Biogeochemistry (2024) 167:759–773
https://doi.org/10.1007/s10533-024-01144-0

# Bison and cattle grazing increase soil nitrogen cycling in a tallgrass prairie ecosystem

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Received: 9 January 2024 / Accepted: 15 April 2024 / Published online: 30 April 2024


**Abstract** Nitrogen (N) is a necessary element of soil fertility and a limiting nutrient in tallgrass prairie but grazers like bison and cattle can also recycle N. Bison and cattle impact the nitrogen (N) cycle by digesting forage that is consumed, and recycled back to the soil in a more available forms stimulating soil microbial N cycling activities. Yet we do not know how both grazers comparatively affect N cycling in tallgrass prairie. Thus, we investigated if bison and cattle had similar impacts on N cycling in annually burned tallgrass prairie relative to ungrazed conditions over a 3-year period (2020–2022) at the Konza Prairie Biological Station. We examined: soil pH, soil water content, mineralized N, nitrification potential, denitrification potential and extracellular enzyme assays. Interannual variability in precipitation controlled soil water and N cycling microbial activities but grazing effects had a stronger influence on N cycling. We found significant differences and increased soil pH, nitrification and denitrification potential and less N limitation in bison vs cattle grazed soils where bison grazed soils exhibited faster N cycling. Differences between the grazers may be attributed to the different management of bison and cattle as both can impact N cycling. Overall, these data provide some evidence that bison and cattle affect N cycling differently at this study site, and improve the ecological understanding of grazer impacts on N cycling dynamics within the tallgrass prairie ecosystem.

**Keywords** Nitrogen · Tallgrass prairie · Bison · Cattle · Soil

Responsible Editor: Edith Bai.

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## Introduction

Nitrogen (N) is a limiting nutrient in many terrestrial ecosystems, including tallgrass prairies (Blair 1997; Schlesinger and Bernhardt 2020). In this ecosystem, frequent fire volatilizes N from plant litter, slowing the accumulation of soil organic N; therefore, fire maintains conditions in which N-limited plants and soil microbes rapidly assimilate and immobilize, and effectively retain, soil available inorganic N (Dodds et al. 1996; Dell and Rice 2005; Dell et al. 2005). In addition, tallgrass prairies were historically grazed by large mammalian herbivores, which often enhance soil N cycling rates, N heterogeneity, and soil fertility (Hobbs 1996; Frank and Evans 1997; Blair et al. 1998; Knapp et al. 1999; Bakker et al. 2003). Bison grazing can increase soil N cycling rates in areas managed with annual fire to levels equivalent to areas experiencing infrequent fire (Groffman et al. 1993; Johnson and Matchett 2001).

Plains bison, also known as the American Buffalo (*Bison bison*), are keystone herbivores that once ranged across the whole North American continent (Knapp et al. 1999; Lott 2002; Anderson 2006; List et al. 2007). However, in the 1880s, extermination through settler colonization (Dunbar-Ortiz 2014) decimated bison populations which numbered in the millions to less than 1000 individuals, driving societal collapse and negative economic impacts on Native American communities that remain until this day (Hornaday, 1913; Flores 1991; Shaw 1995; Lott 2002; Feir et al. 2021). Domesticated cattle (*Bos taurus*) now outnumber bison by an estimated 950 million individuals (Samson et al. 2004; Kohl et al. 2013). In the 1980s and 90 s, ecological researchers recognized bison as major ecosystem drivers in maintaining Great Plains grasslands and their nutrient cycles (Hulbert 1986; Vinton et al. 1993; Ojima et al. 1994; Coppedge et al. 1998a, b; Woodmansee and Duncan 1980; Risser and Parton 1982; Blair 1997). All things considered, replacement of the keystone bison with cattle raises concerns of whether both animals occupy the same functional roles on the landscape (Allred et al. 2011; Kohl et al. 2013), mirroring global concerns and studies on the alteration of nutrient cycling following the replacement of native megaherbivores with domesticated cattle in managed rangeland (Enquist et al. 2020; Abraham et al. 2023; Roy et al. 2023).

Cattle and bison have certain redundant roles in ecosystem N-cycling function, but also differ physiologically in potentially influential ways. They both graze similar grasses in tallgrass prairie (Allred et al. 2011) and excrete dung and urine which in turn increases bioavailable N for soil microorganisms and plants (McNaughton 1983; Detling 1988; Schlesinger and Hartley 1992; Anderson 2006). However, bison are hardier and tolerant of extreme hot and cold weather temperatures, enabling them to travel and spend more time grazing away from streams on upland prairie (Christopherson et al. 1978; Allred et al. 2011; Larson et al. 2013; McMillan et al. 2021; McMillan et al. 2022), while cattle are less weather hardy and tend to travel infrequently by comparison choosing to spend more time near riparian areas (Kohl et al. 2013; McMillan et al. 2021). Therefore, the distribution of N across the landscape by bison and cattle depends on decisions to travel and forage and drink water (Plumb and Dodd 1993; Augustine and Frank 2001; Raynor et al. 2021). Many comprehensive studies in tallgrass prairie focus on aboveground plant responses to grazing by bison and cattle, with less emphasis on soil microbial functions that allow N to become available for forage regrowth (Plumb and Dodd 1993; Coppedge and Shaw 1997; Coppedge et al. 1998a, b; Towne et al. 2005; McMillan et al. 2011; McMillan et al. 2019; Ratajczak et al. 2022). To our knowledge, no studies have directly assessed whether bison and cattle similarly influence N cycling in tallgrass prairie soils.

Therefore, we investigated soil microbial N cycling activities in annually burned tallgrass prairie, in bison grazed, cattle grazed, and ungrazed areas, focusing on microbially mediated N cycling transformations. We predicted that all soil N cycling rates would be higher, and that soil microbial N limitation would be lower, in grazed relative to ungrazed treatments, and that bison and cattle would have similar magnitudes of influence on soil N cycling. In addition, we considered that the influence of grazing on soil N cycling rates may vary due to differences in soil water availability stemming from precipitation variability, which is a primary control over ecosystem and N cycling dynamics in tallgrass prairie and other grasslands around the world, with higher water generally promoting more plant production and faster N cycling (Groffman et al. 1993; Broderick et al. 2022; Chen et al. 2022). To assess the predictions, we sampled upland soils in annually burned watershed-scale experimental grazing treatments at the Konza Prairie Biological Station (KPBS), each summer from 2020 through 2022, and measured resin-bound inorganic N (a proxy for the amount of mineralized N available for plant and soil microbial uptake through a growing season), nitrification potential rates, denitrification potential rates, denitrification enzyme activity rates, and hydrolytic extracellular enzyme activity rates (which were also used to calculate an index of soil microbial N limitation).

