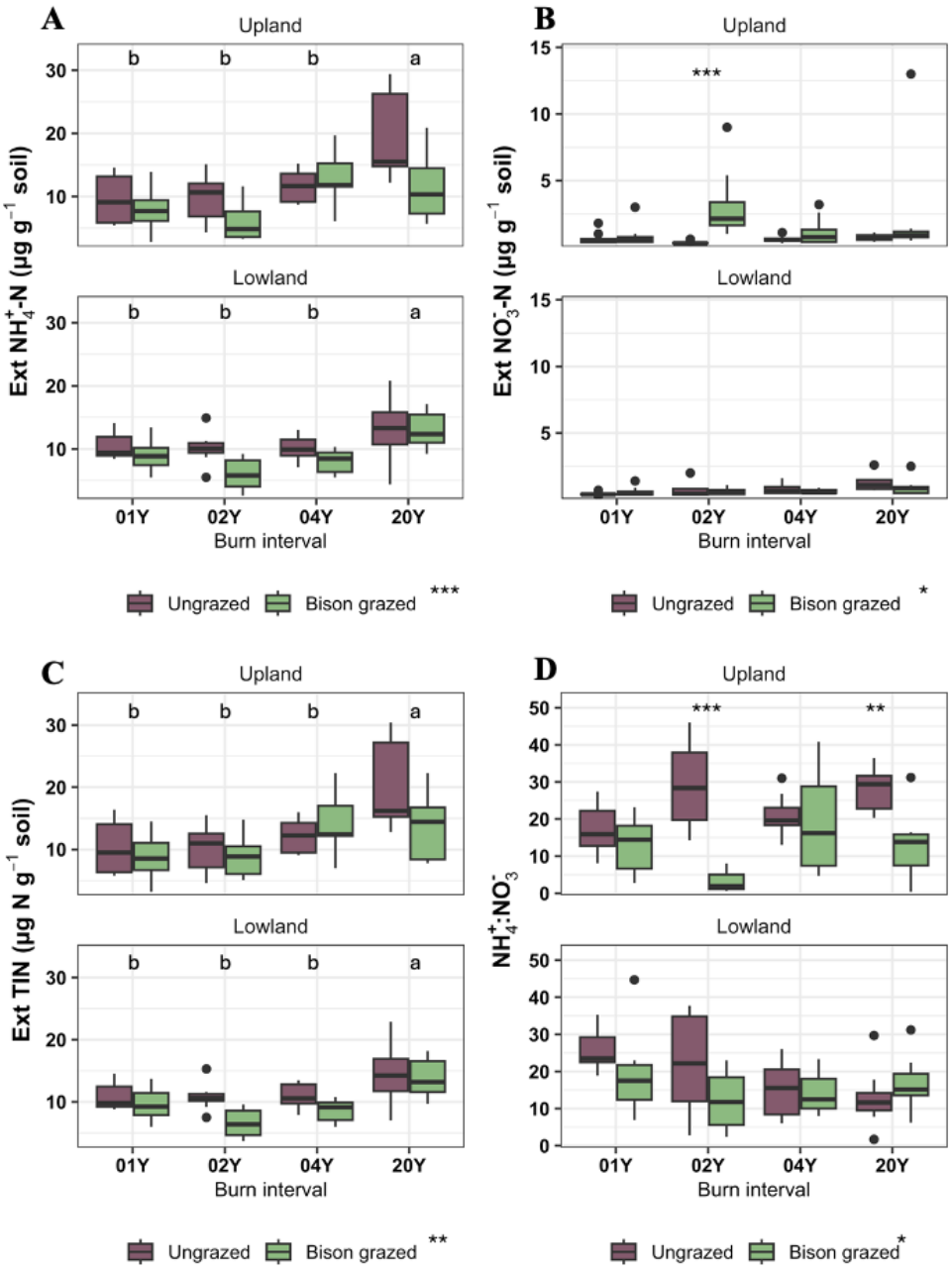


Figure 2.4. A) Potentially mineralizeable N through a 7-day incubation (PMN_{7d}) and B) through a 28-day incubation (PMN_{28d}), and C) autoclavable citrate-extractable protein (ACE protein) across burn intervals (01Y, 02Y, 04Y, 20Y), grazing treatments (ungrazed, grazed), and landscape topography (uplands, lowlands). Box plots show medians and interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at $P < 0.05$, and main topographic and main or interactive grazing effects with $P < 0.05$, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

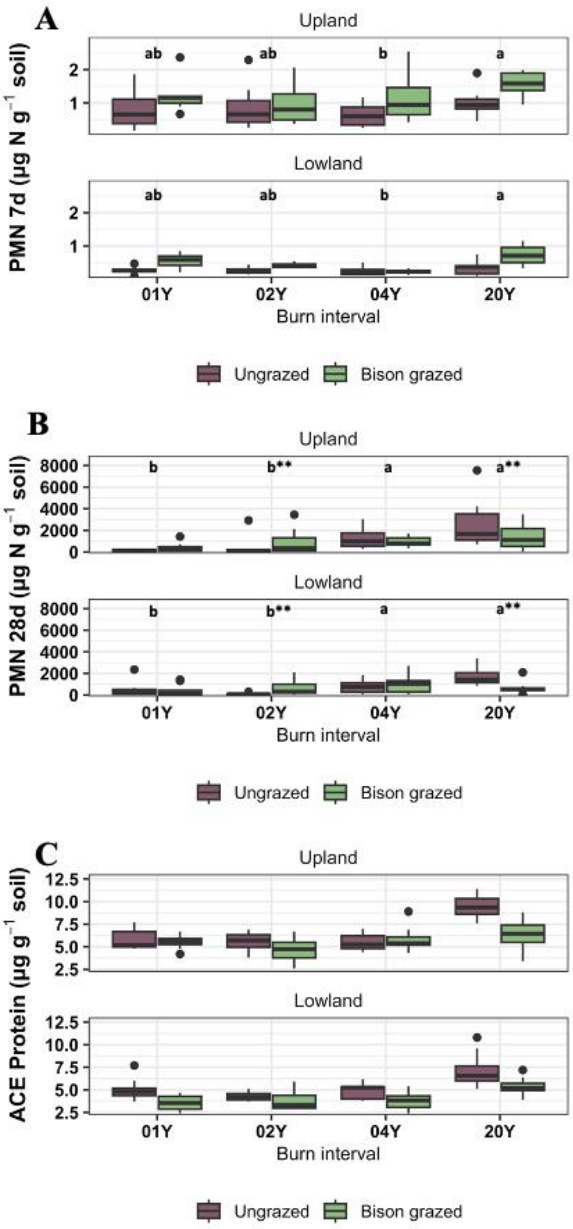
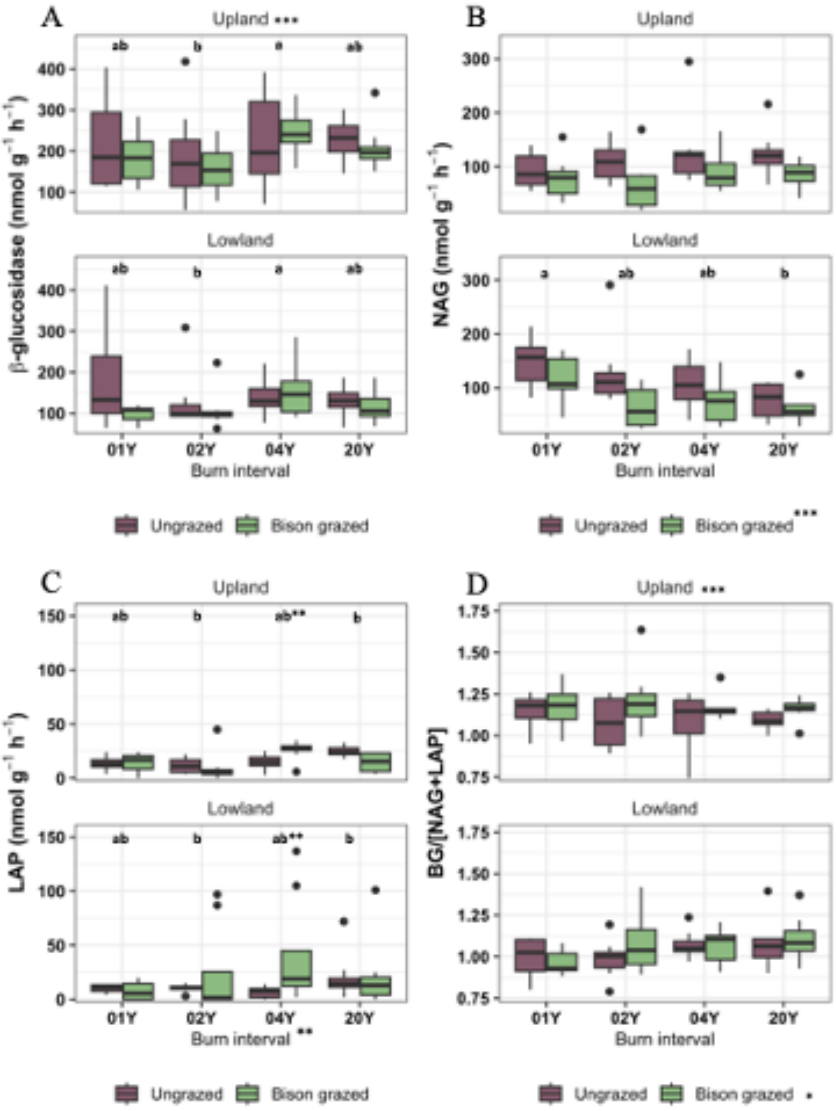


Figure 2.5. A) Soil β-glucosidase activity (βG, nmol g^{-1} dry soil h^{-1}), B) N-acetyl-glucosaminidase activity (NAG, nmol g^{-1} dry soil h^{-1}), C) L-aminopeptidase activity (LAP, nmol g^{-1} dry soil h^{-1}), and D) (ln βG) / (ln (NAG+LAP)), the index of microbial N-limitation, across burn intervals (01Y, 02Y, 04Y, 20Y), grazing treatments (ungrazed, grazed), and landscape topography (uplands, lowlands). Box plots show medians and interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**



Chapter 3 - Interactive Effects of Fire and Bison Grazing on Forage Nitrogen Dynamics within a Tallgrass Prairie Ecosystem

Abstract

Fire frequency, grazing, and seasonal timing collectively shape forage nitrogen (N) dynamics in tallgrass prairie, but their effects are context-dependent and mediated by landscape position. This study evaluated forage carbon (C), nitrogen, and C:N molar ratios alongside soil properties and extracellular enzyme activities across three scales of comparison: A factorial fire interval, topographic position (upland, lowland), and grazing treatment (ungrazed, bison-grazed) comparison at Konza Prairie Biological Station (KPBS); a growing season comparison (early, late) across ungrazed, bison-grazed, and cattle grazed treatments, all under annual fire, at KPBS; and a cross-site comparison of ungrazed, bison-grazed, and cattle grazed at KPBS, Tallgrass Prairie Preserve (TPP), and Tallgrass Prairie National Preserve (TPNP), late in the growing season.