## Methods

### Study site and sampling design

The KPBS is a 3487-ha tallgrass prairie preserve located in the Flint Hills region of northeastern

Kansas near Manhattan, KS, USA (39° 05′ N, 96° 35′ W). KPBS is situated on one of the last remaining tracts of tallgrass prairie, was established as a research station in 1971, and became host to a Long-Term Ecological Research (LTER) project in 1980. KPBS maintains watershed scale treatments of differing fire intervals. Bison were reintroduced to a subset of these experimental watersheds between 1987 and 1992, and cattle were introduced to another subset of watersheds in the 1990s. Bison are stocked at 0.4 ha per animal unit month (AUM: the forage required to feed a 454 kg animal or its equivalent for 1 month), or 0.98 acres per animal, reproduce on-site, and are present year-round (Blair 2023); while cattle graze annually as cow-calf pairs between May 1 to October 1 and are stocked at 0.7 ha per AUM or 1.7 acres per cow-calf pair (Olson 2023), but for a shorter period of the year (April through October). Dominant plants of this area include *Sorghastrum nutans*, *Andropogon gerardii*, *Schizacryum scoparium*, *Panicum virgatum*, *Amorpha canescens* and *Rhus glabra*. Mean annual precipitation (MAP) at this site is 899 mm and mean annual temperature (MAT) is 12.5 °C.

For this study, research was restricted to upland soils, to control for variability in soil type. Samples were collected on the Florence-Benfield complex soil map unit (Clayey-skeletal, smectitic, mesic Udic Argiustolls and Fine, mixed, superactive, mesic Udertic Argiustolls), which is widespread across the Flint Hills of Kansas. Soil sampling was undertaken once late in each summer growing season from 2020 to 2022. These years spanned a range of above-average rainfall (2020) to well below average (2021) and slightly below average (2022) (Fig. 1). We sampled along four 10-m transects, parallel to long-term plant sampling transects in each experimental watershed, in two bison grazed (N1A and N1B), two cattle grazed (C1A and C1B), and two ungrazed (1D and SpB) watersheds, all of which are burned annually. We acknowledge that landscape-scale replication of grazing treatment is low in this study; however, the field experimental design provides good standardization of soil type, which often affects baseline levels of microbial N-cycling (Zeglin et al. 2007).

Each transect covered six sampling points at 0, 0.1, 0.5, 1, 5, and 10 m, from which 2-cm diameter mineral soil samples were collected with an Oakfield corer (Oakfield, WI, USA), to a depth of up to 15 cm. Sample locations at 0, 1, 5, and 10 m were

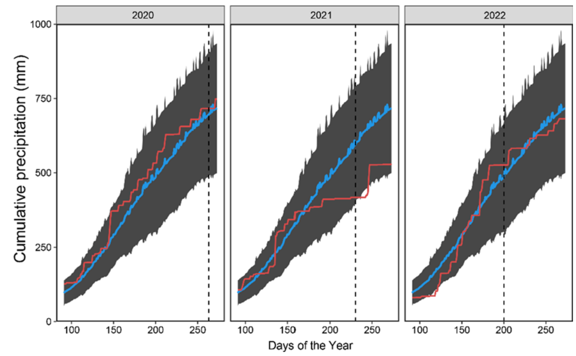


**Fig. 1** Cumulative precipitation on KPBS in the 2020, 2021, and 2022 growing seasons. Historic (30-year) average precipitation is denoted by a blue line overlaying the gray one standard deviation boundaries. Accumulated precipitation is denoted by the red line for each respective year, and dashed vertical lines indicate soil sampling dates for the year

geolocated using the WGS84 datum with a Garmin GPSMAP 64x (Garmin, Olathe, KS, USA). Samples were taken using sterile technique, i.e., while wearing nitrile gloves, and by washing the corer in ethanol between each sample. Samples were stored in a cooler on ice and transported to the lab, where all samples were aseptically sieved using a No. 4 (4 mm) sieve, to remove rocks and plant roots while largely retaining soil aggregate structure. A portion of each soil sample was frozen and stored at – 20 °C before soil physical analysis, and the remaining fresh soil was stored at 4 °C for no more than 48 h before measuring N cycling activity potential rates.

## Soil physical characteristics and N-cycling rates

Soil water content was measured gravimetrically by drying soil at 105 °C for 24 h. Soil pH was measured from a 1:3 slurry of field-moist soil and DI $H_2O$, on samples collected in 2021 and 2022. Soil available N was measured using ion exchange resin bags installed from June to September (Baer and Blair 2008; Nieland et al. 2021) in 2021 and 2022. Resin bag sorbed $NH_4^+$–N and $NO_3^-$–N was quantified using a modified indophenol method and $VCl_3$/Griess reagent method (Hood-Nowotny et al. 2010), respectively, and measured spectrophotometrically with a Filtermax F5 Multimode Microplate Reader (Molecular Devices, San Jose, CA, USA).

We measured rate potentials of two microbially-mediated N transformation processes, nitrification and denitrification, and we measured denitrification potential in two ways, to learn about different limiting factors on soil N cycling. Nitrification is the process of oxidation of ammonium to nitrate, and is an important consideration in soil fertility since fewer types of plant can readily assimilate both ammonium and nitrate, while nitrate is more easily lost from the soil due to leaching or denitrification. Denitrification is the process of reduction of nitrate to dinitrogen gas or nitrous oxide, atmospheric gases that are not useful to plants. Nitrification and denitrification processes bring energy to different types of specialist microorganisms, and are thus limited by the abundance of those organisms, as well as the availability of ammonium and oxygen (for nitrification), and the availability of nitrate, dissolved organic carbon, and anoxic conditions (for denitrification). We assayed both processes in lab incubations with saturating levels of substrates and optimal levels of oxygen, effectively measuring an index of maximum microbial potential for nitrification and denitrification, and also measured denitrification rates in assays with no substrate added to understand process rates under ambient soil conditions.

Nitrification potential (NP) rates were measured in an aerobic soil slurry amended with saturating concentrations (250 μM) of $NH_4^+$-N, and shaken at 120 rpm. After 0.25 and 24 h, 1 ml of each soil slurry sample was transferred into a 1.5 ml tube, centrifuged at 15,000 rpm, and the supernatant was frozen at -20° C until measuring $NO_3^-$–N as described above (Taylor et al. 2010). The resulting increase in $NO_3^-$–N over time, due to ammonia oxidation by soil microorganisms, was used to calculate the maximum nitrification potential rate of each soil sample.

Denitrification potential activity (DNP) and denitrification enzyme activity (DEA) were measured in parallel (Groffman et al. 2009; Nieland et al. 2021). Both are estimates of the reduction of $NO_3^-$–N to $N_2O$–N in an aerobic soil slurry in the presence of acetylene, which prevents the transformation of $N_2O$–N to $N_2$–N. DEA is defined as the maximum enzymatic potential at which denitrification can occur over 1 h, measured with the addition of both glucose and $KNO_3$, which provides the optimal resources necessary for bacterial denitrification. In contrast, DNP assays were not amended, reflecting denitrification rates attainable under levels of nitrate and carbon availability in the soil sample, and measured over a 4-h period. The production of $N_2O$–N used to calculate DNP and DEA was measured using a Shimadzu 2014 GC analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA).

## Soil extracellular enzyme activity (EEA) and microbial N limitation

The enzymatic hydrolysis of amino acids and amino sugars from soil organic matter controls soil fertility by limiting the rate of net N mineralization from biologically inaccessible soil N into forms that can be assimilated by plants and microorganisms (Schimel and Weintraub 2003; Sinsabaugh et al. 2009). The expression of these enzymes is generally regulated by product suppression, i.e., if soil N availability is higher, then N demand is lower, and fewer enzymes to produce available N are synthesized relative to synthesis of enzymes catalyzing hydrolysis of bioavailable C or P (Allison and Vitousek 2005; Nieland et al. 2024).

For this study, we measured the activity potential of two common N acquiring enzymes, β-N-acetylglucosaminidase (NAG; EC 3.2.1.14, 4-MUB-N-acetyl-β-D-glucosaminide) and leucyl aminopeptidase (LAP; EC 3.4.11.1, L-leucine-7-amido-4-MC), as well as one carbon acquiring enzyme, β-glucosidase (βG; EC 3.2.1.21, 4-MUB-β-D-glucoside). Hydrolytic enzyme activity rates were measured using fluorometric substrates (methylumbelliferone (MUB) for NAG and BG, and methylcoumarin (MC) for LAP). Soil samples were thawed and 1 g of each soil sample was added to a solution of 100 ml 50 mM sodium acetate buffer (pH 5), forming a slurry. We combined 200 μl of soil slurry and 50 μl of the target substrate in 96 well assay plates, with six analytical replicates and triplicate quench standards per sample and replicate blanks, negative controls, and 200 μM reference standards. Assays for NAG were incubated for 3.5 h, LAP for 16 h, and βG for 2 h. After incubations, reactions were halted with the addition of 10 μl of 0.5 M NaOH, raising the pH to $>8$. Fluorescence of hydrolyzed substrate was measured at excitation/emission of 360/450 nm with a Filtermax F5 Multimode Microplate Reader (Molecular Devices, San Jose, CA, USA). Finally, we calculated indices of soil microbial N limitation: (ln(βG))/(ln(NAG + LAP)), which decreases under conditions of higher N- than C-limitation, and ((ln(NAG + LAP)/

ln(Phos)), which increases under conditions of higher N- than P-limitation (Sinsabaugh and Shah 2012).

### Data analysis

All statistical analysis was done using the R programing language in the R studio interface for statistical analysis (R Core Team 2022). While sampling was performed using a log-distance design to assess spatial heterogeneity patterns, this structure was surprisingly weak, so we proceeded with a standard statistical approach. To test for the direct and interactive effects of grazing treatment and year on soil characteristics and N cycling rates, we used two-way analysis of variance (ANOVA) models, with post-hoc Tukey's honest significant difference (HSD) tests for pairwise comparisons of within-group differences. Coefficient of correlation ($R^2$) was used to assess linear relationship strength between soil characteristics, N cycling rates, and EEAs. For each variable, diagnostic Q-Q plots and histograms were used to assess assumptions of statistical normality; if these assumptions were not met, a square root or natural log transformation was used to shift the data distribution to better satisfy normality assumptions. Statistical results with a P-value of $<0.05$ are reported in the text.

## Results

### Soil water content, pH, and available N

Soil gravimetric water content and pH varied with both grazing treatment and year, independently of one another (Table 1). Mean soil water content at the time of sampling was highest in 2020 and lowest in 2022, and was also higher in ungrazed soils than in bison or cattle grazed soils (Fig. 2). Soil pH was measured higher in 2022 than 2021, and was also consistently higher in bison grazed soils than in cattle grazed or ungrazed soils (Table 1, Fig. 2). Resin-sorbed N responses to grazing and year were also independent of one another (Table 1): In 2022, resin-sorbed $NH_4^+$–N, $NO_3^-$–N, total inorganic N, and $NO_3^-$–N:$NH_4^+$–N were all higher than in 2021 (Fig. 3); also, bison grazed treatments had higher resin-sorbed nitrate and total inorganic N than ungrazed and cattle treatments, but resin-sorbed $NH_4^+$–N and the ratio of $NO_3^-$–N:$NH_4^+$–N did not respond to grazing (Fig. 3).

### Nitrification and denitrification potentials

Soil nitrification potential (NP) responded to grazing and year independently, such that NP was lower in 2022 than in 2020 or 2021, and was also

**Table 1** Two-way ANOVA results (F statistic and P values) showing the direct and interactive effects of grazing and sampling year on soil GWC and pH, resin-bound N, N-cycling potential rates, and extracellular enzyme activities