Results partially supported the hypothesis that fire frequency and grazing jointly regulate forage N content, but this regulation was spatially and temporally structured. Results showed that at KPBS, frequently burned and grazed systems did have higher forage %N and lower C:N molar ratios, primarily in annually burned uplands and in late-season forage, but these patterns were not uniform across burn intervals and topography. Across sites, forage quality did not vary with bison or cattle grazing, which may be attributable to either differences in plant growth after variable time since prescribed fire, or to differences in grazing intensity. However, soil N availability was higher on average, based on soil extracellular enzyme activity ratios, in both bison- and cattle-grazed prairies across all three study sites.

These results demonstrate that fire and grazing regulate forage N through context-dependent interactions with landscape position and grass phenological stage, rather than as uniform additive drivers, and that among-site variation in forage quality can override grazing effects at broader spatial scales, despite consistent grazing effects on soil microbial N availability. Specifically, because grazing effects on forage quality are more likely to emerge in more late season forage, timing of sampling must be considered when designing and interpreting any study. In addition, differences in grazing intensity among sites or within topographically complex landscapes are likely to moderate forage quality feedback.

Introduction

Soil nitrogen (N) availability is a primary constraint on productivity in tallgrass prairie ecosystems and plays a central role in regulating plant growth and forage quality (Blair, 1997). The quantity and form of N available to prairie plants is shaped by interactions between fire and grazing (Blair, 1997; Blair et al., 1998; Knapp et al., 1998; Robertson et al., 1997). Because herbivores directly consume plant biomass, variation in plant N concentration and carbon (C) to N ratios represents a critical link between ecosystem processes and trophic dynamics (Collins & Steinauer, 1998; Owen-Smith, 2002; Raynor et al., 2015).

Forage N concentration (%N) and C:N molar ratios are indicators of forage quality, with higher %N and lower C:N ratios generally reflecting great nutritional value and digestibility for grazing herbivores (Mattson, 1980; Owen-Smith, 2002). These properties are not static but instead reflect underlying N cycling processes and plant growth dynamics that regulate the balance between plant N uptake and biomass C accumulation. N availability to plants depends on the microbial depolymerization of organic matter, mineralization, and subsequent plant-microbe competition for bioavailable N forms (Schimel & Bennett, 2004; Schimel & Weintraub, 2003).

When accessible N is readily available, more resources are allocated to the production of protein-rich biomolecules (such as extracellular enzymes) and tissues, resulting in higher %N and lower C:N ratios (Mattson, 1980). Conversely, when N is limiting, plants allocate relatively more C to structural compounds like cellulose and lignin, increasing C:N ratios and reducing forage quality (Knapp et al., 2012; Raven et al., 2005).

Microbial processes regulate plant-available N through a series of coupled biochemical transformations that control the depolymerization, mineralization, and mobilization of organic N pools (Johnson & Matchett, 2001). In grassland soils, most N is bound in complex organic forms such as proteins and chitin, which must first be cleaved by extracellular enzymes before becoming accessible to microbes and plants (Johnson & Matchett, 2001; Schimel & Bennett, 2004). N-acetylglucosaminidase (NAG) is a chitin-degrading enzyme that cleaves the larger molecule into smaller compounds like amino acids and amino sugars (Saiya-Cork et al., 2002; Schimel & Weintraub, 2003). These lower-molecular-weight compounds can then be metabolized by microbes. Mineralization reactions convert organic N into inorganic forms, primarily ammonium (NH_4^+) through microbial deamination; this compound can be taken up by plants directly or further oxidized to nitrite (NO_2^-) and nitrate (NO_3^-). Ammonium can also be immobilized into microbial biomass. Carbon-acquiring enzymes, like β-glucosidase (βG), interact with these processes by regulating access to C substrates, both as a response to reduced N limitation and increased C limitation (Schimel & Weintraub, 2003; Sinsabaugh & Follstad Shah, 2012). Fire can alter these microbial-mediated stages of the N cycle by modifying available organic N pools.

Combustion of surface litter reduces both C and N soil inputs from aboveground sources, and can shift soil microorganisms towards increased investment in nutrient-acquiring enzymes

(Blair, 1997; Johnson & Matchett, 2001; Ojima et al., 1994). Fire reduces surface detrital layers and encourages young plant growth by warming soils, depositing nutrients through ash fall, and increasing access to sunlight (Blair, 1997; Wan et al., 2001). Reduced surface litter inputs combined with increased plant growth reduces localized pools of inorganic N (Blair, 1997; Ojima et al., 1994). In frequently burned systems, these changes may promote increased soil microbial N cycling, increasing soil inorganic N availability to plants, thus linking fire-driven microbial processes to increased forage N concentrations and lower C:N molar ratios (Ojima et al., 1994; Seastedt et al., 1991; Wan et al., 2001).

Pyric herbivory, the interactive process by which fire and grazing jointly structure grassland systems, provides another mechanism linking disturbance to N cycling and forage quality (Blair, 1997; Fuhlendorf & Engle, 2004; Knapp et al., 1999; Raynor et al., 2015). After a fire, recently burned patches produce plant regrowth that is relatively higher in %N and lower in C:N ratios (Blair, 1997; Raynor et al., 2015). Large grazing herbivores like bison preferentially graze these recently burned areas, concentrating grazing pressure where forage quality is highest (Allred et al., 2011; Fuhlendorf et al., 2009; Raynor et al., 2015). This selective grazing reinforces spatial patterns of plant growth and nutrient distribution by repeatedly removing surface biomass, stimulating compensatory growth, and accelerating nutrient return through dung and urine deposition (Bardgett & Wardle, 2003; Johnson & Matchett, 2001). These inputs create localized zones of elevated N availability, enhancing microbial activity and further promoting N mineralization and plant uptake (Groffman et al., 2009; Johnson & Matchett, 2001). Over time, coupled fire and grazing generates a shifting mosaic of patches at different stages of landscape recovery, each with distinct N dynamics and forage quality (Fuhlendorf et al., 2009; Fuhlendorf & Engle, 2004). Through this process, pyric herbivory integrates fire-driven pulses of N

availability with grazing-mediated redistribution and recycling of N, creating strong spatial and temporal heterogeneity in plant-available N across the landscape.

Grazing not only removes plant biomass but also redistributes nutrients across the landscape through dung and urine deposition, creating spatially discrete patches of elevated N availability (Bardgett & Wardle, 2003; Frank & Groffman, 1998; Van De Vijver et al., 1999). Urine deposition, in particular, produces localized zones of high NH_4^+ and NO_3^- concentrations, accompanied by elevated soil pH and steep gradients of nutrient availability that decline rapidly with distance from the deposition site (Frank & Groffman, 1998; Van De Vijver et al., 1999). These microsites can persist for extended periods and represent important hotspots of plant-available N, although some of this N may be lost over time through volatilization and leaching. As a result, grazing introduces strong spatial heterogeneity in soil N availability (Bakker et al. 2003), which can enhance plant N uptake in some locations while leaving others relatively nutrient-limited. This patchy redistribution of N provides a key mechanism linking herbivore activity to forage quality and helps explain why grazing effects on plant N concentration are often variable across space and time.

Spatial and temporal heterogeneity are features of N cycling and forage quality in tallgrass prairie ecosystems, emerging from the interaction of disturbance, landscape position, and plant growth patterns (Blair, 1997; Seastedt et al., 1991). Topography creates consistent spatial gradients in soil moisture, organic matter accumulation, and microbial activity, with upland positions typically supporting greater N mineralization than lowlands (Turner et al., 1997). These differences influence plant N uptake, often resulting in higher forage %N and lower C:N ratios in more resource-rich landscape positions (Blair, 1997; Turner et al., 1997), although the magnitude of these effects can vary with disturbance regimes. Superimposed on these spatial

patterns are strong temporal dynamics associated with the growing season. Early-season plant growth is characterized by relatively high N concentrations due to rapid tissue production and active N uptake, compared with later in the season as N is diluted with accumulated biomass carbon (Mattson, 1980; Raynor et al., 2015). The result is a decrease in forage quality as the growing season progresses. Fire and grazing interact with both spatial and temporal gradients by redistributing nutrients and altering plant growth trajectories, but the expression of these effects depends on when and where they occur (Fuhlendorf et al., 2009; Hobbs et al., 1991b; Vinton et al., 1993). As a result, forage quality and plant-available N are not uniform across the landscape or through time, reflecting an ever-shifting mosaic driven by topography and seasonal progression, with disturbance regimes modulating the strength and pattern on these gradients.

This study evaluates how fire frequency, grazer identity, and seasonal timing interact to regulate forage N dynamics in tallgrass prairies. While previous work has demonstrated that fire and grazing independently influence forage quality, fewer studies have explicitly integrated these drivers across multiple prairie systems while also accounting for temporal and topographic variation in plant and soil N content (Fuhlendorf & Engle, 2004; Kerby et al., 2007; Raynor et al., 2015). As a result, it remains less resolved how consistent grazing effects are across landscapes and whether they are expressed similarly throughout the growing season. By comparing forage %N and C:N molar ratios across sites with differing grazing regimes, this study aims to provide a broader framework for understanding how disturbance and herbivore identity shape plant nutrient dynamics. Additionally, by incorporating both early and late-season sampling, this work explicitly evaluates how temporal changes in plant growth interact with grazing status to influence forage quality. Building on the soil N dynamics evaluated in Chapter 2 of this thesis, forage quality represents aboveground expression of belowground processes.