| Response variable | Graze F, P | Year F, P | Graze * Year F, P |
|---|---|---|---|
| $GWC^{Sqrt}$ | **16.0, < 0.001** | **83.1, < 0.001** | 1.17, 0.32 |
| pH | **22.3, < 0.001** | **6.52, 0.012** | 2.36, 0.10 |
| $NH_4^+$–$N^{ln}$ | 1.32, 0.27 | **262.2, < 0.001** | 1.18, 0.31 |
| $NO_3^-$–$N^{ln}$ | **12.8, < 0.001** | **24.9, < 0.001** | 2.37, 0.10 |
| $NO_3^-$–N + $NH_4^+$–$N^{ln}$ | **13.1, < 0.001** | **34.3, < 0.001** | 2.16, 0.12 |
| $NO_3^-$–N: $NH_4^+$–$N^{ln}$ | 2.49, 0.086 | **84.2, < 0.001** | 0.37, 0.69 |
| $NP^{ln}$ | **20.4, < 0.001** | **6.36, 0.002** | 0.37, 0.83 |
| $DNP^{ln}$ | 2.29, 0.11 | **36.8, < 0.001** | 1.35, 0.25 |
| $DEA^{sqrt}$ | **13.7, < 0.001** | **28.6, < 0.001** | **1.35, < 0.001** |
| $BG^{ln}$ | 2.20, 0.11 | **62.5, < 0.001** | 0.67, 0.62 |
| $CBH^{ln}$ | 3.03, 0.050 | **47.6, < 0.001** | 1.72, 0.15 |
| $NAG^{ln}$ | **5.70, 0.004** | **60.1, < 0.001** | **2.72, 0.030** |
| $LAP^{ln}$ | **14.5, < 0.001** | **138.2, < 0.001** | **9.41, < 0.001** |
| $Phos^{ln}$ | 2.21, 0.11 | **47.4, < 0.001** | 2.37, 0.053 |
| lnBG:ln(NAG + LAP) | **11.0, < 0.001** | **4.03, 0.019** | **2.93, 0.021** |
| ln(NAG + LAP):ln(Phos) | **6.90, 0.001** | 1.89, 0.15 | **3.23, 0.013** |

Bolded values indicate P < 0.05

Sqrt denotes square root transformation and ln denotes natural log transformation

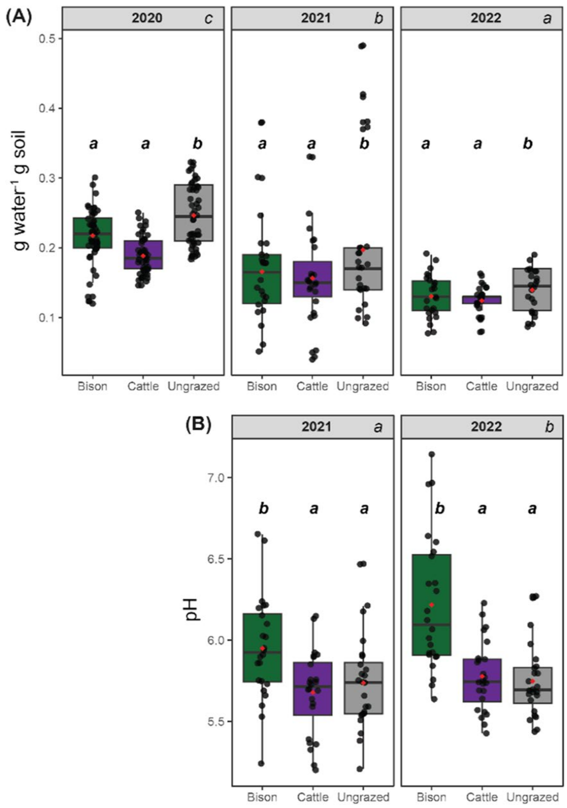


**Fig. 2** **A** Soil gravimetric water content over a 3-year period in grazed and ungrazed soils and **B** soil pH over a 2-year period. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (x-axis) that differed from each other at P < 0.05

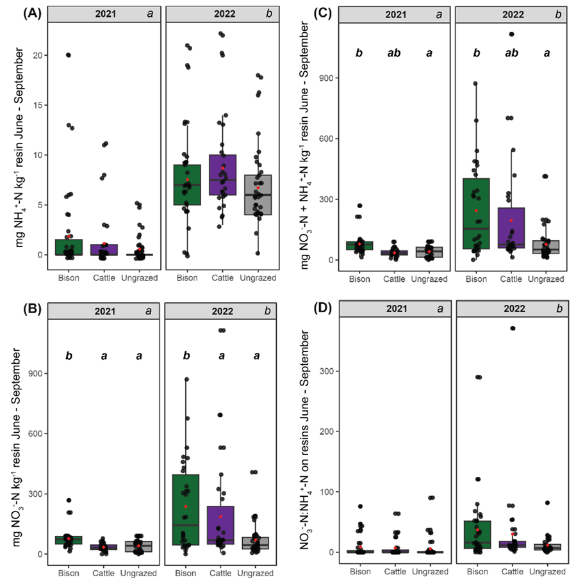


**Fig. 3** **A** $NH_4^+$–N, **B** $NO_3^-$–N, **C** $NO_3^-$–N + $NH_4^+$–N, and **D** $NO_3^-$–N: $NH_4^+$–N sorbed to resin bags through the summer growing season in two study years. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (center) that differed from each other at P < 0.05

consistently higher in bison grazed soils than cattle grazed or ungrazed soils (Table 1; Fig. 4). Soil denitrification potential (DNP) did not respond to grazing treatment, but was higher in 2020 than in 2021 or 2022 (Table 1; Fig. 4). Notably, DNP rates were only 14% (on average) of the denitrification enzyme activity (DEA) when detectable, and were below detectable limits in 2022 and in cattle and ungrazed treatments in 2021. DEA responses to grazing varied interannually (Table 1). In 2020, soil DEA in bison grazed treatments was greater than in both cattle grazed and ungrazed treatments; in 2021, DEA was higher in cattle grazed than in ungrazed soils, and intermediate in bison grazed soils; and in 2022, DEA was higher in bison grazed than in ungrazed soils, and intermediate in cattle grazed soils (Fig. 4).

## Soil extracellular enzyme activities and relative N limitation

Extracellular enzyme activity potentials varied interannually, tending to be highest in 2022 and lowest in 2020 (Table 1; Fig. 6). Only N-acquiring enzyme activities (NAG and LAP) and the indices of N demand relative to C demand (lnBG:ln(NAG + LAP)) and relative to P demand (ln(NAG + LAP):lnPhos) responded to grazing treatment (Table 1; Figs. 5, 6). In 2020, the ungrazed treatment soils were most N-limited relative to C and P, cattle grazed soils were least, and bison grazed soils were intermediate; in 2021, bison grazed soils were less N limited relative to C and P than either cattle grazed or ungrazed soils; and in 2022, bison grazed soils were less N limited relative to C than either cattle grazed or ungrazed soils, and less N limited relative to P than ungrazed soils (Fig. 5).

## Correlations

Many linear model correlations among soil characteristics and N cycling parameters were statistically

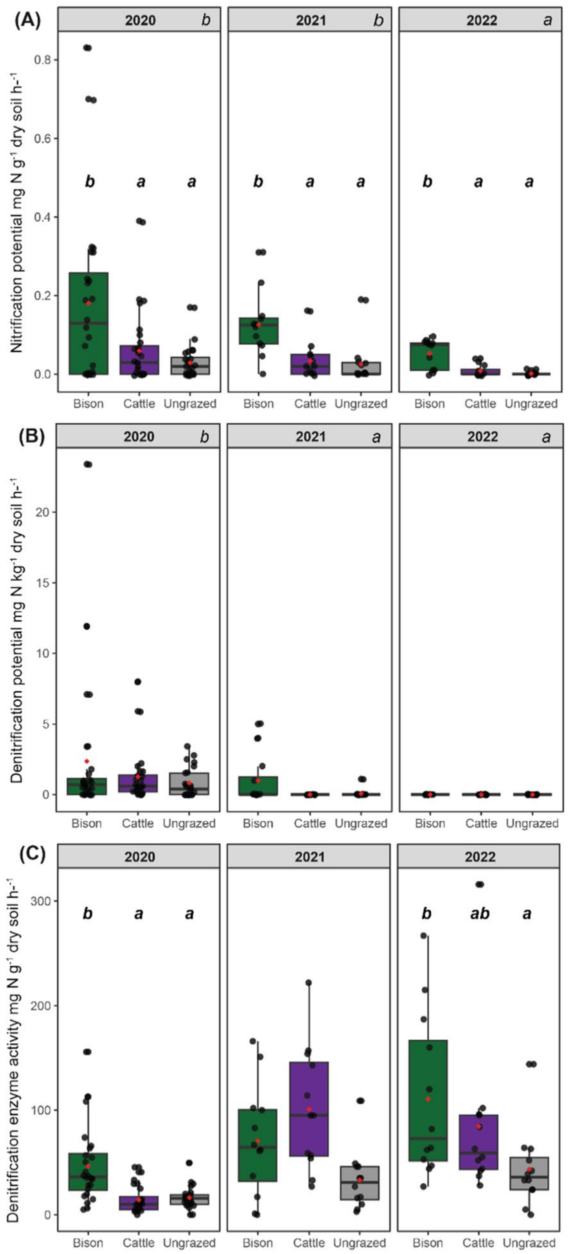


**Fig. 4** **A** NP, **B** DNP, and **C** DEA in soils from different grazing treatments over 3 years. Tukey's HSD post-hoc results are shown with different letters indicating years (top) or grazing treatments (center) that differed from each other at P < 0.05

significant, but none had an $R^2$ value higher than 0.15 (Table 2). Soil water content was positively correlated with DNP and negatively correlated with DEA, and DNP and DEA were negatively correlated with one another. Soil pH was positively

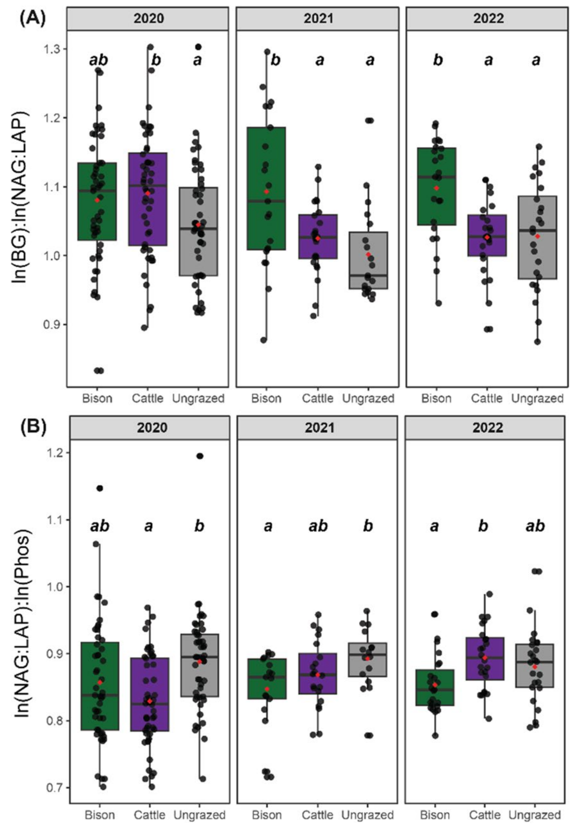


**Fig. 5** **A** ln(BG):ln(NAG:LAP) and **B** ln(NAG:LAP):ln(Phos), indicators of relative soil microbial demand for C and N, and N and P, respectively, in soils from different grazing treatments over 3 years. Tukey's HSD post-hoc results are shown with different letters indicating grazing treatments that differed from each other at P < 0.05 level each year

correlated with resin-sorbed $NO_3^-$–N, NP, and lnBG:ln(NAG + LAP). Resin-sorbed $NH_4^+$–N was positively correlated with resin-sorbed $NO_3^-$–N, but negatively correlated with NP. Microbial N-limitation index values (lnBG:ln(NAG + LAP)), which are higher in less N-limited conditions, were positively correlated with both NP and DNP.

## Discussion

We wanted to learn whether bison and cattle influence soil microbial N cycling activities in tallgrass prairie similarly, and investigated this question over a three-year period. Our results show that bison and cattle grazing have qualitatively similar but