Although this chapter focuses on forage N dynamics, soil C and N measurements were included to provide context for plant nutrient responses. Also, soil microbial enzyme activity measurements were included in the cross-site comparison to evaluate expectations about grazing effects on soil N availability. Forage N reflects integration of belowground N availability and plant dynamics, therefore concurrent soil measurements allow evaluation of whether changes in plant N content correspond with shifts in soil N pools and microbial activity (Malhi et al., 2010; Olson-Rutz & Jones, 2015).

I hypothesized that grazing and fire frequency jointly regulate forage N content through their effects on plant growth and available inorganic nitrogen. I predicted that grazed systems would exhibit higher forage %N and lower C:N ratios due to the volatilization of surface organic matter and resultant increase in subsurface SOM decomposition and the dual grazing activities of mechanical removal of aboveground biomass (ingestion) and the resupply of N through dung and urine deposition (egestion). I further predicted that grazing effects would not necessarily vary with herbivore identity. Finally, I predicted that seasonal progression would mediate these responses, with early-season forage exhibiting higher %N and lower C:N ratios than late-season forage, and that grazing effects would be most apparent later in the growing season as plant N becomes more limiting.

Methods

Site descriptions and experimental design

This study was conducted across three tallgrass prairie locations in the central United States: Konza Prairie Biological Station (KPBS; Kansas), Tallgrass Prairie Preserve (TPP; Oklahoma), and Tallgrass Prairie National Preserve (TPNP; Kansas). These sites represent remnant tallgrass prairie ecosystems managed under long-term fire and grazing regimes. Across

all sites, dominant vegetation includes warm-season C_4 grasses such as *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachryium scoparium*.

KPBS is a 3,487-ha tallgrass prairie research site in the Flint Hills of northeastern Kansas, USA (39 05'N, 96 35'W). KPBS land is co-owned by The Nature Conservancy and Kansas State University and hosts an NSF Long-Term Ecological Research (LTER) project. The site sits on the largest remaining tract of native tallgrass prairie in North American and has been the location of research on experimentally controlled fire and grazing regimes for more than four decades. Watersheds sampled for the investigations within this chapter have been historically burned at 01-, 04-, and 20-year intervals (Table 3.1, 3.2). Prescribed burns were applied to the landscape in March and April 2025, approximately three months prior to early season sample collection and five months prior to late season sample collection. Watersheds within KPBS are either bison- or cattle-grazed, or ungrazed. Bison are present throughout the year, stocked at a rate of 0.4 ha per animal unit month (AUM) (Blair, 2026). Cattle are present from late spring to autumn in cow-calf pairs at a rate of 0.7 ha per AUM (Olson, 2023). Climate at KPBS is continental, with mean annual precipitation (MAP) of approximately 850 mm and mean annual temperature (MAT) of 12.4 °C (National Ecological Observatory Network (NEON), 2026a). Soils are derived from shale and limestone residuum. Uplands are characterized by shallow, well-drained silty clay loams (National Cooperative Soil Survey, 2020), whereas lowland soils are deeper, with greater organic matter accumulation and water retention (Oviatt, 1998).

TPNP is co-managed by the National Park Service and owned by The Nature Conservancy, encompasses approximately 11,000 acres, and is 60 miles south of KPBS; similar to KPBS, the hilltops of TPNP uplands are comprised of shallow, cherty loams atop limestone deposits. MAP is 850 mm and MAT is 13.5 °C (US Department of Commerce, 2024). Prescribed

burns were conducted in April 2025, approximately four months prior to sampling for this study. Bison inhabit the preserve year-round, while cattle are grazed seasonally from April through October, with recent stocking levels averaging between 0.4 and 0.6 AUM per acre (Leis & Morrison, 2018).

TPP is 205 miles south of KPBS near Pawhuska, Oklahoma. This site is maintained by The Nature Conservancy, with management focusing on sustaining a functioning tallgrass prairie ecosystem through the combined use of prescribed fire, free-ranging bison, and seasonal cattle grazing; with stocking levels of approximately 0.27 animals per hectare (McMillan et al., 2022). The preserve encompasses approximately 40,000 acres of contiguous prairie, making it one of the largest in North America. MAP at TPP is approximately 1047 mm and MAT is 15 °C (US Department of Commerce, 2024). Fire was applied to the landscape from late July to early August 2025. Soils at TPP are primarily Mollisols formed under prairie vegetation, with silty clay loam textures.

This study evaluated forage N dynamics across three complementary analytical frameworks designed to isolate specific drivers while maintaining comparability. Cross-site comparisons (KPBS, TPP, TPNP) were restricted to upland positions under annual burning during late the growing season and included ungrazed, bison-grazed, and cattle grazed treatments to evaluate grazing identity effects. Within KPBS, early-season analysis examined burn interval, grazing and topographic position, while seasonal comparisons evaluated early- versus late-season dynamics within upland sites.

Forage sampling and processing

At KPBS, aboveground forage biomass was collected early (June) and late (August) during the summer 2025 growing season to measure seasonal variation in forage quality.

Sampling targeted dominant graminoid vegetation and was conducted using a 20 x 50 cm quadrat and utility shears at three equidistant locations along established 50 m transects. Early season sampling was conducted across 12 long-term experimental watersheds presenting combinations of burn interval and grazing treatment (Tables 3.1, 3.2). Within each watershed, four established LTER transects were accessed in both upland and lowland positions to capture topographic variation, and at each transect three forage replicate quadrats were collected. In June and August, samples were also collected in a similar fashion along transects in upland cattle-grazed prairies at KBPS, to investigate the influence of grazer identity on forage N dynamics (Table 3.1). Finally, in August only, samples were collected for a comparison of forage N across the three study sites (Table 3.1). Upland sampling locations at TPP were distributed across the connected landscape in ungrazed, bison-grazed, or cattle-grazed areas bounded by fences or cattle guards. Ungrazed sampling locations at TPNP were separated from the grazed locations by roads and streams, while bison and cattle were separated by fences or cattle guards.

Forage samples were transported in paper bags and collected plant material was dried at 60°C for 24-36 hours to remove moisture and ensure consistent dry-weight measurements. Dried samples were then ground to a fine, homogenous powder using a mechanical grinder. Ground forage samples were analyzed at the Kansas State University Soil Testing Laboratory (Manhattan, KS) for total %C and %N using a combustion-based LECO elemental analyzer (St. Joesph, MI). C:N molar ratios were calculated from these measurements to provide an integrated metric of forage quality and nutrient stoichiometry.

Soils were collected concurrently with all forage samples, as three composite mineral soil cores up to 15-cm in depth from each transect using a 2-cm diameter Oakfield soil corer (Oakfield Apparatus Co., Oakfield, WI, USA). Corers were cleaned with 95% ethanol between

transects, and nitrile gloves were worn during sampling to minimize biological contamination. Samples were transported to the laboratory, where they were sieved (<4 mm) to remove rocks and root material while preserving aggregate structure, and split into subsamples for different analyses. For extracellular enzyme activities, which were run on cross-site comparison samples only, soil subsamples were stored at -20°C before analysis.

Soil samples were oven-dried at 60 °C and ground using a pulverizing bench-top grinder. The dried and ground samples were tested for total C and N at the Kansas University Soil Analysis Service Center (Lawrence, Kansas, USA) using a Thermo Scientific Flashsmart Elemental Analyzer (ThermoFisher Scientific) in the NC Soil configuration; this employs the modified Dumas method.

Extracellular enzyme activity

Extracellular enzyme activities (EEA) were measured to assay decomposition potentials and microbial production of enzymes for N acquisition relative to C acquisition (Saiya-Cork et al., 2002; Sinsabaugh, 2000). Three hydrolytic enzymes were assayed: β-glucosidase (βG), which produces C-containing glucose from cellulosic organic matter; N-acetylglucosaminidase (NAG), which produces N- and C-containing amino sugars from bacterial and fungal cell wall compounds, and leucine aminopeptidase (LAP), which produces N-containing amino acids from proteinaceous organic matter. EEAs followed standard fluorometric protocols, in 50 mM sodium acetate buffer (pH 5.0), with analytical replicates, substrate blanks, reference standards, and quench controls; βG assays were incubated for 1.5-3 hrs, NAG for 3-4 hrs, and LAP assays ran for 16-18 hours. Fluorescence of the substrate reporters methylumbelliferyl (βG and NAG) and methyl coumarin (LAP) was measured at 360 nm excitation and 450 nm emission using a FilterMax F5 microplate reader (Molecular Devices, San Jose, CA, USA), and activities were

expressed as nmol substrate hydrolyzed g^{-1} dry soil h^{-1}. To estimate microbial N demand relative to C acquisition, the ratio was calculated as:

$$\ln(\beta G) \,/\, \ln(NAG + LAP)$$

Lower ratios indicate greater microbial investment towards N-acquisition relative to C-acquisition.

Statistical analysis

All statistical analyses were conducted in R (R Core Team, 2025). Three-way analysis of variance (ANOVA) was used to evaluate the effects of burn interval, grazing treatment, and topographic position on all response variables, using the *aov()* function. Model assumptions were evaluated by inspecting residual plots and conducting Shapiro-Wilk tests for normality and Levene's tests for homogeneity of variance. Log transformations were applied where necessary to improve normality and variance structure prior to analysis. Post hoc comparisons among treatment combinations were conducted using estimated marginal means with Tukey-adjusted pairwise comparisons, using the *TukeyHSD()* function. Data visualization was conducted using the *ggplot2* package.

Results

Topographic position and fire frequency comparisons

Soil C concentration was higher in uplands and under the longest fire return interval; soil N concentration was higher in uplands, in 20y fire interval treatments, and in grazed prairies; and soil C:N was highest in ungrazed, annually burned prairies (Table 3.2, Figure 3.1 A-C). Forage C concentration was higher in uplands, in 20y fire interval treatments, and in ungrazed prairies; and forage N concentration was higher and C:N was lower in grazed uplands under longer fire intervals (Table 3.2, Figure 3.1 D-F). Soil water content showed idiosyncratic patterns that may

be more related to time of sampling than field treatment; while pH was lower in 4y fire interval treatments and in grazed 20y fire interval treatments (Table 3.2, Figure 3.2).

Intraseasonal comparisons

Soil C and N concentrations were higher in bison-grazed than cattle-grazed or ungrazed prairies in the early season samples, and higher in cattle-grazed than ungrazed prairies in the late season samples; soil C:N was lower in bison-grazed than ungrazed prairies in both seasons (Table 3.4, Figure 3.3 A-C). Grazing effects on forage quality emerged in the late season samples, when N concentrations were higher, and forage C:N was lower, in bison- and cattle-grazed prairies relative to ungrazed prairies (Table 3.4, Figure 3.3 D-F). Soil water content was lowest in bison-grazed prairies and later in the growing season, and soil pH was highest in bison-grazed prairies (Table 3.4, Figure 3.4).

Cross-site comparisons

Forage C content did not differ significantly by location, grazing treatment, or their interaction (Table 3.6, Figure 3.5 A). Forage N content and forage C:N molar ratio both differed significantly by location, but grazing treatment had no significant effect on either variable, and no significant location by grazing interactions were detected (Table 3.5, Figure 3.5 B, C). Specifically, forage N content was higher, and forage C:N was lower, at TPP. Soil water content was lower at TPP at the time of sampling, and soil pH was higher in bison-grazed areas at KPBS (Table 3.5, Figure 3.6). Soil BG and NAG enzyme activities were lower at TPP than at TPNP or KPBS, and higher in bison-grazed than ungrazed prairies; soil LAP activity was higher in ungrazed than grazed prairies at TPP; the ln(BG):ln(NAG+LAP) ratio was lower at TPP than at TPNP or KPBS, and lower in ungrazed prairies than bison- or cattle-grazed prairies across all three sites (Table 3.5, Figure 3.7).

Discussion

Our results partially supported the hypothesis that fire frequency and grazing jointly regulate forage N content, but this regulation was spatially and temporally structured. The prediction that frequently burned and grazed systems would exhibit higher forage %N and lower C:N molar ratios was supported primarily in annually burned uplands (Figure 3.1) and in late-season forage (Figure 3.3), rather than uniformly across burn intervals and topography at the focal study site, KPBS. Across sites, forage quality did not increase under grazing, whether bison- or cattle-grazing (Figure 3.5), which may be attributable to either differences in plant growth after variable time since prescribed fire, or to differences in grazing intensity. However, soil N availability was higher on average, based on soil extracellular enzyme activity ratios, in both bison- and cattle-grazed prairies across all three study sites (Figure 3.7). Therefore, while large ungulate herbivores can have relatively consistent influences on soil N availability, expression of grazing feedback linking greater soil N availability to higher forage quality may rely primarily on factors regulating plant growth.

The effects of fire and grazing on N cycling in tallgrass prairie are relatively well documented, but predicting how those effects will play out in any given place or time remains difficult when each driver is considered on its own (Collins & Calabrese, 2012; Craine, 2006; Frank et al., 1994). The results reported here show that burn interval and grazing do not impart simple, additive controls on forage N, but instead interact with the landscape position and seasonal timing to produce variation that is structured in both space and time (Figure 3.1). This matters for how frameworks like pyric herbivory are applied across heterogeneous landscapes and suggests that N dynamics in tallgrass prairie are better understood as the product of overlapping controls like fire, grazing, topography, and growing season, rather than

straightforward responses to any one of them (Collins & Smith, 2006; Fuhlendorf et al., 2009; Fuhlendorf & Engle, 2004; Robertson et al., 1997).

The topographic dependence of fire and grazing effects on forage N observed here (Table 3.3, Figure 3.1 D, F) aligns most closely with Turner et al. (1997), who showed that N cycling varies with landscape position at KPBS, with upland soils turning over N up to five times faster than lowland soils. Within this framework, fire-vegetation feedbacks are expressed differently across the landscape, rather than uniformly through time. This spatial structuring differs from the transient maxima hypothesis (Seastedt and Knapp 1993), which describes short-term pulses in resource availability, but does not account for topographic differences in how fire effects accumulate. Consistent with this, Blair (1997) found that inorganic soil N and net mineralization rates at KPBS were highest in intermediate fire intervals and declined with biennial and annual burning, supporting a model in which fire reduces N availability over time. The upland-specific expression of higher forage quality in bison grazed, longer burn interval prairies in this study is consistent with these perspectives: Upland soils, which turn over N more rapidly under baseline conditions, appear more sensitive to cumulative fire effects on N availability, while lowland plots are less differentiated across burn intervals, likely because lower mineralization rates and greater moisture retention buffer post-fire N losses. At broader scales, Pellegrini et al. (2020) found that repeated burning reduced both bulk soil N concentrations and potential decomposition rates across multiple ecosystem types, reinforcing the view that long-term annual burning trends toward dampened rather than enhanced N cycling. This trajectory is corroborated by Chapter 2 data from the same plots, where PMN was lowest in annually burned sites, consistent with reduced biologically available N. Taken together, these findings suggest that frequent fire's influence on forage N reflects gradual depletion, and changing internal feedbacks between plant

inputs and microbial demand (i.e., lower C:N inputs and enhanced immobilization), rather than episodic stimulation (Table 3.3, Figure 3.1).

Seasonal declines in forage %N and increases in C:N molar ratios are primarily attributable to plant growth and physiology. As grasses grow through the growing season, plant N concentrations are diluted as tissues accumulate structural carbon (Craine, 2006; Knapp et al., 1999; Mattson, 1980). Raynor et al. (2015) connected this pattern to the forage maturation hypothesis, arguing that bison track spatial variation in vegetation phenology as a strategy for maintaining access to high-quality forage through the season. Furthermore, grazing and fire influence the time and extent of these changes by altering plant regrowth dynamics (Blair, 1997). Similarly, in grazed grassland systems such as Yellowstone, herbivory maintains younger, N-rich tissues, whereas ungrazed vegetation accumulates structural biomass and exhibits lower N concentrations as the season progresses (Frank & Evans, 1997). Fire and grazing influence the timing and intensity of regrowth, but plant phenology ultimately determines when elevated N availability is expressed in plant tissues.

Temporally conditional expression of grazing effects, which were most pronounced in late-season forage independent of fire treatment (Figure 3.3 D, F), supports the idea that herbivory enhances plant N availability most effectively when plant demand is high relative to background supply (Bardgett & Wardle, 2003). The finding here, that grazing effects on forage quality emerged in late-season but not in early-season forage, extends this interpretation: Herbivory appears to influence N availability most where and when plant uptake has reduced the background supply below what plants can meet through microbial mineralization rates alone. This temporal contingency has practical implications for cross-study comparisons, as the

measured effect of any given grazing treatment on forage quality will depend considerably on when in the growing season sampling takes place.

Grazer identity, whether large herbivores are bison or cattle, has been proposed as a potential driver of N cycling in tallgrass prairie, although isolating identify effects from associated differences in grazing behavior and management remains challenging. Vega Anguiano et al. (2024) reported that bison-grazed soils at KPBS exhibited higher nitrification and denitrification potentials, elevated soil pH, greater plant-available N, and lower extracellular enzyme N limitation than cattle-grazed soils, with cattle-grazed conditions often intermediate between bison- and ungrazed treatments. They also documented higher forage N content and larger soil N stocks under bison grazing, suggesting differences in both microbial process rates and plant N uptake. However, these differences may reflect not only grazer identity per se, but also variation in grazing behavior, landscape use, and management context. In contrast, the present study found no consistent effect of bison or cattle grazing on forage N or C:N molar ratios across KPBS, TPP, and TPNP (Table 3.5, Figure 3.5). This discrepancy likely reflects differences in both spatial scale and experimental design. Vega Anguiano et al. (2024) used watershed-scale treatments with moveable exclosures to isolate grazer effects under controlled conditions at a single site, whereas the cross-site design used here incorporated variation in soil properties, grazing intensity, and fire management that may obscure differences associated with grazer type.

In contrast to forage quality responses, bison and cattle grazing consistently lowered soil microbial N limitation at all three study sites, based on microbial extracellular enzyme responses (Table 3.5, Figure 3.7 D). In the cross-site analysis, variation in individual enzyme activities were largely site-driven (Table 3.5), suggesting sensitivity to local environmental conditions

(Figure 3.7 B, C). Grazing introduces localized pulses of labile C and N through dung and urine deposition, providing direct and immediate substrates for microbial metabolism. As a result, microbes shift their resource allocation and expression of N demand, causing microbial ln(BG):ln(NAG+LAP) ratios to respond consistently to grazing across sites. This pattern reinforces that microbial enzyme indices are a signal of changes in N availability, while plant N concentration reflects both cumulative supply and plant maturity. The contrasting plant and microbial N responses observed across all three study systems are consistent with a system in which localized herbivore inputs transiently elevate plant-available N, suggesting that plant uptake responded more directly to changes in bioavailable N than bulk soil metrics. However, grazing-induced increases in total soil N observed in Chapter 2 indicate that some of this redistributed N is retained in soil N pools, likely in organic forms not fully captured by ACE protein assays. This discrepancy suggests that herbivore-derived N inputs, rather than accumulating in the proteinaceous N fraction as it does under fire suppression, may be incorporated into particulate or mineral-associated organic matter pools, or cycled rapidly through microbial biomass. In summary, consistent grazing effects on extracellular enzyme activity across sites, despite weak or absent grazing effects on forage quality, highlight fundamental differences in how microbes and plants respond to elevated N availability.