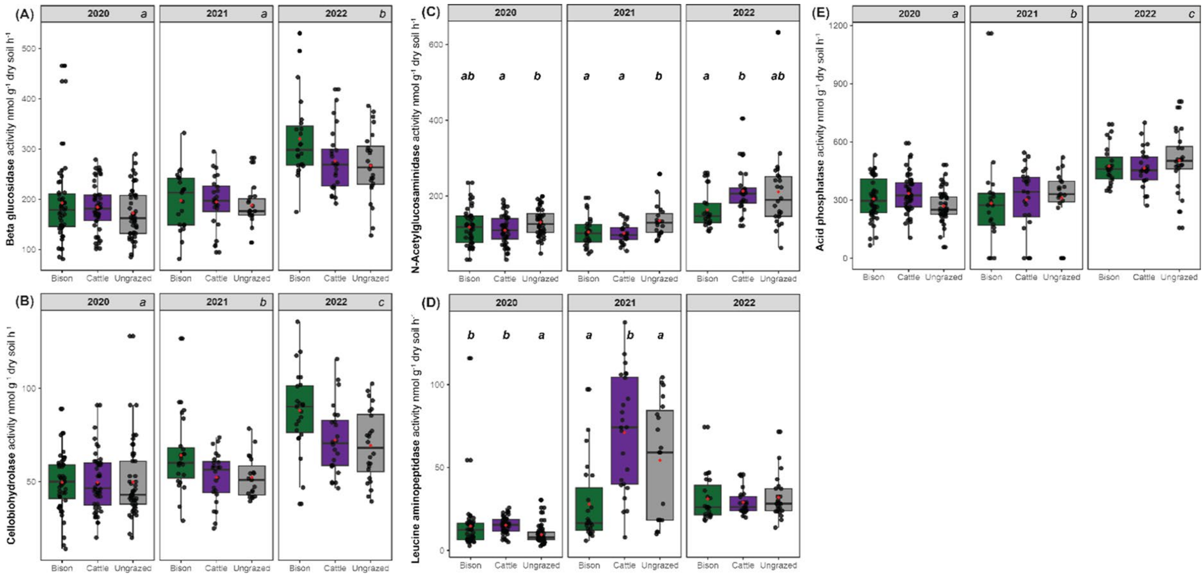


**Fig. 6** **A** Beta glucosidase, **B** cellobiohydrolase, **C** N-acetyl glucosaminidase, **D** leucine aminopeptidase, and **E** acid phosphatase enzyme activity rates (nmol substrate released $g^{-1}$ dry soil $h^{-1}$) through the summer growing season in 3 sampling years. Tukey's HSD post-hoc results are shown with different letters indicating years or grazing treatment units within year that differed at the $P<0.05$ level

**Table 2** Correlation test results (r statistic and P values) among soil GWC and pH, N-cycling rates, and the microbial N limitation index

| r, P | $GWC^{Sqrt}$ | pH | resin-$NH_4^+$–$N^{ln}$ | resin-$NO_3^-$–$N^{ln}$ | $NP^{ln}$ | $DNP^{ln}$ | $DEA^{sqrt}$ |
|---|---|---|---|---|---|---|---|
| pH | − 0.06, 0.44 | | | | | | |
| resin-$NH_4^+$–$N^{ln}$ | − 0.17, 0.12 | 0.01, 0.96 | | | | | |
| resin-$NO_3^-$–$N^{ln}$ | − 0.14, 0.20 | **0.27, 0.013** | **0.35, < 0.001** | | | | |
| $NP^{ln}$ | − 0.02, 0.80 | **0.36, 0.002** | **− 0.27, 0.031** | 0.15, 0.25 | | | |
| $DNP^{ln}$ | **0.23, 0.005** | 0.15, 0.20 | − 0.24, 0.050 | 0.11, 0.35 | 0.14, 0.09 | | |
| $DEA^{sqrt}$ | **− 0.34, < 0.001** | 0.09, 0.47 | − 0.02, 0.89 | 0.03, 0.81 | 0.13, 0.11 | **− 0.24, 0.004** | |
| lnBG:ln(NAG + LAP) | 0.02, 0.74 | **0.28, 0.001** | 0.08, 0.46 | 0.12, 0.30 | **0.24, 0.004** | **0.39, < 0.001** | 0.02, 0.86 |

Bolded values indicate $P<0.05$

Sqrt denotes square root transformation and ln denotes natural log transformation

quantitatively different effects: Bison and cattle both tended to increase N availability and N cycling activities in comparison to ungrazed soils, but the influence of bison tended to be stronger than that of cattle. Specifically, soil pH, resin-sorbed nitrate and nitrification potentials (NP) were consistently highest in bison-grazed soils, and microbial N-limitation was lowest in bison-grazed soils in two of the three sampling years. Also, despite interannual variability in cattle versus bison responses, microbial N-limitation was always highest, and denitrification enzyme activity (DEA) was always lowest, in ungrazed soils relative to both cattle- and bison-grazed soils. However, the magnitude of temporal variation was stronger than grazing effects for soil water content, resin-sorbed N, nitrification potential (NP), and denitrification potentials (DNP). Resin-sorbed N was lowest in the driest year of the study, and both NP and DNP were highest, while DEA was lowest, in the wettest year of the study. Finally, while coarse relationships among measured variables support mechanistic discussion points at the interannual and grazing treatment scale, correlations were not strong enough to suggest predictive relationships among soil water content, pH, and microbial N-cycling variables at the soil sample scale.

### Interannual variation in soil water and microbial N cycling

Soil water content at the time of sampling was higher in 2020 than in 2021 and 2022, while summer precipitation was within the historic 95% confidence interval range in 2020 and 2022, but not in 2021, which was notably dry (Figs. 1, 2). Levels of $NO_3^-$–N, $NH_4^+$–N, and $NH_4^+$–N + $NO_3^-$–N sorbed to resin bags coincided with this variability in water, such that with less than average precipitation there was less mineralized inorganic N (Fig. 3). In 2020, both NP and DNP were highest in magnitude, suggesting that wetter soil conditions supported higher mineralization and mobilization of the ammonium and nitrate substrates driving these two microbial metabolic activities (Fig. 4). Furthermore, extracellular enzyme activities (particularly N-acquiring activities) were lowest, indicating greater product availability (particularly of soluble nitrogenous compounds) and lower investment into enzyme production acquisition (Sinsabaugh and Follstad Shah 2012; Burns et al. 2013), during the wet year (Figs. 5, 6). The resin-sorbed N and microbial N-cycling datasets support the conclusion that interannually, N availability and N-cycling rates are positively associated with seasonal precipitation and soil water content.

The process of denitrification has complex controls, including limitation by nitrate availability, C availability, or low anoxia (Wallenstein et al. 2006; Robertson and Groffman 2014), and because DNP rates never reached DEA rate potential levels (Fig. 4), at least one of these factors must have limited the process. Anoxic conditions would have been highest in the wettest year, when oxygen diffusion into the soil pore space was most restricted. While we did not measure soil C availability, at the landscape level, growing season precipitation has a stronger effect than grazing on annual forage growth measured as aboveground net primary productivity (ANPP), as well as on root production (Johnson and Matchett 2001; Fay et al. 2003), so C availability from plant production should also have been highest in the wettest year. However, DNP rates remained only 9% (on average) of DEA rate potentials in the 2020 sampling year. Further, while DEA rate potentials did not decrease during the dry year of 2021, DNP dropped substantially, and was only detectable in bison-grazed soils where N availability was highest. Based on these observations, denitrification in this system is likely more limited by nitrate than by water, anoxia, or carbon, and only conditionally high, in agreement with conclusions made using the in situ amended core incubation technique by Groffman et al. (1993). The lack of recovery of DNP in 2022, despite a wetter summer overall, could be related to the long period of time following a precipitation event preceding the sampling time (Fig. 1), since as soils dry, soluble nutrient availability also declines, until rewetting stimulates pulses of microbial activity (Schimel 2018).