The decoupling we observed between soil N pools, microbial enzyme signatures, and forage %N fits the depolymerization-centered concept of Schimel and Bennett (2004), in which bioavailable N is produced by extracellular-enzyme cleavage of organic polymers and then partitioned between plants and microbes within spatially heterogeneous soil. In this view, net mineralization and bulk inorganic N are indirect indices of supply, not of plant uptake. Further, because plants and microbes are in active competition for available N (Schimel and Bennett

2004), this framing also dovetails with the broader argument that herbivory enhances plant N availability most effectively where plant demand is high relative to background supply (Bardgett and Wardle, 2003). Because depolymerization produces a continuous but uneven flux of monomers, whether plants capture them depends on when and where their demand is high enough to outpace microbial recapture, which is why grazing-driven increases in N availability tend to register most strongly during periods of active growth and fade as tissues accumulate structural C and shift toward reproduction. Variation among sites likely reflects differences in growing-season progression at the time of sample collection, including timing of green-up and duration of active growth, which shape cumulative N uptake and allocation (Craine et al., 2009; Knapp et al., 1999). Considered together, these results suggest that forage quality integrates N availability over the growing season and is shaped by plant physiological state at the time of sampling: the aboveground summation of many small, transient pulses of monomer release rather than a snapshot of any single N pool.

In summary, rather than reflecting a simple response to N availability, patterns in forage N observed here (Figures 3.1, 3.3, and 3.5) suggest that plant physiological processes mediate how fire- and grazing-driven changes in N cycling are expressed as forage quality. Although fire and grazing influence N availability in soils, these changes did not translate directly into forage N patterns across treatments (Table 3.5). Forage N concentration and C:N molar ratios varied among sites but showed limited and inconsistent responses to grazing (Table 3.5; Figure 3.5), suggesting that plant N status was not determined solely by soil N supply. Instead, forage quality reflects the interaction between N availability and plant development. As plants mature, increasing structural biomass leads to dilution of tissue N, resulting in lower %N and higher C:N molar ratios even under conditions of elevated N availability (Greenwood et al., 1990; Jarrell and

Beverly, 1982; Risser and Parton, 1982). Consequently, differences in forage N among treatments and locations may be better explained by variation in plant growth stage and regrowth dynamics than by differences in soil N pools alone.

Table 3.1. Summary of KPBS soil sample locations collected in June 2025 including watersheds, burn intervals (years), time from last burn (years (y) and months (mos)).

KPBS Watershed	Burn Interval (years)	Time from Last Burn	Note
1D	1	2 mos	
SpA	1	2 mos	
SpB	1	2 mos	
N1A	1	3 mos	
N1B	1	3 mos	
C1SB	1	3 mos	
CIA	1	1 y + 4 mos	
4B	4	2 mos	
4F	4	2 y	
N4B	4	4 mos	
N4D	4	3 mos	
20B	20	7 y	
20C	20	4 y + 7 mos	Autumn burn
N20A	20	3 mos	
N20B	20	3 mos	Historical burn interval = 20y, however, 1y intervals since 2020.

Table 3.2. Summary of sampling locations, watersheds, burn intervals, grazing treatments, and sample sizes for forage and soil collections. Values represent the number of individual replicate forage samples and composite soil samples collected per treatment combination at each site.

Location	Watersheds	Season	Grazing	Burn Interval	Topographic Position	Forage (n)	Soil (n)
KPBS	1D, SpA	Early	Ungrazed	1Y	Upland	24	8
KPBS	4B, 4F	Early	Ungrazed	4Y	Upland	24	8
KPBS	20B, 20C	Early	Ungrazed	20Y	Upland	24	8
KPBS	N1A, N1B	Early	Bison	1Y	Upland	24	8
KPBS	N4B, N4D	Early	Bison	4Y	Upland	24	8
KPBS	N20A,N20B	Early	Bison	20Y	Upland	24	8
KPBS	1D, SpA	Early	Ungrazed	1Y	Lowland	24	8
KPBS	4B,4F	Early	Ungrazed	4Y	Lowland	24	8
KPBS	20B,20C	Early	Ungrazed	20Y	Lowland	24	8
KPBS	N1A,N1B	Early	Bison	1Y	Lowland	24	8
KPBS	N4B,N4D	Early	Bison	4Y	Lowland	24	8
KPBS	N20A,N20B	Early	Bison	20Y	Lowland	24	8
KPBS	SpA, SpB	Early	Ungrazed	1Y	Upland	24*	8*
KPBS	N1A, N1B	Early	Bison	1Y	Upland	24*	8*
KPBS	C1SB, C1A	Early	Cattle	1Y	Upland	36	12
KPBS	SpA, SpB	Late	Ungrazed	1Y	Upland	24	8
KPBS	N1A,N1B	Late	Bison	1Y	Upland	24	8
KPBS	C1SB,C1A	Late	Cattle	1Y	Upland	24	8
KPBS	SpA, SpB	Late	Ungrazed	1Y	Upland	24*	8*
KPBS	N1A, N1B	Late	Bison	1Y	Upland	24*	8*
KPBS	C1A, C1SB	Late	Cattle	1Y	Upland	24*	8*
TPNP	Ungrazed	Late	Ungrazed	1Y	Upland	9	3
TPNP	Bison	Late	Bison	1Y	Upland	9	3
TPNP	Cattle	Late	Cattle	1Y	Upland	9	3
TPP	Ungrazed	Late	Ungrazed	1Y	Upland	18	6
TPP	Bison	Late	Bison	1Y	Upland	18	6
TPP	Cattle	Late	Cattle	1Y	Upland	9	3
TOTAL		—	—	—		**468**	**156**

Note. Sample batches with * were also used in the previously listed comparison model.

Table 3.3. Summary of Type III ANOVA results evaluating effects of burn interval (01Y, 04Y, 20Y), grazing treatment (ungrazed, bison-grazed), and landscape topography (upland, lowland) on forage carbon, nitrogen, and carbon-to-nitrogen ratio; soil carbon, nitrogen, and carbon-to-nitrogen ratio; gravimetric water content; and soil pH at Konza Prairie Biological Station. Forage carbon, nitrogen, and carbon-to-nitrogen ratio; soil carbon, nitrogen, and carbon-to-nitrogen ratio; and gravimetric water content were $\log_{10}$ transformed. Reported values are F-statistics with associated numerator and denominator degrees of freedom. Significance codes: *** p < 0.001; ** p < 0.01; * p < 0.05; . p < 0.10; ns = not significant.

Response variable	Burn interval	Topography	Grazing	Burn interval × Topography	Burn interval × Grazing	Topography × Grazing	Burn interval × Topography × Grazing
[l]Forage C	**$F_{2,83}$ = 4.71***	**$F_{1,83}$ = 9.62****	**$F_{1,83}$ = 5.89***	$F_{2,83}$ = 1.55	$F_{2,83}$ = 0.38	$F_{1,83}$ = 0.57	$F_{2,83}$ = 0.059
[l]Forage N	$F_{2,83}$ = 7.25**	$F_{1,83}$ = 3.14.	$F_{1,83}$ = 14.46***	$F_{2,83}$ = 17.26***	$F_{2,83}$ = 4.60*	$F_{1,83}$ = 1.29	**$F_{2,83}$ = 4.17***
Forage C:N	$F_{2,83}$ = 6.05**	$F_{1,83}$ = 4.65*	$F_{1,83}$ = 16.77***	$F_{2,83}$ = 17.40***	$F_{2,83}$ = 4.89**	$F_{1,83}$ = 1.51	**$F_{2,83}$ = 4.03***
[l]Soil C	**$F_{2,82}$ = 5.58****	**$F_{1,82}$ = 22.24*****	$F_{1,82}$ = 0.26	$F_{2,82}$ = 1.13	$F_{2,82}$ = 0.28	$F_{1,82}$ = 0.57	$F_{2,82}$ = 1.00
[l]Soil N	**$F_{2,82}$ = 6.97****	**$F_{1,82}$ = 20.51*****	**$F_{1,82}$ = 4.07***	$F_{2,82}$ = 0.75	$F_{2,82}$ = 2.17	$F_{1,82}$ = 0.030	$F_{2,82}$ = 0.76
Soil C:N	$F_{2,82}$ = 3.90*	$F_{1,82}$ = 0.68	$F_{1,82}$ = 17.69***	$F_{2,82}$ = 0.82	**$F_{2,82}$ = 6.98****	$F_{1,82}$ = 2.86.	$F_{2,82}$ = 1.82
GWC	$F_{2,84}$ = 6.39**	$F_{1,84}$ = 11.78***	$F_{1,84}$ = 10.26**	$F_{2,84}$ = 4.09*	$F_{2,84}$ = 0.14	$F_{1,84}$ = 0.016	**$F_{2,84}$ = 6.19****
pH	$F_{2,84}$ = 14.76***	$F_{1,84}$ = 2.73	$F_{1,84}$ = 0.39	$F_{2,84}$ = 0.89	**$F_{2,84}$ = 9.26*****	$F_{1,84}$ = 0.003	$F_{2,84}$ = 0.14

Note. . p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001. F subscripts are numerator and denominator degrees of freedom. Some variables were log transformed to approach model assumptions of normality and noted with superscript "l" if so. Bolded values represent results used to interpret data: When interactions emerge, these supersede main effects for results interpretation.

Table 3.4. Summary of Type III ANOVA results evaluating effects of sampling season (early, late) and grazing treatment (ungrazed, bison-grazed, cattle-grazed) on forage carbon, nitrogen, and carbon-to-nitrogen ratio; soil carbon, nitrogen, and carbon-to-nitrogen ratio; gravimetric water content; and soil pH at Konza Prairie Biological Station under annual (1-year) fire return intervals. Forage carbon, nitrogen, and carbon-to-nitrogen ratio; soil carbon, nitrogen, and carbon-to-nitrogen ratio; and gravimetric water content were $\log_{10}$ transformed. Reported values are F-statistics with associated numerator and denominator degrees of freedom. Significance codes: *** p < 0.001; ** p < 0.01; * p < 0.05; . p < 0.10; ns = not significant.

Response	Season	Grazing	Season × Grazing
[l]Forage C	$F_{1,46} = 11.18$, p = 1.65×10^{-3} **	$F_{2,46} = 5.66$, p = 6.33×10^{-3} **	**$F_{2,46} = 6.37$, p = 3.61×10^{-3} ****
[l]Forage N	$F_{1,46} = 91.14$, p = 1.74×10^{-12} ***	$F_{2,46} = 6.82$, p = 2.55×10^{-3} **	**$F_{2,46} = 4.54$, p = 1.59×10^{-2} ***
Forage C:N	$F_{1,46} = 97.32$, p = 6.26×10^{-13} ***	$F_{2,46} = 6.82$, p = 2.55×10^{-3} **	**$F_{2,46} = 5.25$, p = 8.84×10^{-3} ****
[l]Soil C	$F_{1,45} = 1.49$, p = 0.23	$F_{2,45} = 1.26$, p = 0.29	**$F_{2,45} = 3.62$, p = 0.035 ***
[l]Soil N	$F_{1,45} = 3.46$, p = 0.069 .	$F_{2,45} = 6.10$, p = 4.53×10^{-3} **	**$F_{2,45} = 7.29$, p = 1.80×10^{-3} ****
Soil C:N	$F_{1,45} = 1.10$, p = 0.30	**$F_{2,45} = 8.35$, p = 8.27×10^{-4} *****	$F_{2,45} = 2.44$, p = 0.099 .
GWC	**$F_{1,45} = 8.30$, p = 0.0061 ****	**$F_{2,45} = 15.82$, p = 6.26E-06 *****	$F_{2,45} = 1.07$, p = 0.35
pH	$F_{1,45} = 0.08$, p = 0.79	**$F_{2,45} = 8.60$, p = 0.00069 *****	$F_{2,45} = 1.94$, p = 0.16

Note. . p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001. F subscripts are numerator and denominator degrees of freedom. Some variables were log transformed to approach model assumptions of normality and noted with superscript "l" if so. Bolded values represent results used to interpret data: When interactions emerge, these supersede main effects for results interpretation.

Table 3.5. Summary of Type III ANOVA results evaluating effects of site (Konza Prairie Biological Station, Tallgrass Prairie Preserve, Tallgrass Prairie National Preserve) and grazing treatment (ungrazed, bison-grazed, cattle-grazed) on forage carbon, nitrogen, and carbon-to-nitrogen ratio; extracellular enzyme activities; gravimetric water content; and soil pH during late-season sampling under annual (1-year) fire return intervals. Forage carbon, nitrogen, and carbon-to-nitrogen ratio and gravimetric water content were log_{10} transformed, and β-glucosidase, N-acetyl-β-glucosaminidase, and leucine aminopeptidase activities were natural log transformed where necessary to satisfy model assumptions. Reported values are F-statistics with associated numerator and denominator degrees of freedom. Significance codes: *** p < 0.001; ** p < 0.01; * p < 0.05; . p < 0.10; ns = not significant.

Response variable	Site	Grazing	Site × Grazing
[l]Forage C	$F_{2,37}$ = 1.98	$F_{2,37}$ = 1.12	$F_{4,37}$ = 0.69
[l]Forage N	**$F_{2,37}$ = 4.85***	$F_{2,37}$ = 1.00	$F_{4,37}$ = 1.11
Forage C:N	**$F_{2,37}$ = 4.63***	$F_{2,37}$ = 1.04	$F_{4,37}$ = 1.15
[l]Soil C	**$F_{2,37}$ = 8.44*****	$F_{2,37}$ = 1.22	$F_{4,37}$ = 0.86
[l]Soil N	**$F_{2,37}$ = 15.59*****	**$F_{2,37}$ = 7.76****	$F_{4,37}$ = 1.49
Soil C:N	$F_{2,37}$ = 6.30**	$F_{2,37}$ = 12.64***	**$F_{4,37}$ = 5.67****
[l]BG activity	**$F_{2,38}$ = 24.48*****	**$F_{2,38}$ = 7.00****	$F_{4,38}$ = 2.11.
[l]NAG activity	$F_{2,38}$ = 10.87***	$F_{2,38}$ = 3.60*	**$F_{4,38}$ = 2.74***
[l]LAP activity	$F_{2,26}$ = 7.25**	$F_{2,26}$ = 1.48	**$F_{3,26}$ = 11.21*****
ln(BG)/ln(NAG+LAP)	**$F_{2,38}$ = 10.44****	**$F_{2,38}$ = 4.53***	$F_{4,38}$ = 1.27
GWC	**$F_{2,38}$ = 6.69****	$F_{2,38}$ = 0.67	$F_{4,38}$ = 1.64
pH	**$F_{2,38}$ = 18.18*****	$F_{2,38}$ = 2.29	**$F_{4,38}$ = 3.32***

Note. . p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001. F subscripts are numerator and denominator degrees of freedom. Some variables were log transformed to approach model assumptions of normality and noted with superscript "l" if so. Bolded values represent results used to interpret data: When interactions emerge, these supersede main effects for results interpretation.

Figure 3.1. A) Soil carbon (%), B) forage carbon (%), C) soil nitrogen (%), D) forage nitrogen (%) E) soil carbon-to-nitrogen ratio (molar), and F) forage carbon-to-nitrogen ratio (molar) across burn intervals (01Y, 04Y, 20Y), grazing treatments (ungrazed, bison-grazed) and landscape topography (upland, lowland) at Konza Prairie Biological Station. Box plots show medians with interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

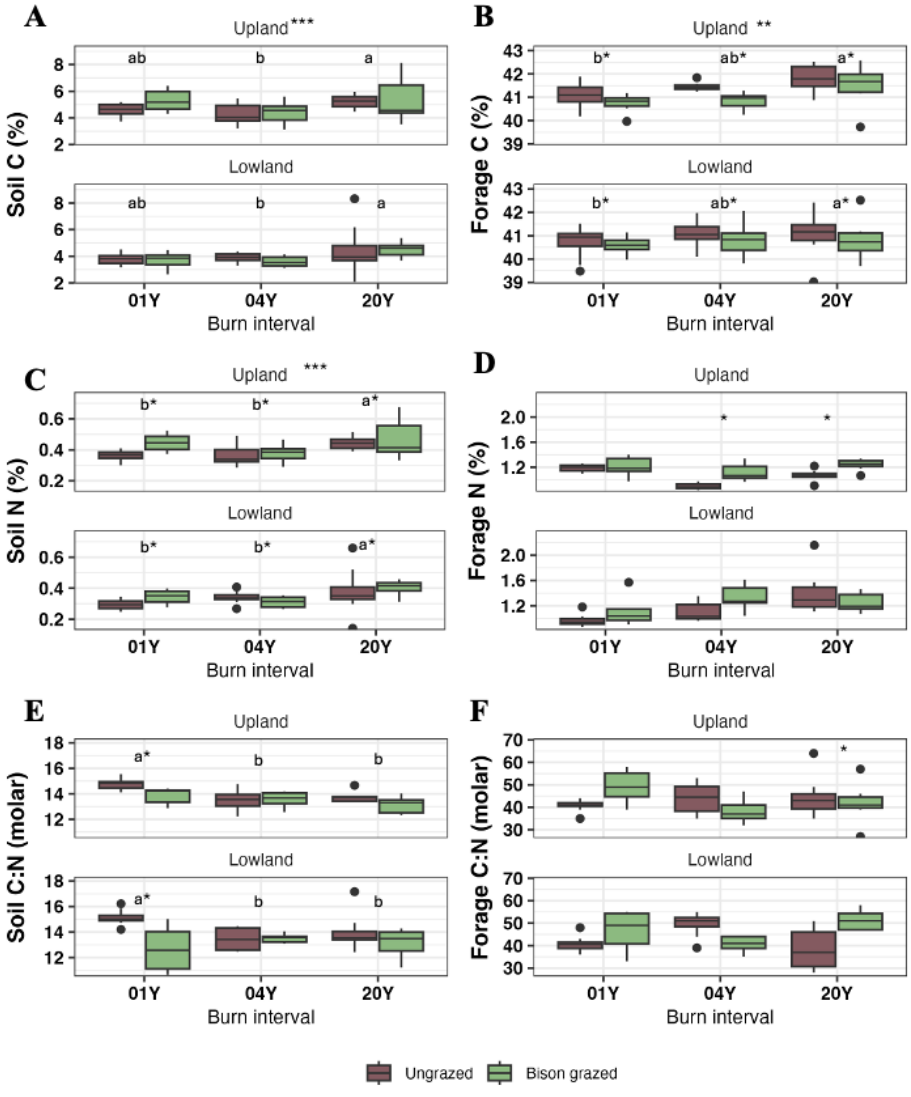


Figure 3.2. A) Soil gravimetric water content (g g^{-1}) and B) soil pH across burn intervals (01Y, 04Y, 20Y), grazing treatments (ungrazed, bison-grazed) and landscape topography (upland, lowland) at Konza Prairie Biological Station. Box plots show medians with interquartile ranges of data points within each treatment combination. Main fire (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05, and main topographic and main or interactive grazing effects with P < 0.05, <0.01, <0.001 are denoted by asterisks (*, **, *, respectively).**