### Grazing and N availability

Mineralization of N from soil organic matter is a primary microbial mechanism that makes N available for plant and microbial uptake, and is controlled by microbial enzyme activity (Tabatabai et al. 2010). Resin bags are an index, not an in situ measure of mineralization, yet they can provide a reliable indicator of N mineralized during the growing season (Baer and Blair 2008; Nieland et al. 2021). Further, ratios of extracellular enzyme activities are indicative of relative microbial investment in N acquisition (Sinsabaugh and Follstad Shah 2012). N availability was higher in bison-grazed soils than ungrazed soils using both of these indicators in 2021 and 2022 (Figs. 3, 5). It is plausible that higher water availability was connected to the weaker bison effect on soil microbial N limitation in 2020, if wetter conditions supported greater inorganic N mineralization and lower N limitation overall, but unfortunately, we do not have resin-sorbed N data from 2020 to corroborate this interpretation. Still, the evidence points to increased N availability in bison treatments for microbial immobilization and plant assimilation, relative to both cattle grazed and ungrazed treatment soils.

Despite the general bison grazing effect on N availability, specific N-cycling enzyme activities did not all respond the same. For example, in cattle and ungrazed treatments, polypeptide-decomposing

(LAP) activity supported higher N demand in 2021, while microbial cell wall-decomposing (NAG) activity did in 2022, suggesting that different components of the soil organic N pool were microbial N sources in each year (Fig. 6). Also, nitrate–N rather than ammonium-N responded to grazing treatment, suggesting that either ammonium uptake was lower, or nitrification was higher, in bison grazed soils (Fig. 3). Notably, despite interannual variation, bison grazed soils maintained a higher (less acidic) pH than cattle grazed soils (Fig. 2). Soil pH broadly constrains soil chemical transformations and the microbial enzymatic activities which drive N cycling, such that different N sources may support soil microbial N demand under different pH conditions due to changes in available N and microbial enzyme production (Sharpley 1991; Zeglin et al. 2007; Sinsabaugh et al. 2008; Nannipieri et al. 2018; Barber et al. 2023). In this study, soil pH was likely affected by bison grazing activity through urine and dung inputs, which add alkalinity to the soil (Somda et al. 1997; Hong et al. 2021).

## Grazing and nitrification potential

Both NP and resin-sorbed $NO_3^-$–N were consistently higher in bison treatments (Figs. 3, 4), suggesting that under bison grazing, there is a higher likelihood of $NO_3^-$–N becoming mobile in soil solution, and subsequently being taken up by plants or microorganisms, reduced by microorganisms and denitrified, or leached out of the soil. Overall, even though nitrification is a precursor for N loss through either nitrate leaching or denitrification, lower denitrification rates relative to nitrification rates suggest loss of soil N to the atmosphere in tallgrass prairie is a comparatively small factor in the N cycle at this site (Groffman et al. 1993; Blair et al. 1998). While nitrate leaching has not been constrained, we do know that stream water nitrate concentrations are low in this watershed despite the long-term grazing pressure (Dodds et al. 1996), and that local grasses can rapidly assimilate nitrate (Dell and Rice 2005); so while possible, nitrate production is not necessarily strongly tied to N leaching losses at the study site.

In addition to higher inorganic N availability overall, the consistently higher pH in bison grazed soils may boost NP through direct effects on ammonium availability. Chemically, soil pH controls the proportion of ammonia ($NH_3$) in soil solution as ammonium ($NH_4^+$), which in turn affects N availability for the process of nitrification (Kemmitt et al. 2006; Sahrawat 2008): With a higher pH, the non-protonated form (ammonia) is favored, which supplies more of the substrate for ammonia monooxygenase, the rate limiting enzyme of nitrification found in both ammonia oxidizing bacteria and archaea (Nicol et al. 2008). This could help explain the positive relationships between pH and NP, and pH and resin-bound nitrate (Table 2). This finding is notable because nitrifier metabolism is the least functionally redundant in soils of all of the N-cycling processes measured in this study (Prosser and Nicol 2012) suggesting in turn that bison treatments could have higher abundance and/or a pH-specialized population of soil nitrifiers (Prosser and Nicol 2012), or a higher NP due to a more optimal soil pH.

## Grazing and denitrification potential

Denitrification, the process of $NO_3^-$–N reduction to gaseous form, was measured in two ways: Under in situ soil N and C availability conditions (DNP) and with nitrate and DOC added to the assay to measure maximum denitrification enzyme activity (DEA). We found that DNP was much lower than DEA, especially in the drier years of 2021 and 2022 (Fig. 4). This indicates that while microbial biomass with enzymatic potential for denitrification exists, because either soil N or C substrate was limiting, little denitrification potential was realized. Only in bison grazed soils, where NP and nitrate availability was higher, was any DNP detected in 2021. In contrast, DEA was not different between bison and cattle treatments in 2021 or 2022, despite differences in NP and nitrate availability, while ungrazed treatments exhibited consistently lower NP, resin-sorbed nitrate, and DEA (Figs. 3, 4). This suggests that grazing intensity in general impacts soil DEA in some biologically similar way. Because many bacterial taxa carry the genetic potential to produce enzymes in the denitrification pathway (Nelson et al. 2016), whether or not conditions allow these enzymes to be used, general changes in the soil microbial community are more likely to affect DEA than the substrate-limited DNP (Wallenstein et al. 2006). At the same tallgrass prairie field site, bison dung is a microbial dispersal vector that increases soil microbial diversity and changes microbial community composition (Hawkins and Zeglin 2022), and other investigations show that grazing by cattle, sheep, and goats can affect soil microbial composition (Clegg 2006; Eldridge et al. 2017; Wang et al. 2019). However, the redundancy of bison and cattle effects on the soil microbiome, and implications for denitrification, are not yet understood.

### Grazing and grassland soil N cycling

Bison grazing substantially increased soil N-cycling rate potentials at this site in the North American Great Plains, and these elevated rates (NP of 0.05–0.5 μg N $g^{-1}$ dry soil $h^{-1}$, DEA of 30–100 μg N $kg^{-1}$ dry soil $h^{-1}$) were similar to those measured in other grazed grasslands worldwide. In Mongolia, grasslands that were grazed by sheep had an estimated NP of 0.5–1 μg N $g^{-1}$ dry soil $h^{-1}$ and DEA of 400–700 μg N $kg^{-1}$ dry soil $h^{-1}$ (Yingjin et al. 2022); in tropical savanna in the Ivory Coast, NP was 0–10 μg N $g^{-1}$ dry soil $h^{-1}$and DEA was 0–100 μg N $kg^{-1}$ dry soil $h^{-1}$ (Srikanthasamy et al. 2018). In cattle grazed Australian grasslands, NP was 0–0.5 μg N $g^{-1}$ dry soil $h^{-1}$ and DEA was 20–40 μg N $kg^{-1}$ dry soil $h^{-1}$ (Mehnaz and Dijkstra 2016). Comparatively, our N cycling rates were close to those estimated in Australian grasslands but a bit lower than those estimated in Mongolia or West African savanna, suggesting that higher grazing intensity and more N recycling is occurring at those two sites and/or the physical and chemical soil properties and biological conditions for NP or DEA were different in the other grassland types. Historically, at the same tallgrass prairie site that we studied, but using different methods, rates were in a similar range as currently estimated: NP was 0.17–0.23 μg N $g^{-1}$ dry soil $h^{-1}$ and DEA was 180–286 μg N $kg^{-1}$ dry soil $h^{-1}$ (Groffman et al. 1993). Comparatively, given the three decades of additional grazing pressure between these two studies, it is somewhat surprising that rates are so similar. However, because variable precipitation mediates the magnitude of N-cycling, rates could be more strongly linked to the direct and indirect effects of soil water than to grazing intensity over time.

This tallgrass prairie region and world as a whole is predicted to have more variable climactic conditions in the future (IPCC 2022) impacting soil water and thus large grazers and microbial N cycling activities in this region and many others (Nippert et al. 2022; Abraham et al. 2023). Our nitrification potentials and denitrification estimates suggest losses of N from the soil are possible, and that higher losses are possible in bison-grazed areas. A recent meta-analysis of greenhouse gas emissions on grasslands showed that heavy grazing intensity did not increase $N_2O$-N emissions, but instead overgrazing severely degraded rangeland habitat leading to soil runoff (Tang et al. 2019). This result suggests that management is the key to mitigating grazing animal effects on N loss from rangelands, specifically, maintaining proper stocking densities based on set carrying capacity (Holechek et al. 2011) and considering how the physical movement of animals controls N export and spatial heterogeneity (Coetsee et al. 2023).

Despite the clear enhancement of soil N-cycling rates, particularly nitrification potential, by bison grazing in this study, we do not know whether nitrate was subsequently leached out, assimilated by plants, or immobilized by microorganisms and retained in soil organic matter. Because the physical properties of these soils promote the retention of N in mineral-associated fractions (Soong and Cotrufo 2015) and the native biota conserve N tightly in both soil microbial biomass and plant tissue (Dell et al. 2005), elevated internal cycling rates may be coupled with N turnover through biotic pools, rather than linked to N losses from the ecosystem. Compared to ungrazed and annually burned tallgrass prairie, N cycling is not as "open" i.e., N recycling is slower and more closed in ungrazed tallgrass prairie even though there are larger inorganic N pools then grazed prairie (Connell et al. 2020). As such, ecosystem N retention may be high in all treatments of this study because they are burned annually (Dell et al. 2005). However, additional research is necessary to directly measure grazing effects on soil and ecosystem N retention.

## Conclusion

We found that, despite interannual variability, bison impacted nitrification differently than cattle because of greater available soil inorganic N, higher soil pH and less N limitation. Also, annual variation in N cycling was apparent because of variability in summer precipitation and soil water availability controlling N cycling. As such, tracking soil characteristics and N cycling activities over time gives primary diagnostic information of N recycling and should strongly be considered in rangeland monitoring health assessments, as it is currently lacking (Pellant et al. 2020). However, due to concomitant differences in bison and cattle behavior (e.g., bison tendency to maintain grazing lawns) with differences in management (i.e., bison graze year-round while cattle are on pasture May–October), we cannot discern whether N cycling effects are inherent to the different grazers per se or to the human-grazer interaction. Human societies past and present around the world have always depended on large grazing animals, in particular the bison, and more recently cattle, in North America. Bison are tied to the existing cultural identity of tribal nations that have lived on grasslands for at tens of thousands of years (Kornfeld et al. 2016), and while in the past bison movement was not directly constrained by humans, bison numbers were orders of magnitude higher across their continental range. Thus, it is also difficult to speculate the extent to which soil fertility changed following the replacement of bison with cattle; however, our data suggest that a significant change was possible. Also knowing that bison grazing can dramatically shift grassland plant diversity (Ratajczak et al. 2022), it is further possible that the interactions between grazers, soils, and plants that maintained Great Plains grassland ecosystems pre-colonialization may have been different from what we understand today. Long term data on N cycling activities are sparse, and considering the data here in the context of grazer management and changing climate will provide useful information for future N cycling and budgeting in grassland ecosystems.

**Acknowledgements** Thank you to Dr. Debra Bolton, Dr. Zak Ratajczak, and Dr. Arnaud Temme for comments and revisions to this manuscript. Thank you to Dr. Matthew Nieland, Brooke Vogt, Domnique Byrd, Harley-Payj Leatherman, and Caroline Gatschet for help with soil sampling, lab work, statistical advice, and comradery. Thank you to the Konza Prairie Biological Station and Long-Term Ecological Research program personnel who have maintained the watershed-scale fire and grazing treatments for decades. The grasslands where we conducted this research are the ancestral homelands of many Indigenous peoples who lived and hunted here before European settlement for thousands of years, including the Kaw (Kaánze), who inhabited and stewarded this area until their forced removal between 1846 and 1872 to their current location in Oklahoma. We acknowledge that since time immemorial, there is a connection of Indigenous peoples to these lands and we pledge to respect and honor their past and current legacy, cultural history, and knowledge. Funding for this research was provided by the National Science Foundation (DEB Ecosystem Science #1943492) and the Grassland Heritage Foundation.

**Author contributions** Authors NVA and LHZ contributed to the study conception and design. Material preparation, data collection and analysis were performed by NVA, KF, JF and JH. The first draft of the manuscript was written by NVA and LHZ. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** This work was supported by NSF-DEB-Ecosystems #1943492 and the Grassland Heritage Foundation.

**Data availability** The data generated and analyzed in this study are publicly available within the Environmental Data Initiative Repository (https://doi.org/https://doi.org/10.6073/pasta/34b4b622191f222c90e7d227291951c8).

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.



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