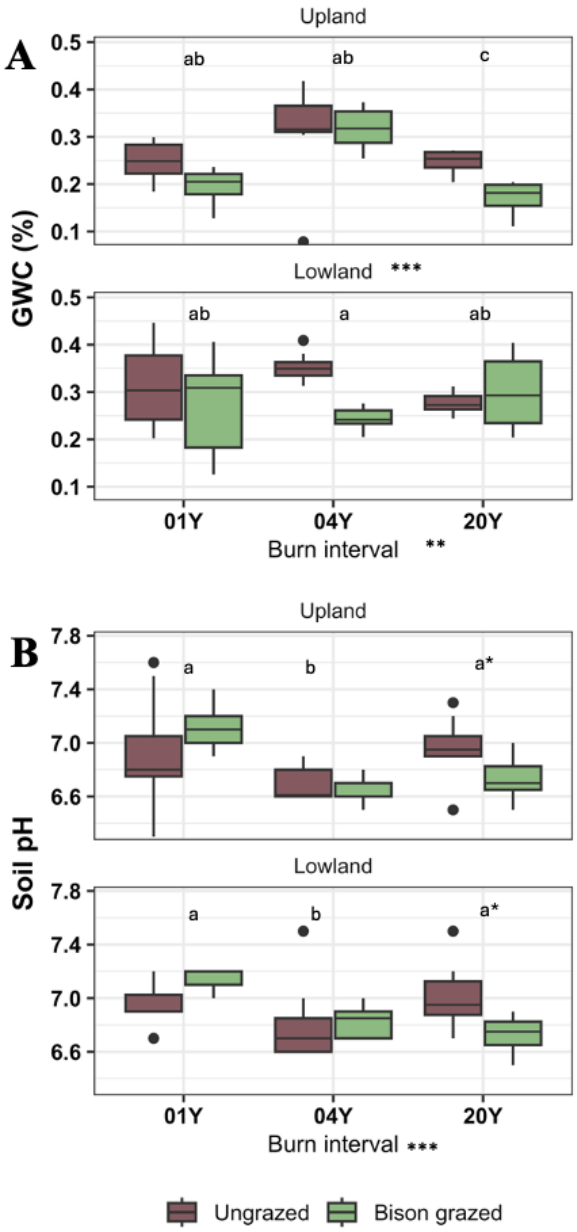


Figure 3.3. A) soil carbon (%), B) forage carbon (%) C) soil nitrogen (%), D) forage nitrogen (%), E) soil carbon-to-nitrogen ratio (molar), and F) forage carbon-to-nitrogen ratio (molar) across sampling seasons (early, late) and grazing treatments (ungrazed, bison-grazed, cattle grazed) at Konza Prairie Biological Station under annual fire return intervals. Box plots show medians and interquartile ranges of data points within each treatment combination. Main or interactive grazing effects (Tukey's HSD post-hoc) are denoted by letters indicating treatments that differed from one another at $P < 0.05$.

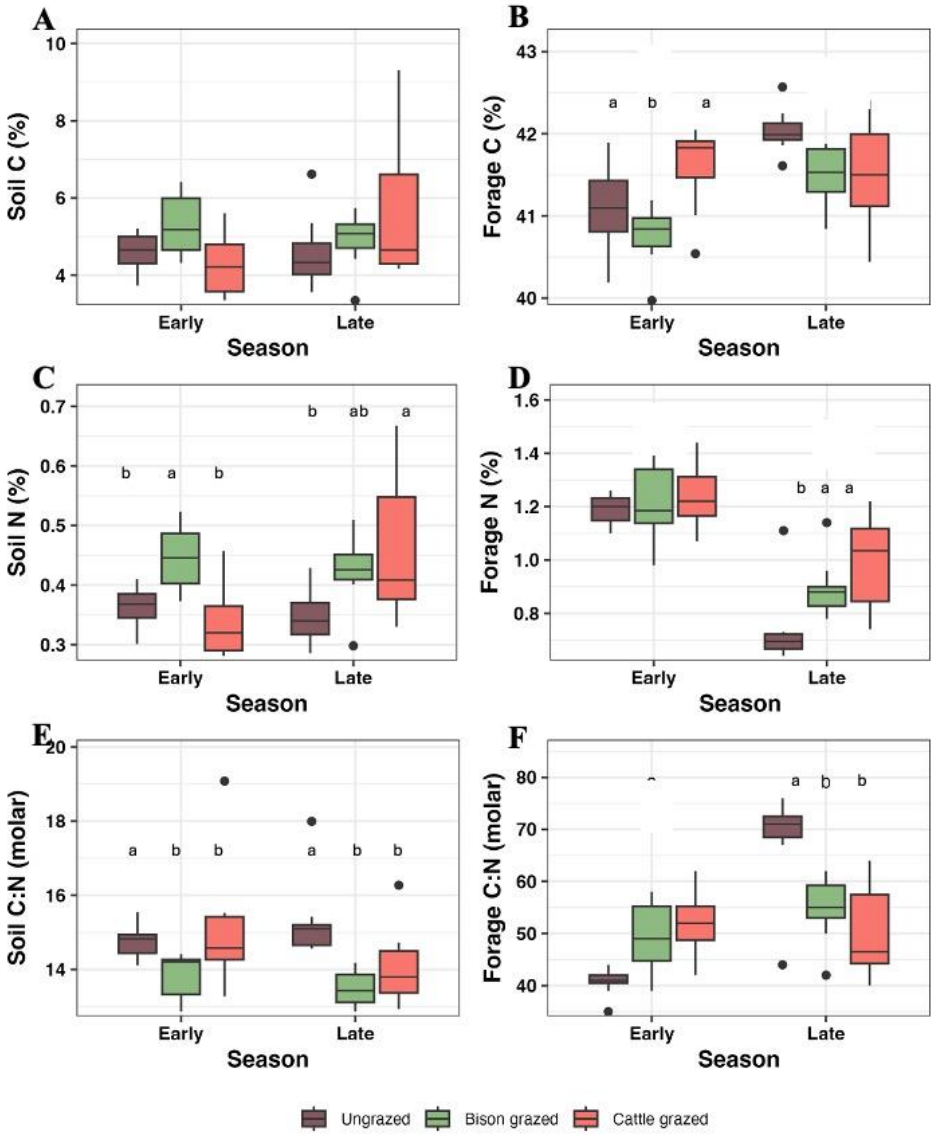


Figure 3.4. A) Soil gravimetric water content (g g^{-1}) and B) soil pH across sampling seasons (early, late) and grazing treatments (ungrazed, bison-grazed, cattle grazed) at Konza Prairie Biological Station under annual fire return intervals. Box plots show medians and interquartile ranges of data points within each treatment combination. Direct seasonal (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05.

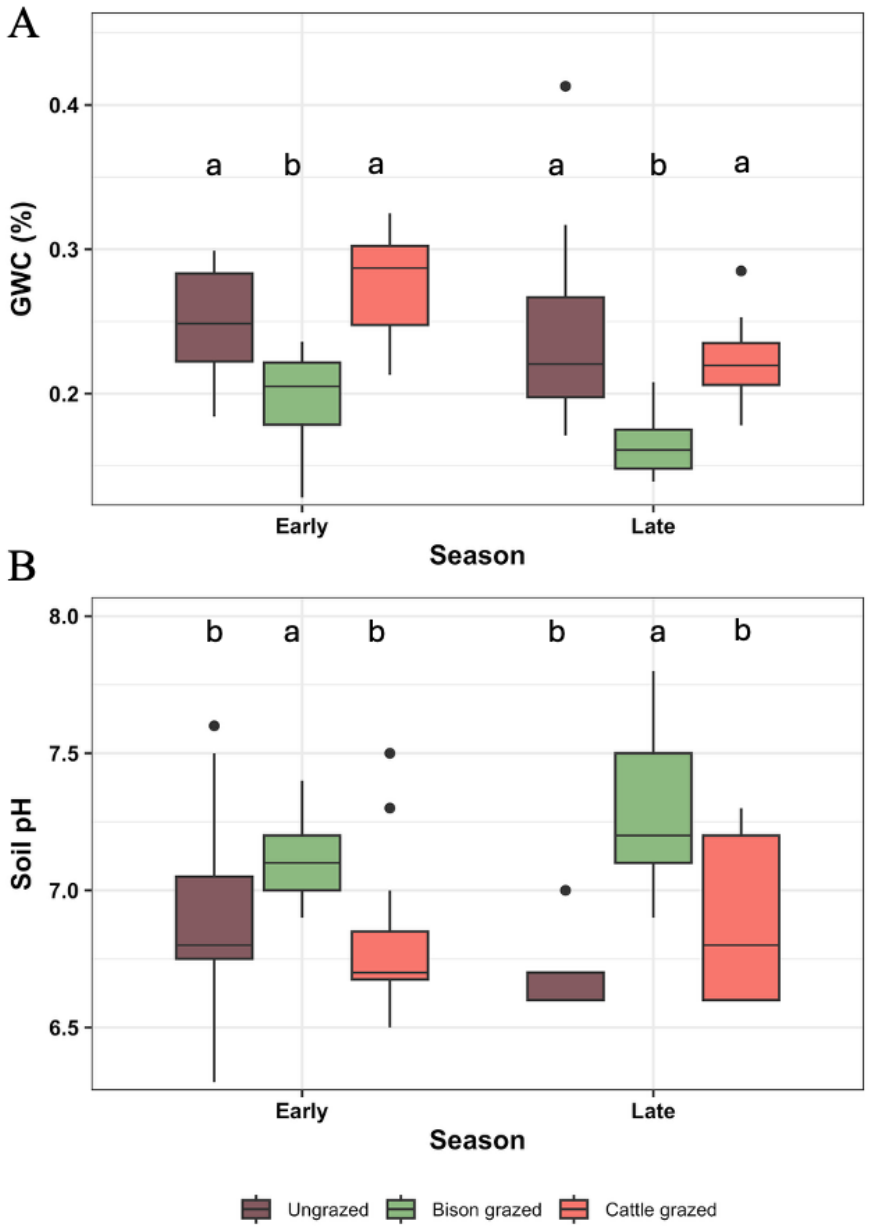


Figure 3.5. A) Forage carbon (%), B) forage nitrogen (%), and C) forage carbon-to-nitrogen ratio (molar) across sites (Konza Prairie Biological Station, Tallgrass Prairie Preserve, Tallgrass Prairie National Preserve) and grazing treatments (ungrazed, bison-grazed, cattle grazed) during late-season sampling under annual fire return intervals. Box plots show medians and interquartile ranges of data points within each treatment combination. Direct site (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at $P < 0.05$.

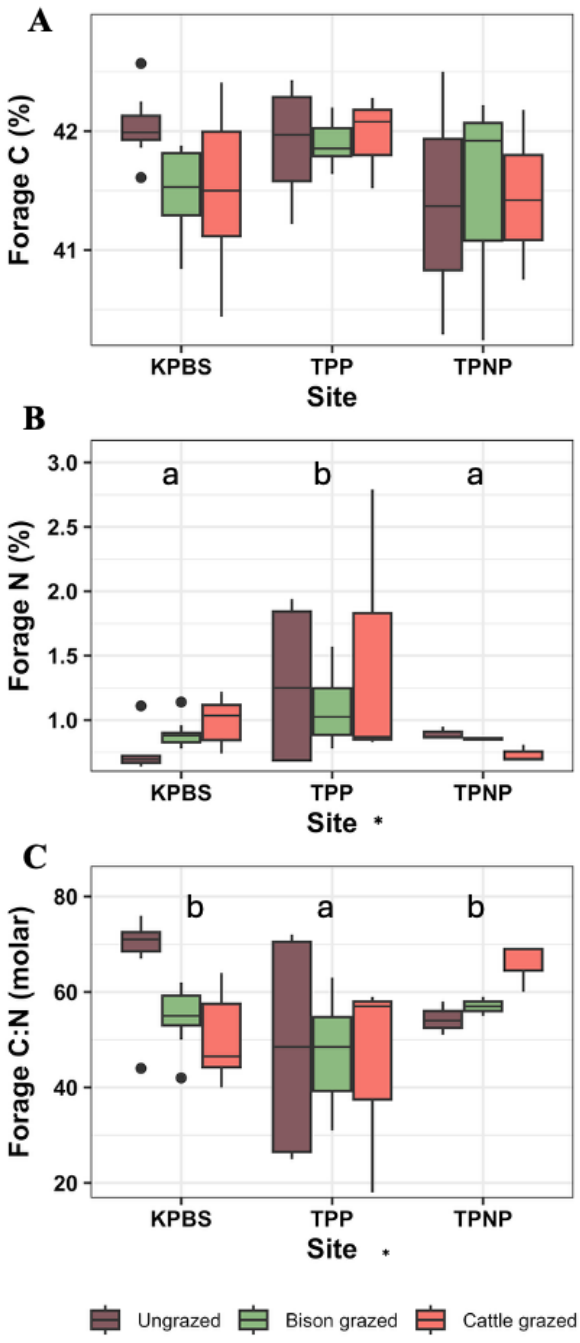


Figure 3.6. A) Soil gravimetric water content (g g^{-1}) and B) soil pH across sites (Konza Prairie Biological Station, Tallgrass Prairie Preserve, Tallgrass Prairie National Preserve) and grazing treatments (ungrazed, bison-grazed, cattle grazed) during late-season sampling under annual fire return intervals. Box plots show medians and interquartile ranges of data points within each treatment combination. Direct site (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at P < 0.05.

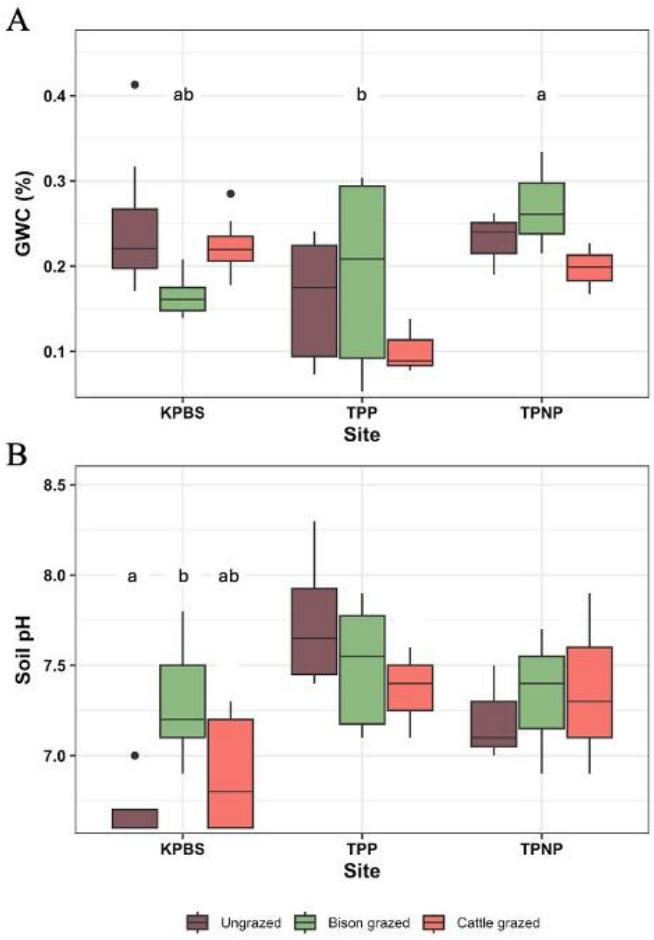
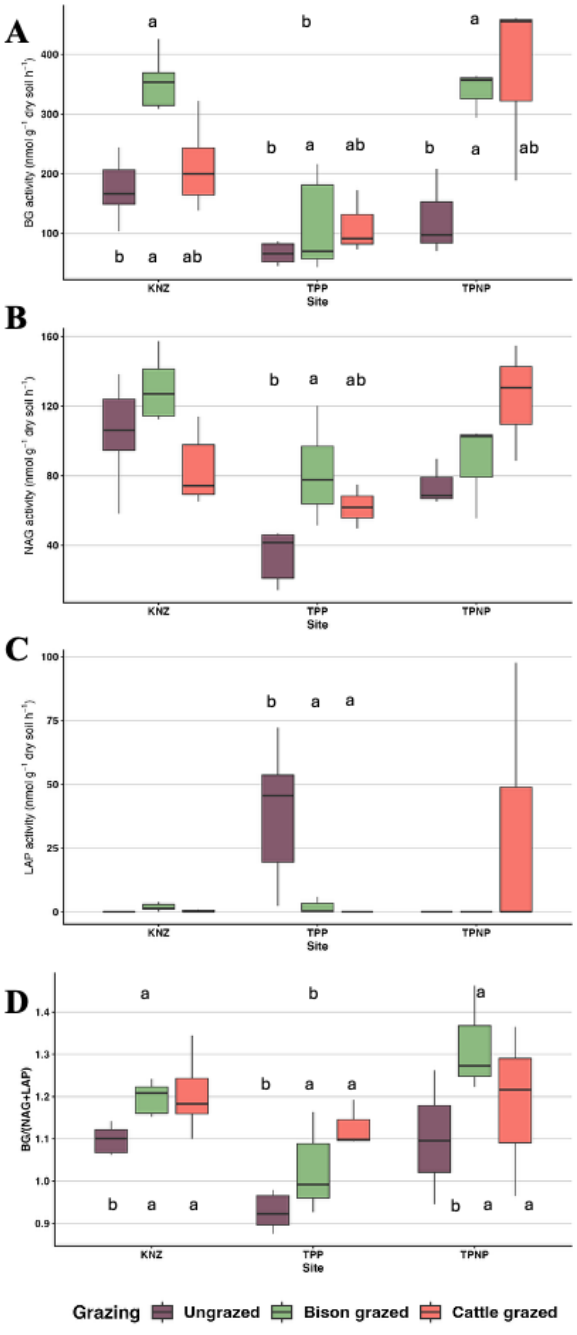


Figure 3.7. A) β-glucosidase activity (BG; nmol g^{-1} h^{-1}), B) N-acetyl-β-glucosaminidase activity (NAG; nmol g^{-1} h^{-1}), C) leucine aminopeptidase activity (LAP; nmol g^{-1} h^{-1}), and D) BG:[NAG+LAP] enzyme ratio across sites (Konza Prairie Biological Station, Tallgrass Prairie Preserve, Tallgrass Prairie National Preserve) and grazing treatments (ungrazed, bison-grazed, cattle grazed) during late-season sampling under annual fire return intervals. Box plots show medians and interquartile ranges of data points within each treatment combination. Direct site (Tukey's HSD post-hoc) effects are denoted by letters indicating treatments that differed from one another at $P < 0.05$.



Chapter 4 - Conclusions

This thesis demonstrates that N cycling in tallgrass prairie is governed by the combined and context-dependent effects of fire frequency, grazing, and landscape position, rather than by any single driver acting in isolation. Across soil and forage systems, fire and grazing did not produce uniform increases or decreases in N pools, but instead shifted N among storage, mineralization, and plant uptake pathways. Fire primarily influenced longer-term patterns of N accumulation and substrate availability, while grazing altered the accessibility and turnover of N through plant-microbe interactions, changes in litter inputs, and localized nutrient return. These processes were mediated by landscape position, reinforcing that spatial heterogeneity is fundamental to understanding N dynamics in tallgrass prairie ecosystems.

Patterns observed across soil and forage responses highlight an important distinction between plant and microbial pathways of N acquisition. Microbial extracellular enzyme production responded primarily to substrate availability and stoichiometric constraints, reflecting longer-term controls on C and N balance. In contrast, forage N responded more directly to localized and transient increases in plant-available N, particularly under grazing. This divergence suggests that upland and microbial processes are linked, but not tightly synchronized, operating on different temporal scales and through different resource pathways. As a results, changes in N availability may be expressed on different time scales in plant tissues than in bulk soil pools or microbial indicators.

Integration across chapters reveals that forage quality and soil N dynamics are connected, but not necessarily equivalent. Higher forage N concentrations and lower C:N molar ratios were associated with conditions that promote N availability and rapid plant regrowth, particularly under grazing and reduced fire frequency. However, microbial indicators such as extracellular

enzyme activity, PMN, and ACE protein demonstrate that N availability, turnover, and storage represent distinct components of the N cycle. Shifts in fire and grazing regimes altered the balance between rapid N cycling through plant and microbial biomass and longer-term stabilization in SOM, suggesting that no single metric adequately captures ecosystem N status.

Together, these findings support a conceptual model in which the interaction of fire and grazing generates a mosaic of N availability across the prairie landscape. Grazing concentrates nutrient inputs and plant uptake in actively utilized areas, while variation in fire frequency regulates the accumulations and turnover of organic N pools. These patterns are further structured by topography; this spatial and temporal variability is a defining feature of how N is cycled and retained in tallgrass prairie ecosystems.

The tallgrass prairie is among the most diminished ecosystems in North American, yet it remains disproportionately important for biodiversity, C storage, and nutrient cycling. Understanding how fire and grazing interact to regulate N dynamics is therefore critical not only for advancing ecological theory, but also for informing management of the remaining prairie systems. Maintaining the coupling between fire and grazing processes is essential for sustaining the heterogeneity that underpins both soil function and forage quality.

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